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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.

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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.

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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.

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Full name/s: Supervision

Full name/s: Software, Validation

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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."

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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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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Fishing by numbers: Empowering Muara Kintap fisheries with data-driven fishing area forecast maps

Sayılarla balıkçılık: Muara Kintap balıkçılığını veri odaklı balıkçılık alanı tahmin haritalarıyla güçlendirmek

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Abstract: The paper aims to enhance the fishing efficiency and sustainability of Muara Kintap fishers by using data-driven Fishing Area Forecast Maps (FAFM). This study makes significant contributions to the field of fisheries management by demonstrating the effective use of satellite data for local-scale fisheries management, bridging the gap between scientific research and practical applications, as well as promoting sustainable fishing practices and improving the fishers' livelihoods. The research ingeniously combined the wisdom of local fishers captured through the Fishing Points app with cutting-edge technology. Aqua MODIS satellite imagery captured detailed Muara Kintap waters condition, revealing sea surface temperature (SST) between 28.4 °C and 29.7 °C and chlorophyll-a (Chl-a) concentrations ranging from 0.38 to 6.27 mg/m³. The results strongly indicate that the distribution of Chl-a is a more influential predictor of fish catch than SST. This discovery underscores the intricate relationship between marine parameters and fish distribution. By providing FAFM, informed by Chl-a data, the fishers were able to make data-driven decisions, optimizing catches and promoting the long-term sustainability of their livelihoods. The study's impact transcends data analysis, highlighting the importance of collaboration and knowledge sharing among researchers, fishers, and policymakers in fostering sustainable fishing practices in Muara Kintap and beyond.

Keywords: FAFM, fishing efficiency, Muara Kintap, oceanographic data, sustainability

Öz: Bu çalışma veri odaklı Balıkçılık Alanı Tahmin Haritaları (FAFM) kullanarak Muara Kintap balıkçılarının balıkçılık verimliliğini ve sürdürülebilirliğini artırmayı amaçlamaktadır. Bu çalışma, yerel ölçekli balıkçılık yönetimi için uydu verilerinin etkili kullanımını göstererek, bilimsel araştırma ile pratik uygulamalar arasındaki boşluğu kapatarak, sürdürülebilir balıkçılık uygulamalarını teşvik ederek ve balıkçıların geçim kaynaklarını iyileştirerek balıkçılık yönetimi alanına önemli katkılarda bulunmaktadır. Araştırma, Fishing Points uygulaması aracılığıyla yerel balıkçıların bilgilerini son teknoloji ile ustaca birleştirdi. Aqua MODIS uydu görüntüleri ile Muara Kintap sularının ayrıntılı durumunu izlendi ve 28,4 °C ile 29,7 °C arasında deniz yüzey sıcaklığı (SST) ve 0,38 ila 6,27 mg/m³ arasında değişen klorofil-a (Chl-a) konsantrasyonları tespit edildi. Sonuçlar, Chl-a dağılımının SST'ye göre balık avının daha etkili bir öngörücüsü olduğunu güçlü bir şekilde göstermektedir. Bu bulgu, deniz parametreleri ile balık dağılımı arasındaki karmaşık ilişkiyi vurgulamaktadır. Chl-a verileriyle bilgilendirilen FAFM sağlanmasıyla, balıkçılar veri odaklı kararlar alabildi, avları optimize edebildi ve geçim kaynaklarının uzun vadeli sürdürülebilirliğini destekleyebildi. Çalışmanın etkisi veri analizinin ötesine geçerek, Muara Kintap ve ötesinde sürdürülebilir balıkçılık uygulamalarını teşvik etmede araştırmacılar, balıkçılar ve politikacılar arasındaki iş birliğinin ve bilgi paylaşımının önemini vurgulamaktadır.

Anahtar kelimeler: FAFM, balıkçılık verimliliği, Muara Kintap, oseanografik veri, sürdürülebilirlik

INTRODUCTION

The global fishing industry is crucial for supporting fish processing and meeting the demands of both local and international markets. While commercial fisheries face challenges related to resource competition, small-scale fisheries grapple with the impacts of climate change (Heck et al., 2023). Various aid programs have been implemented to assist the small-scale fishers, including subsidized fuel (Shafari et al., 2019), fishing insurance (Syarif et al., 2019), and other technical assistance initiatives (Rusdiana et al., 2022). The success of fishing operations depends not only on the availability of fishing fleets, gear, and techniques, but also on the effective utilization of information and communication technologies (ICT) to locate and map potential fishing grounds (Natsir et al., 2020). The acceptance of new technologies by fishers is shown to be influenced by their socio-economic background (Rafi et al., 2020).

The capture fisheries sector is driven by digital innovation and is under Geographic Information System (GIS) software has become increasingly prevalent among capture fisheries, leveraging remote sensing applications to enhance operational efficiency and productivity (Sasmito et al., 2022). For instance, ArcGIS harnesses satellite data to generate fishing maps and disseminates valuable information directly to fishers and their vessels, exemplified by the Fishing Points app, which is accessible on Android smartphones. This app, available for free with basic features or through a premium subscription offering advanced functionality, enables users to locate fishing spots at sea using GPS without requiring an internet connection. It provides real-time data on maps, locations, catch records, fish activity forecasts, wave conditions, and weather updates, as well as significantly reduced fuel consumption and operational costs (Sukresno and Kusuma, 2021). QGPS, an open-source Quantum Geographic Information System, shares similar purposes and functionalities used for spatial analysis, visualization, and mapping for sustainable fisheries management (Yen and Chen, 2021).

At national level, fishing area forecast map (FAFM) served as a valuable tool, providing insight for fishers to predict potential fishing zones. However, FAFM requires a specific map-reading skill and the lack of high-resolution data that is critical to the success of local fishers highlights the need for user-friendly spatial data tools. Briefly, the preparation process for FAFM can be visualized in Figure 1.

FAFM is developed using satellite data on sea surface temperature and chlorophyll-a concentration, key indicators for identifying fish aggregation locations. These data are processed through an algorithm considering wave height and wind speed to predict potential fishing zones. Subsequently, FAFM information is disseminated to fishers, stakeholders, and policymakers via both print and electronic medias. Any feedback from users to enhance the quality of FAFM content is welcome. User feedback can offer valuable insights into the usability, accuracy, and relevance of the FAFM data. Suggestions for additional data sources, improved visualization techniques, or enhanced accessibility features can contribute to a more comprehensive and user-oriented FAFM experience. Moreover, feedback on FAFM's accuracy in predicting potential fishing zones is essential for refining forecasting algorithms and improving the tool's effectiveness.

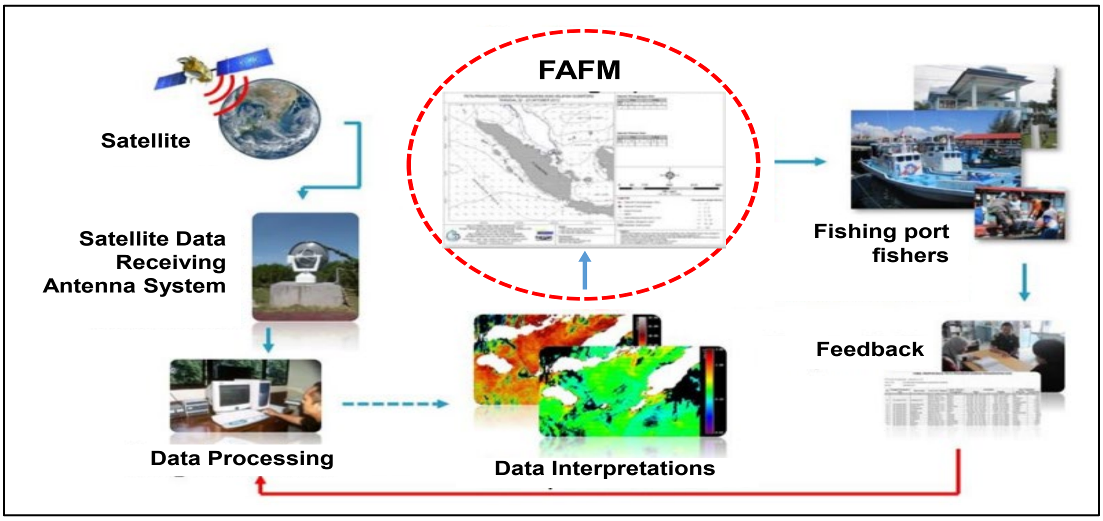

Figure 1. The preparation process for the national FAFM (Sukresno and Kusuma, 2021)

As other artisanal fishing communities (Rafi et al. 2020; Rusdiana et al. 2022), fishers in Muara Kintap Village also face crucial challenges in digital information technology, in addition to limited access to resources, skill gaps, affordability concerns, lack of trust in new technologies, inadequate infrastructure, and connectivity issues, which impact their ability to capitalize on regional economic potential. Limited access to oceanographic data and information hinders many fishers from understanding and sustainably managing their fishing grounds. Our field study empowered fishers to bridge this knowledge gap. By equipping the participants with GPS and user-friendly software (e.g., SeaDas, and ArcGIS/ArcMaps), we transformed them from passive observers into active participants. This newfound ability to analyze real-time data allows researchers to create personalized maps, promoting sustainable management of fishing grounds.

MATERIALS AND METHODS

This research was conducted in Muara Kintap Village, Tanah Laut Regency, Indonesia (Figure 2). The village borders Mulia Village to the north, the Java Sea to the south, Kebun Raya and Sungai Cuka Villages to the east, and Pandansari and Kintap Villages to the west. With a total area of approximately 4,900 m^2, Muara Kintap boasts a coastal location that naturally attracts a large fishing community. Approximately 80% of Kintap sub-district's population relies on fishing for their livelihood. Although the village residents are not indigenous Banjar people of Borneo, most hail from the Bugis tribe. Despite their diverse origins, the community has a strong sense of cultural integration.

By virtue of its direct access to both the Java Sea (WPP-712) and the Makassar Strait (WPP-713), Muara Kintap Village boasts strategically located waters, making it a prime fishing area. This unique position allows fish populations from both sea regions to flourish in the surrounding waters. From November to April, westerly winds usher in a prime fishing season, while July to August, marked by south-easterly winds, brings a bountiful harvest of shrimp seeds, a valuable source of income for the local community. However, September and October represent a transitional period with rough seas and high waves, making fishing less ideal.

There were two types of data collected in this study, i.e. primary and secondary data. Primary data were collected directly from the surveyed within KUB Dermaga Bersama

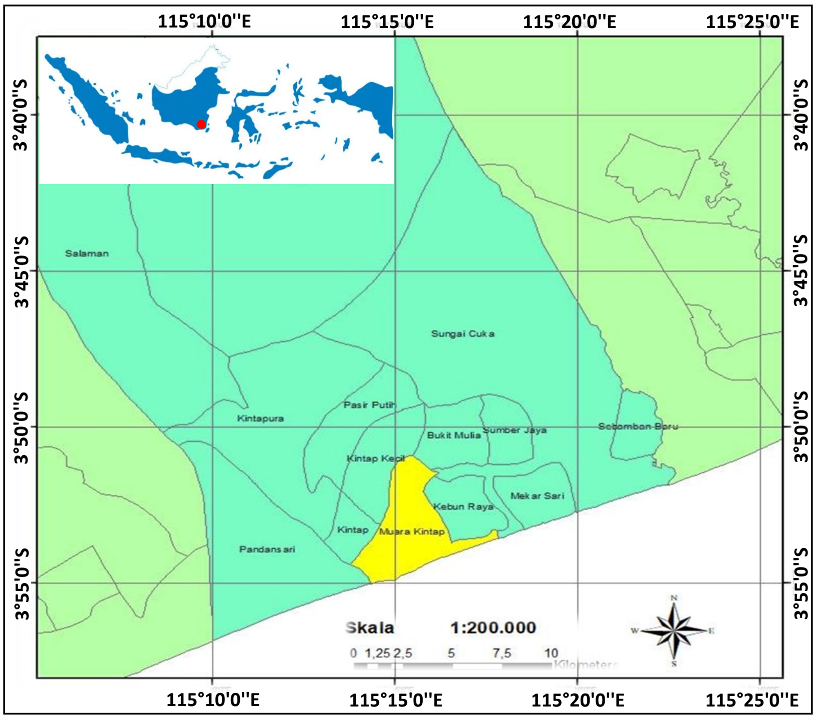

Figure 2. A map showing the study site in Muara Kintap Village, Indonesia

through questionnaires, interviews, and on-board observations (vessel dimension, fishing gear, season, main catch, coordinates of fishing areas), and also documentation (photos and videos). Secondary data were obtained from literature reviews and relevant government agencies (e.g., oceanographic data). Notably, KUB is officially registered with the Food Security and Fisheries Services of Tanah Laut District, and its members hold fishers' insurance cards. KUB was established on April, 3, 2020 with 10-member fishing group. A research vessel, KM. FATIH (15 GT), served as the platform for on-board data collection (Figure 3).

Figure 3. Mini purse seiner 'KM. FATIH' used for data collection

This fishing vessel, constructed from a blend of teak and ironwood, boasts impressive dimensions: 15.85 m long, 3.10 m wide, and 1.42 m depth. Powering the FATIH is a reliable Dongfeng main engine, ensuring efficient navigation throughout the research area. For seamless communication and precise positioning, the FATIH is well-equipped with a radio, compass, and GPS. Fishers usually use mini purse seine and drift gillnets to catch fish such as yellow mackerel (*Selaroides leptolepis*), and little tuna (*Euthynnus affinis*), Hairtail (*Trichiurus lepturus*). Unfortunately, KM. FATIH did not provide monthly catch data for this study. According to fisher's accounts, the fishing season for yellow mackerel peaked in March-April, while the season for little tuna occurs in September-October. Hairtail is considered incidental catch throughout the year.

Using the Fishing Points app, fishers can mark their own waypoints (coordinate points) with their Android phone's GPS to capture their fishing spot's location. Here is how to do this (Figure 4):

1. Download and Launch: Open the Fishing Points app and ensure it has permission to access the phone's location. The Fishing Points app collects data by capturing coordinates, time, and date of each location. These data are essential for subsequent analysis and mapping, and even optimize fishing strategy.

2. Dive into the Menu: Explore the app's menu and navigate to the "Add Location" function to mark the chosen fishing spot.

3. Capture the Coordinates: Now, pinpoint the perfect location. Navigate the map and tap the magnified image to capture the specific coordinates.

4. Share and convert: After confirming the coordinates, tap the "Share" button to export them as a GPX file, compatible, with most mapping software.

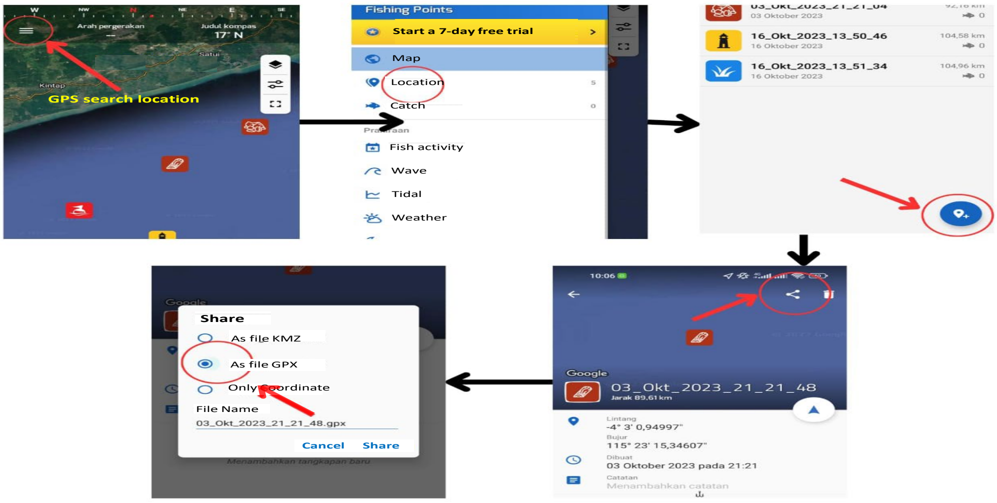

Figure 4. How to use Fishing Point on an Android phone (self-documentation)

GIS is a computer-based system used to collect, store, combine, organize, transform, manipulate, and analyze geographic data. It plays a vital role in environmentally sound resource management by providing insights for informed decision-making. Thematic maps visually represent specific themes or topics with a geographic component, and standardization ensures consistency and clarity in their creation (Darmawan, 2011).

The data requires a specialized software called SeaDAS (SeaWiFS Data Analysis System), established by NASA's ESDS Program (https://www.earthdata.nasa.gov/). The tool is made freely available through open-source software licensing. This program processes satellite imagery to extract valuable information for oceanographers, including sea surface temperature (SST) and chlorophyll-a (Chl-a) concentration. To extract valuable data from satellite imagery, we present a breakdown of the SeaDAS software workflow:

a. Data acquisition

This stage involved collecting Aqua MODIS (Moderate Resolution Imaging Spectroradiometer) satellite imagery from September 15 to October 15, 2023. The specific images chosen corresponded to the month of data collection and focused on capturing SST and Chl-a concentration.

b. Image cropping

To facilitate focused, detailed, and optimized data processing, the acquired Aqua MODIS images were cropped using SeaDAS software. This cropping process narrowed the image area to a specific research location within the Kintap District Fishing Ground.

c. SST and Chl-a data processing

The following image cropping, the relevant sea surface temperature data were extracted from the Aqua MODIS images. This involved processing the cropped data in ArcGIS to convert it from a raster format (gridded data) to vector format (points, lines, or polygons), or the data is exported to Mask Pixels. This conversion allows for easier manipulation and analysis of specific SST and Chl-a metadata within the research area.

ArcGIS, another powerful tool from ESRI (Environmental Systems Research Institute), complements the workflow. This comprehensive GIS software suite integrates various functionalities, allowing users to analyze, visualize, and manage spatial data.

a. Data integration and projection

This stage prepares all spatial data for analysis, which involves:

- *Georeferencing*: All data, including line maps (administrative boundaries) and fish catch coordinates from fishers, must have accurate geographic reference points.
- *Transformation*: All data are converted to a common coordinate system (datum) and projected for consistent spatial representation. This allows for a seamless overlay of different data layers.

b. Fishing ground analysis

The analysis phase uses the prepared data sets as follows:

- *Fish catch overlays*: The fish catch coordinates, obtained, from the "Fishing Points" application, were overlaid on the 1-month sea surface temperature map generated in the previous stage.
- *Field accuracy test*: The fish catch coordinates served as reference points for field studies, allowing researchers to verify the accuracy of the September-October 2023 Sea surface temperature data.
- *Spatial analysis boundary*: The coordinates also define the outer boundary of the Kintap sub-district fishing grounds. This boundary acts as a limit for spatial analysis,

helping to determine and measure the potential fish catch zone within these waters.

Based on the SST and Chl-a concentrations, the suitability for fishing areas can be categorized. Waters with SSTs between 24 ^{0}C and 27 ^{0}C or Chl-a concentrations exceeding 0.2 mg/m^{3} are considered most suitable (potential). Areas with SSTs between 27 ^{0}C and 30 ^{0}C or Chl-a levels between 0.1 and 0.2 mg/m^{3} were categorized as moderately suitable (moderate potential). Conversely, conditions outside these ranges (SST below 24 ^{0}C or above 30 ^{0}C; Chl-a less than 0.1 mg/m^{3}) were considered less suitable (less potential), as described in Table 1.

Table 1. Assessment of potential fishing areas (PFAs) based on SST and Chl-a indicators

No	PFA category	[1] SST (^{0}C)	[2] Chl-a (mg/m^{3})
1	Potential	24-27	>0.2
2	Moderate potential	27-30	0.1-0.2
3	Less potential	<24 or >30	<0.1

Source: [1]Laevastu and Hela (1993), [2]Gower (1972)

RESULTS

GPS application

Muara Kintap fishers have successfully built their own personalized fishing resources and experiences using the Fishing Points app, which allows them to mark waypoints (coordinate points) using their Android phone's GPS. As shown in Figure 5, the integration of digital technologies in fisheries, particularly the Fishing Points app, is central to the workflow of small-scale fishers. This integration demonstrates how app data can enhance traditional fishing practices, potentially optimizing strategies and offering tangible benefits to fishers. This visualization most likely serves to illustrate the practical impact and potential advantages of incorporating digital tools within small-scale fishing operations. The Fishing Points app can track fishing locations and behavior, providing valuable insights into fish populations and migration patterns. By understanding these dynamics, fishers can adjust their practices to reduce bycatch, avoid overfishing certain species, and operate within sustainable limits.

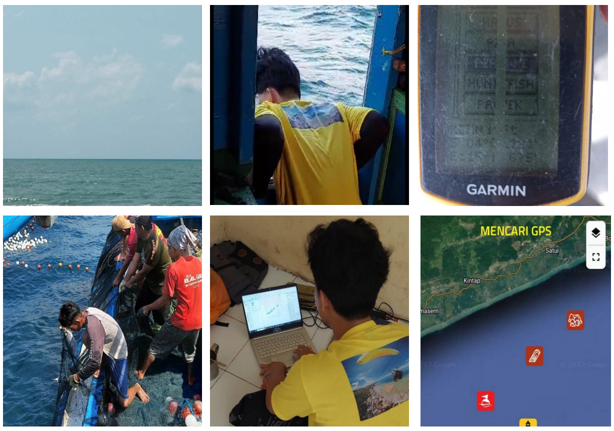

Figure 5. How digital technology is integrated into traditional fishing practices (self-documentation)

In contrast, the fishing area forecast map (FAFM) generated by the mini purse seiner KM. FATIH pinpointed five promising fishing locations within Muara Kintap's waters (Table 2). These measured fishing areas were relatively close together, with distances between the coordinate points ranging from 0.8 mile to 1 mile. The radius of the predicted fishing areas varied between 2.0 miles and 7.7 miles, consistent with the spatial dimensions of traditional fishing grounds. Meanwhile, fishing bases were located farther away, approximately 12-20 miles from these areas. The waters depth varied between 15 m and 20 m. Practically, FAFM stands as a crucial tool for fishers, providing precise guidance, improved efficiency, and long-term environmental benefits, enabling them to refine their fishing strategies, recognize patterns, and make well-founded decisions based on solid data rather than instinct alone.

Table 2. On-site coordinates of the predicted fishing areas in Muara Kintap waters

On-site Coordinates	Actual measurements of fishing areas	SST (^{0}C)	Chl-a (mg/m^{3})	Radius of fishing areas (mile) ^	Water depth (m)
A	3^{0}57'32.48"S - 115^{0}30'16.78"E	29.4	1.9	4.3	15
B	4^{0}03'00.95"S - 115^{0}23'15.34"E	29.3	2.2	7.4	18
C	4^{0}19'30.31"S - 115^{0}12'13.50"E	28.9	1.8	7.7	18
D	4^{0}14'04.70"S - 115^{0}22'08.46"E	28.8	0.6	2.4	20
E	4^{0}09'53.34"S - 115^{0}14'51.15"E	28.7	0.5	2.0	20

^ aligning with the radius of commonly fished areas by fishers.

SeaDas and ArcGIS applications

The foundation for our predicted fishing area maps was the analysis of monthly SST and Chl-a concentration data for Tanah Laut Regency. We used Aqua MODIS Level 3 satellite images, which are readily accessible through the NASA's OceanColor web (https://oceancolor.gsfc.nasa.gov/). The SeaDAS (Sea-viewing Wide Field-of-view Data Analysis System) software emerges as a critical asset in this mapping process. Its functionalities significantly enhance our workflow:

- *Efficient data collection*: SeaDAS streamlines the acquisition of time specific SST and Chl-a data, ensuring that we use the most up-to-date information.
- *Precise image cropping*: This software allows us to precisely crop satellite images, focusing solely on the relevant areas of Tanah Laut Regency.
- *Data processing versatility*: SeaDAS offers robust tools for processing both SST and Chl-a vector data, enabling effective analysis and integration into our mapping process.

This research also leverages ArcGIS, a comprehensive GIS software developed by ESRI (Environmental Systems

Research Institute). The proposed goes beyond being a single program; it acts as a platform that integrates functionalities from various specialized GIS software tools. For fishers, ArcGIS can revolutionize their understanding of fishing grounds. The software's ability to generate maps based on spatial information provides valuable insights into a seasonal, monthly, and even daily basis. This enables fishers to make data-driven decisions about where and when to deploy their efforts.

ArcGIS played a central role in preparing potential FAFMs. This involved incorporating data obtained from SeaDAS software, likely including processed SST and Chl-a data, for spatial analysis within ArcGIS. This analysis contributed to the creation of informative and actionable FAFM. The development of the FAFM relies on a two-pronged approach:

- *Satellite imagery analysis*: Aqua/Terra MODIS satellite data plays a crucial role. By analyzing the SST and Chl-a derived from these images, we can identify promising fishing areas. The Single Image Edge Detection (SIED) method is particularly effective in pinpointing thermal fronts, which often mark the convergence of water masses with differing temperatures. These thermal zones are often associated with intensified currents and fluctuations in sea level within the surrounding waters.

- *Oceanographic data integration*: National and port-specific FAFMs were further refined by incorporating additional oceanographic factors. Satellite-derived data provided insights into parameters like SST, Chl-a concentration, and sea level anomalies. SST data are especially valuable for analyzing thermal fronts, as these zones often indicate cooler waters rich in nutrients, a prime indicator of high fish productivity.

SST and Chl-a distributions

Our analysis of Aqua MODIS satellite images provided informative fishing area forecast maps depicting the distribution of SST and Chl-a concentration in Muara Kintap's waters. The SST across the study area spanned 28.4 °C to 29.7 °C (Figure 6), while the SSTs measured within the five fishing areas ranged from 28.7 °C to 29.4°C (Table 2), and were categorized as moderately suitable (moderate potential). This slight variation can be attributed to the use of different images within the analysis timeframe.

As shown in Figure 7, the predicted Chl-a concentrations across the study area ranged from 0.38 mg/m³ to 6.27 mg/m³, with Chl-a levels within the identified five fishing areas varying from 0.5 mg/m³ to 2.2 mg/m³, as outlined in Table 2. High Chl-a concentration in Muara Kintap waters indicates a productive marine environment. The abundance of phytoplankton in the area plays a crucial role as a primary food source for zooplankton, which further sustains populations of pelagic fish. This interdependent ecosystem highlights the high potential for sustainable fisheries in the region, presenting promising opportunities for long-term productivity and prosperity for Muara Kintap's fishing communities.

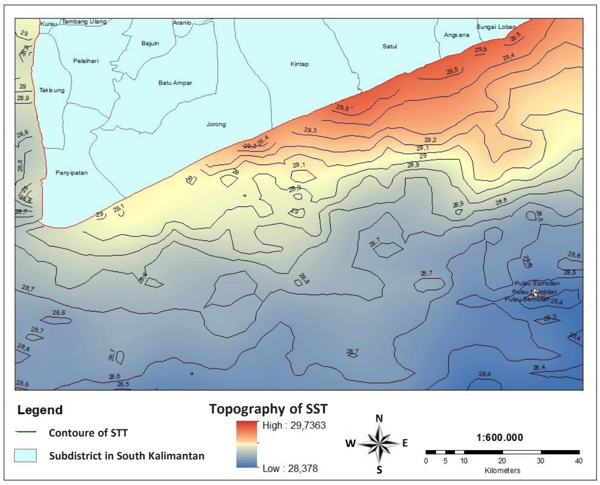

Figure 6. Sea surface temperature distribution in Muara Kintap waters

Emphirical studies confirmed that sustainable fisheries are essential for small-scale fishers, providing livelihoods, boosting local economies, and ensuring food security for communities, even potentially contributing to national food supplies that was fully underpinned by information technology, government policy, and additional technical support (Stacey et al., 2021; Simmance et al., 2022). However, small-scale fishers confront a multitude of challenges, including limited access to essential resources, infrastructure, and technology. They are also exposed to risks associated with climate change and overfishing (Ferrer et al., 2022; Heck et al., 2023). Additionally, small-scale fishers often experience a lack of social and economic protection (Giron-Nava et al., 2021; Virdin et al., 2023).

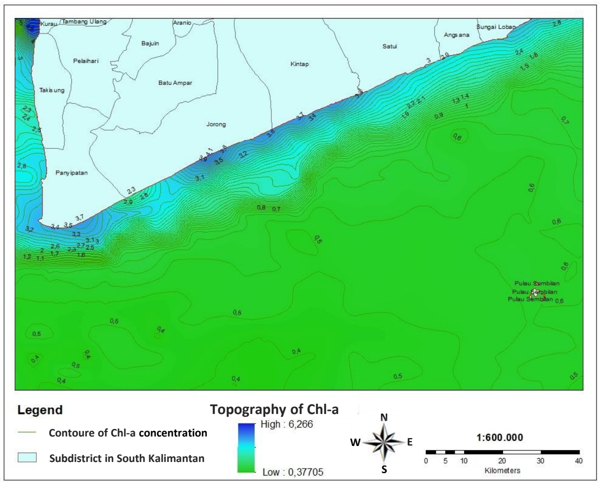

Figure 7. Chlorophyll-a distribution in Muara Kintap waters

Mapping of predicted fishing areas

Aqua MODIS satellite imagery was used to generate fishing area forecast maps (FAFM) for the waters surrounding Muara Kintap (Figure 8). These FAFMs were meticulously constructed by analyzing two critical parameters: SST and Chl-a concentration. The shift in SST patterns in the study area is largely driven by global climate change, while Chl-a concentration remains relatively stable due to a consistent flow of nutrients across the study area. Chl-a content may hold a greater influence on fish catch than SST and, presents an opportunity to refine FAFMs for enhanced effectiveness.

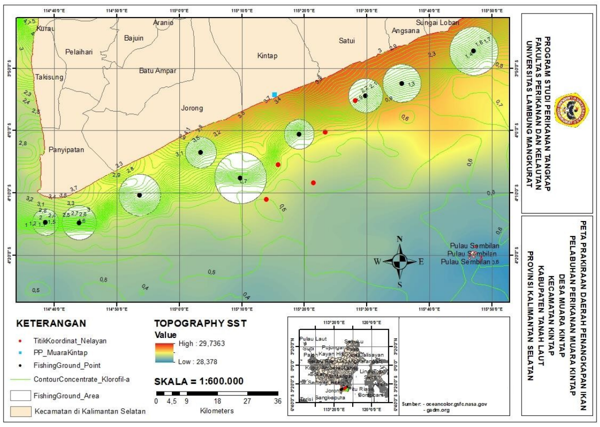

Figure 8. FAFM for small pelagic fish in Muara Kintap waters

DISCUSSION

Muara Kintap's fishers have adopted FAFMs, a transformative digital tool that empowers them with data-driven insights, which promise a brighter future for fisheries. However, despite the potential benefits, local fishers in Muara Kinta Village still contend with some obstacles that hinder their ability to fully embrace these new technologies, including:

1. Access and Skills

- *Technology access*: Not all fishers have access to mobile devices or an adequate internet connection to use fishing apps.
- *Digital skills*: A lack of digital literacy and training for using new technologies can hinder the adoption of apps and digital platforms by fishers.

2. Costs

- *Subscription fees*: Subscription fees for premium apps or certain digital platforms can be a burden on local fishers.
- *Technology costs*: The upfront cost of purchasing mobile devices and internet data plans can also be a barrier for fishers.

3. Trust and Adoption

- *Doubt in information*: Some fishers are still skeptical of the information and recommendations provided by digital apps, preferring to rely on their knowledge and experience.
- *Lack of education*: Lack of education and awareness of the benefits of digital technology for fishers may hinder wider adoption.

4. Infrastructure and Connectivity

- *Internet network*: In some coastal areas, internet connectivity may be limited or unstable, making it difficult to use application and digital platforms.
- *Lack of infrastructure*: A lack of supporting infrastructure, such as mobile device charging stations in ports and fishing villages, can hinder the use of digital technology.

5. Environmental Impact

- *Overfishing concerns*: Some parties worry that using fish finder apps can make it easier to overfish, leading to damage to marine ecosystems.
- *Reliance on technology*: Overreliance on digital technology to find fish is feared to cause fishers to lose their knowledge and local wisdom in sustainable fishing practices.

Bridging the gap in the fishing industry requires a collaborative effort from various parties, such as governments, NGOs, technology companies, and fishing communities to work together to create a more sustainable and prosperous future for fisheries. Alternative solutions to the problem can be considered as follows:

a. Increase access and education: Provide training programs and affordable internet access for fishers to improve digital literacy and encourage technology adoption.

b. Develop fisherman-friendly apps: Design easy-to-use apps and digital platforms that consider the needs and limitations of fishers.

c. Build trust: Provide accurate and transparent information about the benefits of digital technology and involving fishers in the technology development process.

d. Improve infrastructure: Build supporting infrastructure such as internet networks and charging stations in coastal areas.

e. Promote sustainable fishing: Encourage the use of digital technology to support sustainable and responsible fishing practices.

One of the key datasets employed in this research was Aqua MODIS imagery, which provided valuable insights into both the SST and Chl-a distributions. The Muara Kintap coastline has warm waters, ranging from 28.4 °C to 29.7 °C, and high Chl-a concentrations between 0.38 and 6.27 mg/m³. These characteristics, particularly high Chl-a content, serve as valuable indicators of productive fishing grounds for local fishers. Sasmito et al. (2022) pointed out that over 60% of fish caught in the Java Sea came from areas with Chl-a concentrations of 0.2-0.5 mg/m³ and SST of 28-31 °C. These studies reinforced the importance of mapping coastal suitability based on biophysical parameters like SST and Chl-a content. Nugraha et al. (2019) found a strong positive correlation between these parameters and catch per unit effort (CPUE) of the Spanish mackerel (*Scomberomorus commerson*) caught in the coastal region Kejawanan Cirebon, West Java. Time series data like this, encompassing SST and Chl-a variations over time, can be instrumental in developing descriptive models to assess long-term potential fishing area (Clinton et al., 2022).

The Chl-a concentration in Muara Kintap's waters exceeded the 0.1-1.9 mg/m³ threshold established by Clinton et al. (2022). It excellently highlights the richness of biological activity in the area. This is due to geophysical processes that play a significant role in regulating nutrient flow from land through rivers. Although these processes can cause fluctuations in specific nutrient levels, the overall Chl-a concentration in the Muara Kintap remained relatively stable. The distribution of Chl-a, a key indicator of marine productivity, exhibits a distinct pattern within the bay. The concentrations are highest in waters closest to land, such as rivers, river mouths, and bay margins. As we moved toward the center of the bay and then outwards, Chl-a levels gradually decreased (Marlian et al., 2015). This trend mirrors the distribution of nitrate and phosphate, essential nutrients for phytoplankton growth. Ayuningsih et al. (2014) demonstrated a strong positive correlation between Chl-a levels and these nutrients, with higher concentrations found in the estuaries and progressively lower values toward the open sea. These rich nearshore waters benefit not only from riverine nutrient runoff but also from the presence of mangrove vegetation. As mangrove leaves decompose and fall into the water, they release vital nutrients, further enriching the coastal environment and promoting phytoplankton growth (Hidayah et al., 2016).

The compelling evidence from this study strongly indicates that the distribution of Chl-a is a more influential predictor of fish catch than SST, highlighting the ecological importance of Chl-a in relation to fish populations. This finding underscores the importance of prioritizing Chl-a data in future FAFMs to enhance the effectiveness of fishing practices. The positive impact of Chl-a on fish catches can be attributed to a phenomenon known as upwelling (Narvekar et al., 2021). Upwelling occurs when wind and water currents cause the mixing of deep, nutrient-rich cold-water masses with the surface layer. This process increases Chl-a concentration, which is often accompanied by a decrease in SST due to the influx of cooler water from below. Although strong winds can contribute to a decrease in the SST (the uppermost warm layer), the overall impact on fish seems less pronounced than the surge in Chl-a. By analyzing the SST and Chl-a distributions, researchers can pinpoint areas where upwelling might occur, providing valuable insights into targeted fishing practices.

The FAFM, utilizing in-situ SST data, identified a temperature range of 28.9°C to 29.5°C along the Muara Kintap coastal waters. The analysis indicates that the southern region has slightly higher temperatures than the southwest. The SST significantly influences the schooling behavior and migration pattern of small pelagic fish, which prefer warmer waters between 29 °C and 33 °C (Safruddin et al., 2014). These fish inhabit the surface layer of the water column and often reside in relatively shallow areas like estuaries (Safruddin, 2013). The SST values fell within the empirical range (25-31°C) considered optimal for high pelagic fish presence (Clinton et al., 2022). Sobatnu and Irawan (2022) also conducted similar research using Aqua MODIS image processing to generate fishing zone maps.

The importance of comprehensive and up-to-date data for informed decision-making on fisheries management is also patamount. Here are some suggestions to be built upon in future studies:

1. Detailed data collection: This step expands data collection efforts to capture more granular details. This involves the following step.

- Employing standardized data collection protocols across the entire research area.

- Recording a wider range of parameters than those currently measured (e.g., fish species composition, catch sizes, gear types used).

- Finer spatial and temporal resolutions are implemented for data collection to account for potential variations within the Muara Kintap waters.

2. Direct observation and source diversification: Satellite data are supplemented with on-the-ground observations and a broader range of data sources:

- Regular field surveys were conducted to validate satellite data and gather real-time information on fish behavior and distribution.

- Collaborate with local fishers to leverage their practical knowledge and experience of the fishing grounds.

- Explore the potential of citizen science initiatives to engage the community in data collection.

3. Data management and sharing establish robust data management practices:

- Implement a centralized data repository to realize efficient storage, organization, and accessibility of collected information.

- Develop data sharing protocols to facilitate collaboration between researchers, fisheries management agencies, and the fishing community.

CONCLUSION

The implementation of digital technology such as the Fishing Area Forecast Map (FAFM) has enhanced the ability of Muara Kintap's fishers to identify productive fishing grounds, leading to more targeted and efficient fishing practices that promote sustainable fisheries and improve the fisher's livelihoods. The results strongly indicate that the distribution of Chl-a is a more influential predictor of fish catch than SST.

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AUTHORSHIP CONTRIBUTION

All authors contributed equally, ensuring the accuracy, authenticity, and ethical integrity of the research.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ETHICS APPROVAL

Ethics Committee approval certificate was not required for this study.

DATA AVAILABILITY

All relevant data are in the article.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Computational analysis of superoxide dismutase genes (*sod1, sod2*, and *sod3*) and comprehensive tissue-specific gene expression profiling in Tetraodon (*Tetraodon nigroviridis*)

Tetraodon (*Tetraodon nigroviridis*) süperoksit dismutaz genlerinin (*sod1, sod2* ve *sod3*) in siliko analizi ve dokuya özgü gen ekspresyon profili

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Abstract: The objective of this investigation was to conduct in silico analyses on superoxide dismutase (*sod1, sod2,* and *sod3*) genes in tetraodon (*Tetraodon nigroviridis)*, employing bioinformatics tools, and to assess the gene expressions in various tissues such as the intestine, brain, kidney, liver, muscle, heart, eye, spleen, gills, stomach, ovary, and testis of tetraodon. To achieve this, tissue samples were obtained from both male and female tetraodon, spanning the aforementioned organs, with the purpose of acquiring cDNA. Total RNA was isolated from each tissue, and subsequently, the transcripts of *sod*s genes were assessed using qPCR, while transcript quantities were determined through RT-qPCR. The in silico analyses encompassed the examination of gene structure, conserved gene synteny, phylogenetic tree analyses, and the identification of similarity-identity ratios with other vertebrates. When examining the transcriptional differences between male and female tissues for the Tetraodon *sod1* gene, it was noted that, except for the heart tissue, all other tissues studied (including the liver, intestine, muscle, brain, eyes, spleen, gills, kidney, stomach, and gonads) exhibited significantly higher expression levels in male fish. Examining the results for the *sod2* gene in male and female tetraodon, significant upregulation was observed in the liver, muscle, gills, intestine, ovary, and testis, with no statistical significance in tissues like the intestine, heart, and gonads. Regarding the *sod3* gene in male and female tetraodon, heart, spleen, and stomach tissues did not show statistical significance, but the liver, intestine, gills, kidney, stomach, and gonads exhibited significantly higher expression in male fish ($p<0.05$).

