Ege Journal of
Fisheries and Aquatic Sciences

www.egejfas.org
E-ISSN 2418-3140

EgeJFAS

Su Ürünleri Dergisi

Volume 41 Number 3 **2024**

Ege University Faculty of Fisheries

Authors Guidelines

Thank you for deciding to submit your article to the Ege Journal of Fisheries and Aquatic Sciences (EgeJFAS). The journal welcomes the submission of articles that are of interest and high scientific quality. Authors should check the "Author Guidelines" very carefully before submitting their manuscripts. The instructions given here will ensure that your article's evaluation process (referee, publication, etc.) can proceed smoothly. Make sure your article is prepared and submitted in accordance with journal rules.

Submitted manuscripts will be checked primarily for compliance with journal subjects and rules. Manuscripts not complying with required formatting will be returned for correction. Papers outside the scope of the journal will be rejected.

GENERAL INFORMATION

Aim & Scope

Ege Journal of Fisheries and Aquatic Sciences (EgeJFAS) is open access, international, double-blind peer-reviewed journal publishing original research articles, short communications, technical notes, reports, and reviews in all aspects of fisheries and aquatic sciences.

The journal does not charge any submission and publication fees.

All articles receive DOI, are citable, published in PDF format.

The journal focuses on interdisciplinary studies that present new and useful information to the international scientific community/readership, and contribute to scientific progress. Before submitting your article, make sure it is suitable for the journal scopes.

The main functional areas accepted into the journal are listed as follows:

Marine and freshwater fisheries, Aquaculture, Vertebrate and invertebrate aquaculture (marine/freshwater), Planktonology and plankton culture, Living resources, Management and economics, Aquaponic, Seafood processing technology, Feeding and feed technologies, Fishing technology, Fisheries management, Population dynamics, Disease and treatment, Aquatic microbiology, Biology, physiology, Macroalgae, Biotechnology, Conservation and sustainability, Environments and ecology, Biogeography, Biodiversity, Climate effects, Pollution studies.

Ege Journal of Fisheries and Aquatic Sciences (EgeJFAS) (Su Ürünleri Dergisi) published quarterly (March, June, September, December) by Ege University Faculty of Fisheries since 1984.

The journal is published only as an e-journal since the 1st issue of 2020.

Language

Although articles in English and Turkish are accepted, priority is given to articles prepared in English in order to increase international readability and citation. Limited Turkish articles are published in each issue.

Manuscripts should comply with the standard rules of grammar and style of the language (English or Turkish) with appropriate spelling and punctuation in which they are written.

Editorial Policy and Referee Process

Manuscripts should not be copied elsewhere or submitted to another journal for parallel evaluation. Only original manuscripts are considered. It is evaluated with the understanding that the content is approved by all co-authors. Submitted manuscripts are first checked in terms of journal scope, language, presentation, and style. Manuscripts that are not suitable for these aspects will be returned without review.

In order to evaluate the appropriate articles, at least 2 or 3 external and independent referees who are experts in their fields are appointed by a member of the editorial board/section editor. Each manuscript is reviewed through a double-blind peer-review process (identities of neither authors nor peer reviewers are disclosed). Manuscripts returned to authors with referee reports should be revised and sent back to the editor as soon as possible.

Editor-in-chief/editors take the final decision (Accept, Reject) of the manuscript in line with the reviewer's opinions. All responsibility for the scientific content and expressions in the published article belongs to the authors. In accordance with the publication policies of EgeJFAS, the plagiarism report for the relevant manuscript is requested to be uploaded to the submission system by the responsible author.

Article Types

The types of articles accepted include original research articles (priority), short communications, reviews, reports, and technical notes in all aspects, focusing on interdisciplinary studies in the field of fisheries and aquatic sciences.

Original research papers: These are the article type that the Journal gives the most importance and priority. Should contain data obtained from original studies such as experimental results, field data, and/or theoretical studies.

Short communication: It should include original results and headings, like research papers. Articles provide important new research results/methods or discoveries that do not possible to publish as a full research paper. These articles that are narrowly focused deserve to be published faster than other articles.

Review: Reviews may summarize current research areas of broad importance or provide the readers with an insightful introduction to new and groundbreaking areas of research. It should be examined and discussed in-depth and comprehensively written by the author(s) who have expertise in the subject area, not just the literature surveys. Only invited reviews (in English) are considered for publication. If you would like to submit an invited review, please contact the editor-in-chief (editor@egejfas.org) and upload a review cover letter containing the requested information. As of 2023, reviews in Turkish will not be accepted. Publication of those accepted in the previous year will be completed in 2023.

Reports

Case reports encourage the submission of reports containing feature novel findings or new management strategies. Well-written and illustrated reports are taken into account.

Brief reports are short, observational studies that report the initial results or completion of a study or protocol.

Technical notes: They are short articles that focus on a new technique, method or procedure. It should identify significant changes or unique applications for the method described.

MANUSCRIPT SUBMISSION

The manuscript, when submitted together with the Cover Letter (Submission declaration and verification) and Copyright Form signed by the corresponding author on behalf of all authors, warrants (confirms) that it is original and has not been published elsewhere, has been approved - tacitly or expressly - by all co-authors and the responsible authorities at the institute where the work was carried out. The publisher will not be held legally responsible in case of any claim for compensation.

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Authorship Contributions, Conflict of Interest Statement, Ethics Approval, Data Availability should be written in the article after Acknowledgements and Funding section.

While starting

For submission of your manuscript prepared in accordance with the guideline to EGEJFAS please click here and after logging into your account (if you don't have an account please register at https://dergipark.org.tr/en/ . Your default login ID is your email address. Use your existing account; do not create new accounts with new submissions) use the "Submit Article" button on the home page of the journal to start submission. Before submitting a manuscript, do not forget to check the Submission Checklist.

After log in, the article submission process is completed in 5 steps. Upload your article information, article file, and other necessary documents step by step correctly. There is no transition to the next step until a step is completed.

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Please see our information on Ethical Principles and Publication Policy. Before submission, do not forget to read the "Ethical Responsibilities of the Authors".

Please ensure that any manuscript you submit to this Journal conforms to the Committee on Publication Ethics (COPE) recommendations for ethics, Best Practice Guidelines and as well as to the rules of Egejfas.

PREPARATION OF MANUSCRIPTS

Papers must be clearly written in Turkish or English. Manuscripts should be typed double spaced on A4 size paper in 12-point Times New Roman font including the references, table headings and figure captions with standard margins (25 mm) all around. The author's name should appear centred under the title. Numbered (1) note should give the author's institutional address and an asterisked (*) note should indicate the corresponding author's e-mail address. Degrees and qualifications should not be included.

Line and page numbers should be given from the first page of the manuscript.

Please prepare your typescript text using a word-processing package (save in .doc or .docx).

The complete manuscript should be in a single file containing full text, references, figures and tables. Figures and tables should be inside the manuscript placed properly (not at the end of manuscript). The line number should be given to the whole manuscript.

- Research papers and reviews must not exceed 25 manuscript pages including tables and figures (except systematic checklists).
- Short communications, technical notes, and reports which are results of brief but significant work, must not exceed 10 manuscript pages including tables and figures.

Papers must be clearly written in Turkish or English. Manuscripts should be typed double spaced on A4 size paper in 12-point Times New Roman font including the references, table headings and figure captions with standard margins (25 mm) all around. The author's name should appear centered under the title. Numbered ([1]) note should give the author's institutional address and an asterisked (*) note should indicate the correspondence author's e-mail address. Degrees and qualifications should not be included.

Please prepare your typescript text using a word-processing package (save in .doc or .docx).

The complete manuscript should be in a single file containing full text, references, figures and tables. Figures and tables should be at the end of the manuscript file and the locations should be indicated in the text.

- Research papers and reviews must not exceed 25 manuscript pages including tables and figures (except checklists).
- Short communications, technical notes and reports which are results of brief but significant work, must not exceed 10 manuscript pages including tables and figures.

First Page

The title should be short concise and informative, and be a statement of the main result/conclusion presented in the manuscript. The title should not contain abbreviations. Do not forget to add English title for Turkish article. The title should be written in sentence order.

Author Names and Affiliation

The first name and surname of each author should be clearly listed together and separated by commas. Provide exact and correct author names (forenames-surnames) as these will be indexed in official archives. Occasionally, the distinction between surnames and forenames can be ambiguous, and this is to ensure that the authors' full surnames and forenames are tagged correctly, for accurate indexing online.

Present the authors' affiliation addresses should be indicated at the author's name with superscript numbers immediately after the author's name. The full postal address of each affiliation at the time of research should be listed in order: Department, institution, city with postcode, and country name.

Please clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Provide an active e-mail address of the corresponding author. It is editorial policy to list only one author for correspondence.

ORCID numbers of all authors should be listed on the article title page as of June 2017. Authors who do not have an ORCID number are required to register their number at www.orcid.org. The orcid number is mandatory. Articles that do not have an ORCID number or are incorrect will not be evaluated.

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Abstract

English and Turkish abstracts (contributors who are not native Turkish speakers may submit their manuscripts with an English abstract only) of a maximum of 300 words should be included in all submissions. The abstract should be comprehensible to readers before they have read the full paper, and reference citations must be avoided. In the abstract, the importance of the work should be clearly stated; what, why, how it was done should be answered and the contribution of the results to the scientific world should be expressed. It should not contain undefined abbreviations.

Abstract should clearly the importance of the work described in the paper and reflect what was done, why it was done and what important results were achieved. It should not contain any undefined abbreviations and not be written in the first person.

Keywords

Below the abstract, please provide 4-6 keywords related to the study that will help to increase the discoverability of your manuscript. It is especially important to include words that are fundamental to your manuscript but are not included in the manuscript title or abstract to increase discoverability by indexing services.

Following pages

Following pages should contain the rest of the paper and should be organized into an Introduction, Material and Methods, Results, Discussion, Conclusion(s), Acknowledgements and Funding, Authorship Contributions, Conflict of Interest Statement, Ethics Approval, Data Availability, References. These should be capitalized. Please note that submissions without required documents/statements will not be accepted.

Introduction

Provide clearly and an adequate background, avoiding a detailed literature survey or a summary of the results. State the specific objective or hypothesis of the study.

Material and Methods

Provide adequate detail to allow the work/experiment to be reproduced. Methods already published should be mentioned by references. Significant modifications of published methods and new methods should be described in detail.

If the study requires "Ethics Committee Permission Certificate", be sure to report after the "Acknowledgements" section that permission has been obtained from the relevant institution. A copy of the "Ethics Committee Permission Documents" should be uploaded to the system. A detailed explanation on this subject has been made in the "Ethics Approval" heading above.

Results

Results should be clear and concise. Results for different parameters should be described under subheadings or in separate paragraph. Present your results in a logical sequence in the text, tables, and figures.

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The discussion should not repeat the results, but should provide a detailed interpretation of the data. The discussion should highlight the importance of the work and the resulting new insights. Only in exceptional cases may the results and discussion be combined with the editor's consent. Avoid extensive citations and discussion of published literature.

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This should briefly state the major findings of the study.

Acknowledgements and Funding

Acknowledgements including people, grants, funds, projects, etc. should be kept brief and placed after conclusion section. Names of contributing people should be written clearly and fully.

Examples:

"The authors are grateful to John Nare, for his friendly collaboration and hospitality during the lipid analysis."

"The authors would like to thank Ken More for language revision."

Please clearly and fully specify the relevant funding information (name) with the grant number or codes.

Financial support acknowledgwment should be written like the example given:

"This study was supported by the Turkish Scientific and Technological Research Institution (Grant number: ....)."

"This work was supported by Ege University Scientific Research Projects Coordination Unit. Project Number: …."

"Author Mary Lee has received research support from Company A."

If the research has no specific financial support, please include the following statement:

"This research has not received a specific grant, fund or other support from any funding agency in the public, commercial, or not-for-profit sectors."

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Identifying individual author contributions (CRediT - Contributor Roles Taxonomy, ICMJE-Defining the Role of Authors and Contributors, Transparency in authors' contributions) is important to reduce authorship disputes and facilitate collaboration. The publisher recommends that authors include statements of contribution stating each author's contribution to the work to promote transparency. This gives authors the opportunity to share an accurate and detailed description of their various contributions to the work. The corresponding author is responsible for ensuring that the disclosures are correct and accepted by all authors.

The roles of all authors should be listed. Authors may have contributed to more than one role. These contributions should be placed in the text with the heading of "Authorship Contributions", after the "Acknowledgements" section of the article. See below examples:

Example: All authors contributed to the idea and design of the study. Material preparation and investigation were performed by [full name], [full name] and [full name]. The writing/editing was carried out by [full name] and all authors have read and approved the article.

Example: CRediT author statement (Click for more information about CRediT)

Full name/s: Conceptualization, Methodology, Software

Full name: Data curation, Writing- Original draft preparation

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Full name/s: Supervision

Full name/s: Software, Validation

Full name/s: Project administration, Resources, Funding acquisition

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For review article; it should be stated whose idea, who did the literature survey and data analysis, who wrote the draft, and who revised the criticisms.

For articles produced from student's dissertations or thesis, it is generally recommended that the student is listed as the principal author (A Graduate Student's Guide-APA Science Student Council 2006).

Changes to Authorship

At the time of submission, the author (s) information, the corresponding author and the order of the authors must be correct. Changing the author order, adding/deleting are not allowed during the revision phases. However, in rare cases, it can be applied when detailed and acceptable reasons are presented. All authors must agree with any addition, removal or rearrangement and the reasons for changes should be explained in detail. After the article is accepted, no changes can be made to the authorships.

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Authors should declare if they have any financial or personal relationships with any institution/organization or person that may adversely affect their work. Conflict of interest statement should be attached to the article after the Acknowledgements section.

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In the event of a potential conflict of interest, the authors must state: "The following financial interests / personal relationships may be potential competitive interests."

Conflict of interest statement should be provided even if the authors have no competition or conflict of interest.

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All animal and human experiments conducted in the manuscript research should comply with the ARRIVE guidelines, EU Directive 2010/63/EU, The Code of Ethics of the World Medical Association (Declaration of Helsinki), and National Ethics Committee for Animal Experiments (HADMEK, HADYEK). If there is a human study in the article, it must comply with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

If the submitted article involves the use of animal (vertebrate) and human subjects, authors should prove that they have carried out the manuscript studies in accordance with the relevant laws and regulations and they have received the approval of the authorized institutional committee (s) (including the ethics committee name and reference number, if possible). If a study was granted exemption or did not require ethics approval, this should also be detailed in the manuscript.

Copies of approval should be uploaded to the system under the subheading "Ethics Committee Approval". In addition, an explanation should be added to the article with the title of "Ethics Approval" after the Acknowledgements section.

Examples:

"Approval was granted by the Ethics Committee of University B (Date.../No....)."

"This is an observational study. The ABC Research Ethics Committee has confirmed that no ethical approval is required."

"This article does not contain any human or animal studies performed by any authors."

"The authors declare that all applicable guidelines for sampling, care, and experimental use of animals in this study have been followed."

"Sampling and handling procedures of the fish were in accordance with an …… protocol approved by University of ……..".

"No specific ethical approval was necessary for this study."

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If necessary, an application should be made to the ethics committee and approval should be obtained before starting a study. Generally, retrospective ethical approval cannot be obtained. It may not be possible to consider such articles for peer review. In such cases, it is at the Editor's discretion to decide whether to proceed with the peer review.

Data Availability

Articles are open access and free to use. Published articles are archived permanently. Proper citation is required when using an article published in a journal.

In order for the datasets reflecting the results of the article should be accessible to the readers; the journal encourages that datasets may be stored in public repositories (where available and appropriate) and addressed in the article, provided in the article, or in supplementary files whenever possible, or available from the corresponding author upon request. Regarding data availability, authors can follow one of the ways described. Enquiries about data availability should be directed to the authors. This information should be placed in the text with the heading "Data Availability" after the "Acknowledgements" section of the article. See examples below:

Examples:

Data availability: All of the data summarized in the study are available in the (name) Data Repository, (link address).

Data availability: The data sets generated during and/or analysed during the current study will be provided by the corresponding author upon the request of the editor or reviewers.

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Scientific Style

In writing of systematic /biological papers, international terminology such as "International Codes of Zoological Nomenclature (ICZN), and International Code of Nomenclature for Algae Fungi and Plants (ICNAFP)(Formerly known as the International Code of Botanical Nomenclature - CBN) International Code of Botanical Nomenclature (ICBN)" must be strictly followed. The first mention in the text of any taxon must be followed by its authority including the year. The names of genera and species should be given in italics. Clearly write the full genus name at the first occurrence in the text, and abbreviate it when it occurs again. When referring to a species, do not use the genus name alone; Be careful when using 'sp' (singular) or 'spp.' (plural).

Equations and units

Please ensure that equations are editable. Leave a space on both sides of the <, ±, =, etc. equations used in the text. For units and symbols, the SI system should be used.

Abbreviations

Please define non-standard abbreviations at first use in the text with full form followed by the acronym in parentheses. Use only the acronym for subsequent explanations.

Footnotes

Footnotes should be numbered consecutively. Those in tables or figures should be indicated by superscript lower-case letters. Asterisks should be used for significance values and other statistical data. Footnotes should never include the bibliographic details of a reference.

References

Full references should be provided in accordance with the APA style. The usage of reference managers as Mendeley© or Endnote© or an online reference manager as Citefast with the output style of APA 7th edition is advised in organizing the reference list.

Please ensure that every reference cited in the text is also present in the reference list (and vice versa) and avoid excessive referencing.

In-Text Citation

In-text citation to the references should be formatted as surname(s) of the author(s) and the year of publication (also known as the author-date system).

If a specific part of a source (book, article, etc) is cited directly, a page number should also be included after the date. If the full source is used, the citation page number is not displayed.

For example: Kocataş, 1978, p. 3

Citation can be shown in two ways: Parenthetical Citation or Narrative Citation.

References to be made at the end of the sentence should be shown in parentheses. If the cited reference is the subject of a sentence, only the date should be given in parentheses. There should be no parentheses for the citations that the year of the citation is given in the beginning of the sentence.

Citation examples according to the number of authors are given below.

One author:

Consider the following examples:

-……………(Kocataş, 1978)

- Kocataş (1978) states…….

- In 1978, Kocataş's study of freshwater ecology showed that….

Two authors:

If there are two authors, the surnames of both authors should be indicated and separated from each other by "and", (Geldiay and Ergen, 1972).

Consider the following examples:

-……….(Geldiay and Ergen, 1972)

- Geldiay and Ergen (1972) states……

- Similar results were expressed by Geldiay and Ergen (1972), Kocataş (1978).

More than two authors:

For citations with more than two authors, only the first author's surname should be given, followed by "et al." –in Turkish article 'vd.'- and the date (Geldiay et al.,1971; Geldiay vd., 1971).

See below examples:

-Geldiay et al. (1971) state…….

-…………………………(Geldiay et al., 1971).

There are few studies on this subject (Geldiay et al.,1971).

Two or more works by different author:

When its needed to cite two or more works together, in-text citations should be arranged alphabetically in the same order in which they appear in the reference list and used semicolons to sparate citations.

For example: Several studies have reported similar results (Geldiay and Ergen, 1972; Kocataş 1978; Thurry 1987).

Two or more works by the same author:

If there are two or more works by the same author, list the years of publication in order, earliest first. For example: (Kocataş, 1978, 1979, 1981) or Kocataş (1978, 1979, 1981)

Citation to authors with more than one work in the same year:

The works should be cited as a, b, c, etc. after the date. These letters must be listed alphabetically according to the surname of the first author in the bibliography list.

For Example:

-Geldiay and Ergen, 1972a

-Geldiay and Ergen, 1972a, b

No authors:

If the author is unknown, the first few words of the source should be used and dated.

For example: (A guide to citation, 2017).

In some cases, "Anonymous" is used for the author, accept this as the name of the author (Anonymous, 2001). Use the name Anonymous as the author in the reference list.

No publication date:

If the publication date is unknown, write "n.d." (no date) in the in-text citation.

Example: (Geldiay, n.d.).

Citation to secondary sources:

In scientific studies, citation should be made to the original primary sources. Cite secondary sources when the original work is out of print, not available, or only available in a language you do not understand. If you want to cite a work that you can't find yourself, through a citation from another source, using the phrase "…………as cited in".

For Example:

(Geldiay and Ergen 1972, as cited in Kocataş, 1978)

Personal communication and unpublished results:

Personal communications, such as phone calls, emails, and interviews, are not included in the reference list because readers can't access them. The in-text citation is also formatted slightly differently as follow:

Example:

- Demands have been increasing lately. (A. Kale, personal communication, May 10, 2021).

General use of websites and software:

It should be showed as below.

-The website of Egejfas (www.egejfas.org) includes author guidelines.

-Statistical software SPSS (version 25) was used to analyze the data.

In References

All citations should be listed in the reference list, with the exception of personal communications and unpublished results.

All references must be written in English. If an article is written in a language other than English, give the title in English and indicate the language in which the article is in parentheses at the end of the source. Example: (in Turkish)

If the article has only an English abstract, indicate it in parentheses (English abstract) or (only English abstract)

References should be listed alphabetically ordered by the author's surname, or first author's surname if there is more than one author.

Hanging indent paragraph style should be used.

The year of the reference should be in parentheses after the author name(s).

The correct arrangement of the reference list elements should be in order as "Author surname, first letter of the name(s). (publication date). Title of work. Publication data. DOI

Article title should be in sentence case and the journal title should be in title case. Journal titles in the Reference List must be italicized and spelled out fully; do not abbreviate titles (For example; Ege Journal of Fisheries and Aquatic Sciences, not Ege J Fish Aqua Sci). Article titles are not italicized. If the journal is paginated by issue the issue number should be in parentheses.

DOI (Digital Object Identifier) information (if available) should be placed at the end of the reference as in the example. After added DOI information, "dot" should not be put. The DOI information for the reference list can be retrieved from CrossRef © Simple Text Query Form (https://doi.crossref.org/simpleTextQuery) by just pasting the reference list into the query box. After copying and pasting all the references of your article in the query box on this page, the DOI information is listed as added to the relevant reference. It is strongly recommended to provide DOI information of the references.

- For a reference with up to 20 authors, ALL authors (up to 20) are spelled in the reference list. When the number of authors is more than 21, "......" is used between the 19th author and the last author (APA 7th edition).

For example:

Bolotov, I.N., Kondakov, A.V., Konopleva, E.S., Vikhrev, I. V., Aksenova, O. A, Aksenov, A. S., Bespalaya, Y. V., Borovskoy, A. V., Danilov, P. P., Dvoryankin, G. A. Gofarov, M. Y., Kabakov, M. B., Klishko, O. K., Kolosova, Y. S., Lyubas, A. A., Novoselov, A. P., Palatov, D. M., Savvinov, G. N., Solomonov, N. M., ……..& Vinarski, M. M., (2020). Integrative taxonomy, biogeography and conservation of freshwater mussels (Unionidae) in Russia.Scientific Reports, *10*, 3072. https://doi.org/10.1038/s41598-020-59867-7

- In the reference list starting with the same surname and names (initials), works with a single author are put in chronological order first; Then, two-author works are taken into account in alphabetical order of the second author. Multi-author works are listed only chronologically.

For example:

Kocataş, A. (1978)

Kocataş, A., & Ergen, Z. (1972).

Kocataş, A., & Geldiay, R. (1972)

Kocataş, A., Ergen, Z., & Geldiay, R. (1980)

The citation of journals, books, multi-author books and articles published online etc. should conform to the following examples:

Journal Articles

Öztürk, B. (2010). Scaphopod species (Mollusca) of the Turkish Levantine and Aegean seas. Turkish Journal of Zoology, *35*(2), 199-211. DOI:10.3906/zoo-0904-23

Özbek, M., & Ulutürk, E. (2017). First record of Spongilla lacustris (Porifera: Demospongiae) from the Eastern Black Sea (Uzungöl Lake, Trabzon) (in Turkish with English abstract). Ege Journal of Fisheries and Aquatic Sciences, *34*(3), 341-346. https://doi.org/10.12714/egejfas.2017.34.3.14

Books

Parsons, T.R., Matia, Y., & Lalli, C.M. (1984). A manual of chemical and biological methods for seawater analysis. New York, Pergamon Press.

Kleiner, F.S., Mamiya, C.J., & Tansey, R.G. (2001). Gardner's art through the ages (11th ed.). Fort Worth, USA: Harcourt College Publishers.

Chapter in books

Gollasch, S. (2007). Is ballast water a major dispersal mechanism for marine organisms? In W. Nentwig (Ed.), Biological Invasions (pp. 29-57). Berlin: Springer.

E-books and chapter in e-books

Mitchell, J.A., Thomson, M., & Coyne, R.P. (2017). A guide to citation. Retrieved from https://www.mendeley.com/reference-management/reference-manager

Troy, B.N. (2015). APA citation rules. In S.T, Williams (Ed.). A guide to citation rules (2nd ed., pp. 50-95). Retrieved from https://www.mendeley.com/reference-management/reference-manager

Proceedings

Soultos, N., Lossifidou, E., Lazou, T., & Sergedilis, D. (2010). Prevalence and antibiotic susceptibility of Listeria monocytogenes isolated from RTE seafoods in Thessaloniki (Northern Greece). In Ş. Çaklı, U. Çelik, C. Altınelataman (Eds.), West European Fish Technologists Association Annual Meeting 2010 (pp. 94-98). İzmir, Turkey: Proceedings Book.

Websites

Mitchell, J.A. (2017, May 21). How and when to reference. https://www.howandwhentoreference.com

If the resource was written by a group or organization, use the name of the group/organization as the author. Additionally, if the author and site name are the same, omit the site name from the citation.

American Society for the Prevention of Cruelty to Animals. (2019, November 21). Justice served: Case closed for over 40 dogfighting victims. https://www.aspca.org/news/justice-served-case-closed-over-40-dogfighting-victims

Thesis

Acarli, S. (2005). Larval production of oyster. Doctoral dissertation, Ege University, Turkey.

Tables and Figures

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Corresponding Address Su Ürünleri Dergisi Ege University Faculty of Fisheries 35100 Bornova-İzmir, Turkey Phone: +90 232 311 3838 Fax: +90 232 388 3685 E-mail: editor@egejfas.org	**E- ISSN 2148-3140**

Su Ürünleri Dergisi
Ege Journal of Fisheries and Aquatic Sciences

Su Ürünleri Dergisi yılda dört sayı olarak yayınlanır. Ege Journal of Fisheries and Aquatic Sciences is published in four issues annually.

T.C. Kültür ve Turizm Bakanlığı Sertifika No: 18679
Ministry of Culture and Tourism Sertificate No:18679

Yayımlanma Tarihi **Publishing Date**
15 Eylül September 15th, 2024

İletişim **Contact**
Ege Üniversitesi Su Ürünleri Fakültesi, 35100, Bornova, İzmir Ege University Faculty of Fisheries, 35100, Bornova, İzmir, Türkiye
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Su Ürünleri Dergisi

Ege Journal of Fisheries and Aquatic Sciences

Volume 41 Number 3 **ISSN 1300-1590 / E-ISSN 2418-3140**

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Published by
Ege University Faculty of Fisheries, İzmir, Türkiye

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 41(3), 166-178 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.01

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Analysis of the ocean and marine health performances of 18 countries in the G20 countries: An application using the CEBM-based TOPSIS method

G20 ülkeleri içinde 18 ülkenin okyanus ve deniz sağlığı performanslarının analizi: CEBM tabanlı TOPSIS yöntemi ile bir uygulama

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Received date: 15.02.2024 *Accepted date: 04.06.2024*

How to cite this paper:
Altıntaş, F.F. (2024). Analysis of the ocean and marine health performances of 18 countries in the G20 countries: An application using the CEBM-based TOPSIS method. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 166-178. https://doi.org/10.12714/egejfas.41.3.01

Abstract: This study investigates the critical role of G20 nations in maintaining ocean health, given the significant influence their economic activities have on global maritime ecosystems. Employing the most recent Ocean Health Index (OHI) data (2023) and the CEBM-TOPSIS Multi-Criteria Decision Making (MCDM) method, the research assesses the ocean health performance of 18 countries G20 countries. The CEBM analysis identifies biodiversity, carbon sequestration capacity, fisheries sustainability, water quality, and coastal protection as the most important OHI criteria, respectively. According to the CEBM-TOPSIS method, Russia, Brazil, and France are the top three countries with the highest ocean health performance, while China, India, and South Africa are ranked lowest among the first three countries. Notably, the average performance score indicates that Russia, Brazil, France, the United Kingdom, Australia, Mexico, South Korea, the United States, Germany, Saudi Arabia, and Canada all exceed the average. This suggests a need for improvement among G20 countries with below-average performance to ensure a more substantial contribution to the global economy and interconnected dimensions. Finally, sensitivity, comparison, and simulation analysis validate the CEBM-TOPSIS MCDM method as a reliable tool for evaluating national ocean health performance.

Keywords: CEBM, CEBM based TOPSIS, G20, large economies, ocean/marine health performance, ocean/marine pollution

Öz: Bu çalışma, ekonomik faaliyetlerinin küresel deniz ekosistemleri üzerindeki önemli etkisini göz önünde bulundurarak, G20 ülkelerinin okyanus sağlığını korumadaki kritik rolünü araştırmaktadır. En yeni Okyanus Sağlık Endeksi (OHI) verilerini (2023) ve CEBM-TOPSIS Çok Kriterli Karar Verme (MCDM) yöntemini kullanan bu çalışma, 18 tane G20 ülkesinin okyanus sağlığı performansını değerlendirmektedir. Yapılan CEBM analizi sonucunda en önemli OHI kriterleri sırasıyla; biyoçeşitlilik, karbon tutma kapasitesi, balıkçılık sürdürülebilirliği, su kalitesi ve kıyı korumayı belirlemektedir. CEBM-TOPSIS analiz sonucuna göre, okyanus sağlığı performansı en yüksek olan ülkeler sırasıyla Rusya, Brezilya ve Fransa, en düşük ülkeler ise Çin, Hindistan ve Güney Afrika'nın ise en düşük ilk üç sırada yer aldığını ortaya koymaktadır. Ayrıca, Rusya, Brezilya, Fransa, Birleşik Krallık, Avustralya, Meksika, Güney Kore, ABD, Almanya, Suudi Arabistan ve Kanada'nın ortalama puanlarının ortalama değerleri aştığı belirlenmiştir. Bu sonuç, küresel ekonomiye ve birbiriyle bağlantılı paydaşlara (Boyut yerine başka geniş bir ifade kullanılabilir) daha etkin katkılar sağlamak için ortalama altında performans gösteren G20 ülkelerinin deniz sağlığı performanslarının iyileştirilmesinin gerektiğini gözler önüne sermektedir. Buna benzer şekilde düzenlenebilir. Son olarak, bu çalışma kapsamında yapılan duyarlılık, karşılaştırma ve simülasyon sonuçları, CEBM-TOPSIS MCDM yönteminin ülkelerin veya ülke bazında okyanus/deniz sağlığı için güvenilir bir araç olarak doğrulamaktadır.

Anahtar kelimeler: CEBM, CEBM tabanlı TOPSIS, G20, büyük ekonomiler, okyanus/deniz sağlığı performansı, okyanus/deniz kirlenmesi

INTRODUCTION

Marine and ocean/marine pollution pose a critical global environmental challenge with far-reaching implications for ecosystems, human health, economies, biodiversity, and food security. Recognizing this, international collaboration and effective policies are essential for the sustainable management of seas and oceans (Karim, 2015). Monitoring the marine health performance of countries is crucial to prevent pollution and ecosystem degradation, fostering global cooperation and transparency.

The escalating use of seas and increasing global interconnections between countries have elevated marine and ocean health as a critical global concern (Kennish, 1997; Neto et al., 2017; Krushelnytska, 2018; Cusine and Grant, 2019; Pei and Junaid, 2019; Yao et al., 2023). However, the presence of harmful substances endangering ecological balance impedes the sustainability of ocean/marine health, leading to declining water quality and atmospheric/climatic changes (Arias and Marcovecchio, 2018; Niceforo, 2019).

Internationally, ocean/marine pollution is defined by the United Nations Convention on the Law of the Sea as any human activity that introduces substances or energy into the marine environment, causing deleterious effects such as harm to living resources and marine life, impairment of water quality for human use, and interference with legitimate sea uses (Proelß, 2017). Ocean/marine pollutants include nutrients, sediments, pathogens, alien species, persistent toxins (PCBs, heavy metals, DDT), oil, plastics, radioactive substances, thermal, and noise (Potters, 2013). The literature classifies factors influencing ocean/marine pollution, such as land-based activities, industrial waste disposal, radioactive pollution, ship-borne pollutants, and mineral exploitation (Hardy, 1971; Tornero and Hanke, 2016). Specific pollutants in oceans and marines include POPs, EDCs, mercury, heavy metals, pesticides, pharmaceuticals, oil, plastic wastes, BPA, phthalates, personal care products, and industrial/agricultural emissions. Understanding these pollutants is crucial for collaborative policies to achieve sustainable marine and ocean health (Lloyd-Smith and Immig, 2018).

Countries depend on oceans for fisheries, tourism, transportation, water supply, and wastewater discharge, necessitating the evaluation of marine health performance to ensure economic benefits. The Ocean Health Index (OHI) is the sole metric measuring countries' ocean/marine health performance, emphasizing sustainability in food, cultural, economic, and social aspects.

OHI raises awareness and aids countries in planning pollution protection strategies, comprising 10 components and 8 sub-components measured through arithmetic means (Halpern et al., 2012). The explanations of the components and sub-components are shown in Table 1.

Table 1. OHI components and sub-components (Rintaka et al., 2023; Ocean Health Index, 2023)

Components	Sub-Components	Explanation
Food Provision	Wild Caught Fisheries	Measures the sustainability of wild-caught and farmed seafood.
	Mariculture	Measures the sustainability of farmed seafood.
Artisanal Fishing Opportunities	------	Measures the level of opportunity for people to fish for subsistence or local-scale fishing.
Natural Products	------	Measures how well countries maximize the sustainable harvest of non-food marine resources.
Carbon Storage	------	Measures the amount of carbon storage provided by oceans.
Coastal Protection	------	Measures the level of coastal protection.
Livelihoods and Economies	Livelihoods	Measures the quality and quantity of ocean-related jobs.
	Economies	Measures the value of income generated from the ocean.
Tourism and Recreation	------	Measures the level of sustainable tourism.
Sense of Place	Iconic Species	Measures the level of protection of important marine species.
	Lasting Special Places	Measures the level of protection of culturally significant marine places.
Clean Waters	------	Measures the performance of countries in providing clean water.
Biodiversity	Habitat	Measures the performance of countries in protecting the natural habitats of marine species.
	Species	Measures the performance of countries in protecting marine species.

Rapid economic growth, industrialization, and rising consumption intensify ocean/marine pollution through industrial waste, pesticides, carbon emissions, and plastic waste. These factors threatens ecosystems, marine life, and human health. Sustainable development, integrating economic growth with environmental protection, offers mitigation strategies. Further research and policy development are crucial (Sachs et al., 2023).

Reducing ocean/marine pollution drives economic growth through new opportunities, innovation, and solutions to global challenges like food security and resource availability (Mitra et al., 2021). Conversely, inaction incurs greater costs, harming sectors like tourism, fisheries, health, and coastal development (Diez et al., 2019). In the second dimension, Economic growth often correlates with increased ocean/marine pollution, as seen across various studies (Zhang and Chen, 2022; Li, 2024). Marine resource depletion further emphasizes the need for innovative solutions.

G20 countries, being major economies, significantly contribute to global ocean/marine pollution, with the top plastic waste producers and rivers carrying plastic waste located within the G20 (World Ocean Initiative, 2024). Recognizing this, G20 countries have developed action plans and initiatives, such as the Marine Litter Action Plan and the Osaka Blue Ocean Vision, to address ocean/marine pollution comprehensively (Ministry of Foreign Affairs of Japan, 2017; Kojima et al., 2022). The policies and actions of G20 countries in this regard have global implications for human health, economic development and growth, social well-being, biodiversity, and food security (OECD, 2019). Therefore, it can be considered relevant to analyze the ocean/marine performance of G20 countries. According to the Ocean Health Index (OHI) report for the year 2023, it has been determined that among the G20 countries, the top three countries with the highest ocean/sea health performance are Russia, Brazil, and Australia, while the bottom three countries with the lowest performance are India, China, and South Africa. Furthermore, as per the Ocean Health Index (2023) report, countries with ocean/sea health performance scores above the average include Russia, Brazil, Australia, Germany, South Korea, France, the United Kingdom, Mexico, and Saudi Arabia. Conversely, it has been observed that Türkiye and the United States are closely aligned with the average ocean/sea health performance value of the G20 countries (Ocean Health Index, 2023).

Monitoring the marine health performance of countries is crucial to prevent pollution and ecosystem degradation, fostering global cooperation and transparency. The interplay between economic growth and ocean/marine pollution emphasizes the importance of evaluating the ocean/marine health performance of the G20 countries, as they represent the world's major economies (Ullah et al., 2023). Therefore, it is important for countries to prioritize specific ocean/sea health performance criteria in order to contribute to global ocean/sea health and economic development. Identifying the ocean/marine health performance criteria that major economies should prioritize, and determining which major economies need to improve their ocean/marine health performance, is crucial (Mitra et al., 2021).

Methodologically, the CEBM method is effective in measuring the weights of criteria in a non-linear structure. CEBM (Cubic Effect Based Measurement) is a novel method for weighting criteria. It captures complex relationships by analyzing cubic interactions between them, using integral calculus and normalized data. This functional approach offers a distinct advantage and leads to more effective outcomes for complex problems compared to other methods (Altıntaş, 2023). On the other hand, the TOPSIS method is a reliable approach for measuring the performance of alternatives and is frequently utilized for this purpose. TOPSIS (Technique for Order Preference by Similarity to Ideal Solution) was introduced to the MCDM literature in 1980 by Hwang and Yoon (1981). In TOPSIS, alternatives are assessed based on two key points: the positive ideal solution and the negative ideal solution (Ayçin, 2019). The positive ideal solution maximizes benefit criteria and minimizes cost criteria. Conversely, the negative ideal solution minimizes benefit criteria and maximizes cost criteria (Paksoy, 2017). According to the method, as a decision point moves away from the negative ideal solution, it approaches the positive ideal solution. Consequently, a decision point that moves closer to the positive ideal solution and farther from the negative ideal solution gains an advantage over other decision points in the ranking (Aktaş et al., 2015). A review of the literature reveals that the TOPSIS method is frequently utilized to evaluate the performance of decision alternatives and in selection problems. The current literature on the TOPSIS method is presented in Table 2.

Table 2. TOPSIS literature

Author(s)	Method(s)	Theme
Korucuk et al. (2022)	FFS–TOPSIS	Evaluation green approaches and digital marketing strategies
Asadabadi et al. (2023)	BWM based TOPSIS	Supplier selection to support environmental sustainability
Badi et al. (2023)	Grey TOPSIS	Solar farm location selection
Das and Kumar (2023)	TOPSIS	Assessment of electric two-wheeler ecosystem
Korucuk et al. (2023)	BN-TOPSIS	Assessment of agile supply chain management
Singh et al. (2023)	AHP based TOPSIS	Selecting parameter-influencing testing
Tanveer et al. (2023)	Fuzzy TOPSIS	Selecting digital technologies in circular supply chains
Yavari et al. (2023)	Genetic Algorithm and TOPSIS	Selection of optimal well trajectory
Zhao et al. (2023)	GRA and TOPSIS	Social and economic impact assessment of coal power
Zhu et al. (2023)	SWOT and TOPSIS	Investigation of West Lake ecotourism capabilities
Dharmawan (2024)	TOPSIS	Assessing teachers performance
Kolsara (2024)	TOPSIS	Project proposal selection

In this context, the study assessed the ocean/marine health performance of 18 countries G20 countries in 2023 using the Ocean Health Index (OHI) criteria and the CEBM-based TOPSIS method. Three key findings emerged from the quantitative analysis. Firstly, the study identified priority OHI criteria for countries to enhance their overall marine health performance, contributing to global economic and related dimensions. Secondly, it pinpointed countries that need to improve their marine health performance to make more significant contributions to the global economy. Lastly, the study evaluated the applicability of the CEBM-based TOPSIS method within the OHI framework for measuring countries' ocean/marine health performance. The methodology details the research analysis and dataset, and the results section provides insights and discussions based on the quantitative findings.

MATERIALS AND METHODS

Dataset and analysis of the research

The research dataset consists of the OHI criteria data for the year 2023, which is the latest and most up-to-date data for 18 countries in the G20 group. The weights (importance levels) of the OHI criteria for each country were measured using the CEBM method, and the ocean/marine health performance of the countries was measured using the CEBM-based TOPSIS method. As It is known, the G20 group consists of 19 countries and the European Union as an organization (Öztaş and Öztaş, 2024). Japan was not included in the study because the numerical performance value of the Tourism and Recreation criterion was not available in the OHI report for Japan. For convenience, the abbreviations of the OHI criteria are shown in Table 3.

Table 3. Abbreviations of OHI components (Ocean Health Index, 2023)

Components	Abbreviations
Food Provision	OHI1
Artisanal Fishing Opportunities	OHI2
Natural Products	OHI3
Carbon Storage	OHI4
Coastal Protection	OHI5
Livelihoods and Economies	OHI6
Tourism and Recreation	OHI7
Sense of Place	OHI8
Clean Waters	OHI9
Biodiversity	OHI10

The CEBM method, known for its interactive nature and non-linear structure based on cubic functions, proves effective in solving complex problems, setting it apart from other criteria weighting methods (Altıntaş, 2023). The TOPSIS method, widely utilized in decision and selection problems, gains popularity due to its simultaneous evaluation of ideal and non-ideal solutions, mathematical simplicity, algorithm clarity, and adaptability to different weighting methods (Çakır and Perçin, 2013; Öztel and Alp, 2020). Leveraging the advantages of MCDM methods, this study employed CEBM to determine OHI criterion weights and CEBM-based TOPSIS to measure ocean/marine health performance.

This research stands out as the first to utilize MCDM methods to elucidate the ocean/marine health performance of G20 countries. The novelty is further emphasized by the application of the CEBM-based TOPSIS method in decision alternative performance measurement, contributing to both ocean/marine health and MCDM literature.

CEBM method

Step 1: Retrieving the Decision Matrix (Altıntaş, 2023)

i: 1, 2, 3...n, where n represents the number of decision alternatives

j: 1, 2, 3...m, where m represents the number of criteria

D: Decision matrix

C: Criterion

d_{ij}: The decision matrix is constructed according to Equation 1, where "i_j" represents the $i-th$ decision alternative on the $j-th$ criterion.

$$D = [d_{ij}]_{nxm} = \begin{bmatrix} C_1 & C_2 & \dots & C_m \\ x_{11} & x_{12} & & x_{1m} \\ x_{21} & x_{22} & \dots & x_{2m} \\ \vdots & \vdots & \vdots & \vdots \\ x_{n1} & x_{n2} & \dots & x_{nm} \end{bmatrix} \quad (1)$$

Step 2: Establishment of Decision Matrix Normalization (d^*_{ij}) (Altıntaş, 2023)

The decision matrix is normalized using the following equation. Equation 2 is applied for normalizing benefit criteria, while Equation 3 is utilized for the normalization of cost criteria.

$$d^*_{ij} = \frac{x_{ij} - x_j^{min}}{x_j^{max} - x_j^{min}} \quad (2)$$

$$d^*_{ij} = \frac{x_j^{max} - x_{ij}}{x_j^{max} - x_j^{min}} \quad (3)$$

Step 3: Obtaining of Cubic Equations (Altıntaş, 2023)

Utilizing SPSS assistance (CURVE ESTIMATION), cubic functions $(y = ax^3 + bx^2 + cx + d)$ are formulated for the variables, where the number of criteria, denoted as 'm', determines the quantity of $\left\{2.C(m,2) = 2.\frac{m!}{2!.(m-2)!}\right\}$ up to which the functions are generated, taking into account the cubic relationship between them.

$$(1)\ f(C_1) = C_2, f(C_1) = C_3, \dots\dots f(C_1) = C_m \quad (4)$$

$$(2)\ f(C_2) = C_1, f(C_2) = C_3, \dots\dots f(C_2) = C_m \quad (5)$$

$$(3)\ f(C_3) = C_1, f(C_3) = C_2, \dots\dots f(C_3) = C_m \quad (6)$$

$$\vdots \quad \vdots \quad \vdots$$

$$(m)\ f(C_m) = C_1, f(C_m) = C_2, \dots\dots f(C_m) = C_{m-1} \quad (7)$$

Step 4: Calculation of Cubic Impact Value between Criteria (Altıntaş, 2023)

During this phase, assessing the impact or modification of a dependent a criterion by an independent variable (another criterion) involves evaluating the independent variable's influence across its minimum and maximum values through definite integral calculation. In this context, the symbol "k" represents the cubic impact value of one criterion on the other. It is essential to verify the absolute values of the impact post-integral calculation.

$$(1)\ f(C_1) = C_2,\ \int_{C_{1min.}}^{C_{1maks.}} (f'(C_1))\,dx = |k_{C_1 \to C_2}| \quad (8)$$

$$(2)\ f(C_1) = C_3,\ \int_{C_{1min.}}^{C_{1maks.}} (f'(C_1))\,dx = |k_{C_1 \to C_3}| \quad (9)$$

$$(3)\ f(C_1) = C_4,\ \int_{C_{1min.}}^{C_{1maks.}} (f'(C_1))\,dx = |k_{C_1 \to C_4}| \quad (10)$$

$$\vdots \quad \vdots \quad \vdots$$

$$\left(\frac{m!}{(m-2)!}\right) f(C_m) = C_{m-1},\ \int_{C_{mmin.}}^{C_{mmaks.}} (f'(C_m))\,dx = |k_{C_m \to C_{m-1}}| \quad (11)$$

The significance of the absolute value of the impact value of one criterion on another is highlighted. This is crucial in this approach, as the focus lies not on the direction of influence between criteria but rather on the magnitude of the influence.

