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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.

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.

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.

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When log into the system (Dergipark) with user information, the related journal appears when the dashboard is clicked. By clicking on the journal, the status of the article can be followed.

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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.

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

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

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

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

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

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

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

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

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

First Page

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

Author Names and Affiliation

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

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

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

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

Please refer to the journal's "Ethical Responsibilities of Authors" policy in the Ethical Principles and Publication Policy section for details on eligibility for author listing.

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.

Discussion

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: …."

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

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

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

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

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

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

Full name/s: Conceptualization, Methodology, Software

Full name: Data curation, Writing- Original draft preparation

Full name/s: Visualization, Investigation

Full name/s: Supervision

Full name/s: Software, Validation

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

Full name/s: Writing- Reviewing and Editing

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.

Conflict of Interest Statement

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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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."

Retrospective Ethics Approval

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.

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

Data availability: All relevant data is in the article.

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. DOI: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. DOI: 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

All illustrations (drawing, photograph, image, graphics, etc.), except tables, should be labeled 'Figure'. Tables and figures should be numbered using consecutive Arabic numbers, and referred to as "Table 1, Figure 1" in the text, unless there is only one table or one figure.

Each table and figure should contain a short title. If the paper is prepared in Turkish, table and figure titles should be written in 2 languages, both English and Turkish. Table and figure captions should be placed in appropriate places.

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İÇİNDEKİLER CONTENTS

ARAŞTIRMA MAKALELERİ RESEARCH ARTICLES

KISA MAKALELER SHORT COMMUNICATION

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Seasonal changes in antioxidant defense system indicators in the tissues of *Cyprinion macrostomus* (Heckel, 1843) caught from Göynük Stream (Bingöl, Turkey)

Göynük Çayı'nda (Bingöl) yakalanan *Cyprinion macrostomus* dokularında antioksidan savunma sistemi göstergelerindeki mevsimsel değişiklikler

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Abstract: In this study, antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase GPx), glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PD)) and malondialdehyde (MDA) levels occurring throughout the year were examined in *Cyprinion macrostomus* tissues (kidney, gill, liver and gonad) captured from Göynük Stream (Bingöl, Turkey). For this purpose, two locations (Ilıcalar and Garip) where fish can be caught regularly in summer, autumn, winter and spring were determined. Fish were caught regularly from these two locations every month and brought to the laboratory. Spectrophotometric methods were used to determine enzyme activities and MDA levels in the study. As a result of the study, it was determined that the MDA level and enzyme activities between Ilıcalar and Garip stations, in general, were statistically different from each other in all tissues. However, it was observed that there were important differences in general between the seasons at both stations. In addition, while GR and G6PD activities were lower than other enzyme activities throughout the study, CAT and SOD activities were higher.

Keywords: Doctor fish, freshwater systems, Murat River, Göynük Stream, oxidative stress

Öz: Bu çalışmada, Göynük Çayı'ndan (Bingöl, Türkiye) yakalanan *Cyprinion macrostomus* dokularında (böbrek, solungaç, karaciğer ve gonad) yıl boyunca meydana gelen antioksidan enzim aktiviteleri (süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz GPx), glutatyon redüktaz (GR) ve glukoz 6-fosfat dehidrojenaz (G6PD)) ve malondialdehit (MDA) seviyelerindeki değişimler mevsimsel olarak incelenmiştir. Bu amaçla yaz, sonbahar, kış ve ilkbaharda düzenli olarak balık yakalanabilecek iki lokasyon (Ilıcalar ve Garip) belirlenmiştir. Bu iki lokasyondan her ay düzenli olarak balıklar yakalanarak laboratuvara getirilmiştir. Çalışmada enzim aktivitelerini ve MDA düzeylerini belirlemek için spektrofotometrik yöntemler kullanılmıştır. Çalışma sonucunda genel olarak Ilıcalar ve Garip istasyonları arasındaki MDA düzeyi ve enzim aktivitelerinin tüm dokularda istatistiksel olarak birbirinden farklı olduğu belirlendi. Bununla berarber, her iki istasyonda da mevsimler arasında genel olarak anlamlı farklılıkların olduğu gözlemlenmiştir. Ayrıca çalışmada, GR ve G6PD aktiviteleri diğer enzim aktivitelerinden daha düşük iken, CAT ve SOD aktivitelerinin diğer enzim aktivitelerinden daha yüksek olduğu tespit edilmiştir.

Anahtar kelimeler: Doktor balık, tatlısu sistemleri, Murat Nehri, Göynük Çayı, oksidatif stress

INTRODUCTION

Today, one of the most important dangers for all living things in the ecosystem is environmental pollution. Environmental pollution has increased especially in parallel with the start of urban life and the realization of the industrial revolution. As a result, the aquatic ecosystem is affected the most from this pollution in our country as in the whole world (Sökmen et al., 2018; Güneş et al., 2019; Taysı et al., 2021; Kirici et al., 2022). Different pollutants that enter the aquatic environment and pose a great threat to fish, catalyze oxidative reactions; They lead to the formation of reactive oxygen compounds such as hydrogen peroxide, superoxide, singlet oxygen and hydroxyl radical. These radicals are highly reactive compounds and cause oxidation and impairment of the functions of important biological molecules such as deoxyribonucleic acid (DNA), protein and lipid (Yu, 1994; Castillo et al., 2002). The harmful effects of reactive oxygen compounds are neutralized by antioxidant defense systems. This system, known as antioxidant defense, includes the enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PD) (Figure 1), which are low molecular weight structures. It includes non-enzymatic structures such as vitamins A, E, C, carotenes, ubiquinone10. In a healthy cell, there is a physiological balance between reactive radicals formed as a result of metabolic reactions and the level of antioxidant molecules formed by various defense mechanisms (Finkel and Holdbrook, 2000; Yonar et al., 2016). Disruption of this balance towards oxidants is defined as oxidative stress (Sies, 1997). This may result in impairment of cell functions, apoptosis or necrosis. Therefore, the functionality of antioxidant defense systems and ensuring the balance of oxidants/antioxidants are vital for the cell (Nordberg and Arner, 2001). Increased free radicals also cause lipid peroxidation, causing impairment of cell membrane functions. As a result, malondialdehyde (MDA), which is the breakdown product of lipid peroxidation, is formed and is used as an indicator in determining the oxidative damage of lipids (Kasai, 1997).

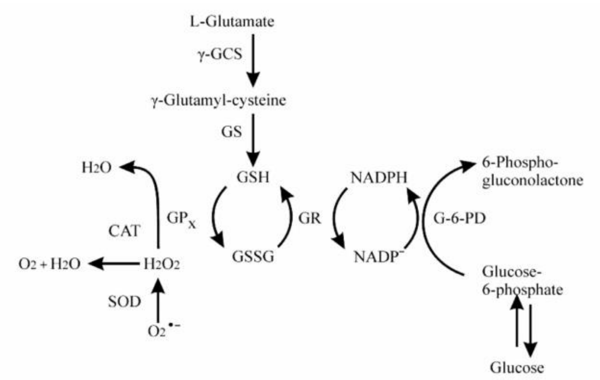

Figure 1. Key antioxidant enzymes and the reactions they catalyze are shown (Kehinde et al., 2016)

Oxidative stress in fish is affected by many factors. Temperature, salt adaptation, hunger, xenobiotics and diseases are the main ones. Increase in temperature increases metabolic activities in all living things. With increasing metabolism, the amount of oxygen needed increases and as a result, the total oxidant level increases. It has been reported that oxidative stress increases depending on the temperature in different living groups (Almroth et al., 2015). Fish with poikilotherm are strongly affected by water temperature; therefore, they constantly adjust their bodies to environmental conditions. They are widely used in biomonitor studies (Aleshko and Lukyanova, 2008). Many physiological changes are observed during salt adaptation in fish. These are increased energy metabolism, adjustment of ion balance, molecular and cellular changes, and hormonal regulations. Reactive oxygen species are formed in the tissues of fish during salt adaptation, both experimentally created and in the natural environment, causing oxidative damage (Liu et al., 2007; Wilson et al., 2014). Prolonged starvation has caused oxidative damage, particularly in the liver, where energy metabolism occurs in fish as well as in mammals (Morales et al., 2004; Bayir et al., 2011). It has been reported that foods with different contents in fish have an effect on the oxidative status in the liver. Especially foods with high lipid content increased antioxidant enzyme levels (Rueda-Jasso et al., 2004).

Cyprinion macrostomus (Figure 2) is distributed in West Asia, India, Afghanistan, Iran, Syria and Mesopotamia and is located in the Euphrates-Tigris system in our country. These two species are widely distributed, especially in thermal hot springs in the Euphrates and Tigris River Basin (Çelik and Güzel, 2017). It is known that these fish can easily survive in wide temperature ranges, they can survive in waters with a pH level of about 7.3 and isothermal, even in waters with temperatures around 35ºC throughout the year (Değirmenci and Ünver, 2021). It is known that such fish species help to heal some skin diseases (psoriasis, eczema and purulent wounds), the origin of their use for therapeutic purposes in spas goes back thousands of years and such practices continue today (Demir, 2009; Çelik and Güzel, 2017).

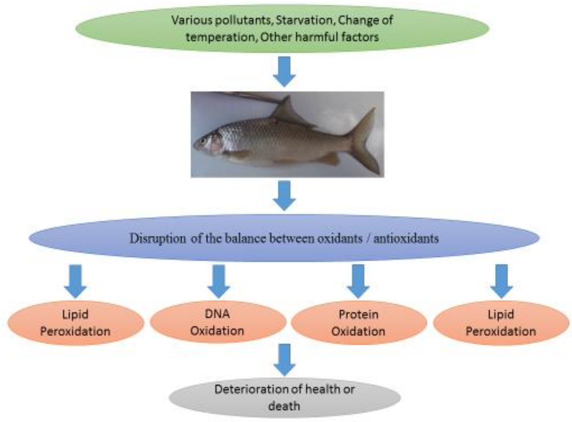

Figure 2. Schematic representation of major events in accrued damage of various harmful factors in *Cyprinion macrostomus*

In this study, 2 stations were determined from the Göynük Stream, which is a branch of the Murat River, which passes through the north of Bingöl Province Genç District and flows into the Keban Dam. These two stations were chosen because the fish could be caught regularly throughout the year. In addition, Ilıcalar station is a region where thermal springs are located and therefore has temperature values above seasonal norms. The other station is Garip station, where the water temperature is relatively cold, passing mostly through the residential area and close to Genç district. These stations were selected based on the migration criteria of these species, which tend to migrate to warm waters. The aim of this study was to investigate the seasonally the changes occurring antioxidant enzyme (SOD, CAT, GPx, GR and G6PD) activities and MDA levels in kidney, gill, liver and gonad tissues of *C. macrostomus* fish caught from Ilıcalar and Garip stations in the Göynük Stream. In addition, the suitability and sensitivity of fish oxidative stress biomarkers for early detection of the health of the freshwater ecosystem were evaluated.

MATERIAL AND METHODS

Study area and stations

In this study, 2 stations (Ilıcalar and Garip) were determined from the Göynük Stream, which is a branch of the Murat River, which passes through the north of Bingöl Province Genç District and flows into the Keban Dam (Figure 3).

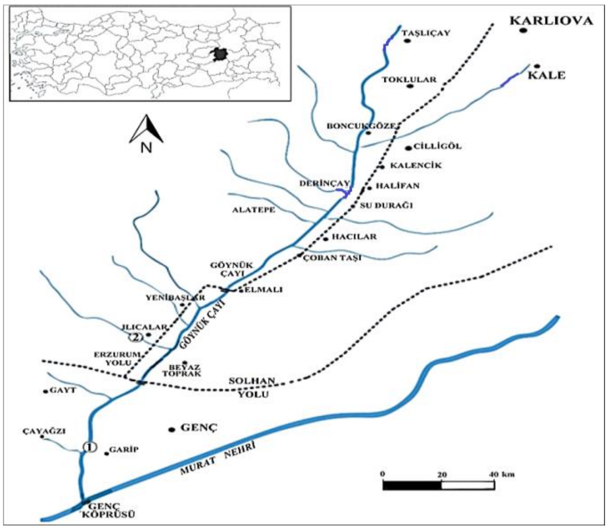

Figure 3. Stations (1. Garip; 2. Ilıcalar) (Modified from Koyun et al., 2018)

Homogenate preparation for enzyme activity determination

The fish were anesthetized using anesthetics. The abdomen was opened and the kidney, gonad, liver and gills were removed and treated with 0.9% NaCl to remove the blood from the tissues. After these procedures, the tissues were stored at -80ºC in the deep freezer until the time of use (Kirici et al., 2017).

10 g of each kidney, gonad, liver and gill tissue were weighed on a precision scale. The weighed tissue samples were thoroughly cut into small pieces with scissors or a scalpel. The fragmented tissue samples were taken into porcelain mortar cooled at -80ºC beforehand, and some liquid nitrogen was added on it and crushed until it became a dough. 3 times the amount of KH_2PO_4 buffer solution was added to the pulp tissue sample. After the samples were taken into the centrifuge tube, they were centrifuged at 13000 rpm for 2 hours at 4ºC. Enzyme activities were studied by taking the supernatant after centrifugation (Beutler, 1975).

Determination of lipid peroxidation

Lipid peroxidation product MDA level, absorbances at maximum 532 nm were measured in Shimadzu UV / VIS-1201 spectrophotometer as a result of color reaction with TBA reagent. In a tube, 200 µl of extracted tissue samples were taken and suspended on it with 800 µl phosphate buffer and 25 µl BHT. Then 500 µl of 30% TCA was added. The tubes were kept in a refrigerator at -20ºC for 2 hours by vortexing. It was then centrifuged at 2000 rpm for 15 minutes. 1 ml of the supernatant was taken and transferred to other tubes. 75 µl EDTA Na_2H_2O and 250 µl TBA were added on top of this. After vortex mixing, the tubes were kept in a hot water bath (90ºC) for 15 minutes. Then it was brought to room temperature and its optical densities were read at 532 nm (Slater, 1984).

Measuring the levels of antioxidant enzymes

The amount of NADP reduced during its formation from glucose-6-phosphate and 6-phosphoglucanolac is directly proportional to the activity of the G6PD enzyme that catalyzes this reaction. The measurement of enzyme activity is based on the determination of the absorbance difference of NADPH formed during the reaction at 37ºC and 340 nm wavelength per unit time (Beutler, 1975).

GR activity was determined by the method of Carlberg and Mannervik (1985). The reaction mixture consisted of 100 µL 50 mM Tris-HCl + 1 mM EDTA (pH 8.0), 100 µL 20 mM GSSG, 100 µL 2 mM NADPH substrate in 600 µL distilled water in a total volume of 1 ml and supernatant containing 100 µL enzyme. The reaction was carried out by observing the absorbance change in every 1 minute for 3 minutes in quartz cuvettes with a light path of 1 cm at 340 nm wavelength of NADP, which is formed when the enzyme reduces GSSG in the presence of NADPH at 25ºC.

The SOD enzyme was determined by the method modified by Sun et al. (1988). The principle of this method is based on the reduction of nitroblue tetrazolium (NBT) by the xanthine-xanthine oxidase system, which is the superoxide producer. SOD activity was expressed as units $gram^{-1}$ ($U\ g^{-1}$) tissue protein.

GPx activity was studied according to the method of Beutler (1975). GPx catalyzes the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) in the presence of hydrogen peroxide. In the presence of hydrogen peroxide, the GSSG formed by GPx is reduced to GSH with the help of GR and NADPH. GPx activity was calculated by reading the absorbance decrease at 340 nm during the oxidation of NADPH to NADP + and was expressed as units $gram^{-1}$ ($U\ g^{-1}$) tissue protein.

CAT activity was determined according to Aebi (1983) method. The method is based on reading the enzymatic degradation of the H_2O_2 substrate with CAT at 240 nm (Aebi, 1983).

Protein Determination

Tissue protein determination is performed by Lowry et al. (1951) was done spectrophotometrically by the method described. This method is based on the complex formation of peptide bonds of proteins with copper ions in an alkaline environment. The copper-peptide complexes react with the folin reagent to form a blue-purple color and are read in the spectrophotometer against blank at 750 nm.

Statistics

Data are expressed as mean ± standard error. Data were subjected to One-Way ANOVA and Duncan test was used to determine the significant difference between control and experimental groups using the SPSS 23.0 computer program. $P<0.05$ value was considered statistically significant.