Keywords: Tetraodon, in silico analyses, *sod*s genes, gene expression

Öz: Bu araştırmanın amacı, tetraodon (*Tetraodon nigroviridis*) süperoksit dismutaz (*sod1, sod2* ve *sod3*) genleri üzerinde in siliko analizler yapmak, biyoenformatik araçlar kullanarak bu genlerin çeşitli dokulardaki (bağırsak, beyin, böbrek, karaciğer, kas, kalp, göz, dalak, solungaçlar, mide, yumurtalık ve testis) gen ekspresyonlarını değerlendirmektir. Bu hedefe ulaşmak için, yukarıda belirtilen organlardan cDNA elde etmek amacıyla erkek ve dişi balıklardan doku örnekleri alındı. Her dokudan toplam RNA izole edildi ve ardından *sods* genlerinin transkriptleri qPCR kullanılarak değerlendirildi. Transkript miktarlarının belirlenmesi amacıya ise RT-qPCR yapıdı. İn siliko analizler, gen yapısının incelenmesini, korunmuş gen sentenisi analizlerini, filogenetik ağaç analizlerini ve diğer omurgalılarla benzerlik-özdeşlik oranlarının tespitini kapsamaktadır. Tetraodon *sod1* geninin erkek ve dişi dokularındaki transkripsiyon farklılıkları incelendiğinde, kalp dokusu dışında çalışılan tüm diğer dokularda (karaciğer, bağırsak, kas, beyin, gözler, dalak, solungaçlar, böbrek, mide ve gonadlar dahil) erkek balıklarda anlamlı derecede daha yüksek ekspresyon seviyeleri gözlendi. Erkek ve dişi tetraodonlarda *sod2* geninin sonuçları incelendiğinde, karaciğer, kas, solungaçlar, bağırsak, yumurtalık ve testiste anlamlı bir yukarı düzenleme gözlendi; bağırsak, kalp ve gonadlar gibi dokularda ise istatistiksel olarak anlamlı bir fark görülmedi. Erkek ve dişi tetraodonlarda *sod3* geni ile ilgili olarak, kalp, dalak ve mide dokuları istatistiksel olarak anlamlılık göstermedi, ancak karaciğer, bağırsak, solungaçlar, böbrek, mide ve gonadlar erkek balıklarda anlamlı derecede daha yüksek ekspresyon sergiledi ($p<0.05$).

Anahtar kelimeler: Tetraodon, in silico analizler, *sod*s genleri, gen ekspresyonu

INTRODUCTION

Superoxide dismutase genes (*sod*s) are essential for antioxidant defense mechanisms, as they catalyze the dismutation of superoxide radicals into less hazardous molecular oxygen and hydrogen peroxide (Chatzidimitriou et al., 2020). These genes are particularly important for teleost fishes, especially Tetraodon , due to their ability to withstand high levels of oxidative stress (Kim et al., 2021). SODs can be classified into three distinct groups based on their redox-active metals: copper/zinc SOD, manganese SOD, and iron SOD (Chen et al., 2022). Superoxide dismutases (SODs; EC 1.15.1.1), considered the first line of defense, are a family of redox-active metalloenzymes that catalyze the conversion of superoxide radicals into molecular oxygen and hydrogen peroxide. It has been demonstrated through homology and phylogenetic data that different SOD isoforms have diverse evolutionary histories within the animal kingdom (Sheng et al., 2014). Superoxide radicals, which are normal byproducts of metabolic oxidation, can cause extensive cellular damage if not neutralized. Both extracellular (secreted) superoxide dismutase (*sod3*) and intracellular superoxide dismutase (*sod1* in the nucleus and cytoplasm, and *sod2* in the mitochondria) play important roles in neutralizing superoxide radicals (Fujii et al., 2022). Scientific names play a crucial role in biological informatics, providing precision in labeling biodiversity information. However, their utility is limited by semantic ambiguity and syntactic changes that don't always reflect taxonomic modifications (Remsen, 2016). This is evident in the genus Tetraodon, a group of pufferfish species used in bioinformatics research. The Tetraodon nigroviridis genome, characterized by its compact size and reduced intergenic and intronic sequences, has been analyzed for its repeat content and organization (Roest Crollius et al., 2000). Pufferfish genomes, including Tetraodon, are valuable for comparative genomics due to their small size yet complex structure, with preserved gene structures despite reduced intron sizes (Koop and Nadeau, 1996). Tetraodon (*Tetraodon nigroviridis*) is a remarkable species due to its unique biological characteristics, particularly its ability to withstand high levels of oxidative stress (Wang et al., 2016). Investigating the genetic basis of antioxidant defense in Tetraodon can offer valuable insights into their adaptation mechanisms and contribute to a better understanding of the evolution of *sod* genes in aquatic vertebrates (Stump et al., 2018; Ahn et al., 2018). *sod* genes play a crucial role in teleost fish adaptation mechanisms by helping maintain redox balance within cells, ensuring that oxidative stress does not lead to cellular damage in response to environmental perturbations or pathogenic infections (Kim et al., 2021). Research on the river pufferfish, *Takifugu obscurus*, has identified a robust antioxidant system in its liver, with genes such as catalase, glutathione reductase, and superoxide dismutase being significantly induced in response to cadmium exposure (Kim et al., 2010). Similarly, the Japanese pufferfish, *Takifugu rubripes*, has been found to possess functional NADPH oxidase components, which play a crucial role in host defenses against microbial infection (Inoue et al., 2004). The compact genome of the Japanese pufferfish has also facilitated the isolation and characterization of serine/threonine phosphatase genes (Koh et al., 1997). Furthermore, the identification of novel genes related to tetrodotoxin intoxication in pufferfish, such as fibrinogen-like proteins, suggests a potential role in detoxification processes (Lee et al., 2007). The green pufferfish, *Tetraodon nigroviridis*, is an important genetic model organism, and various studies have been conducted on its molecular characteristics, gene expression, and development (Rothenburg et al., 2008; Watson et al., 2009; Bayır and Arslan, 2020; Bayır, 2020).

In this study, we aimed to gain valuable insights into the adaptation mechanisms and evolution of sod genes in Tetraodon, by understanding the genetic basis of antioxidant defense. To achieve this goal, we performed an in silico analysis of the *sod1, sod2*, and *sod3* genes in Tetraodon, examining their gene expressions across various tissues. Through the analysis of genomic data, our objective was to reveal the structural and functional aspects of the *sod/SOD* genes and their crucial role in maintaining redox balance in this ecologically significant species.

MATERIALS AND METHODS

Fish sampling and experimental designs

The material for the study consists of 3 female and 3 male Tetraodon (*Tetraodon nigroviridis*) obtained from the Faculty of Fisheries at Atatürk University. The molecular analyses were conducted at the Agricultural Biotechnology Laboratory. Tissue samples, including intestines, brain, kidneys, muscles, liver, heart, eyes, spleen, gills, stomach, and gonads, were collected from all the fish and preserved in 1 ml RNA later in 2 ml Eppendorf tubes at +4°C for 24 hours and then at -80°C until the day of analysis. Before sampling, the Tetraodon were anesthetized with clove oil. The entire study was conducted in accordance with the Local Ethics Committee for Animal Experiments at Atatürk University.

RNA isolation and cDNA synthesis and real-time PCR (qPCR) analysis

Tissue samples were initially extracted from RNAlater and then placed into nuclease-free tubes with 1 ml of trizol reagent (Life Technologies) for homogenization. The Trizol protocol was employed to isolate RNA. Subsequently, RNA concentrations were assessed using a Nanodrop 8000 spectrophotometer, and the quality of total RNA was determined through agarose gel-electrophoresis. To prevent genomic contamination, all RNAs underwent DNase treatment (DNase I, Amplification Grade, Life Technologies) prior to cDNA synthesis. The High-Capacity cDNA Reverse Transcription Kit (Life Technologies) was utilized for the cDNA synthesis.

Quantitative PCR was conducted using the Rotor-Gene 6000 thermal cycler system (Qiagen GmbH, Düsseldorf, Germany) and the QuantiTect SYBR Green PCR kit (Qiagen) to determine the tissue-specific distribution (copy number/µL) of Tetraodon target genes (*sod1, sod2,* and *sod3*) and reference genes (*rpl7* and *rpl13a*). Each Quantitative PCR reaction for a tissue sample, including a negative control, comprised 20µl (10 µL SYBR Green, 4 µL forward and reverse primer, 5 µL nuclease-free water, and 1 µL cDNA). The RT-qPCR steps involved initial denaturation (95.0 °C for 15 min), followed by 40 cycles of denaturation (95.0°C for 20 s), primer annealing at the optimum temperature for each primer (Table 1) for 30 s, and elongation (72.0°C for 30 s). The mRNA transcript levels of *sod1, sod2,* and *sod3* genes in Tetraodon tissues were normalized to *rpl7* and *rpl13a* to assess tissue-specific distribution post qPCR. The gene expression levels were reported relative to the mean value of the control groups (Anderson and Elizur, 2012).

Primer optimisation

The forward and reverse primers were created using NCBI Primer-BLAST for the real-time quantitative PCR (qPCR) amplification of Tetraodon target genes (*sod1, sod2,* and *sod3*) as well as reference genes (*rpl7* and *rpl13a*) (Table 1). The primers were designed based on an exon-exon junction model to prevent the PCR amplification of products originating from any contaminating heterogeneous nuclear

RNA (hnRNA) or genomic DNA. The lyophilized primers were ordered and then reconstituted in TE buffer (10mM Tris, 1mM EDTA, pH 8.0) in a manner that achieved a stock concentration of 100 pmol/µl for each primer.

Table 1. Primer sequences for Tetraodon genomic (*sod1, sod2,* and *sod3*), Target Genes (*sod1, sod2,* and *sod3*), and reference genes (*rpl7* and *rpl13a*)

Tetraodon		Forwardprimer(5´→3´)	Reverseprimer(5´→3´)	Tm(°C)
sod1	Target	ATGTTTGGTTTTCCAGCAAGCGCAG	CGGGGACACGGTAGTTGTAG	60.6
sod2	Target	ACAGCGTTCGCCTCTGCTGTC	CTCTTTTTGGCAGTTTGGAGACG	61.4
sod3	Target	CGTTGACGATGCGTCTGCAC	GCCGGATACAAAGATGGAAT	61.5
rpl7	Reference	CGAGAAAAAGGCCCGCAAG	GGCTGACACCGTTGATACCT	59.7
rpl13a	Reference	TCCACCCTACGACAAGAGGAA	GTACTTCCAGCCAACCTCAT	60.20
sod1	Genomic	ATGTTTGGTTTTCCAGCAAGCGCAG	CTGTTTACTGAGTGATGCCGATG	61
sod2	Genomic	ACAGCGTTCGCCTCTGCTGTC	CTACTTTTTGGCAGTTTGGAGACG	63.20
sod3	Genomic	CGTTGACGATGCGTCTGCAC	GCCGGATACAAAGATGGAAT	60.5

The process of identifying and determining the structure of Tetraodon *sod1, sod2,* and *sod3* genes

The Ensembl database was used for bioinformatic identification of *sod1, sod2* and *sod3* genes. To confirm the accuracy of the obtained cDNA from this database, a BLAST search was performed in the NCBI database (https://www.ncbi.nlm.nih.gov/). This study revealed that the superoxide dismutase gene, utilized as the target gene, possesses three isoforms, specifically *sod1, sod2,* and *sod3*.The Ensembl gene IDs and amino acid numbers are provided in the Table 2.

The similarity and identity rates of Tetraodon *sod1, sod2*, and *sod3* genes with those of other teleost fish and vertebrates were determined using the BLOSUM62 matrix algorithm (Gromiha 2010). Protein sequences synthesized by *sods*/*SODs* genes from Tetraodon (*Tetraodon nigroviridis*), fugu (*Fugu rubripes*), stickleback (*Gasterosteus aculeatus*), zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), goldfish (*Carassius auratus*), human (*Homo sapiens*), and mouse (*Mus musculus*) were utilized for calculating similarity-identity rates. This analysis was performed using the BioEdit program, and the results are presented in Figures 1, 2 and 3.

Table 2. Tetraodon *sod1, sod2,* and *sod3* genes with ENSEMBL accession numbers and amino acid numbers

Gene	Organism	Ensembl gene ID	Amino acit numbers
sod1	Tetraodon	NSTNIT00000013030.1	179
sod2	Tetraodon	ENSTNIT00000015459.1	225
sod3	Tetraodon	ENSTNIT00000015540.1	209

```
Tetraodon Sod1    1   ------------------------------------------MFGFPASAVLPCVSFLEV
Fugu Sod1         1   ------------------------------------------------------------
Stickleback Sod1  1   ------------------------------------------------------------
Zebrafish Sod1    1   ------------------------------------------------------------
Medaka Sod1       1   ------------------------------------------------------------
Mouse Sod1        1   ------------------------------------------------------------
Human SOD1        1   ------------------------------------------------------------
Goldfish Sod1     1   MKAFRQVQNLGIILKSLTSLIVSRQTLTQPTSTSLFCLLIGQFDSRSS.FIKLFF.LFWL

Tetraodon Sod1    19  TT----AKMVIKAVCVLKGAGETSGTVYFEQQDEKAPVKLTGEIKGLTAGEHGFHVHAFG
Fugu Sod1         1   --------.AM..........D..........--.S............P...........
Stickleback Sod1  1   --------..L........S..V....H....NGD....V..K.S...P.D.........
Zebrafish Sod1    1   --------..N........T..VT.....N.EG..K...V....T...P.K.........
Medaka Sod1       1   --------..L........T...N.V.N...ESDS....V........P.K....I.VY.
Mouse Sod1        1   --------.AM........D.PVQ..IH...KASGE..V.S.Q.T...E.Q......QY.
Human SOD1        1   --------.AT........D.PVQ.IIN...KESNG...VW.S.....E.L......E..
Goldfish Sod1     61  GHNSQFDS.AM........T..VI.N......N.TSS...S.K.T...P.K.........

Tetraodon Sod1    75  DNTNGCISAGPHYNPHNKTHAGPNDENSLKRHVGDLGNVTAEADQIAKIDITDSVISLHG
Fugu Sod1         51  .......................T.AD---..L........G..N......K..MLT.T.
Stickleback Sod1  53  ......V.............G..R...---...........GE.KV.N.N.E.KH.T.T.
Zebrafish Sod1    53  ............F...D...G..T.SV---...........D.SGV...E.E.AMLT.S.
Medaka Sod1       53  ......V.....F..Y..N.G..E.AE---...........GDNNV.......KL.R.S.
Mouse SOD1        53  ...Q..T.....F...S.K.G..A..E---...........GK.GV.NVS.E.R....S.
Human SOD1        53  ...A..T.....F..LSRK.G..K..E---...........DK.GV.DVS.E......S.
Goldfish Sod1     121 ............F..FG.D.G..T.SD---.........I.DDNGV.....E.KIVT.L.

                                                               Similarity(%)  Identity(%)

Tetraodon Sod1    135 KFSIIGRTMVIHEKADDLGKGGNEESLKTGNAGGRLACGVIGITQ    100            100
Fugu Sod1         108 PY...........................................    74             79
Stickleback Sod1  110 Q.....................DD...................A.    69             77
Zebrafish Sod1    110 QH............E..............................    68             75
Medaka Sod1       110 PD..V...V.V...V........D.........A.........A.    63             73
Mouse SOD1        110 EH........V...Q...........T......S.........A.    60             68
Human SOD1        110 DHC.....L.V...............T......S.........A.    59             66
Goldfish Sod1     178 QH............E..................S...........    52             60
```

Figure 1. The similarity-identity ratios between the protein sequence of the Tetraodon (*Tetraodon nigroviridis*) *sod1* gene and the Sod1 protein sequences of some other vertebrates. (The dots in the table indicate similarities, while short dashes represent missing amino acids)

```
Tetraodon Sod2   1   ---MLCRVGQIH---------------RCAASLSQAIR-QVGASRQKHTLPDLTYDYGAL
Medaka Sod2      1   ---...K.W.MR---------------S..SI.H.TVSWK..S.................
Zebrafish Sod2   1   ---......YVR---------------....TFNPLLG--AVT.....A...........
Goldfish Sod2    1   ---RTR.ACCVESD-------------M...T.NPILG--AV...R.......P......
Stickleback Sod2 1   SCSV.LLLKA.KG----------AQLYL..GG.G.T.S-..A..K........P......
Human SOD2       1   ---..S.AVCGT-----------------SRQ.APVLG--YLG.....S....P......
Fugu SOD2        1   -MNT........SSRGNTCFSCPNIHTK........V.-......H..............
Mouse Sod2       1   ---....AACST-----------------GRR.GPVAG--AAG..H..S....P......

Tetraodon Sod2   42  EPHISAEIMQLHHSKHHATYVNNLNVTEEKYQEALAKGDVTAQVALQPALKFNGGGHINH
Medaka Sod2      43  ....C.......................................T...............
Zebrafish Sod2   41  ....C....................................T..S...............
Goldfish Sod2    43  ....C...................................IT..S...............
Stickleback Sod2 50  ...VN......................................I................
Human SOD2       39  ....N.Q...........A........................I................
Fugu Sod2        59  .....................................R............R.........
Mouse SOD2       39  ....N.Q...........A......A.....H.........T..................

Medaka Sod2      103 .....................A...........Q..L.................D.ES.R
Zebrafish Sod2   101 ...................L.A..............I................FE.ES.R
Goldfish Sod2    103 ...................L.A..............I................FD..S.R
Stickleback Sod2 110 .........K..D..K...L.A................................D.ES..
Human SOD2       99  S..............K...L.A........D.F...LT..S.G..........FN.ER.H
Fugu Sod2        119 .....................A..................................E..R
Mouse SOD2       99  .........K.....K...L.A........E.F...LT.VS.G..........FN.EQ.R

Tetraodon Sod2   162 LCIAACGNQDPLQGTTGLIPLLGIDVWEHAYYLQYKNVRPDYVKAIWNVINWENVSERLQ
Medaka Sod2      163 .RV...A..........................................V..........
Zebrafish Sod2   161 .R....A..........................................V........F.
Goldfish Sod2    163 .R....S...........T..............................V..G.......
Stickleback Sod2 170 .RV...A.....H.....V..............................V..........
Human SOD2       159 .Q....P...................................L............T..YM
Fugu Sod2        179 ............................................................
Mouse SOD2       159 .Q....S...................................L............T..YT

                                                     Similarity(%)  Identity(%)

Tetraodon Sod2    222 TAKK-----------------------------------     100         100
Medaka Sod2       223 I...-----------------------------------      87          92
Zebrafish Sod2    221 A...-----------------------------------      86          91
Goldfish Sod2     223 A...-----------------------------------      82          87
Stickleback b Sod2 230 S...-----------------------------------     81          87
Human SOD2        219 AC..-----------------------------------      78          86
Fugu Sod2         239 ...NVCCRINHLVGKNTMLHLQIEAHFELYLSDSASQI      77          79
Mouse SOD2        219 AC..-----------------------------------      76          83
```

Figure 2. The similarity-identity ratios between the protein sequence of the Tetraodon (*Tetraodon nigroviridis*) *sod2* gene and the Sod2 protein sequences of some other vertebrates. (The dots in the table indicate similarities, while short dashes represent missing amino acids)

```
Tetraodon Sod3    1   MRLHGWV-IASAVLLLLLAGCQDCGSAHGDPAA---------------------------
Stickleback Sod3  1   ..VGSTF-PLGSA..V......P.V..DSETL.---------------------------
Fugu Sod3         1   ..P..SA-S.L.A.M....D..R.VL.E.NGS.---------------------------
Medaka Sod3       1   .S..RS.RVLEMAF.VW..CS.Q.F.T.T.HLL---------------------------
Zebrafish Sod3    1   --------MIRFNI.P...FLLS.HVLFCGSGS---------------------------
Goldfish Sod3     1   --------MK.FYI.P...FLFS.HVHFCGSLS---------------------------
Human SOD3        1   ------MLALLCSC...A..AS.AWT--.EDS.EPNS-----------DSAEWIRDMYAK
Mouse SOD3        1   ------MLAFLFYG...A.CGSVTM.NP.ESSFDLADRLDPVEKIDRLDLVEKIGDTHAK

Tetraodon Sod3    32  --------PPEASQNNGSLYAACNMRPSALLPEDLPKVHGHVLFKQDHPQGGLSALLQLG
Stickleback Sod3  32  --------...V..Y..T.....K....TS.ADG....Y.Q..L...Y.L.K.N...RFN
Fugu Sod3         32  --------L............S....R.SA....V.QLY........R...I.RV.FHV.
Medaka Sod3       33  --------...F..Y..T.....KVS..TS..D.....Y.QA......G..K.QV....A
Zebrafish Sod3    25  --------GLAYAS.SD..Q.V.R.Q.NTR.EPGM.R.Y..I..R.SG.KEK..VTFR.Y
Goldfish Sod3     25  --------GLVYAS.-----------------PVS.P.SSNS-LRASG.KEK..VTFR.H
Human SOD3        42  VTEIWQEVMQRRD-DD.A.H...QVQ...T.DAAQ.R.T.V...R.LA.RAK.D.FFA.E
Mouse SOD3        55  VLEIWMELGRRREVDAAEMH.I.RVQ...T..P.Q.QIT.L...R.LG.GSR.E.YFS.E

Tetraodon Sod3    85  GFLSDGEPT--AVHIHQYGDLSQGCGSTGGHYNPHGKNHPNHPGDFGNFEPQEGKVD-AA
Stickleback Sod3  85  ..P.E.D.QPR...........R..A.........AH..................IN-.M
Fugu Sod3         85  ...T.-----------------P.....R.....Y.E............V....IIS-.V
Medaka Sod3       86  ..PE.ES.QSR.I............I........Y.VD...........VAH..RIS-EQ
Zebrafish Sod3    78  .LPA.-SQQPR.M...E.....R..D........LNV...Q........V.VNK.IR-QS
Goldfish Sod3     60  .LPVY-SQQPR.M...E.....K...........L.V...Q........V.VN..IR-LS
Human SOD3        101 ..PTEPNSSSR.I.V..F.......E...P....LAVP..Q........AVRD.SLWRYR
Mouse SOD3        115 ..PAEQNASNR.I.V.EF.......D...P....MEVP..Q........VVRN.QLWRHR

Tetraodon Sod3    142 VESNATLFGATSVIGRAVVVHEKRDDLGQGGDAGSLLHGNAGRRLACCVIGISSSDLWNT
Stickleback Sod3  144 I..E..V...L............M....S................G..I...A.PEP..M
Fugu Sod3         127 ...K.....GM.A..........N..............................P....L
Medaka Sod3       145 I..E.....GL..L........TI........V..................M......EE
Zebrafish Sod3    136 L..P.....KL.IV..S..I..GK....R..NV....N....G........LRNPQN---
Goldfish Sod3     118 MN.S.....RL.IL..S..I..GE....R..NV....N....G........LANPQN---
Human SOD3        161 AGLA.S.A.PH.IV.......AGE....R..NQA.VEN...........V.VCGPG..ER
Mouse SOD3        175 .GLT.S.A.PHAIL..S....AGE....K..NQA..QN...........V.T...AA.ES

                                                  Similarity(%)    Identity(%)
Tetraodon Sod3    202 S-KEFTERG-----------                 100              100
Stickleback Sod3  204 HY.LYNR.LRRI--------                  66               76
Fugu Sod3         187 NYPK.A..MKKN--------                  66               73
Medaka Sod3       205 QQ.LQSS-------------                  63               70
Zebrafish Sod3    192 --------------------                  44               59
Goldfish Sod3     174 --------------------                  39               51
Human SOD3        221 QAR.HS..KKRRRESECKAA                  37               51
Mouse Sod3        235 QT..---.KKRRRESECKTT                  35               46
```

Figure 3. The similarity-identity ratios between the protein sequence of the Tetraodon (*Tetraodon nigroviridis*) *sod3* gene and the Sod3 protein sequences of some other vertebrates. (The dots in the table indicate similarities, while short dashes represent missing amino acids)

The determination of the nucleotide sequences of the *sod1, sod2*, and *sod3* genes in the Tetraodon (*Tetraodon nigroviridis*) has been accomplished. The nucleotide sequences, including intron and exon sequences, of the *sod1, sod2,* and *sod3* genes in the pufferfish have been identified in the ENSEMBL database.

The acquisition of cDNA was facilitated using the ENSEMBL database. The nucleotide sequences have been designed to depict the exons, introns, amino acids synthesized by the exons, 5' and 3' ends, TATA box, poly-A signal, and stop codon of the *sod1, sod2*, and *sod3* genes (Figures 4, 5 ,6).

ENSTNIT00000013030.1 *sod1*

```
5'atgaaatcatcaatgtttcagccttaggaaattgttttaataaaatattttttttaa
caagatgtttctttggactgatggttttatgtttacagctcaggagaagtcgaccatgtt
catttaataaaaagtctcaatatTATActaaaaattctgattttcgaagtcaaattgaac
gcaccataatgtagaagaaccaagtcattaaacctttaccctgctaaccagttaaaatta
           +1
aacgctccaaCGAGCTCTCGTTCTGATTGGCTTACCGATCCTTAAACACTCCCACCTAGC
ATGTTTGGTTTTCCAGCAAGCGCAGTATTGCCGTGTGTGTCGTTTCTTGAAGTGACAACT
-M--F--G--F--P--A--S--A--V--L--P--C--V--S--F--L--E--V--T--T-
GCGAAGATGGTGATAAAAGCTGTTTGCGTGTTAAAAGGAGCCGGGGAGACCAGCGGAACG
-A--K--M--V--I--K--A--V--C--V--L--K--G--A--G--E--T--S--G--T-
GTTTATTTTGAGCAGCAGgtgaa'N882'cccagGATGAAAAGGCTCCTGTCAAGTTGAC
-V--Y--F--E--Q--Q-                -D--E--K--A--P--V--K--L--T
GGGGGAGATTAAAGGGCTGACCGCTGGTGAACACGGGTTCCATGTCCACGCTTTTGGAGA
--G--E--I--K--G--L--T--A--G--E--H--G--F--H--V--H--A--F--G--D
CAATACCAATGgtaag'N95accagGTTGCATCAGTGCAGGCCCTCACTACAATCCCCAC
--N--T--N--            G--C--I--S--A--G--P--H--Y--N--P--H-
AACAAGACCCATGCTGGGCCTAACGATGAAAACAGgtaaa'N543'ttaaaTCTAAAAAG
-N--K--T--H--A--G--P--N--D--E--N--S                --L--K--R
GCACGTTGGAGACCTGGGAAATGTGACCGCTGAAGCAGACCAGATCGCCAAGATTGACAT
--H--V--G--D--L--G--N--V--T--A--E--A--D--Q--I--A--K--I--D--I
AACCGATTCAGTAATAAGCCTCCATGGCAAGTTTTCTATAATTGGCAGAACCATGGTGgt
--T--D--S--V--I--S--L--H--G--K--F--S--I--I--G--R--T--M--V-
Gag'N85'cttagATCCACGAGAAGGCCGATGACCTGGGAAAAGGAGGCAACGAAGAGAG
               -I--H--E--K--A--D--D--L--G--K--G--G--N--E--E--S
CCTTAAAACAGGAAACGCTGGTGGGCGTTTGGCCTGTGGAGTCATCGGCATCACTCAGTA
--L--K--T--G--N--A--G--G--R--L--A--C--G--V--I--G--I--T--Q--*
Acagtcggcaaggacagaaagttctggaaactattcttgtcaacgcctaataagaccaat
-

ctagttgttctttaaccttgtggatttactggggtcacaggtcgggtgtgtaggagactc
agcttcaccctgtctgctctttgtgacagtgtttccaaggtttccatgtctgctgtttaa
gttttgattccaaagaattggaaacgcacaagtaacacacatgtagacgttaattagatc
cAATAAAtgtcaagttca3'
```

Figure 4.. Exon-intron organization of the Tetraodon (*Tetraodon nigroviridis*) *sod1* gene*

ENSTNIT00000015459.1 *sod2*

```
5'atatttcatttgcatcccgtatggaatgcatcgtggtaatgactagaagtattttgaa
aatataaaggcattaaacgacgtattgtggaaaaccaacaagatgcataacgtaacgtgt
tcaaatttatgcagatatatcacgtttgtttaaagacgtgcatttagactgaaatattga
gtTATAgctgttatttcgaaatagtttgctgaaaagctctgccccctattcacacccta
tggactgataatggtacggcccttgctgtgtcacgttgaaattgcacatcaaggacagtc
+1
ACAGCGTTCGCCTCTGCTGTCCCGCCTGCTAAACCAACACTATCAACATGTTGTGCAGAG
                                               -M--L--C--R--
TTGGTCAGATACACAGgtaaa'N439'ttcagATGTGCAGCCAGCCTTAGCCAGGCTATA
V--G--Q--I--H--R                --C--A--A--S--L--S--Q--A--I-
AGGCAGGTGGGAGCTTCTCGACAAAAGCACACGCTCCCAGACCTGACCTACGACTATGGG
-R--Q--V--G--A--S--R--Q--K--H--T--L--P--D--L--T--Y--D--Y--G-
GCCCTGGAGCCCCACATCAGTGCAGAGATCATGCAGCTGCACCACAGCAAGCACCACGCC
-A--L--E--P--H--I--S--A--E--I--M--Q--L--H--H--S--K--H--H--A-
ACATATGTCAACAATCTTAACGTCACAGAGGAGAAATATCAGGAGGCATTAGCAAAGGgt
-T--Y--V--N--N--L--N--V--T--E--E--K--Y--Q--E--A--L--A--K--
atg'N86'gttagGAGATGTGACTGCACAAGTTGCTCTGCAGCCTGCTCTGAAGTTTAAC
              G--D--V--T--A--Q--V--A--L--Q--P--A--L--K--F--N-
GGAGGAGGCCACATAAACCACACCATCTTCTGGACGAACCTTTCTCCAAACGGTGGAGGC
-G--G--G--H--I--N--H--T--I--F--W--T--N--L--S--P--N--G--G--G-
GAGCCTCAGGgtaat'N93'tctagGGGAGCTGATGGAGACCATTAAGCGGGACTTTGGC
-E--P--Q--                G--E--L--M--E--T--I--K--R--D--F--G-
TCTTTCCAGAAGATGAAGGAGAAGATGTCTGCTGCTACTGTTGCAGTACAGGGTTCAGGC
-S--F--Q--K--M--K--E--K--M--S--A--A--T--V--A--V--Q--G--S--G-
TGGGGATGGCTGGGCTACAGCAAAGACACTGGAAGTCTTTGTATTGCTGCCTGTGGCAAC
-W--G--W--L--G--Y--S--K--D--T--G--S--L--C--I--A--A--C--G--N-
CAGGACCCCCTCCAAGGAACTACAGgtcgg'N76'ctcagGTCTCATCCCGCTCCTCGGT
-Q--D--P--L--Q--G--T--T--                G--L--I--P--L--L--G-
ATTGATGTGTGGGAACACGCTTACTATCTTCAGTACAAAAATGTGCGGCCAGACTATGTT
-I--D--V--W--E--H--A--Y--Y--L--Q--Y--K--N--V--R--P--D--Y--V—
AAGGCCATCTGGAATGTGATCAACTGGGAGAATGTGAGCGAACGTCTCCAAACTGCCAAA
-K--A--I--W--N--V--I--N--W--E--N--V--S--E--R--L--Q--T--A--K-
AAGTAGtgcaaaggagcaaaagctgttgcatgctacttctgtacactggaaaaataatta
-K--*-
ttcaaatcaaaacgatctgtacactggAAAAATAAttattcaaatcaaaacgatgtgtat
tagtaaaaagaatagagtcagtttacttttaaatattcatcctaccagaagaaacacttg
cttgaaaacaggtattacatcgaaaggaaaattaattaacaacagactgatgtaatgagc
aggttttgaacggaaaagcaaattaattttaaagcagtttttggttaaaacgaacgcggg
cgaatgagagctgcaatattcaatctggccgaaagagggcag3'
```

Figure 5. Exon-intron organization of the Tetraodon (*Tetraodon nigroviridis*) *sod2* gene*

ENSTNIT00000015540.1 *sod3*

```
5’ttaaacaagaacaggctattgccttttatttatttatcaaatattgcctaatttctat
ggatagaaaTATAtttttaaagacatgggagctttaactggtcgtgttacctttcatgga
aagcaggtttctttttgctgttgacggtttcttttttaaaaagcagtcattaaaaatta
aacattgcaatttctcataaaaaacatgtgtgtaaacaaagtgtccacccttggaaaatg
agccccaccttcatgtttcattggtctgctcagaacagcgagcagcggtcggattgatcc
+1
AGACTGAAAGTCCACCTTCGTTGACGATGCGTCTGCACGGgtaag’N785’ttcagGTGG
                          -M--R--L--H--G                --W-
GTGATCGCGTCGGCAGTGCTGCTGCTTCTGCTGGCCGGTTGTCAAGATTGCGGCTCAGCT
V--I--A--S--A--V--L--L--L--L--L--A--G--C--Q--D--C--G--S--A--
CACGGTGACCCTGCAGCTCCGCCGGAGGCCTCTCAGAACAATGGCAGCCTGTATGCGGCC
-H--G--D--P--A--A--P--P--E--A--S--Q--N--N--G--S--L--Y--A--A-
TGCAACATGAGACCCAGCGCCTTGCTGCCAGAGGACCTGCCCAAAGTGCACGGTCACGTG
-C--N--M--R--P--S--A--L--L--P--E--D--L--P--K--V--H--G--H--V-
CTGTTCAAGCAGGACCACCCTCAGGGAGGACTCTCGGCCCTCCTTCAGCTTGGCGGCTTT
-L--F--K--Q--D--H--P--Q--G--G--L--S--A--L--L--Q--L--G--G--F-
CTCAGCGACGGCGAGCCCACGGCCGTCCACATCCATCAGTACGGGGACCTGAGCCAGGGG
-L--S--D--G--E--P--T--A--V--H--I--H--Q--Y--G--D--L--S--Q--G-
TGCGGCTCCACCGGTGGGCACTACAACCCACACGGCAAAAACCACCCCAACCACCCCGGA
-C--G--S--T--G--G--H--Y--N--P--H--G--K--N--H--P--N--H--P--G-
GACTTTGGTAACTTTGAGCCTCAGGAGGGGAAGGTCGACGCCGCGGTAGAGTCAAACGCC
-D--F--G--N--F--E--P--Q--E--G--K--V--D--A--A--V--E--S--N--A-
ACGCTCTTTGGAGCGACGTCTGTGATCGGAAGGGCAGTGGTGGTCCACGAGAAGAGAGAT
-T--L--F--G--A--T--S--V--I--G--R--A--V--V--V--H--E--K--R--D-
GACCTGGGCCAGGGTGGAGACGCCGGGAGCCTCCTGCACGGAAACGCAGGACGGAGGCTT
-D--L--G--Q--G--G--D--A--G--S--L--L--H--G--N--A--G--R--R--L-
GCCTGCTGCGTTATTGGAATTTCCTCTTCCGATCTGTGGAACACCTCCAAGGAGTTTACA
-A--C--C--V--I--G--I--S--S--S--D--L--W--N--T--S--K--E--F--T-
GAAAGGGGTAAaaaatacagtaatttacatgcaaaacataaacagctgagacggaggtt
-E--R--G--*-
ctttaagaaaacgtcgcttgagtatcttttggttttaaagatgttcagcagaaaacagca
gctgtgccatcgcctacgactcacctcatctaccaacgtctatcagagtttgacaagcta
cggtggtgattctgtcctgctcagcgctgttgatcaatgctttacacatatggtttcttc
agaggtaggaagATAAAAgaaaagaggtttcatccgacagaaactgtcggaaaaaccgcc
gttattgctacaag3’
```

Figure 6. Exon-intron organization of the Tetraodon (*Tetraodon nigroviridis) sod3* gene*

*The exons of the Tetraodon superoxide dismutase (*sod1, sod2,* and *sod3*) genes are indicated in uppercase letters. In transcription, the starting point is denoted as +1, and the 5' and 3' sequences are indicated in lowercase letters. The TATA box and poly-A signal (ATAAAA) are highlighted in green and represented in uppercase letters.

Phylogenetic analysis

The alignment of Tetraodon *sod*s genes was conducted using the CLUSTALW algorithm (Thompson et al., 1994) within the BioEdit software. The phylogenetic tree constructed using the Maximum Likelihood Method (Felsenstein, 1981) includes the following organisms, protein sequences, and accession numbers for the *sod1* gene: Tetraodon ENSTNIT00000013030.1, zebrafish (*Danio rerio*) ENSDART00000064376.5, Japanese goldfish (*Carassius auratus*) ENSCART00000041002.1, Medaka (*Oryzias latipes*) ENSORLT00000027902.1, human (*Homo sapiens*) ENST00000270142.11, and mouse (*Mus musculus*) ENSMUST00000023707.11 protein sequences were used. For the *sod2* gene: Tetraodon (*Tetraodon nigroviridis*) ENSTNIT00000015459.1, zebrafish (*Danio rerio*) ENSDART00000062556.4, Japanese goldfish (*Carassius auratus*) ENSCART00000055705.1, Medaka (*Oryzias latipes*) ENSORLT00000016614.2, human (*Homo sapiens*) ENST00000337404.8, and mouse (*Mus musculus*) ENSMUST00000007012.6 protein sequences were used. For the *sod3* gene: Tetraodon (*Tetraodon nigroviridis*) ENSTNIT00000015540.1, zebrafish (*Danio rerio*) ENSDART00000112150.4, Japanese goldfish (*Carassius auratus*) ENSCART00000099621.1, medaka (*Oryzias latipes*) ENSORLT00000024189.2, human (*Homo sapiens*) ENST00000382120.4, and mouse (*Mus musculus*) ENSMUST00000101208.6 protein sequences were used. ENSEMBL and NCBI databases were used for data acquisition (Figure 7).

Conserved gene synteny

The conserved gene synteny has been manually designed using the ENSEMBL database, and for this purpose, the chromosomes and chromosomal regions where the superoxide dismutase *(sod1, sod2,* and *sod3*) gene is located in Tetraodon have been recorded. The *sod1* gene is found on the chromosome 10 in zebrafish, on the chromosome 7 in Tetraodon, and on the chromosome 21 in human. It has been observed that the genes *ltn1, paxbp1, grik1, tiam1, sod1, scaf4, synj1, cxadr* are also located on the same chromosomes in zebrafish, Tetraodon, and humans. The *sod2* gene is identified on the chromosome 20 in zebrafish, on the chromosome 14 in Tetraodon, and on the chromosome 6 in humans. Other genes found on the same chromosomes as *sod2* in these three organisms include *kif25, acat2, wtap, sod2, slc22a16, cep57l1, sesn1, snx3.* Finally, for *sod3*, it is located on the chromosome 1 in zebrafish, on the un_random chromosome in Tetraodon, and on the chromosome 4 in humans. Other genes found on the same chromosomes as *sod3* in these three organisms include *fgb, fga, exosc9, fabp2, vegfc, ing2, sod3, clgn.* A conserved gene synteny has been created based on the common genes present in the genomes of these three organisms and their chromosomal locations. These findings allowed us to create a conserved gene synteny map that showed the relationship among the *sod1/SOD1, sod2/SOD2* and *sod3/SOD3* genes of Tetraodon, zebrafish, and human (Figure 8).