Step 5: Calculation of the Total Cubic Impact Values of Each Criterion (T_c) (Altıntaş, 2023)

During this stage, the cubic impact values originating from a criterion on other criteria are aggregated to assess the comprehensive cubic impact value of a criterion on the remaining criteria.

$$(1)\ for\ C_1\ |k_{C_1 \to C_2}| + |k_{C_1 \to C_3}| + |k_{C_1 \to C_4}| \dots\dots + |k_{C_1 \to C_m}| = \left(\sum_{j=1}^{m-1} |k_{C_1 \to C_{j+1}}|\right) = T_{C_1} \quad (12)$$

$$(2)\ for\ C_2\ |k_{C_2 \to C_1}| + |k_{C_2 \to C_3}| + |k_{C_2 \to C_4}| \dots + |k_{C_2 \to C_m}| = \left(\sum_{j=0, j\neq 1}^{m-1} |k_{C_2 \to C_{j+1}}|\right) = T_{C_2} \quad (13)$$

$$(3)\ for\ C_3\ |k_{C_3 \to C_1}| + |k_{C_3 \to C_2}| + |k_{C_3 \to C_4}| \dots + |k_{C_3 \to C_m}| = \left(\sum_{j=0, j\neq 2}^{m-1} |k_{C_3 \to C_{j+1}}|\right) = T_{C_3} \quad (14)$$

$$\vdots \quad \vdots \quad \vdots$$

$$(m)\ for\ C_m\ |k_{C_m \to C_1}| + |k_{C_m \to C_2}| + |k_{C_m \to C_3}| .. + |k_{C_m \to C_{m-1}}| = \left(\sum_{j=1}^{m-1} |k_{C_m \to C_j}|\right) = T_{C_m} \quad (15)$$

Step 6: Establishing Criterion Weight Values (w_j) (Altıntaş, 2023)

During this phase, the division of the cumulative cubic impact value of each criterion on the remaining criteria by the sum of the cumulative cubic impact values of all criteria takes place. This calculation enables the determination of the weight coefficient for each criterion.

$$w_j = \frac{T_{c_j}}{\sum_{j=1}^{m} T_{c_j}} \quad (16)$$

TOPSIS method

Step 1: Formation of the Decision Matrix (Kaya and Karaşan, 2020)

The matrix consisting of m decision alternatives and n criteria is defined in Equation 17.

$$A_{ij} = \begin{bmatrix} a_{11} & x_{12} & \cdots & k_{1n} \\ a_{21} & x_{22} & \cdots & k_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ a_{m1} & x_{m2} & \cdots & k_{mn} \end{bmatrix} \quad (17)$$

Step 2: Attainment of the Standard Decision Matrix (Atan and Altan, 2020)

In the TOPSIS method, normalization is generally calculated through vector normalization. Accordingly, the normalized values (r_{ij}) are initially measured. The r_{ij} values are provided with Equation 18.

$$r_{ij} = \frac{a_{ij}}{\sqrt{\sum_{k=1}^{m} a_{kj}^2}} \quad i = 1,2,\ldots,m \; ve \; j = 1,2,\ldots,n) \quad (18)$$

After calculating the r_{ij} values, the standard decision matrix (R_{ij}) is obtained using Equation 19.

$$R_{ij} = \begin{bmatrix} r_{11} & r_{12} & \cdots & r_{1n} \\ r_{21} & r_{22} & \cdots & r_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ r_{m1} & r_{m2} & \cdots & r_{mn} \end{bmatrix} \quad (19)$$

Step 3: Attainment of the Weighted Standard Decision Matrix (Özarı and Eran, 2019)

At this stage, the sum of the weights of the criteria (w_{ij}) must be equal to 1 $(\sum_{j=1}^{n} w_j)$. Accordingly, the obtained weighted standard decision matrix (V_{ij}) is shown in Equation 20.

$$V_{ij} = \begin{bmatrix} w_1 r_{11} & w_2 r_{12} & \cdots & w_n r_{1n} \\ w_1 r_{21} & w_2 r_{22} & \cdots & w_n r_{2n} \\ \vdots & \vdots & \vdots & \vdots \\ w_1 r_{m1} & w_2 r_{m2} & \cdots & w_n r_{mn} \end{bmatrix} \quad (20)$$

Step 4: Determination of the Positive Ideal (A^+) and Negative Ideal (A^-) Solution Values (Kaymaz et al., 2020)

For criteria oriented towards maximization, the positive ideal solution set is formed by selecting the column values in the weighted evaluation criteria matrix V_{ij} where the criteria are maximized, and for criteria oriented towards minimization, the smallest column values are preferred. In this context, the positive ideal solution values are calculated as shown in Equations 21 and 22.

$$A^+ = \{mak_i v_{ij} | \; j \; \in J), (min_i v_{ij} | \; j \; \in J'\} \quad (21)$$

$$A^+ = \{v_1^*, v_2^*, \ldots, v_n^*\} \quad (22)$$

For criteria oriented towards maximization, the negative ideal solution set is formed by selecting the column values in the weighted evaluation criteria matrix V_{ij} where the criteria are minimized, and for criteria oriented towards maximization, the largest column values are preferred. In this context, the negative ideal solution values are calculated as shown in Equations 23 and 24.

$$A^- = \{min_i v_{ij} | \; j \; \in J), (min_i v_{ij} | \; j \; \in J'\} \quad (23)$$

$$A^- = \{v_1^-, v_2^-, \ldots, v_n^-\} \quad (24)$$

Step 5: Measurement of Distance to Positive and Negative Points (Kaymaz et al., 2020)

Initially, to determine the deviations in the positive and negative ideal solution sets, Equation 25 is utilized for the detection of the distances of x and y values in the coordinate plane using the Euclidean Distance Approach.

$$d_{ij} = \sqrt{\sum_{k=1}^{n} (x_{ik} - x_{jk})^2} \quad (25)$$

In Equation 25 x_{ik} explains the kth variable value of the ith observation and x_{ik} explains the jth observation's kth variable value, along with the number of variables (criteria), denoted by n. Additionally, the number of measurements for the positive ideal distance (S_i^+) and negative ideal distance (S_i^-) metrics is equal to the number of decision alternatives. Thus, in the TOPSIS method, Equations 26 and 27 are utilized for calculating the distances to ideal and non-ideal points for each decision alternative.

$$Positive\ Ideal\ Distance: S_i^+ = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^*)^2} \quad (26)$$

$$Negative\ İdeal\ Distance: S_i^- = \sqrt{\sum_{j=1}^{n} (v_{ij} - v_j^-)^2} \quad (27)$$

Step 6: Measurement of Relative Proximity to the Ideal Solution (Atan and Altan, 2020)

The proximity of each decision point to the positive ideal solution (C_i^*) is calculated utilizing measurements of positive and negative ideal distances. The main criterion here is the ratio of the negative distance measurement to the total distance measurement. Equation 28 is employed for measuring the proximity values to the positive ideal solution.

$$C_i^* = \frac{S_i^-}{S_i^- + S_i^+} \quad (28)$$

The value of C_i^* specified in Equation 18 should be within the range of $0 \leq C_i^* \leq 1$ when $C_i^* = 1$, it indicates the absolute proximity of the corresponding decision alternative to the positive ideal solution. On the other hand, if $C_i^* = 0$, it signifies the absolute proximity of the respective decision alternative to the negative ideal solution.

RESULTS

Computational analysis

In the study, initially, the weighting values of the OHI criteria were calculated using the relevant equations specified in the CEBM method, and then they were ranked. The weighting values (degrees of importance) of these OHI criteria and the rankings of the weighting values are described in Table 4.

Table 4. Weighting values of OHI criteria

Criteria	Total Effect	Weights	Ranking
OHI1	1.672	0.066	10
OHI2	3.013	0.119	3
OHI3	2.137	0.085	7
OHI4	3.299	0.131	2
OHI5	2.684	0.106	5
OHI6	2.26	0.090	6
OHI7	2.063	0.082	8
OHI8	1.711	0.068	9
OHI9	2.908	0.115	4
OHI10	3.49	0.138	1
Total	25.237	Mean: 0.100	

Upon examining Table 4, it can be observed that the weighting values of the criteria are ranked as follows: OHI10, OHI4, OHI2, OHI9, OHI5, OHI6, OHI3, OHI7, OHI8, and OHI1. Additionally, the average weight of the criteria was calculated according to countries, and it was observed that the OHI criteria with weights exceeding the calculated average weight are OHI10, OHI4, OHI2, OHI9, and OHI5. Therefore, based on this result, it has been evaluated that countries need to develop strategies for the improvement of OHI10, OHI4, OHI2, OHI9, and OHI5 criteria to enhance global ocean/marine health and thus contribute to the global economy. In the continuation of the study, the ocean/sea health performance of countries was calculated using the CEBM-based TOPSIS method and the equations described in the methodology section and presented in Table 5.

Table 5. Countries' ocean/sea health performance values (Ci^*)

Countries	(Ci^*)	Rank	Countries	(Ci^*)	Rank
Argentina	0.519269	15	**Italy**	0.531301	14
Australia	0.610531	5	**Mexico**	0.608984	6
Brazil	0.673539	2	**Russia**	0.731705	1
Canada	0.564713	11	**S.Arabia**	0.566239	10
China	0.400724	18	**South Africa**	0.428932	16
France	0.621205	3	**South Korea**	0.590257	7
Germany	0.577571	9	**Türkiye**	0.562318	12
India	0.406321	17	**United Kingdom**	0.611918	4
Indonesia	0.537598	13	**USA**	0.589594	8
			Mean: 0.562929		

Upon examining Table 5, it is noted that Russia, Brazil, and France rank as the top three countries with the highest ocean/sea health performance, while South Africa, India, and China rank at the bottom. Moreover, the countries surpassing the average ocean/sea health performance are ranked as follows: Russia, Brazil, France, the United Kingdom, Australia, Mexico, South Korea, the USA, Germany, Saudi Arabia, and Canada. Additionally, Türkiye's ocean/sea health performance closely aligns with the average value, as indicated in Table 5.

Sensibility analysis

This study explores the sensitivity of the CEBM-based TOPSIS approach in MCDM. We used various criteria weighting methods on the same dataset and compared the resulting values and rankings. We expect the rankings from our chosen sensitivity analysis to differ from those generated by other methods, confirming MCDM sensitivity in weight coefficient calculations (Gigovič et al., 2016).

Adhering to this methodology, we employed established objective weighting techniques to calculate and organize the weighting coefficients associated with the FIW components. These techniques, widely recognized in scholarly literature, included ENTROPY, CRITIC, SD, SVP, MEREC, and LOPCOW. The corresponding numerical results are meticulously presented in Table 6.

Table 6. Values of Objective Criterion Weighting Methods

Criteria	ENTROPY		CRITIC		SD		SVP		MEREC	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank
OHI1	0.1032	2	0.1512	2	0.1201	1	0.1488	3	0.1380	3
OHI2	0.0996	5	0.1140	4	0.0917	7	0.1079	4	0.1429	2
OHI3	0.0985	7	0.0796	7	0.1045	5	0.0701	6	0.0617	4
OHI4	0.0979	8	0.0568	9	0.0897	8	0.0334	9	0.0237	9
OHI5	0.0996	4	0.0873	5	0.1011	6	0.0756	5	0.0266	8
OHI6	0.0977	9	0.0824	6	0.1127	2	0.0466	8	0.0284	7
OHI7	0.1047	1	0.1727	1	0.1116	3	0.2465	1	0.0295	6
OHI8	0.1019	3	0.1509	3	0.1104	4	0.2026	2	-0.0115	10
OHI9	0.0992	6	0.0782	8	0.0775	10	0.0620	7	0.0327	5
OHI10	0.0976	10	0.0269	10	0.0808	9	0.0064	10	0.5279	1

Upon examining Table 6, it is observed that the rankings of OHI criteria under the CEBM method differ from the rankings of OHI criteria determined under other methods. Furthermore, in the continuation of sensitivity analysis, countries' ocean/sea health performances were measured using the ENTROPY, CRITIC, SD, SVP, and MEREC-based TOPSIS methods and presented in Table 7.

Upon examination of Table 7, it has been observed that the rankings of countries' ocean/sea health performance values determined using the CEBM-based TOPSIS method differ from the rankings of countries' ocean/sea health performance values obtained using ENTROPY, CRITIC, SD, SVP, and MEREC-based TOPSIS methods. Based on this finding, it is evaluated that the CEBM-based TOPSIS method is sensitive in measuring ocean/sea health performance within the scope of OHI.

Table 7. Countries' Ocean/Sea Health Performance Values and Rankings using ENTROPY, CRITIC, SD, SVP, and MEREC-based TOPSIS

Countries	ENTROPY TOPSIS		CRITIC TOPSIS		SD TOPSIS		SVP TOPSIS		MEREC TOPSIS	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank	Value	Rank
Argentina	0.5193	15	0.5994	9	0.5567	11	0.6058	9	0.5067	8
Australia	0.6105	5	0.5915	10	0.5668	9	0.6612	6	0.4091	16
Brazil	0.6735	2	0.6432	4	0.6272	3	0.7034	4	0.5029	9
Canada	0.5647	11	0.5021	15	0.5059	13	0.5272	13	0.4221	15
China	0.4007	18	0.4277	17	0.4631	17	0.3545	18	0.6142	4
France	0.6212	3	0.6020	8	0.6224	4	0.5735	10	0.6845	2
Germany	0.5776	9	0.6146	6	0.5792	8	0.6892	5	0.3993	17
India	0.4063	17	0.3805	18	0.3633	18	0.4074	17	0.4528	14
Indonesia	0.5376	13	0.5091	13	0.4675	16	0.5668	11	0.4859	12
Italy	0.5313	14	0.6204	5	0.5804	7	0.6572	7	0.3903	18
Mexico	0.6090	6	0.4883	16	0.5026	14	0.5080	14	0.5387	7
Russia	0.7317	1	0.8354	1	0.8056	1	0.8480	1	0.8011	1
Saudi Arabia	0.5662	10	0.5213	12	0.5018	15	0.5617	12	0.4604	13
South Africa	0.4289	16	0.6054	7	0.5594	10	0.6380	8	0.4969	11
South Korea	0.5903	7	0.7551	2	0.6922	2	0.7965	2	0.5645	5
Türkiye	0.5623	12	0.5044	14	0.5189	12	0.4837	15	0.5486	6
United Kingdom	0.6119	4	0.6475	3	0.5982	5	0.7133	3	0.5010	10
USA	0.5896	8	0.5418	11	0.5805	6	0.4797	16	0.6561	3

Comparative analysis

In the comparative analysis, the proposed model is evaluated alongside other MCDM calculation methods to assess similarities and differences. The objective is to ensure the credibility and reliability of the proposed model by establishing positive and significant relationships with various MCDM methods (Keshavarz-Ghorabaee et al., 2021). To this end, the ocean/sea health performances of countries were initially assessed using commonly employed methods such as CEBM-based SWA, ARAS, EDAS, WASPAS, GRA (Grey Relation Analysis), MAUT, ROV, and COCOSO. These assessments are summarized in Table 8.

In the second part of the comparative analysis, correlation values between the ocean/sea performance values of countries calculated under the OHI and CEBM-based SWA, ARAS, EDAS, WASPAS, GRA, MAUT, ROV, and COCOSO methods were computed. These correlation values are presented in Table 9, and a visual representation of the correlation values is depicted in Figure 1.

Drawing from Walters (2009) research, Keshavarz-Ghorabaee (2021) suggests that a correlation value falling within the range of 0.400-0.600, as evaluated between the MEREC method and others (SD, ENTROPY, and CRITIC), indicates a moderate association between the variables. Notably, correlations surpassing 0.600 signify a strong and significant relationship. Upon examining Table 9 and Figure 1 concurrently, it is evident that the CEBM-based TOPSIS method demonstrates a positive, significant, and high correlation with other methods except for COCOSO. These findings lead to the conclusion that the proposed model (CEBM-based TOPSIS) for assessing countries' OHI criteria values is both credible and reliable.

Simulation analysis

Simulations explore the proposed method's behavior under diverse scenarios by varying decision matrix values. We expect its behavior to increasingly diverge from other methods as scenarios multiply. Ideally, its average variance across scenarios should surpass others, demonstrating its strength in differentiating weightings based on context. ADM (ANOM for variances with Levene) analysis further delves into this, visually assessing the consistency of variances across scenarios. Deviations from established limits suggest heterogeneity, while consistency within them affirms uniformity (Keshavarz-Ghorabaee et al., 2021).

Table 8. Country rankings of ocean/marine health performance values using CEBM-based SWA, ARAS, EDAS, WASPAS, GRA, MAUT, ROV, and COCOSO methods

Countries	SWA		ARAS		EDAS		WASPAS	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank
Argentina	0.3190	7	0.7511	15	0.3709	15	0.7706	15
Australia	0.2953	14	0.8275	3	0.6957	3	0.8474	3
Brazil	0.3457	2	0.8597	2	0.8269	2	0.8848	2
Canada	0.2805	17	0.7955	10	0.5591	10	0.8139	9
China	0.3031	13	0.7115	17	0.2210	17	0.7132	17
France	0.3343	5	0.8233	4	0.6728	4	0.8434	4
Germany	0.3080	11	0.8086	7	0.6101	8	0.8307	7
India	0.3031	12	0.6885	18	0.1078	18	0.7017	18
Indonesia	0.2928	15	0.7584	14	0.4052	13	0.7775	14
Italy	0.2829	16	0.7592	13	0.3939	14	0.781	13
Mexico	0.3309	6	0.8190	5	0.6614	5	0.8405	5
Russia	0.3758	1	0.8933	1	0.9746	1	0.9178	1
Saudi Arabia	0.3168	8	0.7905	11	0.5383	11	0.8125	10
South Africa	0.2488	18	0.7164	16	0.2367	16	0.725	16
South Korea	0.3138	9	0.8081	8	0.6104	7	0.8261	8
Türkiye	0.3427	3	0.7904	12	0.5283	12	0.8083	12
United Kingdom	0.3105	10	0.8113	6	0.6152	6	0.8358	6
USA	0.3412	4	0.8009	9	0.5857	9	0.8112	11
Countries	**GRA**		**MAUT**		**ROV**		**COCOSO**	
	Value	**Rank**	**Value**	**Rank**	**Value**	**Rank**	**Value**	**Rank**
Argentina	0.5019	16	0.2036	16	0.2090	15	2.7895	18
Australia	0.6481	4	0.4340	4	0.3058	5	256.27	7
Brazil	0.6880	2	0.4846	2	0.3547	2	319.98	5
Canada	0.5967	8	0.3344	9	0.2975	6	160.87	16
China	0.5285	14	0.2375	14	0.2321	14	190.9	15
France	0.6109	6	0.3551	6	0.3119	4	304.07	6
Germany	0.6006	7	0.3425	7	0.2969	7	248.76	10
India	0.4795	18	0.1697	18	0.1914	18	248.05	11
Indonesia	0.5557	13	0.2887	13	0.2385	13	204.12	13
Italy	0.4929	17	0.1857	17	0.2058	16	199.89	14
Mexico	0.6660	3	0.4570	3	0.3349	3	249.58	9
Russia	0.7031	1	0.5059	1	0.3710	1	427.21	1
Saudi Arabia	0.6175	5	0.3846	5	0.2752	10	394.58	2
South Africa	0.5111	15	0.2302	15	0.1925	17	57.701	17
South Korea	0.5715	11	0.3024	11	0.2635	12	240.6	12
Türkiye	0.5957	9	0.3392	8	0.2842	9	371.43	3
United Kingdom	0.5786	10	0.3057	10	0.2866	8	254.2	8
USA	0.5684	12	0.2981	12	0.2729	11	358.44	4

Table 9. Correlation values of CEBM-based TOPSIS method with other methods

Methods	SWA	ARAS	EDAS	WASPAS	GRA	MAUT	ROV	COCOSO	OHI
TOPSIS	0.678*	0.985**	0.982**	0.991**	0.850**	0.831**	0.884**	0.589*	0.965**

p*<.05, p**<.01

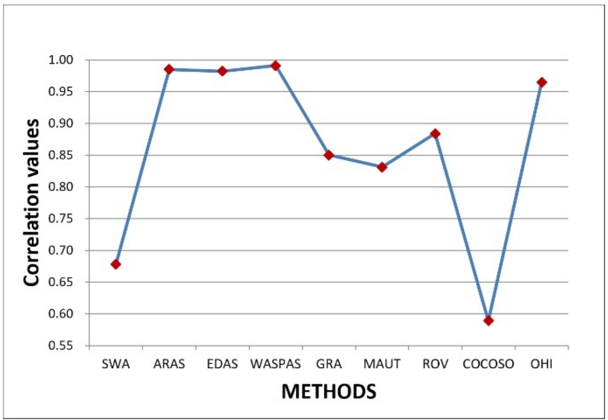

Figure 1. Correlation values of methods

ADM offers a visual representation to assess variance evenness, incorporating parameters such as the overall average ADM, upper decision limits (UDL), and lower decision limits (LDL). Deviation of a group's standard deviation from the decision limits suggests heterogeneity in variances, while consistency within the LDL and UDL affirms uniformity (Keshavarz-Ghorabaee et al., 2021). In this regard, in the simulation analysis, initially, a total of 10 scenarios (decision matrices) were created, with 3 in the first group and 7 in the second group. Subsequently, correlation values between the CEBM-based TOPSIS method and the CEBM-based SWA, ARAS, EDAS, WASPAS, GRA, MAUT, and COCOSO methods were measured for each created scenario. The correlation values measured for these scenarios are described in Table 10.

When Table 10 and Figure 2 are examined together, it is observed that especially the CEBM-based TOPSIS method shows positive, significant, and high relationships with CEBM-based SWA, ARAS, EDAS, WASPAS, GRA, MAUT, and ROV methods across scenarios, while it exhibits a moderate level of relationship with the CEBM-based COCOSO method. Furthermore, upon evaluation of Table 10, it is noted that as the number of scenarios increases, the correlation values of the CEBM-based TOPSIS method with other CEBM-based methods decrease. Additionally, within the scope of the simulation analysis, the variance values of the methods were calculated according to the created scenarios. The variance values of these methods are presented in Table 11.

Table 10. Correlation values of the CEBM-based TOPSIS method with other methods across scenarios

Scenarios		SWA	ARAS	EDAS	WASPAS	GRA	MAUT	ROV	COCOSO
First Group	1	0.680*	0.991**	0.990**	0.995**	0.875**	0.845**	0.890**	0.610*
	2	0.700**	0.983**	0.989**	0.990**	0.848**	0.839**	0.881**	0.575*
	3	0.725**	0.978**	0.980**	0.996**	0.870**	0.850**	0.884**	0.569*
Second Group	4	0.635*	0.962**	0.965**	0.988**	0.820**	0.800**	0.870**	0.577*
	5	0.641*	0.955**	0.950**	0.900**	0.845**	0.821**	0.879**	0.546*
	6	0.663*	0.956**	0.954**	0.984**	0.833**	0.811**	0.872**	0.539*
	7	0.635*	0.978**	0.967**	0.980**	0.835**	0.815**	0.863**	0.548*
	8	0.618*	0.948**	0.942**	0.991**	0.822**	0.803**	0.855**	0.532*
	9	0.594*	0.961**	0.955**	0.973**	0.801**	0.795**	0.833**	0.527*
	10	0.644*	0.937**	0.948**	0.969**	0.812**	0.803**	0.812**	0.542*

p*<.05, p**<.01

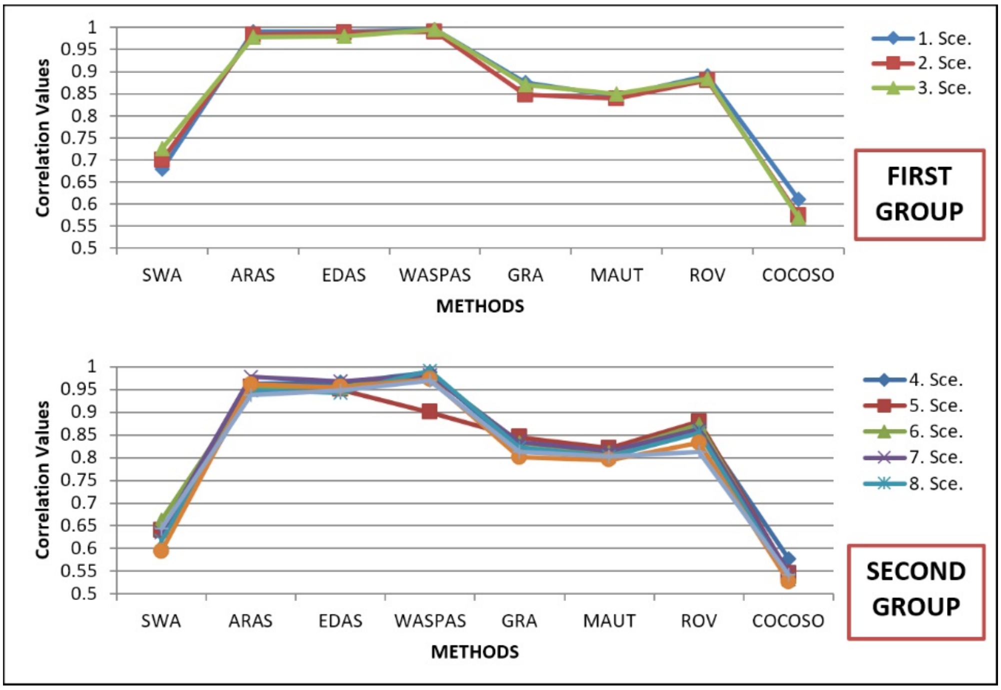

Figure 2. Position of correlation values of the CEBM-based TOPSIS method with other CEBM-based methods (Sce.: Scenario)

Upon reviewing Table 11, it's evident that the CEBM-based TOPSIS method exhibits higher variance compared to other CEBM-based MCDM methods. This suggests its superior ability to differentiate decision alternatives within the OHI criteria context. In the subsequent simulation analysis, an ADM evaluation of countries' ocean/sea health performance values was conducted specifically for the CEBM-based TOPSIS method across various scenarios. Relevant data for each scenario are detailed in Figure 3.

As illustrated in Figure 3, the computed ADM values for each scenario are positioned below the upper decision limit (UDL) values and above the lower decision limit (LDL) values. Consequently, the variances in the identified weights for each scenario demonstrate uniformity. This verification was further validated through the implementation of the Levene Test. The essential statistics for the Levene Test are presented in Table 12.

Derived from the data in Table 12, the observed p-value (p=0.328) exceeds the critical threshold of 0.05, confirming the uniformity of variances in criterion weights across scenarios. In summary, the results obtained from the simulation analysis suggest the robustness and stability of the CEBM based TOPSIS method.

Table 11. Variance values of CEBM-based MCDM methods across scenarios

Scenarios	SWA	ARAS	EDAS	WASPAS	GRA
1	0.000025546	0.000012265	0.000477377	1.4212E-05	3,7235E-05
2	0.000025435	0.000012756	0.000477461	1.4567E-05	3,7278E-05
3	0.000025237	0.000012476	0.000477487	1.4287E-05	3,7451E-05
4	0.000025678	0.000012876	0.000477167	1.4483E-05	3,7535E-05
5	0.000025568	0.000012686	0.000477781	1.4496E-05	3,7812E-05
6	0.000025789	0.000012365	0.000477387	1.4461E-05	3,7665E-05
7	0.000025276	0.000012851	0.000477218	1.4764E-05	3,7496E-05
8	0.000025349	0.000012199	0.000477569	1.4512E-05	3,7571E-05
9	0.000025587	0.000012571	0.000477435	1.4645E-05	3,7768E-05
10	0.000025836	0.000012941	0.000477476	1.4328E-05	3,7223E-05
Mean	2.55301E-05	1.25986E-05	0.000477436	1.4476E-05	3,7503E-05

Scenarios	MAUT	ROV	COCOSO	TOPSIS
1	0.000275666	0.000111882	0.000553709	0.000067812
2	0.000275712	0.000111768	0.000553634	0.000067749
3	0.000275756	0.000111671	0.000553671	0.000067843
4	0.000275812	0.000111596	0.000553471	0.000067666
5	0.000275561	0.000111682	0.000553575	0.000067705
6	0.000275468	0.000111699	0.000553684	0.000067645
7	0.000275669	0.000111866	0.000553901	0.000067712
8	0.000275802	0.000111878	0.000553875	0.000067684
9	0.000275799	0.000112101	0.000553913	0.000067688
10	0.000275682	0.000121411	0.000554015	0.000067586
Mean	0.000275693	0.000112755	0.000553745	0.000067709

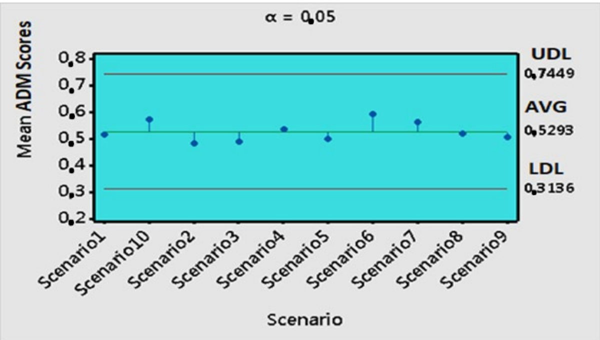

Figure 3. ADM visual

Table 12. Levene test

Levene Statistic	df1	df2	Sig.
0,345	2	16	0,388

p**<.05

DISCUSSION

Due to the intensification of inter-country relations and the direct and indirect activities it brings, the health levels of oceans and seas worldwide have become a significant contemporary and global issue (Gilmour et al., 2021). This is because countries can benefit more efficiently from oceans and seas through the policies and strategies they develop for the sustainability of ocean/sea health (Halpern et al., 2012). Therefore, the awareness of countries regarding their performance in ocean/sea health is of great importance (Frid and Caswell, 2017). Particularly, acknowledging the correlation between economic growth and ocean pollution (Chen et al., 2017), G20 nations require precise assessments of sea health. This is because the activities and methodologies of G20 countries regarding ocean/sea health can influence the global economy and other dimensions associated with the economy (OECD, 2019). In this context, this study evaluates the ocean/marine health of 18 countries in the G20 group using the CEBM-based TOPSIS method, aiming to inform policy-making, environmental sustainability, and global marine ecosystem protection.

Upon reviewing the literature, no research examining the ocean/marine health performance of G20 countries through any numerical method other than the Ocean Health Index (2023) has been encountered. In this context, it has been determined that within the scope of the Ocean Health Index (2023), the top three countries exhibiting the highest marine/ocean health performance are Russia, Brazil, and Australia. However, in the current research, this ranking has been observed as Russia, Brazil, and France. Therefore, based on this finding, it is assessed that Russia and Brazil demonstrate a notable performance in ensuring ocean/sea health compared to other countries. Additionally, according to the Ocean Health Index (2023), countries that surpass the average marine health performance include Russia, Brazil, Australia, Germany, South Korea, France, Italy, Mexico, and Saudi Arabia. In the present study, however, this ranking has been realized as Russia, Brazil, Australia, the United Kingdom, Mexico, South Korea, the United States, Germany, Saudi Arabia, and Canada. Thus, based on the results of the Ocean Health Index (2023) and the current research, consistency is observed in terms of surpassing the average marine/ocean health performance value for Russia, Brazil, France, Germany, South Korea, the United Kingdom, Mexico, and Saudi Arabia.

CONCLUSION

Especially the major economies prioritize activities related to ocean/sea health primarily because they affect ocean/sea health, the global economy, and other economic dimensions. Therefore, the efforts of G20 countries in the field of ocean/sea health are of vital importance. Because the activities of especially large economies regarding ocean/sea health affect global ocean/sea health and the global economy, it is important to determine which ocean/sea health criteria large economies should prioritize and which large economies need to improve their ocean/sea health performance for the global ocean/sea health to reach better levels and for the global economy to thrive. In this context, in the study, the ocean/sea health performances of 18 countries in the G20 group for the year 2023 were measured using the latest Ocean Health Index (OHI) data and the CEBM-based TOPSIS Multi-Criteria Decision Making (MCDM) method. According to the findings, using the CEBM method, the most important OHI criteria for countries were determined to be biodiversity, carbon capacity, fishing opportunities, clean water, and coastal protection. According to this, it has been evaluated that countries need to place more importance on the biodiversity criterion in order to contribute more to global ocean/sea health and the economy. Subsequently, based on the CEBM-based TOPSIS method, the top three countries with the highest ocean/sea health performance were determined to be Russia, Brazil, and France, while the bottom three countries were ranked as China, India, and South Africa. In addition, the average of the performance values calculated using the CEBM-based TOPSIS method showed that the countries with performance values above the average were Russia, Brazil, France, the United Kingdom, Australia, Mexico, South Korea, the United States, Germany, Saudi Arabia, and Canada. Based on this result, it is considered that G20 countries whose ocean/sea health performance values are below the average need to improve their performance to contribute more to the global marine/ocean health in the global economy and other relevant dimensions. Finally, sensitivity, comparison, and simulation analysis of the method show that the CEBM-based TOPSIS method can be used to measure countries' ocean/sea health performances. Recommendations suggest G20 countries focus on biodiversity (OHI10), carbon storage (OHI4), artisanal fishing opportunities (OHI2), clean waters (OHI9), and coastal protection (OHI5) for global ocean/marine health. Countries with lower-than-average performance according to this study, like United States, Türkiye, Indonesia, Italy, Argentina, South Africa, India, and China, are encouraged to enhance their marine health to contribute global economy. Methodologically, comparing CEBM-based other MCDM methods ensures a comprehensive examination of marine health performance measurement. In addition, the ocean/sea health performances of other international economic organizations (G20, OECD, European Union, E7, etc.) can be measured, allowing for a comparison of the ocean/sea health performances of these organizations and their member countries.

ACKNOWLEDGEMENT AND FUNDING

The author expresses their gratitude to the anonymous reviewers for their valuable input during the manuscript review process. This study did not receive any financial support, grant, or assistance from any public, commercial, or nonprofit funding organization.

AUTHOR CONTRIBUTIONS

The data collected and findings obtained for this article were provided by Furkan Fahri Altıntaş.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

DATA AVAILABILITY

For any questions, the corresponding author should be contacted.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 41(3), 179-186 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.02

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Length-weight relationships and condition factors of *Mystus vittatus* (Hamilton, 1822) in natural and culture habitat

Mystus vittatus (Hamilton, 1822)'un doğal ve kültür ortamındaki boy-ağırlık ilişkisi ve kondisyon faktörü

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Received date: 19.05.2024 *Accepted date: 03.07.2024*

How to cite this paper:
Das, P., Jana, A., Sit, G., Mandal, B., & Chanda, A. (2024). Length-Weight relationships and condition factors of *Mystus vittatus* (Hamilton, 1822) in natural and culture habitat. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 179-186. https://doi.org/10.12714/egejfas.41.3.02

Abstract: The Asian catfish *Mystus vittatus* is very popular food and ornamental fish in India. Length- weight relationships and condition factors of this fish are crucial component in fishery managements. The objective of this study is to investigate the relationships between the length, weight, condition factor (K), relative condition factor (Kn) and modified condition factor of *Mystus vittatus* (Hamilton, 1822). The study is particularly focus on analysing the sex wise variations in growth patterns, productivity, stocks, and conservation in two different habitats, natural habitat and biofloc system. The findings show that the species did not strictly follow the predicted cube law and showed allometric growth patterns in both habitats. The "b" value varies from 2.72 to 3.12, the condition factor (K) ranges from 0.587-1.50, modified condition factor ranges 0.54-2.26 and relative condition factor (Kn) ranges from 0.71-1.65. Natural habitats had the highest mean values for the regression parameter (b), condition factor (K), and relative condition factor (Kn). For male, female, and combined sexes in two different habitats, the R^2 values show a statistically significant relationship between weight and length. The correlation analysis indicates some positive and negative significant relationships among sex, length, weight, condition factor, and relative condition factor. Therefore, there is significant relationship between condition factor, relative condition factor length, weight in two different habitats and it varies between male and female within same habitat. Thus, this finding will help to fishery managers for long term strategy development of *Mystus vittatus* in both natural as well as biofloc system.

Keywords: Length-weight relationship, condition factors, *Mystus vittatus*, natural growth, biofloc growth

Öz: Asya kedi balığı *Mystus vittatus* Hindistan'da çok popüler bir yiyecek ve süs balığıdır. Bu balığın boy-ağırlık ilişkileri ve kondisyon faktörleri balıkçılık yönetiminde önemli bir bileşendir. Bu çalışmanın amacı *Mystus vittatus*'un (Hamilton, 1822) boy, ağırlık, kondisyon faktörü (K), nispi kondisyon faktörü (Kn) ve modifiye kondisyon faktörü arasındaki ilişkileri araştırmaktır. Çalışma özellikle iki farklı habitatta (doğal habitat ve biyoyumak sistemi) büyüme kalıpları, üretkenlik, stoklar ve korumadaki cinsiyete dayalı farklılıkları analiz etmeyi odaklamaktadır. Bulgulara göre tür öngörülen küp yasasına tam olarak uymamış ve her iki habitatta da allometrik büyüme göstermiştir. "b" değeri 2,72 ile 3,12 arasında, kondisyon faktörü (K) 0,587-1,50 arasında, modifiye kondisyon faktörü (Kn) 0,54-2,26 arasında ve nispi kondisyon faktörü 0,71-1,65 arasında değişmektedir. Regresyon parametresi (b), kondisyon faktörü (K) ve nispi kondisyon faktörü (Kn) açısından en yüksek ortalama değerler doğal habitatlarda bulunmuştur. İki farklı habitattaki erkek, dişi ve tüm cinsiyetler için gözlenen R^2 değerleri, ağırlık ve boy arasında istatistiksel olarak anlamlı bir ilişki olduğunu göstermektedir. Korelasyon analizi cinsiyet, boy, ağırlık, kondisyon faktörü ve nispi kondisyon faktörü arasında bazı pozitif ve negatif anlamlı ilişkileri göstermiştir. Buna bağlı olarak, iki farklı habitatta kondisyon faktörü, nispi kondisyon faktörü, boy ve ağırlık arasında önemli bir ilişki bulunmuştur ve bu aynı habitatta erkek ve dişi arasında farklılık göstermektedir. Dolayısıyla bu bulgu, *Mystus vittatus*'un hem doğal hem de biyoyumak sisteminde uzun vadeli strateji geliştirilmesinde balıkçılık yöneticilerine yardımcı olacaktır.

Anahtar kelimeler: Boy-ağırlık ilişkisi, kondisyon faktörü, *Mystus vittatus*, doğal gelişim, biyoyumak gelişim

INTRODUCTION

The high protein content of indigenous fish makes it a crucial dietary component. Regrettably, there is a significant decline in fish stocks globally, This decline may be attributed primarily due to two different factors: the over exploitation of the fish species and the degradation of the ecosystem, which includes pollution as a contributing factor (Zhou et al., 2010; Coll et al., 2010; Chanda, 2017). Fisheries management include the consideration of economic, social, and biological aspects that impact fish populations. Its objective is to develop a strategy that meets the nutritional needs of societies while avoiding the overexploitation of fish stocks (FAO, 2003). A crucial instrument for inquiry and management is the utilization of biometric studies, which provide data on fish species to estimate their biomass (Zargar et al., 2012). When conducting biometric research, it is crucial to assess the growth features of fish, such as weight and length (Morato et al., 2001).

Furthermore, it is essential to assess the species' well-being, which can be affected by a variety of biological and environmental conditions. The length weight relationship (LWR) information also provide details on the fish's growth pattern, habitat conditions, life history, overall health, fatness and condition, as well as its physical traits, several studies have emphasized that (Schneider et al., 2000, Froese, 2006). In fishery studies, length-weight relationships and condition factors are most significant (Jin et al., 2015). The length-weight relationship is valuable for evaluating morphometric parameters, comprehending fish health, and assessing the composition of fish landing and overall growth potentiality of fish (Gupta and Tripathi, 2017). On the other hand, the condition factor is used to assess the state of the individual fish (Le Cren, 1951; Froese, 2006). Based on the premise the parameter is predicated that larger fish of a specific length exhibit better physiological condition. In addition, it can be utilized to determine the development and feeding rate of fish, as well as the condition of the aquatic environment in which they thrive (Fagade, 1979). The LWRs of fish species vary based on their inherent body form and physiological parameters, such as maturity and spawning (Schneider et al., 2000). The dynamics of this association may fluctuate during different seasons or even over a span of days (De Giosa et al., 2014). According to Flura et al. (2015), there is a contention that b can vary over distinct time periods, reflecting factors such as stomach fullness, overall hunger, and phases of gonad development. Furthermore, the growth process might vary within the same species inhabiting different areas, as it is influenced by a multitude of biotic and abiotic factors. The relative condition factor (Kn) is calculated by using the methods of LWRs (Le Cren, 1951), is another significant biometric instrument. Calculating the deviation of an organism's weight from the average weight in a specific sample is known as Kn measurement. It is used to evaluate if a specific water condition is suitable for fish growth (Yılmaz et al., 2012; Mensah, 2015). A fish species is considered to have a high level of overall fitness when its Kn values are approximately greater than 1 or equal to 1. *Mystus vittatus* is an important food as well as ornamental fish species in the present study area and market acceptance is very high (Paul and Chanda, 2017; Chakraborty et al., 2019; Sit et al., 2021) population size of the species is gradually facing threats to extinction locally and need immediate action for proper conservation policy and procedure as well as market availably of fish species. Biofloc is an advance fish culture technology in respect to fishery production and supply as well as minimizing environmental pollution due to aquaculture. The measurement of length weight relationship in relation is crucial for proper culture and production in relation to the natural habitat as well as cultural medium. Various researchers (Paul and Chanda, 2017; Chakraborty et al., 2019; Sit et al., 2020; Jana et al., 2022a; Jana et al., 2022b; Sit et al., 2022; Sahil et al., 2023; Sit et al., 2023, Jana et al., 2024) have also studied some aspects of various indigenous fish species in West Bengal, but none of these observed the length-weight relationship in natural and cultural environment of *M. vitatus*. Therefore, the main aim of the present work is to determine the relationship between length and weight of *M. vittatus* both male, female and combined sex wise in natural and biofloc culture system and a comparison between natural and biofloc system has been done to achieve the goal.

MATERIAL AND METHODS

Study area

For this study a total of 331 fish were sampled from various freshwater habitats and biofloc culture system during the March, 2022 - February, 2023. The Natural habitat was located in the southeastern region of West Bengal, namely in the Paschim Medinipur district (Figure 1). The fishes that were captured by using a cast net, gill net, hand net from rivers, pond and canals are shown here as natural habitats. Then they were transported to the laboratory for morphometric analysis. The identification of fish was done based on the classification system established by Talwar and Jhingran (1991).

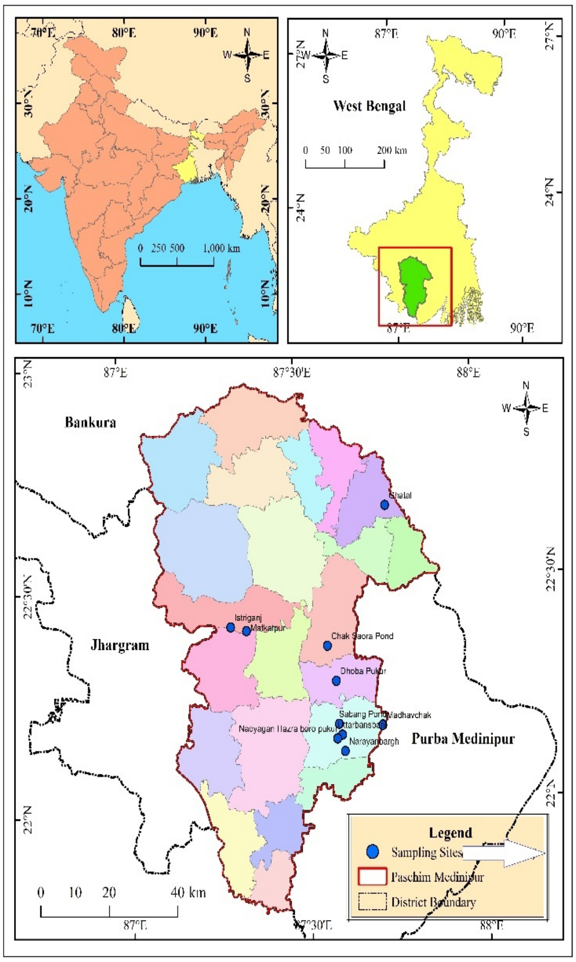

Figure 1. Study site

Length and weight measurements

The length measurements were done with an accuracy of 0.1 cm vernier calliper, measuring the total length (TL) from the snout to the end of the caudal fin and standard length (SL) from the snout to base of the caudal fin. The weight measurement was done with an accuracy of 0.001 g by keroy11 balancer.

Length and weight relationship (LWR)

The Le Cren log transformation formula has been used to establish length-weight relationships (Le Cren, 1951) or known as Cubes Law (Kalita et al., 2017). The equation was used $W = aL^b$ to evaluate the correlation between the fish weight (g) and overall length (cm) of the fish. Applying linear regression to the equation after log transformation

$$\text{Log}(W) = \log(a) + b\log(L)$$

In the equation, 'a' indicates the coefficient of body shape and 'b' is the slope of the relationship or growth coefficient. The values of 'b' can be smaller or larger, indicating negative or positive allometric growth, or equal to 3, indicating isometric growth. The equation relates weight (W) in grams to total length (L) in centimeters. To determine LWR, fish were divided into male and female groups in two different habitats.

Fulton's condition factor (K)

The condition factor exhibits a negative correlation with length. Therefore, it is possible to compare the differences in the physiological condition of fish populations that live in various feeding or climatic situations. By using the following formula, the condition factor was calculated:

$K = 100\times(W/L^3)$ (Fulton, 1904)

Here, W= Weight of the fish (g), L= Length of the fish (cm)

Relative condition factor (Kn)

The relative condition factor (Kn=W/w) is determined by dividing the observed fish weight (W) at a specific length by the expected fish weight (w) of the same length, which is calculated using Le Cren (1951) formula. This factor has been calculated for both male and female specimens of *Mystus vittatus* in two different habitats. The fish is considered to be in a state of good growth when the Kn value is equal to or more than 1. Conversely, if the "Kn" value is less than 1, the fish is in a state of poor growth relative to an average individual of the same length. It is important to mention that the stomachs of fish were not removed prior to being weighed.