RESULTS

While the difference between MDA level, CAT, GR, G6PD and GPx activities in kidney tissue between Ilıcalar and Garip stations was statistically significant in all seasons, the difference between SOD activities was not significant ($P<0.05$). The highest activity value in kidney tissue was detected in CAT activity in Ilıcalar station in summer, and the lowest in G6PD activity in Ilıcalar station in spring. However, the differences between the seasons in the levels of MDA and enzyme activities at the two stations were found to be statistically significant. Only at Garip station, there was no statistical difference between seasons in G6PD activities ($P<0.05$) (Table 1).

Table 1. Levels of oxidative stress parameters in *C. macrostomus* kidney caught from Göynük Stream

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	51.76 ± 8.04^{a,*}	4.10 ± 1.09^{a}
	Autumn	56.53 ± 11.29^{a,*}	10.20 ± 2.71^{b}
	Winter	40.14 ± 9.20^{b,*}	1.71 ± 0.82^{c}
	Spring	73.03 ± 15,61^{c,*}	2.90 ± 0.91^{c}
SOD	Summer	7.75 ± 0.87^{a}	8.06 ± 0.64^{a}
	Autumn	5.24 ± 0.70^{b}	4.98 ± 0.24^{b}
	Winter	4.75 ± 0.87^{b}	5.33 ± 0.41^{b}
	Spring	4.06 ± 0.57^{b}	4.41 ± 0.32^{b}
CAT	Summer	254.27 ±	30.72 ± 3.21^{a}
	Autumn	121.15± 24.11^{b,*}	62.74 ± 5.77^{b}
	Winter	150.44 ±	45.69 ± 4.45^{c}
	Spring	117.82 ±	48.63 ± 4.80^{c}
GR	Summer	0.21± 0.02^{a,*}	4.41 ± 0.43^{a}
	Autumn	0.33± 0.03^{a,c,*}	8.43 ± 0.72^{b}
	Winter	0.59± 0.04^{b,*}	2.14 ± 0.44^{c}
	Spring	0.39± 0.03^{c,*}	7.72 ± 0.59^{b}
G6PD	Summer	0.024± 0.001^{a,*}	1.56 ± 0.09
	Autumn	0.023 ± 0.004^{a,*}	1.59 ± 0.09
	Winter	0.039 ± 0.001^{b,*}	2.01 ± 0.23
	Spring	0.009± 0.0003^{c,*}	1.34 ± 0.56
GPx	Summer	68.31 ± 9.26^{a,*}	13.53 ± 1.29^{a}
	Autumn	71.32 ± 13.88^{a,*}	40.76 ± 2.72^{b}
	Winter	45.37 ±11.03^{b,*}	11.90 ± 1.84^{a}
	Spring	63.44 ± 12.49^{a,*}	38.89 ± 2.03^{b}

*$P<0.05$ when compared with values at Garip

a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters ($P<0.05$)

In the gill tissue, there was no statistically significant difference in MDA level between stations in all seasons ($P<0.05$). However, the difference between seasonal values of SOD, CAT, G6PD and GPx activities between Ilıcalar and Garip stations was statistically significant ($P<0.05$). Although there was no statistically significant difference between the stations in the summer, autumn and winter seasons in GR activity, it was found higher at Garip station than at Ilıcalar station in the spring season, and the difference between them was found to be statistically significant. At the Garip station, there is a statistically significant difference between the values of MDA levels and enzyme activities between seasons. At Ilıcalar station, there were no statistically significant differences between the values of GR and G6PD activities between seasons, while the differences in other parameters were found to be statistically significant ($P<0.05$) (Table 2).

Table 2. Levels of oxidative stress parameters in *C. macrostomus* gill caught from Göynük Stream

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	7.86 ± 1.31^{a}	7.09 ± 1.64^{a}
	Autumn	4.15 ± 0.84^{b}	4.22 ± 0.85^{b}
	Winter	5.49 ± 1.19^{b}	4.09 ± 0.35^{b}
	Spring	4.09 ± 0.98^{b}	3.36 ± 0.29^{b}
SOD	Summer	34.46 ± 3.30^{a,*}	243.24 ± 21.04^{a}
	Autumn	15.71 ± 1.19^{b,*}	231.11 ± 29.84^{a}
	Winter	19.91 ± 1.24^{b,*}	317.00 ± 39.04^{b}
	Spring	11.60 ± 2.00^{b,*}	226.71 ± 21.01^{a}
CAT	Summer	111.49 ± 10.61^{a,*}	64.73 ± 24.39^{a}
	Autumn	318.58 ± 23.17^{b,*}	22.29 ± 7.91^{b}
	Winter	128.18 ± 11.72^{a,*}	43.89 ± 10.47^{c}
	Spring	141.82 ± 15.29^{a,*}	28.05 ± 9.87^{b}
GR	Summer	1.23 ± 0.19	1.56 ± 0.44^{a}
	Autumn	0.97 ± 0.20	1.53 ± 0.25^{a}
	Winter	1.57 ± 0.28	2.02 ± 0.39^{a}
	Spring	1.09± 0.33*	4.96 ± 0.97^{b}
G6PD	Summer	0.21 ± 0.002*	2.67 ± 0.35^{a}
	Autumn	0.19 ± 0.001*	1.10 ± 0.11^{a}
	Winter	0.21 ± 0.002*	4.28 ± 0.42^{b}
	Spring	0.26 ± 0.002*	6.85 ± 0.92^{c}
GPx	Summer	75.06 ± 8.20^{a,*}	37.68 ± 4.32^{a}
	Autumn	53.34 ± 4.11^{b,*}	17.49 ± 1.11^{b}
	Winter	72.33 ± 7.91^{a,*}	15.53 ± 1.75^{b}
	Spring	70.61 ± 7.73^{a,*}	20.97 ± 3.03^{b}

*$P<0.05$ when compared with values at Garip

a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters ($P<0.05$)

Although no statistically significant difference was found between the stations in the spring season in liver tissue, MDA level and GR activity, it was found higher at Ilıcalar station than at Garip station in summer, autumn and winter seasons, and the difference between them was found to be statistically significant. Significant differences were found between stations in SOD and GPx activities in all seasons. In G6PD activity, the difference between the stations in the summer and autumn seasons is statistically significant, while the difference between the winter and spring seasons is not statistically significant. In addition, no difference was detected between stations in CAT activity. At Garip station, there was no significant seasonal difference in GPx activity. However, statistically significant differences were determined between the MDA level and the activities of enzymes in all stations as seasons ($P<0.05$) (Table 3).

Table 3. Levels of oxidative stress parameters in *C. macrostomus* liver caught from Göynük Stream

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	$2.59 \pm 0.53^{a,*}$	0.65 ± 0.11^{a}
	Autumn	$1.08 \pm 0.69^{b,*}$	0.40 ± 0.12^{a}
	Winter	$4.12 \pm 0.53^{c,*}$	0.32 ± 0.09^{a}
	Spring	2.41 ± 0.45^{a}	1.58 ± 0.26^{b}
SOD	Summer	$3.19 \pm 0.74^{a,*}$	18.53 ± 1.99^{a}
	Autumn	$3.15 \pm 0.59^{a,*}$	39.76 ± 3.35^{b}
	Winter	$2.97 \pm 0.37^{a,*}$	10.54 ± 1.09^{a}
	Spring	$1.02 \pm 0.70^{b,*}$	14.76 ± 1.35^{a}
CAT	Summer	95.53 ± 21.19^{a}	101.78 ±
	Autumn	86.22 ± 16.37^{a}	84.97 ± 8.05^{b}
	Winter	58.79 ± 13.04^{b}	63.28 ± 2.66^{c}
	Spring	85.90 ± 22.48^{a}	$72.80 \pm 5.17^{b,c}$
GR	Summer	$0.25 \pm 0.009^{a,*}$	5.15 ± 0.82^{a}
	Autumn	$0.71 \pm 0.054^{b,*}$	6.71 ± 1.49^{a}
	Winter	$0.31 \pm 0.005^{a,*}$	2.22 ± 0.27^{b}
	Spring	1.45 ± 0.177^{c}	2.09 ± 0.55^{b}
G6PD	Summer	$4.28 \pm 0.59^{a,*}$	1.04 ± 0.29^{a}
	Autumn	$6.72 \pm 0.43^{b,*}$	0.88 ± 0.08^{a}
	Winter	2.39 ± 0.09^{c}	2.80 ± 0.08^{b}
	Spring	2.51 ± 0.27^{c}	2.79 ± 0.57^{b}
GPx	Summer	$51.19 \pm 4.23^{a,*}$	11.7±0.71
	Autumn	$45.28 \pm 7.11^{b,*}$	10.4±0.57
	Winter	$38.05 \pm 8.10^{c,*}$	9.7±0.38
	Spring	$45.19 \pm 10.06^{a,*}$	10.7±1.08

*P<0.05 when compared with values at Garip

a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P < 0.05)

Table 4. Levels of oxidative stress parameters in *C. macrostomus* gonad caught from Göynük Stream

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	$11.71 \pm 0.92^{a,*}$	7.05 ± 1.02^{a}
	Autumn	$26.54 \pm 1.96^{b,*}$	4.91 ± 0.49^{b}
	Winter	$15.77 \pm 1.00^{a,*}$	1.22 ± 0.37^{c}
	Spring	$10.30 \pm 0.75^{a,*}$	2.19 ± 0.25^{c}
SOD	Summer	$27.92 \pm 4.18^{a,*}$	14.28 ± 0.91^{a}
	Autumn	38.93 ± 6.30^{b}	36.01 ± 3.74^{b}
	Winter	19.48 ± 5.47^{c}	17.79 ± 1.07^{a}
	Spring	13.54 ± 4.13^{c}	14.29 ± 1.78^{a}
CAT	Summer	23.09 ± 2.09^{a}	25.47 ± 8.54
	Autumn	29.21 ± 3.10^{b}	26.12 ± 3.19
	Winter	21.45 ± 2.17^{a}	22.99 ± 1.37
	Spring	23.40 ± 2.32^{a}	21.74 ± 1.54
GR	Summer	$3.25 \pm 0.64^{a,*}$	0.64 ± 0.27^{a}
	Autumn	$5.76 \pm 1.03^{b,*}$	0.53 ± 0.14^{a}
	Winter	$6.82 \pm 1.48^{b,*}$	0.20 ± 0.01^{b}
	Spring	$3.72 \pm 0.76^{a,*}$	0.22 ± 0.01^{b}
G6PD	Summer	1.64 ± 0.12	1.56 ± 0.03
	Autumn	1.87 ± 0.15	1.60 ± 0.03
	Winter	1.94 ± 0.39	2.04 ± 0.02
	Spring	1.78 ± 0.42	1.24 ± 0.01
GPx	Summer	5.80 ± 1.37^{a}	6.12 ± 0.93
	Autumn	6.30 ± 1.03^{a}	6.07 ± 0.33
	Winter	$9.72 \pm 1.76^{b,*}$	5.04 ± 0.54
	Spring	5.85 ± 0.64^{a}	5.75 ± 0.47

*P<0.05 when compared with values at Garip

a, b, c: Different letters in same column as superscripts show statistical importance of values among terms in same site and parameters (P<0.05)

The difference between stations in gonad tissue, MDA level and GR activity was found to be statistically significant. However, the difference between stations in CAT and G6PD activities was not found to be statistically significant. In addition, only the difference in SOD activity in summer was found significant among the stations, while the difference in GPx activity in winter was found to be statistically significant. Although the seasonal differences were determined to be significant in the stations in general, it was determined that the seasonal differences between the G6PD activities at the Ilıcalar station and the CAT, G6PD and GPx activities at the Garip station were not statistically significant (P<0.05) (Table 4).

DISCUSSION

Murat River is one of Turkey's most important water resources. It is polluted by domestic wastewater along with natural pollution and pesticides, which have cumulative negative effects. Biomarkers are frequently employed in ecotoxicology to assess the interaction of the biological system with a chemical, physical, or biological environmental agent. *In vivo* inhibition or induction of biomarkers can be used to evaluate xenobiotic exposure and potential effects on living organisms (Yonar et al., 2011; Yildirim et al., 2014; Kirici et al., 2016a). The use of a biochemical technique to provide early warning of potentially harmful alterations in stressed fish has been promoted. In the field of ecotoxicology, the use of oxidative stress biomarkers has exploded. Antioxidant enzymes are recommended as biomarkers because the first response to environmental effects is given by the antioxidant defense system and they are suitable and reliable for ecotoxicological risk assessment (Farombi et al., 2007; Alak et al., 2011; Yonar et al., 2012; Topal et al., 2014).

Fish are extremely sensitive to anthropogenic contamination, and certain of them can be used as biomonitors to assess the aquatic environment's ecological status. Many variables influence aquatic creatures' resistance to pollution, including their phylogenic location, ecological and biological traits, physiological circumstances, and the presence of effective detoxifying mechanisms (Hotard and Zou, 2008; Kirici et al., 2015; Kirici et al., 2016b). Detection of seasonal biomarker changes of *Cyprinion macrostomus* fish, which are a common species in the Göynük Stream, may be an indicator of their reproductive potential and river health. *C. macrostomus* was selected as sentinel organisms in this study due to their ease of sampling, good adaptability to environmental conditions, and high ecological and economic convenience.

Changes in concentrations and enzyme activity frequently represent cell damage in specific organs in toxicological investigations of acute exposure. The liver is an important organ for metabolic activities and xenobiotic detoxification. Heavy metals can build up to dangerous amounts in the liver and induce pathological alterations in some people. Fish liver tissues have been proposed as a better indicator of water pollution than other organs. Toxic compounds create a change in the fish's physiological state, which has an impact on enzyme activity. Later, it causes disruption in cell organelles, which may result in an increase in enzyme activity (Vinodhini and Narayanan, 2009; Kaptaner and Dogan, 2019).

As a result of the study, statistically significant differences were determined between MDA levels and enzyme activities in all tissues between stations in general and between seasons in both fish. In the study, in *C. macrostomus* fish, the lowest activity was detected in G6PD enzyme in kidney tissue at Ilıcalar station in the spring season (0.009 ± 0.0003), and the highest activity value in CAT activity in gill tissue in Ilıcalar station in autumn (318.58 ± 23.17).

Gabryelak et al. (1983) and Palace and Klaverkamp (1993) suggested that the antioxidant defense in fish is stronger in spring and summer compared to colder winters. However, Ronisz et al. (1999) did not report an interaction between GPx and CAT activities and water temperature in eelpout *Zoarces viviparous* (L). In this study, it was found that SOD activity increased in the summer, especially in the liver, while the highest GPx activity was obtained in the summer, and Gabryelak et al. (1983) and Palace and Klaverkamp (1993). On the other hand, it was determined that there is a significant increase in CAT activity in muscle tissue in summer and autumn, in gills in winter, in liver in summer and winter, which differed from these studies. In another study, it was found that the basic liver antioxidant enzyme activities were not related to the rising temperature, but changed in autumn in all 3 fish species studied. It has been suggested that fish experience oxidative stress during this period and this has been associated with the pre-breeding period. In addition, it has been mentioned that a rapid decrease in temperature, an increase in daylight and an increase in precipitation can cause stress in fish (Aras et al., 2009). Again, the same researchers (Aras et al., 2009) found that SOD, GPx, CAT, G6PD, GR and GST activities were generally higher in the livers of the 3 different species studied compared to the gills in their study. In our study, especially SOD and GPx activities in the liver were found to be higher than other organs studied in all seasons. Can et al. (2017) examined the seasonal changes of CAT, GPx and SOD activities in their study with Munzur Alası in Munzur Stream. As a result of the study, they found that CAT, GPx and SOD activities increased in summer. However, they found that the difference between seasonal values of SOD activities in liver tissue was significant. They found the difference in GPx activities in muscle, gill and liver tissues between summer and other seasons statistically significant. In addition, they stated that the difference between the seasonal CAT activities in muscle, gill and liver tissues was significant (P<0.05).

CONCLUSION

The selected parameters are valuable biomarkers for monitoring aquatic systems, as they provide an early warning signal of xenobiotics that help counteract their adverse effects on aquatic organisms at molecular levels. This approach used in this study has significant potential for use in routine monitoring or evaluation studies of all other aquatic environments. The findings obtained in this study will shed light on the studies to be carried out on the cultivation of *C. macrostomus* in natural conditions, which are used as an alternative treatment method in the treatment of some skin diseases such as psoriasis, eczema and pus. Although oxidant and antioxidant enzyme activities vary according to the seasons, they have also been affected by location, gender, age, date, sexual maturity, contaminants, climatic parameters and reproductive period. Therefore, regular monitoring and evaluation should be done, and surveys should be used to discover the unknowns. It should focus on similar studies, taking into account the factors.