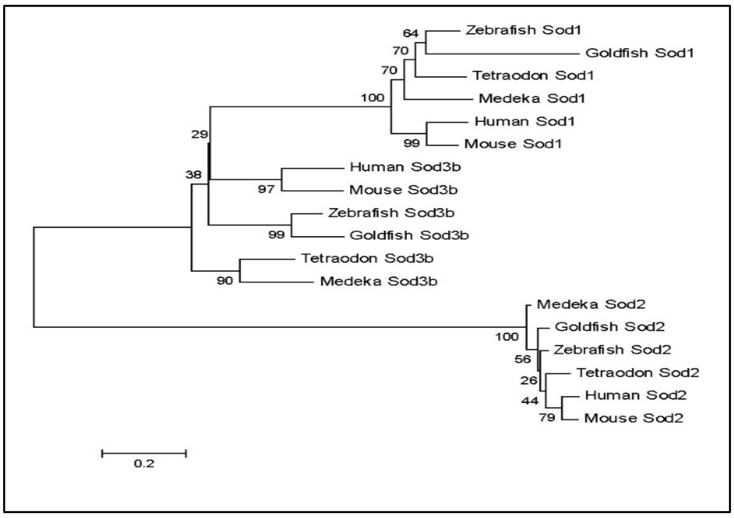

Figure 7. The phylogenetic relationships of the *sod1, sod2*, and *sod3* genes of Tetraodon (*Tetraodon nigroviridis*) with those of other teleost fishes and tetrapods were examined to understand their evolutionary context

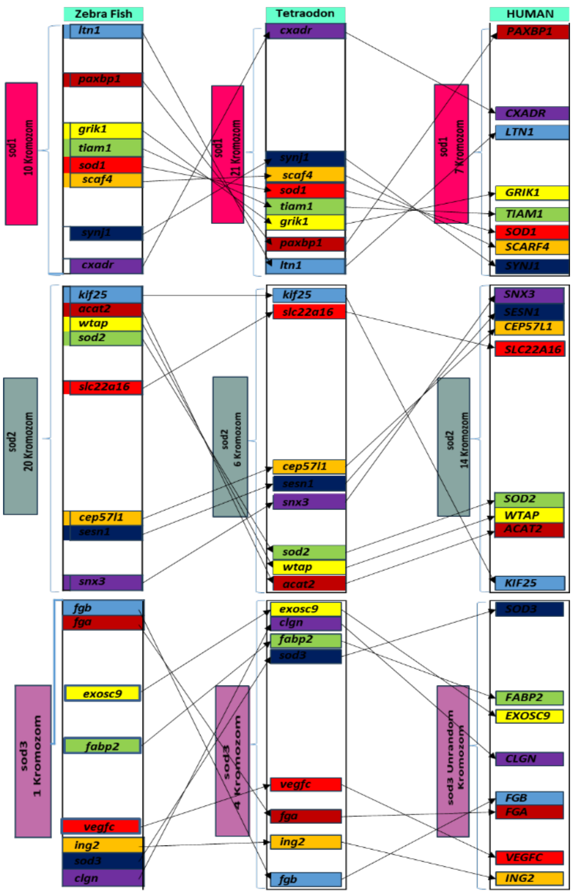

Figure 8. Conserved gene synteny of the Tetraodon *sod1, sod2,* and *sod3* genes with *sod1/SOD1, sod2/SOD2*, and *sod3/SOD3* genes from zebrafish and human

Statistical analysis

In this research study, the results normalized after qPCR application were evaluated through statistical analysis. The statistical analysis utilized the SPSS statistical program, and differences were determined to be statistically significant ($P<0.05$) by applying ANOVA (Duncan's multiple comparison test) to the results (SPSS 1996).

RESULTS

Gender-specific expression of *sod1, sod2* and *sod3* Genes in different tissues of Tetraodon (*Tetraodon nigroviris*)

In this study, tissue-specific distributions of the *sod1, sod2,* and *sod3* genes were determined in female and male Tetraodon through qPCR transcription measurements (Figure 9, 10, and 11). For the female Tetraodon, tissue-specific distribution of the *sod1* gene was determined as follows: liver 28.74 ± 1.21; intestine 13.88 ± 0.5; muscle 5.09 ± 0.42; brain 2.72 ± 0.24; heart 3.41 ± 0.32; eye 1.88 ± 0.1; spleen 1.52 ± 0.1; gill 6.37 ± 0.85; kidney 1.62 ± 0.11; stomach 2.01 ± 0.14; ovary 7.16 ± 0.96. For male Tetraodon, tissue-specific distribution of the *sod1* gene was determined as follows: liver 40.46 ± 3.22; intestine 22.68 ± 1.63; muscle 8.86 ± 1.04; brain 5.14 ± 0.96; heart 3.79 ± 0.55; eye 3.08 ± 0.42; spleen 3.64 ± 0.88; gill 10.91 ± 1.45; kidney 4.08 ± 1.27; stomach 3.84 ± 0.99; testis 10.84 ± 1.08. The results showed that the liver had higher gene expression compared to all other tissues, and the intestine, ovary, and gill had significantly lower the *sod1* gene expression compared to the liver, while all other tissues had significantly higher expression. When examining the transcriptional differences between male and female tissues for the Tetraodon *sod1* gene, it was observed that the intestine, gill, kidney, stomach, muscle, and gonads were significantly higher in male fish, but the differences among other tissues were not statistically significant (Figure 9).

In female tetraodon, tissue-specific distribution of the *sod2* gene is determined as follows: liver 32.93 ± 3.01; intestine 19.29 ± 1.99; muscle 16.64 ± 1.52; brain 4.91 ± 1.01; heart 5.6 ± 1.44; eye 3.79 ± 0.62; spleen 3.77 ± 0.85; gill 25.46 ± 1.89; kidney 2.85 ± 0.55; stomach 2.89 ± 0.22; ovary 13.46 ± 1.07. For male tetraodon, tissue-specific distribution of the *sod2* gene is determined as follows: liver 35.54 ± 3.08; intestine 19.72 ± 2.11; muscle 12.07 ± 1.19; brain 4.14 ± 0.09; heart 5.32 ± 1.11; eye 2.07 ± 0.52; spleen 1.99 ± 0.28; gill 30.63 ± 3.24; kidney 4.81 ± 0.35; stomach 4.17 ± 0.96; testis 14.43 ± 1.33.

When examining the results for both male and female tetraodon for the *sod2* gene, it is observed that the liver, muscle, gill, intestine, ovary, and testis have significantly higher the *sod2* gene expression compared to all tissues, while the differences among the intestine, heart, and gonad tissues are not statistically significant (Figure 10).

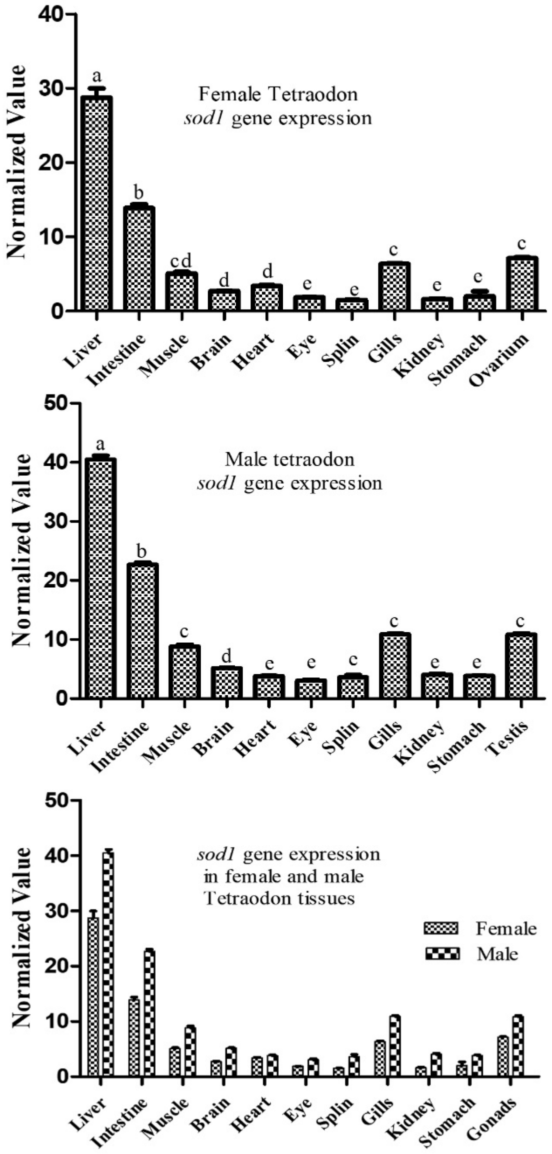

Figure 9. Tissue-specific expression of the *sod1* gene in female and male Tetraodon

For female tetraodon, the tissue-specific distribution of *sod3* gene is determined as follows: liver 33.04 ± 1.17; intestine 23.2 ± 1.11; muscle 6.82 ± 0.09; brain 1.85 ± 0.01; heart 2.99 ± 0.02; eye 1.05 ± 0.01; spleen 1.42 ± 0.01; gill 16.70 ± 2.07; kidney 0.94 ± 0.01; stomach 1.05 ± 0.02; ovary 10.85 ± 0.99. For male tetraodon, the tissue-specific distribution of the *sod3* gene is determined as follows: liver 42.31 ± 2.71; intestine 24.76 ± 2.01; muscle 5.70 ± 1.11; brain 2.67 ± 0.08; heart 3.90 ± 0.08; eye 2.12 ± 0.06; spleen 1.6 ± 0.04; gill 19.17 ± 1.10; kidney 1.60 ± 0.05; stomach 1.29 ± 0.04; testis 20.67 ± 1.27. When examining the results for both male and female tetraodon for the *sod3* gene, it is observed that the heart, spleen, and stomach tissues do not show statistically significant differences, but all other tissues are significantly higher in male tetraodon (Figure 11).

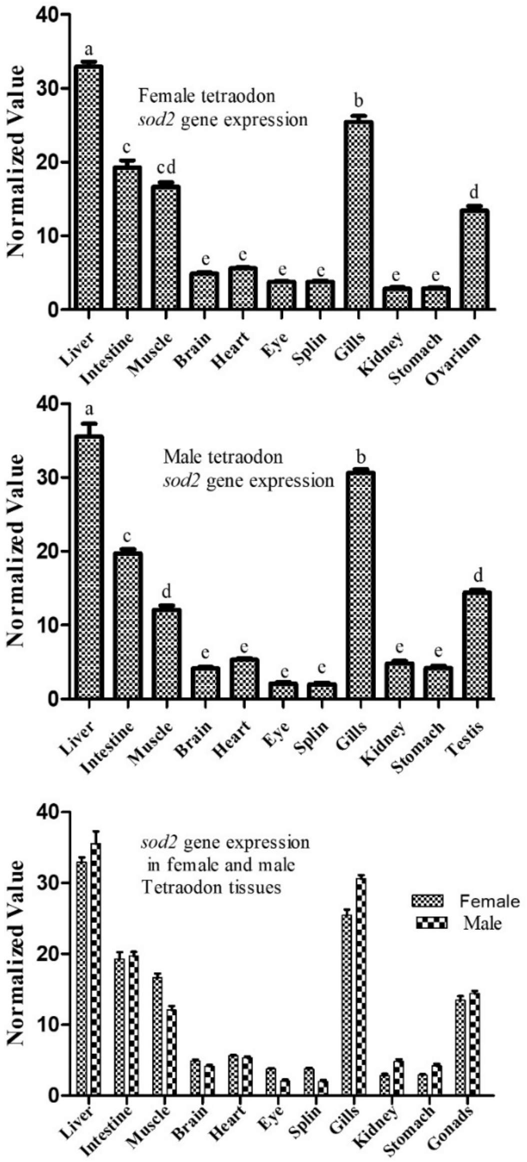

Figure 10. Tissue-specific expression of the *sod2* gene in female and male Tetraodon

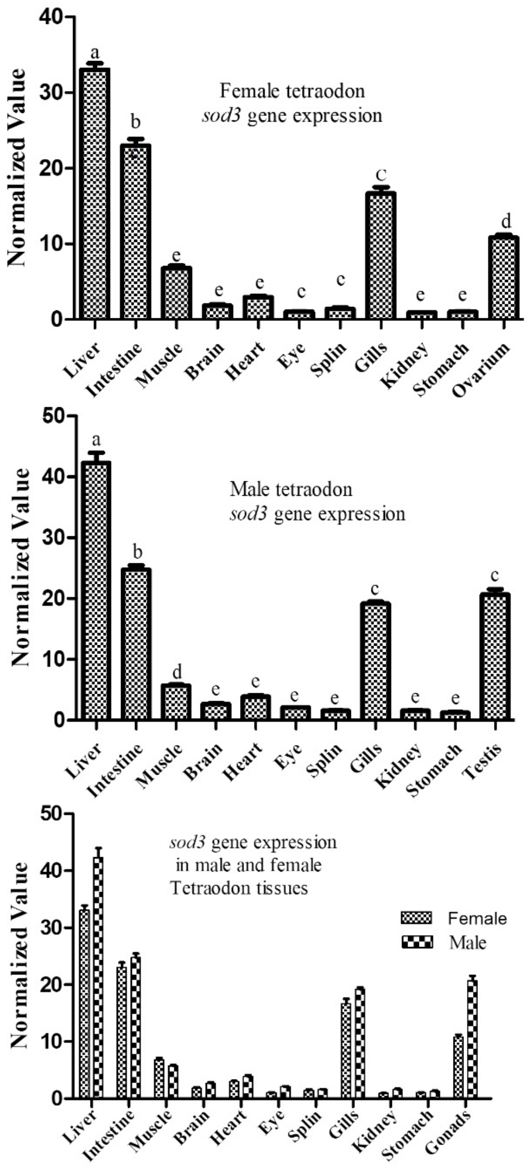

Figure 11. Tissue-specific expression of the *sod3* gene in female and male Tetraodon

3.2. Bioinformatics studies of *sod1, sod2,* and *sod3* genes in Tetraodon (*Tetraodon nigroviridis*)

The bioinformatics studies conducted for the characterization and identification of *sod1, sod2,* and *sod3* genes in tetraodon aim to establish foundational information for the development of contemporary strategies to mitigate the adverse effects of oxidative stress in both fish and other vertebrates. The analysis revealed that not only tetraodon but also other fish species such as zebrafish, goldfish, medaka, and stickleback exhibited a structure for *sod1* and *sod2* genes consisting of 5 exons and 4 introns, while the *sod3* gene comprised 2 exons and 1 intron, demonstrating a highly conserved exon-intron organization. Using CLUSTAL W for sequence alignment analysis (Thompson et al., 1994), it was noted that the levels of polypeptide identity and similarity between tetraodon and various species including zebrafish, medaka, goldfish, stickleback, fugu, mouse, and human were notably elevated. Furthermore, the analysis indicated that the tetraodon *sod1* gene exhibited the highest similarity (74%) and identity (79%) rates with the fugu, *sod2* gene exhibited the highest similarity (87%) and identity (92%) rates with the medaka, while the *sod3* gene displayed the highest similarity (66%) and identity (76%) rates with stickleback.

The phylogenetic relationship can be seen in the tree created using protein sequences of tetraodon (*Tetraodon nigroviridis*), zebrafish (*Danio rerio*), medaka (*Oryzias latipes*), goldfish (*Carassius auratus*) human (*Homo sapiens*), and mouse (*Mus musculus*) according to the maximum-likelihood method using the MEGA11 program. It was observed that the *sod1*, *sod2*, and *sod3b* genes were clustered in different regions (Figure 7).

DISCUSSION

Tissue-specific transcriptional activity of *sod1, sod2,* and *sod3* genes in male and female Tetraodon (*Tetraodon nigroviridis*)

Fish, due to their adaptation to a broad range of habitats and stressful environmental conditions associated with life strategies, tend to be exposed to harmful reactive oxygen species (ROS)-mediated oxidative stress conditions (Carney Almroth et al., 2015; Wang et al., 2016; Chatzidimitriou et al., 2020). Therefore, understanding how fish have evolved to cope with oxidative stress, including normal cellular metabolism, environmental changes, and/or pathogenic infections through various mechanisms, is particularly intriguing.

The expression of the *sod1, sod2, and sod3* genes in different tissues of *Tetraodon nigroviridis* is influenced by gender, with significant differences observed in the liver, intestine, gill, and kidney (Isensee and Noppinger, 2007). This gender-specific expression is consistent with the sexually dimorphic gene expression observed in mammalian somatic tissue (Guan et al., 2000). The role of these genes in reproductive function is further supported by the subfertility of female mice lacking SOD1 (Matzuk et al., 1998). The response of these genes to environmental stressors, such as the down-regulation of *sod* genes in platyfish exposed to diazinon, highlights their potential as biomarkers for environmental toxicity (Bayır and Özdemir, 2023). Uzun and Bayır (2023) investigated the expression differences of the *gsr* and *g6pd* genes, which are antioxidant enzyme genes, between genders in zebrafish. They found that the expression of the *gsr* gene was significantly higher in the liver, intestine, heart, eye, gills, and reproductive organs of male zebrafish compared to female fish. Additionally, they observed that the transcription of the *g6pd* gene was significantly higher in the male liver, intestine, muscle, brain, eye, gills, kidney, stomach, and reproductive organs. When examining the transcriptional differences between male and female tissues for the Tetraodon *sod1* gene, it was noted that, except for the heart tissue, all other tissues studied (including the liver, intestine, muscle, brain, eyes, spleen, gills, kidney, stomach, and gonads) exhibited significantly higher expression levels in male fish. Upon analyzing the *sod2* gene results in male and female Tetraodon, a significant increase in expression was observed in the liver, intestine, muscle, gills, spleen, eyes, kidneys, and stomach, with no notable statistical significance in tissues such as the intestine, heart, and gonads. Regarding the *sod3* gene in male and female Tetraodon, tissues like the heart, spleen, and stomach showed no statistical significance, yet the liver, intestine, gills, kidneys, stomach, and gonads displayed markedly higher expression levels in male fish.

Superoxide dismutases (SODs) play crucial roles in antioxidant defense across various organisms. In fish, SOD genes exhibit differential expression patterns between sexes and tissues (Ferrão et al., 2024; Bayır and Özdemir, 2023). The analysis of transcriptional differences between male and female tissues for the Tetraodon *sod1* gene revealed intriguing findings. Notably, except for the heart tissue, all other tissues studied displayed significantly higher expression levels in male fish. This observation suggests a potential sex-dependent regulation of the *sod1* gene expression across various tissues in Tetraodon. This phenomenon is observed in various organisms, including fish, where superoxide dismutase (SOD) genes exhibit differential expression between sexes (Bayır and Özdemir, 2023). In cichlid fishes, sex-specific gene expression is more pronounced in gonads than in the brain, with a trend towards male-biased expression, particularly in mouth-breeding species (Böhne et al., 2014). The higher expression levels of the *sod1, sod2,* and *sod3* genes in male fish across multiple tissues could be indicative of several underlying factors. Firstly, it may reflect inherent physiological differences between male and female Tetraodon individuals, possibly related to their reproductive roles or metabolic demands. For instance, male fish may require elevated antioxidant defenses in tissues such as the liver, intestine, muscle, and gonads to cope with oxidative stress associated with mating behaviors or territorial disputes. Additionally, the differential expression of the *sod*s genes in various tissues could be attributed to sex hormone-mediated regulatory mechanisms (Uzun and Bayır, 2023). Sex-biased genes often show elevated rates of protein sequence and gene expression divergence between species, which may be influenced by factors such as sexual selection and sexual antagonism (Grath and Parsch, 2016). These transcriptional differences can contribute to sex-specific traits and disease susceptibilities, highlighting the importance of considering sex as a biological variable in gene expression studies across tissues and species. Testosterone, for example, has been shown to influence antioxidant enzyme activity and gene expression in fish (Elsevar and Bayır, 2023). Therefore, the observed transcriptional differences may be linked to the modulatory effects of sex hormones on the *sod*s genes expression in male Tetraodon. Moreover, the *sod*s genes's role in protecting tissues from oxidative damage suggests potential functional implications of its differential expression between male and female fish. Elevated expression levels in male fish may confer greater antioxidant capacity and resilience to oxidative stress, which could be advantageous in environments characterized by fluctuating oxygen levels or exposure to environmental toxins. However, it's important to consider the limitations of the study, such as the sample size and potential confounding factors that were not accounted for. Further research, including experimental manipulation of sex hormone levels or environmental stressors, may provide deeper insights into the mechanisms underlying the observed transcriptional differences in *sods* genes expression between male and female Tetraodon individuals.

In conclusion, our findings highlight the complexity of sex-dependent regulation of antioxidant defenses in Tetraodon fish and underscore the importance of considering tissue-specific differences in gene expression when studying oxidative stress responses in vertebrates. Further investigation into the molecular mechanisms governing sod1 gene expression in different tissues and under various physiological conditions is warranted to elucidate its functional significance in antioxidant defense and overall health of Tetraodon populations.

Bioinformatics studies of *sod1, sod2,* and *sod3* genes in Tetraodon (*Tetraodon nigroviridis*)

Organisms share genetic closeness, identities, and similarities, enabling studies conducted on one species to serve as a model for different species. Consequently, conducting in silico analysis of sod genes in Tetraodon, a model organism in this study, will provide pivotal data for molecular investigations in other fish species.

Fish possess three isoforms of the sod gene: copper-zinc SOD, which is encoded by the sod1 gene; manganese SOD, encoded by the sod2 gene; and extracellular SOD, encoded by the sod3 gene (Sheraz et al., 2023). Previous research has indicated that teleost fish typically possess duplicated copies of numerous genes, a characteristic not commonly observed in other vertebrates, which usually have single copies of these genes (Braasch and Postlethwait, 2012; Taşbozan et al., 2022). *Tetraodon nigroviridis* genome has only one copy of the sod genes (*sod1, sod2,* and *sod3*), in contrast to the zebrafish, common carp (*Cyprinus carpio*), and goldfish (*Carassius auratus*) genomes, which have two copies of sod3 (*sod3a* and *sod3b*). The loss of *sod3a* in the tetraodon genome is thought to be due to non-functionalization, which is a common outcome in the variation of duplicated genes (Glasauer and Neuhauss, 2014; Bayır and Özdemir, 2023). A search of the Ensembl database revealed single copies of *sod1* and *sod2* genes not only in tetraodon but also in many other fish species, such as zebrafish, platyfish, Amazon molly, brown trout, common carp, fugu, Nile tilapia, goldfish, and stickleback. This finding suggests that the loss of other coppy of *sod*s genes in the tetraodon genome is not unique to this species and may be a common occurrence in various fish genomes. Asymmetrical selective pressure refers to the differential selection of gene copies, which can lead to the retention of specific copies while others are lost over time. Biased gene loss, on the other hand, involves the preferential loss of certain gene copies due to factors such as gene dosage, expression levels, or functional redundancy. This process can lead to an enrichment of specific gene functions, such as developmental, signaling, or behavioral genes, in certain species or lineages.

The search results indicate that the tetraodon identity/similarity rate of Sod1, Sod2, and Sod3 sequences with their respective orthologous is higher than with their respective paralogous. This is likely due to Sod/SOD polypeptides being related to the ancestral gene, and the phylogenetic tree shows a strong evolutionary relationship between tetraodon Sods and Sods/SODs from vertebrates, suggesting that tetraodon sods are orthologs of *sod*s/*SOD*s.

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AUTHORSHIP CONTRIBUTIONS

The manuscript, produced from Büşra Kaya's master thesis, involves collaborative contributions from the authors. Büşra Kaya was responsible for the literature review, drafting, writing, conducting laboratory experiments, and managing and analyzing data. In contrast, Mehtap Bayır contributed through conceptualization, drafting, writing, reviewing, editing, and supervision. All authors have reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICS APPROVAL

The research adhered to all relevant international, national, and institutional guidelines for the ethical care and use of animals.(Ankara University, Date: 30.07.2021/No: 177)

DATA AVAILABILITY

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

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Assessment of polonium-210 bioaccumulation in Mediterranean limpet *Patella caerulea* (Linnaeus, 1758) and sea urchin *Paracentrotus lividus* (Lamarck, 1816) from different coastal areas of Türkiye: Inclusion of a seasonal investigation

Türkiye'nin farklı kıyı bölgelerinden Çin şapkası, *Patella caerulea* (Linnaeus, 1758) ve deniz kestanesinde *Paracentrotus lividus* (Lamarck, 1816) polonyum-210 biyoakümülasyonunun mevsimsel olarak değerlendirilmesi

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Abstract: This study investigated the seasonal variations of polonium-210 (^{210}Po) activity concentrations in two marine invertebrate species: Mediterranean limpet *(P. caerulea)* and the sea urchin *(P. lividus)*. Seasonal sample collection was conducted across three Aegean and Sea of Marmara coastal stations from December 2018 to October 2019. The stations included İzmir-Urla, Karaburun, and İstanbul Island-Kınalıada. To assess the size-dependent bioaccumulation of ^{210}Po, individuals were categorized into size groups. The activity concentrations in both species exhibited seasonal fluctuations, ranging from 4.9 ± 3.4 Bq kg^{-1} dry weight to 28.0 ± 8.4 Bq kg^{-1} dry weight in Mediterranean limpets and 8.7 ± 6.1 Bq kg^{-1} dry weight to 58.0 ± 18.5 Bq kg^{-1} dry weight in sea urchins. The highest ^{210}Po activity concentrations were consistently observed in spring across all sampling locations.

Keywords: Polonium-210, bioaccumulation, environmental monitoring, marine invertebrates

Öz: Bu çalışma, iki deniz omurgasız türünde polonyum-210 (^{210}Po) aktivite konsantrasyonlarının mevsimsel değişimlerini incelemiştir: Çin şapkası *(Patella caerulea)* ve deniz kestanesi *(Paracentrotus lividus)*. Aralık 2018'den Ekim 2019'a kadar olan dönemde, Ege ve Marmara Denizi kıyılarında mevsimsel örnek toplama çalışmaları üç farklı istasyonda gerçekleştirildi. Bu istasyonlar İzmir-Urla, Karaburun ve İstanbul Adaları-Kınalıada olarak belirlendi. ^{210}Po'nun boyuta bağlı birikimini değerlendirmek için numuneler boy gruplarına ayrılmıştır. Her iki türdeki aktivite konsantrasyonları mevsimsel dalgalanmalar göstermiş olup, Çin şapkası örneklerinde 4,9 ± 3,4 Bq kg^{-1} kuru ağırlık ile 28,0 ± 8,4 Bq kg^{-1} kuru ağırlık arasında, deniz kestanelerinde ise 8,7 ± 6,1 Bq kg^{-1} kuru ağırlık ile 58,0 ± 18,5 Bq kg^{-1} kuru ağırlık arasında değişmiştir. Tüm örnekleme istasyonlarında en yüksek ^{210}Po aktivite konsantrasyonları sürekli olarak bahar aylarında gözlenmiştir.

Anahtar kelimeler: Polonyum-210, biyoakümülasyon, çevresel izleme, deniz omurgasızları

INTRODUCTION

Marine pollution presents a significant environmental challenge for developed nations, demanding global efforts for control and prevention. Contemporary strategies integrate traditional chemical analysis with biological indicators to assess the impact of pollution on living resources (Beiras et al., 2003). Among these biological tools, embryo-larval bioassays with marine invertebrates, particularly sea urchins and bivalves, are highly developed and extensively employed for global pollution monitoring and evaluation (His et al., 1999).

Sea urchins *(P. lividus)* have emerged as valuable tools for acute bioassays in marine pollution studies due to their sensitivity to pollutants (Dorey et al., 2018; Kobayashi, 1971, 1972, 1990, 1995; Warnau et al., 1996). As bioindicators, Mediterranean limpets *(P. caerulea)* hold particular significance. These widely distributed gastropods, known as Chinese hat shells, exhibit ideal characteristics for pollution monitoring. Their herbivorous diet, sedentary lifestyle on intertidal hard surfaces, and limited mobility simplify the interpretation of pollutant accumulation (Bu-Olayan and Thomas, 2001; Campanella et al., 2001; Cravo et al., 2002; Nakhlé et al., 2006; Pérez et al., 2019; Reguera et al., 2018; Storelli and Marcotrigiano, 2005). Additionally, their documented sensitivity to metal contamination has led to their widespread use in marine pollution monitoring programs (Reguera et al., 2018).

The ever-present threat of environmental pollution in coastal ecosystems necessitates the use of diverse indicator species for effective biomonitoring programs. This approach ensures a wider range of organisms can be utilized to detect a broader spectrum of potential toxic substances and exposure pathways. In this context, limpets (*Patella* spp.) have emerged as promising candidates for biomonitoring, prompting a comprehensive literature review. While not traditionally consumed for human food in Türkiye, limpets play a vital role in the marine food web as a primary food source for fish (Xu and Barker, 1990). They occupy intertidal rocky shores across the Mediterranean and Black Sea basins (Çulha and Bat, 2010).

Among marine contaminants, radioactive isotopes (radionuclides) are of particular concern. This study focuses on ^{210}Po, a naturally occurring radioisotope with a high alpha energy (5.3 MeV) and a relatively short half-life (138.4 days). Due to its bioaccumulation in marine organisms, ^{210}Po is the primary radionuclide responsible for internal radiation exposure in both marine life and seafood consumers (Carvalho et al., 2017; Hansen et al., 2022; Kül et al., 2020; Makmur et al., 2020; McDonald et al., 1986; Putri et al., 2022).

Several studies have shed light on the complex interplay between seasonal variations and bioaccumulation processes in marine invertebrates, particularly sea urchins. Lök and Köse (2006) identified peak gonad development in *P. lividus* during February and May, with gonads reaching up to 8.84%-8.97% of their body weight. These findings align with those of Rithu et al. (2022), who observed that fluctuations in ^{137}Cs activity within sea urchins mirrored dietary changes rather than variations in seawater concentrations. This suggests that seasonal changes in food availability and composition, coinciding with peak gonad development, may significantly influence radionuclide uptake. Reeves et al. (2019) further emphasized this complexity by demonstrating a link between uranium bioaccumulation and both seasonal variations in gonad quantity and protein content. This suggests that internal physiological factors, beyond simply dietary intake, can play a crucial role in radionuclide uptake and accumulation within sea urchins.

By combining these findings, we gain a more comprehensive understanding of the multifaceted nature of bioaccumulation in sea urchins. In general, seasonal variations in both external environmental factors and internal physiological states play a significant role in shaping radionuclide uptake and accumulation patterns (Brown et al., 2024) Further research is needed to fully elucidate the intricate interplay between these factors and their impact on the health and well-being of marine ecosystems.

Sánchez-Marín et al. (2022) explored the potential of limpets as substitutes for mussels in monitoring metal pollution. Their findings suggest that limpet-to-mussel metal concentration ratios can be employed to compare metal concentrations across different regions. This method has been shown to be effective for several metals (As, Cu, Hg, Pb, Cr, Ni, and Zn). However, a notable exception is cadmium (Cd), where no correlation was observed between limpet and mussel concentrations, likely due to differing feeding strategies or detoxification mechanisms for Cd in these organisms.

This study aims to:

1. Determine ^{210}Po concentrations in the sea urchin *(P. lividus)* and Mediterranean limpet *(P. caerulea)* from three coastal regions of Türkiye: İstanbul Adaları-Kınalıada, İzmir-Urla, and Karaburun.

2. Evaluate the obtained ^{210}Po data to assess the marine pollution status in these regions.

MATERIALS AND METHODS

Specimens of the Mediterranean limpet, *P. caerulea*, and the sea urchin, *P. lividus*, were collected from December 2018 to October 2019 across a total of three coastal stations (Figure 1). These stations included two locations in the Aegean Sea: İzmir-Urla (Latitude: 38.370760, Longitude: 26.793942) and Karaburun (Latitude: 38.428651, Longitude: 26.491126), and one station in the Sea of Marmara: İstanbul Island-Kınalıada (Latitude: 40.914077, Longitude: 29.058763). Kınalıada ranks as the fourth-smallest inhabited island amongst the Princes' Islands Archipelago. The Aegean region harbors numerous sprawling industrial centers and major urban populations. Additionally, intensive agricultural practices characterized by substantial fertilizer application are prevalent within the region. Furthermore, several rivers, including the Bakırçay, Gediz, and Menderes, act as conduits, discharging industrial and agricultural pollutants directly into the Aegean Sea.

Figure 1. The sampling locations

Field collections of Mediterranean limpet specimens were conducted from December 2018 to October 2019. For each experimental trial, 30–50 adult Mediterranean limpets were collected. Specimens were carefully detached from the substratum using a sterilized knife and transported alive to the laboratory.

Sea urchins were collected seasonally from December 2018 to October 2019. In each season, a sample of greater than fifty adult *P. lividus* individuals was collected from each designated sampling station. SCUBA diving techniques facilitated manual specimen collection at a depth ranging from one to two meters. Upon capture, live animals were promptly transported to the laboratory for processing. Following collection, field samples were transported to the laboratory in labeled plastic bags on ice to maintain sample integrity. Subsequently, the collected individuals were grouped based on their size measurements. Each sample underwent thorough cleaning with distilled water to eliminate any surface impurities. For each sampling season, thirty pooled samples of Mediterranean limpet and sea urchins were homogenized and subsequently divided into different groups based on sizes. Soft tissues from these subsamples were weighed and oven-dried at 70°C, followed by grinding and sieving through a 2 mm mesh. Three 1 g subsamples were prepared for analysis.

Following the addition of a standard polonium-209 tracer, complete sample dissolution was achieved through treatment with HCl and HNO_3. Subsequently, polonium underwent spontaneous electrodeposition onto copper discs immersed in 0.5 M HCl. Ascorbic acid's reducing ability is essential for converting ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), preventing thick plating and ensuring efficient deposition with high resolution (Baskaran, 2011, Flynn, 1968).

Quantification of ^{210}Po relied on the detection of its characteristic 5.30 MeV alpha particle emission. Importantly, an internal tracer consisting of polonium-209 (4.88 MeV alpha emission, $t_{1/2}$ = 109 years) was employed to ensure accuracy. Specific alpha activities were measured by Ortec Octete Plus spectrometry system. ^{209}Po (4.88 MeV alpha emission, $t_{1/2}$ = 103 years) was used as the internal tracer (Standard Reference Material 4326). The chemical yields using the ^{209}Po tracer ranged between 70 and 90%. The detection limit of the alpha spectrometry system is 0.0003 Bq.

The results were analyzed with one-way ANOVA test via IBM SPSS 23 statistical program and the Microsoft Excel packages. The differences were evaluated at the 5% significance level (P<0.05).

RESULTS

The ^{210}Po activity concentrations in the Mediterranean limpet *(P. caerulea)* and sea urchin *(P. lividus)* species samples are given in Figure 2, Figure 3. The average dry weight to wet weight ratios for the Mediterranean limpet *(P. caerulea)* and sea urchin *(P. caerulea)* species are 0.25 and 0.23, respectively.

The ^{210}Po activity concentrations in the Mediterranean limpet *(P. caerulea)* species samples varied between 4.9±3.4 Bq kg^{-1} dry weight – 28.0±8.9 Bq kg^{-1} dry weight.

For the Mediterranean limpet samples, while preliminary observations suggested highest values in spring and lowest in summer for all stations, a more detailed examination incorporating error bars indicates a different pattern. Specifically, the lowest concentrations were detected in both autumn and summer, whereas the highest values occurred during winter and spring. Furthermore, a notable exception to this trend was observed for Mediterranean limpets in the 4 cm-<5 cm size category at Kınalıada station, which exhibited elevated ^{210}Po levels in autumn. A one-way ANOVA was employed to determine if significant differences existed in specific ^{210}Po activity concentrations among stations, seasons, and size categories. Statistical analysis indicated no significant correlation (P > 0.050) between specific ^{210}Po activity concentrations and station or size. However, there was a significant correlation (p < 0.000) between ^{210}Po activity concentrations and season. Descriptive statistics of ^{210}Pb activity concentrations in *P. caerulea* individuals are given in Table 1. To the best of our current knowledge, no prior studies have reported ^{210}Po activity concentrations in Mediterranean limpet samples.

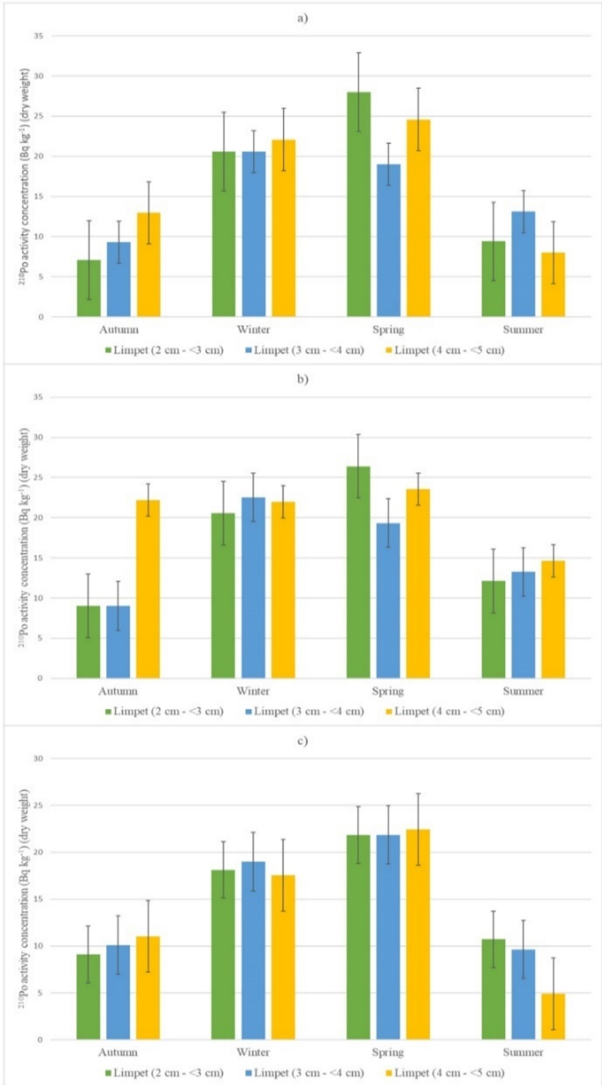

Figure 2. a) Seasonal variations of the ^{210}Po activity concentrations in the Mediterranean limpet *(P. caerulea)* samples Urla station, b) Seasonal variations of the ^{210}Po activity concentrations in the Mediterranean limpet *(P. caerulea)* samples Kınalıada station, c) Seasonal variations of the ^{210}Po activity concentrations in the Mediterranean limpet *(P. caerulea)* samples Karaburun station

The ^{210}Po activity concentrations in the sea urchin *(P. lividus)* species samples varied between 8.7±6.1 Bq kg^{-1} dry weight – 58.0±18.6 Bq kg^{-1} dry weight.