Modified condition factor

$K = 100\ W/L^b$ (Ricker, 1975)

Where, W=fish weight in gram, L= fish length in centimeter and b is b value of length- weight relationships.

Data analysis

The data was analysed by using Microsoft Excel 2019. To study the data of LWRs, one-way ANOVA was applied to assess the statistical analysis identified up to the P value below 0.05. The t-test was applied to compare b value for each sex with the predictions made for isometric growth (b = 3) in order to determine whether the difference is statistically significant. When the value b is lower than 3 ($P < 0.05$) indicates negative allometric and when it is greater than 3, indicates positive allometric growth. Correlation was performed for establishing statistical relationships among habitat and sexes.

RESULTS

The male and female individuals of *M. vittatus* in natural habitat system ranged in length from 4.1 to 16.0 cm and weight from 0.7 to 42.8 g (Table 1). Simultaneously, in biofloc culture system, length ranges from 6.1 to 13 cm and weight 1.8 to 24.6 g in the present study (Table 1). The 'b' values and 'R^2' values in the natural habitat vary from 2.93 to 3.12 and 0.965 to 0.978, respectively, whereas in the biofloc system it ranges from 2.72 to 2.85 and 0.870 to 0.892 (Table 2 and Figure 2).

Table 1. Length and weight values of *Mystus vittatus* individuals used in this study

Habitat	Sex	Total length (cm)				Total weight (g)			
		Min.	Max.	Average	SD	Min.	Max.	Average	SD
Natural Habitat	Male	4.3	12	7.798	±1.730	0.7	14.5	4.794	±3.086
	Female	4.1	16	8.268	±2.643	1	42.8	7.586	±8.959
	Combined	4.1	16	7.999	±2.171	1	42.8	5.987	±6.420
Biofloc culture	Male	6.4	11.5	9.385	±1.331	2.1	12.7	7.395	±2.788
	Female	6.1	13	9.978	±1.674	1.8	24.6	9.339	±4.283
	Combined	6.1	13	9.664	±1.527	1.8	24.6	8.310	±3.691

Table 2. Estimated parameters of length-weight relationships for *Mystus vittatus* from two different habitats

Habitat	Sex	a	b	R^2
Natural Habitat	Male	0.01011	2.934	0.965
	Female	0.00739	3.123	0.978
	Combined	0.00861	3.029	0.969
Biofloc culture	Male	0.01575	2.723	0.870
	Female	0.01201	2.858	0.892
	Combined	0.01297	2.817	0.889

In comparison to the natural habitat, the biofloc system exhibited the lowest 'b' value, while females show highest 'b' value in natural habitat. The condition factor (K) value ranges 0.699 to 1.50 in natural habitat and 0.587 to 1.260 in biofloc culture system in two sexes (Table 3). The relative (Kn) and modified condition factor ranges 0.739 to 1.65, 0.546 to 1.400 in natural system and from 0.696 to 1.44, 0.865 to 2.269 in biofloc culture system in two sexes (Table 3). Pearson correlation analysis revealed significant relationships between sex, length, condition factor, relative condition factor, and modified condition factor, in two habitats (Table 4). One-way ANOVA has represented statistical variations in length and weight values between two habitats and between the two sexes, at the significance level of P < 0.05 (Table 5).

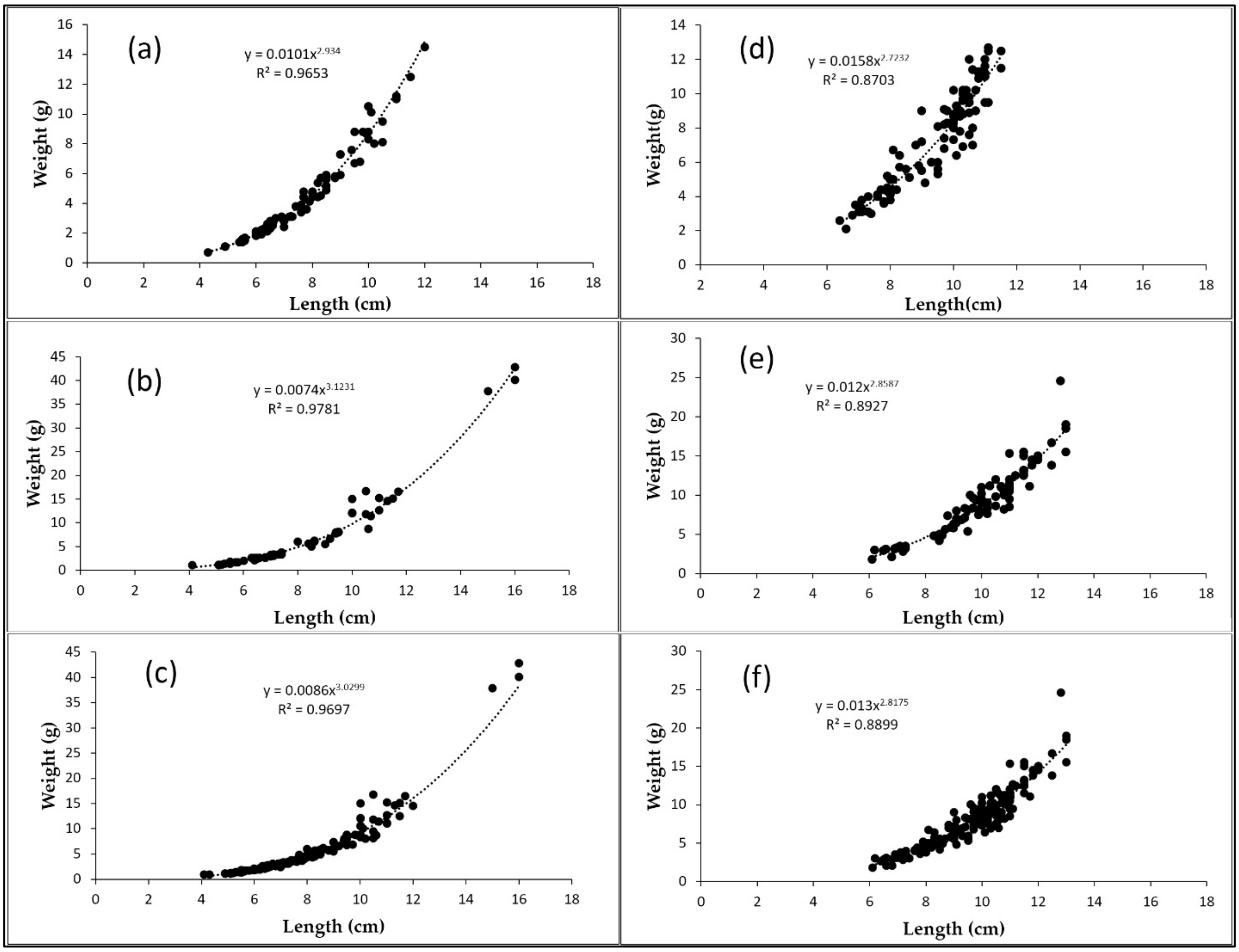

Figure 2. Length-weight relationship of *M. vittatus* in natural habitat (a-c) and culture system (d-f): a and d are male; b and e are female; c and f are combined sex

Table 3. Habitat wise condition factors of *M. vittatus*

Habitat	Sex	Condition factor (K) (Fulton,1904)				Modified condition factor (Ricker, 1975)				Relative condition factor (Kn) (Le Cren,1951)			
		Min.	Max.	Average	SD (±)	Min.	Max.	Average	SD (±)	Min.	Max.	Average	SD (±)
Natural Habitat	Male	0.699	1.051	0.888	0.082	0.795	1.222	1.015	0.094	0.786	1.20	1.004	0.093
	Female	0.730	1.50	0.963	0.154	0.546	1.219	0.746	0.117	0.739	1.65	1.010	0.158
	Combined	0.699	1.50	0.923	0.127	0.652	1.400	0.868	0.120	0.758	1.62	1.010	0.139
Biofloc culture	Male	0.587	1.260	0.858	0.123	1.130	2.269	1.591	0.218	0.717	1.44	1.01	0.138
	Female	0.629	1.258	0.878	0.124	0.865	1.682	1.213	0.168	0.720	1.39	1.01	0.140
	Combined	0.587	1.260	0.868	0.124	0.905	1.870	1.311	0.182	0.696	1.43	1.00	0.140

Table 4. Habitat wise (natural & biofloc) correlations among total length, total weight, fulton condition factor, modified condition factor and relative condition factor of male, female and combined sex of *M. vittatus*

		Total length	Total weight	Fulton cf	Modified cf	Relative cf
Natural habitat - Male	Total length	1				
	Total weight	0.96476347	1			
	Fulton cf	-0.15003376	0.003988	1		
	Modified cf	0.00598071	0.152644	0.987448	1	
	Relative cf	0.00598071	0.152644	0.987448	1	1
Natural habitat - Female	Total length	1				
	Total weight	0.926774	1			
	Fulton cf	0.263461	0.35827	1		
	Modified cf	0.026116	0.155714	0.968705	1	
	Relative cf	0.026116	0.155714	0.968705	1	1
Natural habitat - Combined	Total length	1				
	Total weight	0.898399	1			
	Fulton cf	0.114742	0.303436	1		
	Modified cf	0.056327	0.254315	0.998163	1	
	Relative cf	0.056327	0.254315	0.998163	1	1
Biofloc - Male	Total length	1				
	Total weight	0.9264	1			
	Fulton cf	-0.296	0.050241	1		
	Modified cf	-0.00682	0.335117	0.956307	1	
	Relative cf	-0.00682	0.335117	0.956307	1	1
Biofloc - Female	Total length	1				
	Total weight	0.925727483	1			
	Fulton cf	-0.187297354	0.121827	1		
	Modified cf	-0.005033676	0.296061	0.982452	1	
	Relative cf	-0.005033676	0.296061	0.982452	1	1
Biofloc - Combined	Total length	1				
	Total weight	0.92337102	1			
	Fulton cf	-0.2162123	0.107314	1		
	Modified cf	0.00055482	0.315375	0.975402	1	
	Relative cf	0.00055482	0.315375	0.975402	1	1

Table 5. Habitat wise (natural & biofloc) one-way ANOVA test among total length, total weight, fulton condition factor, modified condition factor and relative condition factor of male, female and combined sex of *M. vittatus*

Habitat	Sex		Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Natural habitat	Male	Intercept	5.20546239	0.096302846	54.05305	2.08126E-62	5.01365865	5.397266131
		Total weight	0.540839395	0.01691969	31.96509	8.06866E-46	0.507140912	0.574537877
	Female	Intercept	6.194242561	0.173018379	35.80106693	2.7921E-40	5.847645099	6.540840022
		Total weight	0.273486208	0.014812075	18.46373398	1.7523E-25	0.243814057	0.303158359
	Combine	Intercept	6.18032978	0.112407963	54.98125	8.16689E-94	5.958006412	6.40265314
		Total weight	0.30378871	0.012828999	23.67985	9.94219E-50	0.27841519	0.32916224
Biofloc culture	Male	Intercept	6.113763887	0.14418185	42.40314387	2.05445E-64	5.827602819	6.399924955
		Total weight	0.442340357	0.01825506	24.23110477	6.21747E-43	0.406109109	0.478571605
	Female	Intercept	6.597980203	0.163671951	40.31222298	1.25091E-57	6.272611132	6.923349274
		Total weight	0.361939095	0.015944636	22.69974071	4.45978E-38	0.330242208	0.393635982
	Combine	Intercept	6.48831769	0.106178694	61.10753	1.3E-124	6.27884093	6.69779444
		Total weight	0.38216512	0.011681994	32.71403	7.66E-79	0.35911806	0.40521217

DISCUSSION

Weight and length are positively correlated and hence, it is significant, according to the R^2 values, in both environments (Table 2 and Figure 2). Male in the natural habitat and female species in the biofloc system exhibit negatively allometric development or it is nearly isometric (≤3) in both environments; only the females and combined group in the natural habitat with the value of "b" show positive allometric growth. When fishes are insufficiently feeding or their environmental parameters and maturity is not favourable for their growth, then strongly negative allometric growth (< 2) is noticed, (Le Cren, 1951; Deka and Gohain, 2015). In Bangladesh, Hossain et al. (2006) documented that "b" value was 3.05, 2.96, and 3.13, for mixed sex, male, and female individuals of *M. vittatus*. In contrast, from Tamil Nadu, India, Victor et al. (2014) observed that "b" values of same species were 2.732, 2.405 and 2.873, respectively. The "b" value of the mixed sex of *M. vittatus* and had been documented as 2.88 by Srivastava et al., (2013) from India and as 3.27 by Hossain et al., (2009) from Bangladesh. Venkateshwarlu et al., (2007) obtained the "b" values for females and males of same species were 2.740 and 2.493, respectively. Krishna Rao (2007), Sani et al. (2010), Karna and Panda (2012) and Hossain et al. (2012) had documented on mixed sex of *M. cavasius* the "b" values was 2.83, 2.91, 3.009 and 3.21 accordingly. In *M. gulio* the same was measured by Begum et al. (2010) as 1.388 and 1.468 for male and female specimens whereas, Karna and Panda (2012) reported 3.032. Naeem et al., (2012) reported the same *M. bleekeri* was 2.62, 2.63 and 2.70. For *M. tengara* 'b' value was 2.071 and 0.74 to 1.39 was the Kn value (Kalita et al., 2017). The "b" values for *M. tengara* for the same variation ware 3.071, 3.119, and 2.941 (Gupta and Banerjee, 2013). Jana et al. (2022a) similarly reported a seasonal allometric growth trend in *M. tengara*, in their study the 'b' values range 2.00-3.29, the condition factor (K) ranges 0.33-1.49, and the Kn ranges 0.44- 1.77. This study also records quite similar results with the earlier researchers for *M. vittatus*, which ranges from 2.72 to 3.12. The "b" value changes mainly for the shape and fatness of the specimens, one or combination of above mention factors such as area or season, durations of sample collection, number of specimens examined etc., can create difference in "b" value (Moutopoulos and Stergiou, 2002). The parameter b, and a value, may vary according to seasonal change, or even diurnal, as well as habitat to habitat (Gonçalves et al., 1997 and Özaydin et al., 2007). According to Muchlisin et al., (2010) also suggested the same may be changed due to environmental parameters. So, at present, no data on the association between length, weight, 'K', and 'Kn' of *M. vittatus* have been investigated in sexual or habitat-related study. Hence, it is unfeasible to thoroughly compare the current outcome with prior data.

The study found that the 'K' values ranged 0.58 to 1.50, whereas 'Kn' values ranged 0.54 to 2.26 (Table 3). In female species of two habitat the highest average 'K' and 'Kn' values was observed. Kalita et al. (2017) founded the values 0.74-1.39, indicating lower 'Kn' results than the current study in different species. "Kn" values >1 for the majority of the fish indicated that they were in good health. The length of all specimens in biofloc culture system exhibited a significant negative correlation with 'K'. However, weights of the specimens show positive correlation with 'Kn' across all habitats (Table 4).

The relative condition component was shown to remain rather constant as the fish got larger or lighter, clearly shows good health of the fish. A reversal scenario was reported by Bhatta and Goswami (2014), wherein the medium-sized *Channa aurantimaculata* fish exhibited a high "Kn" value. Das et al. (2015) and Rahman et al. (2015), found the "Kn" value for *Heteropneustes fossilis* and *Anabas testudineus* in a gradient like the tendency for the same is in a degraded fashion as the size of the fish is increasing up to medium size and it is again highest in the larger fishes.

CONCLUSION

The length-weight relationship and the condition of fish are very useful parameters for understanding the general state, growth, survival, maturity, and reproduction of fish populations and also it is crucial to understand the suitability of the environment for fish, as well as playing an important role in fishery management, conservation and sustainability.

The present study represents the first data on the weight-length relationship and the condition of the *M. vittatus* species in two habitats viz. natural and culture habitat. The result of this study shows an allometric growth pattern where 'b' value ware < 3 in the Paschim Medinipur districts of West Bengal, India in both the natural and cultural habitat. The intended cube law may have been significantly deviated from by this species. In both ecosystems, the species' length and weight are significantly correlated. Significant relationships exist between weight, length, 'K', and 'Kn'; the same parameters also have habitat-dependent relationships. It was found that the 'K' and 'Kn' were in an optimal position for maintaining the health of the fish species. The obtained data could be valuable for guiding the design of future biometric studies for fish captured and culture in the study area.

Therefore, the present study certainly be helpful in assisting fishery personals in formulating significant methods for the sustainable maintenance of *M. vittatus* in natural as well as culture system also.

ACKNOWLEDGEMENTS AND FUNDING

Authors are indebted to the Principal of Raja Narendra Lal Khan Women's College (A) and departmental faculty and staff members of Postgraduate Zoology for their constant inspiration and help to conduct sustainable research work for the benefit of science and society. The author is also extremely grateful to the Department of Fishery Sciences, Vidyasagar University, for providing him with facilities as a Research Scholar. The study was supported by a Major research Project [35(Sanc.)/ST/P/S&T/17G-13/2018], Department of Science & Technology and Biotechnology, Government of West Bengal, India.

AUTHORSHIP CONTRIBUTIONS

Angsuman Chanda & Basudev Mandal: Designing, monitoring, communication, reviewing; Purnachandra Das: Specimen collection, observation, data analysis, manuscript preparation; Arun Jana: Data analysis, manuscript preparation, reviewing; Godhuli Sit: Data analysis, manuscript preparation

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

Ethical clearance from Institutional Animal Ethics Committee (IAEC), Approval no. 18/IAEC (05)/RNLKWC/2019, dated-27/07/2019

DATA AVAILABILITY

For questions regarding datasets, the corresponding author should be contacted.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 41(3), 187-193 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.03

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Heavy metals concentration in mud crab (*Scylla serrata*) and related soil at Chattogram and Cox's Bazar area of Bangladesh

Bangladeş'in Chattogram ve Cox's Bazar bölgesindeki çamur yengeci (*Scylla serrata*) ve ortam çamurundaki ağır metal konsantrasyonu

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Received date: 27.03.2024 *Accepted date: 08.07.2024*

How to cite this paper:
Hossen, S., Chowdhury, M.A.S., Das, R., & Rahman, Md.H. (2024). Heavy metals concentration in mud crab (*Scylla serrata*) and related soil at Chattogram and Cox's Bazar area of Bangladesh. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 187-193. https://doi.org/10.12714/egejfas.41.3.03

Abstract: Heavy metal levels have increased due to increased industrialization, which has a negative impact on crab, an essential source of protein. This study aimed to measure the level of certain heavy metals (Pb, Cd, and Hg) in edible tissue from mud crab (*Scylla serrata*) and related soil collected from Chattogram and Cox's Bazar in Bangladesh. The concentration of heavy metals in mud crab and the soil were determined using an Atomic Absorption Spectrophotometer (AAS) (Model ICE 3300). While the mean values of heavy metals in crab tissue samples from Chattogram were 0.06 mg/kg for Cd and 0.45 mg/kg for Pb, in the related soil samples these values were determined as 0.52 mg/kg for Cd and 7.24 mg/kg for Pb. In Cox's Bazar, the mean Pb content was 4.1 mg/kg in related soil, but the levels of Cd and Hg were below the detection limit. In *S. serrata* tissues, all heavy metal concentrations were below the detection limit. The heavy metal values obtained in the study were compared with the Food and Agriculture Organization (FAO), World Health Organization (WHO), European Union (EU) and Food and Drug Administration (FDA). Results concluded that metal concentrations were within permissible limits and did not pose an immediate risk to public health or human consumption. Specifically, the measured concentration levels are considered poor when compared to national and international requirements.

Keywords: Metal pollution, coastal area, concentration, *Scylla serrata*

Öz: Sanayileşmenin artması nedeniyle ağır metal seviyeleri artmakta ve bu da önemli bir protein kaynağı olan yengeç üzerinde olumsuz etki yaratmaktadır. Bu çalışma, Bangladeş'teki Chattogram ve Cox's Bazar'dan toplanan çamur yengecinin (*Scylla serrata*) yenilebilir dokusunda ve ilgili çamurda bazı ağır metallerin (Pb, Cd ve Hg) düzeyini ölçmeyi amaçlamıştır. Yengeç ve çamurdaki ağır metallerin konsantrasyonu, Atomik Absorbsiyon Spektrofotometresi (AAS) (Model ICE 3300) kullanılarak belirlenmiştir. Chattogram'dan alınan yengeç doku örneklerinde ağır metallerin ortalama değerleri Cd için 0,06 mg/kg ve Pb için 0,45 mg/kg iken ilgili çamur örneklerinde bu değerler Cd için 0,52 mg/kg ve 7,24 mg/kg olarak saptandı. Cox's Bazar'da ilgili toprakta ortalama Pb içeriği 4,1 mg/kg iken Cd ve Hg düzeyi tespit sınırının altındaydı. *S. serrata* dokularında ise tüm ağır metal konsantrasyonları tespit sınırının altındaydı. Çalışmada elde edilen ağır metal değerleri Gıda ve Tarım Örgütü (FAO), Dünya Sağlık Örgütü (WHO), Avrupa Birliği (AB) ve Gıda ve İlaç İdaresi (FDA) ile karşılaştırılmıştır. Sonuçlar, metal konsantrasyonlarının izin verilen sınırlar dahilinde olduğu ve halk sağlığı veya insan tüketimi açısından acil bir risk oluşturmadığı sonucuna varmıştır. Spesifik olarak ölçülen konsantrasyon seviyeleri, ulusal ve uluslararası gerekliliklerle karşılaştırıldığında zayıf kabul edilmektedir.

Anahtar kelimeler: Metal kirliliği, kıyısal alan, konsantrasyon, *Scylla serrata*

INTRODUCTION

Pollution of marine environments is a persistent worldwide issue. The issue of aquatic environment contamination by trace metals is drawing international attention, especially in developing nations such as Bangladesh (Ahmed et al., 2015). The main human-caused sources of heavy metal contamination of water, sediment, and aquatic life include industrial activities, mining, and the disposal of toxic, partially treated effluents containing metals. Trace metals are common and may accumulate in the environment, in fish, crustaceans, water, and sediments. They can then make their way up the food chain to humans. As a typical benthic organism, crustaceans particularly crabs may be regarded as a completely distinct aquatic species (Ololade et al., 2011). They may also serve as useful indicators of the levels of pollution in surface debris. Depending on the environmental conditions of the waters, heavy metal pollution that enters the aquatic ecosystem will dissolve in the water, collect in sediments, and may even get worse with time. Through the food chain, heavy metals can pass from one creature to another as well as from the environment to living things (Sugiarti et al., 2016). Some contaminants that pose a threat to aquatic biota include non-essential metals including Pb, Cd, and Hg (Setiawan, 2013). Crabs *Scylla serrata* are commonly found in mangrove zone, estuarine area, and coastal regions. Because of their increased ingesting, crabs are becoming more and more important, and this has made biological studies of them important (Sangun et al., 2009). Bangladesh's coastal regions are home to a variety of river mouth types, which contribute to the development of an estuary ecology close to the coastline (Kamal and Khan, 2009). Due to their reputation as deposit feeders in aquatic environments, mud crabs have the ability to collect heavy metals (Suprapti et al., 2012). Bangladesh's second-biggest metropolis and industrial hub is Chattogram. The banks of the Karnafuly river system are home to thousands of companies and industries. Bhatiary, Salimpur, Barabkunda, Kumira, and Sitakunda are important shipbuilding locations. They are situated on the Karnafully River's bank and along the Bay of Bengal shore. The Karnafully River and other bodies of water are contaminated by shipbreaking firms' releases of dangerous metal, persistent organic and inorganic contaminants, untreated solid waste, and liquid effluent. The shipbreaking industry is one of the major sources of trace metal on the Chattogram coast. This shipbreaking pollutes the air, sea, land, and vegetation (Das et al., 2002). There are about 30 shipbreaking yard in Bhatiary-Sonaichari shipbreaking area. Heavy metal found in different part of ship such as painting, coating, engines, electric equipment etc. (Hossain et al., 2016). Bangladesh's largest ship breaking industry is located at Fozdarhat in Chittagong, which covers about 18 km of coastal area. Abandoned oil from ships, harmful chemicals are polluting the sea water. (Rahman et al., 2023). The severity of metal pollution in Sitakunda shipbreaking area is strongly noticeable. Heavy metals emitted from home, industrial, and other man-made activities may pollute natural aquatic systems to a large extent (Velez and Montoro, 1998). The biological balance of the recipient habitat and a variety of aquatic creatures may be severely impacted by heavy metal pollution (Farombi et al., 2007). According to Hugget et al. (1973), the identification of hazardous and poisonous compounds in water, sediments, and biota would thus provide direct insight into the extent of contamination in the aquatic environment. Estuaries and coastal zones are subject to pollutant input from both particular and non-specific sources. Specifically, these environments get metal input on a chronic basis from industrialised coastal areas such as seaports and cities. Since many different species of crustaceans live in estuaries, a great deal of research has been done to look at how different toxicants affect these creatures and how they bioaccumulate them (Bryan, 1971; Rainbow et al., 1990). According to Kamaruzzaman et al. (2012), heavy metals' toxicity, protracted persistence, and capacity to bioaccumulate and biomagnify throughout the food chain make them a serious threat to aquatic ecosystems. So, more research needed in this sector to know the present status of heavy metal in this area. For humans, crabs are a vital dietary and protein source. Comparatively speaking, it's critical to keep an eye on the metal levels in the reservoir because there is no official regulation over the wastewater that households and businesses dump into the river. To evaluate the safety of eating crabs, the concentrations of arsenic and chromium were tested in their gills, muscles, hepatopancreas, and whole bodies. Additionally, it could create a baseline for upcoming research on heavy metal contamination. These include the length of exposure, the environment's temperature, interacting agents, the animal's age and metabolic rate, and the metals' biological half-lives. There are more pathways for metal removal than absorption; yet, metal accumulates more quickly than it is eliminated, most likely due to tissues' existence of metal-binding proteins (Soegianto et al., 1999). For a long time, the effects of heavy metal exposure at work or in the environment on public health were mostly ignored, despite the fact that the abundance of these substances in our environment is still increasing (Mudipalli, 2008). Unfortunately, this problem has gone unnoticed by the relevant authorities and the general public in Bangladesh. Because eating crustaceans may put coastal residents' health at risk, it is crucial to ascertain the quantities of trace metals in these organisms. Some research works have been done in Bangladesh on heavy metal pollution in some important rivers of Bangladesh (Hossain et al., 2005; Hossain et al., 2016; Ahmed et al., 2015; Siddique and Aktar, 2012). But There is little information available on the bioaccumulation of trace metals in the most popular crustacean species and associated soil in the Bay of Bengal region. So the current study is monitoring heavy metal of *S. serrata* in Bhatiary area, Chattogram, and Chakaria, Cox's Bazar in Bangladesh. The study about edible crabs and related soil in Sitakunda shipbreaking area is noticeable. So, the current study aims to analyse heavy metals (Pb, Cd and Hg) in edible crab (*S. serrata)* and related soil. Different health-hazard parameters were also calculated to assess the marine environmental quality in this region concerning these pollutants, in addition to the health risks resulting from the consumption of the studied seafood.

MATERIALS AND METHODS

The levels of heavy metals were determined using an Atomic Absorption Spectrophotometer (AAS) and a standard analytical procedure. For the purpose of examining metals, the sample-collecting stage is essential. The majority of the time, samples were handled carefully to avoid contamination. The current study was conducted in Bangladesh's coastal areas (Bhatiary, Shitakunda, and Foujdarhat) and in Cox's Bazar (Figure 1). The area of Chattogram is located between 22.51^0 N to & 23.17^0 N latitude and 91.75^0 E to 91.98^0 E. The latitude of Cox's Bazar is between 21.42^0 N to 21.56^0 N and the longitude is between 92.0058^0 E to 92.0282^0 E. The samples were collected from Bhatiary, Shitakunda, Foujdarhat (Chattogram) and Cox's Bazar.

Sample collection

Adult mud crabs measuring 100 mm or more in carapace were procured from Bhatiary, Faujdarhat, Sitakunda, and Cox's Bazar. Following collection, the samples were completely cleaned in clean water, sealed in plastic bags, stored in an ice box, and then sent right away to the University of Chittagong's Faculty of Marine Sciences and Fisheries Laboratory. Then, the surface dirt from the collecting site—which was just a few centimeters deep—was packed into separate plastic bags, refrigerated, and delivered to the Faculty of Marine Sciences and Fisheries Laboratory at the University of Chittagong.

Preparation and digestion of tissue

Samples of abdomen muscle weighing 25 g were obtained by combining tissue from a total of 150 crabs (5 replicate samples from every point). After being oven-dried for 48 hours at 50°C, the tissues were ground into a powder. 1% nitric acid was used for the two rounds of digestion, with 2 mL and 10 mL in each cycle. Samples were heated to 60°C to aid in tissue breakdown and solvent evaporation. The samples were de-pigmented using hydrogen peroxide, and the products were then fully evaporated and redissolved in 25 mL of distilled deionized water (Jumawan et al., 2010).

Soil collection and preparation

Two g of soil from each duplicate was used after samples were taken using Van Veen Grab Sampler. Then, a three-step digestion procedure was carried out using 10 mL of concentrated hydrochloric/nitric acid (1:1), 10 mL of 3:1 hydrochloric/nitric acid, and 10 mL of 5% nitric acid. To aid in the breakdown and evaporation of acids at each step of digestion, a temperature of 40°C was applied. After being redissolved in 50 mL of 1% nitric acid, the final products were filtered (Jung, 2001).

Sample analysis

In order to optimise the flame condition and absorbance for the analysis, the atomic absorption apparatus was built. Next, inhaling into the flame in the AAS (Model-ICE 3300, Thermo Scientific, UK-designed, China-made) involved sample blanks as well as sample, standards, and blanks (deionized water). When calculating the concentration of different elements, an additional blank reagent was used, and the appropriate corrections were applied.

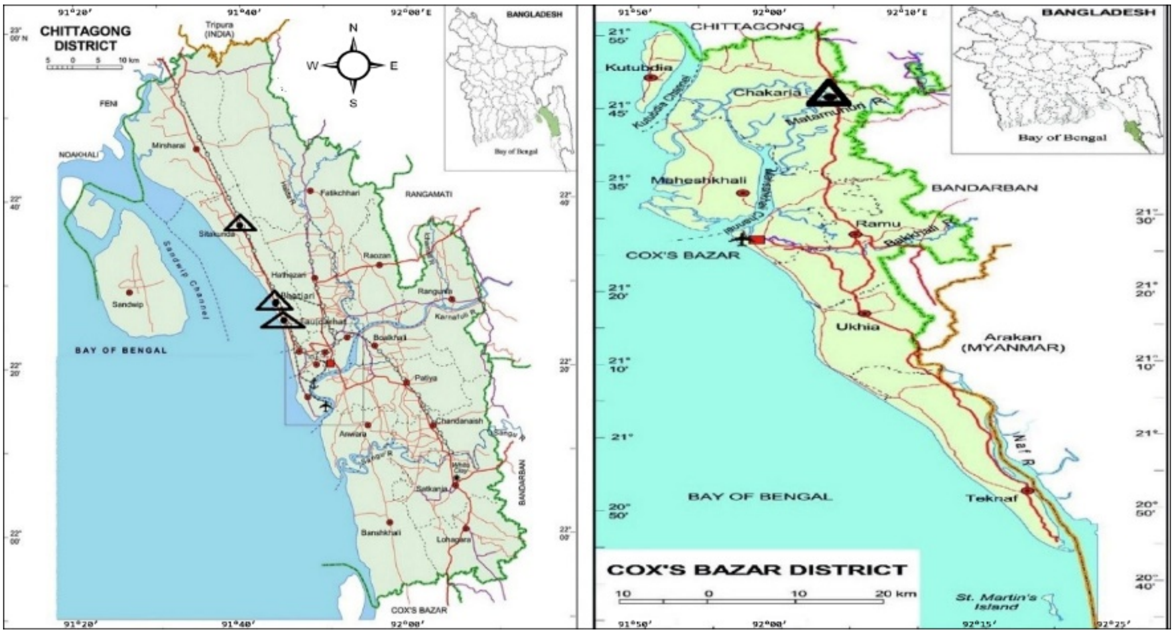

Figure 1. Study area map indicating sampling locations

Limit of detection and limit of quantification

The limit of detection (LOD) and the limit of quantification (LOQ) were used to determine the sensitivity of the flame AAS method. Based on the slope and response standard deviation, the LOD and LOQ were computed (Table 1).

Table 1. Limit of detection (LOD) and limit of quantification (LOQ) of the flame atomic absorption spectroscopy method (Vlad et al., 2019)

Parameter	Element		
	Pb	Cd	Hg
Linear working range (mg/L)	0-1	0-1	-
Limit of detection (mg/L)	0.012	0.083	-
Limit of quantification (mg/L)	0.039	0.276	-

Statistical analysis

To determine whether there are statistically significant differences in the concentration of heavy metals, one-way ANOVA was employed in this investigation. Using the statistical program of Microsoft Excel, the risk factors such as EDI & THQ were analyzed. All measurements were carried out at least five times.

Health risk assessment

The values of heavy metals concentrations in *Scylla serrata* were used to calculate the estimated daily and weekly intake, Target hazard quotient for metals and target cancer risk for the study area's people.

Estimated daily and weekly intake

The metals estimated daily intake (EDI) was determined using the daily intake in grams of mud crab soft tissue. The ingesting of seafood by coastal people was acquired from a previous study conducted by Soegianto et al. (2020), which showed a food ingestion rate (FIR) of around 140 g person^{-1}day^{-1}. The following equation was used for EDI:

$$\text{EDI} = \frac{C_m \times FIR}{BW_b}$$

Where C_m is the metal concentration in mud crab (mg.kg^{-1}), FIR is the daily food ingestion rate in grams per person per day, BW_b is an average body weight of Bangladeshi people which is taken as 49.5 kg (Ara et al., 2018; Uddin et al., 2019). The weekly intake of heavy metals (EWI) is derived by multiplying the EDI by seven days. To evaluate the risk of heavy metal food intake, the EWI values were compared to the provisional tolerated weekly intake (PTWI) standards presented.

Target hazard quotient for metals

The target hazard quotient (THQ) is the ratio of toxic metal intake to the reference dosage, which is the highest quantity at which no negative health effects occur. The THQ recognizes the hazardous metal in question's noncarcinogenic health risk. There should be no non-carcinogenic health consequences when the THQ is less than one. Negative effects on non-cancerous health outcomes might occur if the THQ is more than one. The THQ was determined using the U.S. EPA (1989) approach. THQ value was calculated using the equation-

$$\text{THQ} = \frac{C_m \times FIR \times E_f \times D_e}{RfD \times BW_b \times T_{avncar}}$$

Where, E_f denotes the frequency of trace element exposure (365 days year^{-1}), the exposure period is denoted by D_e (71.8 years), the food intake rate in grams per day for the corresponding type of food (mud crab) is denoted by FIR, C_m denotes the trace element's concentration in wet weight in the supplied food item, and RfD is the trace element's oral reference dosage. RfD values of Hg, Cd, Cr, Cu, and Zn were 0.3, 1, 3, 40, and 300 (g kg^{-1} BW day^{-1}) correspondingly (U.S. EPA, 1989). We implemented the RfD value for Pb from Hang et al. (2009), which is 3.5 g kg^{-1} BW day^{-1}. BW_b denotes the reference body weight of Bangladeshi people 49.5 kg, T_{avncar} denotes the averaged exposure period (365 days × 71.8 years).

RESULTS

Heavy metal concentration in the mud crab (*S. serreta)* and soil sample are diverse among stations.

Heavy metal concentration in the mud crab (wet weight)

The body tissue of the mud crab *S. serrata* varied somewhat in quantity depending on the site. The Pb, Cd, and Hg levels in crabs that were collected from several places in Chattogram and Cox's Bazar are shown in Table 2 and 3. Lead (Pb) concentrations in mud crab from the Chattogram coast region varied from 0.40 to 0.50 mg/kg (wet weight basis), whereas cadmium (Cd) concentrations ranged from 0.06 mg/kg (wet weight basis).

Table 2. The concentration of Lead (Pb), Cadmium (Cd), Mercury (Hg) in the body of Crab *S. serrata* (wet weight basis, mg/kg) collected from Chattogram coast

Station	Pb	Cd	Hg
1. Bhatirary	BDL	0.06	BDL
2. Shitakunda	0.40	BDL	BDL
3. Foujdarhat	0.50	BDL	BDL
4.Mean concentration	0.45	0.06	-

BDL= Bellow Detection Limit

Among the three metals tested for *S. serrata* from Chattogram coast area found that, Lead concentration was the highest (0.45 mg/kg) and Mercury concentration was the lowest (BDL). The sequence of heavy metal concentration were Pb (0.45 mg/kg) > Cd (0.06 mg/kg) > Hg (BDL). The highest concentration of Pb (0.50 mg/kg) in Foujdarhat area, Cd (0.06 mg/kg) in Bhatirary area. The sequence of metals in the station were represented in Foujdarhat (3) > Shitakunda (2) > Bhatirary (1) for *S. serrata*.

Table 3. The concentration of Lead (Pb), Cadmium (Cd), Mercury (Hg) in the body of carb *S. serrata* (wet weight basis) collected from Cox's Bazar coast

Station	Pb	Cd	Hg
1. Cox's Bazar	BDL	BDL	BDL

BDL= Below Detection Limit

Among the three metals tested for *S. serrata* from Cox's Bazar area, the concentration of all the three metals were found below detection limit.

Heavy metal concentration in the soil (wet weight)

There were some differences in the amount of heavy metals present in the soil sample between the locations. The levels of Pb, Cd, and Hg in crabs were taken from several locations in Chattogram and Cox's Bazar. In the Chattogram coast area, the mean concentration of heavy metals in the soil varied from 6.85 to 7.52 mg/kg for Pb (Table 4) and from 0.49 to 0.56 mg/kg for Cd.

Table 4. The concentration of lead (Pb), cadmium (Cd), mercury (Hg) in soil sample collected from Chattogram coast (mg/kg)

Station	Pb	Cd	Hg
1. Bhatirary	7.52	0.56	BDL*
2. Shitakunda	7.34	0.52	BDL*
3. Foujdarhat	6.85	0.49	BDL*
4. Mean concentration	7.23	0.52	-

*BDL: Below detection limit

Among the three metals tested for *S. serrata* from Chattogram coast area found that, load concentration was the highest 7.52 mg/kg and Mercury concentration was the lowest (BDL). The sequence of heavy metal concentration were Pb (7.23 mg/kg) > Cd (0.52mg/kg) > Hg(BDL).

Table 5. The concentration of lead (Pb), cadmium (Cd), mercury (Hg) in the soil collected from Cox's Bazar coast (mg/kg)

Station	Pb	Cd	Hg
1. Cox's Bazar	4.1	BDL	BDL

Among the three metals tested for soil samples from Cox's Bazar area, the concentration of all three metals was found that lead concentration was the highest (4.1 mg/kg) and other metal concentrations were found below the detection limit (Table 5).

Table 6. One-way ANOVA Result

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.169	2	0.0844	3.556	0.0958	5.143
Within Groups	0.142	6	0.0237			
Total	0.311	8				

DISCUSSION

The Chattogram Coast region (Bhatiary, Shtakunda, Faujdarhat) is a potential area of heavy metal pollution as it is the point of major ship-breaking yard and the coastal area of Sitakunda is polluted by both industrial and ship-breaking garbage, seeping traps and hence mangrove plant and fauna are susceptible to potential heavy metal pollution. The area of Cox's Bazar is also polluted by excessive tourist and human waste, waste of ships and trawlers mainly oil waste. Although the mean concentration of Pb in crab was higher than the other heavy metals, the study found that the mean concentrations of Pb (0.45 mg/kg), Cd (0.06 mg/kg), and Hg (0.45 mg/kg) in Chattogram and Cox's Bazar for crab were lower than the permissible limit of FAO/WHO (1995), Anonymous (2006) and FDA (1990-2012). The concentrations of heavy metal at the study area was not significantly high ($p>0.05$) compared to all stations (Table 6).

Target values are specified to indicate desirable maximum levels of elements in unpoluted soils compared to the FAO/WHO (1995) standard limit for Pb of 85.00 mg/kg (Table 7). All of the mean levels of these metals from the sample sites in the current investigation (mg/kg, wet weight basis) were less than the limit, with the exception of Cd (0.8 mg/kg). Table 8 lists the guidelines on trace metals for food safety for crab that have been established by several organisations.

Table 7. The heavy metal concentration (mg/kg) of soil in Chattogram coast Cox's Bazar and its comparison with International Standard and other regional studies

Elements	Target Value of Soil (mg/kg)	Chattogram Coast	Cox's Bazar
Pb	85	7.23	4.1
Cd	0.8	0.52	BDL
Hg	BDL	BDL	BDL

BDL= Below Detection Limit

Compared with the allowable limits for Pb (0.50 mg/kg), Cd (0.50 mg/kg), and Hg (0.50 mg/kg) established by the Ministry of Fisheries and Livestock Government of Republic Bangladesh (2014). Every sampling station's mean result for these metals in the current investigation was below the standard limit. The metal level is likewise below the FDA (1990–2012) and FAO/WHO's (1995) recommended guidelines for Pb, Cd, and Hg (Table 8).

Table 8. The heavy metal concentrations (mg/kg) in crab *S. serrata* and its comparison with national and international standards

Heavy metal	Ministry of Fisheries and Livestock (2014)	FAO/WHO (1995)	FDA (1990-2012)	EC (1881/2006)	Present study
Pb	0.50	0.50	-	0.50	0.45
Cd	0.50	2.00	-	0.50	0.06
Hg	0.50	0.50	0.65	0.50	-

The overall content of Pb and Cd in the soil was shown to be greater than that of the crab. This result confirmed that the Chattogram and Cox's Bazar costal region had clearly experienced bioaccumulation. It is evident that some heavy metals might be absorbed and stored by soil. Even while Chattogram and the Cox's Bazar coastal area have acceptable levels of heavy metal concentration in the soil and crabs, the rising levels of metals pose a serious threat to the ecosystem going forward.

Hutton (1987) suggested that Pb may be the cause of health issues in the hematological, neurological, and renal systems—the three organ systems that make up the human body. The amount of crab an individual consumes (g/weight) will likely determine the possible risks of metal transmission to humans. Pb exposure may result in a coma or perhaps death for the afflicted individual (Hutton, 1987). If an adult takes 10g of *S. serrata* per day from the Chattogram coast area, their daily intake of lead would be about 4.5 mg/kg ($0.45 * 10 = 4.5$ mg/kg). The user would ingest 31.50 mg/kg of lead if he consumed the crab for seven days. This is less than the FAO/WHO (1995) recommended threshold for the provisionally tolerated weekly intake of lead (50.00 mg/kg/adult). Likewise, an adult who eats crab from the same region will take in 4.2 mg/kg Cd over the course of seven days. Once more, this amount of intake is below the FAO/WHO (1995) permitted limits (6.70–8.30 mg/kg for adults). The human body eliminates cadmium at a relatively slow rate. The human body will accumulate copper if it is consumed over an extended period of time (Filov et al., 1993). According to Patnaik (1992), nausea, vomiting, diarrhea, headaches, stomach discomfort, muscle aches, salivation, and shock are the acute toxic symptoms of concentration of Cd intake. The concentration level of Pb (7.23mg/kg) in the soil of the Chattogram coastal area is lower than the permitted standards (85mg/kg) of FAO/WHO. But activity in Chattogram coastal area is concerning about increasing number of Pb and Cd in soil. In other hand Pb concentration level is lower in Cox's

Bazar coast. But it should need monitoring and awareness to control heavy metal pollution in Cox's Bazar coastal region.

All metal levels in mud crabs (*Scylla serrata*) from our study areas were lower than the maximum permissible amounts established by regulatory standards , PTWI (Table 9), and THQ regulations (Table 10). As a result, it seems safe for human health to consume mud crab (*Scylla serrata*) from this location based on the current available criteria.

Table 9. The EWI of metals by consuming mud crab (*Scylla serrata*)

Location	EWI of metal (µg kg^{-1})	
	Pb	Cd
Chattogram coast	8.90	1.19
The PTWI requirement (µg kg^{-1} BW)	25	7
References	EC (2001)	EC (2001)

Table 10. Estimated target hazard quotient (THQ) for studied metals examined in mud crab (*Scylla serrata*)

THQ	Metal	
	Pb	Cd
Chattogram coast	0.364	0.170
RfD (µg kg^{-1} BW day^{-1})	3.5	1

CONCLUSION

Crabs accumulate heavy metals (Pb, Cd, and Hg) to a significant degree. The purpose of this study was to find out more about the concentrations of heavy metals in crab and related soil from the southeast region of Bangladesh. Crabs showed a greater average content of cadmium, lead and mercury due to a unique bioaccumulation. This investigation made it clear that *S. serrata* and soil had heavy metal accumulations that were below nearly all international standard values. The quantity of heavy metals in Cox's Bazar is rather high, and soil, crustaceans, and the environment should all be monitored to guarantee safety. Although greater than in Cox's Bazar, the concentration levels along the Chattogram shoreline were still tolerable. If the concentration of heavy metals increases on a regular basis, it will become concerning. To identify the bioaccumulative heavy metal, this matter has to be thoroughly investigated.

ACKNOWLEDGEMENT AND FUNDING

The authors would like to thank to all the lab members of the Faculty of Marine Sciences and Fisheries, University of Chittagong.

AUTHORSHIP CONTRIBUTIONS

Shipan Hossen: Conceptualization, Funding acquisition, Methodology, Writing- Original draft preparation. Mohammad Abu Sayed Chowdhury: Analysis, Writing- Review and editing. Rimu Das: Analysis, Writing- Reviewing and Editing. Md. Habibur Rahman: Supervision, Validation, Writing- Reviewing and Editing.