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AUTHORSHIP CONTRIBUTIONS

All authors took part in designing the research, collecting and writing the manuscript. Muammer Kırıcı analysed all data of the study statistically and writing the manuscript. Muammer Kırıcı and Mustafa Koyun prepared their field studies and references. Nurgül Şen Özdemir undertook the editing and application of the article. Muammer Kırıcı and Fatma Caf have edited the graphics and figures of the article. All authors took part in a part of the article. All authors approved the submission and publication of this manuscript.

CONFLICTS OF INTEREST

The author declares that there is no conflict of interest on this manuscript.

ETHICS APPROVAL

The research was approved by Bingöl University Animal Experiments Local Ethics Committee in terms of sampling and use of experimental animals with the decision number 06/5 at the meeting held on 13.10.2016. All researchers declare that all trials were conducted in accordance with ethical values.

DATA AVAILABILITY

The data supporting the conclusions of this paper are available in the main paper.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Trace element bioaccumulation and health risk assessment derived from leg consumption of the marsh frog, *Pelophylax ridibundus* (Pallas, 1771)

Ova kurbağası, *Pelophylax ridibundus*'un (Pallas, 1771) bacak tüketiminden elde edilen eser element biyobirikimi ve sağlık riski değerlendirmesi

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Abstract: Amphibians, which can live in aquatic and terrestrial environments, are a good indicator of pollution in these areas. Although frog leg is not consumed frequently in some cuisines, including Turkey, it is important in terms of evaluating metal accumulation since it is preferred as human food in many European countries. In this study, the quantities of trace elements (Cd, Pb, Cu, Zn, As, Co, Cr, Ni, Mn, V) were measured in the edible tissues (muscles) of an amphibian species by sampling from two frog farms in Turkey. It was aimed to assess possible health hazards for humans by frog legs consumption comparing with the toxicological limit values, including provisional tolerable weekly intake (PTWI), target hazard quotient (THQ), and Hazard Index (HI). In general, the average values (µg kg^{-1}) of trace elements were Zn (3.437.62)> Pb (69.22)> Cu (66.72)> Mn (35.07)> As (24.24)> Cr (11.47)> Ni (6.94)> Cd (6.51)> Co (2.97)> V (<0.001). The results indicated that concentrations of the analyzed trace elements were determined below the European Commission's permitted levels and edible tissues of the marsh frog posed no carcinogenic health risk to humans.

Keywords: Amphibians, environment, human health, pollution, marsh frog

Öz: Hem sucul hem de karasal ortamlarda yaşayabilen amfibiler, bu alanlardaki kirliliği gösteren önemli biyoindikatör canlılardandır. Kurbağa bacağı, Türkiye'nin de dahil olduğu bazı mutfaklarda sıklıkla tüketilmemesine rağmen pek çok Avrupa ülkesinde insan gıdası olarak tercih edilmesi nedeniyle metal birikiminin değerlendirilmesi açısından önemlidir. Bu çalışmada, Türkiye'deki iki kurbağa çiftliğinden örnek alınarak bir amfibi türünün yenilebilir dokularında (kaslarında) eser element (Cd, Pb, Cu, Zn, As, Co, Cr, Ni, Mn and V) miktarları ölçülmüştür. Geçici tolere edilebilir haftalık alım (PTWI), hedef risk katsayısı (THQ) ve Tehlike İndeksi (HI) dahil olmak üzere birçok değer toksikolojik sınır değerlerle karşılaştırılarak kurbağa bacağı tüketiminin insanlar için olası sağlık tehlikelerinin değerlendirilmesi amaçlanmıştır. Genel olarak eser elementlerin ortalama değerleri (µg kg^{-1}) Zn (3.437.62)> Pb (69.22)> Cu (66.72)> Mn (35.07)> As (24.24)> Cr (11.47)> Ni (6.94)> Cd (6.51)> Co (2.97)> V (<0.001) olarak sıralanmıştır. Sonuçlar, analiz edilen eser elementlerin konsantrasyonlarının Avrupa Komisyonu'nun izin verdiği seviyelerin altında belirlendiğini ve ova kurbağasının yenilebilir dokularının insanlar için kanserojen bir sağlık riski oluşturmadığını gösterdi.

Anahtar kelimeler: Amfibiler, çevre, insan sağlığı, kirlilik, ova kurbağası

INTRODUCTION

Amphibians are poikilothermic, vertebrate animals that develop through metamorphosis and are used in many sectors, especially food (Alpbaz, 2009, Çiçek et al., 2021). Although frog meat is not consumed frequently in some cuisines including Turkey, it is important to evaluate metal accumulation because it is preferred as human food in many European countries (Şereflişan and Alkaya, 2016). Similarly, there are studies examining the effects of heavy metal accumulation on human health in some marine species such as *Solen marginatus* (Taş and Sunlu, 2019), *Rapana venosa* (Bat et al., 2016), which are not widely consumed in Turkey. Today, meeting the demand for frogs through farms has become even more critical (Helfrich et al., 2009). Although frog muscles are used in many cultures, they are not widely used in metal accumulation assessments. Indeed, it is crucial to evaluate metal accumulation due to its use in the human diet (Prokić et al., 2016a).

Heavy metals or potential harmful elements are produced by both natural, geogenic, lithogenic and anthropogenic factors (Ali et al., 2019). Determining the accumulation of trace elements in the body due to a metal combination in organisms aids in assessing its effect (Rainbow, 2018). However, the interaction between trace elements can affect accumulation and toxicity (De Medeiros et al., 2020). Therefore, it is crucial to figure out how trace elements interact with one another in tissues because trace elements have increased concentrations in the environment (Briffa et al., 2020).

Contaminated water contains many metal mixtures rather than a single metal, and aquatic organisms are influenced by mixed metals (Zeng et al., 2019; Fettweis et al., 2021). Heavy metal concentrations are toxic to mg L^{-1} levels for most organisms due to heavy metal ions irreversible suppression of particular enzymes (Henczova et al., 2008). Amphibians assimilate these metals from water and water-suspended food through adsorption by tissue and membrane surfaces with ion exchange on the skin (Birungi et al., 2007). Amphibians are more sensitive bioindicators than the other water vertebrates in showing pollution in water because they quickly absorb substances from the environment thanks to their permeable skin (Hecnar, 1995; Altunışık et al., 2021a, b).

How heavy metals in the environment affect amphibians as a bioindicator has attracted the attention of many researchers. The effects of various metals (Lee and Stuebing, 1990; Loumbourdis and Wray, 1998; Loumbourdis et al., 2007; Stolyar et al., 2008; Shaapera et al., 2013; Prokić et al., 2016a, b, 2017; Aguillón-Gutiérrez and Ramirez-Bautista, 2020), such as cadmium (Nebeker et al., 1995; Vogiatzis and Loumbourdis, 1998; Othman et al., 2009; Medina et al., 2016), lead (Kaczor et al., 2013), and copper (Papadimitriou and Loumbourdis, 2002), on amphibian tissues and surrounding waters are among the most frequently studied topics. However, heavy metal studies on amphibians and human health risk assessment studies are scarce (Thanomsangad et al., 2019). This study used the marsh frog, *Pelophylax ridibundus* (Pallas, 1771), a frog species widely distributed in Europe and Asia and frequently consumed, as a bioindicator organism since it is one of the most pollution-resistant species (Zhelev et al., 2014; Zhelev et al., 2020, Mani et al., 2021). Since it is unknown whether the edible tissues of *P. ridibundus* that supplied from frog farms pose a health risk due to potentially harmful elements, this research was undertaken.

In this regard, it was aimed to determine the concentrations of trace elements (Cd, Pb, Cu, Zn, As, Co, Cr, Ni, Mn and V) in the edible tissues (muscles) of 20 samples taken from two frog farms in Adana and Istanbul in Turkey, and to assess potential health risks for humans from frog leg consumption compared with the toxicological limit values, including provisional tolerable weekly intake (PTWI), target hazard quotient (THQ), and Hazard Index (HI).

MATERIAL AND METHODS

Sampling procedure

In this study, 20 samples of the Marsh frog, *Pelophylax ridibundus* (Pallas, 1771) was purchased from two separate frog farms (Kemal Balıkçılık, İstanbul and Sasu Su Ürünleri, Adana) in Turkey between August and September 2020. Since these examples were purchased from frog farms, there is no need for ethics committee permission in Turkey. The samples were packaged in iceboxes inside Ziploc containers, transported to the laboratory and frozen at -20 °C for subsequent laboratory experiments. After the frogs were thawed at room temperature, they were washed with pure water. Snout-vent length (SVL; range: 64-104 mm, mean: 87 mm) of the adult individuals was measured using a digital vernier caliper (500-706-11, Mitutoyo, Tokyo, Japan) with an accuracy of 0.01 mm. The body mass (range: 28-125 g, mean: 80 g) of the adult individuals was weighed with an accuracy of 0.01 g using microbalances. Then the muscle tissue, the edible part of frog's hind legs, was removed using a stainless-steel dissection set.

Sample digestion

Approximately 2 grams (fresh weight) of the muscle tissue were weighed and placed in glass tubes using sensitive laboratory scales (75 ml). 5 ml HNO_3 (Suprapure 65%) was added into each test tube, which was then covered with a watch glass and stored at room temperature overnight. Then, the glass tubes were then placed in the block heater, which was heated to 120 °C, and left for 8 hours. After filling the tubes with 2.5 mL H_2O_2 (Suprapure ≥ 30%) and holding them at 120 °C for another 8 hours, the watch glass was withdrawn from the tubes and placed in the heater until the quantity of solution in it was ~1.5 mL. The resulting solutions were diluted to 50 ml with the help of ultra-pure water, then passed through a 0.45 µm PTFE syringe filter and held at +4 °C until trace element analysis (Gedik, 2018).

Determination of heavy metals and quality control

ICP-MS (Agilent Technologies 7700X) was used to determine the concentrations of trace elements (As, Cd, Co, Cr, Cu, Mn, Ni, Pb, V, and Zn) in diluted and filtered solutions. It was calibrated primarily by diluting ICP-MS multi-element stock solution (1000 ppm) (0-100 ppb) (US EPA, 1994).

For the verification of ICP-MS measurements, reference solutions and metal extraction results were used as standard reference material (BCR 185R, Bovine Liver) (US EPA, 1996). Recovery values for As, Cd, Cu, Mn, Pb, and Zn from the BCR 185R ranged from 93-97% (Table 1).

Table 1. Results of the certified reference material (BCR-185R bovine liver) analysis: reference material values versus measured values (mean±SD)

	Detection Limit*	References values mg kg^{-1}	Analyzed values mg kg^{-1}	Recovery (%)
As	0.040	0.033	0.03	93.94
Cd	0.003	0.544	0.53	97.43
Cu	0.140	277.000	268.00	96.75
Mn	0.311	11.070	10.75	97.11
Pb	0.040	0.172	0.16	93.02
Zn	0.128	138.600	131.00	94.52

*ppb, N=5

Statistical analyses

We used Shapiro-Wilk and Levene test for the normality and homogeneity of the variances, respectively. The t-test was conducted to determine the differences between the areas because the data exhibited a normal distribution ($p > 0.05$). The association between trace elements was then established using the Pearson correlation test. In addition, the relationship between the concentration of trace elements and body measurements (weight and SVL) was analyzed by applying linear regression analysis. All statistical calculations were performed in R programming with a 95% confidence interval (Wickham, 2016; R Core Team, 2020).

Health risk assessment

To protect the health risk of consumers, some institutions and countries have set guidelines for maximum TE levels allowed in seafood. The European Commission (EC, 2006) and Turkish food Codex (TFC, 2011) reported that the Pb and Cd values in seafood products should not exceed 1.5 and 1.0, respectively, in terms of food safety. Therefore, the values obtained in the study were compared with these values and the suitability of the examined frog in terms of consumer health was evaluated. In addition, the effects of weekly and lifetime exposure based on average consumption were also examined in the study. For this purpose, comparisons with weekly metal intake limit values specified by JECFA (1982, 2011a,b) were made by calculating As, Cd, Cu, Pb, and Zn intakes depending on the weekly and average portion size. For this purpose, Estimated Weekly Intake (EWI, µg/kg/week) was determined by using trace elements concentrations in frog muscles to compare with Provisional tolerable weekly intake (PTWI) values (JECFA, 1982, 2011a,b), which represent the pollutant concentration present in the ingested food that would offer no health hazards if consumed weekly over the course a person's lifetime. The Target Risk Coefficient (THQs) was also utilized to quantify the hazards associated with consuming frog legs. Calculated by comparing exposed concentrations of trace elements with reference dose values, THQs are used to describe the probabilities of long-term exposure that is not carcinogenic (US EPA,2015). It means that if the value of THQs is >1, the metal can probably show negative impacts on health. EWI and THQs are calculated in the following way:

EWI: (C x FCR)/BW

THQ= (C x EF x ED xFCR)/(RfD x BW x EF x ET) x 10^{-3}

C indicates the of trace elements in frog muscle tissue (mg kg^{-1} wet weight), FCR indicates the weekly consumption of frog muscles (g). BW indicates average body weight (72.5 kg), EF specifies exposure time in one year (365 days) and ED refers to exposure time (mean life expectancy in Turkey 78 years) (Basara et al., 2016). RfD refers to the amount of reference dose (As: 0.3; Cd: 1; Co: 20; Cr:3; Cu: 37.1; Ni: 20; Pb: 3.5; Zn: 300 µg/kg/day) and finally ET is the average exposure time for non-carcinogens (365 d $year^{-1}$ x number of exposure years (average life expectancy)). Since we do not have data on the daily average consumption of the frog legs, THQ, EWI and HI values were computed according to the weekly consumption of 225 g (US EPA 2000).

All trace elements in the leg muscle tissue were assessed using the Hazard Index (HI) to determine their potential non-carcinogenic health hazard (Newman and Unger, 2002). The total of all THQs is HI, which is expressed as follows.

$$HI = \sum_{i}^{x} \text{THQi}$$

If the HI value is > 1, it indicates that trace elements in the frog's leg may indicate a potential non-carcinogenic health risk.

RESULTS AND DISCUSSION

Spatial distribution of trace elements detected in the muscle tissue of *P. ridibundus*

The distributions of Cd, Pb, Cu, Zn, As, Co, Cr, Ni and Mn concentrations in *P. ridibundus* edible hind leg tissues sampled from two different regions, the average and confidence range are given in Figure 1. There were no significant differences ($p<0.05$) between the two-frog farm regarding trace element concentrations. Vanadium concentrations were found to be below the detection limit on both sides. In the Adana location, average trace element concentrations were 26, 7, 0.4, 11, 70, 36, 11, 75, 2900 µg kg^{-1} for As, Cd, Co, Cr, Cu, Mn, Ni, Pb, and Zn, respectively. In İstanbul location, these concentrations were detected as 23, 6, 6, 12, 63, 34, 3, 63, and 3977 µg kg^{-1}, respectively.

The average values (µg kg^{-1}) of trace elements detected in general were found in the order of Zn (3437.62)> Pb (69.22)> Cu (66.72)> Mn > (35.07)> As (24.24)> Cr (11.47)> Ni (6.94)> Cd (6.51)> Co (2.97)> V (<0.001). According to our findings, only Ni was shown to be positively correlated with the SVL of the marsh frog, but the other elements (Zn, Pb, Cu, Mn, As, Cr, Cd, and Co) were not (Figure 2). Furthermore, the weight of marsh frogs was favorably associated with Ni and Zn, but not with the remaining elements (Figure 2). The mean Pb, Cu, As, Cr, Ni, Mn, and Cd concentrations did not differ between the sites ($p<0.05$). In contrast, the Zn and Co concentrations were significantly higher ($p<0.01$) in the İstanbul site compared to the Adana site. There was a positive relationship between Cu-As, Cr-Cd, Cr-Pb, Cu-Pb and Cd-Pb as while other elements were not found to have a significant relationship with each other (Table 2).