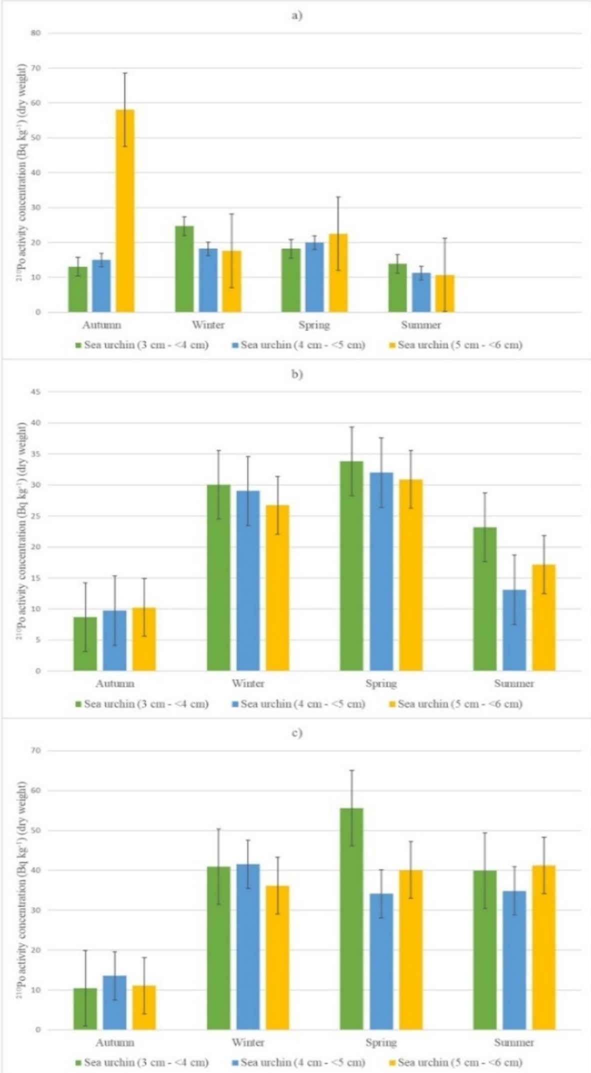

Figure 3. a) Seasonal variations of the ^{210}Po activity concentrations in the Sea urchin *(P. lividus)* samples Urla station, b) Seasonal variations of the ^{210}Po activity concentrations in the Sea urchin *(P. lividus)* samples Kınalıada station, c) Seasonal variations of the ^{210}Po activity concentrations in the Sea urchin *(P. lividus)* samples Karaburun station

Table 1. Descriptive statistics of *P. caerulea* ^{210}Pb activity concentrations and stations, seasons, sample sizes

Station	Mean	±SE	±SD	Minimum	Maximum	P
Urla	16.2192	2.04853	7.0963	7.1	28	
Kınalıada	17.8825	1.72596	5.9789	9.01	26.40	0.482
Karaburun	14.6950	1.75007	6.0624	4.91	22.45	
Season	**Mean**	**±SE**	**±SD**	**Minimum**	**Maximum**	**P**
Autumn	11.0899	1.48950	4.4685	7.1	22.21	
Winter	20.3289	0.59014	1.7704	17.56	22.54	0.000*
Spring	23.0010	0.99823	2.9947	19	28	
Summer	10.6424	1.00914	3.0274	4.91	14.64	
Sample Size	**Mean**	**±SE**	**±SD**	**Minimum**	**Maximum**	**P**
2 cm-<3 cm	16.0808	2.12171	7.3498	7.1	28	
3 cm-<4 cm	15.5584	1.53148	5.3052	9.01	22.54	0.829
4 cm-<5 cm	17.1574	1.93192	6.6924	4.91	24.58	

*There are statistically differences between the ^{210}Pb activity concentrations and stations, seasons sample sizes; Mean, average ^{210}Pb activity concentration, SE, Standard Error; SD, Standard Deviation; P, Significance

The highest ^{210}Po activity concentrations in sea urchin (*P. lividus*) samples were observed in individuals measuring 5-6 cm in the autumn at Urla station. While lower concentrations were found in other size categories, the data indicate a clear peak in this particular group during this season. Conversely, the lowest ^{210}Po activity concentrations were recorded in autumn at Kınalıada station. No significant correlation ($p > 0.05$) was observed between specific ^{210}Po activity concentrations and seasonal variations or sample sizes within the sea urchin population. A significant difference ($p < 0.029$) in ^{210}Po concentrations was observed among stations for sea urchin samples. Descriptive statistics of ^{210}Pb activity concentrations in *P. lividus* individuals are given in Table 2. In a study conducted by Hurtado-Bermúdez et al. (2019) on Sea Urchin samples collected in Spain, the authors reported ^{210}Po activity concentrations ranging from 38 to 61.5 Bq kg^{-1} dry weight.

Table 2. Descriptive statistics of *P. lividus* ^{210}Pb activity concentrations and stations, seasons, sample sizes

Station	Mean	±SE	±SD	Minimum	Maximum	P
Urla	20.2742	3.64895	12.6404	10.72	58.01	
Kınalıada	22.0579	2.78508	9.6478	8.69	33.81	0.029*
Karaburun	33.2957	4.07404	14.1129	10.44	55.58	
Season	**Mean**	**±SE**	**±SD**	**Minimum**	**Maximum**	**P**
Autumn	16.6537	5.21246	15.6404	8.69	58.01	
Winter	29.4444	2.92950	8.7885	17.62	41.55	0.057
Spring	31.9187	3.82481	11.4744	18.25	55.58	
Summer	22.8202	4.18922	12.5677	10.72	41.23	
Sample Size	**Mean**	**±SE**	**±SD**	**Minimum**	**Maximum**	**P**
3 cm-<4 cm	26.0396	4.16800	14.4383	8.69	55.58	
4 cm-<5 cm	22.7065	3.16645	10.9622	9.73	41.55	0.729
5 cm-<6 cm	26.8817	4.29628	14.8828	10.27	58.01	

*There are statistically differences between the ^{210}Pb activity concentrations and stations, seasons sample sizes; Mean, average ^{210}Pb activity concentration, SE, Standard Error; SD, Standard Deviation; P, Significance

DISCUSSION

The study investigated the bioaccumulation of Polonium-210 (^{210}Po) in two marine invertebrate species: the Mediterranean limpet (*P. caerulea*) and the sea urchin (*P. lividus*). This could be attributed to seasonal changes in phytoplankton abundance, a primary food source for sea urchins known to accumulate these radionuclides. It is important to acknowledge, however, that limitations in our understanding of uptake pathways and bioaccumulation capacities for PAHs prevent a definitive attribution of the observed peak spring concentration solely to biological and physiological processes. (Bartolomé et al., 2011) reported a similar seasonal pattern in PAH concentration profiles for other sentinel organisms like mussels and oysters, suggesting broader environmental factors may be at play.

The review process, encompassing 88 studies identified on the Web of Science platform, further strengthens the case for limpet suitability in biomonitoring programs. Numerous field studies have documented the capacity of limpets to accumulate both metals and hydrocarbons. In many cases, a clear link exists between the level of a pollutant in the surrounding environment and the corresponding body content of the pollutant within the limpet's soft tissues. Additionally, research has revealed various physiological responses in limpets exposed to pollutants. These responses include DNA damage induction, metallothionein induction, oxidative stress, reduced Neutral Red retention, and variations in heart rate. While some Patella species exhibit varying responses to disturbances (e.g., oil spills, wastewater discharge), the overall trend suggests their sensitivity is comparable to, or even surpasses, that of mussels. This, coupled with their demonstrated ability to accumulate pollutants, makes limpets strong candidates for inclusion as sentinel organisms in regional monitoring plans (Reguera et al., 2018).

There was an observed seasonal variation in the activity concentrations of ^{210}Po for both Mediterranean limpet and sea urchin species. Higher activity concentrations were consistently detected in samples collected during the springtime. However, these seasonal variations were not statistically significant. This likely reflects seasonal changes in phytoplankton abundance, a primary food source known to accumulate these radionuclides. Existing research suggests that internal factors beyond diet, such as gonad development cycles, may influence radionuclide uptake in sea urchins. A comprehensive understanding of bioaccumulation in marine organisms like sea urchins is critical for assessing the health of marine ecosystems. Seasonal variations in both environmental factors (e.g., food availability) and internal physiological states significantly impact radionuclide uptake patterns. Further research is needed to fully elucidate the complex interplay between environmental factors, internal physiology, and their impact on radionuclide bioaccumulation in marine organisms. Investigating the impact of anthropogenic activities, such as pollution from disused mines, on marine life using sensitive bioassays can be valuable for environmental monitoring and mitigation efforts (Jewel et al., 2002; Santhanabharathi et al., 2023; Stewart et al., 2008; Thiessen at al., 1999). While not traditionally consumed in Türkiye, limpets (*Patella* spp.) serve as a vital food source for fish. Their declining populations due to pollution and habitat loss necessitate further investigation to ensure their long-term sustainability.

This study documented elevated ^{210}Po concentrations within both *Paracentrotus lividus* and *Patella caerulea* during the spring and winter seasons. It is noteworthy that the observed elevation in ^{210}Po levels during spring did not achieve statistical significance. Seasonal fluctuations in phytoplankton abundance, a primary food source for these sea urchins and known concentrators of ^{210}Po, may be a contributing factor. The timing of sea urchin spawning is hypothesized to be strategically linked to peak phytoplankton blooms, ensuring a readily available food source for their developing offspring. This coincides with a seasonal cycle where spring ushers in phytoplankton blooms, followed by sea urchin reproduction in spring/summer to capitalize on this abundance (Padilla-Gamiño et al., 2022; Peck et al., 2005). Phytoplankton declines in summer/fall as nutrients are depleted, and winter finds sea urchins utilizing alternative food sources or entering a period of reduced activity (Khaili et al., 2024). However, limitations in our current understanding of PAH uptake pathways and bioaccumulation capacities in these species hinder a definitive attribution of the observed springtime peak solely to biological processes (Bartolomé et al., 2011). Bartolomé et al. (2011) reported a similar seasonal trend in PAH concentrations within other sentinel organisms, such as mussels and oysters, suggesting the influence of broader environmental drivers.

A review conducted by (Reguera et al., 2018) analyzing 88 studies identified on the Web of Science platform strengthens the argument for the suitability of limpets in biomonitoring programs. Extensive field research has documented the ability of limpets to bioaccumulate both metals and hydrocarbons (Pérez et al., 2019; Viñas et al., 2018). This bioaccumulation often demonstrates a positive correlation between the level of a pollutant in the surrounding environment and the corresponding concentration found within the limpet's soft tissues (Nuñez et al., 2012). Additionally, research has revealed various physiological responses in limpets exposed to pollutants, including DNA damage induction, metallothionein induction, oxidative stress, reduced Neutral Red retention, and variations in heart rate (Prusina et al., 2014; Sun et al., 2023; Virgin and Schiel, 2023). While some Patella species may exhibit differing sensitivities to specific disturbances (e.g., oil spills, wastewater discharge), the overall trend suggests their sensitivity to pollutants is comparable to, or potentially exceeds, that of mussels (Viñas et al., 2018). This, combined with their documented bioaccumulation capabilities, positions limpets as strong candidates for inclusion as sentinel organisms in regional monitoring plans.

CONCLUSIONS

This study highlights the importance of multi-faceted research approaches in understanding and managing marine ecosystems. By combining investigations of dietary influences on bioaccumulation with monitoring of pollutant impacts, we can ensure the well-being of these ecologically and economically valuable marine organisms.

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AUTHORSHIP CONTRIBUTIONS

Duygu Arslantürk: Field work, lab analysis, formal analysis, methodology. Aysun Uğur Görgün: Conceptualization, formal analysis, methodology, funding acquisition, investigation, project administration, resources, supervision, writing - review & editing, Işık Filizok: Conceptualization, investigation, validation, writing - review & editing.

CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

ETHICS APPROVAL

Ethics Committee approval certificate is not required for materials used in this study. For this reason, Ethics Committee Certificate was not obtained in this study.

DATA AVAILABILITY

All relevant data is in the article.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Enhancing goldfish reproduction: Role of substrates in optimizing fertilization and hatching rates under controlled conditions

Japon balığı üremesinin iyileştirilmesi: Kontrollü koşullar altında döllenme ve yumurtadan çıkma oranlarının optimize edilmesinde substratların rolü

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Abstract: Ornamental fish production is significantly impacted by whether fish spawn naturally under controlled conditions. Therefore, goldfish (*Carassius auratus*) were allowed to breed naturally using various types of substrates to investigate their effects on ovulation, fertilization, and hatching rates in an experimental setup. The goldfish were subjected to five different substrate treatments: T1 (water hyacinth), T2 (jute rope), T3 (polythene), T4 (net), and T5 (no substrate). The optimal pH and dissolved oxygen levels for goldfish spawning were found to be 7.14 to 7.24 and 5.65 to 6.22 mg/L, respectively. Results indicated that the highest number of eggs (356.66±40) was observed in the polythene substrate (T3), while no eggs were found in the absence of substrate (T5). The polythene substrate also yielded the highest fertilization rate (93%) and hatching rate (95.01%). Notably, goldfish exhibited no spawning behavior without any substrate, suggesting that substrate may act as both a spawning substrate and an essential cue for ovulation in goldfish. Furthermore, the study's findings support the recommendation of goldfish as substrate breeders. This research offers valuable insights for small-scale fish farmers, entrepreneurs, and hatchery owners looking to enhance goldfish spawning techniques in aquarium settings.

Keywords: Goldfish, substrate, ornamental fish, fertilization, hatching

Öz: Süs balığı üretimi, balıkların kontrollü koşullar altında doğal olarak yumurtlayıp yumurtlamadıklarından önemli ölçüde etkilenir. Bu nedenle, Japon balıklarının (*Carassius auratus*) yumurtlama, döllenme ve yumurtadan çıkma oranları üzerindeki etkilerini araştırmak için çeşitli substrat türleri kullanılarak doğal yollarla üremelerine izin verilmiştir. Süs balığı üretimi, balıkların kontrollü koşullar altında doğal olarak yumurtlayıp yumurtlamadıklarından önemli ölçüde etkilenir. Bu nedenle, Japon balıklarının (*Carassius auratus*) yumurtlama, döllenme ve yumurtadan çıkma oranları üzerindeki etkilerini araştırmak için çeşitli substrat türleri kullanılarak doğal yollarla üremelerine izin verilmiştir. Sonuçlar, en yüksek yumurta sayısının (356,66±40) polietilen substratta (T3) gözlendiğini, substrat yokluğunda (T5) ise hiç yumurta bulunmadığını göstermiştir. Polietilen substrat aynı zamanda en yüksek döllenme oranını (%93) ve kuluçka oranını (%95,01) vermiştir. Özellikle, Japon balıkları herhangi bir substrat olmadan yumurtlama davranışı sergilememiştir, bu da substratın Japon balıklarında hem yumurtlama substratı hem de yumurtlama için önemli bir ipucu olarak işlev görebileceğini düşündürmektedir. Ayrıca, çalışmanın bulguları Japon balıklarının substrat yetiştiricileri olarak önerilmesini desteklemektedir. Bu araştırma, akvaryum ortamlarında Japon balığı yumurtlama tekniklerini geliştirmek isteyen küçük ölçekli balık yetiştiricileri, girişimciler ve kuluçkahane sahipleri için değerli bilgiler sunmaktadır.

Anahtar kelimeler: Japon balığı, substrat, süs balığı, döllenme, kuluçka

INTRODUCTION

In recent times, a growing number of people across various societal segments have embraced the practice of maintaining aquariums in commercial, public, and residential settings. This trend highlights the potential of domestic ornamental fish production to contribute significantly to export revenues and foster cost savings (Rahaman et al., 2011). Bangladesh is renowned for its extensive inland water bodies and diverse indigenous fish species (Ali et al., 2017; Mia et al., 2017), ranging from large to small. Some indigenous species like *Trichogaster fasciata*, *T. lalia*, *Badis badis*, *Esomus danricus*, and *Ompok bimaculatus* have been popular choices for aquariums or ornamental purposes. Additionally, several exotic species have been imported from other countries. The goldfish (*Carassius auratus*) stands out as the most widespread cyprinid fish in freshwater aquariums globally.

In contrast, both common carp and goldfish have become invasive on a global scale (Chan et al., 2019; Halas et al., 2018). Their life history strategies, including broad feeding habits, high reproductive rates (Tang et al., 2020), early maturation, rapid growth compared to native species (Jones and Stuart, 2009; Morgan and Beatty, 2007), and tolerance to extreme environmental conditions (Tang et al., 2020), contribute to their success in unfamiliar habitats. These fish are generally hardy, peaceful towards other tank inhabitants, and well-suited for aquarium environments. Originating from the Prussian gibel carp (*Carassius gibelio*), goldfish were first domesticated in China around 1000 AD and are native to China (Komiyama et al., 2009; Vasil'eva and Vasil'ev, 2000). Breeders have developed a variety of ornamental goldfish breeds with distinctive features such as fringed, veil, or finned tails, double or triple fins, and bulging "telescope" eyes. Many of these varieties, known as scaled goldfish, exhibit metallic hues ranging from scarlet, gold, and white to silver or black.

The physiological and behavioral responses of certain aquarium fish can be significantly influenced by the presence of substrate. When deprived of substrate, some species may exhibit immobility, indicating unmet behavioral needs (Smith and Gray, 2011; Galhardo et al., 2008; Stenberg and Persson, 2005). Substrate plays a crucial role in facilitating egg adhesion during spawning in aquarium environments. Haniffa et al. (2007) demonstrated the use of substrate for breeding koi carp, where it served both as a hiding place and a surface for egg attachment. Certain fish species naturally deposit their eggs on the tank floor; without substrate, these eggs are vulnerable to predation by mature fish. The introduction of variegated substrate helps to camouflage the eggs, and larger substrate sizes can provide protective gaps where eggs can settle securely. Goldfish eggs, known for their transparency and adhesive properties, typically adhere to aquatic vegetation (Battle, 1940).

In Bangladesh, the number of well-established goldfish hatcheries is quite limited, with most breeding operations conducted on a small scale. Goldfish are valued as experimental subjects due to their ability to adapt well to various environmental conditions (Battle, 1940). This study aims to explore specific aspects of natural reproduction within Bangladesh's ecological context. While artificial breeding methods for goldfish are established, understanding substrate preferences for their natural reproduction remains a new area of investigation. The use of substrate may enhance ovulation in goldfish, providing a simpler and cost-effective alternative to induced reproduction methods. Therefore, this study aims to develop a protocol for the controlled production of goldfish larvae under confined conditions, focusing on identifying an optimal substrate that promotes successful ovulation, fertilization, and hatching of goldfish.

MATERIALS AND METHODS

Study area

The three-month study took place in the wet laboratory of the Faculty of Fisheries, BAU, Mymensingh, Bangladesh, spanning from February to April.

Accumulation of specimens

Adult goldfish were sourced from various pet shops in the Katabon market, Dhaka city. Males and females were collected in groups of twenty pairs for breeding purposes. Two oxygenated polythene containers were used to transport these pairs, with each container accommodating ten pairs of fish. The broods exhibited colors ranging from red, orange, yellow, to black. Fish were acclimatized by submerging them in water within poly sacks for two hours. After conditioning, the fish were transferred to a glass aquarium for further rearing, where they were fed twice daily and had their water changed once daily. After seven days of acclimatization, the brood fish were moved to the breeding aquarium.

Determination of broods

Male and female goldfish were distinguished based on specific physical characteristics, such as abdominal condition, pigmentation of genital organs, and their ability to release sperm or eggs when gently pressed on the lower abdomen. Females could be identified by the presence of abdominal edema (43.35±2.2g), while mature males typically exhibited a slender physique and flattened abdomens (36.52±1.8g) (Figure 1).

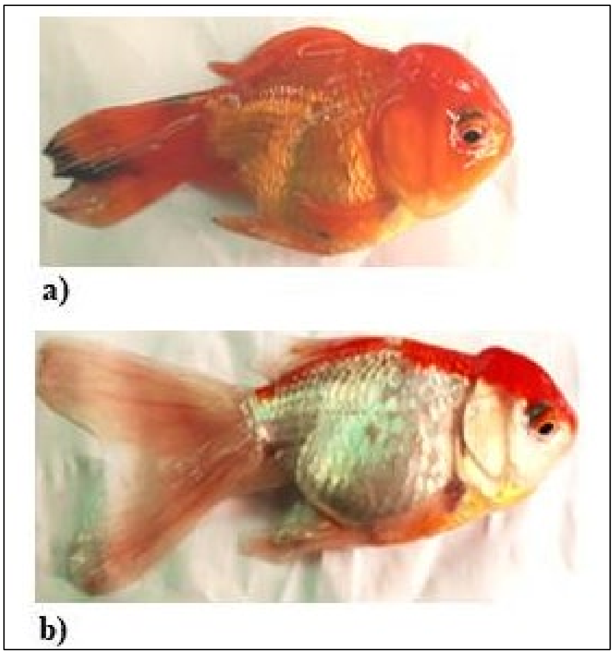

Figure 1. Broods of goldfish (a) Mature male and (b) Gravid female

Selection of substrates for spawning

Four types of floating substrates were employed in the study, namely water hyacinth, polythene, net, and jute rope (Figure 2). Goldfish naturally deposited their eggs near these floating substrates, which effectively adhered to their surfaces due to their sticky and adhesive nature. The presence of substrate induced ovulation without the need for external stimulation. The substrates were inspected twice daily to monitor egg deposition. To prevent filial cannibalism, brood fish were promptly removed from the breeding tank after spawning.

The breeding tank preparation

The spawning tanks were assembled using fifteen plastic drums. Prior to each drum being used in the experiment, they underwent a thorough cleaning process involving washing with detergent, rinsing with tap water, and air-drying. Each drum had a water capacity of 80 liters. To simulate natural conditions, a plastic hose was installed in each breeding tank for water circulation. At the base of each container, an outlet was created to facilitate drainage of excess water. The drums were arranged in a row and filled with ground water. Additionally, an aerator was affixed to each drum to ensure adequate oxygen supply.

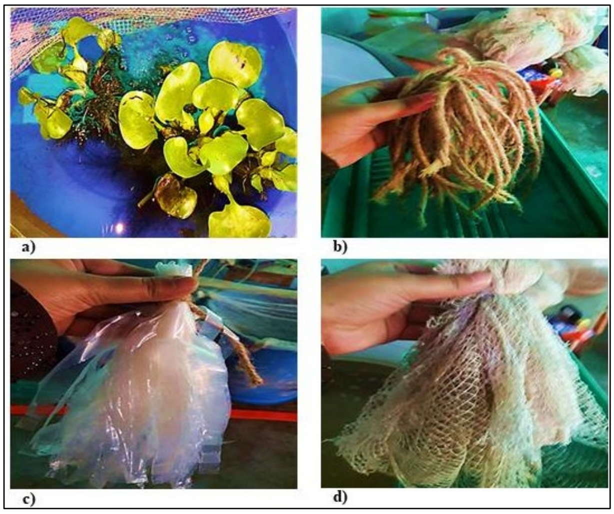

Figure 2. Photographs showing substrates used for breeding (a) water hyacinth, (b) jute rope, (c) polythene and (d) net

Experimental design

Goldfish spawning was evaluated across five different experimental conditions using paired adult broods at a 1:1 ratio. The five conditions were considered as different treatments. Fish were assigned to substrates such as water hyacinth, jute rope, polythene, net, or no substrate (control), and were labeled as T1, T2, T3, T4, and T5, respectively. Three replicates were conducted for each treatment.

Observation of spawning behavior

The male and female goldfish were placed together in a spawning drum. During this period, the female laid a large number of eggs, which adhered to the substrates. The male released sperm to fertilize the eggs. The substrates of each treatment were inspected daily, and after spawning, the breeders were promptly removed. The eggs were then left to hatch in the breeding drum. Both male and female fish were housed together in the spawning tank, allowing them to naturally release eggs and sperm for fertilization in the plastic drums.

Determination of fertilization rate

To assess the fertilization rate, the eggs were examined approximately 1 to 2 hours after collection. Water samples from the base of the plastic drums were transferred onto a small steel plate for inspection. Using a microscope, we distinguished between fertilized and unfertilized eggs based on their appearance. Fertilized eggs typically displayed a transparent shell with a grey or black patch inside, whereas unfertilized eggs appeared opaque. We calculated the fertilization rate using the following formula.

$$\text{Fertilization rate (\%)} = \frac{\text{Number of fertilized eggs}}{\text{Total no.of egg}} \times 100$$

Collection of fertilized eggs and incubation

The fertilized eggs were transferred to a container designed for hatching, ensuring continuous water flow. After 48 hours of fertilization, the eggs were removed from the incubator and left in the container for three days without feeding, allowing absorption of the yolk sac. To promote optimal larval growth, the hatchlings were subsequently transferred to a circular tank with gentle water circulation. Water temperatures were kept between 20–23ºC using a NETONDA Aquarium Heater 50 W Heating Rod, while air temperatures were ranged around 23–26ºC throughout the period. The water circulation rate was maintained slowly using an aerator, ensuring even distribution of heat and consistent temperature throughout the water body. The hatchlings were fed commercial powder feed (Nova, Osaka, turtle) twice daily.

Determination of hatching rate

The hatching rate was calculated by visually counting the number of fertilized eggs in the samples and the resulting hatchlings. After counting, the hatchlings were removed from the hatching jar. The hatching rate was determined using the following formula:

$$\text{Hatching rate (\%)} = \frac{\text{No.of Hatchlings}}{\text{Total no.of fertilized eggs}} \times 100$$

Measurement of water quality parameter

Twice daily, we monitored water temperature, dissolved oxygen (DO), and pH levels to maintain water quality. Data were reported as mean averages (Mean±SD) for consistency. A pH meter (Hanna ISO 9001) was used to measure pH levels, while temperatures were recorded using a mercury thermometer and dissolved oxygen levels with a meter (Lutron DO-5510). Water quality parameters in all spawning tanks were carefully maintained within the optimal ranges outlined in Table 1.

Table 1. Water quality parameters in the different treatments throughout the experimental period

Months	Treatments	Temperature (ºC)	pH	Dissolved oxygen (mg/l)
February	T1	22.0±1.10	7.16±0.6	5.65±0.33
	T2	22.0±0.70	7.24±0.5	6.03±0.56
	T3	22.5±0.51	7.17±0.4	5.95±0.30
	T4	21.5±0.8	7.18±0.27	5.99±0.65
	T5	22.2±0.5	7.20±0.1	6.23±0.23
March	T1	22.0±1.10	7.11±0.09	5.85±0.27
	T2	22.0±0.70	7.04±0.7	6.05±0.65
	T3	22.5±0.5	7.07±0.19	5.89±0.32
	T4	21.5±0.8	7.14±0.17	5.92±0.63
	T5	22.2±0.5	7.11±0.21	6.20±0.29
April	T1	22.0±1.10	7.12±0.16	5.62±0.34
	T2	22.0±0.70	7.29±0.05	6.08±0.58
	T3	22.5±0.5	7.13±0.14	5.90±0.30
	T4	21.5±0.8	7.12±0.21	5.90±0.71
	T5	22.2±0.5	7.20±0.13	6.26±0.20

Data analysis

The data were analyzed using SPSS software (IBM® SPSS® Inc., IL, USA, version 20). Shapiro-Wilk's and Levene's tests were employed to assess variance normality and homogeneity. The results are presented as mean ± standard deviation. Differences among treatments were evaluated using one-way analysis of variance (ANOVA) at a significance level of $p<0.05$, with subsequent comparisons made using Duncan's post hoc test.

RESULTS

Courtship and spawning behavior

At the bottom of the spawning tank, courtship behaviors were observed from both male and female goldfish. Males frequently followed and gently nudged the females. During courtship, males displayed a distinctive behavior of circling around the female to keep her in place. The spawning process commenced a few days after initial courtship attempts. During spawning, males continued to follow females around the tank. After a period of two or three hours, females released their eggs. Males then nudged the females to position them over the substrate where the eggs were laid. Males subsequently released their milt to externally fertilize the adhesive eggs. Figure 3 illustrates the process where males fertilized each batch of eggs immediately upon release.

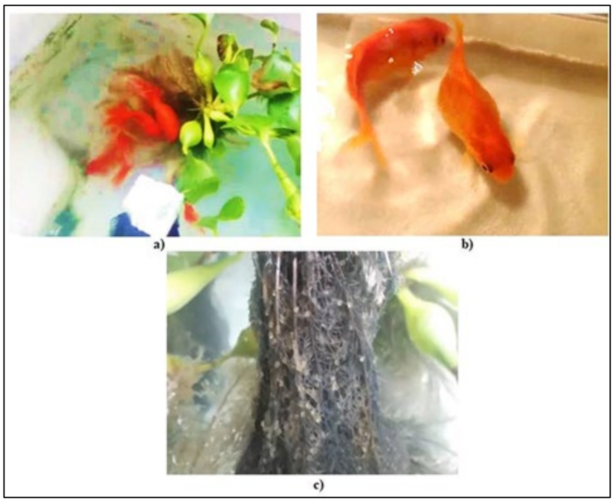

Figure 3. Photographs showing breeding behavior of goldfish; (a) male chase the female, (b) male hitting the vent of female and (c) adhesive eggs at the root of water hyacinth

Fertilization and hatching

Introduction of different substrates in treatments T1, T2, T3, and T4 markedly enhanced the fertilization rate of goldfish (Figure 4). In contrast, goldfish in the absence of substrate (T5) did not ovulate. Among the treatments, the highest number of eggs was observed in treatment T3 (356.67±40.41), while treatment T5 recorded no eggs (0±0). The mean number of eggs in treatment T3 was significantly ($p<0.05$) higher than in other treatments.

Hatching, the process of emerging from the egg envelopes (chorion), marks a critical environmental change in a fish's life. This transition is typically considered the boundary between the embryonic and larval stages. The mean numbers of hatchlings observed were 92.33±20.52, 193.33±14.15, 316.33±51.63, and 112.33±17.78 in treatments T1, T2, T3, and T4, respectively. Treatment T3 recorded the highest number of hatchlings, whereas no hatching was recorded in treatment T5 (Figure 5). Significant differences were found among treatments ($p<0.05$).

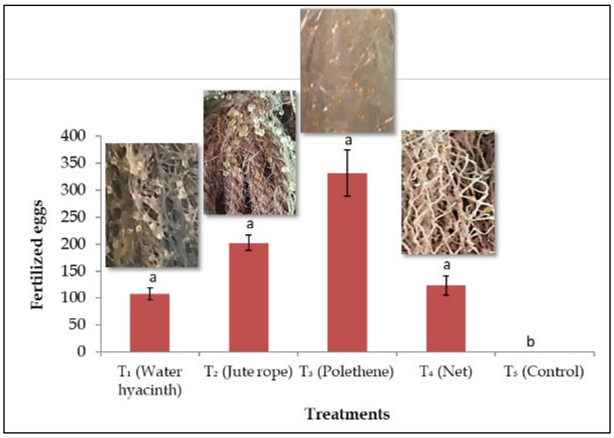

Figure 4. Effect of substrates on fertilized eggs in different treatments. Different superscripts of letters showed significant differences ($p<0.05$)

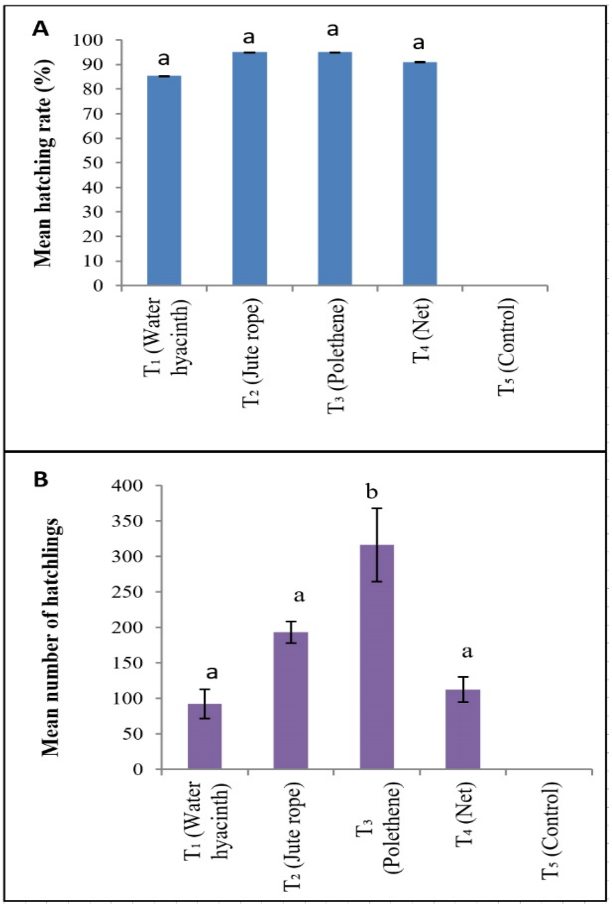

Figure 5. Effect of substrates on mean hatching rate (A) and mean number of hatchling (B) in different treatments. Different superscript letters (a and b) on the bars showed significant differences among treatments ($p<0.05$).

DISCUSSION

This study elucidates the impact of different substrates on goldfish reproduction under controlled conditions, highlighting their significant role in enhancing ovulation, fertilization, and hatching rates. The findings demonstrate that the choice of substrate is crucial for optimizing goldfish breeding, which is essential for ornamental fish production, especially in small-scale operations and hatcheries.

Courtship behavior encompasses the various interactions between male and female goldfish leading up to fertilization. In this study, males displayed a range of courtship behaviors, such as following, nudging, and circling females to position them for spawning. These behaviors, including the patterns of chasing and nudging observed, align with findings from Sharma et al. (2011); Kobayashi et al. (2002); and DeFraipont and Sorensen (1993). The courtship ultimately led to spawning, where females released eggs that were immediately fertilized by the males as they were deposited on substrates. The males' role in nudging females to ensure eggs were deposited on suitable substrates, such as the roots of water hyacinth, and the immediate fertilization of these eggs, underscore the critical interaction between behavior and substrate in goldfish reproduction. This behavior not only facilitates egg adhesion but also ensures that fertilization occurs efficiently, emphasizing the importance of substrate presence in optimizing breeding success.

The results underscore that substrates play a pivotal role in stimulating ovulation in goldfish. Specifically, the polythene substrate proved to be the most effective, resulting in the highest number of eggs (356.67±40.41), a fertilization rate of 93%, and a hatching rate of 95.01%. These outcomes are significantly higher compared to other substrates used and the control group with no substrate. The absence of a substrate led to a complete lack of egg deposition, indicating that substrates are essential not only for the physical attachment of eggs but also as a necessary cue for ovulation. The role of substrates in facilitating egg adhesion and subsequent fertilization aligns with previous research. Myriam et al. (2022) and Haniffa et al. (2007) highlighted the importance of substrates in koi carp breeding, where substrates provided both a physical surface for eggs and camouflage to protect them from predation. Similarly, our findings suggest that the goldfish's reproductive success is highly dependent on the presence of suitable substrates, which facilitate the deposition of eggs and enhance their fertilization.

Among the substrates tested, polythene emerged as the most effective, followed by jute rope, water hyacinth, and net. The high performance of polythene could be attributed to its smooth, non-absorbent surface, which likely provided an ideal environment for eggs to adhere and be fertilized. Jute rope, water hyacinth, and net substrates also supported successful reproduction, though to a lesser extent. The differences in effectiveness among substrates could be due to variations in surface texture, buoyancy, and how well these materials simulate natural conditions for egg attachment. These findings align with the observations of Hawkins et al. (2021), and Smith and Gray (2011), who highlighted that substrate characteristics play a crucial role in determining spawning behavior and success. The smooth and consistent surface of polythene might have provided a more stable and secure environment for eggs compared to the more variable surfaces of jute rope, water hyacinth, and net.

The hatching rate was notably high for the polythene substrate, which could be a result of both effective fertilization and optimal egg conditions provided by the substrate (Smith and Gray, 2011). In contrast, the absence of substrate resulted in no hatching, further emphasizing the necessity of substrates for successful egg development and hatching. This result confirms that substrates not only influence ovulation and fertilization but also play a crucial role in the early stages of egg development. Maintaining optimal water quality parameters such as pH, dissolved oxygen, and temperature was essential for successful spawning and hatching (Arindam et al., 2018; Myriam et al., 2022; Mottaa et al., 2023). Our study adhered to the optimal ranges of these parameters, ensuring a conducive environment for goldfish reproduction. Variations in pH and dissolved oxygen among treatments were statistically significant but did not impact the overall reproductive success when substrates were present.

The results provide valuable insights for ornamental fish hatcheries and small-scale fish farmers. Selecting the appropriate substrate can significantly enhance the efficiency of goldfish breeding programs. Polythene, due to its superior performance, could be recommended for use in breeding setups aiming to maximize egg production, fertilization, and hatching rates. However, it is important for hatcheries to consider the cost and availability of substrates, as well as their suitability for specific breeding environments.

CONCLUSION

In conclusion, this study emphasizes the pivotal role that substrates play in enhancing the reproductive success of goldfish in controlled environments. The research revealed that various substrates—such as polythene, jute rope, and water hyacinth—significantly improved ovulation, fertilization, and hatching rates compared to conditions without substrates. This underscores the importance of incorporating substrates to better replicate natural spawning conditions, which is crucial for successful goldfish breeding. Among the substrates tested, transparent polythene was found to be the most effective. Its ability to camouflage eggs and facilitate fertilization likely contributed to its superior performance. These findings not only advance our understanding of goldfish reproduction but also offer practical insights for optimizing breeding protocols.

The advantages of incorporating suitable substrates are clear: they can enhance reproductive outcomes and create a more natural environment for the fish, potentially leading to higher survival rates and healthier offspring. However, there are some considerations to keep in mind. The choice of substrate can affect maintenance and cleaning routines, as well as the overall management of the aquarium. Moreover, while substrates like polythene and jute rope proved beneficial, the long-term impacts of their use on water quality and fish health warrant further investigation. Overall, this study provides valuable guidance for ornamental fish production, highlighting sustainable practices that can improve breeding success. It also suggests potential applications in aquaculture where optimizing spawning conditions can lead to more efficient and effective fish production systems.

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AUTHORSHIP CONTRIBUTIONS

Asma Jaman: Conceptualization, methodology, writing original draft preparation. Umme Ohida Rahman: Data analysis, validation, writing- review and editing. Nahid Sultana Lucky: Reviewing and editing. Md. Sadiqul Islam: Conceptualization; supervision; writing; reviewing & editing.

ETHICAL APPROVAL

All procedures for experiments involving humans and animals (fish) adhered to the ethical standards set by the Ethical Committee of Bangladesh Agricultural University, Mymensingh. Additionally, all survey participants provided informed consent.

STATEMENTS AND DECLARATIONS

The authors declare of no competing interests. The authors alone are responsible for the content and writing of the paper.