CONFLICTS OF INTEREST

I declare that the authors have no competing interests as defined by EgeJfas or other interests that might be perceived to influence the results and/or discussion reported in this paper.

ETHICAL APPROVAL

Not applicable.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 41(3), 194-206 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.04

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Optimization of extraction conditions for obtaining active compounds of *Ulva* sp.

Ulva sp.'nin aktif bileşiklerinin elde edilmesi için ekstraksiyon koşullarının optimizasyonu

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Received date: 05.04.2024 Accepted date: 29.07.2024

How to cite this paper:
Öder, G., Bayraktar, O., & Bıçakçı, B.T. (2024). Optimization of extraction conditions for obtaining active compounds of *Ulva* sp. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 194-206. https://doi.org/10.12714/egejfas.41.3.04

Abstract: *Ulva* sp., a green macroalgae known as sea lettuce, is rich in polysaccharides, proteins, minerals, and bioactive compounds with antimutagenic, anticoagulant, anticancer, anti-inflammatory, antibacterial, and nutraceutical properties. Its abundance along the Aegean Sea coast poses an environmental challenge, as it is often disposed of as waste. However, *Ulva* sp. holds potential for high-value products in cosmetics and dietary supplements. Optimizing the extraction of its bioactive compounds using response surface methodology involved adjusting ethanol concentration, solid/liquid ratio, and extraction time. Key responses evaluated included yield, total polysaccharides, total protein, total phenol, total antioxidant activity, alpha-glucosidase inhibitory activity, and yeast cell glucose uptake. In this study, extraction yields ranged from 0.86% to 22.47% based on variations in extraction conditions. The highest total protein content was 106.88 mg BSA/g dry extract, while the polysaccharide content was determined to be 15.42%. The highest values for total phenol content and antioxidant capacity were found to be 82.15 mg GAE/g dry extract and 63.63 mg Trolox/g dry extract, respectively. The determination of the total amounts of antioxidants and phenolic compounds in extracts may expand their potential applications. In addition, the potential application of *Ulva* sp extracts as inhibitors for the treatment of diabetes has been demonstrated through experiments assessing both alpha-glucosidase enzyme inhibition and glucose uptake in yeast cells. The results support an environmentally friendly approach for the utilization of *Ulva* sp. from waste into valuable antidiabetic products.

Keywords: *Ulva* sp., response surface methodology, optimization, bioactivity, characterization

Öz: Deniz marulu olarak bilinen yeşil bir makroalg olan *Ulva* sp., antimutajenik, antikoagülan, antikanser, antiinflamatuar, antibakteriyel ve nutrasötik özelliklere sahip polisakkaritler, proteinler, mineraller ve biyoaktif bileşikler açısından zengindir. Ege Denizi kıyılarında bol miktarda bulunması, genellikle atık olarak atıldığı için çevresel bir zorluk oluşturmaktadır. Ancak *Ulva* sp. kozmetiklerde ve diyet takviyelerinde yüksek değerli ürünler için potansiyel taşımaktadır. Tepki yüzey metodolojisi kullanılarak biyoaktif bileşiklerinin ekstraksiyonunun optimize edilmesi, etanol konsantrasyonunun, katı/sıvı oranının ve ekstraksiyon süresinin ayarlanmasını içermektedir. Değerlendirilen temel tepkiler arasında verim, toplam polisakkaritler, toplam protein, toplam fenol, toplam antioksidan aktivite, alfa-glukozidaz inhibitör aktivitesi ve maya hücresi glikoz alımı yer almıştır. Bu çalışmada, ekstraksiyon koşullarındaki değişikliklere bağlı olarak ekstraksiyon verimleri %0,86 ile %22,47 arasında değişmiştir. En yüksek toplam protein içeriği 106.88 mg BSA/g kuru ekstre iken, polisakkarit içeriği %15.42 olarak belirlendi. Toplam fenol içeriği ve antioksidan kapasitesi için en yüksek değerler sırasıyla 82.15 mg GAE/g kuru ekstre ve 63.63 mg Trolox/g kuru ekstre olarak bulundu. Ekstraktlardaki toplam antioksidan ve fenolik bileşik miktarlarının belirlenmesi, potansiyel uygulamalarını genişletebilir. Ayrıca, Ulva sp. ekstraktlarının diyabet tedavisinde inhibitör olarak potansiyel uygulaması, hem alfa-glukozidaz enzim inhibisyonunu hem de maya hücrelerinde glikoz alımını değerlendiren deneyler yoluyla gösterilmiştir. Sonuçlar, *Ulva* sp.'nin atıktan değerli antidiyabetik ürünlere dönüştürülmesi için çevre dostu bir yaklaşımı desteklemektedir.

Anahtar kelimeler: *Ulva* sp., tepki yüzey metodolojisi, optimizasyon, biyoaktivite, karakterizasyon

INTRODUCTION

The oceans, teeming with life, offer a wealth of resources with diverse applications in medicine, cosmetics, and food. Among these resources, macroalgae, classified into Chlorophyta, Rhodophyta, and Ochrophyta, stand out for their rich content of dietary fiber, proteins, vitamins, and minerals, alongside unique secondary metabolites (Milchakova, 2011). The marine environment, boasting vast biological diversity, hosts valuable bioactive compounds, that hold potential therapeutic applications in addressing human diseases like cancer, inflammatory conditions, and viral infections (Nayak et al., 2021). This has fueled a growing interest in harnessing macroalgae as the source of bioactive compounds for various health benefits.

Macroalgae extracts, sourced from various species, exhibit potent pharmacological properties against viral, bacterial, and fungal infections, including common ailments like colds and flu. Notably rich in antioxidants, brown algae and other macroalgae species contain compounds such as sulfated polysaccharides, carotenoids, sterols, peptides, and mycosporine-like amino acids, contributing to their remarkable therapeutic potential (Plaza et al., 2008).

Ulva sp., commonly known as sea lettuce, represent a ubiquitous presence worldwide, displaying two main morphologies: tubular monostromatic and leafy distromatic, with some species exhibiting both forms. This diversity arises from genetic variability and environmental conditions, necessitating genetic-based identification methods. Out of approximately 400 identified *Ulva* sp., only about 40 have been taxonomically recognized through genetic analysis (Alsufyani et al., 2020; Fort et al., 2022).

Ulva sp., renowned for their abundant and nutrient-rich biomass, hold immense potential for various industrial applications (Trivedi et al., 2016). Their composition, encompassing proteins, carbohydrates, polysaccharides, minerals, and lipids, exhibits variations influenced by species, populations, and environmental factors like temperature and salinity (Rasyid, 2017).

Ulva sp. also contain flavonoid and phenolic compounds, with water-extracted *Ulva lactuca* exhibiting significant phenol and flavonoid content. Notably, phenolic compound levels exhibit seasonal variations, peaking during the summer months.

In addition to their nutritional composition, *Ulva* sp. demonstrate diverse biological activities, including antioxidant (Hassan et al., 2011; Yaich et al., 2017), anti-inflammatory (Wekre et al., 2019), antibacterial (Alghazeer et al., 2013), antitumor (Abd-Ellatef et al., 2017; Arsianti et al., 2016), anti-hyperlipidemic (Pengzhan et al., 2003), hypocholesterolemic (Hassan et al., 2011), hepatoprotective (Devaki et al., 2009), cytotoxic (Alves et al., 2013), antifungal (Tüney et al., 2006), antiviral (Chiu et al., 2012), anti-parasitic (Spavieri et al., 2010), insecticidal (Abbassy et al., 2014), and plant growth activities (Hassan and Ghareib, 2009).

Ulva sp. exhibit antibacterial potential, offering a renewable and sustainable source of antibacterial compounds crucial for combating antibiotic resistance (Alghazeer et al., 2013). However, the biological activities of Ulva extracts varies significantly, influenced by extraction methods, solvents, physiological conditions of *Ulva* sp., and harvesting times.

In the realm of antidiabetic activity, *Ulva* sp. demonstrate promise as natural antidiabetic agents, with studies highlighting their antioxidant properties and ability to inhibit carbohydrate-hydrolyzing enzymes. Polysaccharides from *Ulva lactuca*, for instance, have been shown to effectively lower blood glucose levels and inhibit enzyme activity in diabetic animal models (Belhadj et al., 2021), while Ulva extract-loaded nanoparticles exhibit anti-inflammatory and hypoglycemic effects (Al-Malki et al., 2019). Moreover, Ulva water-ethanol extracts have demonstrated efficacy in reducing insulin resistance and cholesterol levels, showcasing anti-diabetic and anti-atherosclerotic effects (Labbaci and Boukortt, 2020).

The selection of extraction parameters is based on the most critical conditions which affect the biological activities of *Ulva* sp. The most important parameters for extraction optimization were identified as solid/liquid ratio, time, and solvent type based on a systematic literature review and our preliminary experimental results in extraction processes. In this study, different biological activities were reported for extracts obtained by optimization of each of these conditions. The effective extraction of bioactive compounds from Ulva depends on developing suitable extraction methods. Extraction optimization is crucial to maximize the yield of these substances, as improper conditions can lead to their loss or degradation. Therefore, the quality and bioavailability of Ulva extracts are directly tied to the careful optimization of extraction parameters, ensuring the full utilization of their benefits and facilitating their industrial application.

In this study, the extraction conditions for obtaining chemical and bioactive compounds from *Ulva* sp. were optimized using response surface methodology, with ethanol concentration, solid/liquid ratio, and extraction time as independent variables. Various responses including yield, total polysaccharides, total protein, total phenol, total antioxidant activity, alpha-glucosidase enzyme inhibitory activity, and yeast cell glucose uptake for the obtained extracts were evaluated during the optimization process.

MATERIALS AND METHODS

Materials

In the study, sea lettuce was collected from the Izmir Gulf on Saturday, October 1, 2022, departing from the Karşıyaka/Izmir coast by boat, at the coordinates 38°27'48.0"N 27°04'03.4"E (Figure 1).

Figure 1. Images of sea lettuces collected with nets from seawater

The collected sea lettuce was quickly brought to the laboratory and washed first with tap water and then with distilled water. Afterward, it was spread onto filter papers for drying. The longest intact sea lettuce, found in a single piece, was 71 cm in length. The shortest one was around 30 cm. The examination revealed that all samples exhibited morphological similarities. However, genetic tests are required for species identification.

Design of experiments

The Box-Behnken Design (BBD) was used to perform Response Surface Methodology (RSM) by using Design Expert 13.0.5.0 software from Stat-Ease Inc. Three independent variables (A: Extraction time (hours), B: Solid/liquid ratio, C: Ethanol (%)) at three levels each were investigated for the optimization of the extraction process (Table 1). Following the extraction, the yield (%) (R1), total polysaccharide content (%) (R2), total phenol content (mg GAE/g dry extract) (R3), total protein (mg BSA/g dry extract) (R4), total antioxidant capacity (mg Trolox/g dry extract) (R5), alpha-glucosidase inhibition activity (mg/ml) (R6), and yeast cell glucose uptake inhibition activity (at constant concentration of 10 mg/ml) (R7) were evaluated as responses.

Table 1. Experimental ranges and levels of independent extraction variables

Factor	Factor Levels		
	-	0	+
A: Extraction Time (hours)	8	16	24
B: Solid/Liquid Ratio	20	30	40
C: Ethanol Percentage	0	50	100

Extraction of *Ulva*

The study involved processing dried *Ulva* sp. for grinding to reduce particle size. Extraction experiments, designed via Design-Expert®, varied extraction time, solid-liquid ratio, and ethanol-water ratio while keeping other factors constant. Extraction was performed in a cooled stirred incubator (NB-T205LF, N-BIOTEK), followed by concentration and alcohol removal using a rotary vacuum evaporator (LabTech) before freeze-drying (Telstar Lyoquest). The resulting powder extracts were weighed to calculate yield. Response Surface Methodology (RSM) with statistical analysis determined the suitable extraction model based on significance levels, lack of fit, regression coefficients, and predicted values within confidence intervals, affirming the model's adequacy for the study (Ummat et al., 2021).

Total phenol content

The phenolic content was analyzed using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965) with gallic acid as the standard. Samples were prepared and added to 96-well plates, with a control group using methanol. Folin-Ciocalteu reagent and sodium carbonate solution were added sequentially, followed by incubation and absorbance measurement at 725 nm. The phenolic content was quantified in gallic acid equivalents (GAE), using a gallic acid standard calibration curve (y=5.436x + 0.0095; R^2 = 0.9992).

Total antioxidant capacity

The Trolox equivalent antioxidant capacity was determined following the method described by Miller et al. (1993). An ABTS-potassium persulfate solution was incubated to trigger the reaction, and its absorbance was adjusted to 0.7 using a spectrophotometer. Extracts at the same concentration were added to 96-well plates along with ABTS solution, incubated in darkness, and absorbance was measured at 734 nm after 30 minutes. Trolox Equivalent Antioxidant Capacity (TEAC) was calculated based on the absorbance of a Trolox standard curve (y =8578.8x - 1.5223; R^2 = 0.9935).

Total protein content

The total protein content was determined using the Bicinchoninic Acid Protein based on the assay kit instruction (BCA Assay kit, Sigma-Aldrich, Přerovská et al., 2022). The protein content was calculated using a BSA calibration curve (y = 0.0011x + 0.0314; R^2 = 0.9938).

Total polysaccharide content

The determination of total polysaccharide content was performed using the method proposed by He et al. (2018). After creating a standard curve with a reference glucose solution (103 µg/mL) (y = 11.478x + 0.0449; R^2 = 0.9986), samples were prepared. The samples were treated with a 5% phenol solution and sulfuric acid and then kept in boiling water for 20 minutes. They were subsequently cooled in an ice bath for 5 minutes. Absorbance was then measured at 488 nm.

Alpha-glucosidase enzyme inhibition assay

Samples were dissolved in 5% DMSO, while alpha-glucosidase enzyme and 4-Nitrophenyl-alpha-D-glucopyranoside substrate were prepared in 0.1 M phosphate buffer (pH 6.9). Acarbose and samples were added to a 96-well plate in triplicates, followed by enzyme addition and incubation at 37°C for 15 minutes. The substrate was then added and further incubated for 10 minutes before stopping the reaction with sodium carbonate solution. Absorbance at 405 nm was measured using a microplate reader (Lankatillake et al., 2021). Results were given in terms of IC_{50} values (mg/ml).

Yeast cell glucose uptake test

The experiment involved suspending washed yeast cells in pure water to a 10% v/v final volume. Glucose solution and yeast solution were added to Falcon tubes for standard concentration and positive control, with acarbose solutions added for different concentrations. The tubes were then incubated at 37°C in a shaking incubator, followed by centrifugation and transfer of supernatant to test tubes. Acetate buffer and DNS solution were added to the test tubes, which were then boiled and cooled before measuring absorbance at 540 nm using a spectrophotometer. The results were calculated based on the acarbose standart curve (y= 0.0597 – 0.0033; R^2 = 0.9831).

RESULTS

Table 2 shows the final version of the experimental matrix with 17 runs obtained by implementing the Box-Behnken method, where 3 independent variables and 5 center points.

Yield of extraction

A quadratic model was selected for optimizing yield (%) based on the sequential sum of squares and lack of fit tests. The ANOVA results highlight the model's high significance with a Model F-value of 58.38 and a low probability of occurrence at 0.01%. Key variables like A-Time, C-Ethanol, A^2, and C^2 are deemed important for yield prediction. The equations created with the quadratic model, which was used to determine the independent variables affecting the extraction yield (%) through the sequential model sum of squares and lack of fit test, are provided below in encoded variables:

$$Yield\ \% = 6.03 + 0.2955*A + 0.5973*B - 9.35*C - 0.242*AB + 0.208*AC - 0.8685*BC + 2.74*A^2 + 3.09*C^2$$

The three-dimensional surface plots of yield (%) have been generated using the obtained model from the analysis. These graphs (Figure 2a-b-c) demonstrate the effect of combinations of the two variables on yield while keeping one factor at the center point (0) of the experimental design constant. Upon examining Figure 2a-c, although the changes in solid/liquid ratio and time are similar, it is evident that the percentage of ethanol is the most influential factor on yield.

Table 2. Final version of the experimental matrix with obtained results

	Factors*			Responses**						
Run	F1	F2	F3	R1	R2	R3	R4	R5	R6	R7
1	16	30	50	6.074	7.3	52.51	59.76	52.82	1.2	39.68
2	24	30	100	1.68	4.24	58.2	106.88	37.25	0.82	36.84
3	8	30	100	1.064	1.03	70.14	74.82	28.22	0.61	34.05
4	24	20	50	9.2	7.025	53.38	90.42	57.27	0.8	42.13
5	16	40	0	19.09	13.2	24.91	53.1	55.46	1.28	12.47
6	8	30	0	22.47	11.09	18.4	75.67	42.6	0.65	14.02
7	16	40	100	0.94	6.13	70.39	103.628	34.1	0.64	23.65
8	24	30	0	22.254	12.54	24.38	80.25	56.36	1.6	25.81
9	16	30	50	6.36	7.35	47.81	68.42	47.88	1.67	35.81
10	8	20	50	7.734	6.88	53.72	62.78	45.07	0.45	28
11	16	30	50	6.658	7.1	44.52	56.77	46.13	1.1	38.3
12	16	20	100	0.856	6.93	82.15	69.1	51.96	1.2	38.12
13	16	20	0	15.532	15.42	29.17	94.07	63.63	1.46	18.91
14	16	30	50	6.208	7.5	48.61	56.82	49.98	1.3	36.55
15	8	40	50	8.786	6.34	47.37	49.19	42.63	0.17	26.12
16	16	30	50	4.889	5.08	45.63	60.82	44.12	1.18	35.3
17	24	40	50	9.284	9.96	58.6	90.16	49.71	0.35	18.58

*F1: A: Extraction Time (hours); F2: B: Solid/Liquid Ratio; F3: C: Ethanol %

**R1: Yield %; R2 Total Polysaccharide Content %; R3: Total Phenol Content (mg GAE/g Dry Extract); R4: Total Protein Content (mg BSA/g Dry Extract); R5: Total Antioxidant Capacity (mg Trolox/g Dry Extract); R6: Alpha-glucosidase Inhibition IC50 (mg/ml); R7: Yeast Cell Glucose Uptake %

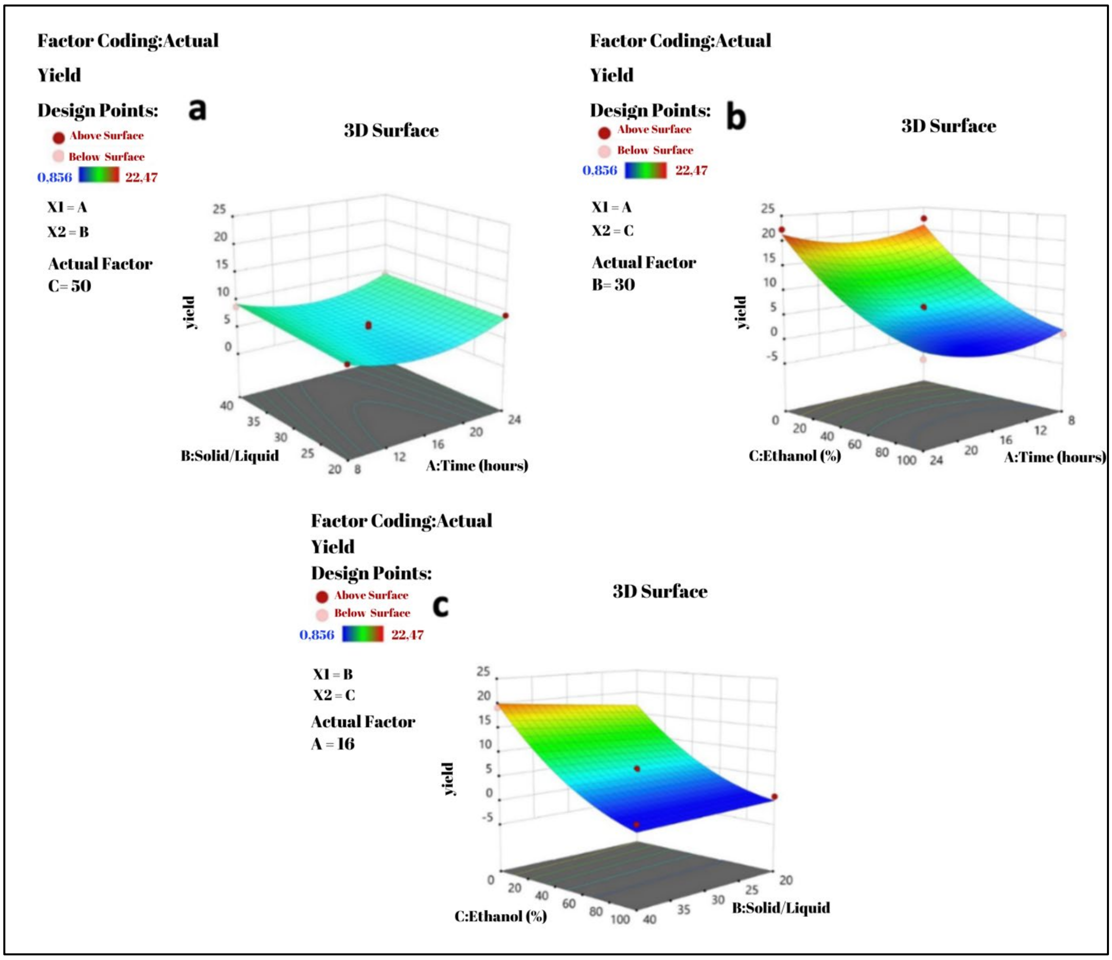

Figure 2. Response surface plots for the effects of independent variables on extraction yield. (a) A (Time, hours) vs B (Solid/Liquid Ratio), C (Ethanol, %):50; (b) A (Time, hours) vs C (Ethanol, %), B (Solid/Liquid Ratio):30; (c) B (Solid/Liquid Ratio) vs C (Ethanol, %), A (Time, hours): 16.

Total polysaccharide content

A quadratic model was chosen for optimizing total polysaccharides (%) based on the sequential model sum of squares and lack of fit tests. The ANOVA results highlight a significant Model F value of 20.34 ($p < 0.01$), indicating the model's robustness, while the lack of fit's F value of 1.29 ($p = 40.55\%$) is deemed insignificant. The quadratic model equations created for the independent variables affecting total polysaccharides (%) and formulated in terms of coded variables are as follows:

$$\text{Total polysaccharides (\%)} = 6.87 + 1.05A - 0.0781B - 4.24C + 0.8687AB + 0.44AC - 1.25A^2 + 1.94B^2 + 1.61C^2$$

Three-dimensional surface curves for total polysaccharides (%) are drawn using the model obtained from the analyses. The graphs obtained from the optimization of the current study are shown in Figure 3. These graphs illustrate the effect of the combination of the other two variables on the total polysaccharides (%) when one factor is kept constant at the center point (0) of the experimental design. Upon examining Figure 3, it can be observed that a decrease in ethanol (%) has a positive effect on the response of total polysaccharides (%). Similarly, an increase in time (hours) also increases the total polysaccharides (%).

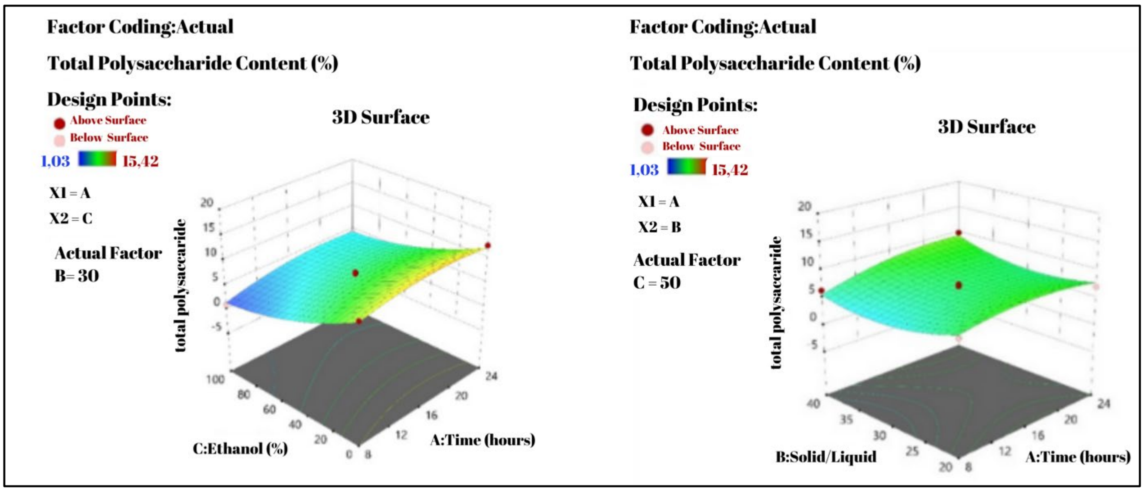

Figure 3. Response surface plots for the effects of independent variables on total polysaccharides. (a) A (Time, hours) vs C (Ethanol, %), B (Solid/Liquid Ratio):30; (b) A (Time, hours) vs B (Solid/Liquid Ratio), C (Ethanol, %):50.

Total phenol content

A quadratic model was used for optimizing total phenol content (mg GAE/g Dry Extract). Significant terms ($p<0.05$) were identified through ANOVA, and insignificant factors were integrated into the model equation. The lack of fit was deemed insignificant at a 95% confidence level, supporting the effectiveness of the simplified model equation due to the significant Model F value (34.54) and insignificant lack of fit (2.52). The optimization study resulted in a model with high R^2 (0.9719) and Adj-R^2 (0.9437) values, indicating a strong fit and absence of statistically insignificant terms. The "Adeq Precision" value exceeding 4 (20.8348) confirms the model's usability. The quadratic model equations, based on sequential model squares and adequacy tests, are provided in coded variable terms for the independent variables impacting total phenolic content (mg GAE/g Dry Extract).

$$\text{Total phenol content} = 47.1 + 0.6163A - 2.14B + 23C + 2.89AB - 4.48AC - 1.88BC + 7.07B^2 - 3.41C^2$$

Three-dimensional surface curves for total phenols (%) were created using the model derived from conducted analyses, showcased in Figures 4a-c. These graphs demonstrate how varying combinations of two variables affect total phenols when one factor remains constant at the center point (0) of the experimental design. Figure 4a,b highlights that while solid/liquid ratio and extraction time show similar variations, the percentage of ethanol stands out as the most impactful factor on total phenol content.

Total protein content

A quadratic model was selected to optimize total protein content (mg BSA/g Dry Extract) based on sequential model squares and lack of fit tests. Significant terms were identified through ANOVA ($p<0.05$), supporting the model's validity with significant F and p-values. Insignificant values were incorporated into the model equation, and the lack of fit was deemed insignificant at a 95% confidence level, with a significant Model F value (18.11) and an insignificant lack of fit value (2.20). The optimized model achieved high R^2 (0.9337) and Adj-R^2 (0.8821) values, meeting expectations with a small difference between Pred-R^2 and Adj-R^2 values (<0.2), indicating a lack of statistically significant and insignificant terms.

The quadratic model equations, based on sequential model squares and adequacy tests, are provided in coded variable terms for the independent variables impacting total protein content (mg BSA/g Dry Extract).

$$\text{The total protein content (mg BSA/g Dry Extract)} = 62.24 + 13.16*A - 2.54*B + 6.42*C + 6.87*A*C + 18.87*B*C + 8.74*A^2 + 15.58*C^2$$

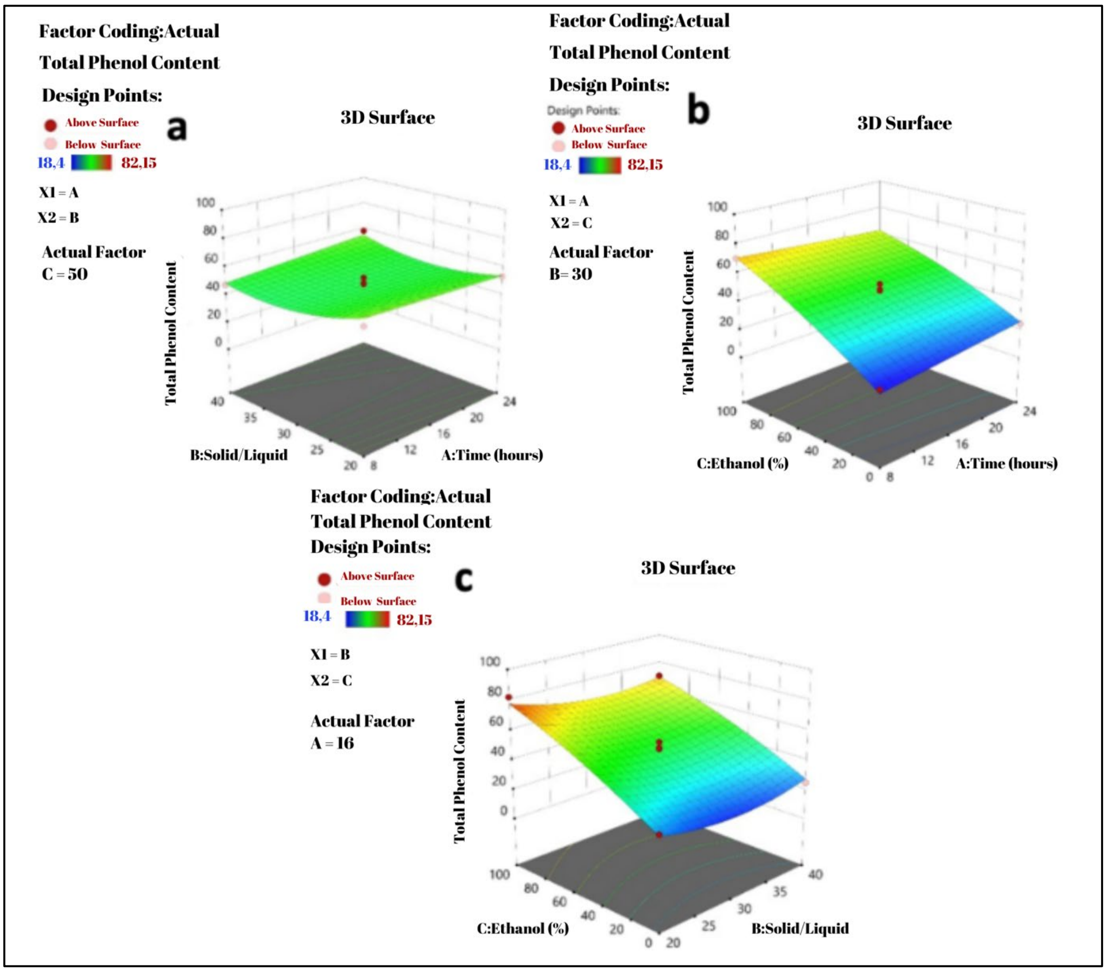

Figure 4. Response surface plots for the effects of independent variables on total phenol content. (a) A (Time, hours) vs B (Solid/Liquid Ratio), C (Ethanol, %):50; (b) A (Time, hours) vs C (Ethanol, %), B (Solid/Liquid Ratio):30; (c) B (Solid/Liquid Ratio) vs C (Ethanol, %), A (Time, hours): 16.

Three-dimensional surface curves were created for total protein content (mg BSA/g Dry Extract) using the obtained model, shown in Figure 5a-c. These graphs illustrate how varying combinations of two variables affect total protein content when one factor remains constant at the center point (0) of the experimental design. Figure 5a indicates that total protein content peaks when the solid/liquid ratio is minimal and the ethanol percentage is maximal, while Figure 5c shows that maximum extraction time and ethanol percentage result in the highest total protein content.

Total antioxidant capacity

The study on total antioxidant capacity (mg Trolox/g Dry Extract) employed a quadratic model selected based on the sum of squares and lack of fit test results, with significant terms identified through ANOVA ($p<0.05$). Main effects, interactions, and second-order terms were found to be statistically significant, supported by an insignificant lack of fit at a 95% confidence level (F value of 0.883) and a Model F value of 13.92 in ANOVA. The optimized model achieved an R^2 value of 0.9330 and an Adj-R^2 value of 0.8659, signifying a good fit of the model to the data.

The coefficients obtained for the coded independent variables for the model equation are as follows:

Total antioxidant capacity (mg Trolox/g Dry Extract) = $48.19 + 5.26{*}A - 4.5{*}B - 8.32{*}C - 4.85{*}A^2 + 5.33{*}B^2 - 2.23{*}C^2$

The optimization study resulted in three-dimensional surface plots illustrating the total antioxidant content (mg Trolox/g Dry Extract) using the obtained model. Figure 6a-c showcase the effects of varying combinations of two variables on the response, with one variable held constant at the center point (0) of the experimental design. In Figure 6a, minimizing the percentage of ethanol and the solid/liquid ratio maximizes total antioxidant content, while Figure 6b indicates that increasing extraction time can compensate for a decrease in ethanol percentage. Additionally, Figure 6c demonstrates that reducing the solid/liquid ratio and increasing time lead to increased total antioxidant content.

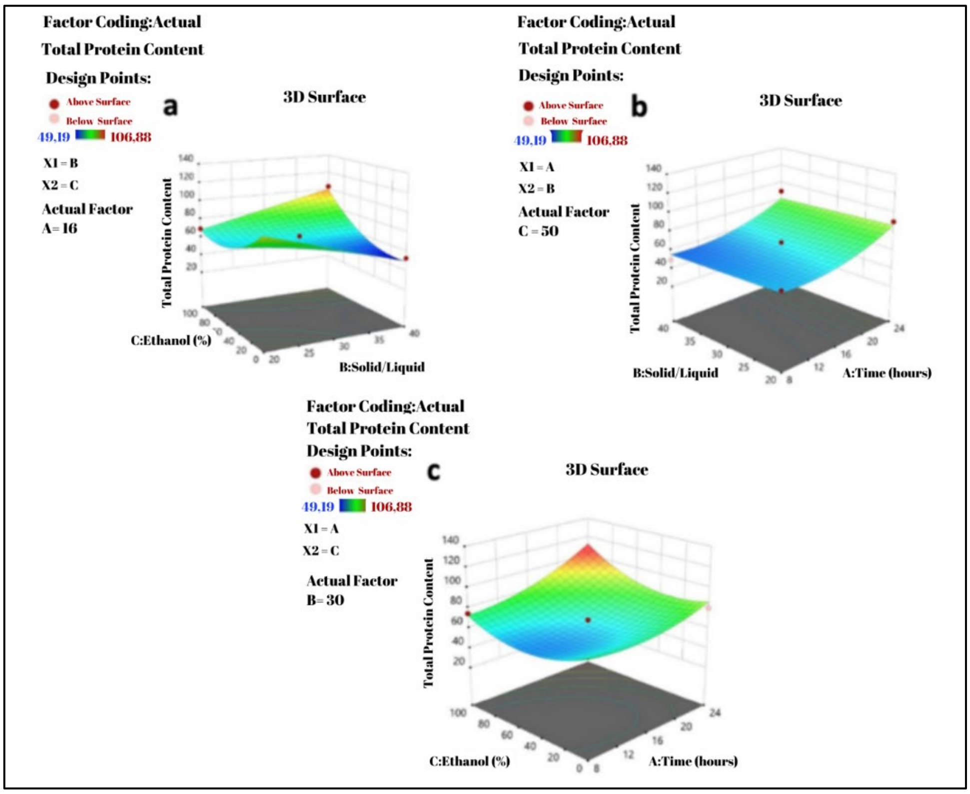

Figure 5. Response surface plots for the effects of independent variables on total protein content. (a) B (Solid/Liquid Ratio) vs C (Ethanol, %), A (Time, hours): 16; (b) A (Time, hours) vs B (Solid/Liquid Ratio), C (Ethanol, %):50; (c) A (Time, hours) vs C (Ethanol, %), B (Solid/Liquid Ratio):30.

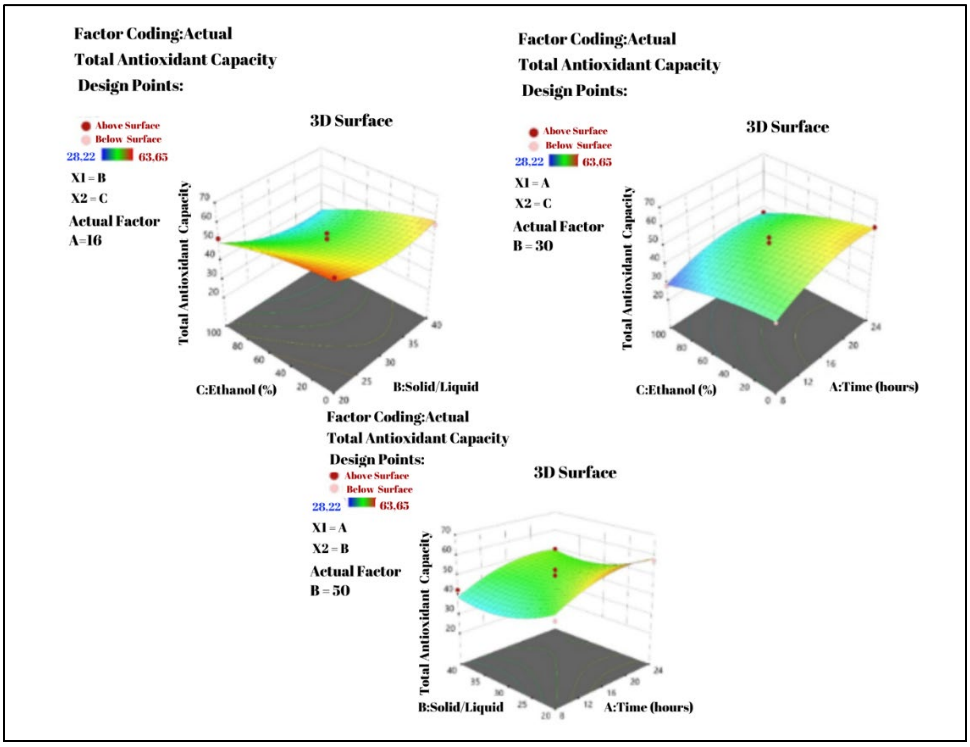

Figure 6. Response surface plots for the effects of independent variables on total antioxidant capacity. (a) B (Solid/Liquid Ratio) vs C (Ethanol, %), A (Time, hours): 16; (b) A (Time, hours) vs C (Ethanol, %), B (Solid/Liquid Ratio):30; (c) A (Time, hours) vs B (Solid/Liquid Ratio), C (Ethanol, %):50.

Alpha-glucosidase enzyme inhibition activity

The optimization of glucosidase inhibitor activity (mg/ml) involved selecting a quadratic model, with significant terms determined through ANOVA and model adequacy, confirmed at a 95% confidence level. Equations based on coded variables were formulated to represent the influential independent factors affecting glucosidase inhibitor activity.

Equations based on coded variables for influential independent factors affecting glucosidase inhibitor activity were formulated, showcasing the model's suitability for optimization purposes.

Glucosidase inhibitor activity (mg/ml) = $1.29 + 0.2112A - 0.1838B - 0.215*C - 0.0425*A*B - 0.185*A*C - 0.095*B*C - 0.5363*A^2 - 0.3113*B^2 + 0.1663*C^2$

The analysis led to the generation of 3D surface plots depicting alpha-glucosidase inhibitor activity (mg/ml) using the obtained model, as shown in Figure 7a-c. These graphs demonstrate the impact of variable combinations on the response while maintaining one variable constant at the center point (0) of the experimental design.

The results, represented in terms of IC_{50} values, reveal that increasing the solid-to-liquid ratio and ethanol percentage positively affect the response, as seen in Figure 7a, while Figure 7b shows a positive effect when time and ethanol percentage are minimized, and Figure 7c indicates a positive effect with an increase in the solid-to-liquid ratio and a decrease in extraction time on alpha-glucosidase enzyme inhibitor activity.

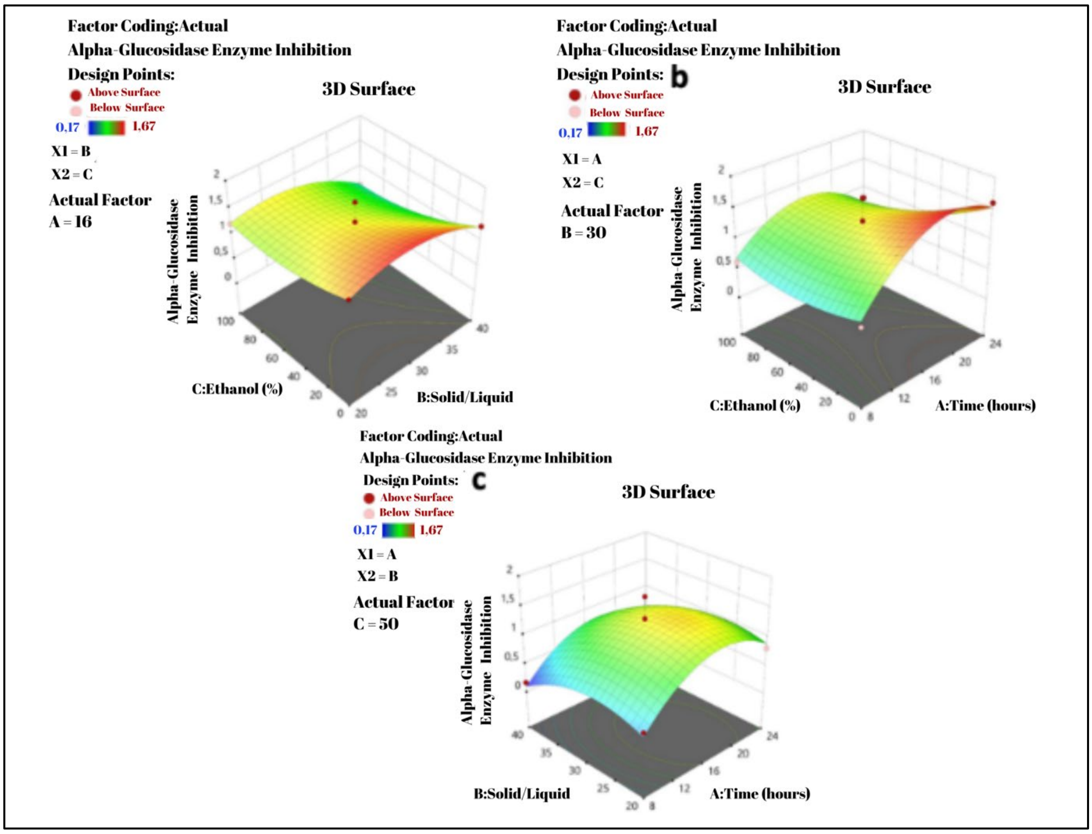

Figure 7. Response surface plots for the effects of independent variables on alpha-glucosidase enzyme inhibition activity. (a) B (Solid/Liquid Ratio) vs C (Ethanol, %), A (Time, hours): 16; (b) A (Time, hours) vs C (Ethanol, %), B (Solid/Liquid Ratio):30; (c) A (Time, hours) vs B (Solid/Liquid Ratio), C (Ethanol, %):50.

Yeast cell glucose uptake

The yeast cell glucose uptake test employed a quadratic model selected based on sequential model sum of squares and model adequacy tests, with significant terms determined via ANOVA ($p<0.05$) for main effects, interactions, and second-degree expressions. Model adequacy was deemed insignificant at a 95% confidence level, indicating a strong fit for the model. The high Model F value (45.93) and insignificant model inadequacy F value (1.06) led to a simplified model equation based on significant effects. Optimization studies resulted in high R-squared (0.9833) and adjusted R-squared (0.9619) values, validating the model's suitability for predictions with a small difference between Pred-R^2 and Adj-R^2 values (<0.2) and an Adeq Precision value (21.6977) exceeding 4. The quadratic model equations in encoded variable terms accurately predict yeast cell glucose uptake inhibition activity (10 mg/ml). The quadratic model equations were derived for independent variables affecting yeast cell glucose uptake inhibition activity (10 mg/ml) in encoded variable terms following sequential model sum of squares and model adequacy testing.

Yeast cell glucose uptake inhibition activity (10 mg/ml): $37.13 + 2.65*A - 5.79*B + 7.68*C - 5.42*A*B - 2.25*A*C - 2.01*B*C - 2.01*A^2 - 6.41*B^2 + 7.43*C^2$

The obtained model was used to generate three-dimensional surface plots for the yeast cell glucose uptake test, depicted in Figure 8a-c, showcasing how varying two variables while keeping one constant affects the response. Results were calculated as % inhibition of glucose uptake at a specific concentration. Figure 8a indicates a positive impact on the response with increased extraction time and ethanol percentage, while Figure 8b shows a greater positive effect from increased extraction time compared to the solid/liquid ratio. Figure 8c reveals a negative effect on the response with decreased solid/liquid ratio and lower ethanol levels.