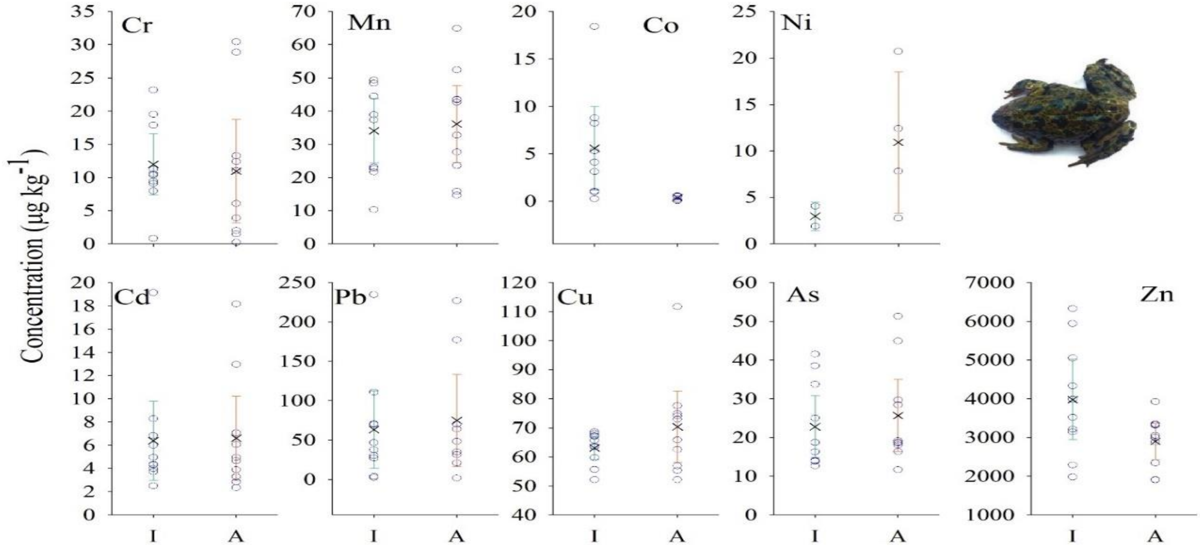

Figure 1. Distribution of trace elements in *Pelophylax ridibundus* muscle tissues. (A) refers to Adana, (İ) refers to İstanbul. Each blank circle represents one sample (n=10 per station). The negative number of empty circles are not shown, as they are below the detection limit. x shows the averages of the stations. Error bars indicate the confidence interval (95%). T-test was applied to determine the statistical differences between stations and no significant difference was detected.

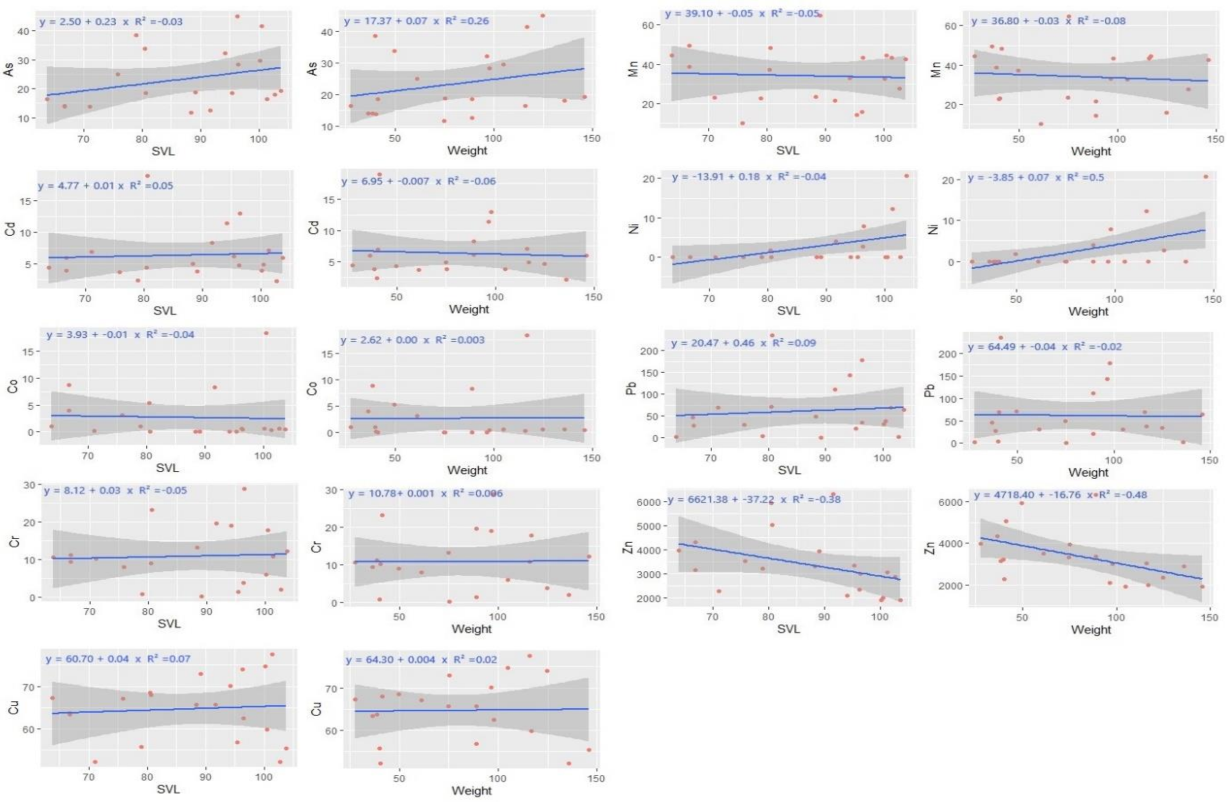

Figure 2. The relationship between the potential toxic element concentrations and SVL, and weight of the individuals belonging to the *Pelophylax ridibundus*

Table 2. The relationship among heavy metal concentrations detected in frog muscle tissue. The numbers are given in bold show the correlation coefficient.

	Cr	Mn	Co	Ni	Cu	Zn	As	Cd	Pb
Cr	**1**	**0.352**	**0.357**	**0.078**	**0.432**	**0.168**	**0.187**	**0.859**	**0.871**
		0.128	0.16	0.883	0.056	0.48	0.43	*0.000	*0.000
Mn			**0.276**	**0.619**	**0.367**	**0.094**	**0.019**	**0.398**	**0.487**
			0.284	0.19	0.112	0.693	0.936	0.081	0.034
Co				**-0.525**	**-0.040**	**0.152**	**0.243**	**-0.002**	**0.018**
				0.285	0.876	0.56	0.348	0.994	0.944
Ni					**-0.492**	**-0.623**	**-0.508**	**0.090**	**-0.058**
					0.322	0.186	0.303	0.864	0.912
Cu						**0.11**	**0.496**	**0.527**	**0.532**
						0.643	*0.026	0.017	*0.019
Zn							**-0.244**	**0.227**	**0.305**
							0.301	0.336	0.204
As								**0.202**	**0.199**
								0.392	0.415
Cd									**0.98**
									*0.0001

Light colored numbers show the p value; * p <0.05

Comparison with literature

Despite the effects of trace elements on amphibians' tissues have been well documented (Lee and Stuebing, 1990; Nebeker et al., 1995; Papadimitriou and Loumbourdis, 2002; Loumbourdis et al., 2007; Othman et al., 2009; Kaczor et al., 2013; Medina et al., 2016; Prokić et al., 2016a, b,2017; Zhelev et al., 2020), there is limited work involving human health risk assessment via consumption of frog (Thanomsangad et al., 2019). Although Turkey is a major supplier of frogs (Çiçek et al., 2021) and Anatolian water frogs (*Pelophylax* spp.) have been gathered for more than 40 years, shipping about 700 t of frogs annually and for frog trade (Akın and Bilgin, 2010; Kürüm, 2015), the low intake of these frogs in Turkey may have slowed the estimation of the health risks associated with their consumption.

Our results for trace elements in the muscle tissue of the marsh frog are given in Table 3. Although trace elements are found in water at lower concentrations, relatively higher concentrations in frog tissues have been reported in several studies (Stolyar et al., 2008; Borković-Mitić et al., 2016; Prokić et al., 2016b;).

Table 3. Estimated Weekly Intake (EWI; µg / kg / week), target risk coefficient (THQ), Hazard Index (HI), arising from consumption of frogs sampled from Adana (A) and İstanbul (İ) regions

		As	Cd	Co	Cr	Cu	Ni	Pb	Zn	HI
Metal Concentration in frog*	A	25.66	6.62	0.36	10.95	70.35	10.91	75.05	2898.52	
	İ	22.81	6.40	5.57	11.99	63.08	2.97	63.38	3976.71	
EC (2006)*			1000					1500		
TFC (2011)*			1000					1500		
EWI	A	0.08	0.02	0.001	0.03	0.22	0.03	0.23	8.90	
	İ	0.07	0.02	0.02	0.04	0.20	0.01	0.20	12.34	
PTWI		15	7			3500		25	7000	
RfD		0.3	1	20	3	37.1	20	3.5	300	
THQs	A	$1.2x10^{-4}$	$9.2x10^{-6}$	$2.5x10^{-8}$	$5.1x10^{-6}$	$2.6x10^{-6}$	$2.1x10^{-7}$	$3.0x10^{-5}$	$1.3x10^{-5}$	1.8x10-4
	İ	$1.1x10^{-4}$	$8.9x10^{-6}$	$3.9x10^{-7}$	$5.6x10^{-6}$	$2.4x10^{-6}$	$7.6x10^{-7}$	$2.5x10^{-5}$	$1.8x10^{-5}$	1.7x10-4

* µg kg^{-1}. EC (2006): European commission regulation "determination of maximum levels for certain contaminants in foodstuffs", TFC (2011): Turkish food codex contaminants regulation, PTWI (µg/kg/weeks): provisional tolerable weekly intake, Cu and Zn (JECFA, 1982), As (JECFA, 2011a), Cd and Pb (JECFA, 2011b). RfD (µg/kg/day): reference dose, from USEPA THQ: Target Risk Coefficient <1 is unlikely to cause any adverse health effects, if THQ> 1 may cause adverse health effects. EWI, THQ and HI values were calculated according to the weekly consumption of 225 g.

The values of Cd, Pb, Cu, Zn, and As obtained in the present study were lower than those of another study (Zhelev et al., 2020) performed on *P. ridibundus* from two sites in Bulgaria. Trace element bioaccumulations in the muscles were determined in the order of Zn > Se > As > Cu > Pb > Cd in those Bulgarian populations (Zhelev et al., 2020). Similar results (since Zn was in the first and Cd in the last order) were also observed in this study in terms of the order of trace elements. In Ibadan (Nigeria), researchers investigated the major and minor element accumulation in *Rana esculentus's* some organs, including muscle tissue and found trace element concentrations in the order of Ni> Zn > Pb> Co> Cu> Cd> As (Tyokumbur and Okorie, 2011), which was different as compared to this study.

Moreover, they found relatively higher mean trace element (Cd, Pb, Cu, Zn, As, Co, Cr, Ni and Mn) concentrations than our study. Besides, the concentrations of eight elements identified in Quereshi et al. (2015)'s experiment for the species *R. tigrina* and *E. cyanophlyctis* were much higher than ours (Table 4).

Table 4. Comparison of trace element concentrations (mg kg^{-1}) in the marsh frog (*Pelophylax ridibundus*)' leg muscle from different countries

	Location	West and South Turkey	NE Turkey	Southern Bulgaria	Ibadan, Nigeria	Sialkot, Pakistan
	Species	***P. ridibundus***	***P. ridibundus***	***P. ridibundus***	***X. laevis, R. esculentus***	***R. tigrina E. cyanophlyctis***
As	Leg	0.012-0.051	0.0002-0.077	0.05-0.80	0.18-0.78	
Cd	Leg	0.002-0.019	0.018-0.18	0.001-0.05	0.33-0.92	6.75-8.31
Co	Leg	0.000018-0.018	0.0013-0.10		1.12-2.43	1.11-5.33
Cr	Leg	0.0002-0.03	0.015-0.30			0.89-2.64
Cu	Leg	0.052-0.111	0.183-0.90	0.23-1.29	0.97-5.12	2.84-6.72
Mn	Leg	0.010-0.065	0.012-0.104		1.28-9.30	0.81-1.64
Ni	Leg	0.000023-0.027	0.019-0.298		3.4811.68	5.02-7.76
Pb	Leg	0.00003-0.235	0.025-0.975	0.007-0.61	0.15-5.12	11.44-37.00
V	Leg	0.0004-0.017	0.0001-0.0019		3.29-102.76	
Zn	Leg	1.901-6.326	1.889-11.450	3.62-7.57	6.93-7.82	10.30-21.09
References		Present study	Mani et al., 2021	Zhelev et al., 2020	Tyokumbur and Okorie, 2011	Qureshi et al., 2015

In another study conducted around an electronic-waste dump, the relative order of some trace elements was Cr>Pb>As>Cd, which was overlapped the data noticed in our study except Cr (Thanomsangad et al., 2019). However, all concentrations (As, Cu, Pb, and Zn) investigated by Thanomsangad et al., (2019) for *H. rugulosus*, *F. limnocharis*, and *O. lima* was higher than the present study. A recent study (Mani et al., 2021) conducted on wild populations of *P. ridibundus* in Turkey, all concentrations (As, Cd, Co, Cr, Cu, Ni, Pb, and Zn) in leg tissue were similar to our study (Table 4).

Risk estimations

As a bioindicator on monitoring water pollution (Rohman et al., 2020), amphibians absorb heavy metals from the aquatic environment and accumulate them in their bodies which can pose a health risk to humans if ingested. The maximum permissible limits for Cd and Pb had been set by some international organizations such as the European Commission (EC, 2006) and Turkish Food Codex (TFC, 2011). Since the maximum permissible limits of Cd and Pb for human consumption in frog have not been set by these organizations, we used the muscle meat of fish instead of frog muscle. For example, the European Commission (EC) and TFC (2011) declared that the Pb and Cd levels in the muscle meat of fish should not exceed 1500 and 1000 µg kg^{-1}, respectively. When we compared our data to these parameters, we noticed that neither Adana nor Istanbul samples exceed the maximum risk limit set by TFC (2011) and EC (2006)/European commission regulation "determination of maximum levels for certain pollutants in foodstuffs" and TFC (2011)/ Turkish food codex contaminants regulation) in terms of Cd and Pb.

THQ, EWI and HI were computed using the estimated weekly intake amount and trace element reference doses (RfD) specified by US EPA (2015) and given in Table 3. EWI values (µg/kg/week) were calculated as 0.08, 0.02, 0.001, 0.03, 0.22, 0.03, 0.23, 8.90 and 0.07, 0.02, 0.02, 0.04, 0.20, 0.01, 0.20, 12.34 for As, Cd, Co, Cr, Cu, Ni, Pb, Zn for samples obtained from Adana and İstanbul, respectively (Table 3). The computed EWI values in our study were much lower than the PTWI values indicated by JECFA (1982, 2011a, b) (Table 3). When the contributions of calculated EWI values to the PTWI were analyzed, these values for Cu, Pb, As, Zn, and Cd were calculated to be 0.006%, 0.8%, 0.53%, 0.17%, and 0.28% for frog samples.

Similarly, THQs were computed to evaluate the hazards that may result from the non-carcinogenic exposure over a lengthy period of time by consuming the *P. ridibundus* leg from two farms in Turkey. Table 1 shows the THQ values derived using trace element concentrations, reference dosage, and daily average frog leg eating. THQ levels detected were below the limit value of 1, indicating that trace elements from *P.*

ridibundus' leg may not have harmful health impacts on humans when consumed regularly. These risk calculations were made entirely according to the raw material results. It is thought that the TE concentration will decrease due to leaching during cooking processes such as boiling and frying. Studies have shown that trace elements in seafood lose their concentration after cooking (Maulvault et al., 2013; Afonso et al., 2015). In addition, it has been determined by modeling that TE intakes were lost throughout the digestive system in humans (Amiard et al., 2008; Afonso et al., 2015; Gedik, 2018;). When such factors are considered, it can be concluded that the TEs obtained from frog consumption will remain at a very limited level.

CONCLUSION

The concentrations of trace elements were determined on edible muscle tissues of the marsh frog samples obtained from two different farms or suppliers in Turkey, and a risk assessment (according to the weekly consumption of 225 g) was evaluated. It was determined that the concentrations of Zn, Pb, Cu, As, Cr, Ni, Cd, and Co detected in the edible tissues of *P. ridibundus* varied spatially, but they were all within the safe consumption limit levels. Comparisons (EWI and PTWI values) could only be made for As, Cd, Cu, Pb and Zn and EWI values were found to be lower than the reported PTWI values. Because the computed THQ and HI values for all of the examined trace elements were less than 1, it is possible to conclude that the trace element concentrations found in *P. ridibundus* samples purchased from two suppliers in Turkey will not pose a health risk to consumers.