DATA AVAILABILITY

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

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Fish species composition and seasonal variations in Lake Sapanca and its tributaries

Sapanca Gölü ve kollarındaki balık tür kompozisyonu ve mevsimsel değişimler

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Abstract: The aim of this study was to determine the current fish fauna and their distribution rates in Lake Sapanca and its tributaries. For this purpose, standard benthic and pelagic nets were used in the lake and a portable electroshock device was used in the streams. As a result of the sampling carried out in two different seasons, wet and dry periods, the presence of 26 species from 14 families was determined and the abundance values for many species were higher in the wet season. In terms of species diversity, the Leuciscidae family ranked first with 9 species, while the Gobiidae family was represented by 4 species, the Clupeidae family by 2 species and the remaining 11 families were represented by one species each. In terms of individual density, *Rhodeus amarus* was the most dominant fish in both lake and streams (78.1% lake; 28.3% stream). In the lake, *Blicca bjoerkna*, *Clupeonella cultriventris,* and *Atherina boyeri* were the most abundant species after *R. amarus* (7.2%, 3.2% and 3.2%, respectively). An important finding of the study was that there were almost no individuals of *Silurus glanis*, *Esox lucius,* and *Cyprinus carpio* (4, 8 and 1 individuals, respectively), which are species of high commercial value in the lake. In addition, the fact that *Carassius gibelio*, one of the invasive species reported in previous studies, was not found in the lake is very pleasing in terms of lake biodiversity. In addition, *Leucaspius delineatus*, caught in both seasons, was identified from the lake basin for the first time.

Keywords: Biodiversity, exotic fish, life below water, population structure

Öz: Bu çalışmanın amacı Sapanca Gölü ve kollarındaki güncel balık faunasını ve dağılım oranlarını belirlemektir. Bu amaçla gölde standart bentik ve pelajik ağlar, akarsularda ise taşınabilir elektroşok cihazı kullanılmıştır. Yağışlı ve kurak olmak üzere iki ayrı mevsimde yapılan örneklemeler sonucunda 14 familyaya ait 26 türün varlığı belirlenmiş olup, birçok türün bolluk değerleri yağışlı mevsimde daha yüksek bulunmuştur. Tür çeşitliliği bakımından Leuciscidae familyası 9 tür ile ilk sırada yer alırken, Gobiidae familyası 4 tür, Clupeidae familyası 2 tür ve kalan 11 familya ise birer tür ile temsil edilmiştir. Birey yoğunluğu bakımından ise *Rhodeus amarus* hem gölde hem de akarsularda en baskın balık olmuştur (%78,1 göl; %28,3 akarsu). Gölde *Blicca bjoerkna*, *Clupeonella cultriventris* ve Atherina boyeri *R. amarus*'tan sonra en bol bulunan türlerdir (%7,2, %3,2 ve %3,2). Çalışmanın önemli bir bulgusu ise ticari değeri yüksek türlerden *Silurus glanis*, *Esox lucius* ve *Cyprinus carpio*'nun (sırasıyla 4, 8 ve 1 birey) gölde neredeyse hiç bulunmamasıdır. Ayrıca önceki çalışmalarda bildirilen istilacı türlerden Carassius gibelio'nun gölde bulunmaması göl biyoçeşitliliği açısından oldukça sevindiricidir. Ayrıca her iki mevsimde yakalanan Leucaspius delineatus türü göl havzasından ilk kez tanımlanmıştır.

Anahtar kelimeler: Biyodiversite, egzotik balık, su altında yaşam, popülasyon yapısı

INTRODUCTION

Whereas their certain importance to be a habitat for aquatic organisms, freshwater lakes also a source of drinking water for us and can be used for domestic, industrial and agricultural activities, energy production, recreation, water sports and tourism. In addition, they are of great importance to mankind, with their including aquatic organisms, by using them in many areas such as food, cosmetics, health, etc. (Messyasz et al, 2018; Hamed, 2016; Mielcarek and Socha, 2022) Therefore, the conservation and sustainable use of all aquatic systems, especially freshwater lakes, is of vital importance (Ferreira et al., 2023). This is an issue that should be considered with the utmost sensitivity, not only for the biodiversity of aquatic organisms, but also for the people who use these services (Özbayram et al., 2022).

Lake Sapanca, one of the most important water bodies in Türkiye, is a freshwater lake of tectonic origin with a maximum depth of 54 m and an average depth of 30 m (Akıner and Akıner, 2021). It is the main source of drinking water for the surrounding settlements and is used for industrial and agricultural activities. The lake, which is also used for water sports and recreation, provides wetland services of high ecological and economic value. It also supports the region's fishing industry, as it is home to many commercially valuable fish species. The lake, which has a water catchment area of about 300 km^2, is well fed by the floods and streams (Keçidere, İstanbuldere, Mahmudiye, Yanıkdere and Kurtköy) that come down from the mountains in the south and discharges its outlet water into the Sakarya River from the east through the Çark Stream.

Sapanca Lake and its basin have been the focus of interest of many researchers in the past and present due to its ecosystem services, and the presence of 46 fish species has been mentioned in ichthyological studies conducted in the region (Deveciyan, 1915; Kosswig and Battalgil, 1943; Numann, 1958; Ladiges, 1960; Ongan, 1982; Rahe and Worthmann, 1986; Ergüven, 1989; Karabatak and Okgerman, 2002; Okgerman, 2006; Özuluğ et al., 2007; Tarkan, 2007; Tarkan et al., 2007, 2008; Saç and Özuluğ, 2015; Saç et al., 2019). However, the distribution of 20 species was reported in the last study on the fish fauna of the lake, which only included in-lake sampling (Okgerman, 2006). The lake environment is in a situation where the human population has increased significantly due to illegal construction and rapid urbanisation, and thus the pressure on the lake and its tributaries in terms of pollution and water balance has also increased (Akıner and Akıner, 2021).

The present study aims to determine the current status of the fish fauna and their distribution rates in Lake Sapanca and the streams flowing into the lake, which have a special position with their social and ecological functions. Another aim of the study is to reveal threats to the fish composition of the basin.

MATERIALS AND METHODS

Field surveys were conducted in two different seasons, wet (May 2022) and dry (September 2022). Fish sampling was carried out using standard benthic and pelagic nets according to TS EN 14757 and 14011 Water Quality criteria in the lake and the SAMUS 725G electro-shocker in the streams. Sampling was carried out at 26 stations, 24 in the littoral parts of the lake with benthic nets and 2 in the deep parts with pelagic nets. Scoop nets and seine net were also used in the littoral areas of the lake. As for the streams, although the study plan provided for sampling in the lower, middle and upper sections of all the streams, due to the low flow rates and being relatively small size of the Değirmen, Maden and Keçi streams, sampling could only be carried out in the lower sections close to the lake. In the other streams, Balıkhane, Yanık, Mahmudiye, İstanbul and Kurtköy streams, sampling was carried out at two stations, one in the upper section and one in the lower section close to the lake, because of the presence of many physical obstacles that degrade the stream continuity (reversing dykes, reclamation benches, base belts, chutes, walls, dykes, stone fortifications, rip-raps, gabion mattresses, prefabricated pavements, industrial pavements, industrial reinforcements, culverts etc.) and prevent fish passage. Sampling was therefore carried out at 12 stations in 8 streams flowing into the lake (Figure 1). Threats to fish species and their habitats were recorded observationally during field surveys.

To determine fish abundance, catch per unit effort (CPUE) was calculated using the following equation: CPUE = $(n/t/a) \times 100$ (n: number of samples, t: time (minute (min) for streams and hour (h) for lakes, a: sampling area) (Jordan & Willis, 2001; Mehner et al., 2005). For streams, the sampling area is the area sampled by electroshock, and for lakes, the sampling area is the area of the gillnet used to catch fish. The time spent on fishing effort was recorded in hours for the lakes and in minutes for the streams.

Alive fish samples were euthanised with pure (99.5%) phenoxyethanol (1 ml/L) and fixed in 4% formaldehyde. Species identification was made by detailed examination of the samples brought to the laboratory, and the number of individuals was determined according to the location where they were caught. Kuru (1980), Miller (1986), Geldiay and Balık (2007), Kottelat and Freyhof (2007), Özuluğ and Freyhof (2011), and Freyhof et al. (2018) were used for species identification.

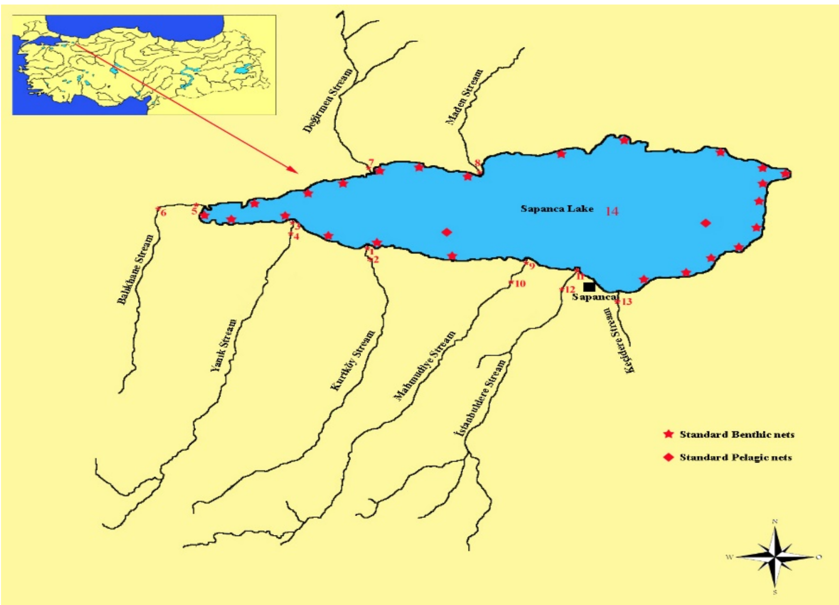

Figure 1. Sampling stations in Lake Sapanca and in the streams flowing into the lake

RESULTS

As a result of the study, the existence of 26 species from 14 families was determined (Table 1). The identified species were described and classified according to Van der Laan et al. (2023). Among these species, seven species, *A. maeotica, C. cultiventris, C. carpio, S. erythrophthalmus, E. lucius, S. glanis,* and *S. abaster* were sampled only from the lake; five species, *G. sakaryaensis, P. borysthenicus, P. strandjae, S. pursakensis,* and *O. mykiss* were sampled only from the streams flowing into the lake (Table 1). *Atherina boyeri, R. amarus, C. emrei, A. istanbulensis, B. bjoerkna, L. delineatus, R. rutilus, V. vimba, T. tinca, G. holbrooki, B. gymnotrachelus, N. fluviatilis, N. melanostomus,* and *P. semilunaris,* and were found both in the lake and in the streams flowing into the lake (Table 1).

Based on field studies conducted during two different seasons at Lake Sapanca, the most abundant species in terms of CPUE values during the wet season were *R. amarus*, *B. bjoerkna*, *A. boyeri*, *C. cultriventris*, *R. rutilus*, *V. vimba*, and various gobiid species. In the dry season, *R. amarus* and *B. bjoerkna* remained dominant, followed by *S. erythrophthalmus*. Other species, represented by only a few individuals, exhibited lower CPUE values (Table 2).

Similarly, field studies conducted on the streams flowing into Lake Sapanca revealed that the most abundant species during the wet season were *P. strandjae*, *S. pursakensis*, *N. fluviatilis*, and *R. amarus*, respectively. During the dry season, the dominant species shifted slightly, with *R. amarus*, *S. pursakensis*, *P. strandjae*, and *P. semilunaris* being the most abundant (Table 3).

When analysing the distribution of the species in the lake, the species *R. amarus* and *B. bjoerkna* were obtained from 23 of the 26 stations studied. *Rutilus rutilus* was also sampled at 18 stations. Among the species of high commercial value in the lake, *E. lucius* was represented by eight individuals in six stations, *S. glanis* by four individuals in three stations and *C. carpio* by one individual in only one station. In addition, *T. tinca, L. delineatus,* and *S. abaster* were found at only one site each. The invasive species, *G. holbrooki*, which was sampled with a scoop net in the coastal area of the lake, was not found in the nets.

In the samplings carried out at 13 stations in 8 streams flowing into the lake, 19 species were identified (Table 1, 2). When the streams were analysed in terms of species diversity, Balıkhane, Yanık, and Kurtköy streams were the richest locations with 16, 15 and 11 species, respectively, while Maden and Keçi streams had only 4 species each (Table 2).

Table 1. Fish species caught from Lake Sapanca and in the streams flowing into the lake. + indicates the presence of fish species in the Sapanca Lake basin

Ordo	Familia	Species	Lake	Streams
Atheriniformes	Atherinidae	*Atherina boyeri*	+	+
Clupeiformes	Clupeidae	*Alosa maeotica*	+	
		Clupeonella cultriventris	+	
Cypriniformes	Acheilognathidae	*Rhodeus amarus*	+	+
	Cobitidae	*Cobitis emrei*	+	+
	Cyprinidae	*Cyprinus carpio*	+	
	Gobionidae	*Gobio sakaryensis*		+
	Leuciscidae	*Alburnus istanbulensis*	+	+
		Blicca bjoerkna	+	+
		Leucaspius delineatus	+	+
		Petroleuciscus borysthenicus		+
		Phoxinus strandjae		+
		Rutilus rutilus	+	+
		Scardinius erythrophthalmus	+	
		Squalius pursakensis		+
		Vimba vimba	+	+
	Tincidae	*Tinca tinca*	+	+
Cyprinodontiformes	Poeciliidae	*Gambusia holbrooki*	+	+
Esociformes	Esocidae	*Esox lucius*	+	
Gobiiformes	Gobiidae	*Babka gymnotrachelus*	+	+
		Neogobius fluviatilis	+	+
		Neogobius melanostomus	+	+
		Proterorhinus semilunaris	+	+
Salmoniformes	Salmonidae	*Oncorhynchus mykiss*		+
Siluriformes	Siluridae	*Silurus glanis*	+	
Syngnathiformes	Syngnathidae	*Syngnathus abaster*	+	

Table 2. Distribution and CPUE values of the species caught in the Sapanca lake basin (1-Kurtköy Stream lower, Kurtköy Stream upper, 3-Yanık Stream-lower section, 4-Yanık Stream-upper section, 5-Balıkhane Stream-lower section, 6- Maşukiye Stream, 7-Değirmen Stream, 8-Maden Stream, 9-Mahmudiye Stream-lower section, 10-Mahmudiye Stream-upper section, 11-İstanbul Stream-lower section, 12-İstanbul Stream-upper section, 13-Keçi Stream, 14-Sapanca Lake; W: Wet period, D: Dry period, ∗: drying up)

Species	**1**		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**		**11**		**12**		**13**		**14**	
	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D
A. boyeri	0.10	-	-	-	1.85	-	-	-	0.35	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	57.04	4.26
A. maeotica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	0.93	0.19
C. cultriventris	-	-	-	-	-	-	-	-	0.25	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	55.74	6.11
R. amarus	0.05	0.10	-	-	0.10	0.40	-	-	4.40	2.15	-	0.20	-	2.20	0.15	∗	-	2.20	-	-	-	8.75	-	-	-	-	1114.07	381.67
C. emrei	-	-	-	-	0.25	-	-	-	-	0.05	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	0.56	-
C. carpio	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	-	0.19
G. sakaryaensis	-	-	-	-	0.05	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	-	-
A. istanbulensis	0.25	-	-	-	0.10	-	-	-	0.35	0.15	0.10	-	-	2.00	0.10	∗	-	0.10	-	-	-	0.10	-	-	-	-	0.74	0.18
B. bjoerkna	-	-	-	-	-	-	-	-	0.30	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	91.85	46.85
L. delineatus	-	-	-	-	0.15	-	-	-	3.70	-	-	-	0.05	-	-	∗	-	-	-	-	-	-	-	-	-	-	1.11	0.56
P. borysthenicus	-	0.80	-	-	-	0.25	-	-	0.15	0.10	-	-	1.80	0.30	0.25	∗	0.05	-	-	-	-	-	-	-	-	-	-	-
P. strandjae	-	0.20	-	0.20	0.05	0.10	2.25	2.10	-	-	-	0.05	-	-	-	∗	2.10	1.30	2.10	1.25	0.10	-	0.85	3.00	-	0.55	-	-
R. rutilus	-	-	-	-	-	-	-	-	0.30	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	19.81	15.93
S. erythrophthalmus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	1.85	30.37
S. pursakensis	1.00	1.30	-	0.85	0.60	-	-	-	-	0.10	0.45	0.10	0.10	0.20	0.20	∗	1.90	1.50	-	3.65	0.70	2.15	-	-	0.65	1.35	-	-
V. vimba	0.05	-	-	-	0.05	-	-	-	0.10	-	0.40	-	0.10	-	-	∗	-	-	-	-	-	-	-	-	-	-	10.19	4.26
T. tinca	-	-	-	-	-	-	-	-	-	-	-	-	0.05	-	-	∗	-	-	-	-	-	-	-	-	-	-	-	0.19
G. holbrooki	-	0.40	-	-	3.00	0.50	-	-	-	0.75	-	-	-	0.45	-	∗	-	2.10	-	-	-	0.90	-	-	-	-	0.19	7.04
E. lucius	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	0.74	0.74
B. gymnotrachelus	-	-	-	-	2.10	-	-	-	0.05	0.35	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	0.05	-	8.33	0.37
N. fluviatilis	1.70	-	-	-	2.80	-	-	-	0.05	0.10	0.10	-	-	-	-	∗	0.10	-	-	-	-	0.05	-	-	0.10	-	36.48	1.85
N. melanostomus	-	-	-	-	0.10	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	7.41	-
P. semilunaris	0.60	0.50	-	-	0.40	-	-	-	0.25	2.10	-	1.00	-	0.40	-	∗	1.20	0.10	-	-	-	1.15	-	-	-	-	2.59	1.85
O. mykiss	-	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	-	-
S. glanis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	0.19	0.56
S. abaster	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	∗	-	-	-	-	-	-	-	-	-	-	0.37	-
Total	**3.75**	**3.35**	**-**	**1.05**	**11.35**	**1.25**	**2.25**	**2.10**	**10.25**	**5.85**	**1.00**	**1.35**	**2.10**	**5.55**	**0.70**	∗	**5.35**	**7.30**	**2.10**	**4.90**	**0.80**	**13.10**	**0.85**	**3.00**	**0.80**	**1.90**	**1410.19**	**504.44**

Table 3. Total CPUE values of fish species collected from streams flowing into Lake Sapanca (W: Wet period, D: Dry period)

Species	W	D	∑
A. boyeri	2.3	-	2.3
A. maeotica	-	-	-
C. cultriventris	0.25	-	0.25
R. amarus	4.7	16	20.7
C. emrei	-	0.05	0.05
C. carpio	-	-	-
G. sakaryaensis	0.05	-	0.05
A. istanbulensis	0.9	2.35	3.25
B. bjoerkna	0.3	-	0.3
L. delineatus	3.9	-	3.9
P. borysthenicus	2.25	1.45	3.7
P. strandje	7.45	8.55	16
R. rutilus	0.3	-	0.3
S. erythrophthalmus	-	-	-
S. pursakensis	5.6	10.35	15.95
V. vimba	0.7	-	0.7
T. tinca	0.05	-	0.05
G. holbrooki	3	5.1	8.1
E. lucius	-	-	-
B. gymnotrachelus	2.2	0.35	2.55
N. fluviatilis	4.8	0.15	4.95
N. melanostomus	0.1	-	0.1
P. semilunaris	2.45	5.25	7.7
O. mykiss	-	0.05	0.05
S. glanis	-	-	-
S. abaster	-	-	-

DISCUSSION

Although the first scientific data about the fish fauna of Sapanca Lake is found in Deveciyan (1915), the great Turkish traveller Evliya Çelebi, who lived in the 17th century, while talking about Sapanca Lake in his famous work, Book of Travels, states that the fishermen make a profit by catching 70-80 different species of fish living in the lake, such as trout, carp, pike and luna fish which are very delicious and nutritious (Danışman, 1969; 1970; 1971). In the following years, the presence of 41 different taxa was reported in the faunistic studies on the fishes of the lake (Kosswig and Battalgil, 1943; Numann, 1958; Ladiges, 1960; Ongan, 1982; Rahe and Worthmann, 1986; Okgerman, 2006) and in addition to these, Ergüven (1989) reported *Lampetra fluviatilis*, Tarkan (2007) *Lampetra* sp.; Özuluğ et al. (2007) *Phoxinus phoxinus* and *Gambusia holbrooki*; Tarkan et al. (2008) *Salmo trutta macrostigma*; Saç and Özuluğ (2015) *Phoxinus strandjae*; Saç et al. (2019) *Gobio sakaryaensis*, as well. Therefore, the distribution of 46 different fish species in Lake Sapanca has been reported in studies carried out between 1915 and the present day (Table 4). In the present study, 27 species belonging to 14 families were identified in the lake and its tributaries. Among these species, *L. delineatus* was identified for the first time from the lake basin and is the first record. Lake Sapanca is within the natural range of *L. delineatus*. The fact that it has not been reported in previous studies is probably due to the morphological similarity of this small-sized fish with the juveniles of some species such as *Squalius* and *Petroleuciscus*.

The eel, *A. anguilla*, which was last recorded from the lake in 1986 (Rahe and Worthmann, 1986), is a species whose range is shrinking due to habitat loss in many parts of Europe (Ağdamar et al., 2021). Lake Sapanca is connected to the Sakarya River by the Çark Stream and thus to the Black Sea. For this fish, which has to use this route for breeding migration, the control gates on the Çark Stream and the fact that part of the stream has been enclosed in a concrete canal can be considered as an obstacle. The species previously reported as *C. muhlisi* from the lake was found to be *C. cultriventris* through genetic and morphological studies (Aydoğan, 2018). In addition, the species *A. albidus* and *A. alburnus* reported in previous studies are considered to be a misidentification of the species *A. istanbulensis*. *Syngnathus nigrolineatus*, previously listed by Kosswig and Battalgil (1943) and Rahe and Worthmann (1986), is now recognised as a synonym of *S. abaster*. *Salmo* species prefer the trout zone of streams and it is thought that these fish were not caught because the upper zones of the streams present in the study could not be sampled.

Kosswig, who carried out the first important limnological study of the lake, compiled unpublished data on fish in 1958 and reported that there were 28 fish species living in the lake. There is no information on the species *Alvonus brunner*, and it is thought that this name included in the study as a typographical error. A similar situation applies to the species *Varicorhinus tri*, which has not been found in subsequent studies conducted in the lake or in other literature worldwide. Subsequently, Ladiges (1960) recorded 13 fish species from the lake. Among these species, *Chondrostoma knerrii* was then identified as *Chondrostoma nasus* in a study of *Chondrostoma* species in Türkiye by the same researcher (Ladiges, 1966). Since then, a single individual of this species was first identified in the lake in 2006 (Tarkan et al., 2007). Elvira (1987) gave the name *Chondrostoma nasus angorensis* to the form of this fish living in the Sakarya basin, and later the same researcher updated the scientific name of this fish to *Chondrostoma angorense* (Elvira, 1997). Examinations conducted on the single individual caught in the lake in 2006 confirmed that this fish was *Chondrostoma angorense* (Tarkan et al., 2007). The species *Alburnus albidus* recorded in Ladiges' study in 1960 has not been found in subsequent studies. *Alburnus albidus* is a fish native to Italy (Kottelat and Freyhof, 2007), but it was mistakenly reported to inhabit in Sapanca Lake. Many years later, in 1982, in the first study published by Ongan on the fish in the lake, 27 fish species were reported. In this study, three gobiid fish species were reported, namely *Neogobius fluviatilis*, *Pomatoschistus caucasicus kosswigi*, and *Proterorhinus marmoratus*, different from Numann (1958). Following this, Rahe and Worthmann (1986) reported 35 fish species from the lake in their study.

Alburnoides bipunctatus, *Atherina boyeri*, *Clupeonella abrau muhlisi*, *Neogobius syrman*, *Oncorhynchus mykiss*, *Syngnathus nigrolineatus*, *Syngnathus tenuirostris*, and *Vimba vimba tenella* are species that have been added to the lists

Table 4. Species identified in Lake Sapanca in the faunistic studies conducted to date (1- Deveciyan (1915), 2- Kosswig and Battalgil (1943), 3- Numann (1958), 4- Ladiges (1960), 5- Ongan (1982), 6- Rahe and Worthmann (1986), 7- Karabatak and Okgerman (2002), 8- Okgerman (2006), 9- Present study)

Species	1	2	3	4	5	6	7	8	9
Anguilla anguilla	-	-	+	-	+	+	-	-	-
Atherina boyeri	-	+	+	-	+	+	-	+	+
Alosa maeotica	-	+	+	-	+	+	+	+	+
Clupeonella cultriventris	-	-	-	-	-	+	-	+	+
Cobitis emrei	-	-	+	-	+	+	-	+	+
Abramis brama	-	-	+	+	+	+	-	-	-
Alburnoides bipunctatus	-	-	-	-	-	+	-	-	-
Alburnus albidus	-	+	-	+	-	-	-	-	-
Alburnus alburnus	-	-	+	-	+	+	-	-	-
Alburnus istanbulensis	-	+	+	+	+	+	-	+	+
Alvonus brunner	-	-	+	-	-	-	-	-	-
Blicca bjoerkna	-	+	+	+	+	+	+	+	+
Carassius carassius	-	-	+	+	+	+	+	-	-
Carassius gibelio	-	-	-	-	-	-	-	+	-
Chondrostoma angorense	-	-	-	+	-	-	-	-	-
Cyprinus carpio	-	+	+	+	+	+	+	+	+
Gobio sakaryaensis	-	-	-	-	-	-	-	-	+
Leuciscus aspius	-	+	+	+	+	+	+	-	-
Petroleuciscus borysthenicus	-	+	+	+	+	+	-	-	+
Phoxinus strandjae	-	-	-	-	-	-	-	-	+
Rhodeus amarus	-	+	+	+	+	+	+	+	+
Rutilus rutilus	+	+	+	+	+	+	+	+	+
Scardinius erythrophthalmus	+	+	+	+	+	+	+	+	+
Squalius pursakensis	-	-	+	-	+	+	+	+	+
Tinca tinca	+	-	-	-	-	+	+	+	+
Varicorhinus tri	-	-	+	-	-	-	-	-	-
Vimba vimba	-	+	+	+	+	+	+	+	+
Esox lucius	+	+	+	-	+	+	+	+	+
Babka gymnotrachelus	-	+	+	-	+	+	-	+	+
Knipowitschia caucasica	-	+	-	-	+	+	-	-	-
Neogobius fluviatilis	-	+	+	-	+	-	-	+	+
Neogobius melanostomus	-	+	+	-	+	+	-	+	+
Ponticola syrman	-	-	-	-	-	+	-	-	-
Proterorhinus semilunaris	-	+	-	-	+	+	-	-	+
Oxynoemacheilus angorae	-	-	+	-	+	+	-	-	-
Perca fluviatilis	+	+	+	-	+	+	-	-	-
Lampetra lanceolata	-	-	-	-	+	-	-	-	-
Gambusia holbrooki	-	-	-	-	-	-	-	-	+
Oncorhynchus mykiss	-	-	-	-	-	+	+	+	+
Salmo labrax	-	-	+	-	-	-	-	-	-
Salmo cf. *macrostigma*	-	+	-	-	-	-	-	-	-
Silurus glanis	+	-	+	-	+	+	+	+	+
Syngnathus abaster	-	-	+	-	+	+	-	-	+
Syngnathus tenuirostris	-	-	-	-	-	+	-	-	-
Syngnathus nigrolineatus	-	+	-	-	-	+	-	-	-
Leucaspius delineatus	-	-	-	-	-	-	-	-	+
Number of species	**6**	**21**	**28**	**13**	**27**	**33**	**14**	**20**	**26**

provided by previous researchers. It is believed that the individuals of the species *Oncorhynchus mykiss* (rainbow trout) found in the wild are rare individuals that have escaped from trout farms established on streams flowing into the lake, that do not have the ability to reproduce on their own, and are therefore unable to form a population. Indeed, all the studies carried out on this species to date have found their numbers to be very low. The species *Atherina mochon* and *Atherina boyeri* (sand smelt) listed in the fish lists of Kosswig and Battalgil (1943) and Numann (1958) are actually the same species and synonyms. Therefore, these two sand smelt records should actually be considered as a single species, namely the valid species *Atherina boyeri*. Therefore, in the studies by Kosswig and Battalgil (1943), Numann (1958), and Rahe and Worthmann (1986), only one species of sand smelt, *Atherina boyeri*, is mentioned. In the study conducted by Karabatak and Okgerman (2002), following Rahe and Worthmann (1986), this species is also given as *Atherina mochon*. Similarly, the species *Vimba vimba tenella* and *Vimba vimba*; *Syngnathus nigrolineatus* and *Syngnathus abaster* are synonyms, and these fish are now accepted as *Vimba vimba* and *Syngnathus abaster*. Therefore, considering them as separate species in the species list provided by Rahe and Worthmann (1986) will contribute incorrect information to the literature.

After the results of Rahe and Worthmann's study were published in 1986, there was no study on the fish composition for a long time, until Ergüven (1989) reported a new record of *Lampetra fluviatilis* (European river lamprey) for the lake in 1989. In this study, it was reported that *L. fluviatilis* was caught in the Kurtköy and Yanık streams, which flow into Lake Sapanca. However, the distribution areas of *L. fluviatilis* reported by Ergüven (1989) does not include the Black Sea coast and more southern latitudes (Kottelat and Freyhof, 2007). The first detailed research results after Rahe and Worthmann (1986) were published in 2002, and 15 fish species were reported in the list. Later, according to the more comprehensive results of Okgerman et al. (2006), there are 20 fish species in the lake, and two invasive fish species (*Carassius gibelio* and *Lepomis gibbosus*) were also identified. The species reported in this study, with the exception of the two invasive species, are species known to have previously occurred in the lake. However, in the study conducted by Okgerman et al. (2006) between 2000-2004, the species *Abramis brama*, *Alburnus alburnus*, *Alburnoides bipunctatus*, *Anguilla anguilla*, *Lampetra fluviatilis*, *Neogobius syrman*, *Nemacheilus angorae*, *Perca fluviatilis*, *Petroleuciscus borysthenicus*, *Pomatoschistus caucasicus kosswigi*, *Proterorhinus marmoratus*, *Syngnathus abaster*, which were previously known to occur in the lake, were not found. The last study on the fish fauna of Lake Sapanca was conducted in 2007, and no comprehensive ichthyofauna study has been conducted since then (Tarkan, 2007; Özuluğ et al., 2007). In this study, the fish fauna of the streams flowing into the lake was examined, and 12 species were identified (Tarkan, 2007). Among these species, *Phoxinus phoxinus* and *Gambusia holbrooki* are new records for the lake basin (Özuluğ et al., 2007).

Later, it was reported that the new record of *P. phoxinus* in Sapanca Lake was *Phoxinus strandjae* (Saç and Özuluğ, 2015). In addition, an individual belonging to the genus *Lampetra* was identified from Yanık Stream in 2007 for the first time by Ergüven (1989) and after Özuluğ et al. (2007). However, it was reported that the juvenile individuals obtained could not be used for species determination because adult individuals are very important for species determination of fishes belonging to this genus. But, recent molecular-based studies have revealed that the species living in Sapanca Lake and recorded as *Lampetra fluviatilis* is actually *Lampetra lanceolata* (Geiger et al. 2014).

Among the fish caught during the study, *G. holbrooki* and *O. mykiss* are exotic fishes to the lake and its tributary. The invasive *G. holbrooki* was found to be dense in the littoral region of the lake and especially in the lower parts of the streams. It is an undesirable fish species due to its predatory and aggressive behaviour on the eggs and larvae of native fish and amphibians, and because it can compete with them for food and habitat. The *O. mykiss* fish caught in the Kurtköy Stream is believed to be one of the fish that escaped from trout farms and settled in the wild. *Carassius carassius* was first reported from the lake by Numann (1958) and caught by other researchers in the following years. The species *C. gibelio*, which is difficult to distinguish morphologically, was only reported by Okgerman (2006). The fact that this invasive species was not caught in the current study is very positive for the biodiversity of the lake.

Among the fish species found in the lake basin are species endemic to Turkish inland waters such as *C. emrei*, *G. sakaryaensis*, *A. istanbulensis,* and *S. pursakensis*. In addition, *E. lucius*, *S. glanis*, and *C. carpio*, which are commercially valuable and preferred for sport fishing, and *V. vimba* and *S. erythrophthalmus*, which are consumed with pleasure by the local population, are important elements of the fauna. However, an important result of the study is that very few individuals of the species *S. glanis, E. lucius,* and *C. carpio*, which are among the species with high commercial value in the lake, were found. This situation is thought to be the result of intense sport fishing and poaching, despite the fact that the lake is closed to commercial fishing. Therefore, in addition to protecting the lake itself, it is important to protect the biodiversity it contains in order to ensure its sustainable use.

When analysing the temporal and spatial distribution of the fishes, it was found that the abundance (CPUE values) of some fishes were higher in the lake and lower sections of the streams, especially during the wet season. It is thought that this may be related to the reproductive behaviour of the fishes; many species such as *A. boyeri, C. cultriventris, R. amarus, B. bjoerkna, A. istanbulensis* and *V. vimba* come to the littoral areas mainly firstly to breed and also to feed after surviving the harsh winter conditions in the deeper parts of the lake. In addition, it has been observed that the fish composition in the lake has changed over the years. Karabatak and Okgerman (2002) determined that *S. erythrophthalmus, B. bjoerkna,* and *R. rutilus* were the dominant fishes in the lake about 20 years ago, followed by *V. vimba, E. lucius,* and *A. maoetica*. The fishing gear they use for their monthly surveys throughout the year is gill nets with mesh sizes ranging from 22 to 50 mm. In the present study, the dominant species was *R. amarus*, with small species such as *A. boyeri* and *C. cultriventris* also present with high CPUE values. This difference between the two studies may be mainly related to the mesh size used. It is also thought that continued fishing pressure on commercial fish such as *E. lucius* and *C. carpio*, which have had high catch rates in recent years (Karabatak and Okgerman, 2002), is responsible for their low abundance today.

When the factors that threaten the sustainable use of Lake Sapanca are examined, despite it being a drinking water basin, the following risk factors stand out: i) domestic waste due to construction on the shoreline almost all around the lake, ii) fertiliser and pesticide waste from intensive farming around the lake, iii) pollution from highways and railways along the northern and southern shores of the lake, iv) numerous water bottling plants around the lake, and v) pollution caused by intense tourism activities around the lake. Besides, constructions such as dams, reclamation benches, base belts, chutes, walls, dykes, stone fortifications, rip-raps, gabion mattresses, prefabricated pavements, industrial pavements, industrial reinforcements, culverts are another factor that prevents fish from migrating to the upper reaches of streams for reproduction. The negative effects of these structures can be seen in the distribution of fish in the streams. While the lower sections of the streams close to the lake are rich in biodiversity, the lack of biodiversity in the upper sections is due to these physical barriers that restrict the migratory mobility of fish in almost all streams.

CONCLUSION

Sapanca is the smallest district of the city Sakarya, but its population increased to 46 080, 26% in the last 23 years (TUİK, 2024). In addition, its population can be three folded in summers because of the native and foreign tourists (Yılmazer, 2022). The tourism activities in the town is clearly seen from the number of bungalovs increased up to 4000 nowadays (Çağlar, 2023). This faunistic study, carried out in a lake basin, which is subject to population growth and therefore urbanisation pressure, has provided current information on the species present and their population densities. The distribution of fish species in the lake and its streams was determined and species reported in previous studies but not caught in this study were interpreted. In the face of threats that are thought to be limiting the ecosystem functioning of the lake basin, it is recommended that monitoring studies are carried out, particularly for fish, which are thought to respond rapidly to water balance and pollution. A further recommendation is to provide urgent solutions to structures that prevent fish from breeding and feeding in streams.

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AUTHORSHIP CONTRIBUTIONS

Ali İlhan: Design of the study, sampling and laboratory studies, writing draft. Gülşah Saç: Sampling and laboratory studies, writing draft. Özcan Gaygusuz: Desing of the study, sampling and laboratory studies, writing draft. Sencer Akalın: Sampling and laboratory studies. Esat Tarık Topkara: Sampling and laboratory studies. Dilek İlhan: Laboratory studies. Çiğdem Gürsoy Gaygusuz: Laboratory studies., Hasan Musa Sarı: Laboratory studies.

CONFLICT OF INTEREST

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

ETHICS APPROVAL

The care and use of experimental animals, sampling and analysis techniques used in this work are approved by “Ege University Animal Experiments Ethics Committee” with decree no “2021/078”.

DATA AVAILABILITY

All relevant data is in the article.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Challenges for revitalizing seafood exports in Hatay's of Türkiye: A comparative analysis (2008-2023)

Hatay'ın Su ürünleri ihracatını canlandırma yolunda zorluklar: Karşılaştırmalı bir analiz (2008-2023)

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Abstract: A comparative analysis of seafood exports in the Hatay region between 2008 and 2023, in relation to overall trends in Türkiye and different regions, was conducted. Seafood export data was obtained from the Turkish Statistical Institute. The export amount has been in a long-term decline fishing and seafood production potential of Hatay, contrary to the general trend in Türkiye. Specifically, the export amount decreased by 62% since 2018, while it decreased by 22.65% in 2023 compared to the previous year. This ongoing downward trend can be attributed to the COVID-19, Syrian civil war, and the recent devastating earthquake in the region. Despite the high potential for seafood production in the region, investments have been limited due to the lack of allocation in organized industrial zones. Additionally, the absence of a wholesale fish market is a major issue preventing the emergence of a regional market. All these factors have caused disruptions in the supply chain and production, increased input costs, and market uncertainties both domestically and internationally. This indicates that infrastructure investments in the region must be prioritized by all stakeholders, the central authority, decisionmakers and policymakers for sustainable seafood exports contributing to regional development. In conclusion, despite the strong industrial and logistics infrastructure in Iskenderun Bay, the seafood sector in Hatay has failed to reach its full potential, unlike the national trend in Türkiye. This shortfall is directly reflected in export values. Therefore, necessary measures must be taken to effectively utilize the region's seafood potential.

Keywords: Seafood exports decline, Hatay fishing industry, supply chain disruptions, infrastructure investment needs

Öz: Bu çalışmada, 2008 ile 2023 yılları arasında Hatay bölgesindeki su ürünleri ihracatının, Türkiye genelindeki ve farklı bölgelerdeki eğilimlerle karşılaştırmalı analizi yapılmıştır. Su ürünleri ihracat verileri Türkiye İstatistik Kurumu'ndan elde edilmiştir. Balıkçılık ve su ürünleri üretim potansiyeli açısından Hatay'da ihracat miktarının Türkiye genelindekinin aksine uzun vadede azalma eğiliminde olduğu tespit edilmiştir. Özellikle 2018 yılından itibaren ihracat miktarı %62 oranında azalmış, 2023 yılında ise bir önceki yıla göre %22,65 oranında düşmüştür. Bu sürekli düşüş eğilimi, COVID-19, Suriye iç savaşı ve bölgedeki son yıkıcı deprem gibi faktörlere bağlanmaktadır. Bölgedeki yüksek su ürünleri üretim potansiyeline rağmen, organize sanayi bölgelerinde yer eksikliği nedeniyle yatırımlar sınırlı kalmıştır. Ayrıca, bu bölgede bir toptan balık halinin olmaması bölgesel bir pazarın oluşmasını engelleyen büyük bir sorundur. Tüm bu faktörler, tedarik zincirinde ve üretimde aksamalara, girdi maliyetlerinde artışa ve hem yurtiçi hem de yurtdışı piyasalarda belirsizliklere yol açmaktadır. Bu durum bölgesel kalkınmaya katkıda bulunacak sürdürülebilir su ürünleri ihracatı için bölgedeki altyapı yatırımlarının tüm paydaşlar ve Tarım ve Orman Bakanlığı tarafından önceliklendirilmesi gerektiğini göstermektedir. Sonuç olarak, İskenderun Körfezi'ndeki güçlü sanayi ve lojistik altyapısına rağmen, Hatay'da su ürünleri sektörü, Türkiye'deki ulusal eğilimin aksine, henüz tam potansiyeline ulaşamamıştır. Dolayısıyla, bölgenin su ürünleri potansiyelinin etkin bir şekilde değerlendirilmesi için gerekli tedbirlerin alınması şarttır.