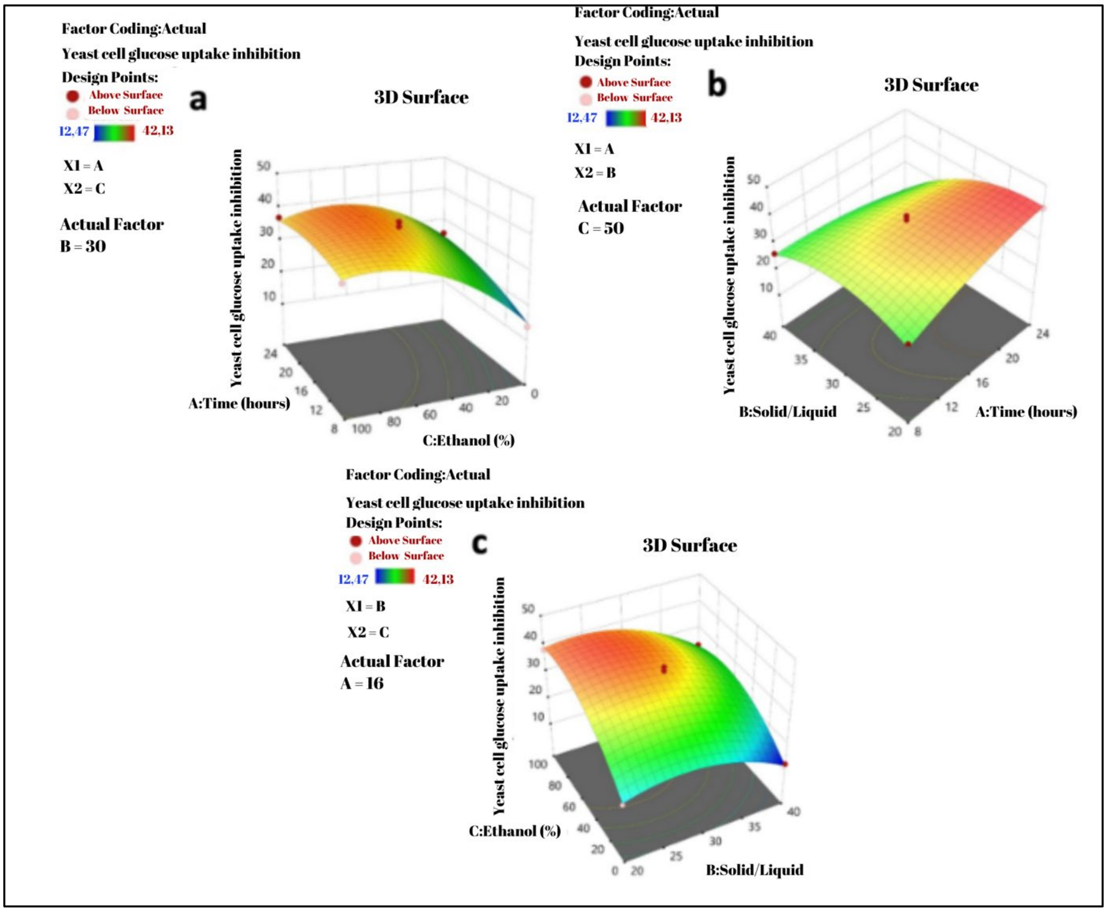

Figure 8. Response surface plots for the effects of independent variables on yeast cell glucose uptake activity. (a) A (Time, hours) vs C (Ethanol, %), B (Solid/Liquid Ratio): 30; (b) A (Time, hours) vs B (Solid/Liquid Ratio), C (Ethanol, %):50; (c) B (Solid/Liquid Ratio) vs C (Ethanol, %), A (Time, hours):16.

DISCUSSION

In the present study, the yield of extraction varied between 0.86% and 22.47% due to changes in extraction process parameters such as ethanol concentration, solid/liquid ratio, and extraction time. Our optimization study resulted in a maximum yield of 22.47% of bioactive compounds from *Ulva* sp. at an ethanol concentration of 0%, a solid/liquid ratio of 1:30, and an extraction time of 24 hours. In the literature, Ulva extraction yield ranging from 13.8% to 26.7% depending on the extraction method and conditions used, was reported. The aqueous mixture of ethanol was found to be the most efficient solvent in the recovery of bioactive compounds, with an extraction yield of 10–15% dry weight (Pappou et al., 2022). The yields obtained by the four extraction methods ranged from 17.88% to 26.77%, and the use of enzymes improved extraction yields, with the maximum yield reaching 26.7% (Juul et al., 2021).

Our results were in accordance with the results reported in the literature. An increase in the yield of extracted protein with the addition of sulfite during protein extraction from *Ulva* sp. was reported (Juul et al., 2021). The extraction yield of Ulva can be optimized by selecting the appropriate extraction method, conditions, and additives such as enzymes or sulfite (Chen et al., 2021).

In the present study, the total polysaccharide content of the extracts varied from 1.03% to 15.42% based on the extraction conditions, with the highest value (15.42%) obtained at an ethanol concentration of 0%, a solid/liquid ratio of 1:20, and an extraction time of 16 hours. Polysaccharides from *Ulva* sp., specifically ulvan, have been extracted using various methods in the literature. Ulvan is a sulfated heteropolysaccharide found in the cell wall of *Ulva* sp. It has been extracted from *Ulva fasciata Delile* collected from the Alexandria coast in Egypt. The ulvan content in *Ulva fasciata Delile* was found to be 43.66% of the total carbohydrate, with a sulfate content of 20.45% (Barakat et al., 2022). *Ulva lactuca* collected from the Alexandria coastline in Egypt also yielded ulvan, with a polysaccharide content of 36.50 g/100 g and a sulfate content of 19.72% (Ibrahim et al., 2022). The extraction of ulvan from *Ulva lactuca* biomass has been optimized using different solvents, with the most efficient solvent being an ethanol/water mixture (Pappou et al., 2022). The extraction parameters, such as time, temperature, and biomass-to-solvent ratio, have been investigated to optimize the extraction yield and antioxidant activity of ulvan (Ning et al., 2022). Ulvan has also been used to synthesize an ulvan/chitosan biomembrane with potential applications in the biomedical field (Ben Amor et al., 2023).

In the present study, the total phenol content of the extracts varied from 18.4 to 82.15 mg GAE/g Dry Extract based on the extraction conditions, with the highest value (82.15 mg GAE/g Dry Extract) obtained at an ethanol concentration of 100%, a solid/liquid ratio of 1:20, and an extraction time of 16 hours. The total phenolic content of the extracts was primarily influenced by the ethanol concentration, with extraction time and solid/liquid ratio having lesser impacts. *Ulva lactuca* and *Ulva linza* macroalgae have been studied for their potential in polyphenol extraction by several researchers. The aqueous mixture of ethanol was found to be the most efficient solvent for extracting bioactive compounds from *Ulva lactuca*, with an extraction yield of 10-15% dw (Pappou et al., 2022). *Ulva linza* extract showed anti-inflammatory effects in TNBS-induced colitis mice, suggesting its potential as a natural therapeutic agent for inflammatory bowel disease (Kim et al., 2018). Another study investigated the protective effect of *Ulva lactuca* polyphenolic extract against heavy metal mixture-induced cardiovascular diseases (Nabil-Adam and Shreadah, 2021). The extract showed antioxidant and anti-inflammatory activities, which were attributed to its high polyphenolic content. A case study explored different methods for quantifying the total phenolic content of *Ulva intestinalis*, including quantitative NMR, HPLC-DAD, and the Folin-Ciocalteu assay (Wekre et al., 2019). Their results showed variations in the quantification of polyphenols, highlighting the challenges in accurately measuring total polyphenolic content (Wekre et al., 2019). Additionally, protein extraction from *Ulva* sp. using double screw pressing and sulfite treatment resulted in improved protein quality, possibly due to inhibited oxidative reactions and improved polyphenol levels (Juul et al., 2021). Finally, pyrolysis of *Ulva lactuca* was investigated for bio-oil production, and the results showed an increase in phenolic compounds with increasing temperature, indicating the potential for producing renewable phenolic resins (Amrullah et al., 2023).

The present study investigated the total protein content of Ulva extracts, ranging from 49.19 to 106.88 mg BSA/g Dry Extract based on the extraction conditions, with the highest value (106.88 mg BSA/g Dry Extract) achieved at an ethanol concentration of 100%, a solid/liquid ratio of 1:30, and an extraction time of 24 hours. Notably, the total protein content of the extracts was significantly influenced by the ethanol concentration, extraction time, and solid/liquid ratio, with ethanol concentration and extraction time showing the most significant effects. In the literatüre various methods have been explored to enhance protein extraction from Ulva macroalgae. For instance, alkaline pretreatment and ultrasonic-assisted extraction improved protein extraction yield from *Ulva rigida* biomass (Pan–Utai et al., 2022). Another study combined enzymatic cell wall degradation with high-voltage Pulsed Electric Fields (PEF), resulting in higher protein extraction yields compared to individual treatments (Steinbruch et al., 2023). Ionic liquids (ILs) assisted mechanical shear followed by two-phase partitioning or ultrafiltration enabled selective extraction of proteins from *Ulva lactuca* (Suarez et al., 2023). Additionally, alkaline extraction at pH 8.5 and double screw pressing with sulfite addition were found to be effective methods for improving protein yield and quality from *Ulva* sp. (Juul et al., 2021).

In the present study, the total antioxidant capacity of Ulva extracts ranged from 28.22 to 63.63 mg Trolox/g Dry Extract, with the highest value (63.63 mg Trolox/g Dry Extract) achieved at an ethanol concentration of 0%, a solid/liquid ratio of 1:20, and an extraction time of 16 hours. Various extraction methods and parameters have been explored to optimize the extraction process and maximize the yield of bioactive compounds from Ulva macroalgae (Pappou et al., 2022). Ultrasonic-assisted extraction (UAE) was found to be highly effective in extracting antioxidants from *Ulva lactuca* biomass (Rashad et al., 2023). Factors such as extraction solvent, time, and temperature significantly affect the extraction process. The extraction of antioxidants from Ulva macroalgae holds promising potential for application in various industries, including food and medicine (Pan-Utai et al., 2022).

Ulva, a green marine seaweed, has been extensively studied for its antioxidant properties. Different extraction methods, such as microwave-assisted extraction, have been explored to optimize the yield and composition of ulvan, a polysaccharide found in *Ulva* sp. Microwave-assisted extraction has shown improved extraction yield and antioxidant activity of ulvan (Chen et al., 2021; Le et al., 2019). Ulva extracts, rich in polyphenols, have demonstrated significant antioxidant and anti-inflammatory activities in various studies, showcasing their potential applications in mitigating oxidative stress-related diseases (Feng et al., 2020; Nabil-Adam and Shreadah, 2021). Furthermore, enzymatic hydrolysis of sulfated polysaccharides extracted from *Ulva lactuca* has resulted in fractions with promising antioxidant and antitumor activities, suggesting their potential in pharmaceuticals (Abou El Azm et al., 2019).

The Alpha-glucosidase Inhibition activity (IC_{50}) of extracts varied from 0.17 to 1.67 mg/ml, with the lowest value (0.17 mg/ml) achieved at specific extraction conditions. *Ulva* sp. demonstrated α-glucosidase inhibitory activity, suggesting their potential as natural inhibitors for controlling blood glucose levels and preventing diabetes (Vega-Gálvez et al., 2022; Nazarudin et al., 2020). *Ulva reticulata* extracts, particularly the methanolic extract, showed significant antidiabetic activity by inhibiting carbohydrate metabolizing enzymes (Unnikrishan et al., 2022; Tong et al., 2020). Additionally, the aqueous extract of *Ulva fasciata* showed hypoglycemic effects and restored hepatic glycogen content in diabetic rats. These findings suggest that *Ulva* sp., including *Ulva reticulata*, *Ulva lactuca*, and *Ulva fasciata*, have potential as antidiabetic agents (Tas et al., 2011). *Ulva lactuca* oligosaccharides were found to have hypoglycemic effects, potentially through a specific pathway (Chen et al., 2022). Additionally, *Ulva australis* exhibited inhibitory effects on α-glucosidase and α-amylase enzymes, indicating its potential as an anti-diabetic agent (Trentin et al., 2020).

In the present study, the Yeast Cell Glucose Uptake Inhibition activity (inhibition %)) of the extracts varied between 12.47 and 42.13 % based on the extraction conditions, with the highest value (42.13 %) obtained at an ethanol concentration of 50%, a solid/liquid ratio of 1:20, and an extraction time of 24 hours. The ethanolic extract of Ulva demonstrated dose-dependent inhibition of glucose uptake in yeast cells, suggesting its potential for treating type 2 diabetes mellitus (Pitchaipillai and Ponniah, 2016). *Ulva reticulata* extracts, including the methanolic extract showed significant antidiabetic activity and were further analyzed to isolate active fractions (Unnikrishnan et al., 2022). Their study focused on identifying and characterizing compounds in the active fraction of *Ulva reticulata* for their antidiabetic potential. Marine algae, such as *Ulva* sp., have been recognized for their medicinal properties and potential as a source of antidiabetic compounds. This study contributes to the search for natural antidiabetic chemicals and emphasizes the importance of investigating marine algae for their therapeutic benefits (Unnikrishnan et al., 2022).

CONCLUSION

The optimization study on Ulva extraction processes successfully pinpointed specific conditions for achieving the highest yield and optimal levels of bioactive compounds, polysaccharides, proteins, phenols, antioxidants, and alpha-glucosidase enzyme inhibitors. It emphasized the significant influence of ethanol concentration, solid/liquid ratio, and extraction time on the extraction yield variability of bioactive compounds. Notably, the study highlighted Ulva's potential in managing blood glucose levels and preventing diabetes, showcasing its medicinal properties as a natural source of antidiabetic compounds. Marine algae like *Ulva* sp. hold promise in the quest for natural remedies for diabetes, given their recognized medicinal properties and potential as a source of beneficial compounds.

In conclusion, the multifaceted pharmacological properties of *Ulva* sp. underscore their potential as valuable sources of bioactive compounds with diverse applications in health, nutraceuticals, and pharmaceuticals. With ongoing research and development, *Ulva* sp. hold promise in addressing various health challenges and contributing to the advancement of therapeutic interventions. As the algae industry expands, there's a growing need to extract bioactive compounds efficiently. However, phenomena like algal blooms pose environmental challenges, calling for research to transform this waste into valuable products.

ACKNOWLEDGMENTS AND FUNDING

We acknowledge the support provided by the Turkish Scientific and Technological Research Institution (Grant number: 223M357) for funding this research, which was performed under WG 4 (Bioactive chemicals and Ulva-associated microorganisms) within the scope of Cost Action CA20106-Tomorrow's 'Wheat of The Sea': Ulva, A Model for An Innovative Mariculture (SEAWHEAT).

AUTHOR CONTRIBUTIONS

Oguz Bayraktar contributed to the idea and design of the study. Material preparation and research were carried out by (Gizem Öder) and (Oğuz Bayraktar). The study was the MS Thesis of Gizem Öder, but she switched to another supervisor, and she waived her rights for the publication of findings resulting from the thesis. The writing and editing of the manuscript were done by Prof. Oğuz Bayraktar`s MS student Beyza Tutku Bıçakçı under his supervision. All authors have read and approved the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest or competing interests.

ETHICAL APPROVAL

Ethical approval is not required for this study.

DATA AVAILABILITY

All relevant data is inside the article. Additional data sets of. the current study will be provided by the corresponding author upon request.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 41(3), 207-212 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.05

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Are potential bisphenol-A substitutes really safe for aquatic life? Impact on primary producers

Potansiyel bisphenol-A ikameleri sucul yaşam için gerçekten güvenli mi? Birincil üreticiler üzerindeki etkisi

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Received date: 29.05.2024 *Accepted date: 26.08.2024*

How to cite this paper:
Benas, K., & Çakal Arslan, Ö. (2024). Are potential bisphenol-A substitutes really safe for aquatic life? Impact on primary producers. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 207-212. https://doi.org/10.12714/egejfas.41.3.05

Abstract: Bisphenol A threat to environmental health and human health and has been added to the Candidate List as Very High Concern Substances by the European Chemicals Agency. This led to the replacement of bisphenol A (BPA) with bisphenol analogues, which were considered "safer". However, there are very few scientific studies on the impact of BPA analogues on the environment. In this study, three analogues bisphenol B (BPB), bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) were selected to investigate their ecotoxicological effects on the marine phytoplankton species *Phaeodactylum tricornutum*, which is representative of primary producers. *Phaeodactylum tricornutum* was exposed to different concentrations (0.5, 0.8, 1.0, 1.5, 2.0 mg/L) of BPB, BADGE and BFDGE analogues for 72 hours and the toxicity values of three BPA analogues were calculated by OECD 201 algal growth inhibition assay (IC_{50}/EC_{50}). In the light of the data obtained, algal growth inhibition (IC_{50}/EC_{50}) values for marine phytoplankton *Phaeodactylum tricornutum* were determined as 3.91 mg-BPA/L, 7.83 mg-BPB/L, 5.69 mg-BFDGE/L, 11.71 mg-BADGE/L. The results revealed that BPB, BFDGE and BADGE showed lower toxicity to *Phaeodactylum tricornutum* compared to BPA algal growth inhibition (3.91 mg-BPA/L). Therefore, it is necessary to share the results of the adverse effects of BPA analogues on aquatic organisms and to conduct ecotoxicological risk assessments.

Keywords: Bisphenol analogues, marine phytoplankton, algal growth inhibition test, toxicity*, Phaeodactylum tricornutum*

Öz: Bisfenol A çevre sağlığı ve insan sağlığı için tehdit oluşturmaktadır ve "Avrupa Kimyasallar Ajansı" tarafından "Çok Yüksek Önem Arz Eden Maddeler" olarak aday listeye eklenmiştir. Bu durum, bisfenol A'nın (BPA) "daha güvenli" olduğu düşünülen bisfenol analogları ile değiştirilmesine yol açmıştır. Bununla birlikte, BPA analoglarının çevre üzerindeki etkisine ilişkin çok az bilimsel çalışma bulunmaktadır. Bu çalışmada, birincil üreticileri temsil eden deniz fitoplankton türü *Phaeodactylum tricornutum* üzerindeki ekotoksikolojik etkilerini araştırmak için üç analog bisfenol B (BPB), bisfenol A diglisidil eter (BADGE) ve bisfenol F diglisidil eter (BFDGE) seçilmiştir. *Phaeodactylum tricornutum* BPB, BADGE ve BFDGE analoglarının farklı konsantrasyonlarına (0.5, 0.8, 1.0, 1.5, 2.0 mg/L) 72 saat boyunca maruz bırakılmış ve üç BPA analoğunun toksisite değerleri OECD 201 alg büyüme inhibisyonu deneyi (IC50/EC50) ile hesaplanmıştır. Elde edilen veriler ışığında, deniz fitoplanktonu *Phaeodactylum tricornutum* için alg büyüme inhibisyonu (IC50/EC50) değerleri 3.91 mg-BPA/L, 7.83 mg-BPB/L, 5.69 mg-BFDGE/L, 11.71 mg-BADGE/L olarak belirlenmiştir. Sonuçlar BPB, BFDGE ve BADGE'nin *Phaeodactylum tricornutum* için BPA alg büyüme inhibisyonuna (3.91 mg-BPA/L) kıyasla daha düşük toksisite gösterdiğini ortaya koymuştur. Bu nedenle, BPA analoglarının sucul organizmalar üzerindeki olumsuz etkilerinin sonuçlarının paylaşılması ve ekotoksikolojik risk değerlendirmelerinin yapılması gerekmektedir.

Anahtar Kelime: Bisfenol analogları, deniz fitoplanktonu, alg büyüme inhibisyon testi, toksisite, *Phaeodactylum tricornutum*

INTRODUCTION

In the plastics industry over the last century, monomeric components have been transformed into plastic polymers in the final product and to improve end-use performance (Deanin, 1975). BPA is an important synthetic chemical widely used in the industrial production of polycarbonate plastics and phenolic resins (Ballesteros-Gómez, 2014; Ou et al., 2006). BPA cause a hazardous effect on both human and environmental health due to its toxicity (Diler et al., 2022; Minaz et al., 2022a; Muhamad et al., 2016). The use of BPA, which has become a global concern, has been restricted by the "European Chemicals Agency and European Food Safety Authority" due to its adverse effects by being placed on the "Candidate List of Substances of Very High Concern" (Andersson, et al., 2018). As a result of these restrictions, manufacturers have developed more than 200 BPA analogues that are structurally similar (Lucarini et al., 2020; Xie et al., 2022).

BPB is used in Europe for the coating of beverage cans and production of polycarbonate resins (Grumetto et al., 2008). In a study conducted in Italy, BPB was detected in canned peeled tomatoes samples between 27.1-85.7 µg/kg (Grumetto et al., 2008). A study by Cunha et al. (2011) in canned beverages in Portugal found BPB levels as 0.06- 0.16 µg/L. In the same study, BPB was found in all cola samples tested and was detected as 0.03-4.70 µg/L (Cunha et al., 2011). Due to their lipophilic properties (logKoc>3), bisphenol analogues have a tendency to accumulate in sediment. In Beibu Bay, South China Sea, BPB was detected in both water (14%) and sediment samples (18%) (Gao et al., 2023). In a study conducted in the Persian Gulf, bisphenol A and its analogues accumulated in 5 different marine organisms and the highest levels were found in Epinephelus coioides as 13.58 µg-BPA/kg.dw and 10.30 µg-BPB/kg.dw on a dry weight basis (dw) (Akhbarizadeh et al., 2020).

BADGE (bisphenol A diglycidyl-ether) and BFDGE (bisphenol F diglycidyl-ether) are used as a monomer in epoxy resin production during the reaction of BPA or BPF (Bello et al., 2021; Poole et al., 2004; Szczepańska et al., 2018). In 2015, annual BADGE production was estimated 9072 tons in the US (Wang et al., 2021). BADGE/BFDGE is also used in the production of dental restorative products (Olea et al., 1996). Organosol polyvinylchloride (PVC) is used as an additive as an initiator in food contact applications (Coulier et al., 2010). BADGE can be easily converted to chlorinated or hydrolysis products and most of the products resulting from this conversion are toxic (Marqueno et al., 2019). Studies have identified that presence of BADGE and BFDGE in the environment (Wang et al., 2012). Due to concerns of mutagenicity, antiandrogenicity and genotoxicity (Poole et al., 2004) the European Union has set the limit for BADGE and their hydrolysis products to enter foods at 9 mg/kg (Lane et al., 2015). Biles et al. (1999) tested liquid concentrates of diet cola, canned fish products and infant formula liquid concentrates from Washington D.C. grocery stores and reported the presence of BADGE in sardine, tuna, anchovy and herring fish samples. According to study by Simoneau et al. (1999) BADGE were found tested 382 canned fish oil samples collected from 15 members of the European Union and Swiss national supermarket. When the literature is examined, there is a lack of data on in vitro toxicological studies on cell types of BADGE and BFDGE chemicals. On the one hand, BADGE has been reported to be able to induce apoptosis or cell death in cell lines (Bishop-Bailey et al., 2000).

Bisphenol A and its analogues are known to cause bioaccumulation in aquatic organisms and are prone to biomagnification at trophic levels (Guo et al., 2017; Kim et al., 2020; Wu and Seebacher, 2020). Despite the increasing number of ecotoxicology studies (Chen et al., 2002; Czarny et al., 2021; Diler et. al., 2022; Guo et al., 2017; Kovalakova et al., 2020; Liu vd., 2010; Mihaich et al., 2009; Minaz et al., 2022a, b; Minaz and Kurtoglu, 2024; Paerl and Justic, 2011; Pascoe et al., 2002; Seoane et al., 2021; Tato et al., 2018; Xiang et al., 2018), data on BPA's environmental risk and its analogues on aquatic organisms and primary producer marine phytoplankton species are extremely scarce.

Phytoplankton are photosynthetic microorganisms mediate the biogeochemical cycles of carbon, nutrients (nitrogen and phosphorus) and oxygen in aquatic ecosystems, while also playing an important role as primary producers of the food web (Cakal Arslan et al., 2024). The presence of BPA and its analogues not only negatively affects the reproduction and biodiversity of phytoplankton, but these can also cause hazard on other organisms by accumulating at higher trophic levels (Kovalakova et al., 2020). Therefore, there is a need to assess the impacts of BPA and its analogues on marine ecosystems in terms of environmental control and management.

In this study, it is aimed to reveal the ecotoxicological effects of BPA and three BPA analogues (BPB, BADGE and BFDGE) on the marine phytoplankton species *Phaeodactylum tricornutum* and to present preliminary data for a better understanding of their effects on aquatic organisms.

MATERIALS AND METHODS

Chemicals

The chemicals that formed the basis of our study in the experiments were purchased from Sigma-Aldrich (St. Louis, MO, USA) bisphenol A (BPA), bisphenol B (BPB), bisphenol A diglycidyl ether (BADGE), bisphenol F diglycidyl ether (BFDGE). Chemicals were dissolved in dimethyl sulfoxide (DMSO), determined to be non-toxic by preliminary experiments, and prepared before starting 100 ppm stock biotests.

Microalgae media and trial culture conditions

The test organism *Phaeodactylum tricornutum* was cultured in F/2 medium (Guillard, 1975) in the Ecotoxicology laboratory of the Faculty of Fisheries, Ege University.

A pre-culture was established before the test and incubated at 20±1°C. The initial cell concentration in the test cultures was approximately 4-5x10^{-4} cells/ml for *Phaeodactylum tricornutum*. The experiments were performed in 20 ml sterile glass tubes with 3 replicates. Samples were illuminated at a photoperiod of 14:10 (light:dark) hours under constant illumination at approximately 2000 lux and kept on a 100 rpm shaker. Cell density was confirmed using a Neubauer hemocytometer counting chamber at 24, 48 and 72-hour time points (Anonymous, 1984).

Algal growth inhibition test

The algal growth inhibition test was performed according to the OECD 201 standard (OECD; 2011). Cell count data at 0 and 72 h were evaluated based on growth rate as described in standard protocols. IC_{50} values were calculated as 50% growth inhibition of the test population compared to the control based on growth rate.

Statistical analysis

Data were analysed by One-Way ANOVA using the IBM software package SPSS v26 for Windows. Growth rate data were compared with controls using the Tukey test. Differences between groups were considered significant ($P<0.05$) (Özdamar, 1999).

RESULTS

Due to the importance of primary production for the aquatic ecosystem, the marine diatom *P. tricornutum* was exposed to increasing concentrations (0.5, 0.8, 1.0, 1.5, 2.0 mg/L) of BPA and its analogues (BPB, BADGE and BFDGE) to determine its algal growth inhibition value. A limiting effect was observed in the cell numbers from the lowest concentration due to toxicants compared to the control (Control=0.74-1.15; 0.5 mg/L=0.57-0.93). As a result of the study, it was determined that there were statistically significant differences between the experimental groups ($P<0.05$) (Figure 1). At the lowest concentration (0.5 mg/L), a limiting effect between 15-22% was observed in all chemicals, while the limiting effect increased in parallel with the increase in concentration. It was observed that all chemicals had a limiting effect on the growth rate depending on the concentration, and the inhibition percentages calculated by the dose-response curve also increased. (Figure 2). According to the EC_{50} values calculated for BPA and its analogues depending on different concentrations, the toxicity of bisphenols were sequenced as BPA>BFDGE>BPB>BADGE, respectively. EC_{50} values for all chemicals were calculated as 3.91, 5.69, 7.83, and 11.71 mg/L for BPA, BFDGE, BPB, and BADGE, respectively (Figure 3).

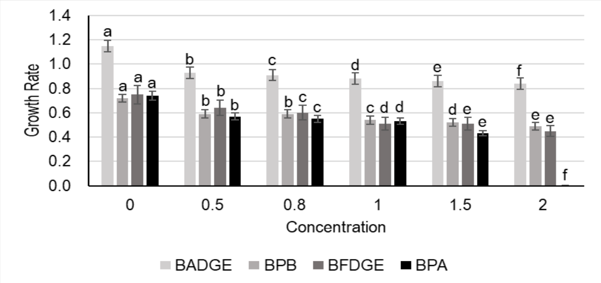

Figure 1. Growth rates of chemicals according to the results of applied concentrations (Different letters (a; b; c; d; e; f) indicate statistical difference. A represents the group with the highest mean and f represents the group with the lowest mean ($P<0.05$) (± standard error) (n=3))

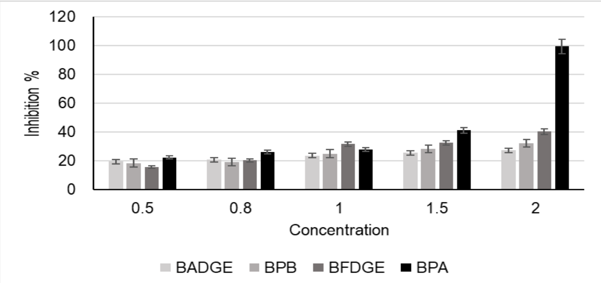

Figure 2. Inhibition percentages of chemicals according to the results of applied concentrations (There is no significant difference between groups sharing the same letter. Different letters (a; b; c; d) indicate that there is a significant difference between the groups ($P<0.05$) (± standard error) (n=3))

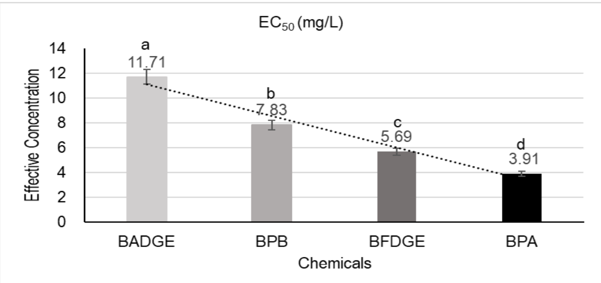

Figure 3. EC_{50} values calculated according to the results of the applied concentrations (Each group (a; b; c; d) is significantly different from the others groups ($P<0.05$) (± standard error) (n=3))

DISCUSSION

Bisphenol A and its analogues are global concern due to their ubiquity in the environment, their endocrine disrupting activity and their negative impact on the aquatic ecosystem (Xing et al., 2022). Many analogues have been confirmed to exert similar or stronger negative effects on aquatic organisms (Liu et al., 2021). Therefore, our study provides an overview of the toxicity of bisphenol A and its analogues BPB, BADGE and BFDGE and their impact on the growth rate of primary producers.

Much less is known about endocrine disruption chemicals (EDCs) such as BPs in terms of their harmful effects on food web organisms compared to organisms at higher trophic levels. Czarny et al. (2021) reported that many BPs penetrate the phytoplankton cell membrane due to their lipophilic properties (log Kow) and negatively affect many physiological processes such as photosynthesis, growth rate. Our study supports the thesis of Czarny et al. (2021) by showing that BPs have a constraining effect on the growth rate of *P. tricornutum* species. Since prokaryotic and eukaryotic primary producers lack endocrine systems, the mechanisms of action of chemicals differ from organisms at higher trophic levels.

Liu et al. (2010) reported that EC_{50} values for BPA as 3.73 and 8.65 mg/L for *Navicula incerta* and *Stephanodiscus hantzschii* respectively. In our study, this value for *P. tricornutum* was determined as 3.91 mg/L. When our study was compared with the 96-hour BPA exposure of Liu et al. (2010), our species was found to be more sensitive than the diatom *Stephanodiscus hantzschii*. The EC_{50} value we determined for *P. tricornutum* is similar to that of *Navicula incerta*. Liu et al. (2010) obtained the EC_{50} value for this species as 7.96 mg/L. Accordingly, it was determined that BPA had a more toxic and limiting effect on *P. tricornutum* than *Cyclotella caspia*.

In a study with the cyanobacterium *Cylindrospermopsis raciborskii* exposed to BPA, Xiang et al. (2018) found that the EC_{50} value is 9.66 mg/L. Also, BPA caused changes in cell morphology. Compared to our study, BPA was more toxic to *P. tricornutum* species.

Czarny et al. (2021) examined the effects of individual and mixed bisphenol analogues on cyanobacteria. Accordingly, BPAF, BPB and BPC were more toxic than BPA on *Anabaena variabilis* and *Microcystis aeruginosa*. The growth and EC_{50} data in our study are similar to this study.

When the data in the current study were compared with previous studies, it was determined that EC_{50} values varied according to the species. There are no studies conducted with *P. tricornutum* species with BPA and its analogues. When the EC_{50} values obtained from studies with different species exposed to several BPA analogues are compared with our study, it is thought that this species (*P. tricornutum*) is more resistant than other species, and the reason for this is thought to be due to the chitin structure of the cell wall of *P. tricornutum* species.

CONCLUSION

Due to the importance of primary production to the aquatic ecosystem, this study evaluated the effects of increasing concentrations of BPA, BADGE, BPB and BFDGE compounds on the marine diatom *P. tricurnitum*. According to the data obtained, it was observed that the toxicants had a limiting effect on cell numbers from the first concentration compared to the control. It was observed that all chemicals had a limiting effect on the growth rate depending on the concentration, and there was an increase in the inhibition percentages calculated by the dose-response curve. According to the EC_{50} values calculated for bisphenol A and its analogues depending on the concentrations applied, the most toxic bisphenols were determined as BPA>BFDGE>BPB>BADGE, respectively.

BPA and its analogues are widely used in our country and in the world. Analogues produced to replace BPA show similar toxicity and estrogenic activity to BPA. Therefore, they may not be as safe as thought and legal regulations should be introduced for their use. The determination of estrogenic toxicity of these chemicals and the development of ecotoxicological tests necessary for environmental and human health should continue. The levels of bisphenolic compounds in Türkiye's rivers, lakes and waste discharge points and in the organisms found there should be determined. The levels of these compounds in drinking water and food should be determined and safety limits should be set. In order to take these measures, previous studies should be examined and new toxicological procedures should be developed in line with these literatures.

ACKNOWLEDGEMENTS AND FUNDING

The study was conducted under the supervision of Özlem Çakal Arslan, "A New Era in the History of Bisphenol-A: Are Potential Chemicals Used Instead of Bisphenol-A Really Safe for Aquatic Creatures?" by TUBITAK (Project no:119Y246). It was carried out within the scope of the 1001 research project titled.

AUTHORSHIP CONTRIBUTIONS

Koray Benas: Conceptualization, resources, investigation, methodology, writing-reviewing and editing. Özlem Çakal Arslan: Conceptualization, resources, methodology, formal analysis, investigation, project administration.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

DATA AVAILABILITY

All relevant data is inside the article.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 41(3), 213-219 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.06

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Entomoneis parikhani sp.nov., a new diatom (Entomoneidaceae, Bacillariophyta) from Aras River (Iran)

Entomoneis parikhani sp.nov., Aras Nehri (İran)'nden yeni bir diatom (Entomoneidaceae, Bacillariophyta)

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Received date: 20.06.2024 *Accepted date: 29.08.2024*

How to cite this paper:
Parikhani, F. (2024). *Entomoneis parikhani* sp.nov., a new diatom (Entomoneidaceae, Bacillariophyta) from Aras River (Iran). *Ege Journal of Fisheries and Aquatic Sciences, 41(*3), 213-219. https://doi.org/10.12714/egejfas.41.3.06

Abstract: The diatom *Entomoneis* has a series of unique features such as connecting lines with different shapes, sigmoid raphe on the bilobate keel, different curtains, and multiple girdle bands. Most species belonging to this genus are found in marine and saline habitats. In fact, the genus *Entomoneis* is an epipelic diatom that is found in streams with high salinity and high electrolyte concentration. To date, no specific type of freshwater has been identified for this species. In this study, a new species of freshwater diatom, *Entomoneis parikhani*, is described based on detailed morphological observations using a scanning electron microscope. The samples were collected the Aras River near Oltan village of Parsabad city in the northwest Iran. A special feature of *Entomoneis parikhani* is its triple valve. The genus *Entomoneis* comprises of diatoms with sigmoidal raphe canals and girdle bands. This taxon is similar to *Entomoneis annagodhei* and *Entomoneis tenera*, as shown by LM and SEM observations, and there are sufficient morphological differences to separate *Entomoneis parikhani.* This new species had very small cells, delicate frustules without valve lines, and wide lanceolate valves. In this study, the wide distribution of this taxon in the Aras River has been evaluated.

Keywords: *Entomoneis*, new taxon, Aras River

Öz: Çift taraflı simetrik bir lobut görümünündeki diyatom türü *Entomoneis*, vücut yapısı üzerinde sigma yapan rafesi, farklı şekillerde görülen bağlantı hatlarıyla diğer diyatom türlerinden şekil olarak ayrılır. Bu cinse ait türlerin çoğu denizel ve tuzlu habitatlarda bulunur. Aslında, *Entomoneis* cinsi yüksek tuzluluk ve yüksek elektrolit konsantrasyonuna sahip akarsularda bulunan bir epipelik diyatomdur. Bugüne kadar, bu tür için belirli bir tatlı su tipi tanımlanmamıştır. Bu çalışmada, taramalı elektron mikroskobu kullanılarak yapılan ayrıntılı morfolojik gözlemlere dayanarak yeni bir tatlı su diyatomu türü olan *Entomoneis parikhani* tanımlanmıştır. Örnekler, İran'ın kuzeybatısındaki Parsabad şehrinin Oltan köyü yakınlarındaki Aras Nehri'nden toplanmıştır. Yeni bir diyatom türü olan *Entomoneis parikhani*'nin özel bir yapısı üçlü valfidir. *Entomoneis* cinsi, sigma yapan rafe kanallarına ve kuşak bantlarına sahip olan bir cinstir. Bu takson, LM ve SEM gözlemleriyle gösterildiği üzere *Entomoneis annagodhei* ve *Entomoneis tenera*'ya benzerdir ve *Entomoneis parikhani*'yi ayırmak için yeterli morfolojik farklılıklar vardır. Bu yeni türün çok küçük hücreleri mevcuttur, früstülleri çok ince yapıda olup kapak çizgileri ise mevcut değildir ve kapakları da geniştir. Bu çalışmada, bu taksonun Aras Nehri'ndeki geniş dağılımı değerlendirilmiştir.

Anahtar kelimeler: *Entomoneis*, yeni takson, Aras Nehri

INTRODUCTION

Diatoms belong to the branch Heterokontophyta and class Bacillariophyceae. They are known for their siliceous cell walls and bivalve structure. *Entomoneis* is one of the most important species of diatom that lives in saline environments. The genus *Entomoneis* is mainly marine, and its representatives are found in saline water (Liu et al., 2018). *Entomoneis* have single rectangular guitar-shaped cells. It is twisted along the longitudinal axis. *Entomoneis* contain one or two chloroplasts. The inserted rims have multiple holes. In *Entomoneis*, the seam was S-shaped. In *Entomoneis*, the shells are thick and have a girdle outline (Mejdandžić et al., 2018). Species belonging to the genus *Entomoneis* have attracted the attention of scientists because of their importance in water quality assessment and biological monitoring (Mejdandžić et al., 2018). The genus *Entomoneis* includes species with a unique morphology. Most species belonging to this genus are salt-loving and live in places with high salinity (Round et al., 1990).

The diatom genus *Entomoneis* Ehrenberg (1846) has species that can be identified by light microscopy because of a series of features, such as multiple girdle bands, sigmoidal raphe, and pandori-shaped cells (Patrick and Reimer, 1975). In most *Entomoneis* species, the basal fibula is visible under an electron microscope (Round et al., 1990). Patrick and Reimer (1975) classified the genus *Entomoneis* as a species that has pandori-shaped cells, sigmoid raphe, multiple striae, and multiple intercardiac bands (Majewska et al., 2019). These results were obtained based on detailed morphological observations and electron microscopy (Ross et al., 1979).

Currently, Entomoneidaceae has two genera: *Entomoneis Ehrenberg* and *Platichthys* (Lange-Bertalot et al., 2015), which these two genera have a series of common morphological characteristics such as the raphe canal and the number and number of valve surfaces. *Entomoneis* belongs to the raphe canal diatoms and is a non-monophyletic group in which the raphe canal evolved independently at two separate locations. According to the phylogenetic analyses and data of three chloroplast genes (SSU, rbcL, and psbC) and the theory of (Ruck et al., 2016) *Entomoneis* has monophyletic origin. *Platichthys* genus, which is morphologically similar to the *Entomoneis*. However, it has unknown phylogenetic position and is monophyletic. During the last two decades, only four new *Entomoneis* species have been identified based on their morphological characteristics.

A number of biomonitoring and floristics studies have been performed over the past few decades to document diversity and ecology of Iranian algae including: Algues des dèserts d'Iran (Compère, 1981), Anzali Lagoon (Nejadsattari, 2005), Caspian Sea (Fatemi et al., 2005), Gharasou River (Atazadeh et al., 2007), Lake Neure (Nejadsattari, 2005), streams in Ramsar (Soltanpour-Gargari et al., 2011), Balikhli River (Panahy Mirzahasanlou et al., 2020) and Karaj River (Kheiri, 2018). In recent studies, the total number of algal species in Iran has reached 1304. These algae species are divided into 8 divisions, 15 classes, 37 orders, 96 families and 262 genera. Among these divisions, Bacillariophyta forms the largest group and is located after Chlorophyta, Cyanophyta and Euglenophyta (Zarei-Darki, 2009). In all investigated waters of Iran, diatoms (Bacillariophyta) were the dominant algae. During recent studies, more than 90% of species and subspecies (i.e. 479 species and 612 infra-specic taxa) have been revealed as new species. *Entomoneis* is a relatively large genus of diatoms, and there is great morphological diversity among the species in the genus *Entomoneis*. So far, different species of *Entomoneis* genus have been reported in the rivers of Iran, and these rivers have salty water. A species of the *Entomoneis* genus was reported in the Etrak River of Bojnord City, which has salty water (Nejadsattari, 2005). 2 species of the *Entomoneis* genus were reported in the Qarasu River (Atazadeh et al., 2007). 3 species of *Entomoneis* genus have been reported in Urmia River (Mehrjuyan and Atazadeh, 2022). So far, no species of *Entomoneis* genus has been reported in Iranian rivers that have fresh water.

In this study, *Entomoneis parikhani* sp. nov, a small and silicified taxon in Ultan village located in Parsabad City of the Aras River in northwestern Iran, is described as a new species, and these descriptions are based on morphological characteristics. In the present study, has tried to compare the similarities and differences *Entomoneis parikhani* with other species. Genus is also discussed.

MATERIAL AND METHODS

Study area

The Aras River originates in the Arpa Yay area of Anatolia, Türkiye (Parikhani et al., 2023). The river has passed through the border of Türkiye, Nakhchivan, Jolfa and Armenian, and after crossing the Iranian border enters the Republic of Azerbaijan and flows into the Kura River. Aras River, is one of the largest rivers in northern Iran and the watershed of the Caspian Sea and supplies an important part of the region's water. In Aras River is one of the longest and water most Border Rivers in Iran. According to Figure 1 the study area is part of the Aras River in the village of Oltan in the city of Pars Abad. Pars Abad Moghan City in the northwest of the country, northeast of Azerbaijan Iran and north of Ardebil province and in the Moghan area. This city covers a vast part of the northern region of the Moghan Plain. Table 1 shows the geographic coordinates of the studied point in Ares River (Parikhani et al., 2023).

Table 1. Location of of the studied point along the Aras River with site GIS coordinates

Site Name	Latitude	Longitude	Altitude (m)
Oltan	47.76384033	39.60737133	63

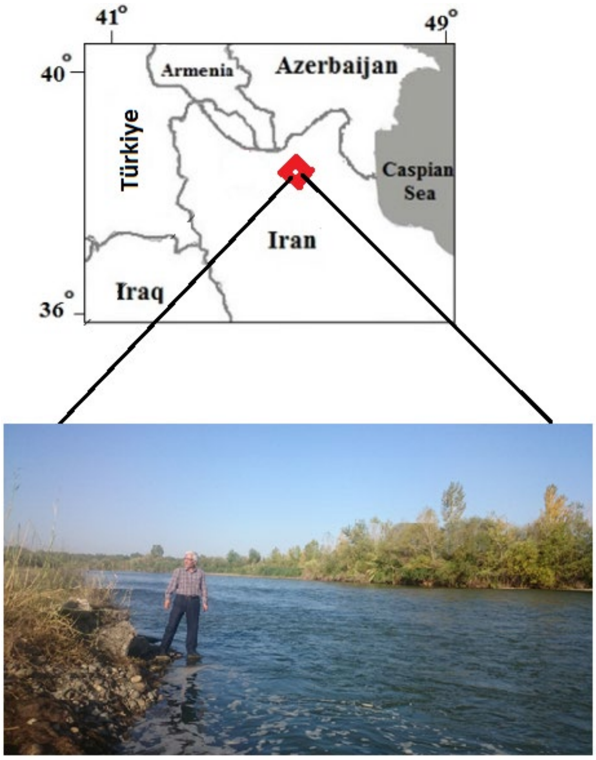

Figure 1. Map of the studied point in Aras River, Northwestern Iran

Table 2 shows the pH, electrical conductivity (EC) and total dissolved solids (TDS) values measured in the sampling point. pH, EC and TDS values were recorded by pH meter, EC meter and TDS meter belonging to Tabriz University Ecology and Environment Laboratory. The method of measurement was in such a way that the sensor belonging to each device was placed in the flowing water of the river. After about twenty seconds, the fixed number was recorded on the device's monitor (Carranzo, 2012).

Table 2. pH, EC and TDS of Aras River in the sampling site

Station	pH	EC (µS/cm)	TDS (mg/L)
Oltan	9.0	1681	1075

Samples were collected from the Aras River at sampling station in northwestern Iran during or period. Diatom samples were collected from cobbles, pebbles, or rocks in a collection bottle (200 mL), and then Lugol's solution (2 mL) was added for preservation (Williams et al., 2016). Temporary slides were prepared for live diatoms to identify the species composition. For diatom species identification and enumeration, the samples were prepared following the method of Battarbee et al. (2001). The samples were digested with 10% hydrogen peroxide in a beaker at 90°C on a hotplate for 2 h, after which two drops of 10% hydrochloric acid were added. The beakers were filled with distilled water and left to settle overnight, after which the supernatants were discarded. This process was repeated four times. The subsamples (400 µl were air-dried on coverslips and mounted using Naphrax (Battarbee et al., 2001). All slides were identified and counted per slide using a Zeiss microscope with differential interference contrast at ×1000 magnification. For scanning electron microscopy (SEM), the rinsed samples were resuspended in a solution of distilled water for 30 min and then rinsed three times in distilled water. Frustule suspensions were dried directly on 22 mm diameter aluminum stubs and gold coated with a Dynavac Xenosput sputter coater. Frustules were imaged using a TESCAN field-emission scanning electron microscope with a working voltage of 2.0 kV and a spot size of 2. The morphology of the new species was compared with that of several described taxa (Flower et al. 1996; Hamsher and Saunders, 2014).