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AUTHORSHIP CONTRIBUTIONS

Abdullah Altunışık, Kenan Gedik: Conceptualization, methodology, software. Abdullah Altunışık, Hale Tatlı: Data curation, writing- original draft preparation. Hale Tatlı, Abdullah Altunışık, Kenan Gedik: Visualization, investigation. Abdullah Altunışık, Kenan Gedik: Supervision. Abdullah Altunışık: Software, validation. Hale Tatlı: Project administration, resources, funding acquisition. Abdullah Altunışık, Kenan Gedik: Writing- reviewing and editing.

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 this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The remarkable observation for a mallard (*Anas platyrhynchos)* food from estuarine/coastal area (İzmir/Turkey): A mosquitofish (*Gambusia holbrooki*)

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Abstract: The purpose of this study is to create awareness about the species of mosquito fishes (*Gambusia holbrooki* Girard, 1859) both vectors and ways of introduction to new aquatic habitats. Because the species creates risks, especially for the circumstance of the native and endemic fish fauna, studies about introduction stories of the species are quite important to prevent the risk. In the current report, mosquitofishes which are known as a highly invasive species were found in the crop content of a hen mallard duck (*Anas platyrhynchos* L., 1758) in an estuarine area, the Gediz River Basin (İzmir, Turkey). This report is the first record of a duck consuming mosquitofish. Although nevertheless, it is known that mallard duck feeding behaviour does not predominantly contain fish (they mainly feed on plant seeds), according to this observation, mosquitofish might be added to the prey list of mallards as a new type of food. Therewith, this feeding behavior also indicates that ducks might be a potential vector for the transport of invasive mosquitofishes.
Keywords: Vector, pathway, long-distance dispersal, waterbirds, invaders, feeding behaviour

INTRODUCTION

The itinerary by which invaders are introduced, transported, or imported into the new habitats are known as pathways. In the literature, natural pathways mean that they are not human-mediated. There are some examples of natural pathways, such as river canals, and birds. Both pathways may naturally bring some fish species or their eggs (invasive- or not) to a new aquatic area. These introduction cases can sometimes even be intercontinental.

The current evidence shows that waterbirds can transport plants and invertebrates as internal and external of their body (Reynolds et al., 2015). To the prevailing knowledge, waterbirds are sometimes cited as playing a role as a vector of alien invasive species (Green and Figuerola, 2005; MacIsaac, 2011; Sánchez et al., 2012). There are some studies that consider the waterbirds being a vector in the introduction of alien species (Green et al., 2008; Brochet et al., 2010; Twigg et al., 2009). Even so, the evidence for waterbird-mediated introduction is restricted. Even not many studies that waterbirds play a role in dispersing the vertebrates, studies focusing on the dispersal of native aquatic plants and invertebrates have established that waterbirds are highly suitable dispersal vectors (Figuerola and Green, 2002; Van Leeuwen et al., 2012; Lovas-Kiss et al., 2020).

Gambusia affinis and *G. holbrooki* species originate from North America, are biological agents in the fight against malaria, and started to be introduced in many different habitats of the world in the 1900s (Courtenay and Meffe, 1989; Walters and Freeman, 2000). They have become a pest in many different habitats following initial introductions in the early 20th century as a biological control agent. They are known as one of the 100 invasive species of the earth and pose a risk to the existence of endemic species (ISSG, 2013). More than this, they are listed as one of the harmful 29 aquarium species (Arthington and Marshal, 1999).

The mosquitofish is highly predatory fish and can cause adverse effects in the habitats they spread, which can lead to the extinction of native fauna members (Pyke, 2008). They prey on endangered rare indigenous fish and invertebrate species and eat the eggs of native fish species. They pose a risk, especially in terms of endemic species persistence (Margaritora et al., 2001; Buttermore et al., 2011). This threat is signally higher, especially for "microendemic" (which have a very limited distribution) fish species (Giannetto and Innal, 2021). In Turkey, many of the endemic fish species have microendemic status, which makes their survival more at risk. For example, Yoğurtçuoğlu and Ekmekçi (2014) stated that *G. holbrooki* species threats the genus *Aphanius* in the aquatic habitats.

It is widely known that mosquitofish are poorly adapted to lotic waters. But in lots of countries stream populations are known including in the introduced range (Wach and Chambers, 2007; Kurtul and Sarı, 2019). The mosquitofish species seem poor dispersers (Pyke, 2005; Zogaris, 2014), because of their characteristics, their dispersal from a point source will depend on other, external factors. Some researchers have reported that mosquitofish can be introduced to new habitats by fishing gear (Zięba et al., 2010). More than this, humans, waterways, and possibly waterbirds are kind of vectors for transfer. However, empirical support for this is lacking, it is considered that fish introduction events are by waterbirds, transporting fish eggs externally (Lovas-Kiss et al., 2020). According to Lovas-Kiss et al. (2020) 10 Prussian carp eggs (ca. 0.25%) and eight intact common carp eggs (ca. 0.2% of those ingested) were recovered from the duck's feces. In the observation, four *Carassius gibelio* eggs and all of the *Cyprinus carpio* eggs had viable embryos.

Gambusia spp., which are known to be used as bait, for mosquito control, as pet fish, etc., can easily create a strong population once introduced to the new freshwater ecosystems. Mosquitofish are live-bearing fish. Therefore, it seems impossible to transport it with birds. Although this seems like a very small possibility, it may be possible if the bird swallowed these fish during feeding and vomited from the crop without digesting them during short-haul flights. Although it's still a theory and there is still no scientific evidence for such transport is; the fact that the species is consumed by ducks indicates that it is still possible to be transported between different waters.

The mallard ducks (*Anas platyrhynchos*) generally prefer subtropical parts of Eurasia, Americas, and Africa continentals (Braithwaite and Miller, 1975). They are known as a median migratory and their speed is approximately 82.5 km h^{-1} (McDuie et al., 2019). It is known that there were some small fish in the feeding behaviour of mallard ducks which is known as omnivorous (Hocaoğlu, 1992). They are known as opportunistic animals and rarely prey on small fish (Swanson et al., 1985; del Hoyo et al., 1992; Snow and Perrins, 1998). The most common knowledge, feeding of mallard duck is formed mainly from plant seeds. According to a study conducted in brackish-water areas and salt marshes, it was found that they mainly ate seeds such as *Salicornin* spp., *Atriples* spp., etc. Also, they consumed animal materials such as molluscs and crustaceans (Olney, 1964). More than this, it has also been reported that a mallard duck eats *Anguilla anguilla* European eel juveniles (Salman, 2017).

Biological invasions come to be following habitat loss as a risk to global biodiversity (Sala et al., 2000; Mooney and Cleland, 2001; Strayer, 2010). Freshwater habitats are in danger because of biological invasions (Ruiz et al., 1999; Green et al., 2008). The biological invasions' negative results, prompted study in terms of management and impacts of invasion (Reynolds et al., 2015). It is known that new management strategies are needed for all invasive species. In order to create the new management strategy for them, both the predators and the vectors of the invasive species should be determined in detail.

Figure 1. A hen (left) and a drake (right) mallard duck (*A. platyrhynchos* from Şirince, Selçuk/İzmir in 2019 (Photo by Irmak KURTUL).

In the current reports, *G. holbrooki* species were recorded in different kinds of water resources in Gediz River Basin- or namely Gediz Delta, where the dead mallard duck was found. This river basin is in the Aegean Region (the west part of Anatolia). The lentic water resources in the area are Marmara Lake, Sülüklügöl Lake, Sazlıgöl Lake; the lotic water resource is Gediz River (Kurtul and Sarı, 2019). Gediz River Basin (or known as Gediz Delta) contains İzmir Bird Paradise and its environs. Gediz Delta is one of the Cultural and Natural Asset; Wildlife Protection Areas and a significant part of the delta is protected by Ramsar Status. The delta has a huge coastal side. It considers bays, salt and freshwater marshes, large saltpans, lagoons located downstream of the Gediz River. It is close to the centre of Izmir.

All of the fauna move through the habitats to obtain resources important for gaining energy (Pianka, 1981). The wetlands are known as one of the most sensitive habitats to the pressures (Kaplan et al., 2005). The communities both fauna and flora permanently change in response to fluctuations in water degree and salinity, in the meantime, periodic droughts induce grand changes in wetland communities (Swanson and Meyer, 1977). The activity of the water regimes, and the changes in the community that it creates, is known as an important parameter influencing the availability to breed waterfowl (Swanson et al., 1985). Also, due to the loss of wetlands, the trophic relationships between fauna and flora members here have become more important for the sustainability of ecosystem health. According to the reports, environmental conditions affect the level of food availability on a marsh (Chura, 1962). Both *G. holbrooki* and mallard ducks use similar water bodies and it seems that they have a trophic relationship with each other. The scope of the study is to give new data and evaluate on review existing literature on the ability of mallard duck to spread *G. holbrooki* which is known as an invasive species.

Figure 2. The map of the dead mallard duck was found (www.earth.google.com)

MATERIALS AND METHODS

The dead *Anas platyrhynchos,* hen mallard duck (highly probably is yet dead- cause of death unknown) was found by a fisherman in the estuarine area of the Aegean Region, Gediz River Basin (İzmir, Turkey) on December 27th 2017. The delta area is approximately 14900 ha. It was found in a location that is quite close to the Gediz River's downstream. The coordinates are 38°30'N, 26°55'E. The locality is given Figure 2 and the area enclosed in a red rectangle.

The species identification processes of individuals were carried out at Ege University, Faculty of Fisheries, Department of Marine–Inland Waters Sciences and Technology, Limnology Laboratory. In the investigation, *G. holbrooki* specimens were identified from the crop content of the mallard duck. Because the species represents sexual dimorphism, the sexes were determined by external examination of the presence of gonopodium. The general body morphology of the individuals were examined and the gonopodium structures of the male specimens have been investigated in species identification (Berg, 1965). In the present study, each *G. holbrooki* specimen was weighed with a digital scale to the ±0.01 g and the total length was measured with a vernier calliper to the ±0.05 mm. The digestibility rates of *G. holbrooki* individuals were determined by visual inspection and given as a percentage.

RESULTS

The mallard duck's crop content was investigated by the naked eye. Totally ten *G. holbrooki* specimens were found in the crop content. In the content, three of the specimens were male, four of the specimens were female, and the sex of three of the specimens was not determined because of the digesting. All of the specimens were adults.

The total lengths of the species were between 2.0 and 3.8 cm. The weight of the specimens varies from 0.12 to 0.49 g. One of the specimens was found partly digested with no head (50%). However, most of the specimens were found as almost not digested (Table 1). They were most likely consumed a very short time ago. *G. holbrooki* specimens which were detected in the crop contents of the mallard duck are given in Figure 3.

Table 1. Fish samples from crop contents of the hen mallard (F:Female, M: Male, U: Unidentified, TL: Total length, TW:Total weight, PIF: Physical integrity of the fishes (%).

No	Sex	TL (cm)	TW (g)	PIF (%)
1	M	2.6	0.14	100%
2	U	2.8	0.25	70%
3	M	2.8	0.17	90%
4	M	2.0	0.25	80%
5	U	2.3	0.12	90%
6	F	3.1	0.36	90%
7	F	3.8	0.49	100%
8	F	2.4	0.18	80%
9	U	2.7	0.24	50%
10	F	2.8	0.26	80%

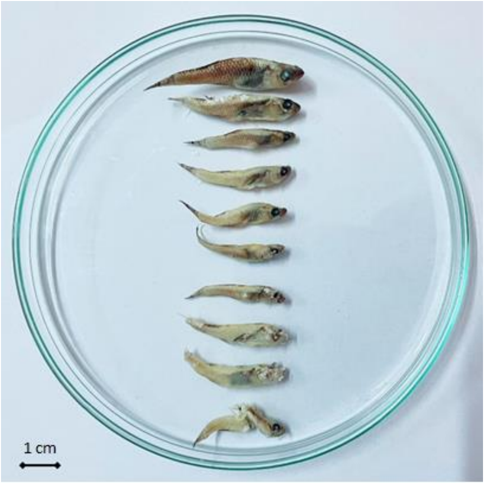

Figure 3. The content of the mallard duck's crop. The undigested *G. holbrooki* individual (on the top), the half-digested *G. holbrooki* individual (on the bottom) from the crop content.

DISCUSSION

Mosquitofishes are small bodied members of the world's freshwater fish. Their total length within their population usually varies between 1-5 cm. However, maximum male and female lengths were found as 3.5 cm and 8.0 cm, respectively (Doadrio, 2002). It means that these fish are small enough to be easily eaten by ducks. As a matter of fact, as they are quite small individuals, they can easily be prey by the predator fishes, aquatic vertebrate species, and birds. The mallard can take this fish species and then release it into another habitat when it is still surviving (vomiting without digesting). In other words, ducks can be vectors for the transportation of this species.

Although *G. holbrooki* seems poor dispersers (Pyke, 2005; Zogaris, 2014), they have a global dispersion today because of their use for biological control. As reported in studies, there may be other vectors besides fishing nets in transporting fish to different habitats (Zięba et al., 2010). Up to date, the aquatic birds, i.e. mallard ducks, might also be a vector which is overlooked affecting this spreading power. It is reported that waterbirds can transport some fauna and flora members as internal and external of their body (Reynolds et al., 2015; Lovas-Kiss et al., 2020) and it is also possible for waterbirds to carry offspring in their feathers. Because the mallard ducks frequently visit different kinds of water sources over short distances and it is very speedy (McDuie et al., 2019). This rapidity means that the fish can stay alive during the mallard duck's transition from one water source to one another.

While both fauna and flora give reactions to the fluctuations in communities, (Swanson and Meyer, 1977) and the wetlands are pretty sensitive habitats (Kaplan et al., 2005), the trophic relationships between fauna members in these regions should be followed carefully all the time. Although it has been reported that mallard ducks rarely prey on small fish (Swanson et al., 1985; Del Hoyo et al., 1992; Snow and Perrins, 1998), it is unclear which of these fish species are. The mallard ducks probably feed on many fish species. According to our observation, it is clear that mallard ducks feed on *G. holbrooki* specimens. While being a vector for a duck is harmless for some fish species, they can create problems if they are vectors of invasive species such as mosquitofishes. In the case of mosquitofishes, it is not easy to eradicate once it creates a population in a new aquatic habitat. In fact, the rotenone (a kind of poison), might be used to eliminate *G. holbrooki* from aquatic habitats. But the rotenone is indiscriminate, so the poison has a negative impact on the native fauna (both vertebrate and invertebrate) (Willis and Ling, 2000). It is almost impossible to eradicate them from the environment they introduced.

As they are invasive species (Arthington and Marshal, 1999; Pyke, 2008; ISSG, 2013), there should be a provision in many different ways to prevent the invasive mosquitofish from dispersing more. Mostly, *G. holbrooki* introduced new habitats through a variety of pathways, including the pet/aquarium trade and deliberate introductions for biological control. Nevertheless, this study predicts that mallard ducks can also be potential carriers. More data is needed to manifest the preferences of the mallard duck about *G. holbrooki* fishes. Because it is known that environmental conditions affect the level of food availability on a marsh, it should be revealed whether the mallard duck consumed this species by preference or whether it was consumed by food deprivation in the wetland area.

CONCLUSION

It is known that ducks are carriers for many species. If these species have invasive properties, then they may cause environmental problems. More data are needed to establish the livebearing carriage status of ducks such as *G. holbrooki*. Therefore, monitoring programs (catch and release) for ducks might be helpful for understanding their feeding behaviours. Thus, this introduction pathway might be understood as the mechanism for further introductions.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea, design, material preparation, investigation, writing/editing of the study and all authors have read and approved the article.