Anahtar kelimeler: Su ürünleri ihracatında düşüş, Hatay balıkçılık endüstrisi, tedarik zinciri aksamaları, altyapı yatırım ihtiyaçları

INTRODUCTION

The fishery and aquaculture industry serves as a significant source of nutrition and is among the most traded food commodities globally (Subasinghe, 2017; Kale, 2020; Acarlı et al., 2022; Cengiz and Paruğ, 2022; Yarkina and Logunova, 2022). This makes it a crucial income source in many countries, driving economic development and contributing to global food security. In 2022, global fisheries and aquaculture production reached approximately 185.5 million tons. Fishery and aquaculture products consistently hold a prominent position among traded food commodities, with a trade volume of 68 million tons and a value of 190 billion dollars in 2022 (Can et al., 2023). Approximately 37% of the total production was exported. Fisheries and aquaculture trade significantly contribute to economic growth in many developing countries, providing economic opportunities and export revenue (FAO, 2023).

In 2023, Türkiye's seafood production increased by 18.6% compared to the previous year, reaching 1,007,921 tons. Production from fishing rose by 39.4%, while aquaculture production saw a 7.6% increase. The total catch from fishing amounted to 454,428 tons, with 387,115 tons from marine fishing and 67,313 tons from inland waters. Aquaculture production reached 553,862 tons, accounting for 55% of the total seafood production in Türkiye. Of this, 72.1% was from marine aquaculture, and 27.9% from inland waters. The most cultivated species were sea bass (160,802 tons) and sea bream (154,011 tons) in marine environments, and rainbow trout (154,006 tons) in inland waters (TurkStat, 2023). In 2023, Türkiye exported fishery products to 103 countries worldwide. The export quantity increased by 5.4% to 251,000 tons compared to the previous year, and the value rose by 20% to

1.652 billion dollars (Aydemir, 2024). Russia is the primary destination for fishery exports, accounting for 18%, with trout exports constituting 76% of this share. In 2022, 66% of fishery product exports were directed to European countries (FAO, 2022).

While fisheries production in the past focused predominantly on fishing, the share of aquaculture in fishery production has increased over the years. This increase is attributed to Türkiye's rich natural resources and strategic geographical location. Türkiye, with its extensive coastline along the Mediterranean, Aegean, and Black seas, as well as numerous inland water sources, provides ideal conditions for both marine and freshwater aquaculture. These natural advantages are strengthened by Türkiye's commitment to developing human resources in this field. Specialized education and training programs in fisheries and aquaculture have created a skilled workforce knowledgeable in modern aquaculture techniques, sustainable fishing practices, and efficient seafood processing. This skilled manpower has played a crucial role in maintaining the quality and sustainability of seafood products, providing Türkiye with a competitive advantage in the global market. In recent years, significant advancements in aquaculture systems have been observed in Türkiye. The relocation of fish farms in the seas to open and deep waters, the adoption of new techniques suitable for local conditions, and the application of technology exceeding global standards in cage sizes and structures, net systems, and feeding systems have all contributed to improvements in the sector (Bilgüven and Can, 2018).

Despite the general upward trend in Türkiye's aquaculture and fisheries exports, regional disparities are evident. Actually, the Hatay region, with its strategic location and the vital Iskenderun Bay, offers significant potential. Iskenderun Bay contains natural stocks of fish and shrimp of high economic value. On the other hand, Hatay province, being a border region, has been active in seafood exports for a long time. Indeed, Hatay has a special place in Türkiye, where the export of seafood products increased especially in the 1950s. This substantial increase highlights Hatay's leading role in the fisheries sector and the rapid development of the industry, supported by various innovations such as the introduction of trawl fishing (Nümann, 1953). It is a well-known fact in the region that many seafood exporting firms operate as family-owned businesses rather than institutional enterprises. None of these companies in Hatay are situated within organized industrial zones; instead, they are located in limited areas outside the city. These businesses commonly face challenges related to infrastructure and high land costs, which have driven investors to consider other regions of Türkiye. This has restricted the region's growth potential in the seafood sector, placing local businesses at a disadvantage. Over time, as a result, many of these companies have scaled down their export operations, with some having ceased activities entirely. Although the Iskenderun Bay has very suitable areas for sea bass and sea bream farming, investments in this area have lagged behind other regions of Türkiye. This delay has hindered investments in hatcheries and feed factories in this region, limiting the infrastructure elements of the marine aquaculture sector (Kumlu et al., 2016).

Additionally, the Hatay region has faced various challenges in recent years; geopolitical issues, the consequences of the COVID-19 pandemic, and the February 6 earthquakes is severe destruction and infrastructure have affected the seafood sector (Şimşek and Can, 2019; Can et al., 2020; Demirci et al., 2020; Demirci et al., 2024). These events have led to supply chain disruptions, production interruptions, increased input costs in fisheries, and market uncertainties (Demirci et al., 2020).

This study aims to investigate these regional disparities by providing an in-depth analysis of the factors affecting growth patterns in Hatay compared to Türkiye's broader seafood environment. The study will provide information on the dynamics of the seafood export sector in Türkiye, focusing on how regional differences and challenges can be addressed to benefit from the full potential of regions such as Hatay in the global seafood market.

MATERIALS AND METHODS

Data

The data encompasses seafood exports from Türkiye between 2008 and 2023. This comprehensive foreign trade dataset is sourced from the Turkish Statistical Institute (TurkStat, 2023), a reputable institution known for its detailed and reliable statistical data on a wide range of economic indicators, by utilizing "Foreign Trade by Province and Region Classification (Province/Region-Chapter)" from Foreign Trade Statistics menu on the website. The export data is presented in US Dollars, facilitating international comparisons.

The dataset includes annual export figures, providing a granular view of the export volumes and values over the 15-year period. This allows for a thorough examination of trends and patterns within Türkiye's seafood export sector. The data is segmented by different types of seafood products, enabling an analysis of which specific products have experienced growth or decline over the years.

Additionally, the dataset includes information on the destination countries for these exports. This geographic segmentation provides insights into how Türkiye's seafood export markets have evolved and shifted during the study period. By analyzing the export destinations, the study can identify key markets and potential opportunities for expansion.

Statistical analysis

In order to accurately analyze the change in Hatay province, export data from Adana-Mersin, Istanbul, and Aydın-Muğla, which are important regions in the production and export of aquatic products in Türkiye, were also taken into account. To capture the time dimension of the data and provide a comprehensive analysis of export amounts, the following two methods were used together:

a) Graphical method: The general trend during the study period was determined based on simple linear regression. This method involves plotting the export data over time and fitting a linear regression line to observe the overall direction and strength of the trend.

b) Proportional determination: The change in export amounts between consecutive years was determined proportionally. This method provides a year-by-year comparison to identify specific periods of growth or decline in export amounts.

The following formula was used to determine the change rate of aquatic products exports (USD) in Türkiye and Hatay province over consecutive years:

$$\boldsymbol{Change\ rate}\ (\%) = \frac{(amount\ year_{\ n} - amount\ year_{n-1})}{amount\ year_{n-1}} X100$$

This formula calculates the percentage change in export amounts from one year to the next, providing a clear measure of annual growth or decline. By applying this formula to the export data, the study aims to identify significant fluctuations and trends in the seafood export sector both in Hatay province and in other key regions of Türkiye.

To understand regional disparities and contextualize the trends observed in Hatay province, the export data from Adana-Mersin, Istanbul, and Aydın-Muğla were compared. This comparative analysis was conducted by:

Trend comparison: Comparing the slopes of the linear regression lines for each region to determine which regions experienced the most significant growth or decline.

Change rate comparison: Analyzing the annual change rates across different regions to identify any common patterns or unique deviations in export performance.

All data analyses were conducted using Microsoft Office Excel software, ensuring accurate computations and visualization of results. The graphical method and proportional determination method were both implemented using appropriate statistical functions and tools available within the software.

This dual-method approach allows for a comprehensive analysis of seafood export trends in Hatay province and other key regions in Türkiye, providing valuable insights into the factors influencing export performance and regional disparities.

RESULTS

The export values in Türkiye and Hatay region between 2008 and 2023 and the change rates of exports over the years compared to the previous year are given in Table 1, Figure 1 and Figure 2. The ratio (%) of Hatay in Türkiye's total exports over the years is given in Table 1.

Table 1. Türkiye and Hatay's seafood export (in Millions USD), Change Rates (%), and the contribution percent of Hatay to Türkiye's total export between 2008-2023

Year	Türkiye (US$)	Hatay (US$)	Türkiye (%)	Hatay (%)	Contribution (%)
2008	382.7	9.33			2.44
2009	317.44	15.43	-17.05	65.38	4.86
2010	312.33	16.32	-1.61	5.77	5.23
2011	395.31	15.50	26.57	-5.02	3.92
2012	413.74	14.56	4.66	-6.06	3.52
2013	473.28	19.06	14.39	30.91	4.03
2014	601.56	19.62	27.10	2.94	3.26
2015	690.14	20.25	14.73	3.21	2.93
2016	730.93	21.99	5.91	8.59	3.01
2017	832.01	22.04	13.83	0.23	2.65
2018	869.56	22.58	4.51	2.45	2.60
2019	912.65	8.58	4.96	-62.00	0.94
2020	1008.99	8.81	10.56	2.68	0.87
2021	1100.75	5.37	9.09	-39.05	0.49
2022	1200.65	4.15	9.08	-22.72	0.35
2023	1359.45	3.21	13.23	-22.65	0.24

While Türkiye's total seafood exports have an increasing trend in the period between 2008 and 2023, in the Hatay region, although there was a fluctuation over the years, it was generally seen to be in a decreasing trend (Table1, Figures 1-3). In this sense, when the results were analyzed in more detail, the following determinations were made regarding the export of aquatic products from Hatay province.

The largest proportional decrease in seafood exports in Hatay province compared to the previous year was 62% in 2019. However, it was seen that there have been many fluctuations regarding exports in Hatay province over the years until this year. After 2018, although there was a small increase (2.68%) in the export amount in 2020 compared to 2019, the decline continued dramatically. This is an indication that Hatay province was not stable in terms of production and export.

The contribution of Hatay province to Türkiye's exports peaked in 2010 with a rate of 5.23%, but it is seen that it decreased continuously (r=-0.54) after that (Table 1 and Figure 3). This was also an indication that, apart from Hatay province, other regions in Türkiye have had a greater position in both production and export over the years.

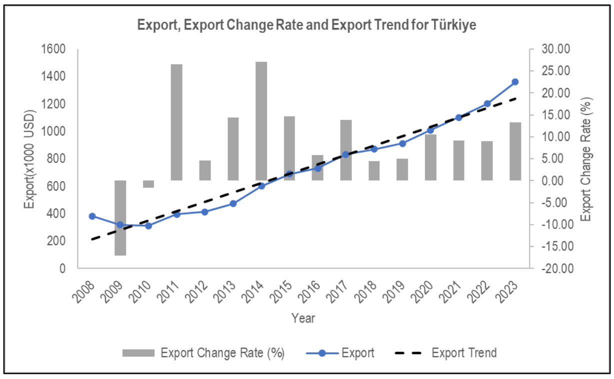

Figure 1. Seafood exports, change (%), and trend in exports for Türkiye between 2008 and 2023

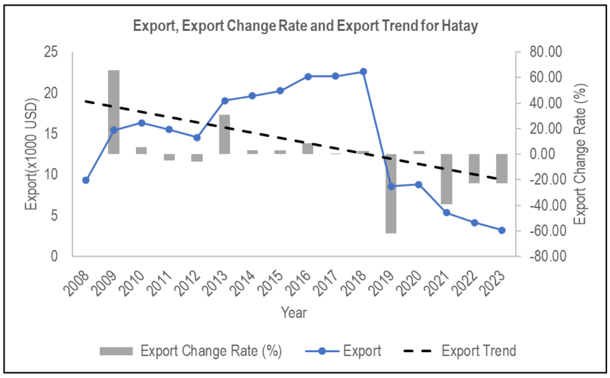

Figure 2. Seafood exports, change (%), and trend in exports for Hatay between 2008 and 2023

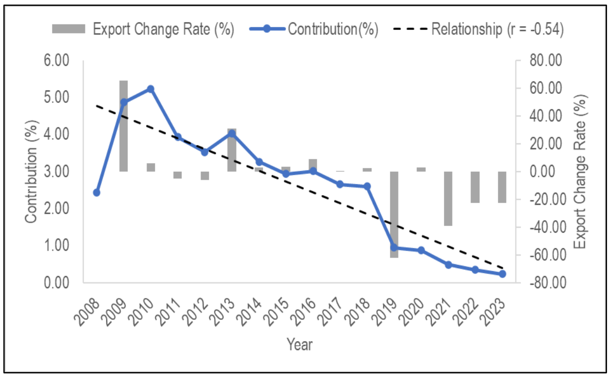

Figure 3. Hatay province's share in Türkiye's seafood exports, amount of change and trend

DISCUSSION

Türkiye's seafood production (fishing and aquaculture) has reached 800 thousand tons by 2023. 500 thousand tons of this production value comes from seafood. Although fisheries production has been almost constant over the years, the amount from aquaculture is increasing slightly every year. There have been significant increases in the production and export of sea bream, sea bass and trout in Türkiye. This is inevitably reflected in export figures. One of the reasons why the share of the Hatay region in Türkiye's seafood exports has decreased over the years is that, although there is production potential in the Iskenderun Bay, the capacities of the seafood production facilities in the region are currently much less than in other regions of Türkiye. For example, in 2022, the increase in seafood exports compared to 2008 was 460.39% in the Adana-Mersin region, 84% in Istanbul, 483.91% in Aydın-Muğla, and 302.24% in the whole of Türkiye, while it decreased by -28.14% in Hatay province. This can also be seen from the proportional contribution (%) of the relevant regions and Hatay province to Türkiye's seafood exports over the years (Table 2).

The reasons for the decrease in seafood exports in the Hatay region can be discussed as follows. For exporting companies in this region, land and infrastructure issues are the primary problems. Despite the presence of numerous organized industrial zones, no space has been allocated for seafood. Therefore, existing facilities struggle primarily with infrastructure issues.

Table 2. Proportional contribution of Hatay, Adana-Mersin, Istanbul and Aydın-Muğla provinces to Turkish seafood exports (%)

Year	Hatay	Adana-Mersin	İstanbul	Aydın-Muğla
2008	2.44	1.46	16.56	32.43
2009	4.86	1.88	14.82	29.50
2010	5.23	1.80	12.48	32.88
2011	3.92	2.22	16.12	44.15
2012	3.52	1.34	15.06	42.76
2013	4.03	2.71	13.69	47.48
2014	3.26	4.29	16.34	60.00
2015	2.93	5.59	13.53	66.43
2016	3.01	6.24	15.26	75.73
2017	2.65	5.74	17.68	80.24
2018	2.60	7.54	18.83	93.88
2019	0.94	6.98	26.05	103.67
2020	0.87	5.28	23.73	113.72
2021	0.49	5.85	22.84	144.75
2022	0.35	8.19	30.49	189.38

Additionally, local municipalities have not established a seafood market, despite the region's significant potential. This is a fundamental reason for the underdevelopment of the seafood sector in Hatay. One reason for the decrease in Hatay's share of Türkiye's seafood exports is the much lower capacity of seafood production facilities in the region compared to other parts of Türkiye, despite the potential in Iskenderun Bay. The increase in exports in these regions reflects the increase in the production of not only fresh seafood but also processed seafood.

The declining exports in the Hatay region can also be attributed to other factors:

Syrian civil war: There are two border gates with Syria in Hatay province. Before the war, trade between the two countries was increasing in all sectors. However, after the Syrian civil war, trade significantly decreased (Collinsworth, 2013; Özenir et al., 2019; Çörekçioğlu et al., 2021). Recent research shows that the war has damaged the aquatic ecosystem and maritime transport in the region (Kılıç, 2018; Özenir et al., 2019; Arslan et al., 2021).

COVID-19 pandemic: Since December 2019, the pandemic has affected every field globally, including the aquaculture sector (Genç et al., 2020; Alam et al., 2022; Sercan, 2022; Demirci, 2024; Koçyiğit and Demiryürek, 2024). The pandemic has had negative effects on the seafood sector globally, such as supply-demand imbalances, restaurant closures, storage inadequacies, plastic pollution, border closures, illegal fishing, inequalities in the sector, and curfews (Can et al., 2020; Kaya and Can, 2022). In a study conducted in Hatay province, the most negative impact of the pandemic in terms of trade (in quantity, kg) was observed in exporters with a 65% decrease, followed by wholesalers (35%), retailers (17% for fishing products and 14% for aquaculture products) (Demirci et al., 2020). However, a study evaluating the effects of the pandemic on Turkish seafood exports found that while fresh seafood exports decreased during the pandemic, frozen and canned seafood exports increased (Can et al., 2020). These results indicate that regions with seafood processing facilities in Türkiye were much less affected by the pandemic than regions without. Since there are not enough processing facilities in the Hatay region, the sharp decrease in 2019 is considered to be due to COVID-19.

Earthquake: The total impact of the disaster caused by the Kahramanmaraş and Hatay earthquakes, which occurred in February 2023 and affected 11 cities in Türkiye, is estimated to be approximately 103.6 billion dollars (Akkuş and Kişlalioğlu, 2023; Yıldız and Kına, 2023; Şenol Balaban et al., 2024). This size, within the scope of the evaluation made by the Presidency's Strategy and Budget Directorate, is estimated to reach approximately 9-10% of the national income of 2023. Hatay province is one of the provinces affected by the earthquake. After the earthquake, not only was the infrastructure damaged, but there was also a migration of qualified personnel out of the city. Like all people in the province, numerous fishermen had to suspend their fishing activities for life care reasons (Demirci et al., 2024). Therefore, the fishing sector was directly and indirectly affected by these disasters. However, the fisheries sector entered the normalization process much earlier than other sectors, about a month after the earthquakes (Demirci et al., 2024). Although this situation cannot be measured exactly for now, it is thought to be inevitably reflected in seafood export data (Can, 2024). These reasons show that the decrease in seafood exports in the Hatay region is due to both regional infrastructure problems and external shocks. Solving these problems is critical for fully realizing the region's seafood potential.

CONCLUSION

Considering the strong industrial and logistics infrastructure in Iskenderun Bay and the region, it is seen that the seafood sector in Hatay has not been able to realize its true potential, contrary to the trend in Türkiye. This situation was inevitably reflected in export figures. It has become inevitable that the already "fragile" sector in the region will be more affected by geopolitical problems, the impact of the epidemic and the effects of earthquakes. An integrated perspective is needed to solve the issue. First of all, the establishment of seafood production facilities in the cages previously planned for Iskenderun Bay should be accelerated. In addition, it will be necessary to establish seafood storage and processing factories, especially fish feed factories, that will support production in the region, taking into account the seafood production capacity. It may not be sufficient to attribute the changes in the seafood sector in Hatay solely to the three factors mentioned. Other elements, such as administrative influences, internal dynamics within the sector, broader economic conditions, and fluctuations in fish stocks in the eastern Mediterranean, may have also impacted production and exports. Considering these additional factors could provide a more comprehensive understanding of the challenges and trends in Hatay's seafood industry. Thus, further analysis into these areas might be valuable for a holistic assessment of the sector. It should not be forgotten that for sustainable export, both qualified human resources and a strong infrastructure are needed. In this sense, universities in the region should also train qualified fishery engineers who can produce added value in the seafood sector, taking into account the needs of the sector.

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AUTHORSHIP CONTRIBUTIONS

Aydın Demirci: Conceptualization, methodology, investigation, supervision. Mehmet Fatih Can: Visualization, methodology, investigation. Yavuz Mazlum: Data collection, writing-reviewing and editing. Emrah Şimşek: Data collection, writing-reviewing and editing. Lastly, all authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors of the article declare that there is no conflict of interest between them.

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

DATA AVAILABILITY

All relevant data is in the article. Even so, for any questions, the corresponding author should be contacted.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Selection of multifilament trammel nets with different mesh width in Lake Erçek

Erçek Gölü'nde farklı ağ göz genişliğine sahip multifilament fanyalı uzatma ağlarının seçiciliği

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Abstract: This study was conducted in Lake Erçek, located in the Van Lake basin. The study examined the selectivity of multifilament trammel nets used in the fishing of pearl mullet (*Alburnus tarichi*, Güldenstädt, 1814) in the lake. The efficiency of nets with mesh sizes of 22 mm, 24 mm, and 26 mm was evaluated in the research. A total of 5.336 pearl mullets were caught in the study carried out between October 2021 and April 2022. 1.296, 1.721 and 2.319 fish were caught from nets with mesh sizes of 22. 24 and 26 mm, respectively. The aim of the study was to determine the most suitable mesh size for sustainable pearl mullet fishing in Lake Erçek. The Holt (1963) method was used to calculate the selectivity parameters. This method considers the fish length and mesh size to determine which size of fish can be caught by the nets. The calculations showed that nets with a 26 mm mesh size had the highest efficiency and contributed to the preservation of fish stocks by allowing smaller fish to escape. The optimum catch lengths for the 22 mm, 24 mm, and 26 mm nets were calculated as 21.15 cm, 22.47 cm, and 23.69 cm, respectively. It was particularly noted that nets with a 22 mm mesh size caught fish below the legal catch size, which could negatively affect the sustainability of the fish stocks. As a result, it was concluded that nets with a 26 mm mesh size are the most suitable option for sustainable fishing in Lake Erçek. The findings of the study provide important data for the conservation of pearl mullet stocks and for the sustainable management of fishing practices.

Keywords: Lake Erçek, Holt method, multifilament trammel nets, pearl mullet, selectivity

Öz: Bu çalışma, Van Gölü havzasındaki Erçek Gölü'nde yürütülmüştür. Çalışmada gölde inci kefali (*Alburnus tarichi*, Güldenstädt, 1814) avcılığında kullanılan multifilament fanyalı uzatma ağlarının seçiciliği incelenmiştir. Araştırmada 22 mm, 24 mm ve 26 mm ağ göz genişliklerine sahip ağlar kullanılarak balık yakalama verimliliği değerlendirilmiştir. Ekim 2021 ile Nisan 2022 tarihleri arasında gerçekleştirilen çalışmada, toplamda 5336 inci kefali yakalanmıştır. 22, 24 ve 26 mm göz genişliğine sahip ağlardan sırasıyla 1296, 1721 ve 2319 adet balık yakalanmıştır. Çalışmanın amacı, inci kefali stokunun sürdürülebilir avcılığına katkı sağlamak için en uygun ağ göz genişliğini belirlemektir. Seçicilik parametrelerinin hesaplanmasında, Holt (1963) metodu kullanılmıştır. Bu metot, balık boyu ve ağ göz açıklığını dikkate alarak ağların hangi boydaki balıkları yakalayabileceğini belirler. Hesaplamalar sonucunda, 26 mm ağ göz genişliğine sahip ağların en yüksek verimliliği sağladığı ve küçük balıkların kaçmasına olanak tanıyarak balık stoklarının korunmasına katkı sunduğu tespit edilmiştir. Çalışma sonucunda, 22, 24 ve 26 mm ağ göz genişliğine sahip ağların optimum yakalama boyları sırasıyla 21,15 cm, 22,47 cm ve 23,69 cm olarak hesaplanmıştır. Özellikle 22 mm ağ göz genişliğine sahip ağların, yasal avlanma boyutunun altında balıkları yakaladığı ve bu durumun stokların sürdürülebilirliğini olumsuz etkileyebileceği belirlenmiştir. Sonuç olarak, 26 mm ağ göz genişliğine sahip ağların Erçek Gölü'nde sürdürülebilir balıkçılık için en uygun seçenek olduğu ortaya konmuştur. Çalışmanın bulguları, inci kefali stoklarının korunması ve sürdürülebilir avcılık yönetimi için önemli veriler sağlamaktadır.

Anahtar kelimeler: Erçek Gölü, Holt metot, fanyalı ağlar, inci kefali, seçicilik

INTRODUCTION

Lake Erçek is the second largest lake after Lake Van in the Lake Van Basin. The lake water is alkaline with pH values ranging from 10.75 to 9 (Yıldız, 1997). The only endemic fish species currently available in Lake Erçek is pearl mullet from the Cyprinidae family (*Alburnus tarichi*, Pallas 1811). Fishing is one of the most important economic activities in the World (Aure et al., 2019) In recent years, overfishing pressure on fish stocks around the world has brought many stocks to the point of collapse (Williams, 1998). Considering the outputs of fishing activities, it is important to use fishing gear with high selectivity (Çınar and Kuşat, 2015; Millar, 1992). Selectivity depends on the type and size of fish caught and their combination during fishing operations; target species or fish individuals of legal size can be selected. Trammel nets and gillnets are used to fish catching a significant proportion of the fishing in Türkiye's inland waters (Kuşat, 1996). Commercial inland fishing occurs on mainly lakes and dam lakes (Cilbiz and Ateşşahin, 2024). In Türkiye inland waters where the use of large-scale fishing gear is prohibited, gillnets and trammel nets are one of the most commonly used fishing gear. Due to their high selectivity, trammel nets are thought to be very important for sustainable fishing (Kocabaş et al., 2018). Selectivity in trammel net is associated with the shape, size, and behavioral characteristics of the fish, and the colour, mesh size, hanging ratio and rigging factor of the net. (Rosman and Maugeri, 1980; Özyurt and Yeşilçimen, 2013). Mesh size in trammel net is the main factor determining selectivity (Von Brandt, 1975). Although Lake Erçek contains the largest pearl mullet stock in the basin after Lake Van, a limited number of studies have been carried out in the lake. In Lake Erçek; studies have been carried out on plankton species (Yıldız, 1997), the pearl mullet population structure

(Gündoğdu, 2010), carrying capacity of lake (Akkuş and Sarı, 2013) and seasonal temperature distribution (Meydan and Akkol, 2020). In Lake Van; the selectivity properties and catch efficiency of trammel nets used in pearl mullet fishing in Lake Van were investigated by Çetinkaya et al. (1995), The catch efficiency of trammel nets with different rigging factors and rope thicknesses used in pearl mullet fishing in Lake Van was investigated by Sarı and Tokaç (2000), The selectivity of multifilament nets used in pearl mullet (*Alburnus tarichi*, Güldenstädt, 1814) fishing was investigated by Pala (2021). Although there are different studies on determining the selectivity of trammel nets used in pearl mullet fishing in Lake Van, there is no selectivity study conducted in Lake Erçek. This situation creates uncertainty in terms of fisheries management in the lake. In this study, the selectivity of multifilament trammel nets with mesh sizes of 22, 24 and 26 mm used in pearl mullet fishing in Lake Erçek was determined. Selectivity parameters of the nets used in the study were calculated by the Holt (1963) method and the effects of different mesh sizes on the fish population were evaluated. It is thought that the findings will contribute to the development of fisheries management strategies and the protection of the pearl mullet stock in the lake.

MATERIALS AND METHODS

The study was carried out in Lake Erçek (Figure 1) between 31.10.2021 and 10.04.2022. Covering an area of 114 km², Lake Erçek is the second largest lake in the basin following Lake Van. It has a maximum depth of 40 m and an average depth of 18.45 m (Sarı and İpek, 1998). The only endemic fish species currently available in Lake Erçek is pearl mullet from the Cyprinidae family.

The nets used in the study are trammel nets traditionally used in Lake Erçek. A total of 27 samplings were made at different points of the lake. The technical plan of the trammel nets used in the study are given in Figure 1, 2 and 3. In this study, the hanging ratio in the outer panels in inner panel sections of the outher panel-type trammel nets are the same and set to 0.5.

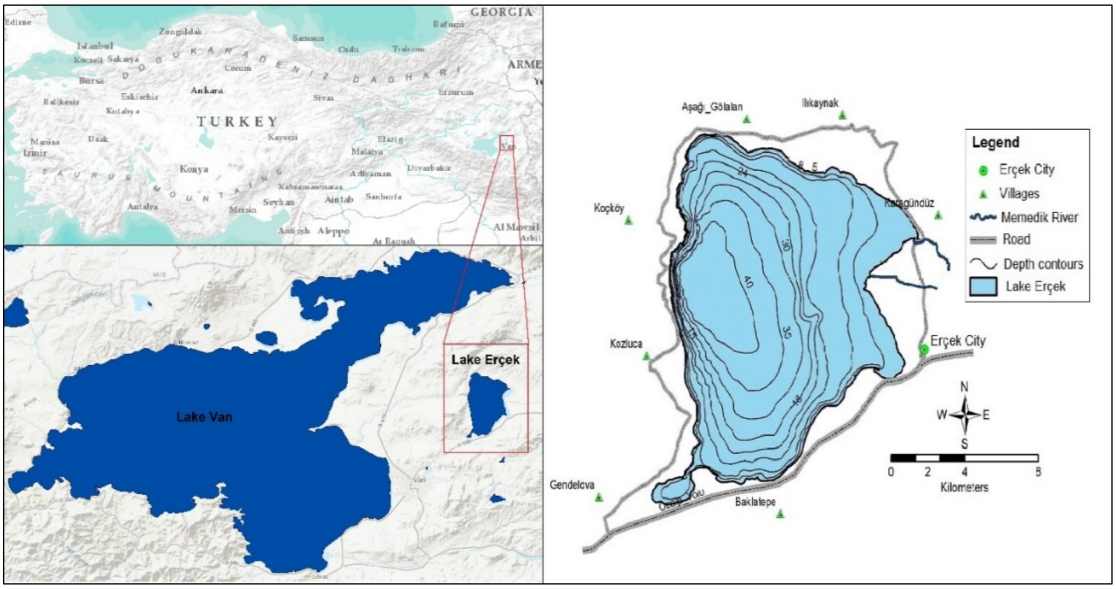

Figure 1. Study area

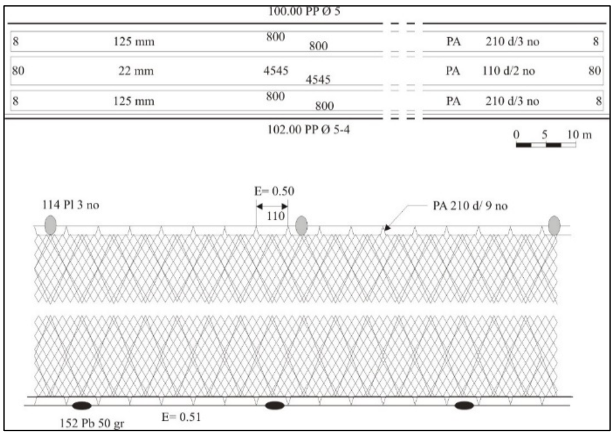

Figure 2. Technical plan of the nets with 22 mm mesh size

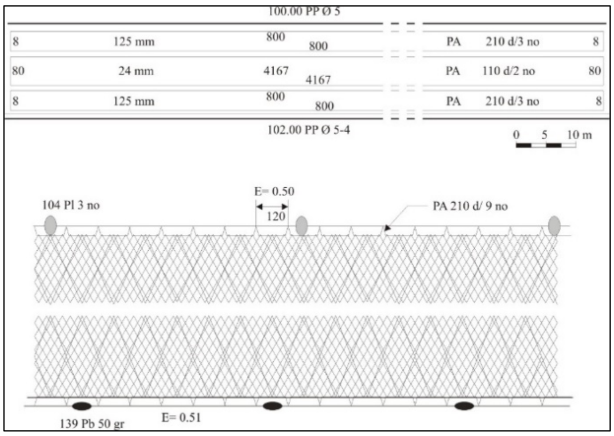

Figure 3. Technical plan of the nets with 24 mm mesh size

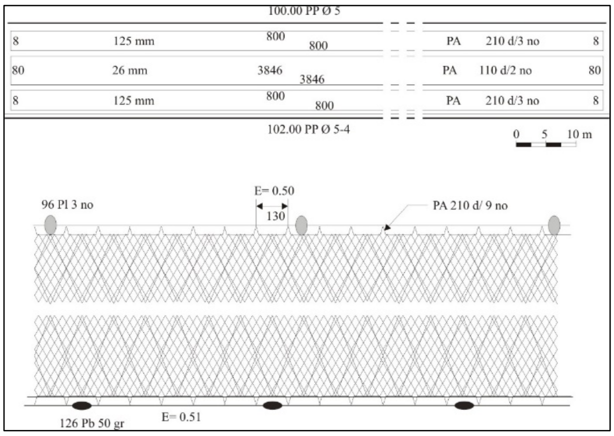

Figure 4. Technical plan of the nets with 26 mm mesh size

Holt (1963) method was used to calculate selectivity. The Holt method, which takes into account fish length and mesh size, was used to calculate selectivity parameters. Fish caught from nets with different mesh sizes were determined according to their size groups; the number of fish caught in large mesh nets was divided by the number of fish caught in small mesh nets to obtain the natural algorithm. Linear regression analysis is used to examine the relationships between size range (L) and logarithmic ratios. As a result of this analysis, parameters such as the slope and intercept between specific independent and dependent variables are calculated.

The formula for calculating the optimum capture size for a small mesh and large mesh net:

$Lm_1 = (-2a * m_1) / (b * (m_1 + m_2))$

$Lm_2 = (-2a * m_2) / (b * (m_1 + m_2))$

m_1, m_2 = Mesh size of small mesh and large mesh net (mm)

Lm_1, Lm_2 = Optimum catch size of small mesh and large net (cm)

Standard Deviation According to Nets:

$SD = \{1 / (n - 1) \Sigma [-2a_i (m_{i_1} - m_i)] / [b_i^2 (m_i + m_{i_1})]\}^\wedge(1/2)$

n: Total number of observations

a_i: Selectivity coefficient

m_{i_1}, m_i: Averages of mesh sizes

b_i: Selectivity curve coefficient

Calculation of the Selection Factor for Two Nets with Consecutive Mesh Sizes:

$SF=-2a/b*(m_1 + m_2)$

The catch rates are calculated as a function of length for each net according to the size group. Using the function s(Li), the selectivity curve is drawn for each net:

$s(L_i) = e^\wedge((L - Lm_1)^\wedge 2 / (2 * s^\wedge 2))$

$s(Li)$= selectivity curve function of the mesh with i mm bar length

Lm_1: optimum catching length of the mesh with i mm bar length

RESULTS

In the study, a total of 5336 pearl mullet were caught; 1296 from 22 mm nets, 1721 from 24 mm nets and 2319 from 26 mm nets with different mesh sizes. It was determined that the fork lengths of the caught fish varied between 17-26 cm (Table 1). It was observed that there were injuries and deformations in the caudal fins of the fish caught especially in 22 mm and 24 mm nets. Therefore, fork length was used in the study.

Table 1. Percentage of fish caught by size groups

Length (cm)	22 mm net		24 mm net		26 mm net	
	Quantity	Percent (%)	Quantity	Percent (%)	Quantity	Percent (%)
17	3	0.231481	0	0	0	0
18	5	0.385802	0	0	0	0
19	58	4.475309	5	0.290528	0	0
20	215	16.58951	123	7.147007	11	0,474342
21	387	29.86111	240	13.94538	140	6,037085
22	412	31.79012	390	22.66124	240	10,34929
23	117	9.027778	756	43.92794	715	30,83226
24	63	4.861111	153	8.890180	1122	48,38292
25	36	2.777778	54	3.137710	86	3,708495
26	0	0	0	0	5	0,21561
Total	**1296**		**1721**		**2319**	**5336**

When the size distribution of fish caught with a 22 mm mesh size, multifilament trammel net is examined, it is seen that 16.59 % of the fish caught are in the 20 cm size group, 29.9 % in the 21 cm size group and 31,8 % in the 22 cm size group. The lowest rates are in the 17 cm size group with 0.23 % and 18 cm size group with 0.38 % (Figure 5). When the size distribution of fish caught with a 24 mm mesh size multifilament trammel net is examined, it is seen that 14% of the fish caught are in the 22 cm size group, 22.7% in the 23 cm size group and 48.5% in the 24 cm size group. The lowest ratio is seen in the 19 cm size group with 0.29% and the 25 cm size group with 3.14% (Figure 6). When the size distribution of fish caught with 26 mm mesh size, multifilament trammel net is examined, it is seen that 10.4% of the fish caught are in the 21 cm size group, 30.9% in the 22 cm size group and 43.9% in the 23 cm size group. The lowest rate is seen in the 20 cm size group with 0.5% and in the 25 cm size group with 3.7% (Figure 7).

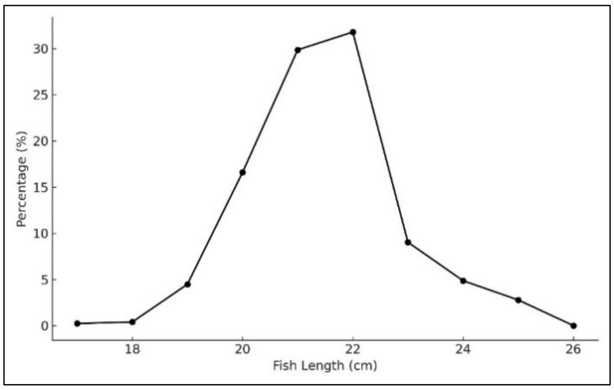

Figure 5. Length-percentage graph of fish caught with 22 mm

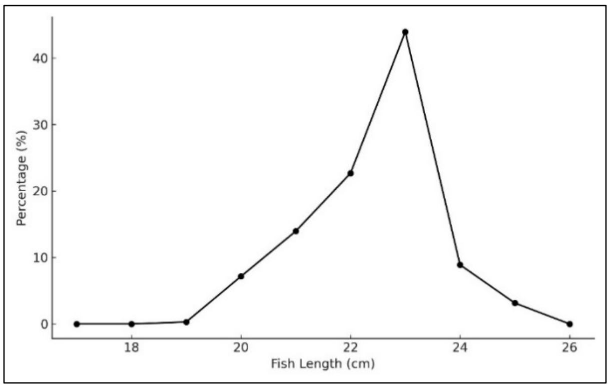

Figure 6. Length-percentage graph of fish caught with 24 mm

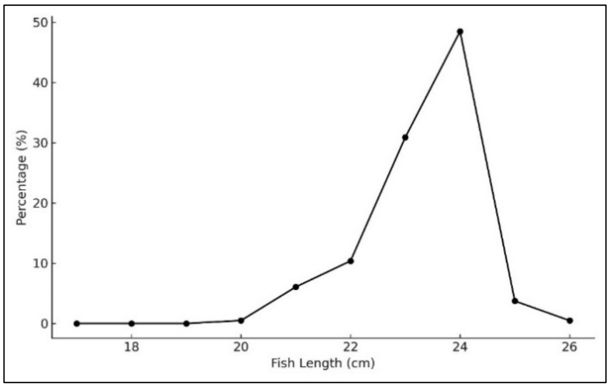

Figure 7. Length-percentage graph of fish caught with 26 mm

Calculation of selectivity parameters of 22-24 mm trammel nets;

It was determined that fish with a maximum length of 22 cm were caught in the net with a mesh size of 22 mm, and fish with a maximum length of 23 cm were caught in the net with a mesh size of 24 mm (Table 2).