RESULTS

The taxonomic classification of the *Entomoneis* as follows:

Division: Bacillariophyta

Class: Bacillariophyceae (Medlin and Kaczmarska, 2004)

Order: Surirellales (Mejdandzic et al., 2017)

Family: Entomoneidaceae (Round et al., 1990) (Patrick and Reimer, 1975)

Genus: *Entomoneis* (Mejdandzic et al., 2018)

Entomoneis parikhani sp.nov. (Figure 2-6)

Description

Morphological, ultrastructural, and ecophysiological characteristics of the species were investigated under the light micrograph (LM). In the belt view, the frustules were two blobs. The frustules are deeply contracted and visible, with a connecting line near the central node. The edges of the wings are thin. Some of these features appear at longer irregular intervals. Finally, it ends at a small point (Figure 2). Scanning electron microscopy revealed membranous valves without ornamentation and a heavily silicified fibular keel. *Entomoneis parikhani* is distinguished from other *Entomoneis* species by the number of distinct striae and the micro morphometric structure of the valve surface. In *Entomoneis parikhani*, the abdomens are rectangular in the girdle view. The valves are lanceolate, linear, and have parallel margins. The raphe canal was unusual. The transapical striae are parallel. Areoles small, fbulae short, delicate, and unevenly distributed. Fascia is shortened due to irregular central striae. The raphe florem expanded with the external central pores. The axial region of the cell was very narrow and linear and hardly extended near the central region. In *Entomoneis parikhani* nov sp, single cells with frustules are well silicified. The valves have a lanceolate line and slightly concave edges in the middle. Two plate-like plastids are arranged parallel to each other in each cell. Pandora-shaped frustules are located in the girdle view. According to Figure 3 the transapical striae are parallel in the middle and slightly radiate towards the apex. Areoles in the raphe canal are distinct and longer than the surface of the valve. Tran's epic is long. Each transapical row Striae corresponds to an elongated halo in the raphe canal. The fibulae are irregular and distributed along the length of the valve.

The external valve view shows a series of fine lines as dense transapical lines according to the SEM. The small indentations near the valve tip correspond to the transition between the valve body and the top. The raphe keel is narrow and high and sigmoid-shaped with a gap like a spear in the middle. The central fissures of the raphe are simple, next to a transapical fascia, and are clear in the inner view of the valve. The end slits of the raphe are hooked.

According to Figure 4 valve apices are slightly elongated in valve view, and it sharpens in the view of the belt. Rafted keel is narrow and sigmoid. The central region of the raphe forms a canal that has a lance-shaped cleft opening, and visible when the transapical fascia is out of focus. The striae are very fine and can be seen under the light microscope. According to Figure 4 the external view of the valve shows a deep channel with a lanceolate slit. The marginal end of the fascia is separated by a small number of areoles. In *Entomoneis parikhani*, the end of the external central raphe is weakly expanded and elongated apically.

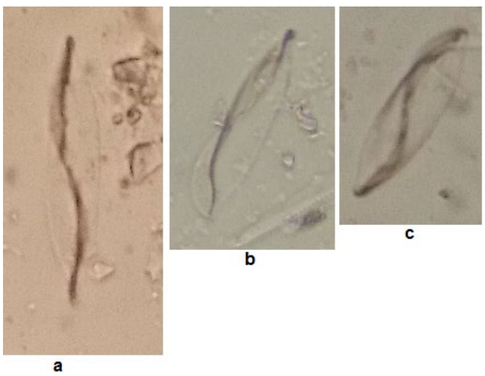

Figure 2. *Entomoneis parikhani* under LM. The central region of the raphe canal shows spear-like slits

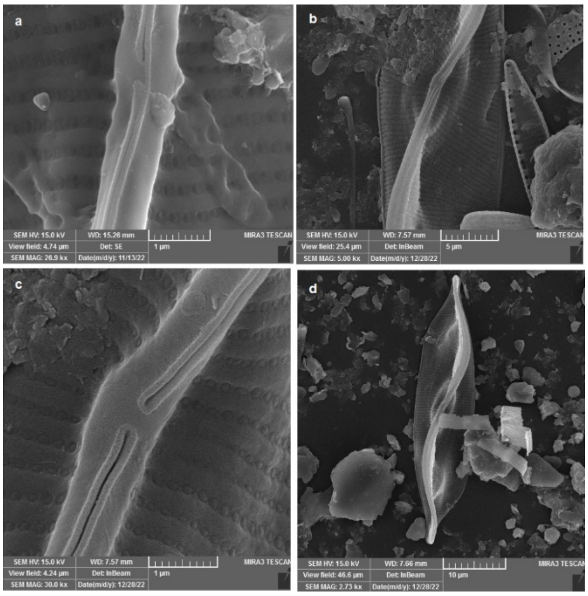

Figure 3. *Entomoneis parikhani* under SEM. a and c) central part of the valve with central node and simple central raphe endings, b and d) show the view of the valve. The sigmoid raphe canal, dense transapical striae, and a spear-like slit opening can be seen in the central raphe canal region

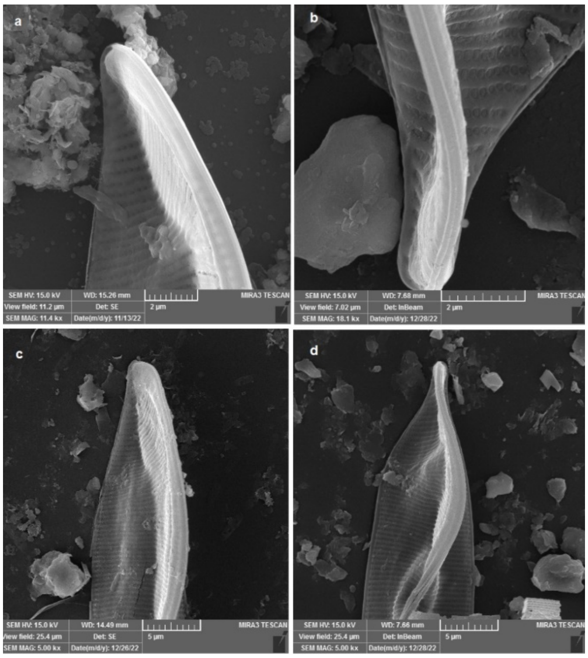

Figure 4. *Entomoneis parikhani* under SEM. a) shows the tip of the valve with the end of the raphe, b and d) show the view of the valve belt indentation in the transition between the valve body and the keel, c) shows the tip of the sharp valve where the terminal raphe slit is hooked

In *Entomoneis parikhani* the valves are highly irregular. The raphe canal is slightly above the level of the valve. Raphe is florum. At the apex, the terminal raphe of the end of the valve is strongly hooked at the same level as the valve, and ends with the mantle (Figure 5). The valve plate is flat. The proximal valve mantle is shallow. A very narrow band forms the distal valve mantle.

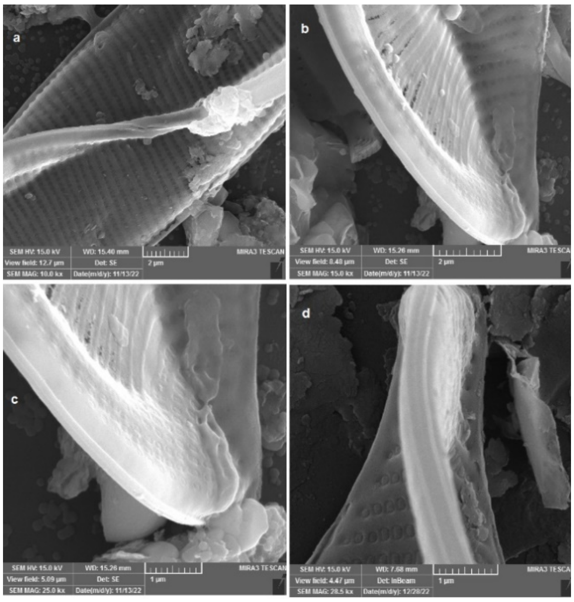

Figure. 5. SEM micrographs of *Entomoneis parikhani* sp. nov.

According to Figure 6 striae are composed of linoleum. Lineole pores are round and prominent. The spaces between the fbulae are variable and tend to become larger towards the apical valve. The fibulae are narrow and similar in shape. At the apex, the raphe ends in smaller helitoglossae. In the inner part, the transapical striae are composed of smaller transapical ones. The areolas are elongated and placed at the same level. The ends of the external central raphe are elongated abically. Valve epic never lasts long. Transapical striae radiate in the middle part of the valves and abruptly converge.

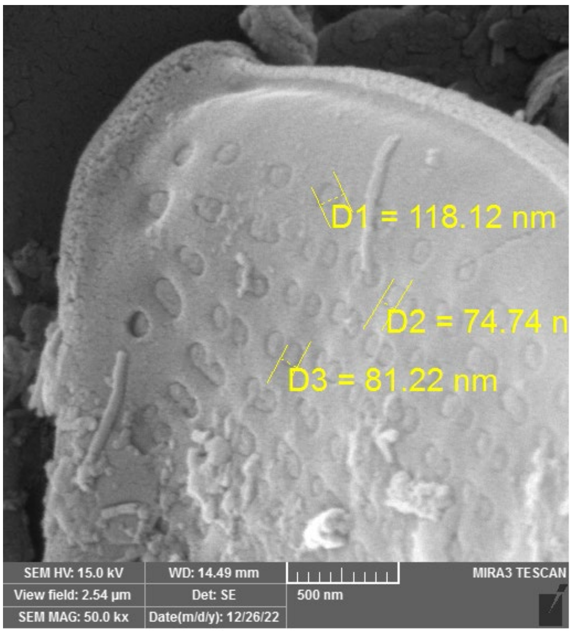

Figure 6. *Entomoneis parikhani*, SEM. Girdle view of the top of the cell showing the end of the apical raphe

The new species *Entomoneis parikhani* has unique shaped valves which are lanceolate, linear, and wide lanceolate. A distinctive feature of *Entomoneis parikhani* is its very delicate appearance, which is because the cells have very little silica.

Other species of *Entomoneis* have very many silicified frustules, which makes these species generally appear stronger. Different species of *Entomoneis* are different from *Entomoneis parikhani* in terms of morphology and shape, as shown in Table 3.

Table 3. Comparison of taxa resembling *Entomoneis parikhani* (np: not present)

Species	Valve length (μ<m)	Valve width (μm)	Valve outline	Striae density (in 10 μm)	Plastids	References
E.aequabilis	47-57 μm	7-9 μm	Linear, weakly sigmoid	32-37	Multi-lobed plastid	(Osada and Kobayasi, 1991)
E. annagodhei	50-82 μm	10-12 μm	Linear-lanceolate with slightly	52-58	Oblique transapical two, axial	(Al-Handal et al., 2020)
E.japonica	75-150 μm	20-40 μm	Linear-lanceolate	11-12	Multi-lobed plastid	(Osada, 1985)
E. parikhani	32-60 μm	8.84-8.61 μm	Linear-lanceolate	33-40	2	This study
E.paludosa	40-130 μm	20-50 μm	Broadly linear	21-26	Two, axial	(Osada, 1990a)
E.punctulata	18-99 μm	10-19 μm	Broadly linear	34-36	Multi-lobed plastid	(Osada, 1990b)
E.tenera	11-22 μm	3-7 μm	Broadly lanceolate	30-50 valve body: 18-42	One, lobed	(Mejdandžić et al., 2017)
E.vertebralis	33-58 μm	10-11 μm	Linear-lanceolate	The keel (SEM) np	Two, palate-like	(Clavero et al., 1999)

DISCUSSION

Entomoneis parikhani Raphe is described based on its morphological characteristics. Of which we can refer to the Pandori frustules that grow on a winged keel. The new species of *Entomoneis parikhani* shows strange morphological characteristics that are very different from other *Entomoneis* species.

Unlike *E. paludosa*, *Entomoneis parikhani* has a multilobed plastid. There are two plastids in each cell. Some authors have reported that species belonging to the genus *Entomoneis* have one to two plastids. A characteristic of *Entamonis parikhani* is that the size of its cells is tiny, the cells are 32-60 μm long, while other species such as *E. japonica*, are 75-120 μm long or species such as *E. paludosa* are 40-130 μm long have. This reduction in cell size is due to the conditions of cell growth and life in fresh water. That maybe the cell has entered a ring in fresh water from living conditions in salt water and this has caused stress in the cells of *Entomoneis parikhani*. There is only one exception among *Entomoneis* species, *Entomoneis vertebralis* also has a delicate appearance and only the silicified part has a hyaline frustule with a raphe system. *Entomoneis vertebralis* is similar in general appearance to *Entomoneis parikhani*. Still, its difference from *Entomoneis parikhani* is that it is not visible in the cells of *Entomoneis vertebralis* in the vent line LM. In *Entomoneis parikhani* the valve has a very fine structure due to the recognizable ornamentation on the valve. The fine structure of the valve in *Entomoneis parikhani* consists of transapical parts and striae with round and circular holes. *Entomoneis vertebralis* no discernible vent ornamentation is seen during SEM examination. *Entomoneis* species have mostly single forms. They have never been seen as a colony in their natural habitat. But sometimes it is seen that these cells form chains in which the chain of cells are connected with their keels. These chain-like cells are mostly seen in laboratory cultures. In any case, we cannot consider this form of life as a defining characteristic of these species, perhaps this chain of cells is formed because there was no separation between the cells after cell division, which caused the formation of a chain in these cells. The general morphology of *Entomoneis parikhani* includes pandora-shaped cells in girdle view, sigmoidal raphe canal and multiple median bands. Girdle view of the top of the cell showing the end of the apical raphe is shown in Figure 6.

In *Entomoneis parikhani* plate-like plastids with other *Entomoneis* species such as *E. alata* (Liu et al., 2018). Cell twisting was not observed in *Entomoneis parikhani*, while it was observed in some species such as *E. alata* and *E. paludosa* (Osada, 1990a). When comparing the shape of the valve, *Entomoneis parikhani* is more similar to *E. alata*. *E. punctulata* has lanceolate linear valves. The apex of the valve is similar in *Entomoneis parikhani* and *E. alata*. However, the species differ in valve length, which is given in Table 2. *E. alata* and *E. punctulata* both have wide bilobate keels with prominent and very wide lobes (Cox, 2001). Which are located on the edge of the valve. While *Entomoneis parikhani* has narrow bilobate keels with relatively long wings that do not exceed the valve margin and valve face (Lobban et al., 2019). The valves of *Entomoneis parikhani* appear hyaline due to the very fine veins in the LM, as do several other species such as *E. vertebralis*, which have hyaline valves. *Entomoneis parikhani* has dense striae with rounded areoles. Which is more similar to the ultrastructure of E. *paludosa* (Mejdandzic et al., 2018).

Recent research on *Entomoneis* has combined morphological observations with phylogenetic observations (Al-Handal et al., 2019). However, the research of (Al-Handal et al., 2019) did not combine these morphological studies with phylogenic. Because the morphological features were specific and unusual enough to define the definite limits of

Entomoneis annagodhei. However, this does not mean that future research should not focus on molecular data collection. Because molecular data are always useful in diatom research. There is still much to do and study (Barkia et al., 2019). In the cells of this diatom, the caps were seen as linear-sharp and rarely swollen in the central part of the caps. In all the observed samples, two blastocysts were seen in the cell. This species was separated based on the length and size of the cells, the shape of the end of the cell (round or sharp tip), and the location of the chloroplasts. The cells in the members of this genus had bilateral symmetry. Rafe is straight. The length of cells in this genus is variable according to its species, and most of the time, their length was measured between 32-60 and their width between 8-9 micrometers. This genus has wavy chloroplasts, and the position and shape of chloroplasts, the length of cells and the position of furrows are important in distinguishing the species of this genus. *Entomoneis parikhani* differs from other *Entomoneis* species in terms of wing shape in girdle view and having central spines. The length of the valves of *E. giginteu* species is reported to be between 160 and 190, while in *Entomoneis parikhani* it is almost half of this size. *Entomoneis parikhani* has smaller valves and closer veins on the valve body. The new species has narrower wings and lacks a central spine. One of the strange features in the new species is the spines on each side of the central nodule, it is also recognizable in LM, and the spine is limited to only one valve. In *Entomoneis parikhani*, the number of spines on both sides of the central nodule is unequal. In *Entomoneis parikhani*, the transapical costae are completely continuous from the margin of the valve to the raphe canal at the top of the valve. The wings of the body are longer and wider than the inner wings. And the wings seem motionless. When viewed using LM, the girdle of *Entomoneis parikhani* shows the actual structure as if a diagonally striated transparent layer is folded in two on top of the wing. In LM observations, it can be seen that the fibulas are located as small dots on the wings. These wings connect the costae to the opposite walls. Finally, *Entomoneis parikhani* was identified as a new species based on the measurement of size (length and height), linear density of fibula, stria, areola, presence or absence of fracture in the raphe gap, central nodule, and the distance between the two middle fibulae, and the following characteristics Among the distinctive features of *Entomoneis parikhani* are considered: an arched connecting line, the valve areolae are separated from each other, and the areolae of the girdle band are large, some of these characteristics, such as the length, width, density of the fibula, and striae, are affected. Physical and chemical parameters such as salinity, nutrients, temperature, and water movement are important for the species and it was concluded that *Entomoneis parikhani*, pH, TDS, and the fresh water of the Aras River have greatly affected these characteristics.

CONCLUSION

To date, all reported species of *Entomoneis* are specific to salt water, and no *Entomoneis* species live in freshwater has been reported (Lobban et al., 2019). The present study is related to a new species of *Entomoneis parikhani*, which is specific to freshwater in the Aras River.

ACKNOWLEDGEMENTS AND FUNDING

I wish to express our appreciation to the University of Tabriz (Iran) for their support. This project is part of a PhD thesis financially supported by the University of Tabriz.

AUTHORSHIP CONTRIBUTIONS

Fatemeh Parikhani performed the experiments and wrote the draft.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICAL APPROVAL

This article does not contain any human or animal studies performed by any authors.

DATA AVAILABILITY

The data for the assessment included in the study is given in the text.

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Ege Journal of Fisheries and Aquatic Sciences, 41(3), 220-225 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.07

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

İskenderun Körfezi kıyı alanlarında sıcaklık ve klorofil-a için uydu ve model temelli veri setlerinin temsil yeteneği üzerine bir değerlendirme

An evaluation on the proximity of satellite- and model-based datasets of temperature and chlorophyll-a in coastal areas of İskenderun Bay

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Received date: 20.06.2024 *Accepted date: 31.07.2024*

How to cite this paper:
Bengil, F., Mavruk, S., Polat, S., & Akbulut, G. (2024). An evaluation on the proximity of satellite- and model-based datasets of temperature and chlorophyll-a in coastal areas of İskenderun Bay. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 220-225. https://doi.org/10.12714/egejfas.41.3.07

Öz: Bu çalışma, İskenderun Körfezi'nde yüzey suyu sıcaklığı (SST) ve klorofil-a (Chl-a) düzeylerinin uydu ve modelleme verileriyle izlenmesini ve bu veri setlerinin deniz ekosistemlerinin izlenmesinde kullanılabilirliğini araştırmaktadır. Araştırmada yerinde ölçüm veri seti, MODIS-Aqua uydu görüntülerinden elde edilen veri seti ve Copernicus MyOcean veri setinden alınan modelleme verileri kullanılmıştır. Körfezdeki SST ve chl-a dağılımı için eşleştirilmiş veri setleri üzerine yapılan analizlerin sonuçlarına göre, SST için uydu ve model veri setlerinin, klorofil-a için ise uydu veri setinin ölçüm verileri ile istatistiksel olarak anlamlı korelasyona sahip olduğunu belirlenmiştir. Veri setlerinin belirsizliği üzerine yapılan değerlendirmeler, SST için uydu veri setinin daha dar bir yayılıma ve daha az aykırı değer dağılımına sahip olduğunu ortaya koymuştur. Klorofil-a için her iki veri setinin de yüksek belirsizlik aralıklarına sahip olduğu ve daha fazla geliştirmeye ihtiyaç duyduğu görülmüştür. Bu çalışma, İskenderun Körfezi'nde SST ve chl-a değişkenlerinin izlenmesi için uydu ve model veri setlerinin kullanılabilirliğini göstermektedir.

Anahtar kelimeler: Uzaktan algılama, sayısal modelleme, temsiliyet, İskenderun Körfezi

Abstract: This study investigates the monitoring of sea surface temperature (SST) and chlorophyll-a (chl-a) levels in Iskenderun Bay using satellite and modeling data and evaluates the possible use of these datasets for monitoring marine ecosystems. Datasets derived from MODIS-Aqua satellite imagery and modeling data obtained from the Copernicus MyOcean and in-situ measurements were used in the study. According to the analysis on paried data sets of the distribution of SST and chl-a, sattelite and model datasets showed statistically significant correlations with in-situ measurements for SST. However, only satellite dataset showed significant correlations for Chl-a. Evaluations on uncertainty of the data sets revealed that the satellite dataset had a narrower range and less outlier distribution for SST. For chlorophyll-a, both datasets had wide uncertainty ranges and required further improvement. This study highlights the potential of satellite and model datasets for monitoring SST and chl-a variations in Iskenderun Bay.

Keywords: Remote sensing, numerical modeling, proximity, İskenderun Bay

GİRİŞ

Okyanus ve atmosferin etkileşimde olduğu tabaka oldukça dinamik bir yapıya sahip olmakla birlikte, bu yapı kıyı bölgelerinde daha da karmaşık bir hal almaktadır. Deniz, kara ve atmosferin etkin olarak birbirini etkilediği kıyı bölgelerinde deniz suyu sıcaklığı ve birincil üretimin bir göstergesi olan klorofil konsantrasyonu gibi önemli ve pratik değişkenlerin izlenmesi zorlaşır (IOCCG, 2000). Standart olan yerinde ölçümler, güvenilirlik konusunda herhangi bir şüphe oluşturmamakla birlikte, kıyısal bölgelerde mekan ve zamansal değişimin yakalanması, her hava koşulunda elde edilmesindeki güçlükler ve maliyet problemleri gibi bazı kısıtlamalara sahiptir (Fettweis ve Nechad, 2011).

Uydu-temelli uzaktan algılama ve deniz ortamının matematiksel modellemesi gibi yaklaşımlar, bu zorluğun üstesinden gelmek için önemli bir imkan oluşturmaktadır. Uydu teknolojisi, geniş denizel alanları düzenli izleyebilme ve değerli veri seti sağlayabilme yeteneğiyle, deniz ekosisteminin anlaşılması konusunda önemli bir potansiyel sağlamıştır (Clementi vd., 2019; Acker ve Leptoukh, 2007). Özellikle, son yıllarda gelişmiş algoritmalar ve zaman-mekan açısından artmış çözünürlükler, karmaşık morfolojiye sahip kıyısal alanlarda veri setinin kullanılabilirliğini arttırmıştır. Benzer şekilde, deniz ortamının matematiksel modellemesi, karmaşık denklemler serisi aracılığıyla denizel koşulları yeniden oluşturmak ve ileriye yönelik tahminde bulunmak için önemli bir araç haline gelmiştir (Le Sommer vd., 2018). Son yıllarda, kullanıma açık servisler olarak sunulan denizel modeller, bilimsel çalışmalar için devamlı veri setleri sağlamaktadır. Bununla birlikte, kıyısal alanda kullanımı konusunda hala bazı kısıtlılıklar mevcuttur Bahsi geçen veri setlerinin kıyı ortamlarında kullanımı, özel bir dikkat gerektirir. Sığ derinlikler, karmaşık kara-su etkileşimleri, kara-atmosfer ve su-atmosfer etkileşimleri hem uydu hem de model tabanlı veriler için kısıtlamalar veya zorluklar oluşturabilir. Ayrıca, nehir girdileri ve karasal kirleticiler gibi faktörler gerek uzaktan algılama gerekse model verilerinin güvenilirliğini veya yorumlanmasını zorlaştırabilir (Fox-Kemper vd., 2019; Baklanov vd., 2011).

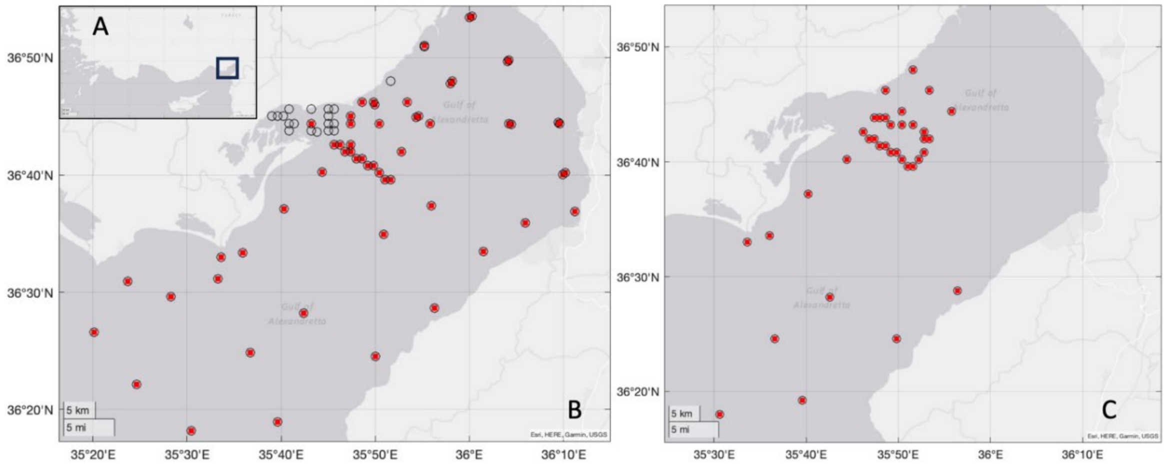
Gerek uydu teknolojisi ile (Agate vd., 2024), gerekse fiziksel modellemeden elde edilen veri setlerinin (Fox-Kemper vd., 2019) kıyısal bölgede kullanımı konusunda dikkate değer gelişmeler olmuştur. Bununla birlikte, daha önceki çalışmalar (Kim vd., 2013; Choi vd., 2022) her iki veri kaynağının entegrasyonunun, veri kalitesini artırma potansiyeline sahip olduğunu göstermektedir. İlgili veri setlerinin kıyısal bölgelerde yapılan çalışmalarda kullanılırlığı günden güne artmaktadır. Özellikle, deniz suyunun ısınmasına veya denizel üretimin zamana bağlı değişimine yönelik değerlendirmeler için vazgeçilmez kaynaklar haline gelmişlerdir (Li vd., 2023).

Doğu Akdeniz kıyılarında yer alan İskenderun Körfezi, bu yenilikçi yöntemlerin uygulanması için ideal bir örnek teşkil etmektedir. Bölge, batimetrik ve jeomorfolojik yapı, biyoçeşitlilik, lesepsiyen göçlerden etkilenme gibi birçok yönden dikkat çekici özelliklere sahipken, insan faaliyetleri ve iklim değişikliği gibi tehditlerle de karşı karşıyadır (Avşar, 1999; Mavruk vd., 2017). Deniz suyu sıcaklığı, bu baskıların biyotik ve abiyotik bileşenler üzerindeki etkilerini yansıtan önemli bir göstergedir (Wang vd., 2023) ve biyolojik çeşitliliğin zaman içindeki değişimini anlamak için kritik bir değişken olarak kullanılabilir (Roland Pitcher vd., 2012). Atmosfer-deniz etkileşiminin oldukça dinamik olduğu ve yerel faktörlerden etkilendiği bu bölgenin daha detaylı bir şekilde mekânsal ve zamansal ölçekte incelenmesine imkan sunan bu veri setinin önemi de buradan kaynaklanmaktadır. Bu nedenle, uydu ve modelden elde edilen SST ve chl-a konsantrasyonu veri setlerinin İskenderun Körfezi'ndeki performanslarını değerlendirmek ve gelecekteki kullanımlarını geliştirmek, bölgenin deniz ekosistemlerinin korunması ve sürdürülebilir yönetimi için önem taşımaktadır.

Bu çalışma kapsamında Doğu Akdeniz'in önemli bir bölgesi olan İskenderun Körfezi'nde SST ve chl-a konsantrasyonunun izlenmesi ve bilimsel çalışmalarda uydu ve modelleme verilerinin kullanılabilirliği araştırılmıştır. Yerinde ölçümler ile, uydu ve modelleme veri setleri istatistiksel yöntemler kullanılarak karşılaştırılmış, uydu ve modelleme verilerinin İskenderun Körfezi'nin izlenmesinde ne kadar kullanışlı olabileceği değerlendirilmiştir.

MATERYAL VE METHOD

Çalışma 2023 yılının Ocak ayında gerçekleştirilen yerinde ölçümler sırasında elde edilen sıcaklık ve klorofil-a veri setlerinin kullanımının yanında, Çukurova Üniversitesi Su Ürünleri Fakülte bünyesinde bugüne kadar gerçekleştirilen deniz araştırması çalışmaları sırasında elde edilen sıcaklık ve klorofil veri setleri kullanılmıştır (Şekil 1). Elde edilen veri setindeki tüm sıcaklık ölçümleri YSI marka 6600 model CTD kullanılarak gerçekleştirilmiştir. Yüzey suyu sıcaklık değerleri ilk derinlik tabakasındaki sıcaklık ölçümlerinin ortalaması olarak kabul edilecek şekilde veri seti oluşturulmuştur. Klorofil-a konsantrasyonu veri setinde kullanılan tüm veri standart bir prosedür izlenerek elde edilmiştir. Klorofil-a ölçümlerini gerçekleştirmek üzere Nansen şişesi aracılığıyla yüzey tabakasının hemen altından su örneği alınmış, alınan su örnekleri 2 litrelik ışık geçirmez polietilen şişeler aktarılmıştır. Klorofil-a analizleri Parsons vd. (1984) tarafından bildirilen yönteme göre analiz edilmiştir. Su örnekleri laboratuvar ortamına taşınarak 47mm'lik GF/F filtrelerden süzülmüştür. Süzme işleminin ardından filtreler spektrofotometrik ölçümler yapılana kadar -20^{o}C ortamda saklanmıştır. Optik ölçümler öncesinde süzüntüyü içeren filtre kağıtları 10ml %90'lık aseton çözeltisine bırakılmıştır. Ekstraksiyon için bir gece buzdolabında bekletilen örnekler daha sonra santrifüj edilmiş ve elde edilen süpernatantlar 1 cm ışık yolun sahip spektrofotometre küvetlerine konularak Schimadzu marka spektrofotometrede 750, 664, 647 ve 630 nm dalga boylarında absorbansları ölçülmüştür. Ölçüm veri setleri ait oldukları tarihlere göre sıralanmış ve eşleştirilecekleri uydu ürünlerine (SST – chl-a) göre gruplandırılmışlardır.



Şekil 1. Çalışma alanın konumu (A); Eşleştirilmiş SST istasyonlarının coğrafi dağılımı (B); Eşleştirilmiş chl-a istasyonlarının coğrafi dağılımı (C). Kırmızı kareler model ile eşleşen istasyonlarını, siyah çemberler uydu ile eşleşen istasyonları göstermektedir.

Uydu veri kaynağı olarak 1 km^2 yersel ve 1 gün zaman çözünürlüklü MODIS-Aqua sensöründen üretilmiş L2 veri setleri hem klorofil konsantrasyonu hem de yüzey suyu sıcaklığı ürünü için seçilerek NASA Ocean Color internet sitesinden indirilmiştir (http://oceancolor.gsfc.nasa.gov/). Örneklemenin gününe ait olan uydu görüntüsünde bulutluluk ve/veya diğer teknik problemlerin (güneş parıltısı vb.) olması durumunda bir gün öncesi veya bir gün sonrası olacak şekilde eşleştirme imkanı değerlendirilmiştir. Bu koşula uymayan veri satırları, genel veri setinden çıkarılmıştır. Yüzey suyu sıcaklığı (SST) ürünü deniz yüzeyini ifade eden çok ince yapıda (film) tabakanın tahmini olmasından dolayı, gün içerisinde olacak ani ısınma ve soğuma etkisini bertaraf etmek için gece ürünü olan (SST-4nm night) L2 veri seti kullanılmıştır.

Denizel model veri seti olarak, Avrupa Birliği Copernicus Deniz Ortamı İzleme Servisi (CMEMS) altında ulaşıma açık olan veri setleri kullanılmıştır. Yüzey suyu sıcaklığı için MEDSEA_MULTIYEAR_PHY_006_004 kodlu model ürününün sıcaklık değişkenine ait veri seti kullanılırken, klorofil-a konsantrasyonu için MEDSEA_MULTIYEAR_BGC_006_008 kodlu model ürünün deniz suyunda klorofil a konsantrasyonu değişkenine ait veri seti kullanılmıştır. Her iki değişken için de günlük oluşturulmuş veri setlerinden deniz yüzeyine en yakın gride ait değerler ölçüm veri seti ile eşleştirmek üzere seçilmiştir. Model ürünlerinin yersel çözünürlükleri uydudan elde edilen veri setine kıyasla daha sınırlı olduğundan eşleştirme model sınırları içerisinde olan veya model sınırlarına en fazla bir grid uzaklıkta olan istasyonlarla sınırlandırılmıştır. Veri setlerinin eşleştirilmesi SeaDAS (8.4.1) programı kullanılarak gerçekleştirilmiştir.

İstatistiksel performans analizi için, yerinde ölçümlerden elde edilen sıcaklık değerlerinin ortalama ve varyansının, model veya uydu verilerinden hesaplanan değerlerle arasındaki farklar değerlendirilmiştir. Bu değerlendirmede, Welch'in iki bağımsız örnek t-testi ve Levene'in varyans homojenliği testi kullanılmıştır (Sokal ve Rohlf, 2012). Ayrıca, ilişkilendirilen veri setleri arasında anlamlı korelasyonlar tespit edilmesi durumunda, veriler için olası bir kalibrasyon yaklaşımı önermek amacıyla lineer regresyon parametreleri hesaplanmıştır. Model ve uydu verilerinin belirsizlik özellikleri de değerlendirilmek üzere yüzdesel hata dağılımı aşağıdaki gibi hesaplanmıştır (Green ve Tashman, 2009).

Yüzdesel hata = 100 * (a - b) / a; denklemde a yerinde ölçüm değerine karşılık gelirken, b uydu veya modelden eşleştirilmiş tahmin ölçüm değerine karşılık gelmektedir.

Çalışmaya ilişkin tüm analizlerin gerçekleştirilmesinde ve görsellerin hazırlanmasında MATLAB R2022a programı kullanılmıştır.

BULGULAR

Körfezdeki klorofil-a ve yüzey su sıcaklığı (SST) dağılımı, yerinde ölçüm meta-verisi ve bölge için veri mevcut uydu görüntüleri kullanılarak eşleştirilmiş veri setleri oluşturulmuştur. Çalışma kapsamında derlenen veri setlerinin eşleştirilmesi sonucunda toplamda 63 adet klorofil-a ve 212 adet SST ölçümü uydudan elde edilen veri seti ile eşleştirilmiştir. Bu veri setinin biyojeokimyasal (chl-a, n=63) ve fiziksel model tahminleri (SST, n=106) ile de eşleştirilmiştir.

Yerinde ölçüm veri setinde sıcaklıklar 11,18°C ile 30,35°C arasında değişmiş olup, ortalama SST değeri 20,56°C (±4,50) olarak belirlenmiştir. Uydu görüntülerinden elde edilen SST verileri ise 10,34°C ile 30,89°C arasında yayılmış ve ortalama 20,88°C (±4,82) değerini göstermiştir. Model veri setinde ise SST değerleri 16,02°C ve 30,36°C arasında dağılmış ve ortalama 19,90°C (±3,30) olarak hesaplanmıştır.

Körfezin klorofil-a dağılımı, yerinde ölçümlerde 0,20mg/m^3 ile 1,30mg/m^3 arasında değişkenlik göstermektedir. Ortalama klorofil-a konsantrasyonu ise 0,59mg/m^3 (±0,27) olarak belirlenmiştir. Uydu görüntülerinden elde edilen klorofil-a verileri ise 0,13mg/m^3 ile 1,32mg/m^3 arasında bir aralıkta yayılmış ve ortalama 0,46mg/m^3 (±0,25) değerini göstermiştir. Model veri setinde ise klorofil-a değerleri 0,07mg/m^3 ile 0,22mg/m^3 arasında dağılmış ve ortalama 0,13mg/m^3 (±0,029) olarak hesaplanmıştır.

Yapılan veri setlerini kıyaslamaya yönelik analiz sonuçları, SST değerleri açısından gerek uydu ve gerekse model veri setlerinin ortalama ve varyans açısından yerinde ölçüm veri seti ile uyumlu olduğunu, istatistik açısından anlamlı farklılıklar göstermediğini ortaya koymuştur (Tablo 1). Diğer taraftan klorofil-a bakımından değerlendirildiğinde hem uydu ($p<0,01$) hem de model ($p<0,001$) veri setleri ile yerinde ölçümler arasında istatistik açıdan önemli farklılıklar dikkat çekmektedir (Tablo 1).

Veri setleri arasındaki bağlantının gücünü değerlendirmeye yönelik yapılan analizlerde, uydu ve ($r=0,99$, $p<0,01$) model temelli veri setlerinin ($r=0,97$, $p<0,01$) yerinde ölçüm SST değerleri ile güçlü bir korelasyona sahip olduğu görülmüştür. Diğer taraftan chl-a konsantrasyonu için gerek ölçüm veri seti ile uydudan elde edilen veri seti arasında ($r=0,70$, $p<0,01$), gerekse modelden elde edilen veri seti arasında ($r=0,45$, $p<0,01$), istatistiksel olarak anlamlı bir korelasyon bulunmakla birlikte bu ilişkinin gücünün daha zayıf olduğu belirlenmiştir.

Tablo 1. SST ve Chl-a konsantrasyonu veri setlerine ait ortalama ve varyans değerlerinin yerinde ölçümler ile kıyaslamak için gerçekleştirilen t-test ve Levene Testi sonuçları

		Welch'in iki bağımsız örnek t-testi		Levene'in varyans homojenliği testi	
		t değeri	p-değeri	F değeri	p-değeri
SST	Uydu verisi	-0,715	0,475^{ns}	1,102	0,295^{ns}
	Model verisi	1,471	0,142^{ns}	0,808	0,37^{ns}
Chl-a	Uydu verisi	2,738	0,007*	0,908	0,343^{ns}
	Model verisi	13,326	<0,001**	79,504	<0,001**

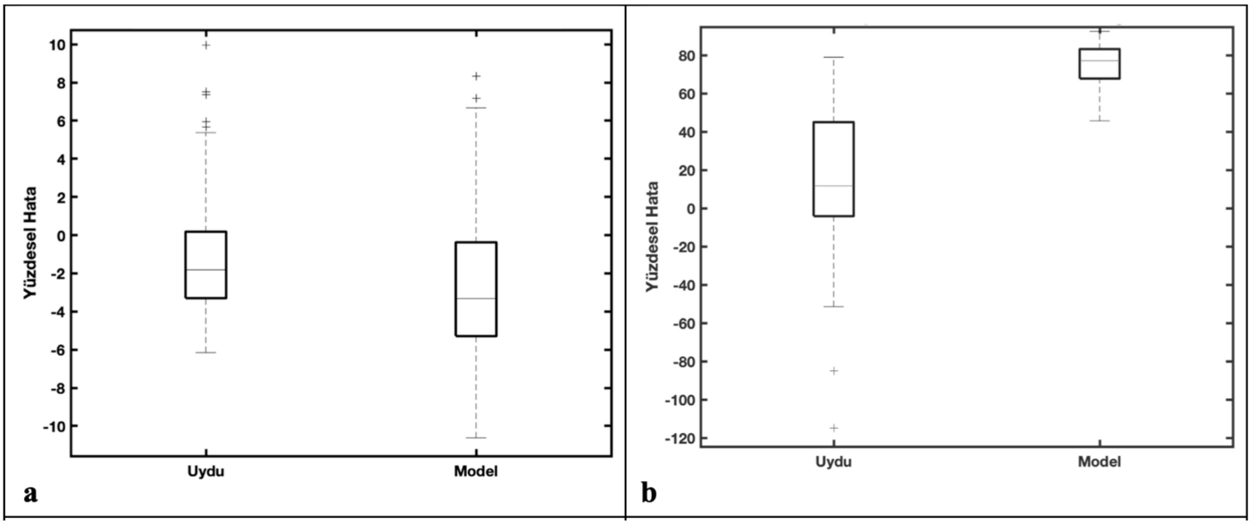
(ns anlamlı farklılık yok; * p<0,01 seviyesinde anlamlı farklılık mevcut; ** p<0,001 seviyesinde anlamlı farklılık mevcut)

Tablo 2 ölçüm veri setleri ile yapılan karşılaştırmalar sonucunda elde edilen lineer regresyon parametrelerine ait tahmin değerlerini göstermektedir. Sonuçlar, SST veri setleri içerisinde uydu verilerinin ölçüm verilerini daha iyi açıkladığını ($R^2=0,99$), ancak, model veri setinin de iyi bir açıklayıcı ($R^2=0,94$) olduğunu göstermektedir. Klorofil-a veri setlerinin ikisi de ölçüm veri setini açıklama konusunda oldukça düşük bir performansa sahiptir ($R^2=0,48$ ve 0,19, sırasıyla).

Tablo 2. Yerinde ölçüm veri seti ile uydu ve model temelli veri setleri arasındaki doğrusal regresyon parametrelerine ait tahmin değerleri

		Kesişim değeri	Eğim değeri	R^2
SST	**uydu**	-1,02	1,07	0,99
	model	-0,79	1,07	0,94
Chl-a	**uydu**	0,09	0,64	0,48
	model	0,1	0,05	0,19

Gerçekleştirilen hata analizleri sonucunda uydu veri setinin %1,8 medyan değeri etrafında (%-3,3 ve %0,2 aralığında) dar bir yayılıma sahip olduğu, aykırı değer dağılımının az sayıda pozitif yönlü olduğu tespit edilmiştir. Model verisi için hesaplanan hataların %-3,3 medyan değeri etrafında nispeten daha geniş (%-0,4 ve %-5,3) yayılıma sahip olduğu görülmüştür. Klorofil-a veri setleri ile yapılan yüzdesel hata değerlendirmelerinde, her iki veri seti için de yüksek hata aralıklarının söz konusu olduğu, özellikle model veri setinin sistemli bir şekilde düşük tahmin ürettiği tespit edilmiştir (Şekil 2).



Şekil 2. Veri setlerine ait hataların dağılımını gösterir kutu grafikleri; a) SST yüzdesel hata dağılımını gösterir kutu diyagramı; b) Chl-a yüzdesel hata dağılımını gösterir kutu diyagramı

TARTIŞMA

Uydu ve model tabanlı tahminlerin, SST ve chl-a gibi değişkenleri temsil etmedeki potansiyellerini gösteren çalışmalar hem küresel hem de bölgesel ölçekte mevcuttur (Smale ve Wernberg, 2009; Wernberg vd., 2012; Ali vd., 2016; Thakur vd., 2018; Bengil vd., 2021). Değerlendirmeler, SST tahminlerinin genellikle yeterli temsiliyet sağladığını, ancak sığ kıyısal alanlarda yetersiz kalabildiğini göstermektedir (Smit vd., 2013). Uydudan elde edilen chl-a tahminlerinin ise kıyısal sularda performansı düşüktür (Schalles, 2006). Bu durum, karmaşık optik yapıya sahip kıyısal su tipleri ve atmosferik etkilerden kaynaklanmaktadır (Schalles, 2006). Performansı geliştirmek için yeni yöntemler geliştirilmiş olsa da, hala bazı sınırlamalar mevcuttur (Ali vd., 2016; Abbas vd., 2019). Model tabanlı chl-a tahminleri, yerel olarak geliştirilmiş karmaşık modellerde daha iyi performans gösterebilmektedir (Zennaro vd., 2023). Fakat bu modeller için düzenli ve ulaşılabilir veri kaynakları bulmak zordur.

Bu çalışma kapsamında SST ile ilgili yapılan değerlendirmeler sonucunda uydu ve model tabanlı tahminlerin ölçüm değerlerine benzer tanımlayıcı istatistiklere sahip olduğunu göstermektedir. Bu durum, iki veri setinin de SST değerleri açısından oldukça uyumlu olduğunu ve her ikisinin de körfezin SST dağılımı hakkında doğru bilgi sağlayabileceğini göstermektedir. Özellikle, korelasyon ve hata analizleri temelinde uydu tabanlı tahminlerin performans olarak model tabanlı tahminlere göre görece daha iyi sonuçlar verdiği belirlenmiştir. Ancak, her iki yöntemin de kendi kısıtlamaları bulunmaktadır ve kullanımları duruma göre dikkatlice değerlendirilmelidir. Bu bağlamda, uydu tabanlı veri setinin yersel çözünürlüğünün sağladığı avantajdan dolayı, kıyısal bölgelerde ve özellikle yarı kapalı alanlarda kullanımının uygun olacağı değerlendirilmesine varılmıştır. Bununla birlikte, atmosferik koşulların uygunluğu gibi kısıtlamalara sahip olması da günlük ölçekte yapılacak zaman serisi değerlendirmelerinde kısıtlayıcı etken olarak ortaya çıkmıştır.

Model tabanlı tahmin veri setlerinin kullanım olanakları değerlendirildiğinde ise, bu verinin düşük yersel çözünürlüğe sahip olması kışın yapılan aşırı düşük ölçümlerin model veri setinde temsil edilememesine neden olmuştur. Dolayısı ile model verisinin bazı durumlarda tam olarak ölçümleri yansıtmayabileceği konusunda kuşkular oluşmuştur. Bu sebeple, farklı mevsimlere ait ölçümlerin model sınırları içerisinde arttırılarak, daha kapsamlı yeni bir değerlendirmenin faydalı olacağı önerilmektedir. Buna rağmen, istatistiksel analiz sonucunda, model tahmin veri setlerinin alternatif bir veri kaynağı olarak belirli bir amaç doğrultusunda kullanılması uygun olacaktır. Diğer taraftan bu veri seti uydu veri setindeki gibi atmosfer koşullarından etkilenmemekte olup zaman serisi analizlerinde kullanımı avantajlıdır.

Akdeniz'in farklı bölgelerinde (Matarrese vd., 2004) ve Doğu Akdeniz özelinde (Bengil ve Bizsel 2014; Bengil vd., 2021) uydu ve/veya model tabanlı veri setlerinin doğrulanması ve kullanım performansını ölçmeye yönelik az sayıda çalışma mevcut olmakla birlikte, bu çalışma Kuzey Levant Denizi için amaca yönelik tasarlanmış ilk çalışma durumundadır. Çalışma sonucunda uydu veya model temelli SST veri setlerinin kullanımı konusunda cesaretlendirici sonuçlar ortaya konmuştur.