CONFLICT OF INTEREST STATEMENT

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Ecological risk assessment of heavy metals after dredging in Mogan Lake, Turkey

Mogan Gölü'nde (Türkiye) sediment tarama ertesi ağır metallerde ekolojik risk değerlendirmesi

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Abstract: The lake management strategy of sediment dredging (removal) is periodically undertaken in shallow, eutrophic Mogan Lake, an important recreation area. This study aims to use certain indices - enrichment index (EF), contamination/pollution index (CF), degree of contamination (C_d), pollution load index (PLI), geoaccumulation index (I_{geo}), potential ecological risk index (Er^i), integrated ecological risk index (PER), and mean probable effect concentration quotient (mPEC-Q) - to evaluate the state of sediment pollution in the lake after dredging. With this in mind, after dredging was completed, two stations in the littoral zone were selected. Station I was located especially where it would be reached by wastewater from mineral processing facilities and domestic areas; and Station II, where it would be reached by residential and agricultural wastewater. Surface sediment samples were taken repeatedly in May and November 2020 using a sediment grabber. According to the study findings, a) Among the heavy metals studied (Hg, As, Cd, Cr, Pb, Ni, Cu, and Zn), Cu, As, and Cd were found to have the highest enrichment and contamination indices; b) The pollution load index (PLI) values (1.30-2.26) suggest heavy metal pollution in the sediment, and the geoaccumulation index (I_{geo}) values show intensive Pb contamination at both stations in both months; c) In terms of potential ecological risk index (Er^i), Cd was found to have a significant level of ecological risk index; d) The integrated risk index (PER) indicates that all heavy metals in the lake were present at moderate ecological risk levels. Ni and As were metals that had negative biological effects. The results indicate that a) Dredging is not a very effective tool for reducing pollution in the sediment; and b) As long as anthropogenic pollutants continue to enter the lake basin, sediment heavy metal levels should be routinely monitored, particularly those with ecological and biological effects on the sediment: Cu, Cd, Pb, Ni and As. It is predicted that the findings of this study will contribute to the sediment-focused monitoring efforts of organizations and local governments.

Keywords*:* Ecological risk assessment, ecological indices, sediment dredging, heavy metals, Mogan Lake

Öz: Rekreaktif öneme sahip ötrofik-sığ Mogan Gölü'nde, göl içi yönetim uygulamalarından biri olan sediment dredging (uzaklaştırımı) zaman zaman uygulanmaktadır. Bu çalışmanın amacı; sediment tarama sonrası, gölde sedimentin kirlenme durumunun bazı indekslerin -zenginleşme indeksi (EF), kontaminasyon/kirlilik indeksi (CF), kontaminasyon derecesi (C_d), kirlilik yük indeksi (PLI), jeoakümülasyon indeksi (I_{geo}), potansiyel ekolojik risk indeksi (Er^i), bütünleşik ekolojik risk indeksi (PER), ortalama olası etki konsantrasyonu oranı (mPEC-Q) - kullanımı ile değerlendirilmesidir. Bu amaçla gölde sediment tarama sonrası, litoral bölgede iki istasyon seçilmiştir. Bunlardan I. istasyon; özellikle maden işleme tesisleri ve evsel kaynaklı atık suların, II. istasyon; evsel atık ve tarımsal faaliyet kaynaklı atık suların ulaştığı bir konumda bulunmaktadır. Yüzey sediment örnekleri, 2020 yılının Mayıs ve Kasım aylarında sediment alma kepçesiyle tekerrürlü olarak alınmıştır. Araştırma bulgularına göre; a) Ele alınan ağır metaller (Hg, As, Cd, Cr, Pb, Ni, Cu ve Zn) içerisinde Cu, As, Cd en yüksek zenginleşme ve kontaminasyon faktörlerine sahip ağır metaller olarak saptanmıştır, b) Kirlilik yük indeksi (PLI) değerleri (1,30-2,26) sedimentin ağır metallerle kirlenmesini desteklemekte, jeoakümülasyon indeks (I_{geo}) değerleri ise, her iki istasyon ve ayda Pb bakımından yoğun kirlenme olduğuna işaret etmektedir, c) Potansiyel ekolojik risk faktörleri (Er^i) açısından, Cd önemli düzeyde potansiyel ekolojik riske sahip bulunmuştur, d) Bütünleşik ekolojik risk faktörleri (PER), gölde tüm ağır metaller bazında orta düzeyli bir ekolojik riski göstermektedir. Ni ve As ise biyolojik açıdan olumsuz etkileri olan metallerdir. Sonuçlar; a) Sediment tarama girişiminin, sedimentteki kirlenmenin indirgenmesi açısından çok etkin olmadığı, b) Göl havzasındaki antropojenik kirleticiler süregeldikçe, sedimentte ekolojik ve biyolojik anlamda önem arzeden başta Cu, Cd, Pb, Ni ve As olmak üzere ağır metal düzeylerinin rutin olarak izlenmesi gereği yönündedir. Bulguların, ilgili kurumların ve yerel yönetimin sediment odaklı izleme çalışmalarına katkı sağlayacağı öngörülmektedir.

Anahtar kelimeler*:* Ekolojik risk tayini, ekolojik indeksler, sediment tarama, ağır metaller, Mogan Gölü

INTRODUCTION

Sediments play an important role as an accumulative environment for heavy metals in fresh- and saltwater ecosystems. In the same way that they can directly affect the benthic ecosystem, sediment heavy metals such as cadmium, mercury, lead, copper, and zinc accumulate in the food chain and can reach levels that threaten human health. Various methods have been developed recently to determine the degree of contamination in the sediment, protect the health of aquatic ecosystems, and facilitate ecological risk assessment. Accordingly, various indices are widely used in sediment contamination studies in freshwater, estuarine, and saltwater ecosystems, such as the enrichment, contamination (pollution), and geoaccumulation indexes, to shed light on potential sources (anthropogenic and/or natural) of heavy metals and their accumulation in the sediment (Wang and Feng, 2007; Hu et al., 2013; Liu et al., 2014; Ghaleno et al., 2015); potential-integrated ecological risk index, to determine the risk that heavy metals pose to human health and the health of aquatic ecosystems or their ecological sensitivity to heavy metals (Guo et al., 2010; Liu et al., 2014); and the mean probable effect concentration quotient, to obtain information on the biological effects of these metals (Kükrer, 2016; Tunca, 2016).

Having recreational importance and located in the Gölbaşı Special Environmental Conservation Area, Mogan Lake is a shallow eutrophic lake. It is under pressure from anthropogenic pollutants due to agricultural activity, residential use, industrial and mineral processing facilities, and various other sectors operating in the lake basin. It has been reported that there are 29 andesite processing facilities in Gölbaşı (24 factories and four workshops) and that in a 2006 inspection of these facilities, many were found to be discharging effluents, especially the watery mud produced while cutting stone, directly into the lake's tributaries without purification, and these facilities were fined. To eliminate anthropogenic pressure on the lake and its negative effects, certain management strategies have continued for nearly 15 years. One of these strategies, sediment dredging, that is, removing sediment from the lake bottom, is a management strategy undertaken on aquatic ecosystems under the threat of eutrophication. The local government reports that dredging mud from the lake bottom started in 2017 and ended in November 2018, with a total of 3,100,000 m^3 of sediment removed.

There has been a limited amount of research undertaken in Mogan Lake before (Olgun and Kocaemre, 2011; Benzer et al., 2013; Topçu and Kaya, 2017) and during (Küçükosmanoğlu and Filazi, 2020) dredging. These investigations will sequence the average metal concentrations in Mogan Lake sediment, examine seasonal and temporal fluctuations, and compare them to sediment quality criteria and TEL/PEL values. Only one of the preceding investigations (Topçu and Kaya, 2017) and two index estimations are available. This is the first study on heavy metal contamination of sediment carried out after lake sediment dredging. In addition, no study on dredging, which is an important in-lake management practice in our country's lakes, was found in the literature review. As a result, the findings highlight the significance of the research topic, particularly at Mogan Lake.

This study aimed to determine the state of post-dredging sediment heavy metal contamination in Mogan Lake using indices such as the enrichment index, contamination/pollution index, degree of contamination, pollution load index, geoaccumulation index, potential ecological risk index, integrated ecological risk index, and mean probable effect concentration quotient. The data obtained from the study will be used to create a roadmap for the development of rational strategies for lake management and to prepare the scientific groundwork for sediment management.

MATERIAL AND METHODS

Study area

Mogan Lake, long under intense anthropogenic pressure, is one of Turkey's important Ramsar candidate wetlands. The alluvial terrace lake, located in the Lower Ankara Creek Basin, 20 km south of Ankara on the Ankara-Konya road, is fed mostly by precipitation and by the waters of more than five small and large rivers (Anonymous, 2016).

Fieldwork

Two stations suitable for obtaining sediment samples and representing the sources of pollution were selected in the littoral zone of Mogan Lake (Figure 1). Station I was established in an area receiving wastewater from mineral processing facilities and domestic wastewater, and Station II in an area reached by domestic waste and agricultural wastewater.

Surface (0-20 cm) sediment samples were taken repeatedly with a Ekman grab at both stations in May and November 2020. Sediment samples were delivered to the laboratory in dark nylon bags and a cold environment.

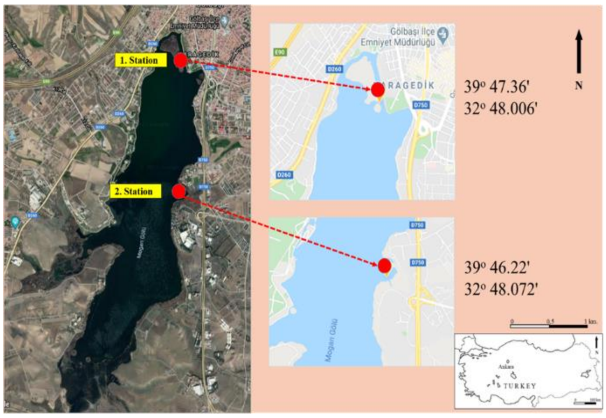

Figure 1. Research area and location of stations (Binici et al., 2021)

Laboratory work

Chemical analyses were performed at the Central Laboratory of Middle East Technical University. Experimental procedures were carried out according to the in-house laboratory methods based on the EPA 6020B method. Sediment samples were dried at 105˚C for 2 hours before digestion. Digestion procedures were carried out with an Anton Paar Multiwave 3000 microwave digestion system (Rotor type 8SXF100).

1. Approximately 0.3 g sample was weighed in a microwave digestion vessel.

2. 4 ml HNO_3, 2 ml HCl and 3 ml HF were added to the vessels, and sample and blanks were digested (Instrument parameters: Power 200 W, ramp 5 min, hold 5 min [step 1]; Power 1000 W, ramp 5 min, hold 15 min [step 2]).

3. Complexation was performed using 18 ml boric acid (Instrument parameters: Power 800 W, ramp 5 min, hold 15 min).

4. Sample/blanks were diluted to a final volume of 50 ml with de-ionized water.

Hg, As, Cd, Cr, Pb, Ni, Cu, and Zn levels were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Perkin Elmer NexION 350D). NIST SRM 8704 Buffalo River Sediment is used for quality assurance and control. The CAS numbers of the acids used during the chemical analyses are 7697-37-2, 7647-01-0, and 7664-39-3, for HNO_3, HCL, and HF, respectively.

The formulas and references for risk assessment of heavy metals and pollution levels per EF, CF, Er^i, PERI, PLI, and mPEC−Q are presented in Tables 1 and 2, respectively.

Table 1. Risk assessment of heavy metals

The contamination level of heavy metals	Symbols	References
Enrichment index $EF= C_i/C_x \div B_n/B_x$	C_i= Measured metal concentration C_x= Geochemical background values of measured (Fe or Al) B_n= Geochemical background values of measured metal* B_{ref}= Geochemical background values of metals (Fe or Al)	Sutherland (2000) Rudnick and Gao (2014) Goher et al. (2017)
Contamination index $CF= C_i/ C_n$ Pollution load index $PLI=(CF_1 x CF_2 x CF_n)^{1/n}$	Ci= Measured metal concentration Cn*= Geochemical background values of metals n= Number of measured metal	Rudnick and Gao (2014) Suresh et al. (2012)
Geo-accumulation index $Igeo = \log_2(C_n/1{,}5 \times B_n)$	C_n= Measured metal concentration B_n= Geochemical background values of the metal (n)	Ghaleno et al. (2015)
Toxic effects of metals	**Symbols**	**References**
Potential risk index $Er^i = T_r^i \times C_i/C_n$ Potential ecological risk index $PERI=\sum Er^i$	T_r^i: Response) index (Hg=40, Cd=30, As=10, Cu=Pb=Ni=5, Cr=2, Zn=1)	Guo et al. (2010) Hakanson (1980) Liu et al. (2014)
Combined biological effects of metals	**Symbols**	**References**
Mean probable effect concentration quotient $mPEC\text{-}Q= \sum_{i=1}^{n} (C_i/PEC_i)/n^{**}$ $TU= C_i/PEC_i$	C_i= Measured metal concentration PEC = Mean probable effect concentration n=Total metal number TU= Toxic unit	Long et al. (2006) Yang et al. (2014)

* Background concentration of the metals (µg/g): Hg:0,05; Cr: 92; Ni:47; Cu:28; Zn:67; As:4,8; Cd:0,09; Pb: 17 (Rudnick and Gao, 2014)
** PEC_i values: Hg:0,486; Cr:90; Ni:36; Cu:197; Zn:315; As:17; Cd:3,53; Pb:91,3 (MacDonald et al., 2000)

Table 2. Pollution levels as per EF, CF, Er^i, PERI, PLI, and mPEC−Q value

EF value	Ecological risk	CF value	Ecological risk	I_{geo} classes	Ecological risk
EF<2	Depletion to mineral	CF<1	Low	0	Unpolluted
2≤EF<5	Moderate	1≤CF≤3	Moderated	1	Unpolluted moderated
5≤EF<20	Significant	3<CF≤6	Considerable	2	Moderated polluted
20≤EF<40	Very high	CF>6	Very high	3	Moderated to high polluted
EF≥40	Extremely high			4	Highly polluted
				5	Highly to extremely polluted
				>5	Extremely polluted
Er^i classes	**Er^i value**	**Ecological risk**	**PERI classes**	**PERI value**	**Ecological risk**
1	Er^i <40	Low	1	PERI <150	Low
2	40<Er^i <80	Moderate	2	150< PERI <300	Moderate
3	80<Er^i <160	Appreciable	3	300< PERI <600	Considerable
4	160<Er^i <320	High	4	600>PERI	Very high
5	320>Er^i	Serious			
PLI value	**Ecological risk**		**mPEC−Q value**		**Ecological risk**
PLI>1	Polluted		mPEC−Q <0.1		Low priority
PLI<1	Non-polluted		0.1< mPEC−Q<1		Low-medium priority
			mPEC−Q >0.1		High priority

RESULTS

Enrichment index (EF)

Enrichment index (EF) values for the metals measured in the sediment of Mogan Lake were determined by month and station: for Station I, Cu>Cd>As>Pb>Zn>Ni>Cr>Hg, in descending order. While Cu levels at Station I were highest in November, indicating a near moderate enrichment level, minimal enrichment was seen for Hg at the same station, also in November (Figure 2). Enrichment indexes for the metals at Station II were found to be Pb>Hg>As>Cd>Cu>Cr>Zn>Ni, in descending order. While Pb and Hg showed moderate enrichment levels in November, Hg showed minimal enrichment in May (Figure 2). Taking general averages into account, enrichment index values were: Cu>Cd>As>Pb>Hg>Cr>Zn>Ni.

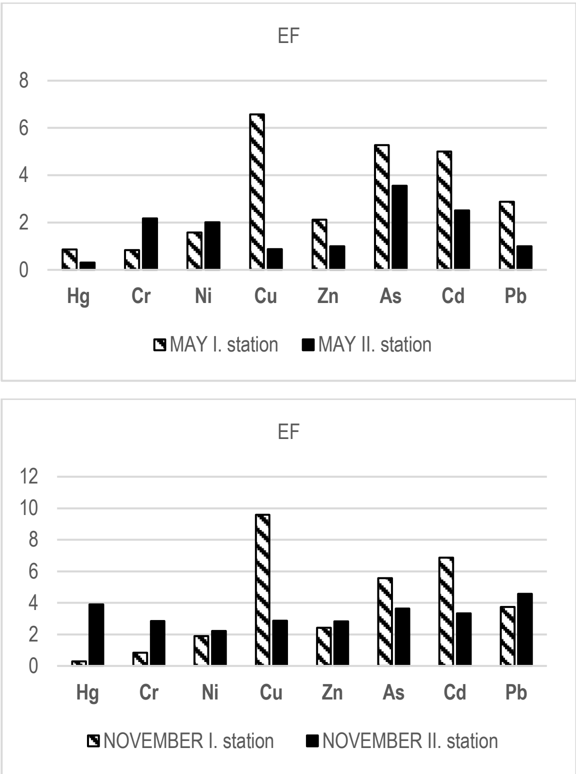

Figure 2. Sediment enrichment indexes at the station I and II in May and November

Contamination (pollution) index (CF)

Sediment contamination index values in Mogan Lake, without considering month and station, were arrayed as Cu>As>Cd>Pb>Hg>Cr>Zn>Ni. High levels of As contamination was found at both stations; for Cd, high contamination levels were detected at Station I. and moderate levels at Station II.; for Pb and Zn, moderate levels were detected at Station I and low levels at Station II; for Cr, moderate levels were detected only at Station II; for Ni, moderate levels were detected at both stations; and for Hg, low levels were detected at both stations (Figure 3).