In the regression analysis; the intersection point with the y-axis (a): -10.901, slope (b): 0.493 was calculated. The optimum catch length of the 22 mm net: 21.150. The optimum catch length of the 24 mm net: 23.072 (Figure 8). Standard deviation: 3.900. Selectivity factor: 0.480. Catch rates of 22 and 24 mm nets; PA: exp[-(L-21.150)2 / 2*(3.900)2] and PB: exp[-(L-23.072)2 / 2*(3.900)2].

Table 2. Selectivity values of 22-24 mm trammel nets

Length (cm)	$C1$(22)	$C2$(24)	$C2/C1$	ln($C2/C1$)	PA	PB
17	3	0	Not Used			
18	5	0				
19	58	5	0.086207	-2.45101	0.859	0.579
20	215	123	0.572093	-0.55845	0.957	0.733
21	387	240	0.620155	-0.47779	0.999	0.868
22	412	390	0.946602	-0.05488	0.976	0.962
23	117	756	6.461538	1.865867	0.893	0.999
24	63	153	2.428571	0.887303	0.765	0.972
25	36	54	1.5	0.405465	0.6143	0.8849

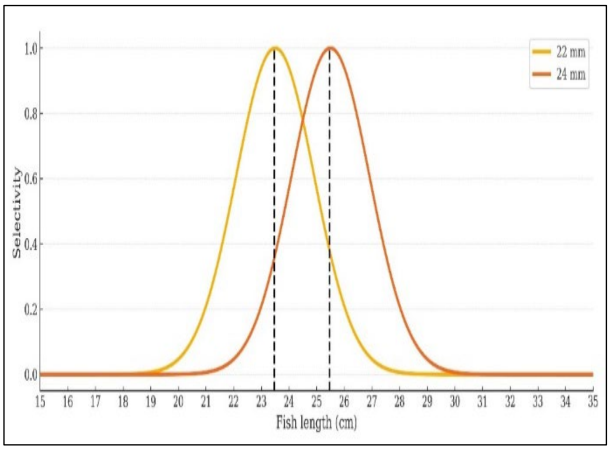

Figure 8. Selectivity curves of 22-24 mm mesh size

In the study, the highest number of fish was caught in the 26 mm trammel net (Table 3).

Table 3. Selectivity values of 24-26 mm nets

Length(cm)	$C2$(24)	$C3$(26)	$C3/C2$	ln($C3/C2$)	PA	PB
19	5	0	Not Used			
20	123	11	0.089	-2.414	0.957	0.431
21	240	140	0.583	-0.539	0.999	0.639
22	390	240	0.615	-0.485	0.976	0.838
23	756	715	0.945	-0.055	0.893	0.970
24	153	1122	7.333	1.992	0.765	0.994
25	54	86	1.592	0.465	0.6143	0.899
26		5	Not Used			
27		2				

In the regression analysis; the intersection point with the y-axis (a): -14.58, slope (b): 0.640 was calculated. The optimum catch size of the 24 mm mesh: 21.870. The optimum catch size of the 26 mm mesh: 23.692 (Figure 9). Standard deviation: 2.847. Selectivity factor: 0.450. The catch rates of 24 and 26 mm meshes; PA: exp[-(L-21.870)2 / 2*(2.847)2] and PB: exp[-(L-23.697)2 / 2*(2.847)2].

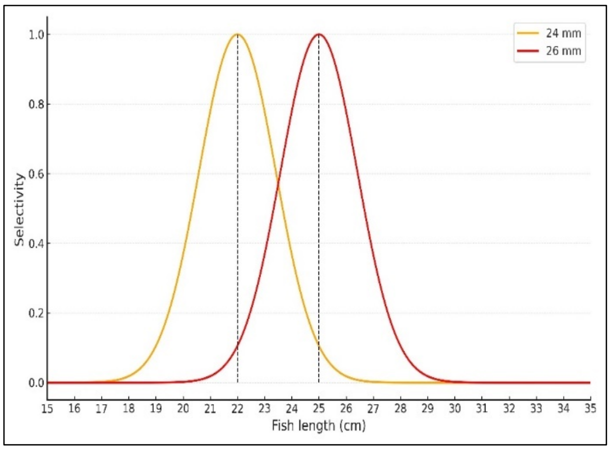

Figure 9. Selectivity curves of 24-26 mm mesh size

DISCUSSION

This study is the first selectivity study conducted in Lake Erçek. When the studies on the selectivity of multifilament trammel nets used in Lake Van pearl mullet fishing were examined; Çetinkaya et al. (1995) used 17 mm, 20 mm and 24 mm mesh widths in pearl mullet fishing. They reported the optimum catch length to be between 15.5-20.6 cm. In the study conducted by Pala (2021) using multifilament trammel nets in Lake Van, the optimum catch length was determined as 20.76 cm for 20 mm mesh widths, 22.07 cm for 22 mm and 24.11 cm for 24 mm. Although the optimum catch lengths determined in the studies conducted in Lake Erçek are similar to the study conducted by Pala (2021), it is seen that they are different from the studies conducted in previous years. Different aquatic ecosystems have different ecological characteristics such as temperature, food and environmental conditions. Therefore, it is expected that different results will be obtained in selectivity studies conducted in different aquatic ecosystems. Langerhans et al. (2003) reported that the same fish species living in different habitats may differ morphologically depending on environmental effects. Another reason for obtaining different optimum sizes in the studies is thought to be due to the fact that the amount of illegal hunting carried out during the pearl mullet breeding season has been largely prevented with conservation efforts in recent years. According to the Communique No. 6/1 on the Regulation of Commercial Aquatic Products Fishing, which regulates fisheries in Türkiye, the minimum catch length of pearl mullet is specified as 18 cm. However, it was determined that the 22 mm net used in the study caught individuals below the minimum catch length of 17 cm (Table 1). Therefore, it is thought that the use of 22 mm nets in the lake will harm the stock. While the 26 mm nets used in the study caught fish with a minimum length of 20 cm, it was observed that they mostly caught fish with a length of 24 cm (Table 1). In addition, it was determined in the study that the 26 mm nets caught the most fish with 2314 individuals. In this respect, the 26 mm nets catch the large fish in the stock and allow the development of small fish. This situation is beneficial for the sustainability of the pearl mullet stock of Lake Erçek. The most important outputs in fisheries management are fuel and labor. Reducing these outputs is one of the basic elements for the continuity of fishing. Since more fish are caught in the same period compared to the other nets used in the study and these fish are in the range of 23-24 cm according to their fork lengths, the use of 26 mm nets will contribute to the fishing carried out in the lake to gain a more economically efficient structure.

CONCLUSION

The results of this study underscore the importance of using highly selective fishing gear, such as multifilament trammel nets, for the sustainable management of the pearl mullet population in Lake Erçek. It was observed that nets with a 26 mm mesh size effectively target larger fish, allowing smaller fish to escape, thus supporting the renewal of the fish stock. On the other hand, the use of 22 mm mesh nets, which were found to capture fish below the legal size limit, poses a threat to the sustainability of the population and could negatively affect the stock if not managed properly. This study provides essential data that can contribute to fisheries management decisions in Lake Erçek, promoting both the long-term sustainability of the pearl mullet population and more economically efficient fishing practices. Future research should focus on determining the selectivity parameters of monofilament nets used in the lake to enhance fisheries management. Additionally, given that pearl mullet rely on two rivers flowing into the lake for spawning, it is crucial to investigate how potential hydrological droughts in the coming years might impact the stock. This would offer valuable insights into preserving the pearl mullet population in the face of environmental changes.

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AUTHORSHIP CONTRIBUTIONS

Seda İlmen Çevik: Data acquisition, writing, validation. Mustafa Akkuş: Data acquisition, statistical analysis, editing.

STATEMENT OF CONFLICT OF INTEREST

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

ETHICS APPROVAL

The research was approved by Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee in terms of sampling and use of experimental animals with decision number 2021/11-11 at the meeting held on 25.11.2021. All researchers declare that all trials were conducted in accordance with ethical values.

DATA AVAILABILITY

The corresponding author should be contacted for questions about datasets.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Fish consumption in restaurants: An investigation on planned behavior theory and food neophobia

Restoranlarda balık tüketimi: Planlı davranış teorisi ve yiyecek neofobisi üzerine bir araştırma

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Abstract: In this study, consumers' intention to consume fish in restaurants was explored by expanding Ajzen's (1985) theory of planned behavior (TPB). The food neophobia (FN) variable was added to the variables of attitude, subjective norm (SN) and perceived behavioral control (PBC), which are the main independent variables of the TPB, and the moderator role of the variable of FN between the variables of attitude and intention to consume fish was also controlled. A questionnaire was used to reach a total of 517 participants in Antalya/Türkiye. For the analysis of the obtained data, exploratory factor analysis (EFA), confirmatory factor analysis (CFA), and multiple linear regression analyses were performed using SPSS and Lisrel package programs. It was found that the variables of attitude, SN and PBC have a significant and positive effect on consumers' intention to consume fish in restaurants. Also, the FN variable had a significant and negative effect on consumers' intention to consume fish in restaurants. Finally, FN variable had a significant moderator effect between the variables of attitude and intention to consume fish. In short, individuals with high FN may not translate a positive attitude toward eating fish into a strong intention to consume it, while those with low neophobia may do so more effectively. FN alters the link between attitude and intention, highlighting its role in shaping fish consumption decisions. This research offers key insights for public health and the food industry. Findings can guide healthy eating campaigns, marketing strategies, product development, and efforts to promote sustainable fish consumption, while also considering the impact of social norms within behavioral economics.

Keywords: Food neophobia, planned behavior theory, seafood consumption, intention of fish consume

Öz: Bu çalışmada, tüketicilerin restoranlarda balık tüketme niyeti, Ajzen'in (1985) planlı davranış teorisi (PDT) genişletilerek incelenmiştir. PDT'nin temel bağımsız değişkenleri olan tutum, öznel norm (ÖN) ve algılanan davranışsal kontrol (ADK) değişkenlerine ek olarak, gıda neofobisi (GN) değişkeni de eklenmiş ve bu değişkenin tutum ve balık tüketme niyeti arasındaki moderatör rolü kontrol edilmiştir. Antalya/Türkiye'de toplam 517 katılımcıya anket uygulanmıştır. Elde edilen verilerin analizi için SPSS ve Lisrel paket programları kullanılarak keşfedici faktör analizi (KFA), doğrulayıcı faktör analizi (DFA) ve çoklu doğrusal regresyon analizleri yapılmıştır. Tutum, ÖN ve ADK değişkenlerinin, tüketicilerin restoranlarda balık tüketme niyetini anlamlı ve pozitif yönde etkilediği bulunmuştur. Ayrıca, GN değişkeninin, tüketicilerin restoranlarda balık tüketme niyetini anlamlı ve olumsuz yönde etkilediği saptanmıştır. Son olarak, GN değişkeninin, tutum ve balık tüketme niyeti arasında anlamlı bir moderatör etkisi olduğu tespit edilmiştir. Kısaca, yüksek gıda neofobisine sahip bireyler, balık yemeye yönelik olumlu bir tutumu güçlü bir tüketime niyete dönüştüremeyebilirken, düşük neofobiye sahip bireyler bunu daha etkili bir şekilde yapabilir. GN, tutum ve niyet arasındaki bağı değiştirerek balık tüketim kararlarını şekillendirmede önemli bir rol oynamaktadır. Bu araştırma, halk sağlığı ve gıda sektörü için önemli bulgular sunmaktadır. Bulgular bağlamında ilgili paydaşlar, sağlıklı beslenme kampanyalarına, pazarlama stratejilerine, ürün geliştirmeye ve sürdürülebilir balık tüketimini teşvik etme çabalarına rehberlik edebilir ve sosyal normların davranışsal ekonomi bağlamındaki etkisini de göz önünde bulundurabilir.

Anahtar kelimeler: Gıda neofobisi, planlı davranış teorisi, su ürünleri tüketimi, balık tüketme niyeti

INTRODUCTION

Nutrition is one of the key factors affecting human health and development. Therefore, the selection and consumption of foods for a healthy diet are critical. Fish has a high nutritional value and beneficial nutrients and is considered a functional food. It contributes to the proper development and functioning of the human body while reducing the risk of certain diseases (Fotea et al., 2012; Sidhu, 2003). Increased fish consumption is in line with healthy eating trends (Kornitzer, 2001; Verbeke and Vackier, 2005). The per capita consumption of seafood products worldwide was 9.0 kg in 1961. It increased at an average rate of 1.5 percent per year, reaching 20.3 kg in 2017 (FAO, 2018), and a record level of 20.5 kg in 2019 (FAO, 2022).

Regional differences in seafood consumption are significant, with Asia emerging as the largest consumer, representing two-thirds of global seafood production. Countries like China, Japan, and Indonesia lead in per capita consumption, often exceeding 30 kg per person each year (Wai et al., 2021). In Japan, for example, fish consumption is an integral part of dietary practices, with average intake reaching about 50 kg annually (Wai et al., 2021). In contrast, European nations display diverse consumption patterns, with Portugal ranking high at approximately 59 kg per capita per year, while countries such as the UK report lower consumption levels (Paolacci et al., 2021). Although there has been an increasing trend in fish consumption and fishing in recent years, studies conducted in various countries have indicated that participants consume fish and seafood products below the recommended levels: at least 2 servings per week (Altintzoglou et al., 2011;

Grieger et al., 2012). This is also valid for Türkiye, which is geographically advantageous in terms of source and proximity to the source. In 2023, per capita seafood consumption in Türkiye has been reported as 7.1 kg, which is well below the world average (TUİK, 2024). The examination of the reasons behind the insufficient consumption of fish and seafood products, despite the increasing interest in healthy nutrition, is still a current and important research topic.

MATERIALS AND METHODS

The study utilized a structured questionnaire as the primary data collection tool. The aim of this study was to evaluate the factors affecting the consumers' intention to consume fish in the restaurant within the scope of TPB. In addition to the attitude, SN and PBC variables that are assumed to be effective in explaining the intention, the FN variable was also included in the research model. Quantitative research design was adopted in the study. In order to collect the data, the scales obtained from the relevant literature were adapted to Turkish and presented to the participants in the form of a questionnaire. EFA, CFA and multiple linear regression analyses were performed on the collected data using IBM SPSS (version 22) and Lisrel (version 8.80) package programs.

Research model and hypotheses

Food neophobia (FN) is the tendency to avoid or hesitate in trying unfamiliar foods (Pliner and Hobden, 1992). It is viewed as a trait that predicts willingness to try new or familiar foods (Caber et al., 2018; Kim et al., 2009). FN negatively impacts the consumption and preference for foods like fish and seafood (Knaapila et al., 2011; Siegrist et al., 2013). Based on this, the study hypothesizes that FN will reduce the intention to consume fish in restaurants. The first hypothesis of the research is as follows.

H1: Food neophobia has a negative and significant effect on the intention to consume fish in the restaurant.

A positive attitude towards a behavior strengthens the intention to perform it (Fishbein and Ajzen, 1975). Consumer attitudes significantly influence food consumption, particularly fish (Tomic et al., 2015; Thong and Olsen, 2012; Verbeke and Vackier, 2005). In restaurants, food quality, service, and environment also shape customer behavior (Canny, 2014; Liu and Jang, 2009; Ryu and Han, 2010). Thus, well-prepared fish dishes, good service, and a favorable environment can positively influence attitudes and increase the intention to consume fish in restaurants. In this direction, the second hypothesis of the research is as follows.

H2: Attitude towards fish consumption has a positive and significant effect on the intention to consume fish in the restaurant.

Subjective norm (SN) refers to the influence of expectations from significant others on a person's behavior (Ajzen, 1991). Social pressure on fish consumption often comes from close social circles, like family and friends (Verbeke and Vackier, 2005). In restaurants, factors such as the presence of others, customer recommendations, and staff suggestions also impact consumption behavior (Canny, 2014; Liu and Jang, 2009; Özdemir, 2010; Pettersson and Fjellström, 2007; Ryu and Han, 2010). Positive impressions and recommendations during dining can enhance SN, thereby increasing the intention to consume fish. The third hypothesis of the research is as follows.

H3: The subjective norm for fish consumption has a positive and significant effect on the intention to consume fish in the restaurant.

Perceived behavioral control (PBC) suggests that a person's intention to perform a behavior increases when they believe they have the necessary resources and face minimal difficulties (Ajzen, 2002; Kocagöz and Dursun, 2010). A person's ease or difficulty in consuming fish, along with available resources, influences their behavior. Restaurant atmosphere and environmental factors also significantly impact consumption (Gustafsson et al., 2006; Hansen et al., 2005; Pettersson and Fjellström, 2007). A positive perception of these factors can enhance PBC, thereby increasing the intention to consume fish in restaurants. The fourth hypothesis of the research is as follows.

H4: PBC for fish consumption has a positive and significant effect on the intention to consume fish in the restaurant.

Attitude plays a key role in explaining fish consumption behaviors (Olsen, 2003; Olsen et al., 2007; Rortveit and Olsen, 2007; Verbeke and Vackier, 2005). While people view fish as healthy, negative sensory factors like smell, texture, and bones may deter consumption. Compared to other TPB variables, food neophobia (FN) is expected to have a negative moderating effect on the relationship between personal attitudes and the intention to consume fish. The fifth and final hypothesis of the research is as follows.

H5: Food neophobia has a negative and significant moderator effect on the relationship between the attitude towards fish consumption and the intention to eat fish in the restaurant.

The research model created is presented in Figure 1

Universe and sample

The study's population includes domestic consumers in Antalya who have dined at any restaurant in the last six months. Since individuals with fish neophobia may avoid fish restaurants, the sample is not limited to those venues. According to Krejcie and Morgan (1970), a population of one million requires a minimum sample size of 384. Although different methods exist for determining sample size for factor analysis, a minimum of 300 participants is generally accepted (Aksu et al., 2017). Therefore, the study aimed for at least 400 participants, utilizing convenience sampling for efficiency and cost-effectiveness.

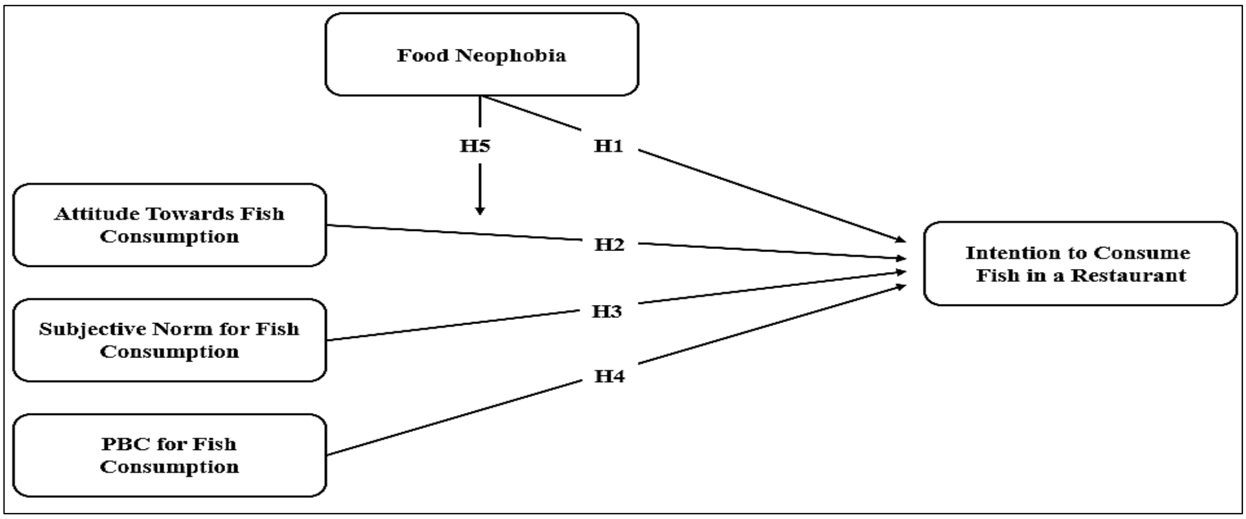

Figure 1. Research model

Data collection tool

A questionnaire was used as the data collection tool in this study. Scales for the dependent and independent variables were adapted to measure the intention to consume fish in restaurants, based on relevant literature. The FN scale by Pliner and Hobden (1992) assessed participants' fish neophobia, while the scale for individual determinants of fish consumption from Verbeke and Vackier (2005) measured attitudes, social norms (SNs), perceived behavioral control (PBC), and intention. Although the original FN scale utilized a 7-point Likert scale, this study employed a 5-point Likert scale (1: Totally Disagree, 5: Totally Agree) to simplify response options for participants (Laureati et al., 2016).

The scales were translated into Turkish by three English experts, with discrepancies checked and consolidated into a single scale. This Turkish scale was then back-translated into English, and differences were compared with the original. The Turkish and English versions were administered separately to a group of 12 bilingual participants, and their total scores were compared. After incorporating feedback, the scale was reviewed by five faculty experts. A pilot test was conducted with 100 participants to validate the measurement tool. The finalized tool was applied to participants from December 15, 2019, to January 15, 2020, yielding 517 valid questionnaires.

RESULTS

Findings regarding the socio-demographic characteristics of the participants

The findings regarding the gender, age, education and monthly income of the participants as frequency and percentage distributions are given in Table 1. As can be seen on the table, 57.3% of the participants in the application were male. Also 35.8% of the participants were between the ages of 18-24, 48% had undergraduate education. Finally, looking at the monthly income, 25.7% of the participants had a monthly income between 0-999 TL, while 22.8% of them had an income of 4000 TL and above.

Table 1. Socio-demographic characteristics of the participants

Category	Frequency(n)	Percentage (%)
Gender		
Woman	221	42.7
Male	296	57.3
Total	517	100
Age		
18-24	185	35.8
24-34	90	17.4
35-44	67	13
45-54	97	18.8
55+	78	15.1
Total	517	100
Education Status		
Primary School	55	10.6
High School	99	19.1
Associate Degree	45	8.7
Undergraduate	248	48
Postgraduate	70	13.5
Total	517	100
Monthly Income Status		
0-999	133	25.7
1000-1999	87	16.8
2000-2999	79	15.3
3000-3999	100	19.3
4000+	118	22.8
Total	**517**	**100**

Findings regarding the validity and reliability of the scales

In this study, the validity of the scale was evaluated using factor analysis to observe to what extent the scale actually measures the construct that is intended to be measured. Factor analysis is one of the methods that helps to reveal the factor structure of the measurement tool instead of giving a single coefficient for the validity of the measurement tool or is used to confirm the factor structure that has been determined before (Aksu et al., 2017). In this context, EFA was used to determine the factor structure of the adapted scale, and CFA was used to confirm the determined factor structure (Büyüköztürk et al., 2018).

To be suitable for factor analysis the analyzed data should have a Kaiser-Meyer-Olkin (KMO) value greater than 0.50 and a Bartlett sphericity test significance value less than 0.05 ($p < 0.05$) (Aksu et al., 2017). All scales showed sufficient ranges of values for factor analysis. Assuming the base value of the factor loadings to be 0.40 (Aksu et al., 2017), expressions with lower loads than this value and expressions showing overlapping problem were excluded from the analysis (Aksu et al., 2017). In determining the number of sub-factors, only the factors with an eigenvalue greater than 1 were considered based on the Guttman-Kaiser rule (Aksu et al., 2017). Cronbach Alpha (α) analysis, which is one of the frequently used methods to calculate the reliability of the measurement results, was used. The fact that the Cronbach Alpha value is between 0.60<α<0.80, shows that the measurement tool is quite reliable (Kalaycı, 2009). According to the findings, it can be stated that all the scales used in the study are quite reliable, the relevant values are presented in Table 2.

Table 2. Results of exploratory factor analysis regarding the scales

Scales and Statements	Factor Load	Explained Variance Percentage
Food Neophobia Scale (α=0.93)		
1. Factor: Food Neophobia		
1. I am constantly sampling new and different foods. (reverse-scaled)	.840	62.096
2. I don't trust new foods.	.798	
3. If I don't know what is in a food, I won't try it.	.749	
4. I like foods from different countries. (reverse-scaled)	.830	
5. Ethnic food looks too weird to eat.	.726	
6. At dinner parties, I will try a new food. (reverse-scaled)	.788	
7. I am afraid to eat things I have never had before.	.826	
8. I am very particular about the foods I will eat.	.734	
9. I will eat almost anything. (reverse-scaled)	.772	
10. I like to try new ethnic restaurants. (reverse-scaled)	.808	
Attitude towards eating fish (α=0.87)		
1. Factor: Positive attitude factor		
1. Eating fish is not trustworthy (reverse-scaled).	.805	60.186
2. Eating fish is healthy.	.886	
3. Eating fish is safe.	.810	
5. Eating fish is nutritious.	.842	
8. Fish has a good taste.	.669	
2. Factor: Negative attitude factor		
6. Fish has an unpleasant smell (reverse-scaled)	.868	15.531
7. The bones in fish are unpleasant (reverse-scaled)	.893	
*The 4th statement was not included in the scale because it had a low factor loading (<.40), and the 9th and 8th statements showed overlap (<.10).		
4. Eating fish is expensive (reverse-scaled).		
9. I am very satisfied when fish is on the menu.		
Subjective norm scale (α=0.91)		
1. Factor: Personal norm (personal responsibility, moral obligation)		
8. To give my family a healthy meal, I buy fish.	.915	55.488
9. To give my family a nutritious meal, I buy fish.	.911	
10. To offer my family a varied meal, I buy fish.	.851	
2. Factor: External social norm		
3. The government stimulates me to eat/buy more fish.	.891	12.831
5. Advertising stimulates me to eat/buy more fish.	.868	
7. The food industry encourages me to eat/buy more fish.	.805	
3. Factor: Internal social norm		
1. My family thinks that I should eat/buy fish.	.749	10.782
2. My friends think that I should eat/buy fish.	.708	
4. Doctors and nutritionists think that I should eat/buy fish.	.774	
6. My partner thinks that I should eat/buy fish.	.612	
Perceived behavioural control Scale (α=0.94)		
1. Factor: Perceived behavioural control		
1. I find it difficult to judge the quality of fish (reverse scaled)	.728	64.198
2. I can make many different meals with fish.	.771	
3. When I buy fish, the chance to make a bad choice is big (reverse-scaled)	.796	
5. Fish is difficult to prepare (reverse-scaled)	.717	
6. When I buy fish, I never know whether I make a good choice (reverse scaled)	.815	
7. I am familiar with eating fish.	.699	
8. I have much experience in buying fish.	.849	
9. I know a lot of fish species that can be prepared.	.851	
10. I have much knowledge about fish.	.877	
11. I am well informed about fish.	.861	
12. I am familiar with preparing fish.	.838	
13. Eating fish is part of my eating habits.	.787	
*4.statement was not included in the scale due to low factor loading (<.40)		
4. Fish is easily available for me.		
Intention scale (α=0.94)		
1. Factor: Behavioural intention		
1. The chance that I eat fish at restaurant for the next weeks is high.	.953	90.519
2. I am planning to eat fish at restaurant during the next weeks.	.970	
3. My willingness to eat fish at restaurant is high.	.931	

The FN scale adapted from the study of Pliner and Hobden (1992) consists of a total of 10 expressions, 5 negatives and 5 positives. Positive expressions (1,4,6,9,10) were analyzed by reverse coding during data processing. In this way, it can be interpreted that the FN will increase as the score increases in the answers given to the related statements. After analysis, 1 factor with an eigenvalue greater than 1 (6.210) and consisting of 10 expressions emerged. The total variance explanation rate of a single factor was 62%. The first analysis on SN, PBC and intention scales adapted from Verbeke and Vackier's (2005) study was made on the attitude scale. The attitude scale consists of 9 statements. 1,4,6 and 7th statements were analyzed by being reverse coded, adhering to the original scale. As a result of the analysis, the 4th statement with a factor load lower than the determined value (0.40) and the 9th statements that caused the overlap problem were removed from the analysis, and 7 statements remained. When the findings were examined, 2 factors with an eigenvalue greater than 1 and a total variance explanation rate of 75.71% have emerged. The variance explanation rates of the first and second factors were 60.18% and 15.53%, respectively. SN scale consisted of 10 statements. When the findings were examined, 3 factors with an eigenvalue greater than 1 and a total variance explanation rate of 79.10% have emerged. The variance explanation rates of the first, second and third factors were 55.48%, 12.83%, and 10.78%, respectively. PBC scale consists of 13 statements. Adhering to the original of the scale, the 1,3,5 and 6th statements were reverse coded and analyzed. Statement 4 with a factor loading less than 0.40 was excluded from the analysis. The rate of explaining the total variance of a single factor with an eigenvalue greater than 1 is 64%. Intention scale consists of 3 statements. The rate of explaining the total variance of a single factor with an eigenvalue greater than 1 is 90.5%.

It can be stated that all scales adapted because of the analyses are suitable in terms of construct validity and reliability. In the next step, CFA was performed with the relevant data in order to verify the factor structures determined as a result of EFA.

The study was based on the fit indices most frequently used in model validation studies (Aksu et al., 2017). In case the model fit indices are not within the acceptable limits, modification (correction) indices were examined, and modifications (corrections) were performed where necessary in order to resolve the discrepancies between the proposed and the predicted model. Within the scope of the analysis results, χ^2/df (4.7), Goodness of Fit Index GFI (0.94), Root Mean Square Error of Approximation RMSEA (0.08), Standardized Error Squares Standardized Root Mean Square Residual SRMR (0.07), Adjusted Goodness of Fit Index AGFI (0.91), Normed Fit Index NFI (0.96), Non-normed Fit Index NNFI or Tucker Lewis Index TLI (0.97), Comparative Fit Index CFI (0.97) were found. According to the underlying indices and the findings, it can be stated that the compatibility index values of the established model are within the acceptable limits (Aksu et al., 2017).

Findings related to the research model

Multiple linear regression analysis was used to test the hypotheses stated in the study. The effect of the independent variables on the dependent variable can be determined using the multiple regression analysis. Some assumptions must be provided to make sound evaluations in regression analysis. In this context, Pearson correlation analysis was used to test the relationship between dependent and independent variables. The analysis results are given in Table 3. According to Table 3, FN has a significant relationship with the intention variable at a rate of -61.9%, attitude 67.4%, SN 61%, and PBC at a rate of 69.2%.

Table 3. Findings related to correlation analysis between variables

Independent Variables	Pearson's Coefficient of Correlation	Significance Level
Food Neophobia	-.619	.000
Attitude	.674	.000
Subjective Norm	.610	.000
Perceived behavioral control	.692	.000

Other assumptions of regression analysis are linearity, normality of distribution, independence of errors (autocorrelation), and non-multilinearity between independent variables. The analyses for the control of the assumptions were made with the methods suggested by Başman et al. (2018). First, the scatter plot of standardized error terms and standardized estimated values was examined. It was seen that the linearity assumption was not partially violated. It has been determined that the standardized error values were normally distributed on the histogram, and the error terms observed in the P-P graph were evenly distributed around the line. Therefore, the assumption of normality of distribution was confirmed. Durbin Watson test was performed to check the assumption of independence of errors, and since its value was 1.791, the assumption of independence of errors was confirmed (Kalaycı, 2009). Finally, tolerance and variance inflation factors (VIF) values were checked to determine whether there was multicollinearity among the independent variables. According to Hair et al. (2006), if the VIF value is below 10 and the tolerance value above 0.10, it can be stated that there is no correlation between the variables. The tolerance value was between 0.432 and 0.567, and the VIF values were between 1.764 and 2.314. Therefore, it can be stated that there is no multicollinearity between the variables, and the last assumption is confirmed. Next, multivariate linear regression analysis was performed to test the hypotheses. Analysis results are given in Table 4.

According to Table 4, while FN (β= -.244; p= .000) negatively and significantly affects the intention to consume fish in the restaurant, the attitude towards fish consumption (β= .394; p= .000) SN (β= .299; p= .000), PBC (β= .410; p= .000) positively and significantly affects the intention to consume fish in the restaurant. When the R^2 value is examined, it is seen that all independent variables in the model (FN, attitude, SN, PBC) explain the dependent variable (intention to eat fish in the restaurant) by 60% and this value is acceptable.

Table 4. Findings related to regression analysis

Independent Variables	BetaCoefficient	t Value	Significance	ToleranceValue	VIF
Food Neophobia	-.244	-4.731	.000	.522	1.914
Attitude	.394	5.663	.000	.432	2.314
Subjective Norm	.299	5.249	.000	.567	1.764
Perceived behavioral control	.410	7.029	.000	.438	2.286
R	.774				
R^2	.600				
Adjusted R^2	.597				
Durbin Watson Value	1.791				

The adjusted R^2 value (0.597) is close to the R^2 value, indicating the suitability of the model. According to the results of the analysis, the H1, H2, H3, H4 hypotheses are supported. Finally, regression analysis was conducted to test the moderator effect of FN on the relationship between attitude and intention variables. In the established model, attitude was assigned as independent, intention dependent, and FN as regulatory variable. Analyses were made in two parts and the results are given in Table 5.

Table 5. Findings on the regulatory role of food neophobia between attitude and intention

Independent Variables	Standardized Beta Coefficient	t Value	Significance
Part 1			
Attitude	.470	11.652	.000
Food Neophobia	-.315	-7.817	.000
R^2	.512		
Adjusted R^2	.510		
Part 2			
Attitude	.530	12.316	.000
Food Neophobia	-.314	-7.881	.000
Attitude * Food Neophobia	-.127	-3.687	.000
R^2	.524		
Adjusted R^2	.521		
R^2 Change	.012		

In the first part, a regression analysis was made by setting up a model with attitude and FN as the independent variables and intention as the dependent variable, and the suitability of this model was checked. Results suggest that this model is significant as a whole (F value 269.279 (p= 0.000)), attitude (dependent) has a positive and significant effect on intention (independent) variable (β= .470; p= .000). FN (dependent) variable has a negative and significant effect on the intention (independent) variable (β=-.315; p= .000). According to R^2, the independent variables (attitude and food neophobia) explain the dependent variable at 51.2%. The independent variable with the highest explanatory power is the attitude variable (47%). In the second part, the centralization process known as the Z score was applied to the attitude (independent) and FN (regulatory) variables (Aksu et al., 2017).

The centralized values are multiplied with each other to produce a new variable (interaction term). The newly obtained value was included as an independent variable in the new model and regression analysis was applied. In Table 5, this model is significant as a whole (F value is 188.449 (p= 0.000)), while the explanatory power of the attitude (dependent) intention (independent) variable increased by 6% to become 53%. However, when the R^2 value is examined, it is seen that the rate of explaining the total variance increased by 1.2% and became 52.4%. Considering the interaction term, which was added to the model later, it can be stated that FN had a negative and significant effect on the relationship between attitude and intention variables (β= -. 127; p= .000). In this context, the H5 hypothesis was also supported by the findings.

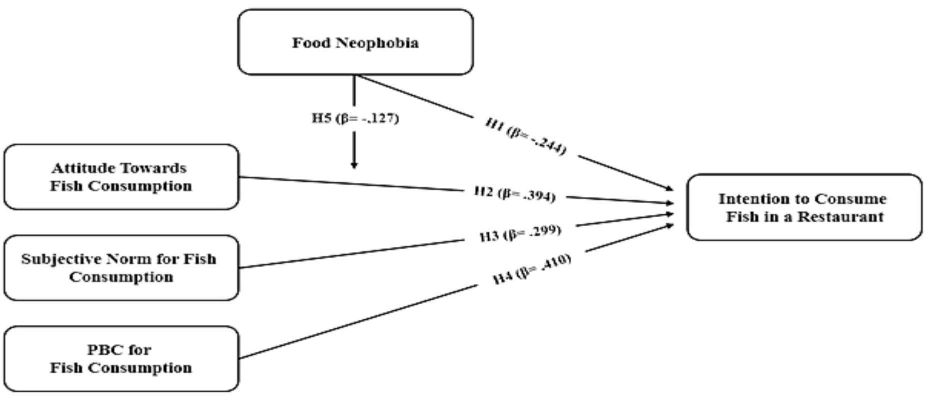

Figure 2. Hypothesis results regarding the research model

DISCUSSION

The variable with the strongest influence on fish consumption intention in restaurants was PBC, followed by attitude, SN, and FN. Similar findings have been reported in other studies on fish consumption, where PBC was the most significant predictor of intention (Olsen et al., 2008; Verbeke and Vackier, 2005). However, some studies found that attitude or SN had a greater effect than PBC (Aghamolaei et al., 2012; Mitterer-Daltoé et al., 2013; Tomic et al., 2015). In contrast, Thong and Olsen (2012) and Siddique (2012) found no significant effect of PBC. These variations may be due to differences in scales, samples, and study variables. The strong PBC effect in this study is likely due to the coastal location, where easy access to fish and familiarity with its consumption positively influence PBC.

The findings of Verbeke and Vackier (2005) and Aghamolaei et al. (2012) support this view. However, this study uniquely examines fish consumption intentions in the context of restaurants. Restaurant dining experiences involve various factors such as food quality, service, and environmental conditions (Canny, 2014; Liu and Jang, 2009; Ryu and Han, 2010). The impact of these components on fish consumption is shaped by customers' quality perceptions and expectations. The stronger influence of PBC and attitude on intention in this study likely stems from positive perceptions of the restaurant atmosphere, food quality, and environmental factors.

Based on the findings, the FN variable negatively and significantly influences the intention to consume fish in restaurants. Many studies (Costa et al., 2020; Jaeger et al., 2017; Knaapila et al., 2011; Laureati et al., 2016; Siegrist et al., 2013) support this, showing that neophobia negatively impacts consumption intentions for various familiar foods, including fish, vegetables, fruits, and poultry. Additionally, the study identified a significant moderating effect of the FN variable on the relationship between attitudes towards fish consumption and intention to consume fish in restaurants. Specifically, while attitudes can influence intentions, a high level of FN weakens this effect (Hsu et al., 2018; Ting et al., 2017).

To enhance the intention to consume fish in restaurants, the food industry should focus on promotional activities highlighting fish's safety, health, nutritional benefits, and taste. To mitigate negative attitudes, restaurants can pre-clean fish bones and use various cooking methods or spices to reduce unpleasant odors. Effective ventilation systems can also help manage indoor smells. For customers with a positive attitude towards fish, the aroma can enhance their dining experience, making it essential to tailor approaches based on the business type and customer profile. Implementing campaigns like discount days or group discounts can encourage fish consumption. Additionally, staff training and customer involvement in fish preparation can further boost familiarity and intention to consume fish.

The level of FN in individuals can change over time and varies with several factors. To reduce FN, service personnel can inform customers about the benefits of fish through menus and promotions. Offering small fish samples can create a positive impression, while visually appealing presentations with spices can enhance sensory motivation. Future research could focus on customers at restaurants offering both fish and other menu items, gathering data to explore factors influencing fish preference, such as food quality, menu variety, atmosphere, price, and service quality. Additionally, the moderating effect of FN could be examined between attitude-intention and specific norm-intention variables. Future studies could also investigate aquatic foods with different sensory properties, like lobster, crab, and octopus, to clarify FN's impact on consumption intentions.

CONCLUSION

The study on fish consumption in restaurants reveals several significant findings regarding the impact of food neophobia (FN) and consumer attitudes on dining choices. It establishes that FN negatively influences the intention to consume fish, indicating that individuals with higher levels of FN are less likely to select fish dishes when dining out. This finding aligns with the Theory of Planned Behavior (TPB), which posits that subjective norms (SN) and perceived behavioral control (PBC) also play crucial roles in shaping consumption intentions.