Klorofil-a veri setleri üzerine yapılan değerlendirmeler sonucunda, uydu temelli tahminlerin nispeten temsil edici değerlere sahip olduğu, model temelli tahminlerin ise oldukça düşük bir temsil yeteneğine sahip olduğu görülmüştür. Ayrıca, özellikle veri setlerinin ortalamasındaki önemli anlamlılık derecelerinde farklılık olması, chl-a veri setlerinin kullanımından önce yerel bir kalibrasyon basamağının uygulanması gerekliliğini ortaya koymuştur. Diğer taraftan bu durum nihai bir değerlendirme olarak kabul edilmemeli ve ilgili çalışmaya özgü doğrulama yapılmasının daha faydalı olacağı görülmektedir.

Bu çalışmanın chl-a için bulguları, uydu temelli veri setlerinin düşük performans gösterdiğine dair mevcut değerlendirmelerle uyumludur (Ali vd., 2016; Abbas vd., 2019). Model veri seti üretimine yönelik yerel performansı yüksek (Zennaro vd., 2023) gibi veri setleri oluşturmak imkanlı olmakla birlikte, bu çalışmada sistemli veri sağlayan ve kolay ulaşılabilirliği veri seti için değerlendirme yapılmıştır. Teknolojik ve yöntemsel gelişmelerin dinamik yapısı göz önünde bulundurulduğunda, bu tür performans ölçümlerinin düzenli aralıklarla ve daha geniş veri setleri ile tekrarlanması önemlidir.

SONUÇ

Sonuç olarak, bu çalışma İskenderun Körfezi gibi karasal etkileşimi yüksek bir bölge için alternatif veri kaynağı sağlayan uydu ve model yaklaşımlarının performansını klorofil-a ve SST için ortaya koymuştur. Bu veri setlerinin deniz ekosisteminin izlenmesi ve ekolojik çalışmalarda destekleyici olarak kullanımı konusunda önemli bir potansiyel sağlamakta, veri kaynaklarının kullanımının avantajlarını ve dezavantajlarını ortaya koymaktadır. Çalışma sonucunda SST için uydu ve model veri setlerinin, klorofil-a için ise uydu veri setinin ölçüm verileri ile istatistiksel olarak anlamlı korelasyona sahip olduğu gösterilmiştir. Bunun yanı sıra hata analizi sonuçları, SST için uydu veri setinin daha dar bir yayılıma ve daha az aykırı değer dağılımına sahip olduğunu göstermiştir. Klorofil-a için ise her iki veri setinin de yüksek hata aralıklarına sahip olduğu ve daha fazla geliştirmeye ihtiyaç duyduğu görülmüştür.

TEŞEKKÜR VE MADDİ DESTEK

Bu çalışma TUBİTAK 2218 Programı tarafından (121C439) desteklenmiştir.

YAZARLIK KATKISI

Çalışma fikri, tasarım, materyal hazırlama, araştırma, yazım Fethi Bengil tarafından ve çalışma fikri, tasarım, materyal hazırlama, araştırma, gözden geçirme Sinan Mavruk tarafından, laboratuvar çalışması, araştırma ve gözden geçirme Sevim Polat, Gürkan Akbulut tarafından gerçekleştirilmiştir. Tüm yazarlar makaleyi okumuş ve onaylamıştır.

ÇIKAR/REKABET ÇATIŞMASI BEYANI

Yazarlar herhangi bir çıkar çatışması ve/veya rekabet eden çıkarlar olmadığını beyan eder.

ETİK ONAY

Bu çalışma için etik onay gerekli değildir.

VERİ KULLANILABİLİRLİĞİ

Veri setleri ile ilgili sorular için, sorumlu yazar ile iletişime geçilmelidir.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 41(3), 226-229 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.08

SHORT COMMUNICATION **KISA MAKALE**

Monocularism (unilateral anophthalmia) in the Sea Toad (*Chaunax abei* Le Danois, 1978) from Suruga Bay, Japan

Kurbağa balığında (*Chaunax abei* Le Danois, 1978) Suruga Körfezi'ndeki (Japonya) tek gözlülük (tek taraflı anoftalmi)

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Received date: 06.07.2024 *Accepted date: 26.07.2024*

How to cite this paper:
Şenbahar , A.M., Eto, A., & Yokota, M. (2024). Monocularism (unilateral anophthalmia) in the Sea Toad (*Chaunax abei* Le Danois, 1978) from Suruga Bay, Japan. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 226-229. https://doi.org/10.12714/egejfas.41.3.08

Abstract: The first case of monocularism (unilateral anophthalmia) was observed in a single specimen of *Chaunax abei* (Le Danois, 1978) during the seasonal trawl sampling in Suruga Bay in 2021. This occurrence marks the first reported incident of such an abnormality within the Chaunacid species. The monocularism was evidenced by photographs, and descriptive statistics of morphometric characters were presented.

Keywords: Abnormality, Chaunacidae, unilateral anophthalmia, one-eyed, Suruga Bay

Öz: 2021 yılında yapılan mevsimsel Suruga Körfezi'nde gerçekleştirilen trol örneklemeleri sırasında bir adet *Chaunax abei* (Le Danois, 1978) bireyinde ilk kez rapor edilmek üzere tek gözlülük (tek taraflı anoftalmi) gözlenmiştir. Bu rapor, Chaunacid türleri içinde böyle bir anormalliğin rapor edilen ilk vakasını işaret etmektedir. Tek gözlülük fotoğraflarla ortaya konulmuş ve morfometrik karakterlerin tanımlayıcı istatistikleri sunulmuştur.

Anahtar kelimeler: Anormallik, Chaunacidae, tek taraflı anoftalmi, tek gözlülük, Suruga Körfezi

INTRODUCTION

The family of Chaunacidae (sea toads or coffinfishes) has two genera, *Chaunacops* and *Chaunax* (Caruso, 1989; Ho and Shao, 2010) and there are 29 Chaunacid species distributed within three groups, *Chaunax fimbriatus* group, *Chaunax pictus* and *Chaunax abei* group (Caruso, 1989; Ho and Shao, 2010; Ho and McGrouther, 2015; Ho and Ma, 2016; Rajeeshkumar et al., 2020). Sea toads are bottom-dwelling species and distributed in Atlantic, Indian, and Pacific oceans, occurs between depths of 90 m to more than 2000 m (Caruso, 1989; Nelson et al., 2016). Over the last decades, studies about the Chaunacid species increased in the introduction of new species and revisions in systematic orders (Ho and Shao, 2010; Ho et al., 2013; Ho and Last, 2013; Ho and McGrouther, 2015; Ho et al., 2015; Ho et al., 2016; Ho and Ma, 2016). Also, the occurrence of new geographical expansion records of the specimens was reported (Quigley et al., 1996; Ragonese and Giusto, 1997; Kobayashi et al., 1999; Lee and Kim, 1999; Ragonese et al., 2001; Rajeeshkumar et al., 2020). However, no abnormality report has been documented and recorded in Chaunacids thus far.

Fish ophthalmology is relatively poorly studied, and few studies examining deformations or pathological changes in fish eye tissues (Dukes, 1975). Thus far, anophthalmia (the congenital anomaly of missing one or both eyes) in fish has been reported by some authors in *Oreochromis mossambicus* (Tave and Handwerker, 1998), in *Sperata seenghala* (Barman et al., 2016), in *Cyprinus carpio* (McElwain et al., 2013) in mostly on freshwater species. However, particularly among marine species, anophthalmia in anglerfishes (*Lophius budegassa* and *L. piscatorius*) has been well-documented in previous reports (Alonso-Allende, 1983; Bucke et al., 1994; Landa et al., 1998; Quigley, 2013; Colmenero et al., 2016; Şenbahar and Özaydın, 2019). Herein, we report the first case of unilateral anophthalmia observed in a sea toad, *C. abei*.

MATERIAL AND METHODS

In October 2021, one of the *C. abei* which have monocularism (unilateral anophthalmia) was obtained from a local fisherman in Heda Port (Numazu City, Shizuoka Prefecture) in Suruga Bay, Japan (34°58'18''N - 138°46'37''E) (Figure 1).

The body morphometric traits were measured following Negzaoui-Garali et al. (2008). The measurements were performed with a 1 mm precision with a digital caliper (Mitutoyo-CDS20C; Kanagawa, Japan). In the measure of the head length (*HL*), the following methodology of Caruso (1989) was used when it described the species of *Chaunax suttkusi*, as the informative definition; from the second neuro spine to the upper jaw (point to point). Fourteen morphometric characters were measured as follows: Total length (*TL*), standard length (*SL*), head length (*HL*) eye diameter (*ED*), pre-orbital length (*PrOL*), post-orbital length (*PsOL*), inter-orbital length (*IOL*), maxillary length (*ML*), pre-pectoral length (*PPecL*), pre-first dorsal fin length (*PD1*), pre-second dorsal fin length (*PD2*), pectoral length (*PecL*), Pre-pelvic length (*PPelvL*) and pre-anal length (*PAnL*).

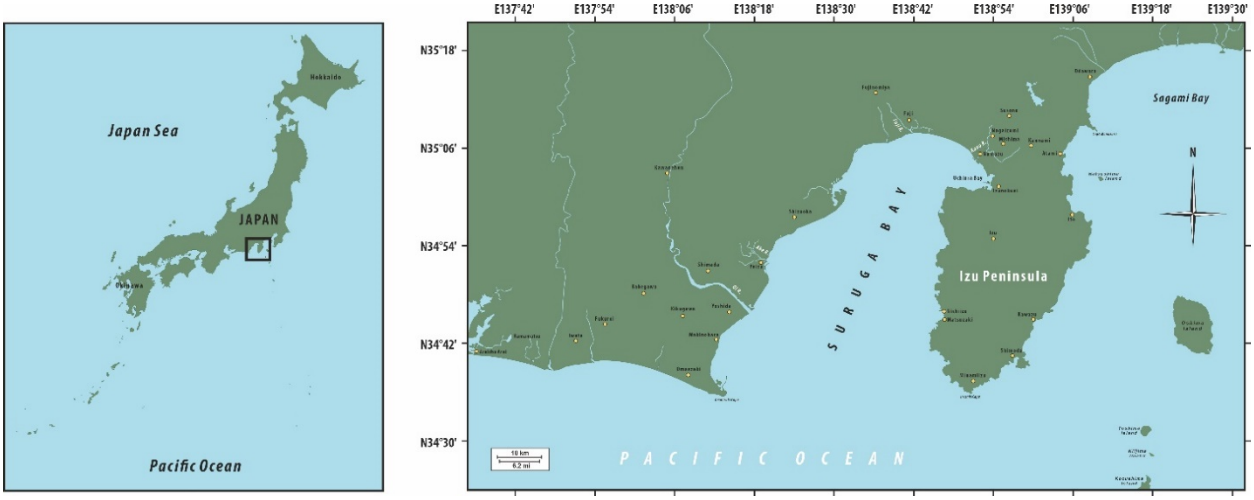

Figure 1. Sampling area

RESULTS AND DISCUSSION

Description: The body is globular and depressiform, the head is large and slightly compressed. Illicium is small and esca is oval-shaped. The surface of the body is a light reddish color with green-yellow dots. Lateral lines can easily be noticeable. The mouth is large and almost horizontal. Teeth of maxilla and mandible are small and sharp. There are numerous spikes/protrusions under the skin tissue. All the fin rays are soft, but the anal fin is branched.

Figure 2. Monocular abnormality in *C. abei* (unilateral absence of right eye), Suruga Bay, 2021

Abnormality reports on morpho-anatomical, coloration (pattern or skin color), eye deformations, growths of tumors, body deformations, lesions and wounds can be seen in wild fish populations since the 1550s (Belon, 1553; Guillaume Rondelet, 1555 by Berra and Au, 1981, cites therein) and it is always referred as anomalies in fishes may be either genetic or epigenetic (Dahlberg, 1970). Besides, eye abnormalities include the following symptoms: exophthalmia, hemorrhaging not caused by trauma or injury, reduction in the size of the pupil, white spot over a pupil or opaque pupil, lack of iris, and

Evidently, an orbital cavity of the monocular specimen is discernible beneath the skin tissue, and the right eyeball's absence is visible in Figure 2. Other than being one-eyed, no distinct features were observed. Therefore, the etiology of monocularism in *C. abei* remains unresolved in this report. The morphometric measurements of the specimen are given in Table 1.

Table 1. Measurements (mm) of *C. abei*

Measurements (mm)	*Chaunax abei* (Monocular)
Number of specimens	1
Total length (*TL*)	182.56
Standard length (*SL*)	141.04
Head length (*HL*)	55.58
Pectoral length (*PecL*)	70.09
Pre-pectoral length (*PPecL*)	64.95
Pre-first dorsal fin length (*PD1*)	5.81
Pre-second dorsal fin length (*PD2*)	70.14
Pre-orbital length (*PrOL*)	5.21
Post-orbital length (*PsOL*)	42.91
Eye diameter (*ED*)	5.23
Maxillary length (*ML*)	39.45
Inter orbital length (*IOL*)	10.93
Pre-pelvic length (*PPelvL*)	40.92
Pre-anal length (*PAnL*)	100.79

a symptom best described as "collapsed" eye (Skinner and Kandrashoff, 1988). According to previous reports, the anglerfishes (Lophiiformes) have quite a proclivity toward eye deformations/abnormalities (Table 2).

Causal factors could be of environmental or biological origin. Examples include possible carnivore attacks, competitive feeding activity behaviors, genetic modifications occurring during embryonic eye development, or genetic mutations. Moreover, the impacts of pollution may be associated with eye abnormalities. On the contrary, the critical concern regarding monocularism is the parasite effect, specifically *Spraguea lophii*, on the dynamics of the host population. Bucke et al. (1994) reported that *S. lophii* possibly causes severe infections in the nervous system, leading to neuropathy and impacting ocular tissues. However, the parasite *S. lophii* (Microsporidia) of the teleost fishes has not been previously documented in Asian anglerfish species thus far.

In future studies, histopathological examinations may be necessary to comprehend the microsporidian ecology in the marine environment, including *Spraguea* spp., especially in the case of a notable rise in reports concerning Chaunacid species.

Table 2. Eye abnormalities on anglerfish (Lophiiformes) species

Author	Alonso-Allende (1983)	Bucke et al. (1994)	Landa et al. (1998)	Quigley (2013)	Colmenero et al. (2016)	Şenbahar and Özaydın (2019)	Present study
Species	*Lophius budegassa*	*Lophius budegassa and L. piscatorius*	*Lophius budegassa*	*Lophius budegassa*	*Lophius budegassa*	*Lophius budegassa*	*Chaunax abei*
Area	Galician waters	Celtic Sea	Bay of Biscay	SE Irish Sea	NW Mediterranean Sea	Central Mediterranean Sea	Suruga Bay
Probable cause	Unresolved	*Spraguea lophii*	Unresolved	Unresolved	Unresolved/*S. lophii* observed	Unresolved	Unresolved
Status	No eye development	-	No eye development	No eye development	No eye development	No eye development	No eye development

ACKNOWLEDGMENTS

All the results were obtained with help of all availability of the Population Biology Laboratory of Tokyo University of Marine Science and Technology. Additionally, we much appreciated the staff of Seishin-Maru trawl vessel for providing the fishes to us.

AUTHOR CONTRIBUTIONS

Ahmet Mert Şenbahar: conceptualization, formal analysis, writing—original draft, writing—review and editing. Akira Eto: resources and sampling. Masashi Yokota: methodology, formal analysis and editing.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary, and no ethical contraventions occurred in this report.

DATA AVAILABILITY

No datasets were generated or analyzed during this report.

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Ege Journal of Fisheries and Aquatic Sciences, 41(3), 230-242 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.09

REVİEW **DERLEME**

Checklist of marine dinoflagellates on the coast of Türkiye

Türkiye kıyılarındaki denizel dinoflagellatların kontrol listesi

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Received date: 01.03.2024 *Accepted date: 02.05.2024*

How to cite this paper:
Çolak Sabancı, F. (2024). Checklist of marine dinoflagellates on the coast of Türkiye. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 230-242. https://doi.org/10.12714/egejfas.41.3.09

Abstract: Using the evaluations of 77 literature studies conducted along the Turkish coasts of the Black Sea, Marmara Sea, Aegean Sea, and the Mediterranean Sea, a checklist has been compiled. It has been observed that the dinoflagellate flora is represented by a total of 330 species belonging to 75 different genera. This literature study has determined that the number of dinoflagellate species reaches up to 181 in the Black Sea, 158 in the Marmara Sea, 206 in the Aegean Sea, and 192 in the Mediterranean Sea. This study has revealed that particularly in the case of new species records, the characteristic structural features, distributions, and photographs of the species are not provided. It has also been observed that many geographical points have not been investigated yet, and some species reported as new records have previously been reported in earlier studies. In future studies, updating the checklist becomes crucial in terms of forming a dataset.

Keywords: Black Sea, Sea of Marmara, Aegean Sea, Mediterranean Sea, marine dinoflagellat, check-list

Öz: Karadeniz, Marmara, Ege ve Akdeniz'in Türkiye kıyıları boyunca yapılan 77 literatür çalışması değerlendirilerek bir kontrol listesi oluşturulmuş ve dinoflagellat florasının 75 farklı cinse ait toplam 330 tür ile temsil edildiği gözlenmiştir. Yapılan bu literatür çalışması ile dinoflagellat tür sayısının Karadeniz' de 181, Marmara' da 158, Ege' de 206 ve Akdeniz' de 192 sayısına kadar ulaştığı saptanmıştır. Bu çalışma ile özellikle yeni tür kayıtlarında türlerin karakteristik yapısal özellikleri, dağılımları ve fotoğraflarının verilmediği, birçok coğrafi noktanın henüz incelenmediği ve bazı türlerin yeni kayıt olarak verilmesine rağmen daha önceki çalışmalarda rapor edildiği saptanmıştır. İleride yapılacak olan çalışmalarda kontrol listelerinin güncellenmesi veri seti oluşturması açısından önem kazanmaktadır.

Anahtar kelimeler: Karadeniz, Marmara, Ege, Akdeniz, denizel dinoflagellat, kontrol listesi

INTRODUCTION

Phytoplankton is a group that includes various prokaryotic and eukaryotic organisms that play an important role in primary production, and climate change (Vadrucci et al., 2008; Cermeño et al., 2008). Dinoflagellates constitute one of the taxonomic groups within the phytoplankton that have an important role in the food web. Dinoflagellates are among the most abundant marine organisms, with an estimated 8000 species of which approximately 2400 have been described to date (De Vargas et al., 2015; Janouškovec et al., 2017), 105 of which are considered toxic and harmful algae (Hallegraeff et al., 2021). Dinoflagellates adapt quickly to a variety of environmental conditions (such as temperature, salinity, light, nutrients, and water movements) that control their distribution, diversity, and migration (Levandowsky and Kaneta, 1987; Behrenfeld et al., 2006) and when environmental conditions are favorable, they can exhibit rapid proliferation within a short period of time, leading to eutrophication. (Delwiche, 2007; Richardson and Schoeman, 2004; Leterme et al., 2006).

Identification and classification studies of dinoflagellates in the world's seas and freshwaters were first started by Schiller (1931–1937). Later, Sournia (1973; 1978; 1982; 1990; 1993) added new taxonomic records of marine dinoflagellates to the literatüre but only at genus level. Comprehensive investigations of the world's dinoflagellates at the species level continued with several different studies (Kofoid and Swezy, 1921; Margalef, 1969; Rampi and Bernhard, 1980; Dodge, 1982; Balech, 1988; Anderson, 1989; Steidinger and Tangen, 1997; Faust and Gulledge, 2002; Gárate-Lizárraga and Verdugo-Díaz, 2007; Ignatiades and Gotsis-Skretas, 2010) including the ones by Gómez (2003; 2005; 2006; 2008; 2012a; 2012b; 2021).

Phytoplankton research in Türkiye started in the 1950s, and the first studies were conducted at the class or family level (Numann, 1955; Acara and Nalbantoglu, 1960). Species-level descriptions began to be made in the early 1960s, and as the time passed, studies on phytoplankton began to increase noticeably. The first study on phytoplankton in the Aegean Sea was carried out by Ergen (1967), in the Marmara Sea by Artüz (1974), in the Mediterranean Sea by Gökalp (1972), and in the Black Sea by Benli (1987).

This study aimed to compile research articles along the Turkish coastline that were approved by a specific referee committee and create a list of dinoflagellate species according to current rules.

MATERIALS AND METHODS

This study is based on the phytoplankton literature study carried out along all Turkish coasts. The total length of Turkey's coastline, including the islands, is 8.333 kilometers, of which

1.067 kilometers are island coasts. The distribution of this total according to the four seas is as follows: Black Sea: 1.701 kilometers (20.4%), Marmara Sea: 1.441 kilometers (17.3%), Aegean Sea: 3.484 kilometers (41.8%) and Mediterranean: 1.707 kilometers (20.5%) and each has its own typical coastal geomorphology in terms of a variety of oceanographic, geological and atmospheric conditions.

The literature review was composed with 77 references containing pelagic dinoflagellates within the scope of studies carried out in Turkish coasts, lagoon and estuary systems. Only articles published in peer-reviewed journals were evaluated in the references. Apart from these, master's theses, doctoral theses, compilation studies, environmental impact assessment reports, monitoring reports and project studies for the private sector were not included. In the literature studies used, it was observed that plankton net and Nansen bottle were used for phytoplankton sampling and formaldehyde and lugol were preferred for fixation of the samples. It has also been stated that standard light microscopy and phase-contrast objectives are mostly used in species determinations. The current nomenclature of the taxa reported in this study was made according to Guiry and Guiry (2024). Some species have been reported with different names depending on the final taxonomic rank. The taxa mentioned as "confer" and "sp." in the species lists have not been included in the list as they require confirmation. In addition, it was observed that in some studies, although the species was given as a new record, the species had been reported before. Therefore, the first records with the oldest date were taken into consideration. Very few studies have included morphological characteristics, pictures or drawings of the species which were reported for the first time. These studies are shown with an asterisk. (*) for light microscopy and with two asterisk (**) for scanning elektron microscopy studies in Table 1.

RESULTS

Although Türkiye has a long coastline, there is a big gap on phytoplankton studies until 1990s. In the present study, a total of 77 literature sources have been utilized, with the Aegean Sea ranking first with 26 studies, followed by the Marmara Sea with 24 studies, the Mediterranean Sea with 19 studies, and the Black Sea with 13 studies (Table 1).

Table 1. List of literature used in this study. Black Sea (B), Sea of Marmara (S), Aegean Sea (A), Mediterranean Sea (M). ("*" refers to light microscopy studies, "**" refers to electron microscopy studies).

Literature	Sampling Period	Sampling Region	Literature	Sampling Period	Sampling Region
1. Ergen (1967)	1967	A	40. Feyzioğlu and Seyhan (2007)	1993-2002	B
2. Gökalp (1972)	1970	A, M	41. Polat and Koray (2007)*	1994-2004	M
3. Koray and Gökpınar (1983)	1983	A	42. Polat (2007)*	2004	M
4. Koray (1984)*	1980-1984	A	43. Çevik et al. (2008)	2000-2001	M
5. Koray (1987)*	1980-1984	A	44. Taş et al. (2009)	1998-2002	S
6. Benli (1987)	1983-1984	B	45. Deniz and Taş (2009)	2000	S
7. Koray and Büyükışık (1988)	1983	A	46. Balkıs (2009)	2000-2001	A
8. Kıdeyş et al. (1989)	1984-1985	M	47. Eker Develi and Velikova (2009)**	2006	B
9. Koray et al. (1994)	1990-1991	A	48. Baytut et al. (2010)	2002-2003	B
10. Koray and Kesici (1994)	1990-1991	A	49. Türkoğlu and Öner (2010)	2004-2005	S
11. Koray (1995)	1978-1990	A	50. Altuğ et al. (2011)	2006-2007	S, A
12. Uysal and Sur (1995)	1990	B	51. Küçük and Ergül (2011)	2009-2010	S
13. Alpaslan et al. (1999)	1997-1998	S	52. İçemer Tugrul (2012)	1999-2001	M
14. Metin and Cirik (1999)	1993-1994	A	53. Özsayman Say and Balkıs (2012)	2005-2006	M
15. Koray et al. (1999)	1999	A	54. Çolak Sabancı and Koray (2012)	2008-2010	A
16. Polat et al. (2000)	1994-1995	M	55. Taş (2014)*	2002-2004	A
17. Türkoğlu and Koray (2000)	1995-1996	B	56. Ağlaç and Balkıs (2014)	2003-2004	A
18. Eker and Kıdeyş (2000)	1995-1997	M	57. Balkıs and Toklu Alıçlı (2014)	2006-2008	S
19. Demir and Atay (2000)	1997	A	58. Güreşen and Aktan Turan (2014)	2009-2010	A
20. Balkıs (2000)*	1998-1999	S	59. Ağırbaş et al. (2015)	2009	B
21. Çolak Sabancı and Koray (2001)	1998-1999	A	60. Baytut and Gönülol (2016)	2007-2008	B
22. Demir (2001)	1999	B, S, A, M	61. Balcı and Balkıs (2017)*	2010-2011	S
23. Polat and Işık (2002)	1998-1999	M	62. Aktan Turan and Keskin (2017)*	2017	A
24. Polat and Koray (2002)*	1999	M	63. Ergül et al. (2018)	2015	S
25. Polat and Piner (2002)	1999-2000	M	64. Aslan et al. (2018)	2016	A
26. Polat and Piner (2002a)	2000-2001	M	65. Çolak Sabancı (2018)*	2017	A
27. Taş and Okuş (2003)	1995	S	66. Balkıs Özdelice and Peynirci (2019)	2012-2013	B
28. Balkıs (2003)	1998-1999	S	67. Uysal (2020)	2002-2003	M
29. Polat and Koray (2003)*	2000	M	68. Taş et al. (2020)*	2004-2007	S
30. Uysal et al. (2003)**	2001	M	69. Balkıs Özdelice et al. (2020)	2006-2008	S
31. Balkıs et al. (2004)	1993-1995	S	70. Şentürk et al. (2020)	2015-2016	B
32. Taş and Okuş (2004)*	1999	S	71. Dursun et al. (2021)	2018-2019	S
33. Polat (2004)*	2001	M	72. Ergül et al. (2021)	2020-2021	B, S
34. Çolak Sabancı and Koray (2005)	1998-2001	A	73. Balkıs Özdelice et al. (2021)*	2021	S
35. Aktan et al. (2005)	1999-2000	S	74. Kayadelen et al. (2022)	2013-2014	S
36. Balkıs (2005)*	2000-2001	A	75. Durmuş et al. (2022)*	2022	S
37. Eker Develi et al. (2006)	2000-2001	M	76. Taş (2023)	2009-2010; 2013-2014	S
38. Taş and Okuş (2006)	2004-2005	B	77. Semin et al. (2023)*	2018-2019	S
39. Taş et al. (2006)*	2006	A			

Based on the results of the researchs on phytoplankton in Türkiye's seas, it has been determined that Türkiye's dinoflagellate flora is represented by a total of 330 species belonging to 75 different genera (Table 2). These results were obtained from scientific studies published in peer-reviewed journals from 1955 to the present. According to the literature, the genera with the most species are *Tripos* Bory (60 species), *Protoperidinium* Bergh (46 species), *Dinophysis* Ehrenberg (23 species), *Gonyaulax* Diesing and *Prorocentrum* Ehrenberg (14 species). With this literature study, it was determined that the number of dinoflagellate species reached up to 181 in the Black Sea, 158 in the Marmara Sea, 206 in the Aegean Sea and 192 in the Mediterranean Sea so far.

Table 2. Check-list of dinoflagellat species reported from previous 77 papers. The numbers following a species name in the checklist refer to the list of literature. (*first new record for single sea, **first new record for the Turkish waters).

Türler	B	S	A	M
Aureodinium pigmentosum Dodge	**60			
Acanthodinium caryophyllum Kofoid		*28	*9	25
Akashiwo sanguinea (K.Hirasaka) Gert Hansen & Moestrup	17	22	22	18
Alexandrium minutum Halim	48	*28	7	
Alexandrium ostenfeldii (Paulsen) Balech & Tangen	59			
Alexandrium tamarense (Lebour) Balech	60	72	4	
Amoebophrya ceratii (Koeppen) J.Cachon		**75		
Amphidinium acutissimum J.Schiller	**60			
Amphidinium carterae Hulburt	**60	*75		
Amphidinium crassum Lohmann	**60	*75		
Amphidinium operculatum Claparède & Lachmann	**60	*75		
Amphidinium sphenoides Wulff	**60	57	64	
Amphidinium steinii (Lemmermann) Kofoid & Swezy	**60			
Amphisolenia bidentata B.Schröder			2	16
Amphisolenia globifera F.Stein	**60			
Amphisolenia laticincta Kofoid		**61		
Amphisolenia palaeotheroides Kofoid				41
Amphisolenia schroederi Kofoid			*9	
Amphisolenia truncata Kofoid & J.R.Michener			*10	16
Amylax triacantha (Jørgensen) Sournia	48	44		
*Archaeperidinium minutum (*Kofoid) Jørgensen	**60	32	14	
Azadinium caudatum (Halldal) Nézan & Chomérat			54	
Balechina gracilis (Bergh) F.Gómez, Artigas & Gast		**69		
Blixaea quinquecornis (Abé) Gottschling		61		
Borghiella pascheri (Suchlandt) Moestrup	**60			
Brachidinium capitatum F.J.R.Taylor			*10	
Centrodinium pavillardii F.J.R.Taylor			*9	41
Centrodinium punctatum (Cleve) F.J.R.Taylor				18
Ceratium contortum var. robustum (Karsten) Sournia			9	18
Ceratium cornutum (Ehrenberg) Claparède & J.Lachmann	60			
Ceratium furcoides (Levander) Langhans	60			
Ceratium gibberum var. *dispar* (Pouchet) Sournia			3	16
Ceratium gibberum var. *subaequale* Jorgensen			3	41
Ceratium hirundinella (O.F.Müller) Dujardin	38		3	
Ceratium pentagonum var. *tenerum* Jørgensen			3	8
Ceratium ranipes f. *furcellatum* (Lemmermann) F.J.R.Taylor			3	
Ceratocorys armata (Schütt) Kofoid		*28	*9	16
Ceratocorys gourretii Paulsen			*5	16
Ceratocorys horrida Stein			1	2
Citharistes regius Stein				**33
Cladopyxis brachiolata F.Stein				8
Cochlodinium archimedis (Pouchet) Lemmermann	**60		64	
Coolia monotis Meunier 1919		*75		
Corythodinium brunellii (Rampi) F.Gómez	17			
Corythodinium compressum (Kofoid) F.J.R.Taylor	17			41
Corythodinium constrictum (F.Stein) F.J.R.Taylor			5	41
Corythodinium diploconus (F.Stein) F.J.R.Taylor	17			
Corythodinium frenguellii (Rampi) F.J.R.Taylor		**74		
Corythodinium milneri (G.Murray & Whitting) F.Gómez	6		1	41

Table 2. (Continued)

Türler	B	S	A	M
Corythodinium tesselatum (F.Stein) Loeblich Jr. & Loeblich III		72	**39	
Cystodinium unicorne G.A. Klebs	6			
Dinophysis acuminata Claparède & Lachmann	6	27	34	
Dinophysis acuta Ehrenberg	6	13	1	18
Dinophysis amandula (Balech) Sournia			*10	41
Dinophysis argus (Stein) Abé			*9	41
Dinophysis caudata Kent	6	13	1	8
Dinophysis elongata (Jørgensen) Abé				41
Dinophysis fortii Pavillard	17	13	5	53
Dinophysis hastata F.Stein	6	13	1	16
Dinophysis infundibulum J.Schiller	17	77		
Dinophysis odiosa (Pavillard) Tai & Skogsberg		**20		23
Dinophysis operculoides (Schütt) Balech				8
Dinophysis ovum F.Schütt	6	28	5	41
Dinophysis parva J.Schiller				67
Dinophysis parvula (F.Schütt) Balech	6		5	23
Dinophysis pulchella (M.Lebour) Balech	**60			
Dinophysis punctata Jørgensen	17	*28		
Dinophysis recurva Kofoid & Skogsberg			1	23
Dinophysis rudgei G.Murray & Whitting	17	74		
Dinophysis sacculus F.Stein	6	13	5	
Dinophysis schroederi J.Pavillard	22		1	
Dinophysis schuettii G.Murray & Whitting			*5	41
Dinophysis sphaerica F. Stein	6	22	1	41
Dinophysis tripos Gourret		32	5	18
Diplopelta asymmetrica (Mangin) M.Lebour ex Balech		*75		
Diplopelta bomba F.Stein ex Jørgensen				41
Diplopsalis lenticula Bergh	17	*28	*5	16
Diplopsalopsis bomba J.D.Dodge & S.Toriumi, nom. inval.				43
Dissodinium pseudocalani (Gonnert) Drebes ex Elbrachter & Drebes		32		
Durinskia agilis (Kofoid & Swezy) Saburova, Chomérat & Hoppenrath	**60			
Glenodinium paululum Lindemann				37
Gonyaulax africana J. Schiller	40			
Gonyaulax birostris Stein, nom. inval.	17		1	16
Gonyaulax diacantha Athanassopoulos	17		*5	
Gonyaulax diegensis Kofoid	17	*31	*5	16
Gonyaulax digitale (Pouchet) Kofoid	40	61		18
Gonyaulax fragilis (Schütt) Kofoid		57	58	
Gonyaulax hyalina Ostenfeld & Schmidt		68		
Gonyaulax milneri (G.Murray & Whitting) Kofoid			*10	41
Gonyaulax monacantha Pavillard	17	28	5	16
Gonyaulax pacifica Kofoid				**42
Gonyaulax polygramma F. Stein	6	61	5	16
Gonyaulax scrippsae Kofoid	48	**74	64	
Gonyaulax spinifera (Claparède & Lachmann) Diesing	17	13	4	16
Gonyaulax turbynei Murray & Whitting	66		*5	16
Gotoius abei K. Matsuoka		50	50	
Gymnodinium agiliforme J. Schiller	**60			
Gymnodinium catenatum H.W.Graham	**60	44		
Gymnodinium elongatum (J.Schiller) Moestrup & Calado, nom. illeg.	**60			
Gymnodinium fuscum (Ehrenberg) F.Stein				37
Gymnodinium herbaceum Kofoid	22	22	19	
Gymnodinium neapolitanum J. Schiller	**60			
Gymnodinium wilczekii Pouchet	**60			
Gymnodinium wulffii J.Schiller	**60			
Gynogonadinium aequatoriale F.Gómez		**61		
Gyrodinium estuariale E.M.Hulbert	**60			
Gyrodinium fusiforme Kofoid & Swezy	6	35	58	18
Gyrodinium lacryma (Meunier) Kofoid & Swezy	60			18

Table 2. (Continued)

Türler	B	S	A	M
Gyrodinium nasutum (Wulff) J.Schiller	**60			
Gyrodinium pellucidum (Wulff) J.Schiller	6			
Gyrodinium pingue (F.Schütt) Kofoid & Swezy	**60		64	
Gyrodinium pusillum (A.J.Schilling) Kofoid & Swezy	6			
Gyrodinium spirale (Bergh) Kofoid & Swezy	60	28	50	
Heterocapsa minima A.J.Pomroy		*75		
Heterocapsa niei (A.R. Loeblich) L. C. Morrill & A. R. Loeblich		*75		67
Heterocapsa pygmaea Lobelich III, R.J.Schmidt & Sherley		*75		**30
Heterocapsa rotundata (Lohmann) Gert Hansen	**60	**61		
Heterodinium angulatum Kofoid & J.R.Michener				**29
Heterodinium inaequale Kofoid				**29
Heterodinium mediocre (Kofoid) Kofoid & Adamson				**29
Heterodinium mediterraneum Pavillard			1	
Heterodinium rigdeniae Kofoid		**69		
Histioneis depressa J.Schiller				**24
Histioneis elongata Kofoid & J.R.Michener				**24
Histioneis expansa Rampi				**24
Histioneis marchesonii Rampi				**24
Histioneis oxypteris J.Schiller				41
Histioneis para G.Murray & Whitting				**24
Histioneis striata Kofoid & J.R.Michener				**24
Kapelodinium vestifici (Schütt) Boutrup, Moestrup & Daugbjerg	**60			
Karenia brevis (C.C.Davis) Gert Hansen & Moestrup	**60		64	
Karenia mikimotoi (Miyake & Kominami ex Oda) Gert Hansen & Moestrup	60			
Karlodinium veneficum (D.Ballantine) J.Larsen	**60			
Kofoidinium velleloides Pavillard	48	13	5	16
Kryptoperidinium triquetrum (Ehrenberg) Tillmann, Gottschling, Elbrächter, Kusber & Hoppenrath	17	*28	55	
Lebouridinium glaucum (Lebour) F.Gómez, H.Takayam, D.Moreira & P.López-García		71		
Lessardia elongata Saldarriaga & F.J.R.Taylor	*47			
Lingulodinium polyedra (F.Stein) J.D.Dodge	6	13	4	16
Margalefidinium citron (Kofoid & Swezy) F.Gómez, Richlen & D.M.Anderson	**60			
Noctiluca scintillans (Macartney) Kofoid & Swezy	6	13	4	41
Ornithocercus carolinae Kofoid			*9	41
Ornithocercus francescae (G.Murray & WHitting) Balech			56	
Ornithocercus heteroporus Kofoid			*10	41
Ornithocercus magnificus F.Stein			*5	23
Ornithocercus quadratus Schütt		*28	5	8
Ornithocercus quadratus var. assimilis			21	
Ornithocercus schuettii T.Wilke & Hoppenrath			*5	
Ornithocercus splendidus F.Schütt			*9	41
Ornithocercus steinii Schütt			*9	41
Ornithocercus thumii (A.W.F.Schmidt) Kofoid & Skogsberg				41
Oxytoxum variabile J.Schiller			50	67
Oxytoxum viride Schiller				67
Oxytoxum adriaticum Schiller	17			
Oxytoxum areolatum Rampi				67
Oxytoxum caudatum Schiller		*75		67
Oxytoxum elegans Pavillard			*10	
Oxytoxum gladiolus Stein				67
Oxytoxum ligusticum Rampi				67
Oxytoxum longiceps Schiller		63	*5	41
Oxytoxum longum J.Schiller				67
Oxytoxum mediterraneum Schiller		*75		
Oxytoxum reticulatum (Stein) Schütt			10	26
Oxytoxum scolopax F.Stein	22	13	1	16
Pachydinium mediterraneum Pavillard			*9	
Peridinium cinctum (O.F.Müller) Ehrenberg	6			
Phalacroma rotundatum (Claparéde & Lachmann) Kofoid & J.R.Michener	6	13	15	25
Phalacroma cuneus F.Schütt			56	

Table 2. (Continued)

Türler	B	S	A	M
Phalacroma doryphorum Stein		61	1	16
Phalacroma favus Kofoid & J.R.Michener			15	41
Phalacroma mitra F.Schütt		61	5	16
Phalacroma ovatum (Claparède & Lachmann) Jørgensen		*28		
Phalacroma oxytoxoides (Kofoid) F.Gomez, P.Lopez-Garcia & D.Moreira	60	**20	*14	
Phalacroma porodictyum F.Stein			1	
Phalacroma rapa F.Stein			21	8
Podolampas bipes F.Stein		57	*5	16
Podolampas elegans F.Schütt		49	1	41
Podolampas palmipes Stein	48	**20	15	41
Podolampas spinifera Okamura	48		1	16
Polykrikos hartmannii W.M.Zimmermann	22	22	62	
Polykrikos kofoidii Chatton	48	44	64	
Polykrikos schwartzii Bütschli	6	28		18
Pronoctiluca pelagica Fabre-Domergue	6	73	46	
Pronoctiluca spinifera (Lohmann) Schiller	6			
Prorocentrum micans Ehrenberg	6	13	4	16
Prorocentrum aporum (Schiller) J.D.Dodge	17	13	21	
Prorocentrum arcuatum Issel		32		
Prorocentrum balticum (Lohmann) Loeblich III	17		46	
Prorocentrum cordatum (Ostenfeld) J.D.Dodge	6	13	34	18
Prorocentrum dentatum F.Stein	17	32		
Prorocentrum gracile F.Schütt	38	32	34	53
Prorocentrum lima (Ehrenberg) F.Stein		32	34	
Prorocentrum maximum (Gourret) J.Schiller	17		21	
Prorocentrum pyriforme (J.Schiller) Hasle ex F.J.R.Taylor	17			
Prorocentrum rotundatum J.Schiller	17		14	
Prorocentrum scutellum B.Schröder	17	13	5	
Prorocentrum shikokuense Y.Hada		**75		
Prorocentrum triestinum J.Schiller	17	13	*5	53
Prosoaulax lacustris (F.Stein) Calado & Moestrup	**60			
Protoceratium areolatum Kofoid	17		*10	41
Protoceratium pepo Kofoid & J.R.Michener				8
Protoceratium reticulatum (Claparède & Lachmann) Bütschli	6	*28	11	43
Protodinium simplex Lohmann	48	*28		
Protoperidinium bipes (Paulsen) Balech	38	*28		
Protoperidinium brevipes (Paulsen) Balech	17	*31		
Protoperidinium brochii (Kofoid & Swezy) Balech	17	13	1	16
Protoperidinium claudicans (Paulsen) Balech	17	28	*5	16
Protoperidinium conicoides (Paulsen) Balech	17	76		41
Protoperidinium conicum (Gran) Balech	17	13	1	16
Protoperidinium conicum var. concavum (Matzenauer) Balech	17			
Protoperidinium crassipes (Kofoid) Balech	6	13	*5	18
Protoperidinium curtipes (Jørgensen) Balech	40	32		
Protoperidinium curvipes (Ostenfeld) Balech		32		
Protoperidinium deficiens (Meunier) Balech	6			
Protoperidinium depressum (Bailey) Balech	6	13	1	8
Protoperidinium diabolus (Cleve) Balech	6	13	1	41
Protoperidinium divergens (Ehrenberg) Balech	6	13	1	16
Protoperidinium elegans (Cleve) Balech	**60	74		
Protoperidinium excentricum (Paulsen) Balech	40	61		
Protoperidinium globulus (F.Stein) Balech	17	*28	1	16
Protoperidinium grande (Kofoid) Balech	17	13	*5	16
Protoperidinium granii (Ostenfeld) Balech	6	13	5	18
Protoperidinium heteracanthum (P.A.Dangeard) Balech	22			52
Protoperidinium leonis (Pavillard) Balech	22	13	5	16
Protoperidinium longipes Balech	17	13	*5	67
Protoperidinium mariebouriae (Paulsen) Balech	17			
Protoperidinium mediterraneum (Kofoid) Balech	60	13	10	16

Table 2. (Continued)

Türler	B	S	A	M
Protoperidinium mite (Pavillard) Balech		45	21	41
Protoperidinium murrayi (Kofoid) Hernández-Becerril			21	8
Protoperidinium oblongum (Aurivillius) Parke & Dodge	59	44	46	18
Protoperidinium obtusum (Karsten) Parke & J.D.Dodge	72			
Protoperidinium oceanicum (Vanhöffen) Balech	38	13	*5	16
Protoperidinium ovatum Pouchet		32	56	
Protoperidinium oviforme (Dangeard) Balech				18
Protoperidinium ovum (J.Schiller) Balech				41
Protoperidinium pallidum (Ostenfeld) Balech	38	13	*5	16
Protoperidinium paulsenii (Pavillard) Balech	40	*28	*9	
Protoperidinium pedunculatum (F.Schütt) Balech			34	16
Protoperidinium pellucidum Bergh	12	28	1	18
Protoperidinium pentagonum	6	28	14	41
Protoperidinium punctulatum (Paulsen) Balech	17	32		41
Protoperidinium pyriforme (Paulsen) Balech	17	*28	*5	16
Protoperidinium pyriforme subsp. *breve* (Paulsen) Balech			14	
Protoperidinium quarnerense (B.Schröder) Balech			*5	16
Protoperidinium simulum (Paulsen) Balech		45	*5	
Protoperidinium solidicorne (Mangin) Balech	6		21	18
Protoperidinium sphaericum (G.Murray & Whitting) Balech			14	
Protoperidinium steinii (Jørgensen) Balech	6	28	4	16
Protoperidinium subinerme (Paulsen) A.R.Loeblich III	17	*28	*5	23
Pseliodinium fusus (F.Schütt) F.Gómez	**60	*69	*65	
Pseliodinium vaubanii Sournia			50	
Pyrocystis elegans Pavillard	17	13	*9	25
Pyrocystis fusiformis C.W.Thomson	72		*9	8
Pyrocystis hamulus Cleve		57	34	41
Pyrocystis lunula (F.Schütt) F.Schütt	**60		50	
Pyrocystis pseudonoctiluca Wyville-Thompson		*75		
Pyrocystis robusta Kofoid		13	*10	41
Pyrophacus horologium F.Stein	17	13	5	16
Pyrophacus steinii (Schiller) Wall & Dale	48	74	*5	8
Scaphodinium mirabile Margalef	70	**20		
Scrippsiella acuminata (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling	6	22	4	23
Sourniaea diacantha (Meunier) H.Gu., K. N. Mertens, Zhun Li & H. H. Shin	60	61		
Spatulodinium pseudonoctiluca (Pouchet) J.Cachon & M.Cachon	**60	*75		
Speroidium fungiforme (Anisimova) Moestrup & Calado	60		64	
Spiraulax kofoidii H.W.Graham			1	16
Torodinium robustum Kofoid & Swezy	**60			
Torodinium teredo (Pouchet) Kofoid & Swezy			64	
Tovellia leopoliensis (Wołoszyńska) Moestrup, K.Lindberg & Daugbjerg	**60			
Triadinium polyedricum (Pouchet) J.D.Dodge	6		5	16
Triadinium sphaericum (G.Murray & Whitting) J.D.Dodge		50	5	16
Tripos aestuarius var. *pavillardii* (Rampi) F.Gómez	17			
Tripos arcuatus (Gourret) F.Gómez			3	16
Tripos arietinus (Cleve) F.Gómez	38	27	3	8
Tripos arietinus f. *gracilentus* (Jørgensen) F.Gómez			3	41
Tripos azoricus (Cleve) F.Gómez		*75		41
Tripos belone (Cleve) F.Gómez	17		*5	41
Tripos brevis (Ostenfeld & Johannes Schmidt) F.Gómez			21	18
Tripos candelabrum (Ehrenberg) F.Gómez	6	*28	1	16
Tripos candelabrum var. *depressus* (Pouchet) F.Gómez			3	8
Tripos carriensis (Gourret) Hallegraeff & Huisman		50	2	2
Tripos claviger (Kofoid) Hallegraeff & Huisman			21	
Tripos coarctatus (Pavillard) F.Gómez			3	41
Tripos compressus (Gran) F.Gómez	17		*10	18
Tripos declinatus f. *brachiatus* (Jørgensen) F.Gómez			*9	
Tripos declinatus var. *major* (Jørgensen) F.Gómez	17		3	16

Table 2. (Continued)

Türler	B	S	A	M
Tripos digitatus (F.Schütt) F.Gómez			*10	41
Tripos eugrammus (Ehrenberg) F.Gómez	17	13	3	16
Tripos extensus (Gourret) F.Gómez	17		46	8
Tripos falcatiformis (Jørgensen) F.Gómez			*10	
Tripos falcatus (Kofoid) F.Gómez		69	2	2
Tripos furca (Ehrenberg) F.Gómez	6	13	1	8
Tripos fusus (Ehrenberg) F.Gómez	6	13	1	2
Tripos fusus var. *schuettii* (Lemmermann) F.Gómez	17	45	*5	
Tripos gallicus (Kofoid) F.Gómez			3	8
Tripos gibberus (Gourret) F.Gómez	22	*31	3	2
Tripos gracilis (Pavillard) F.Gómez	17		1	18
Tripos gravidus (Gourret) F.Gómez			5	41
Tripos hexacanthus (Gourret) F.Gómez	6	*75	2	2
Tripos hexacanthus f. *spiralis* (Kofoid) F.Gómez			*10	8
Tripos hexacanthus var. *contortus* (Lemmermann) F.Gómez				16
Tripos inflatus (Kofoid) F.Gómez	17	*31	2	2
Tripos intermedius (Jørgensen) F.Gómez	17		2	8
Tripos karstenii (Pavillard) F.Gómez			2	2
Tripos limulus (Pouchet) F.Gómez			*10	16
Tripos lineatus (Ehrenberg) F.Gómez	17	32	14	41
Tripos longipes (Bailey) F.Gómez	6	22	3	16
Tripos longirostrum (Gourret) Hallegraeff & Huisman	6	*28	2	2
Tripos longissimus (Schröder) F.Gómez			34	41
Tripos macroceros (Ehrenberg) Hallegraeff & Huisman		22	1	2
Tripos massiliensis (Gourret) F.Gómez		61	1	2
Tripos massiliensis f. *armatus* (Karsten) F.Gómez, nom. inval.	17		21	
Tripos minutus (Jørgensen) F.Gómez		**20	46	
Tripos muelleri Bory	6	22	1	8
Tripos muelleri f. *atlanticus* (Ostenfeld) F.Gómez, nom. inval.	17	13	3	16
Tripos muelleri f. *parallelus* (Schmidt) F.Gómez				16
Tripos paradoxides (Cleve) F.Gómez			3	8
Tripos pavillardii (Jørgensen) F.Gómez			2	2
Tripos pentagonus (Gourret) F.Gómez	6	28	1	8
Tripos pentagonus var. *longisetus* (Ostenfeld & J.Schmidt) Gómez, nom. inval.			*5	
Tripos platycornis (Daday) F.Gómez	60		*9	41
Tripos pulchellus (Schröder) F.Gómez	17	13	1	8
Tripos ranipes (Cleve) F.Gómez			2	8
Tripos schroeteri (B.Schröder) F.Gómez			*10	41
Tripos setaceus (Jørgesen) F.Gómez	17	51	3	8
Tripos subcontortus (Schröder) F.Gómez				8
Tripos symmetricus (Pavillard) F.Gómez			1	8
Tripos teres (Kofoid) F.Gómez	17	51	3	8
Tripos trichoceros (Ehrenberg) Gómez	72	13	2	2
Tripos volans (Cleve) F.Gómez	17		3	8
Tripos vultur (Cleve) Hallegraeff & Huisman		*75	*9	
Triposolenia bicornis Kofoid			**36	

DISCUSSION

There are checklists published by various researchers in the past (Koray 2001; Balkıs 2004). In the checklist made by Koray (2001), a total of 396 phytoplankton species, 193 of which belong to the Dinophyceae class, were identified from the Turkish seas. In the same review study, 87 species belonging to the Dinophyceae class were compiled in the Black Sea, 133 species in the Aegean Sea and 107 species in the Mediterranean Sea. Balkıs (2004) recorded 73 species belonging to the Dinophyceae class out of total 168 species in the phytoplankton species list of the Marmara Sea. With the present study, total number of dinoflagellates recorded along Turkish coasts increased to 330, showing 137 more records of species. However, this number may increase if results of master and doctoral theses are published.