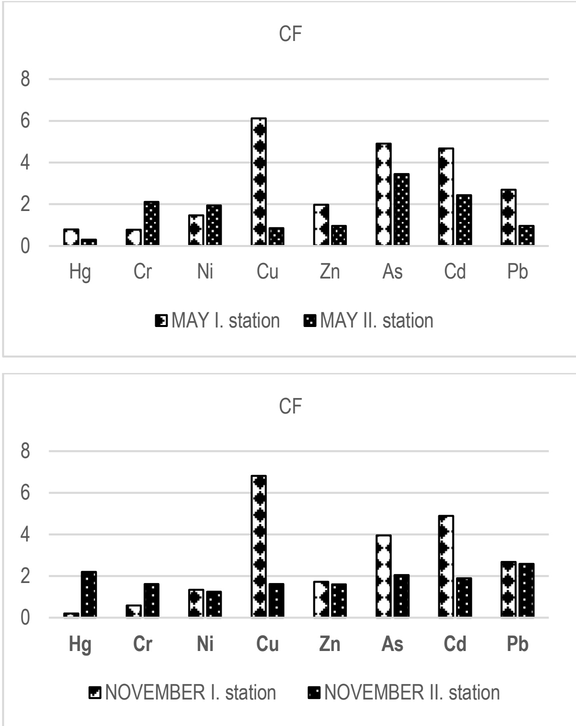

Figure 3. Sediment contamination indexes at the station I and II in May and November

Degree of contamination (pollution) (C_d) and pollution load index (PLI)

An extremely high degree of contamination (C_d) was determined in May at Station I, at 23.42 13.04 mg kg^{-1} dry w and a moderate degree at Station II, at 13.04 mg kg^{-1} dry w. Similarly, in November, while C_d at Station I was found to be extremely high at 22.2 mg kg^{-1} dry w, this value was found to be moderate at Station II, at 14.8 mg kg^{-1} dry w. In light of this data, the C_d levels determined in both months at Station I proved to be extremely high.

PLI values for May were calculated as 2.26 at Station I and 1.30 at Station II, while the values for November were 1.77 at Station I and 1.81 at Station II. Ghaleno et al. (2015) reported that, PLI values of <1 indicate the absence of contamination, and that values greater than 1 indicate advanced pollution. The PLI values of >1 recorded in Mogan Lake point to heavy metal contamination in the sediment.

Geoaccumulation index (I_{geo})

Evaluating the geoaccumulation index by station, values for Station I were recorded as Pb>Cu>As>Cd>Zn>Ni>Hg>Cr, and for Station II, as Pb>As>Cd>Hg>Cr>Ni>Cu>Zn, in decreasing order. In both months at both stations, Pb was determined to have high contamination levels, while Cu had moderate levels in May and higher than moderate levels in November at Station I; and As values for May at both stations and Cd values for the same month at Station I had moderate levels of contamination. The other metals were not detected at levels showing contamination (Figure 4).

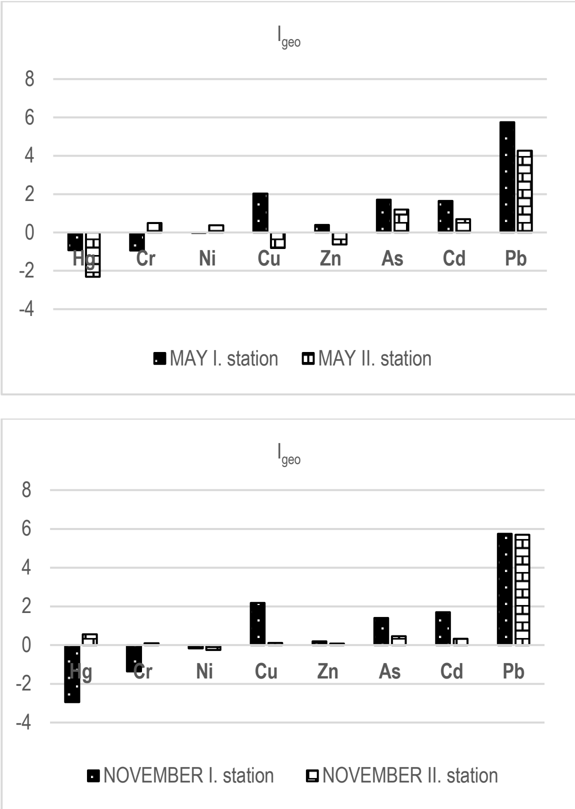

Figure 4. Sediment geoaccumulation indexes at stations I and II in May and November

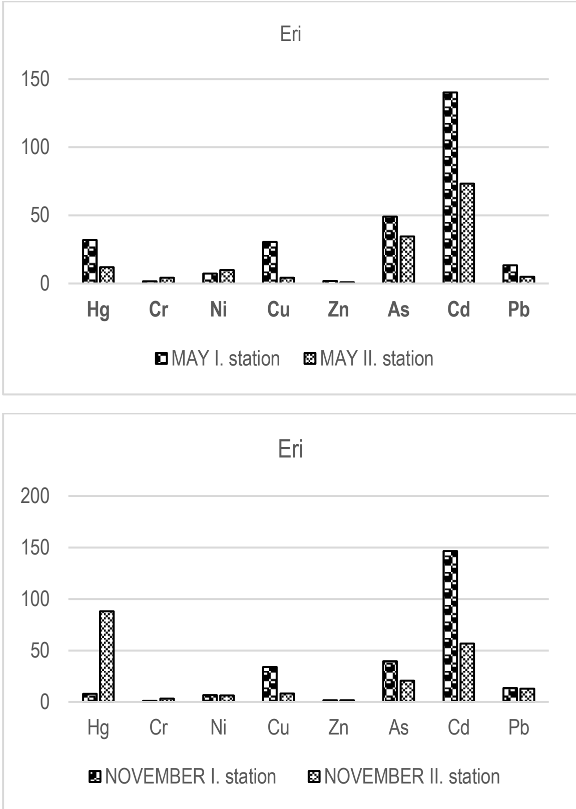

Figure 5. Sediment potential ecological risk indexes at the station I and II in May and November

Findings on the toxic effects of metals

Potential ecological risk index (Er^i)

Arranged according to the calculated ecological risk indices, the metals at Station I are as follows: Cd>As>Cu>Hg>Pb>Ni>Zn>Cr. While Cd had a significant potential ecological risk in both months, As had a moderate ecological risk in May at the same station (Figure 5).

Integrated ecological risk index (PER)

The highest value for the integrated ecological risk index (PER) for the metals was recorded in May at Station I (276.14 mg kg^{-1}), in the moderate ecological risk category. Likewise, in November, as PER values at both stations were at 150≤PER<300, a moderate ecological risk was determined.

Findings on the combined biological effects of metals

Toxic unit (TU)

From a standpoint of toxic units, Ni and As values in May at Station I were higher than those for November, with low values for the other metals. At Station II, Ni and Cr had high values, with those in May higher than those seen in November.

Mean probable effect concentration quotient (mPEC-Q)

The highest and lowest ∑TU values were reported at Station II, in May and November, respectively. The mean probable effect concentration ratio values of lake sediment are determined for May and November respectively; as 0,76-0,68 for me and as 0,79-0,65 for II. There is a high priority for this index.

DISCUSSION

Within the scope of sediment pollution studies, as in many places in the world, many studies have been undertaken in different parts of Turkey using ecological indices in lakes with extremely different hydrographic and nutrient levels.

In a study on Kalimanci Lake in Macedonia, Vrhovnik et al. (2013) found surface sediment enrichment index values of between 0.12-590.3 and reported significant Cd, Pb, Zn, and As sediment contamination. In the study in Mogan Lake, sediment enrichment index values varied between 0.21-6.98; and especially Cu sediment values showed significant enrichment in May and November at Station I. At the same station in Mogan Lake, Zn and Pb were at moderate enrichment levels; and in fact, it is known that wastewater from mineral processing plants reaches this station.

In Veeranam Lake (India), Suresh et al. (2012) reported average PLI values of 2.03 (1.18-4.09) and stated that the lake sediment was significantly contaminated. Likewise, in this study, the lake sediment was found to be contaminated by heavy metals and taking the average PER value of 238.79 (196.86-266.31) into account, it was shown that the lake is facing a moderate ecological risk. The finding in Mogan Lake that sediment Hg and Cd posed a significant potential ecological risk in some months and at some stations is in concordance with the finding at Veeranam Lake that sediment Cd from metals processing plants, agriculture, and residential areas poses a high ecological risk. Similarly, results from studies in China at Dalinouer Lake (Hou et al., 2013), East Lake (Liu et al., 2014), and 35 other surface sediment studies (Yang et al., 2014) show that Cd was the heavy metal posing the highest potential risk, are in parallel with the findings of the study conducted in Mogan Lake.

According to Cheng et al. (2015), mercury is the heavy metal with the greatest ecological hazard in the surface sediments of 16 Chinese lakes, followed by Cd, As, Pb, Cu, Ni, Cr, and Zn. Heavy metals were sequenced as Cd>As>Cu>Pb>Zn in a study conducted by Singh and Upadhyay (2012) in Lake Ramgarh (Upper India), and the average potential ecological risk factor was calculated to be 76.53 (moderate contamination). According to Yin et al. (2011), the potential ecological risk index in the surface sediment of Lake Taihu (China) is moderately polluted, with Cd being the greatest and Hg being the second ecological risk factor. Yuan et al. (2014) discovered that Cd is the heavy metal in the most significant risk group in the sediment of Lake Dianchi (China), a hypereutrophic plateau lake, and that Cr has a very low potential ecological risk index, which is consistent with the data from Lake Mogan. Given the endurance of pollutant inflows from the Lake Mogan basin, long-term monitoring of cadmium in lake sediment is unavoidable for the preservation and maintenance of sediment quality, in this context.

Mamat et al. (2016) reported that Cd, Hg, and Pb were determined to be at low/partially severe contamination levels, while Zn, As, Cr, Ni, and Cu were determined to be at uncontaminated/low contamination levels, based on the enrichment factor and geoaccumulation index value in the Bosten Lake surface sediment located in the arid region of Northwest China. The results partly corroborate the previous observation that Cu, As, and Cd are the most abundant enrichment factors in Lake Mogan sediment. Waheshi et al. (2017) reported that Pb and Cu are not contaminated in Lake Edku sediment according to their I_{geo} values, and the I_{geo} value for Pb was found to be maximum in Lake Mogan. Guo et al. (2018) revealed that 18 lake surface sediments in the Tibetan plateau exhibit moderate contamination in terms of Cd and As, according to the Er^i and I_{geo} indices of the sediment. While the Lake Mogan data partially overlapped with the values of Guo et al. (2018), they were determined to be consistent with As showing moderate-high contamination in terms of both indices. It has been observed that the use of pesticides and chemical fertilizers in agricultural activities causes As contamination in the surface sediment of Lake Dali (North China) (Xu et al., 2019). Given the contamination factors (2.05-3.45) of Mogan Lake Station II, it appears that agricultural wastes could reach this station and contaminate it.

Fan et al. (2019), in a study conducted on Shitang Lake (China), first defined sediment dredging areas using the geoaccumulation index (I_{geo}) and the potential risk index (RI). According to the geoaccumulation index values calculated in the study, it was determined that the sediment was more than moderately contaminated, at Station I in May and November by Cu, and at Station II in November by As. Accordingly, although it may be possible to say that suitable months and locations exist for the aforementioned heavy metals in terms of sediment dredging activities in Mogan Lake since the resultant area would be quite small, this approach, unfortunately, does not appear to be suitable for use in the lake.

According to Imran et al. (2020), elevated I_{geo} and EF values discovered for As, Fe, and Cd in the surface sediment of Lake Keenjhar (Pakistan) are produced by human activity in the lake's surroundings. According to Zhang et al. (2013), the geoaccumulation index (I_{geo}) of six heavy metals in Lake Yangzonghai (China) sediment is Cu>As>Cr>Zn>Mn>Pb in general, with copper reaching a value of 2.42. In the foreground, Pb and Cu were determined in terms of I_{geo} values on the overall average basis of the Lake Mogan sediment, with the maximum I_{geo} value for copper reaching 2.18 at Station I in November. According to Zhang et al. (2013) concluded that potential sources of Pb, Zn, As, and Cr in the sediment are created by human activities such as industry, mining, and tourism, which are also effective contributors in sediment contamination in Lake Mogan.

The finding that average bed sediment Cu, Zn, Ni, Mn, and As values in Kovada Lake were reported to be greater than the shale value, means that values for these elements in Kovada Lake indicated low or moderate enrichment (Şener and Şener, 2015), in parallel to the enrichment index values for the heavy metals other than Cr and Cd in the sediment of Mogan Lake. While Kükrer et al. (2015), reported the potential risk index of two metals, Cd and Hg, in the sediment of Çıldır Lake (Ardahan), in this study, Cd in the sediment of Mogan Lake was also shown to pose a significant potential ecological risk.

While sediment samples from Seyfe Lake, whose sole anthropogenic source is an agricultural activity, were classified according to the geoaccumulation index as moderately polluted in regards to As (Bölükbaşı and Salman Akın, 2016), sediment As in Mogan Lake reached its maximum value (2.64) in November at Station II, indicating contamination above moderate levels.

Similarly, while all sediment geoaccumulation index data in Mogan Lake, except for Cu values for May and November at Station I and As values for November at Station II, indicate a contaminated or moderately contaminated state, in Uluabat Lake (Bursa), according to the geoaccumulation index (I_{geo}), the sediment is moderately contaminated (Hacısalihoğlu and Karaer, 2016).

In a study on Tortum Lake conducted by Kükrer (2016), low-moderate level contamination of the surface sediments was reported, and the highest EF values were found for Cd as a result of the use of fossil fuels in the region. As for PLI and PER values, the lake's low ecological risk due to heavy metals and the mPEC-Q values at 15-29% point to its being a primarily low-moderate area. In Mogan Lake, as the highest sediment EF value was for Cu, and PER values were between 196.86-266.31, this indicates that the heavy metals tested for had a moderate ecological risk; mPEC-Q values showed that the sediment might have been partially impacted by the combined biological effects of the metals.

In Beyşehir Lake (Konya), it has been put forth that the potential toxic effect of sediment As on lifeforms is at a serious level (Tunca, 2016). In Mogan Lake, Ni and As was reported to be the most significant heavy metals in terms of their negative biological impact.

According to the above-mentioned research conducted in various geographical regions of Turkey, heavy metal concentrations in sediment are unavoidably altered by natural or anthropogenic sources. Furthermore, it should be noted that no dredging activity has been carried out for improvement in any of these lakes.

Principal component analysis was used to characterize the types and contributions of heavy metals in the lake's pollutant sources within the scope of a study conducted by Binici et al. (2021) in Mogan Lake after dredging. The researchers observed that the variances in heavy metals originating from diverse anthropogenic sources reaching the lake also indicate the diversity and pressure of environmental pressure for heavy metal accumulation in the lake as a result of principal component analysis. Cu (0.826), As (0.962), and Cd (0.933) in the first basic component, which explains 40.063% of the total variance, in the first station where domestic wastewaters are discharged from the mine processing facilities in the two periods of sampling were quite high compared to the second station in the aforementioned study.

The maximum enrichment index (EF) for all three metals was established at the first station in this study, as were the contamination index values (CF) at the first and second stations. In this regard, both mineral processing-domestic wastewater and agricultural activities might be considered variables in the enrichment and contamination of the sediment with these metals. In the same study, Hg (0.936), Zn (0.831), and Pb (0.900) were shown to have higher positive charges than other heavy metals in the second basic component, which explains 31.625% of the total variation. The high potential ecological risk index (Er^i) value for Hg in November was determined in the present study, particularly at the second station, indicating that the mercury originates from domestic and agricultural wastewaters. Among the Cr (0.779) and Ni (0.935) in the third basic component, which explains 24,452 % variance, Cr was found to be higher in the second station, where domestic wastewater and wastewater from agricultural activities were reached, compared to the first station, in both months of sampling. When the toxic unit (TU) values for both metals are considered, domestic-agricultural wastes have a significant effect on the toxicity of the sediment. Although the interpretations of the indices differ, it is obvious that the heavy metal concentrations in the sediment (Hg, As, Cd, Cr, Pb, Ni, Cu, and Zn) are negatively affected by both point and non-point pollutant sources reaching the lake.