Furthermore, the research underscores the importance of social influences, particularly from family and friends, in shaping consumption behaviors. The study suggests that positive exposure to unfamiliar foods can reduce FN over time, thereby enhancing the rates of fish consumption. Additionally, it highlights the significance of the restaurant atmosphere and environmental factors in facilitating or impeding fish consumption. Overall, the findings emphasize the intricate interplay of psychological barriers, social influences, and environmental factors in determining fish consumption behaviors within restaurant contexts.

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AUTHORSHIP CONTRIBUTIONS

Furkan Dursun: Conceptualization, resources, investigation, methodology, formal analysis, writing-reviewing and editing. Bahar Gümüş: Conceptualization, writing-reviewing and editing, investigation, project administration.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

ETHICAL APPROVAL

The ethical appropriateness of this study was approved by Akdeniz University Social Sciences and Humanities Scientific Research and Publication Ethics Committee with decision number 14 on 07/02/2020.

DATA AVAILABILITY

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

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Potential of leek (*Allium ampeloprasum*) waste for microalgae *Chlorella vulgaris* cultivation: A preliminary evaluation

Pırasa (*Allium ampeloprasum*) atığının mikroalg *Chlorella vulgaris* kültürü için potansiyeli: Ön değerlendirme

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Abstract: Leek is an economical and healthy plant species. It contains rich dietary fibers, amino acids, bioactive compounds that increase its antioxidant capacity and more than 20 different fatty acids. It is rich in potassium, iron and selenium and can be used as a valuable source for microalgae cultivation. For impotance of leek, this study investigated the biomass production of *Chlorella vulgaris* microalgae species with leek leaf waste. To obtain the leek extract to be used for the experiment, leek leaves were dried in an oven at 40 °C and crushed in a mortar and pestle and filtered. Leek leaves were first dissolved with 10 ml DMSO (Dimethylsulfoxide) to 0.1 g/L and diluted with distilled water to a final volume of 100 ml. *Chlorella vulgaris* was exposed to leek extract concentrations of 0.01, 0.025, 0.05, 0.1 and 1.0 g/L for 72 hours and BG-11 enrichment medium was used in the control group. According to the data obtained, when leek leaves were used in the cultivation of *C. vulgaris* microalgae, a very high increase of 160% was observed at a concentration of 0.05 g/L compared to BG-11 enrichment medium. However, in the group where leek leaves were used completely, 64% increase was observed compared to the control group. This study proved that *C. vulgaris* have significant potential for food industries and the biocompost of vegetables is a suitable medium for microalgae cultivation. This study has proven that the use of vegetable wastes is suitable for obtaining a culture with high biomass of C. vulgaris microalgae, which has been used intensively in different areas of the food industry, and that leek wastes in particular provide high biomass growth. Therefore, the lower concentration of leek served as the best medium to increase the growth and biomass of C. vulgaris. This study proves that costs can be reduced and sustainable effective culture techniques can be used in microalgae culture by using vegetable wastes such as leek waste, which provides high biomass growth even at low concentrations.

Keywords: *Allium ampeloprasum*, phytoplankton, biomass, aquaculture, recycling, agricultural waste

Öz: Pırasa ekonomik ve sağlıklı bir bitki türüdür. Zengin diyet lifleri, amino asitler, antioksidan kapasitesini artıran biyoaktif bileşikler ve 20'den fazla farklı yağ asidi içerir. Potasyum, demir ve selenyum açısından zengindir ve mikroalg yetiştiriciliğinde değerli bir kaynak olarak kullanılabilir. Bu çalışmada, pırasa yaprak atıkları ile *Chlorella vulgaris* mikroalg türlerinin biyokütle üretiminin araştırılması amaçlanmıştır. Deneyde kullanılacak pırasa ekstraktını elde etmek için pırasa yaprakları 40 °C'de bir fırında kurutuldu ve bir havan ve tokmakla ezilerek süzüldü. Pırasa yaprakları önce 10 ml Dimetilsülfoksit ile 0,1 g/L'ye kadar çözüldü ve damıtılmış su ile son hacim 100 ml'ye kadar seyreltildi. *C. vulgaris* 0.01, 0.025, 0.05, 0.1 ve 1.0 g/L pırasa ekstraktı konsantrasyonlarına 72 saat süreyle maruz bırakılmış ve kontrol grubunda BG-11 zenginleştirme ortamı kullanılmıştır. Elde edilen verilere göre, pırasa yaprakları *C. vulgaris* yetiştirilmesinde kullanıldığında, 0.05 g/L konsantrasyonda BG-11 zenginleştirme ortamına göre %160 gibi çok yüksek bir artış gözlenmiştir. Ancak pırasa yapraklarının tamamen kullanıldığı grupta kontrol grubuna göre %64 oranında artış gözlenmiştir. Bu çalışma, gıda endüstrisinde farklı alanlarda yoğun olarak kullanılmaya başlanan *C. vulgaris* türünün yoğun biyokütleye sahip kültür elde edilmesinde sebze atıklarının kullanılmasının uygun olduğunu ve özellikle pırasa atıklarının yüksek biyokütle artışını imkan sağladığını kanıtlamıştır. Bu çalışma düşük konsantarasyon da dahi yüksek biyokütle artışını sağlayan pırasa atığı gibi bitkisel atıkların mikro alg kültüründe kullanılarak maliyetlerin düşürülebildiğini ve sürdürülebilir etkili kültür tekniklerinin kullanılabilirliğini ispatlamaktadır.

Anahtar kelimeler: *Allium ampeloprasum*, fitoplankton, biyokütle, su ürünleri yetiştiriciliği, geri dönüşüm, tarımsal atık

INTRODUCTION

Global energy consumption is largely powered by 87% of energy from fossil fuels, which are major contributors to greenhouse gas emissions and climate change. Therefore, energy production from renewable biological sources is important for energy security and environmental sustainability. Reliance on alternative energy sources can increase energy security by stabilizing economic fluctuations. Biomass-based renewable biofuels can be produced from sources such as energy crops, forestry residues and agricultural wastes derived from traditional biomass (Singh et al., 2014). Global challenges such as food security, population growth and environmental pressures require the development of new processes for the utilization of industrial food waste to provide social and economic benefits for the development of global sustainable agriculture (Goula and Lazarides 2015; Wang et al., 2017).

Today, the consumption of vegetables and agricultural products is increasing due to the increasing importance of vegetarian diets. The FAO (Food and Agriculture Organization of the United Nations) estimates the amount of agricultural waste at over 60%. The utilization of powders obtained from fruit waste as a new method has recently attracted the attention of many researchers (Bhandari et al., 2013; Karam et al., 2016; Neacsu et al., 2015). Similarly, Bas-Bellver et al. (2020) produced powders from vegetable waste (carrot, leek, celery and cabbage) and used them as functional food additives.

Leek (*Allium ampeloprasum*) is an economically important plant species of the Amaryllidaceae family. It is widely distributed throughout the Middle East, Russia and the Mediterranean Basin. World leek production has increased steadily since 2001. Turkey, the leading country in Europe in leek production, has an annual production of over 200,000 mmt tons (Celebi-Toprak and Alan, 2021).

Leek is a good source of carbohydrates, protein, fat, dietary fiber and a high source of the minerals. The carbohydrate and protein composition available ranges from 0.5-16.60 and 1.5-2.1 g/100 g. The amino acids (Najda et al., 2016), organic acids and phytochemicals are present in higher amount and increase the positive health effects (Shelke et al., 2020). The edible parts of leeks contain more than 20 different fatty acids such as linoleic, palmitic, oleic and α-linolenic acids. Leek plant, which is a source of Ca (30.24-81.7 mg/100 g) and Fe (0.20-2.1 mg/100 g), has also been reported to be rich in Mg (10-28 mg/100 g), Na (5-54.6 mg/100 g), P (35 mg/100 g) and Cu (0.06-0.30 mg/100 g) elements. (García-Herrera et al., 2014). In addition, leek showed that antibacterial properties against many bacteria, viruses and fungi. (Shelke et al., 2020). Due to the widespread production of leek worldwide and its rich nutritional content, it attracts attention in terms of utilization of agricultural industry wastes. However, there are limited studies on the recycling of leek wastes and the effects of nutrient content.

Microalgae are living organisms with significant potential in areas such as biotechnology and sustainable energy production. Researchers have recognized the importance of algae for the green bioeconomy, considering their functions in the environment and their potential for commercial use that reduces dependence on land-based fossil fuel products (Ahmad et al., 2020; Peter et al., 2022).

In a study, it was found that the use of compost mixture provided higher biomass concentration compared to *Chlorella vulgaris* cultivation in culture media (Chew et al., 2018). Tekin et al. (2021) used carrot pulp as agricultural waste and investigated the effects of carrot pulp on biodiesel production of *Chlorella vulgaris* microalgae. According to the data the biodiesel produced with carrot pulp complies with international standards used for biodiesel production. In addition, the performance of biodiesel production with microalgae cultivation by utilizing the wastes of the palm oil producing plant was also examined and it was reported that these wastes can also be used (Cheah et al., 2018).

The aim of this study was to investigate the potential of using the unused leaf parts of the leek plant, both during and after harvest, as enrichment in the culture medium of the microalgae species *Chlorella vulgaris*. Microalgae cultivation is an important field in terms of biomass production, biofuel production and environmental sustainability, and the use of waste products has great potential in this process. The results will help us to better understand the impression of leek green waste on microalgae cultivation. It will also contribute to waste management by promoting a sustainable approach.

MATERIALS AND METHODS

Preparation of leek extract

The leek leaves to be used for the experiment were first dried in an oven at 40 °C, then crushed in a porcelain mortar and filtered. Leek leaves were first dissolved with 10 ml DMSO (Dimethylsulfoxide) to a value of 0.1 g/L and diluted with pure water to a final volume of 100 ml. During the hot-air drying, a laboratory oven (Nuve FN 400P/500P) was used at 40°C for 12 h. Leek wastes were sliced and dried at 40°C on a basket in a laboratory oven with an air speed of 2.5 m/s (±0.03 m/s) and an adjustable temperature (±1°C) (Doymaz, 2008). Thus, the risk of DMSO-induced inhibition was minimized. The dilutions in which the experiment was performed and the amounts added are shown in Table 1.

Tablo 1. Dilutions made in experiments and amounts added

Dilution Groups (g/L)	Phytoplankton Culture Media (BG11) (ml)	Leek Extract (ml)	Chlorella Culture (ml)
Control Group	1,8	0	0,2
0,01	1,782	0,018	0,2
0,025	1,755	0,045	0,2
0,05	1,71	0,09	0,2
0,1	1,62	0,18	0,2
1	0	1,8	0,2

Cell culture and microalgae media

In the Ecotoxicology laboratory at the Faculty of Fisheries, Ege University, the test organism was cultured *Chlorella vulgaris* using BG-11 medium (OECD, 2011) (Turan and Çakal Arslan, 2023). Prior to the experiment, a pre-culture was established and incubated at a temperature of 25±1°C. The initial cell concentration in the test cultures was approximately 4-5x10^{-4} cells/mL for *Chlorella vulgaris.* The experiments were carried out in 24-well plate containers with a final volume of 2 ml and 3 repetitions. Samples were exposed to a photoperiod of 14:10 (Light: Dark) hours under constant illumination at around 2000 lux, while being agitated at 100 rpm. Cell density was measured at 24, 48 and 72 hour time points on a Biontech plate reader at 660 wavelength (Turan and Çakal Arslan, 2023).

Determining the growth rate

Microalgae growth rate was performed in accordance with the OECD 201 standard (OECD, 2011). At 0 and 72 hours, cell count data were evaluated based on growth rate as described in standard protocols.

The mean specific growth rate (μ), the logarithmic increase in biomass for each control and experimental groups, was calculated as follows,

$\mu i\text{-}j = \ln x_j - \ln x_i / t_j - t_i$ (day^{-1})

$\mu_{i\text{-}j}$: the average specific growth rate from i to j;

X_i: biomass at time i,

X_j: biomass at time j,

The SPSS software was used for probit analysis and the statistical significance of the data on growth rates was compared with controls using ANOVA with the assistance of the SPSS program (Ozdamar, 1999). The cell number averages among the different culture media tested were compared using one-way ANOVA testing The level of significance considered for all the analyses was P<0.05. t-test.

RESULTS

Nutrients, carbon dioxide and light are important contributors to sustain microalgae growth. Among nutrients, especially nitrogen is an important source to increase microalgae biomass (Hsieh and Wu, 2009). In order to examine the effect of leek extract on the growth rate of *C. vulgaris*, different concentrations (0.01, 0.025, 0.05, 0.1 g/L) were diluted with nutrient media. According to the data obtained in the study, when leek extract was used in the cultivation of *C. vulgaris* microalgae, a very high increase of 160% was observed at a concentration of 0.05 g/L compared to the conventional enrichment medium used in the control group. In the group using purely leek extract, an increase of 64% was observed compared to the control group, but not as large as in the groups containing 0.05 g/L and lower leek extract (Figure 1). It is thought that the reason why *C. vulgaris* increases its growth rate and cell density at a concentration of 0.05 g/L (50 mg/L) is due to the amount of nitrogen and ammonium. At low concentrations, it is not sufficient for the increase of algal biomass, and above 0.005, it has a restrictive effect. This depends on the tolerance of the species. It turns out that this is the ideal amount of leek waste for the growth and biomass increase of this species.

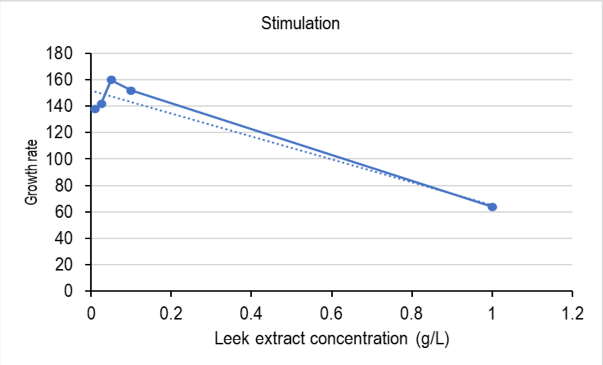

Figure 1. The effect of leek extract on the growth rate of *C. vulgaris*

DISCUSSION

Microalgae need to be produced in a high quality and sustainable manner, both for food supplements and biofuels. For this purpose, the use of agricultural wastes should be investigated and suitable ones should be combined with enrichment media to reduce costs and increase environmentally friendly production processes.

Chew et al. (2018) examined the specific growth rate of *C. vulgaris* by mixing BG-11 and compost media in different ratios. According to the results, the highest specific growth rate was measured in the combination of 75% organic compost + 25% culture medium. *C. vulgaris* grown in 75% compost content was found to increase by 19% compared to the control group. In our study, the highest growth rate was observed at 0.05 g/L, which corresponds to 4.5% of the proportional content of leek extract. Although the percentage of leek extract in the culture medium is low, the 160% increase compared to the control group shows that the nutrient content of leek extract is richer. In terms of nitrogen compounds, algae tend to prefer ammonium over nitrate, and wastewater with high ammonium concentrations promotes rapid algal growth. However, ammonium requirements vary depending on the algae. *Chlorella* and *Scenedesmus* sensivity range reported as 30 to 300 mg/L NH4+–N (Kligerman and Bouwer, 2015; Cai et al., 2013).

Tekin et al. (2021) used carrot pulp hydrolysate to increase the biomass production of *C. vulgaris* to produce biodiesel. For this reason, *C. vulgaris* were grown in BG-11 medium containing carrot pulp at concentrations of 0.25, 0.5, 1 and 2 g/L. It was determined that the addition of 0.5 g/L carrot pulp caused a 1.38-fold higher increase in growth compared to photoautotrophic conditions. When we compared the growth of *C. vulgaris* with carrot pulp, which is 10 times more concentrated than our study with leek extract, the increase rate values were close. These results revealed that leek extract, which is present at lower concentrations in the medium than carrot pulp, is more effective in the growth rate of *C. vulgaris*.

For sustainable environment, Microalgae feedstock is suitable for biodiesel production. However, microalgae biomass has higher biomass productivity compared to terrestrial feedstock production in bioenergy production. However, microalgae production does not have a sufficient market share due to its high-cost production technology (Chung et al., 2017). In order to reduce the high costs of microalgae cultivation, studies on cost-effective and efficient biomass production have increased in recent years (Ak et al., 2013; Benas and Ak, 2022; Cheirsilp et al., 2023; Zhu et al., 2022). According to our study that leek, an agricultural waste, increased the growth rate of microalgae at certain values. The findings suggest that leek waste, traditionally viewed as a byproduct with limited utility, can serve as a valuable resource in the cultivation of microalgae. By converting agricultural waste into a nutrient source, not only is the environmental burden of waste disposal mitigated, but we also pave the way for enhanced production of microalgae. In the light of the results, we think that the use of leek waste in microalgae cultivation will contribute to sustainable microalgae production, reducing resource waste and contributing to green technology for environmental protection.

Leek, which we used in the study, is an important and economic crop widely cultivated in our country and especially in our region. Leek leaves remain as waste in large quantities during and after harvesting. Therefore, utilization of waste leek leaves will contribute to sustainable agriculture. In addition, it is thought to be economically beneficial by reducing the use of chemicals used in microalgae cultivation.

CONCLUSION

In our study, *Chlorella vulgaris* was exposed to leek extract concentrations of 0.01, 0.025, 0.05, 0.1 and 1.0 g/L for 72 hours. According to the results obtained, a very high increase of 160% was observed at a concentration of 0.05 g/L compared to the enrichment medium. However, the growth rate of *C. vulgaris* was 64% in the group with only leek extract in the medium.

This study showed that leek has the potential to be used in microalgae cultivation. With further analysis, the usability of agricultural wastes in the cultivation of *Chlorella vulgaris* species to be used in different fields of use can be revealed more clearly. However, if it is to be used for this purpose, some further research (nutrient analysis, heavy metal and pesticide residue analysis in leaves, etc.) must be carried out.

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AUTHORSHIP CONTRIBUTIONS

Koray Benas: Conceptualization, resources, investigation, methodology, writing-reviewing and editing. Muhammet Ali Karaaslan: Conceptualization, resources, investigation, methodology, writing-reviewing, formal analysis. Özlem Çakal Arslan: Conceptualization, resources, methodology, investigation.

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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SHORT COMMUNICATION **KISA MAKALE**

Confirmation of the presence of *Helicolenus dactylopterus* (Delaroche, 1809), in the Sea of Marmara with morphometrical and bioecological notes

Helicolenus dactylopterus (Delaroche, 1809) türünün Marmara Denizi'nde güncel varlığının doğrulanması, morfometrisi ve biyoekolojisi üzerine notlar

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Abstract: On 9 August 2024 two specimens of *Helicolenus dactylopterus* (Delaroche, 1809) were caught by means of a scientific bottom-trawl hauling towed at a depth range between 143 and 188 m, at the central sector of the Sea of Marmara. After more than 30 years since the last occurrence of *H. dactylopterus* in the region, the capture of only two specimens does not represent more than confirmation of the current presence of the species in the Sea of Marmara. However, this record is also a significant finding revealing that life still exists in the deep regions of the Sea of Marmara and therefore action must be taken to prevent habitat and biodiversity loss.

Keywords: Sebastidae, *Helicolenus*, bathyal, Sea of Marmara, biodiversity

Öz: *Helicolenus dactylopterus* (Delaroche, 1809) türü derin deniz balığının iki örneği 9 Ağustos 2024 tarihinde Marmara Denizi'nin orta kesiminde 143 ila 188 m derinlik aralığında bilimsel amaçlı dip trolü çekimi sırasında yakalanmıştır. *H. dactylopterus* Marmara Denizi'nde en son 30 yıldan uzun süre önce kaydedilmiştir. Yakın zamanda yakalanmış olan bu iki bireyle türün bölgede halen yaşadığı doğrulanmaktadır. Marmara Denizi'nin derin bölgelerinde yaşamın hâlâ devam etmekte olduğunu ortaya koyan bu kayıt, habitat ve biyolojik çeşitlilik kaybını önlemek için harekete geçilmesi gerektiğine vurgu yapan önemli bir bulgudur.

Anahtar Kelimeler: Sebastidae, *Helicolenus*, batiyal, Marmara Denizi, biyoçeşitlilik

INTRODUCTION

The blackbelly rosefish *Helicolenus dactylopterus* (Delaroche, 1809) (Perciformes: Scorpaenoidei) is a member of the family Sebastidae (Froese and Pauly, 2024). *H. dactylopterus* is a bathydemersal deep-sea teleost fish found at depths between 50 and 1,100 m, but the common depth range of the species is known to vary from 150 to 600 m (Froese and Pauly, 2024). The range of the blackbelly rosefish extends from Nova Scotia (Canada) to Venezuela in the western Atlantic, and in the eastern Atlantic it's distribution covers a wide area from Iceland to Norway in the north, and the Mediterranean Sea and the Gulf of Guinea in the south (Froese and Pauly, 2024).

Chronologically the first literature reporting on the presence of *H. dactylopterus* in the Aegean and Mediterranean waters of Türkiye is the ichthyological inventory by Geldiay (1969). In this report Geldiay (1969) considers *H. dactylopterus* as a teleostean found in the Aegean and Mediterranean waters of Türkiye, especially in the waters of Bay of İzmir. The contemporary presence of *H. dactylopterus* in Turkish waters was further confirmed by Altuğ et al. (2011) and Koca (2023). During an extensive survey of the demersal fishery resources of the Turkish seas, *H. dactylopterus* was recorded from the Sea of Marmara for the first time (JICA, 1993), and followed by the second record of the species in the region a few years later (Meriç, 1995). Although the species is being reported as a member of the fish fauna of the Sea of Marmara in several ichthyological checklists published in recent decades (Eryılmaz and Meriç, 2005; Gönülal and Topaloğlu, 2016; Artüz and Fricke, 2019); however, the information on the presence of *H. dactylopterus* in the mentioned region provided in this literature is either based on JICA (1993) or Meriç (1995). Furthermore, *H. dactylopterus* was not been sampled in two recent surveys of demersal fishes of the Sea of Marmara (Torcu Koç et al., 2012; Daban et al., 2021). The absence of *H. dactylopterus* in the ichthyological field surveys conducted in the last two decades (Torcu Koç et al., 2012; Daban et al., 2021) suggests that the species may have been extirpated from the Sea of Marmara, and despite the occurrence information provided in the most recent ichthyological review (Artüz and Fricke, 2019), it's presence in the region requires confirmation. In the present article, the authors report on a recent capture of *H. dactylopterus* in the Sea of Marmara, and provide morphometric data and bioecological notes.

MATERIAL AND METHODS

Study area

The study area of the present study is located in the central sector of the Sea of Marmara (Figure 1), and according to the GFCM's definition of geographical subareas (GSAs) of the Mediterranean Sea, the Sea of Marmara is defined as GSA28 (GFCM, 2018).

Examined specimens

The present study is part of an ongoing governmental large-scale monitoring program of Turkish seas, which is titled as "Denizlerde Bütünleşik Kirlilik İzleme Programı - Integrated Program for Marine Pollution Monitoring" and implemented by Republic of Türkiye, Ministry of Environment, Urbanization and Climate Change. The demersal sampling was carried out onboard of *RV Yunus-S*, a 510 hp stern trawler operated by İstanbul University, Faculty of Aquatic Sciences. According to the MEDITS protocol, a bottom trawl with a codend mesh size of 14 mm and a maximum mesh size of 22 mm was used for the hauls (Anonymous, 2017). The tow duration for the hauls was 30 min at depths ≤200 m and 60 min >200 m depth (Anonymous, 2017). Oceanographic parameters (salinity, temperature and dissolved oxygen) were recorded using the SeaBird CTD probe.

Species identification followed Hureau and Litvinenko (1986), and taxonomic nomenclature followed Kovačić et al. (2021) and Froese and Pauly (2024). After fresh specimens were photographed, morphometric distances, expressed as percentages of standard length (SL) (Table 1), were measured on fresh specimens using either an ichthyometer (for distances >10 cm) or a digital vernier caliper (for distances ≤10 cm) to avoid influence of shape variations or changes in distances due to fixation (Martinez et al., 2013). Morphometric measurements and meristic counts were performed according to the procedure adopted from Kai and Nakabo (2002) and Koca (2023). The definitions of body depths 1 and 2 were adopted from Kai and Nakabo (2002), which are the distance between the anterior origin of the 12th dorsal spine and that of the 1st anal spine, and the distance between the anterior origin of the 1st dorsal spine and that of the pelvic spine, respectively. The total weight (TW) of the examined specimens was weighed to the nearest 0.05 g on a precision balance.The definitions of the maturity stages of the examined reproductive organs follow Follesa and Carbonara (2019). The examined blackbelly rosefishes were fixed and preserved in borax-buffered, 5% seawater formalin solution. According to the evidence-based approach for the confirmed presence of fish species (Kovačić et al., 2021), formalin-preserved specimens are stored at the Department of Fisheries Technologies and Management Laboratory, Faculty of Aquatic Sciences, İstanbul University with barcode numbers PSC20230114-121 and PSC20230114-122.

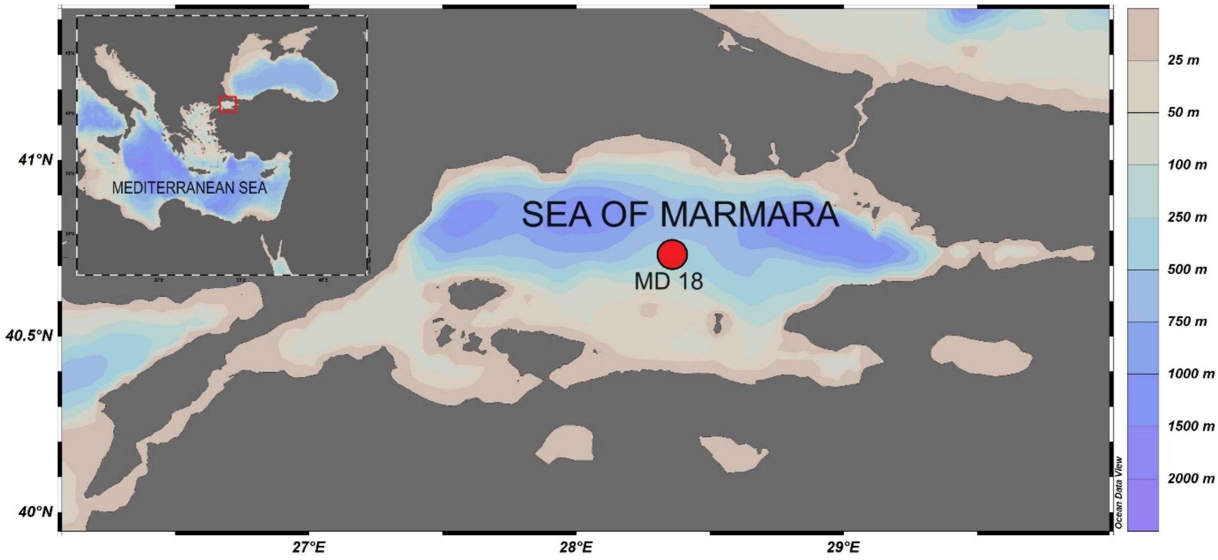

Figure 1. Map shows the approximate locality (red dot) of capture of the examined specimens of *Helicolenus dactylopterus* in the Sea of Marmara

RESULTS

On 9 August 2024, two specimens of *H. dactylopterus* were caught at the station MD18 (haul started at 40º 42.155' N 28º 19.985' E; haul ended at 40º 42.727' N 28º 18.192' E) on a mixed bottom of mud and sand at a depth range of 143-188 m (Figure 1). The following description of *H. dactylopterus* is based on the examined specimens (Figure 2). The morphometric measurements of the examined specimens are given in Table 1.

A large-headed teleostean fish with first suborbital bone without spines, second with 1 spine; nasal spine is present; preocular, supraocular and postocular spines are not highly elevated. The profile of the nape is relatively steep. The mouth is large and dark coloured inside. In both specimens: dorsal fin with 12 spines and 13 rays; anal fin with 3 spines and 5 rays; pectoral fin with 18 rays, of which the lowermost 8 are free for about a third of their length (Figure 2); and pelvic fin with 1 spine and 5 rays. Lateral line with tubular scales. Back and sides are reddish pink, and belly is pink coloured; 5 dark coloured bands are present below anterior, middle and posterior dorsal spines, below soft dorsal rays and at the base of caudal fin; faint dark spot is present on the posterior part of spinous dorsal fin; dark spot is present on the operculum. The abdominal cavity is black. These morphological characters are consistent with those described by Hureau and Litvinenko (1986).

The stomachs of both specimens were empty. Examination of the reproductive organs revealed that both specimens were females and the maturity stage of the ovaries was 2b (recovering) (Follesa and Carbonara, 2019). In terms of total length (TL), both specimens (173 and 201 mm) were well above the 50% mature size (L_{50}; TL 142 mm) reported for females of *H. dactylopterus* (Follesa and Carbonara, 2019). *In situ* dissolved oxygen concentration was 1.99 mg/L at a depth of 140 m, indicating that the seawater at the station MD18 is at the threshold of hypoxia (Howell and Simpson, 1994). Salinity was 38.82‰ and temperature was 15.41ºC at the same depth.

Table 1. Morphometric measurements of the examined specimens of *H. dactylopterus* caught in the Sea of Marmara

Measurements (mm)	SP1	SP2	Mean	±SD	% of SL of mean
TL	201	173	187	14	126.35
SL	158	138	148	10	100
Body depth 1	58.55	48.16	53.36	5.20	36.05
Body depth 2	47.57	39.68	43.63	3.95	29.48
Caudal peduncle depth	15.65	13.44	14.55	1.11	9.83
Predorsal length	50.14	42.26	46.20	3.94	31.22
Postdorsal length	11.70	11.03	11.37	0.34	7.68
Prepelvic length	72.63	55.58	64.11	8.53	43.31
Preanal length	111 .26	97.21	104.24	7.03	70.43
Prepectoral length	61.11	50.39	55.75	5.36	37.67
Between pelvic and pectoral fins	9.74	7.31	8.53	1.21	5.76
Between pelvic and anal fins	27.84	33.15	30.50	2.66	20.60
Dorsal base length	98.60	84.67	91.64	6.97	61.92
Anal base length	23.42	20.28	21.85	1.57	14.76
Pectoral fin length	49.59	44.66	47.13	2.47	31.84
Pelvic fin length	36.97	31.17	34.07	2.90	23.02
Pelvic spine length	17.74	18.15	17.95	0.21	12.13
Caudal fin length	42.95	39.25	41.10	1.85	27.77
1st dorsal spine length	12.73	13.27	13	0.27	8.78
2nd dorsal spine length	21.94	18.27	20.11	1.84	13.58
3rd dorsal spine length	25.32	21.95	23.64	1.69	15.97
4th dorsal spine length	25.04	19.63	22.34	2.70	15.09
5th dorsal spine length	22.55	19.31	20.93	1.62	14.14
12th dorsal spine length	18.91	15.92	17.42	1.50	11.77
11th dorsal spine length	15.91	11.91	13.91	2	9.40
1st anal spine length	11.70	7.67	9.69	2.02	6.54
2nd anal spine length	19.71	17.08	18.40	1.32	12.43
3rd anal spine length	21.33	16.46	18.90	2.43	12.77
Pelvic fin spine length	22.37	17.65	20.01	2.36	13.52
Head length	60.60	50.78	55.69	4.91	37.63
Snout length	12.27	10.66	11.47	0.81	7.75
Orbit length	20.69	17.36	19.03	1.67	12.85
Postorbital length	29.03	26.26	27.65	1.39	18.68
Interorbital length	7.61	7.63	7.62	0.01	5.15
Upper jaw length	34.24	30.10	32.17	2.07	21.74
Weight (g)	136.17	88.68	112.43	23.75	N/A
Gonad weight (g)	4.97	0.41	2.69	2.28	N/A

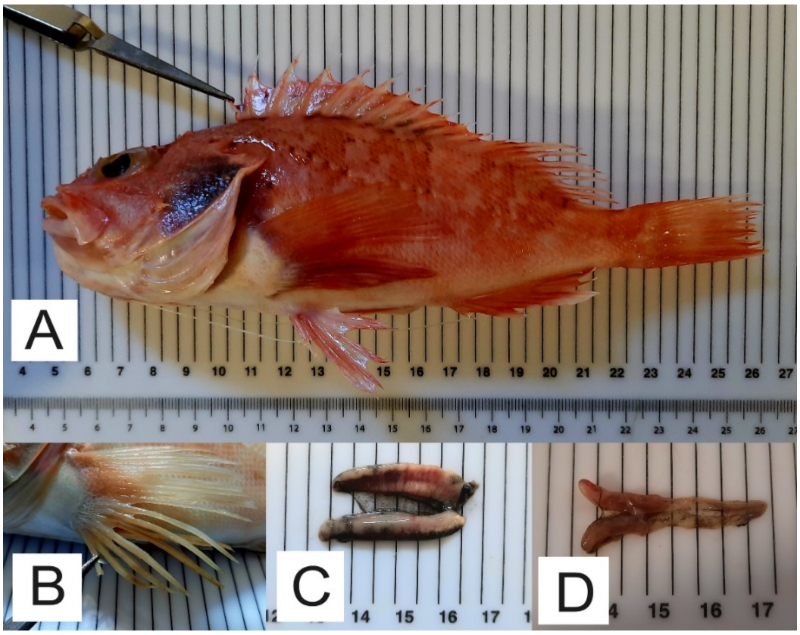

Figure 2. (A) one of the examined specimens of *H. dactylopterus* (specimen 1, TL 201 mm); (B) close-up image depicting lowermost free rays of the pectoral fin (specimen 1); (C) and (D) ovaries of specimen 1 and specimen 2 (TL 173 mm), respectively

DISCUSSION

The contemporary presence of *H. dactylopterus* in the Sea of Marmara is confirmed by the present study. Before further discussing the earlier records of *H. dactylopterus* from the mentioned region, it is necessary to clarify one point that the date of the first record of the species from the Sea of Marmara is contradictory. Although Eryılmaz and Meriç (2005) and Gönülal and Topaloğlu (2016) refer to Meriç (1995) as the first record of *H. dactylopterus* from the Sea of Marmara, contrary to them, Artüz and Fricke (2019) refer to JICA (1993). Presumably based on the sampling dates, the material examined by Meriç (1995) was considered the first record of *H. dactylopterus* from the Sea of Marmara, although this article was published two years after the JICA (1993) report. On the other hand, the demersal fishery resources survey was initiated in May 1991 (JICA, 1993); however, Meriç's (1995) material contains 36 specimens of *H. dactylopterus*, the majority (n=34) of which were caught in 1988. Although this brief anecdote does not change the fact that the first record of the species in the Sea of Marmara was in the early 1990s, it is clear that this discrepancy in the literature should be clarified. In addition, Altun (1997) found postlarvae and juveniles of *H. dactylopterus* in ichthyoplankton samples collected during surveys in the Sea of Marmara in the 1950s and 1960s and preserved at the Department of Hydrobiology, İstanbul University. Therefore, the results of Altun (1997) suggest that *H. dactylopterus* may have been present in the region since earlier years. According to Munoz et al. (2010), *H. dactylopterus* is a zygoparous species, which means that it gives birth in multiple batches by enclosing the embryos in a gelatin sheath. Although the embryos in the gelatin sheath are released to the seafloor, the larvae and juveniles are thought to be planktonic after the gelatin sheath dissolves (Froese and Pauly, 2024). Therefore, *H. dactylopterus* postlarvae found in the previous ichthyoplankton samples from the Sea of Marmara may have been transported from the Aegean Sea by the current. However, in a very recent study, underwater observations conducted by means of a remotly operated underwater vehicle (ROV) showed that both juveniles and adults were mostly standing on their fins on the substratum (93% of the adults and 94% of the juveniles) and in most cases completely inactive (El Vadhel et al., 2024).

Despite the possibility of transport from the Aegean Sea, the sampling period of ichthyoplankton samples from the Sea of Marmara is spread over a long period of time, suggesting that a population of *H. dactylopterus* may have lived in this region in the past. Furthermore, if this assumption is accepted as correct, it is known from the existing literature that *H. dactylopterus* lived under very favourable conditions, especially in terms of dissolved oxygen, in the bathyal zone of the Sea of Marmara in the 1950s and 1960s (Kocataş et al., 1993). According to Kocataş et al. (1993), who emphasized that the oxygen-rich water layer in the Sea of Marmara (mean DO 7.6 mg/L) could reach down to 80 m depth in the past, anaerobic conditions did not occur even if the oxygen level decreased below this layer. Moreover, the abundance of *H. dactylopterus* per unit area (kg/km^2) varied between 3.1 and 15.8 kg/km^2 between 201-500 m depth during the JICA (1993) survey, which supports the assumption that suitable environmental conditions for this species existed in the bathyal zone of the Sea of Marmara in those years.

Considering that *H. dactylopterus* is a member of the family Sebastidae, and sebastids are known to have distributions such as reduced home ranges, suggesting less tolerance to low oxygen than most other taxa (Parnell et al., 2020). Mainly due to anthropogenic impacts, DO levels in the deep regions of the Sea of Marmara have decreased significantly below the hypoxia threshold (DO < 2 mg/L) over the past 40 years, and even anoxia appears to be becoming more widespread (Mantıkçı et al., 2022), suggesting that the living conditions of *H. dactylopterus* have deteriorated in it's natural deep habitat. Contrary to the statement in the JICA (1993) report that the abundance of *H. dactylopterus* in the Sea of Marmara peaked in winter at 20-100 m depth (23.4 kg/km^2) and at 201-500 m depth (15.8 kg/km^2), the absence of the species in the deep zones is presumably due to the environmental degradation of it's habitat. The in situ DO measurements (≤2 mg/L) at station MD18, where only 2 specimens of *H. dactylopterus* were caught, and nearby deeper stations support the link between the absence of the species and deoxygenation.

The morphometric measurements presented in Table 1 provide the first detailed morphometry of *H. dactylopterus* from the Sea of Marmara. According to Koca (2023), who studied the morphometry of *H. dactylopterus* based on 156 samples (97 males and 59 females) caught in Antalya Bay, most of the morphometric characteristics of male fishes differed from female fishes, while such differences were found to be statistically insignificant. Due to the small number of samples (n=2) and the fact that only females were included in the sample, it is currently not possible to make a similar morphometric comparison within the Sea of Marmara population or an interregional comparison with the Antalya Bay population. If environmental conditions do not continue to deteriorate in the future and the species continues to exist in the Sea of Marmara, it may be possible to make such morphometric comparisons in the future when more samples are available.

CONCLUSION

The results of the present study showed that *H. dactylopterus*, a deep-sea teleost of the family Sebastidae, is still present in the Sea of Marmara. After more than 30 years since it's last occurrence in the region following the report of Meriç (1995), the capture of the present specimens raised hopes for life in the bathyal zones of the Sea of Marmara, where environmental degradation is a major challenge for deep-sea life. With regard to the previously reported abundance of *H. dactylopterus* from the Sea of Marmara (JICA, 1993), the capture of only two specimens does not represent more than a confirmation of the current presence. However, this record is also an important finding because it shows that life still exists in the deep regions of the Sea of Marmara, and therefore action must be taken to prevent severe habitat and biodiversity loss.

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AUTORSHIP CONTRIBUTIONS

Material preparation and data collection were performed by all authors. Study conception and drafting of the manuscript were performed by Hakan Kabasakal. All authors commented on previous versions of the manuscript and approved the final version.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

ETHICS APPROVAL

The present study does not raise any ethical issues and no special permissions were required to conduct the study.

DATA AVAILABILITY

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

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