With the latest studies conducted on a regional basis, the number of species continues to increase day by day. Some of the recent studies on phytoplankton composition in distinct regions of Türkiye can be listed as following; the Black Sea (Taş and Okuş, 2006; Baytut et al., 2010; Balkıs Özdelice and

Peynirci, 2019), the Marmara Sea (Balkıs, 2003; Taş et al., 2020; Durmuş et al., 2022), the Aegean Sea (Koray, 1987; Koray, 1995; Çolak Sabancı and Koray, 2001; Çolak Sabancı and Koray, 2012) and Mediterranean Sea (Eker and Kıdeyş, 2000; Polat et al., 2000; Polat and Koray, 2007). By electron microscope analyses, Eker Develi and Velikova (2009) found one species as a new record for the Black Sea. Besides, in the study of Baytut and Gönülol (2016) conducted in the Black Sea, a total of 34 new records species were recorded for Turkish coasts, but the absence of drawings, photographs or morphometric measurements of these species cause that the species to remain as questionable. A total of 51 new records of species were recorded for the Marmara coasts, and 14 of them were noted as new records for the Turkish coasts (Balkıs, 2000; Balcı and Balkıs, 2017; Balkıs Özdelice et al., 2020; Kayadelen et al., 2022; Durmuş et al., 2022). While 2 of these species (*Corythodinium frenguellii* and *Gonyaulax scrippsae*) are only included in the species list, 12 species (*Amoebophrya ceratii*, *Amphisolenia laticincta*, *Balechina gracilis* (=*Gymnodinium dogielii*), *Dinophysis odiosa*, *Gynogonadinium aequatoriale*, *Heterocapsa rotundata*, *Heterodinium rigdeniae*, *Phalacroma oxytoxoides*, *Podolampas palmipes*, *Prorocentrum shikokuense*, *Scaphodinium mirabile*, *Tripos minutus* (=*Ceratium minutum*) were supported by morphometric measurements and photography. Additionally, 2 taxa were recorded as "sp.", 2 taxa were noted as "cf.", and 1 species was not included in the list as its record had been previously provided. It has been observed that out of the 58 new records of species for the Aegean coast, only a few are new records for the Turkish coastline. In the literature study, only *Triposolenia bicornis* and *Corythodinium tesselatum* species were found to be the new records for the Turkish coasts, and these records were reported with morphometric measurements and photography (Balkıs, 2005; Taş et al., 2006). *Tripos gallicus* (=*Ceratium deflexum*, *Ceratium macroceros* var. *gallicum*) and *Tripos longissimus* (=*Ceratium longissimus*) species have not been included in the list as new records since they have been recorded before. New records of 12 species in total have been reported for the Mediterranean, and all of them are the first records for the Turkish coasts (Polat and Koray, 2002; Polat and Koray, 2003; Uysal et al., 2003; Polat, 2004; Polat, 2007). The studies conducted by Polat and Koray (2002) (*Histioneis depressa*, *Histioneis elongata*, *Histioneis expansa*, *Histioneis marchesonii*, *Histioneis para*, *Histioneis striata*), Polat and Koray (2003) (*Heterodinium angulatum*, *Heterodinium inaequale*, *Heterodinium mediocre*), Uysal et al., (2003) (*Heterocapsa pygmaea*), Polat (2004) (*Citharistes regius*), and Polat (2007) (*Gonyaulax pacifica*) have contributed significantly to the flora of Türkiye at both genus and species levels. These studies provide valuable contributions by presenting the characteristic structural features, distributions, and photographs of the species.

CONCLUSION

According to the results obtained from research on phytoplankton, the dinoflagellate flora of Türkiye is represented by 330 species belonging to 75 genera. A total of 2377 species of dinoflagellates belonging to 259 genera have been identified in the world's oceans (Gómez, 2012b). The Mediterranean is represented by 673 species belonging to 104 genera (Gómez, 2003), while the Black Sea is represented by 267 species belonging to 54 genera (Gómez and Boicenco, 2004). The oligotrophic conditions of the Mediterranean lead to an increase in dinoflagellate diversity, whereas the lower salinity (~ 17) of the Black Sea, coupled with its richer organic matter, result in lower species diversity (Gómez, 2003; Gómez and Boicenco, 2004).

Pelagic dinoflagellates show a lower percentage of endemic species compared to macroscopic or benthic species, and studies have concluded that pelagic dinoflagellates cannot be considered endemic (Bianchi and Morri, 2000; Gómez and Boicenco, 2004; Gómez, 2006). In this study, the number of species detected in a single sea constitutes approximately 34.5% of the total species given in the literature. Accordingly, when the single-celled microplankton species compositions of the Black Sea, Marmara, Aegean and Mediterranean were evaluated, it was determined that 40 species in the Black Sea, 19 species in the Marmara, 21 species in the Aegean and 34 species in the Mediterranean were seen in a single sea. It is quite surprising that there are very few endemic species in phytoplankton, and even though most of the species are cosmopolitan, only 20% of the species identified in the literature study are represented in all seas of Türkiye. Variables such as sampling period, sampling method and differencies between salinity levels may be effective in observing this difference between Turkish coasts in the studies conducted, it is also necessary to take into account the possibility of incorrect taxonomic definitions and missing small species during the examination. Detailed studies carried out with electron microscope and DNA sequence analyses can definitely help to identify species correctly. In addition, the species diversity given in the checklist may not accurately reflect reality, since the studies carried out so far have generally been repeated in the same regions and many geographical points have not been investigated. Therefore, it would be more appropriate to plan future studies as ecosystem-wide comprehensive and long-term monitoring studies, and to provide the characteristic structural features, distributions and photographs of the species, especially in new taxonomic descriptions. This study is significant for compiling marine pelagic dinoflagellates from Turkish coasts, reported in 77 differeent literatüre sources and updating their nomenclature, thus serving as a dataset for future research endeavors.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest or competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

DATA AVAILABILITY

For any questions, the corresponding author should be contacted.

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Ege Journal of Fisheries and Aquatic Sciences, 41(3), 243-251 (2024)

Su Ürünleri Dergisi (2024)
DOI: 10.12714/egejfas.41.3.10

REVİEW DERLEME

A systematic review of age, growth and mortality studies in Mediterranean and Black Sea fishes

Akdeniz ve Karadeniz balıklarının yaş, büyüme ve ölüm çalışmalarının sistematik bir derlemesi

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Received date: 15.05.2024 *Accepted date: 29.07.2024*

How to cite this paper:
Cerim, H., Soykan, O., Yapıcı, S., Reis, İ. & Yılmaz, Ö. (2024). A systematic review of age, growth and mortality studies in Mediterranean and Black Sea fishes. *Ege Journal of Fisheries and Aquatic Sciences, 41*(3), 243-251. https://doi.org/10.12714/egejfas.41.3.10

Abstract: Age, growth, and mortality studies (AGMS) conducted in the Mediterranean and the Black Sea were reviewed. The main objective of this study was to find out the gaps on unstudied and less studied species. Names of the all fish species were obtained from fishbase. While the “native” and “endemic” species were taken into consideration, species “introduced”, “questionable”, “misidentified” and “error in a name” were excluded from the data set. Fishbase, semanticscholar and googlescholar were used to obtain the species related studies in June 2023. Graphs and tables were created to represent the results. Totally, 185 of 604 species have AGMS. 22 countries have investigated fish age, growth, and mortalities, and the first three countries are Türkiye, Italy, and Greece, respectively. In the Mediterranean and Black Sea, 796 AGMS were found. The top three of these families with the most species are Sparidae (177), Mullidae (87), and Mugilidae (66). Among the studied species, 86.49% of the species (160) are commercially important for fisheries, and 13.51% of the species (25) are non-commercial. 31 of the 160 species encounter existence problems. All accessible studies were used including studies’ references and it was observed that the most of the species (419) in the Mediterranean and Black Sea have no age or growth studies. This study clearly shows the gaps in AGMS in the Mediterranean and Black Sea regions.

Keywords: Mediterranean, Black Sea, scientific research, age, growth

Öz: Akdeniz ve Karadeniz'de yürütülen yaş, büyüme ve ölüm çalışmaları (AGMS) gözden geçirilmiştir. Bu çalışmanın temel amacı, üzerinde çalışılmamış ve az çalışılmış türlerdeki boşlukları ortaya çıkarmaktır. Çalışmaya dahil olan tüm balık türlerinin isimleri fishbase veri tabanından elde edilmiştir. “Yerli” ve “endemik” türler dikkate alınırken, “yabancı”, “şüpheli”, “yanlış tanımlanan” ve “isim hatası” olan türler veri setinden çıkarılmıştır. Haziran 2023'te türlerle ilgili çalışmaların elde edilmesi için Fishbase, semanticscholar ve googlescholar kullanılmıştır. Sonuçları temsil edecek grafikler ve tablolar oluşturulmuştur. Toplamda 604 türün 185'inde AGMS bulunmaktadır. Balıkların yaşı, büyümesi ve ölümleri 22 ülkede araştırıldığı tespit edilirken ve ilk üç ülke sırasıyla Türkiye, İtalya ve Yunanistan olarak belirlenmiştir. Akdeniz ve Karadeniz'de 796 AGMS yapılmıştır. En çok türün bulunduğu ilk üç familya Sparidae (177), Mullidae (87) ve Mugilidae (66) olarak belirlenmiştir. İncelenen türlerin %86,49'u (160) balıkçılık açısından ticari öneme sahip olup, %13,51'i (25) ticari değildir. 160 türden 31'i varoluş sorunlarıyla karşı karşıyadır. Çalışmaların referansları da dahil olmak üzere erişilebilir tüm çalışmalar kullanılmış ve Akdeniz ve Karadeniz'deki türlerin çoğunun (419) yaş ve büyüme çalışmalarının bulunmadığı görülmüştür. Bu çalışma Akdeniz ve Karadeniz bölgelerinde AGMS'deki boşlukları açıkça göstermektedir.

Anahtar kelimeler: Akdeniz, Karadeniz, bilimsel araştırma, yaş, büyüme

INTRODUCTION

The Mediterranean is surrounded by Asia, Europe and the Africa continents, and since ancient times, the region has numerous civilizations, cultures, and nations (Stavridis, 2017). In its history, the Mediterranean fishery also has its own tradition (Lleonart and Recasens, 1997). However, Bas et al. (1985) draw attention to the technological advances and modernization of fleets in fisheries play a role in the decline of fishery resources.

The global species richness rate is decreasing dramatically compared to past times (Barnosky et al., 2011), and marine fish species are no exception to this threat. Two basins, the Mediterranean and Black Seas (FAO area 37), have the highest percentage (62%) of stocks fished at levels that are biologically unsustainable (FAO, 2018), and one of the regions with the lowest global fisheries management index scores for management and enforcement is the Mediterranean (Hilborn et al., 2020). Anthropogenic activities (overharvesting, introduction of non-native species, pollution, habitat destruction, and human-induced climate change) have long been the underlying causes of species declines (van Treeck et al., 2020). The determination of species that are on the brink of extinction has been a major concern for scientists and environmental agencies (either governmental or private enterprises) worldwide. At the end, a number of threat categories with specified criteria identified for each have been formed to list species and place them into (IUCN, 2023). Also, the efforts to document the species prone to go extinct appear to meet around the essential idea that species that fall into high risk categories are more likely to become extinct than those in low risk categories (van Treeck et al., 2020).

The results of anthropogenic activities, for example, increasing mercury contamination, surface water acidification, and eutrophication, inhibit the growth of algae, reduce hatching success, and increase egg and larval mortality (Driscoll et al., 2001; Bergström and Jansson 2006). Adding to the above-mentioned anthropogenic activities, the complex nature of fisheries complicates the establishment of biotic resource sustainability through decreasing mortality rates and control of other fisheries measures. Unintended by-catches from flawed fishing techniques, fishing gear damaging the natural habitats of the species, and unpredictable ecological consequences of targeting one or key species within the trophic link are some of the known reasons for the complication (Caddy and Agnew, 2004). On the other hand, it is reported that there are numerous fish species that are targeted by small-scale and recreational fisheries, and about which no relevant data is present to assess their current population status (Lloret et al., 2019).

When anthropogenic activities and lack of data are considered together, the exploitation rate of fish species in the Mediterranean and Black Seas and the way this issue is being and will be assessed become even more crucial to be discussed. There are numerous assessment methods suggested to reach the goal of sustainability in fisheries (Hoggarth, 2006; Coll et al., 2013; Goetze et al., 2016; Carvalho et al., 2019). The primary goal of conventional management has been to adjust fishing effort to levels that ensure maximum sustainable yield, or the largest catch that can be taken from a stock during the course without depleting it. Maximum sustainable yield and its related biological reference points, such as stock biomass and fishing mortality rate, are key parameters used for measuring the status of a stock or fishery (Hilborn and Ovando, 2014).

The fish's biological characteristics are important values for the stock assessment studies (Najmudeen and Wilson, 2019). Growth parameters provide some indication of resource utilization and the effectiveness of management strategies. When age and growth are evaluated in combination, it may be easier to understand the relationship between population size and biomass. This understanding is the basis of modern fisheries resource allocation and management, and fisheries management should be designed based on biological data to understand the status of and manage fish stocks (Isely and Grabowski, 2007). Especially the growth parameters L_∞ (asymptotic length), K (growth coefficient and t_0 (theoretical age at zero length) are used almost in all stock assessment models. However, the studies have mostly focused on commercial species. Even if decline in fishery resources is a different topic, ecosystems should be considered as a whole. Therefore, either commercial or non-commercial species should not be considered different components of the fishery resources.

A review study indicates the gaps in a certain area and shows ways for scientists (Dhillon, 2022). Our review idea originated from Dimarchopoulou et al. (2017). The researchers put forward the available studies of Mediterranean fish with different topics. In the brainstorming phase, we thought, "Al*right, how many age, growth, and mortality studies have been conducted up to 2023?*", "*What are the details of these studies in the Mediterranean region?*" and "*Gathering information about the Mediterranean fish species could be helpful for the fisheries scientists*". Thus, this review came out to deeply learn about age and growth studies in the Mediterranean and Black Sea basins. The results obtained are thought to be very important in terms of showing neglected species in fish biology.

MATERIALS AND METHODS

The Mediterranean was separated into sub-regions by FAO (2023) as Mediterranean and Black Sea (Major Fishing Area 37); Western Mediterranean (Balearic, Gulf of Lions, Sardinia), Central Mediterranean (Adriatic, Ionian), Eastern Mediterranean (Aegean, Levant), Black Sea (Marmara Sea, Black Sea, Azov Sea).

In this study, Mediterranean fish species were determined from the Fishbase catalog (June 2023) (Froese and Pauly, 2023). The data set used in the study consisted of between 1920 and 2023. Age, growth, and natural mortality values (L_∞, k, t_0, and M) of all Mediterranean fish species in Fishbase were taken. All literature was searched for non-existing studies in Fishbase. In this process, an artificial intelligence powered research tool, semanticscholar, was used. Studies were also searched on googlescholar to keep sight of any study. The cited literature in the obtained papers was also checked.

According to the results obtained from the literature search, fish species have age, growth, and mortality studies that were conducted in the Mediterranean (from Gibraltar to the Black Sea), were selected and classified. Next, a detailed analysis was conducted to learn about the Mediterranean studies, such as regional, species-specific, number of studies, number of families, etc. Species threatened levels were also determined by the IUCN red list (IUCN, 2023).

In the visualization process, Microsoft Excel and Rstudio were used.

RESULTS

All meta-data was presented in supplementary material by separated tabs. Tabs include different statistics on all Mediterranean species, all age and growth studies on species up to June 2023, selected species for this study, study numbers by species, information on families, red list information of the species, and study information of the countries.

Mediterranean fish and overall distributions

According to Fishbase, the Mediterranean basin includes 778 fish species. In this number, 557 are "native", 135 are "introduced", 47 are "endemic", 23 species are "questionable", 14 are "misidentified" and 2 are "error in a name", or in other words "an incorrect spelling" (Figure 1). All species without native and endemic were excluded from the data set. The total number of the considered fish species in this study is 604 (native+endemic).

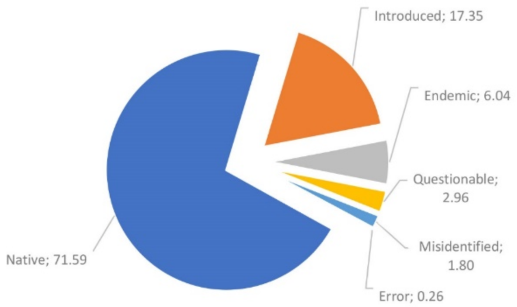

Figure 1. Distribution (%) of the total fish species in the Mediterranean (N=778)

Among the selected species, 518 are Osteichthyes (85.76%) and 86 are Chondrichthyes (14.24%).

Selected species and age, growth, and mortality studies in the Mediterranean and Black Sea

All AGMS were conducted in 22 different countries. The first three countries that have AGMS are Türkiye, Italy, and Greece, respectively (Figure 2). Fish species selected among the total of 604 species which have age, growth, and mortality studies (AGMS), and a total of 796 studies were found that belong to 185 fish species that have AGMS. In some papers, one species was studied, however, in some papers, more than one species was studied. Figure 2 was built according to species numbers in these studies.

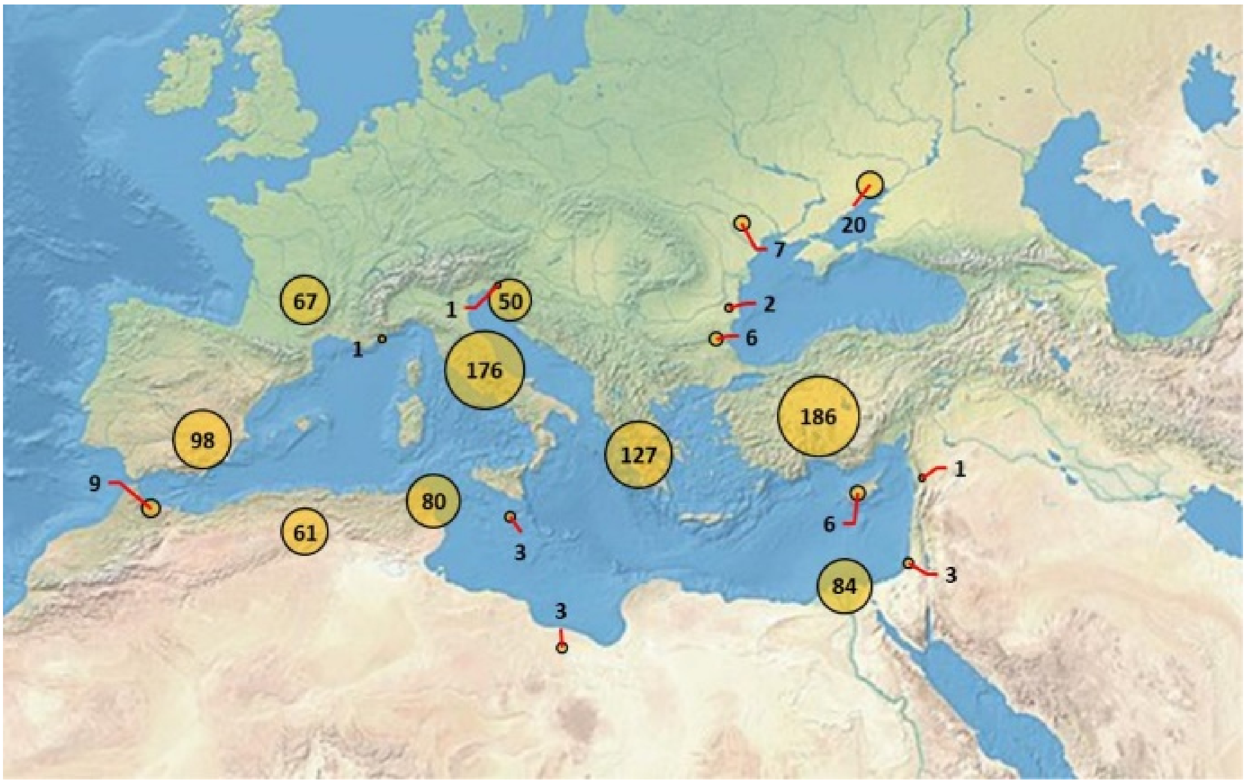

Figure 2. AGMS's distribution by countries in the Mediterranean

The number of studies had started to slightly increase by the late 1940s than a sharp increase in the 1980s (Figure 3).

The selected 185 fish species belonging to 72 families were 161 (87.03%) of Osteichthyes and 24 (12.97%) of Chondrichthyes. On the other hand, in Mediterranean age and growth studies, Osteichthyes were studied 955 times (95.79%) and Chondrichthyes were studied 39 times (3.91%).

The first 10 families and species have the most studies shown in Figure 4. The top three of these families with the most species are Sparidae (177), Mullidae (87), and Mugilidae (66), respectively. In Sparidae, the three most studied species were *Boops boops* (Linnaeus, 1758) (28), *Pagellus erythrinus* (Linnaeus, 1758) (27), and *Spicara smaris* (Linnaeus, 1758) (15) (Figure 5).

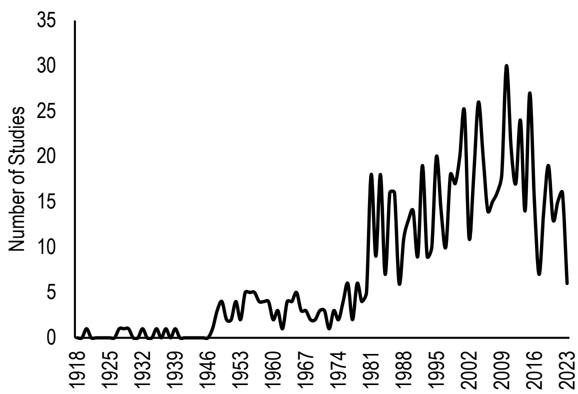

Figure 3. Number of studies by year in the Mediterranean

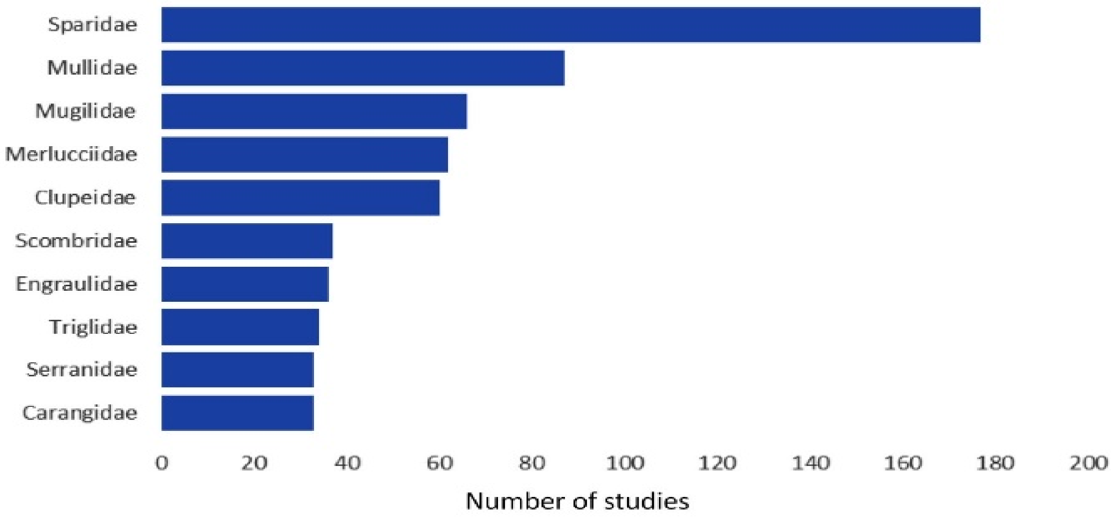

Figure 4. The 10 most studied families in the Mediterranean

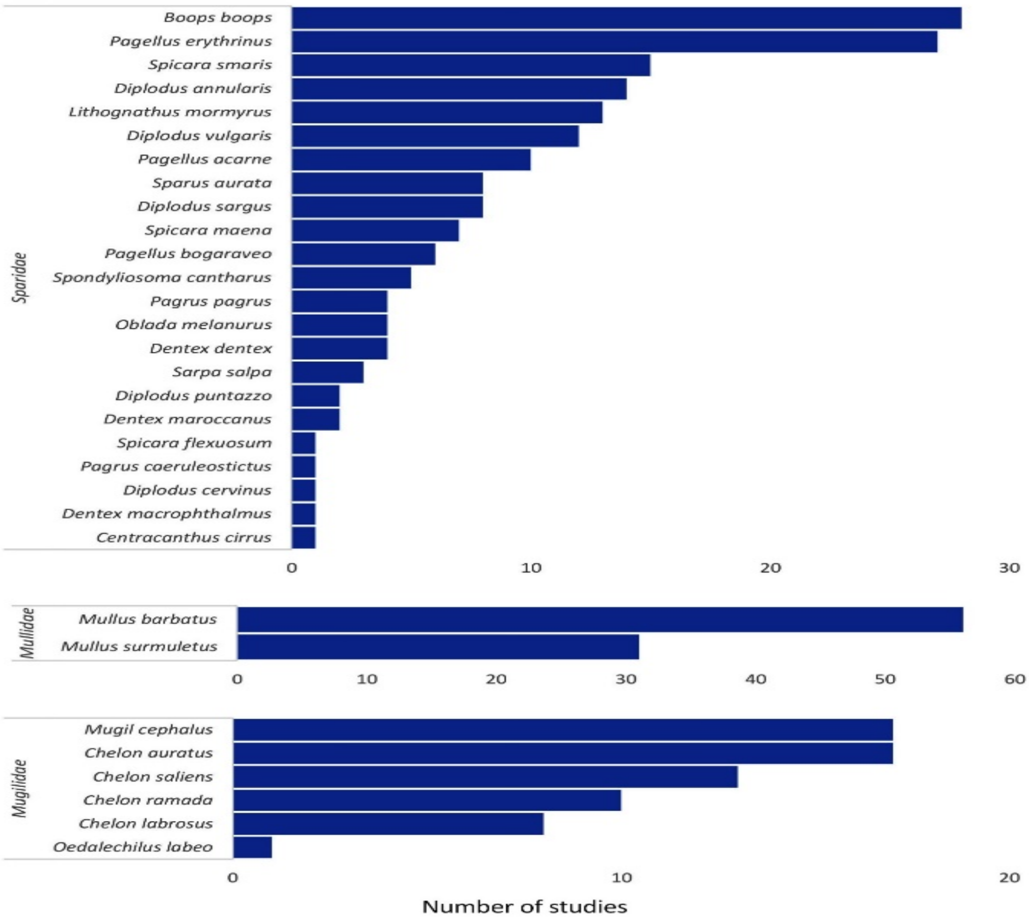

Figure 5. Species of the most studied first three families

Merluccius merluccius (Linnaeus, 1758) is the most studied species (60) among selected species (Figure 6). All species on Figure 6 are commercial for Mediterranean fisheries. On the other hand, while 86.49% of the species (160) are commercial for Mediterranean fisheries, 13.51% of the species (25) are non-commercial (Figure 7). The IUCN Red List Categories for the selected species in this study show that while a great majority of the determined species (70.81%) are in "Least Concern" status, 3.24% are in "Critically Endangered" (six species: *Anguilla Anguilla* (Linnaeus, 1758), *Rhinobatos rhinobatos* (Linnaeus, 1758), *Rhinoptera marginata* (Geoffroy Saint-Hilaire, 1817), *Acipenser stellatus* (Pallas, 1771), *Huso huso* (Linnaeus, 1758), and *Glaucostegus cemiculus* (Geoffroy Saint-Hilaire, 1817)) status (Figure 8). In 160 species that have commercial importance, 31 fish species (19.4% of 160) encounter existence problems (Table 1). 15 of the 31 species are chondrichthyes.

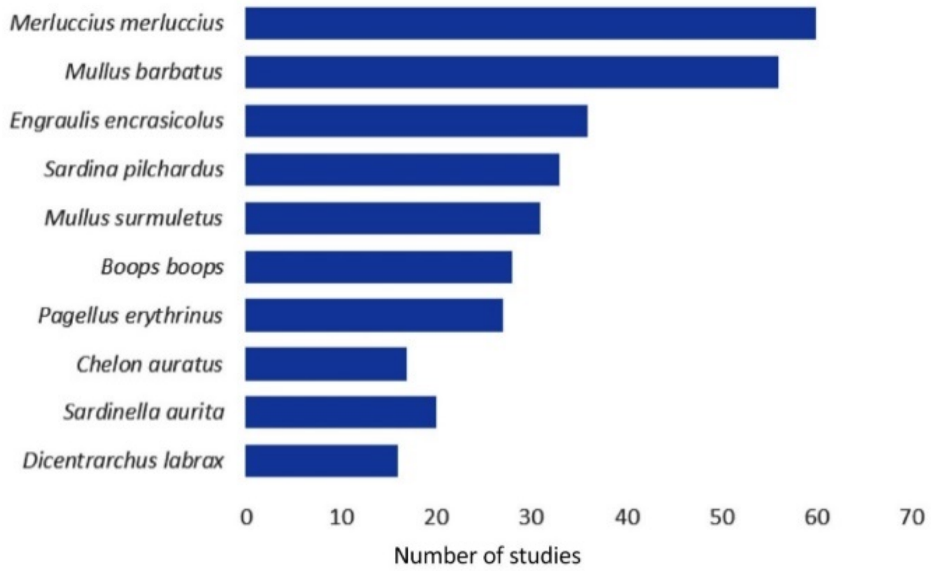

Figure 6. The 10 most studied species in the Mediterranean

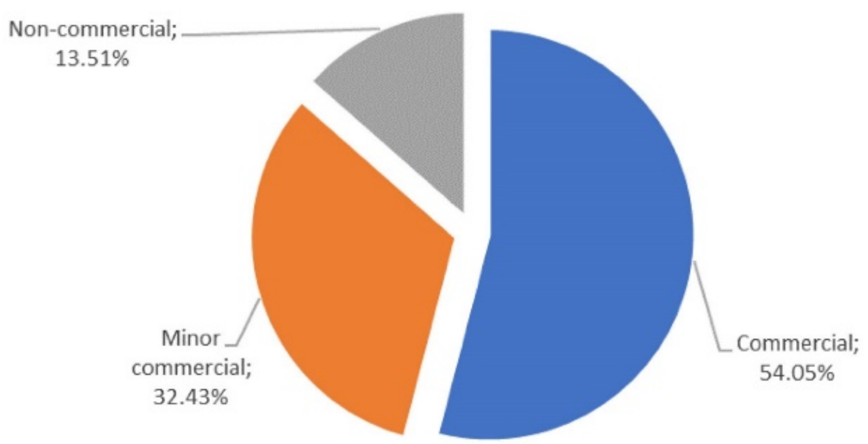

Figure 7. Commercial importance of fish for the Mediterranean fishery

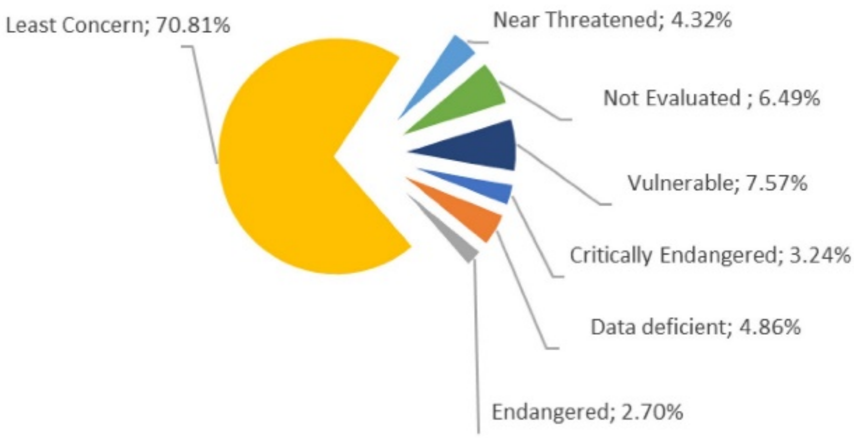

Figure 8. Fish species distributions according to the International Union for Conservation of Nature's (IUCN) Red List (N=185)

Table 1. List of the near threatened, vulnerable, endangered, critically endangered, and species by commercial importance (MC; minor commercial, C; commercial)

	Species	**Commercial Importance for the Mediterranean Fishery**
Near Threatened	*Dipturus oxyrinchus*	MC
	Epinephelus aeneus	C
	Pagellus bogaraveo	C
	Raja asterias	MC
	Raja brachyura	MC
	Raja clavata	C
	Sciaena umbra	C
	Xiphias gladius	C
Vulnerable	*Balistes capriscus*	C
	Dasyatis pastinaca	C
	Dentex dentex	C
	Epinephelusmarginatus	C
	Etmopterus spinax	MC
	Pomatomus saltatrix	C
	Sardinella maderensis	C
	Squalus acanthias	C
	Trachinotus ovatus	MC
	Trachurus trachurus	C
	Umbrina cirrosa	MC
	Uranoscopus scaber	MC
Endangered	*Centrophorus granulosus*	MC
	Gymnura altavela	MC
	Mustelus mustelus	C
	Raja radula	C
	Rostroraja alba	MC
Critically Endangered	*Anguilla anguilla*	C
	Acipenser stellatus	C
	Glaucostegus cemiculus	MC
	Huso huso	C
	Rhinobatos rhinobatos	C
	Rhinoptera marginata	C

DISCUSSION

The Mediterranean Sea has high species richness (Bianchi et al., 2012). The number of native and endemic species constitutes considerable amounts of total fish species in the Mediterranean (77.63% of total species). According to Cramer et al. (2018), the Mediterranean Sea water temperature is 1.4 °C above that of the late nineties. Therefore, an increase in Mediterranean temperature would lead to an increase and to a northward spread of the introduced species (Schickele et al., 2021). Thus, this phenomenon leads to an increased species number in the Mediterranean through the invasion of introduced species.

Türkiye, Italy, and Greece are big peninsulas, and all the shores of these countries are in the Mediterranean and Black Sea regions. On the other hand, France, Spain, and Morocco have shores in both the Atlantic and the Mediterranean and studies in these countries are also relatively high. However, in this study, only Mediterranean studies were collected and sorted by countries. Therefore, study numbers Türkiye, Italy, and Greece are higher than other countries in the Mediterranean and Black Sea regions.

The number of studies has increased since the 1980s. Farrugio et al. (1993) mentioned that, especially in 1980's studies, researchers tended to learn ecological parameters of the exploited populations (besides technical parameters of fishing gear) with direct methods. In addition to this, technical progress has led to an increase in the quality and efficiency of fishing gear. For example, Sardà (1998) stated that the catchability of *Nephrops norvegicus* (Linnaeus, 1758) increased with technological advances. In this finding, it can be said that an increase in the number of fisheries studies in the Mediterranean is attributed to technological advances in fisheries such as the spread of electronic devices (Ferretti, 2011) and high-power engine usage (e.g., Ünal, 2004).

Logically, the increase in fishing capacity and fishing ability has made it easier to reach the wanted species and to do the sampling process for the species.

According to the studied species numbers, most of the species are bony fish (161 species). In contrast, 24 cartilaginous fish species have been studied (24 species). Cartilaginous fish are known mostly as predators, and they are situated at high levels of the food chain. Therefore, they are rarer when compared with other fish species (Bustamante Diaz, 2014). The lesser studies in cartilaginous fish may be attributed to reaching these species is difficult and being lower abundance in catch composition (e.g., Cerim et al., 2022). Furthermore, fishing techniques may be effective in capturing cartilaginous fish species (Bengil and Başusta, 2018). In this study, the cartilaginous species mean depth ranges were min-21 m and max-821 m, and without three species, all 21 species occur at depths above 200 m. As it is known, more depth for studies needs higher fishing equipment and research vehicles (i.e., it is hard to study in deep waters). In our opinion, the most limiting factor for cartilaginous fish studies may be depth. On the other hand, most of the cartilaginous species are on the IUCN red list. This situation may be another restrictive reason for low study numbers.

Sparidae is the most studied family in the Mediterranean basin. Sparidae species are commonly found along the shores (Iwatsuki and Heemstra, 2015). Most of the Sparidae species are caught by different fishing gear. For example, *Boops boops* is captured by different fishing gear such as trawl (İlkyaz et al., 2017), purse-seine (Ceyhan and Tosunoğlu, 2022), and handline (Cerim, 2022). On the other hand, Mullidae species are captured mainly by trawl nets, besides gillnets. Furthermore, even though Mugilidae species are captured by many fishing gears, their mass capture is from traps, especially during migration (Cerim et al., 2021). Therefore, sampling of Sparidae, Mullidae, and Mugilidae species is relatively easy to compare with other species. There is a difference between "doing sampling" and "samples coming to you". Behaviors such as being a school, being in species-specific habitats, and seasonal migration may make the sampling easy. Using these behaviors may be the reason for the aggregation of the studies in particular species.

Papaconstantinou and Farrugio (2000) separated Mediterranean fisheries into three sub-categories as; small-scale fisheries, trawling, and seining fisheries. According to FAO (2022), total 1.19 million tonnes fish were captured in the Mediterranean and Black Sea regions. *Merluccius merluccius* is a demersal species, and even though it is fished with many different fishing gears, its capture is based on trawling (Gül et al., 2019). Moreover, *Mullus barbatus* (Linnaeus, 1758) is also demersal and is mainly captured by trawls. When considered in terms of mobility or lurch, trawl vessels are relatively more stable than many other demersal fishing vessels. On the other hand, *Engraulis encrasicolus* (Linnaeus, 1758) is pelagic, and encircling nets, especially purse-seine, are used for its capture. Purse-seine catch is affected by many different environmental features, such as seasons (Pinello and Dimech, 2013), artificial lights (Tsagarakis et al., 2012), and moonlight (Tosunoğlu et al., 2021). The purse-seine and trawl ships have large horse-power engines and large decks. Therefore, purse-seine and trawl ships are appropriate environments for easy study. In our opinion, most of the species seen in Figure 6 were captured by easy fishing methods. In addition to this, species numbers are higher due to schooling. Mentioned considerations could be the reasons for the high study numbers of these species.

Totally, 86 cartilaginous fish species (sharks, rays, skates, guitarfish, angel sharks, etc.) exist in the Mediterranean basin. According to the IUCN (2023), 68 of the Mediterranean cartilaginous species are under risk (Critically Endangered- 19, Endangered- 15, Vulnerable- 20, Near Threatened- 14; total; 79.07%). Study results showed that 24 cartilaginous species have been studied in terms of age and growth. Among these studied species, 15 have commercial importance (Table 1). On the other hand, GFCM (General Fisheries Commission for the Mediterranean) statistics show that there is a decline in Chondrichthyes capture in the Mediterranean (Figure 9). Ferretti et al. (2008) mention that more than 97% of the shark catch weights have been in decline since the last 200 years, and if this situation continues like this, the extinction will be inevitable. The loss of the top predators could lead to serious ecological effects. Elasmobranchs are caught incidentally. However, by-catch and direct fisheries are not monitored in the Mediterranean (Bradai et al., 2018). Therefore, the biological parameters of cartilaginous fish stocks should be identified for both the ecological and commercial importance of the elasmobranch fishery.

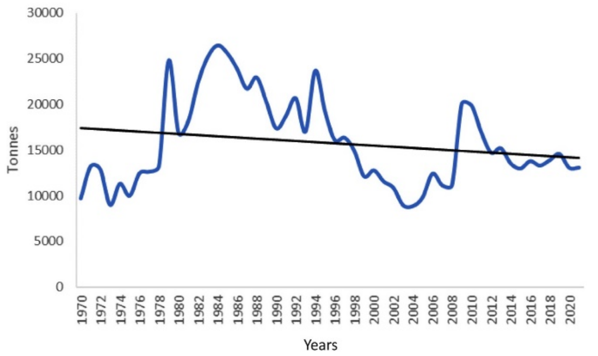

Figure 9. Cartilaginous fish catch trend in the Mediterranean (FAO-GFCM, 2023)

CONCLUSION

In conclusion, most of the species (419) in the Mediterranean have no age or growth studies. Biological identification of the fish stocks serves not only commercial sustainability but also ecological sustainability. In this sense, the construction of a reliable food web is important. Therefore, non-commercial species should also be taken into consideration in terms of age and growth studies.

ACKNOWLEDGEMENTS

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

AUTHOR CONTRIBUTIONS

Hasan Cerim: Writing – original draft preparation, writing – review and editing, data curation, visualization, conceptualization, investigation. Ozan Soykan: Investigation, supervision, writing – review and editing. Sercan Yapici: Supervision, writing – review and editing. İsmail Reis: Conceptualization, data curation, visualization, original draft preparation, writing – review and editing. Özgen Yılmaz: Supervision, writing – review and editing.

CONFLICTS OF INTEREST

The authors of this article declare that they have no financial, professional or personal conflicts of interest that could have inappropriately influenced this work.

ETHICAL APPROVAL

No need for ethical approval for this study.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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