Although the location of the stations was different, within the scope of a study conducted on Mogan Lake before dredging (Topçu and Kaya, 2017), geoaccumulation index I_{geo} values lower than zero indicated extremely low pollution levels. Similarly, enrichment index values generally of <1 indicate a low pollution level. A stark increase can be seen between pre-dredging index values and those recorded in this study. When considering pre-dredging ecological index values, it does not seem possible to evaluate sediment removal as an effective strategy in Mogan Lake.

CONCLUSION

In light of the findings, suggestions regarding Mogan Lake are presented below:

-Sediments such as those in the example of Mogan Lake are important due to the accumulation of heavy metals in the sediment biota and the role they play in the transformation of metals. Heavy metal levels should routinely be monitored, especially for the lake's sustainability and due to the continuation of anthropogenic pollutants.

-Dredging depth appears to be the most important index in laying a foundation for the more effective utility and applicability of sediment dredging activity in the lake. If sediment removal is to be practiced from time to time in the lake, its cost, environmental effects, the process, and other indexes should be examined; the dredging areas should first be prioritized, and other topics such as the dredging technique should be considered. Moreover, monthly or more frequent surface sediment sampling is first of all important to determine dredging areas to maintain the lake's sustainability. Accordingly, it is thought that the findings of existing studies, at least as preliminary scientific data, will shed light on the way to determine dredging areas.

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AUTHOR CONTRIBUTIONS

All authors contributed equally.

CONFLICTS OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS APPROVAL

There are no ethical issues with the publication of this manuscript

DATA AVAILABILITY

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Effects of different thawing methods on fatty acid composition of cultured sea bass (*Dicentrarchus labrax* Linnaeus, 1758)

Farklı çözündürme yöntemlerinin kültür levreği (*Dicentrarchus labrax* Linnaeus, 1758)'nin yağ asidi kompozisyonu üzerine etkisi

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Abstract: This study aimed to determine the potential changes in the fatty acid composition of frozen cultured sea bass (*Dicentrarchus labrax*) thawed at different environmental conditions. Sea bass fillets were thawed using four different methodologies: refrigerator (+4°C), water (+15°C), microwave (defrost mode) and ambient conditions (22±2°C). Some part of the fish was thawed once (on the 7th and 30th days), and the other part was thawed twice (on the 30th day). Thus, crude lipid analysis and fatty acid composition by gas chromatography were carried out in the thawed sea bass fillets. The results showed negative effects on the fatty acid composition caused by the different thawing methods. The most suitable thawing method was determined as refrigerator thawing, especially on the 30th day 1st thawing according to least loss of the lipid values (9.19±0.18%) and unsaturated fatty acids (C18:2 25.92±0.13%, C20:5 5.56±0.02%, C22:6n-3 8.90±0.09%, ∑PUFA 44.70±0.04%). Samples thawed in water and ambient conditions follow the refrigerator thawing method in terms of lipid and fatty acids. The highest lipid and fatty acid loss was observed in microwave thawing. Although the samples thawed in the refrigerator were better than the other groups in terms of lipid content and fatty acid composition, it is recommended to the consumers that frozen foods should be thawed only once and consumed immediately, and that fish should be frozen according to their needs and thaw as much as they can consume.

Keywords*:* Sea bass, *Dicentrarchus labrax*, freezing, thawing, fatty acid composition

Öz: Bu çalışmada, dondurma ve farklı ortam şartlarında çözündürme işlemi uygulanan kültür levrek balığının (*Dicentrarchus labrax* Linnaeus, 1758) yağ asidi kompozisyonunda meydana gelen değişimlerin tespiti amaçlanmıştır. Levrek balığı filetoları, buzdolabı (+4°C), su (+15°C), mikrodalga (buz çözme modu) ve ortam şartları (22±2°C)'nda olmak üzere dört farklı ortamda çözündürülmüştür. Balıkların bir kısmı bir (7. ve 30. günde), diğer kısmı ise iki kez (30. günde) çözündürülmüştür. Çözündürülen levrek balığı filetolarında ham yağ ve yağ asitleri kompozisyonu analizleri gerçekleştirilmiştir. Tüm grupların yağ asidi kompozisyonu gaz kromatografi cihazı kullanılarak belirlenmiştir. Elde edilen bulgular farklı ortamlarda birden fazla çözündürmenin yağ asitleri kompozisyonu üzerinde olumsuz etkilere sebep olduğunu ortaya koymuştur. Uygulanan çözündürme tekniklerinden en uygun çözündürme ortamının özellikle 30. gün ilk çözündürme günü ele alındığında yağ değerleri (%9,19±0,18) ve doymamış yağ asitleri sonuçlarında (C18:2 %25,92±0,13, C20:5 %5,56±0,02, C22:6n-3 %8,90±0,09, ∑PUFA %44,70±0,04) en az kayıp görülmesinden dolayı buzdolabında çözündürme yöntemi olduğu sonucuna varılmıştır. Suda ve ortam koşullarında çözündürülen örnekler yağ ve yağ asitleri sonuçları bakımından buzdolabında çözündürme yöntemini takip etmektedir. Yağ ve yağ asidi miktarında en fazla kayıp mikrodalga çözündürme yönteminde gözlenmiştir. Buzdolabında çözündürülen örnekler her ne kadar yağ miktarı ve yağ asitleri kompozisyonu açısından diğer gruplara göre daha iyi olsalar bile tüketicilere dondurulmuş gıdaların sadece bir kere çözündürülerek hemen tüketilmeleri gerektiği ve ihtiyaçları oranında balıkları dondurup, yiyebilecekleri kadar miktarları çözündürmeleri önerilmektedir.

Anahtar kelimeler*:* Levrek, *Dicentrarchus labrax*, dondurma, çözündürme, yağ asidi kompozisyonu

INTRODUCTION

Seafood is an increasingly important food both in the world and in Turkey. One of the most important factors that determine the quality of food is its digestibility and utilization of its proteins, lipids, carbohydrates, vitamins and minerals by our body. Fish are important food sources with these characteristics. Fish meat is a recommended meal by all health and nutrition experts (Metli, 2006; Murray and Burt, 2011; Chen et al., 2022). Fish have functional effects due to their richness in polyunsaturated fatty acids. As a result of several researches, it has been concluded that dietary supplements containing fish are beneficial in the treatment of many diseases such as cardiovascular diseases, ulcerative colitis and hyperlipidemia

(Chen et al., 2022). The consumption amount of fresh and processed seafood in Turkey is quite low, especially compared to European countries. However, animal protein intake is insufficient in our country. Fish meat and other seafood are important for human nutrition due to their contents of both high-quality protein and especially long-chain unsaturated fatty acids (Gülyavuz and Ünlüsayın, 1999; Anonymous, 2019). In recent years, the Republic of Turkey Ministry of Agriculture and Forestry has been taking steps to increase aquaculture production and export. The main products of our aquaculture exports are sea bass, seabream, rainbow trout and tuna. A large part of our export consists of fresh and chilled fish (Anonymous, 2014).

Freezing is one of the most preferred methods to store fish and other seafood. Although low temperatures protect the food shelf life and quality, incorrect applications can cause negative effects on the quality. If the necessary conditions are not followed, quality losses may occur in fish tissue due to physical, chemical and enzymatic changes, which adversely affect the consumer's preferences (Abraha et al., 2018). The factors mostly causing these quality losses can be storage temperature, freezing and thawing times, temperature changes, incorrect freezing and thawing processes. Various studies demonstrated that multiple freezing and thawing processes lead to physiological and biochemical damage in muscle systems, such as nutrient loss, lipid oxidation, protein denaturation, and hydrolysis (Wu et al., 2021). Food preservation methods aiming to offer fish to consumers under healthier and hygienic conditions can protect the products. Although this, quality losses especially in terms of physical and sensory characteristics may occur in these products due to incorrect applications of the consumers. Bozkır et al. (2014) stated that thawing foods using traditional methods may have some negative effects such as microorganisms development or oxidation (and accordingly color changes). Although the negative effects of traditional methods, consumers use these thawing methods at home in order to save time and consume the products quickly (Konak et al., 2009).

The fact that unconscious storage processes in small businesses and fish shops have a negative effect on the quality of fish is one of the most important reasons why consumers avoid to buy fish. This study aimed to determine the effects of incorrect/unconscious freezing and different thawing processes on the fatty acid composition of sea bass, a species highly consumed in our country and in the world and of very high economic value. Thus, the study evaluated the changes in the amount of lipid and fatty acid composition occurring during the thawing of the fish in different conditions (refrigerator, ambient conditions, water and microwave) after the storage process in the -18°C freezer, which is frequently used in homes and plants.

MATERIAL AND METHODS

Study area and stations

Twenty kg of sea bass (50 fish) with an average weight of 350±20 g and lenght of 30±3 cm, farmed in offshore cage systems in Milas, Bodrum (Turkey), were used for the experiments. Samples were taken from the fish farms in Milas, Bodrum, and brought to Muğla Sıtkı Koçman University, Faculty of Fisheries, Quality Control Laboratory within 1 hour in cold chain conditions with styrofoam boxes in ice.

Methods

The sea bass samples were divided into groups of 5 and stored in refrigerator bags at -18°C. Lipid of fish was extracted before the fresh fish were taken into storage, and methylation was performed to determine the initial fatty acid composition. After 7 days of storage, fish were thawed (7th day 1st thaw) in four different conditions (refrigerator (4±1°C), ambient conditions (22±2°C), water (15±2°C) and microwave (defrost mode). Lipid and fatty acid analyses were applied to some of the thawed fish, and the remaining sea bass were re-frozen. After 30 days of storage, this re-frozen fish group (30th day 2nd thaw) and the fish group that stored for a month (30th day 1st thaw) were thawed with these four different thawing methods. After the 30th day thawing processes, lipid was extracted and the fatty acid composition was determined using gas chromatography for the groups of 30th day thawing.

Crude lipid analysis

The samples were analyzed in triplicate for lipid content following the Bligh and Dyer (1959)'s method. A mixture of methanol and chloroform (1:2, v/v, 100 ml) was added to 5 g of the fish sample (fish/solvent, 1:20, w/v) and homogenized. Then, 20 ml of 0.4% $CaCl_2$ solution was added onto the samples. The samples were filtered on a filter paper into the tared balloons kept in the oven at 105ºC for 2 h. These balloons were kept in a dark environment overnight and the upper layer consisting of methanol+water was separated with the help of a separation funnel. Chloroform from the chloroform+lipid part in the solution remaining in the flask was evaporated using a rotary evaporator (Heidolph) with the help of a water bath set at 60ºC. At the end of the method, balloons were kept in the oven at 60ºC in order to remove the remaining solvent, then they were kept in a desiccator for 30 min and their final weighings were taken after cooling as following:

Yield of crude lipid = [(Final weight - Initial weight) / Sample weight] x 100

Fatty acids analysis

The methyl esters of lipid from the samples were prepared by transmethylation using gas chromatography-flame ionizing detector (GC-FID) according to the method described by Ichihara et al. (1996). 25 mg of extracted oil was dissolved in 2 ml isooctane, followed by addition of 4 ml of 2 M KOH (in methanol). Then, the tube was vortexed for 2 min at room temperature. Separation into methyl esters was performed in triplicate for each sample. After centrifugation at 4000 rpm for 10 min, the isooctane layer was taken for gas chromatography (GC) analysis.

Gas chromatography (GC) conditions

The fatty acid methyl esters were analyzed using gas chromatography of Agilent Technologies model 7820 equipped with a flame ionization detector (FID) and fitted with an HP-88 capillary column (60 m × 0.25 mm × 0.25 µm thickness). Helium was used as the carrier gas at a constant pressure of 16 psi. Injection port was maintained at 220ºC, and the sample was injected in split mode with a split ratio of 50:1. During the analysis, detector temperature was 280ºC. Column temperature was started at 175ºC, and then programmed at 3ºC/min to 220ºC, ramped at 1°C/min to 220ºC, and held for 10 min. The total running time was 26 min. Helium was used as the make up gas at a constant flow of 40 ml/min, and hydrogen and dry air were used as detector gases. Identification of fatty acids was carried out by comparing sample fatty acids methyl esters (FAME) peak relative retention times with those obtained for Supelco standards (Supelco 37 Compounds FAME mix 10 mg/l in $CH_2 Cl_2$–47,885 U, Supelco 1819–1 Ampule FAME mix C4-C24). The results were expressed as percentage of total fatty acid methyl esters (ISO, 1990).

Statistical analysis

All experiments were carried out in triplicate and the results were reported as mean and standard deviation of measurements. Statistics on a completely randomized design were performed with the analysis of variance (ANOVA) using SPSS (Version 21, SPSS Inc., Chicago, IL, USA) software. Tukey's multiple range test (p<0.05) was used to detect differences among mean values of all test intervals.

RESULTS

Crude Lipid Analysis Results

The % lipid analysis results of the fresh material, multiple freezed/thawed and only once freezed/thawed sea bass were reported in Table 1. While the lipid content was 10.29% in fresh sea bass (day 0), a decrease was observed in the lipid content of fish thawed more than once in different mediums (p<0.05). In the refrigerator, a decrease was observed on the 7th day 1st thawing and on the 30th day 2nd thawing with 9.19% and 8.67%, respectively. Also in other thawing mediums, decreases were observed on the 30th day 2nd thawing compared to the 7th day 1st thawing. The highest lipid loss was observed in the microwave thawing method on the 30th day 1st thawing.

Table 1. Lipid analysis results of sea bass thawed in different conditions (%)

	Thawing Medium			
Thawing Time	**Refrigerator**	**Microwave**	**Water**	**Ambient Conditions**
Day 0	10.29 ± 0.85^{aA}	10.29 ± 0.85^{aA}	10.29 ± 0.85^{aA}	10.29 ± 0.85^{aA}
Day 7 (1st Thawing)	9.19 ± 0.08^{aB}	8.18 ± 0.05^{bBC}	9.53 ± 0.44^{aB}	9.48 ± 0.36^{aB}
Day 30 (2nd Thawing)	8.67 ± 0.07^{aC}	8.87 ± 0.48^{aB}	8.38 ± 0.01^{bC}	8.23 ± 0.05^{bC}
Day 30 (1st Thawing)	9.19 ± 0.18^{bB}	7.46 ± 0.57^{cC}	8.87 ± 0.34^{bC}	8.33 ± 0.09^{bC}

Data are expressed as the mean±SD. Lowercase letters indicate the statistical difference between the thawing methods, and uppercase letters indicate the statistical difference between the thawing times of the groups (p<0.05).

According to the results of fatty acid composition, the total saturated fatty acid (∑SFA) of fresh samples was 22.38%, hovewer it decreased to 12.96% in the water thawing method on the 7th day 1st thawing (p<0.05). With regard to the total monounsaturated fatty acid (∑MUFA), the value was 30.07% in fresh sea bass, however the highest increase (33.28%) occurred in water thawing samples. Total polyunsaturated (∑PUFA) was 40.34% in fresh sea bass, but it increased to 44.67% in water thawing. Eicosapentaenoic acid (EPA) (C20:5n-3) and docosahexaenoic acid (DHA) (C22:6n-3) were detected in the range of 4.64±0.03-5.39±0.04% and 7.53±0.23-8.71±0.07%, respectively.

No statistically significant difference emerged between ambient conditions and water thawed groups with fresh material in terms of EPA and DHA (p>0.05). The n-3/n-6 ratio was 0.65 in fresh sea bass, 0.60 in refrigerator, 0.59 in microwave, 0.62 in ambient conditions and 0.61 in water thawed samples (p>0.05) (Table 2.)

Table 2. Fatty acid analysis results of sea bass thawed for the 1st time on the 7th day in different conditions (%)

		Day 7 (1st Thawing)			
	Fresh	**Refrigerator**	**Microwave**	**Ambient Conditions**	**Water**
C12:0	0.03±0.00^{a}	0.02±0.00^{a}	0.02±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}
C13:0	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}
C14:0	2.28±0.03^{a}	2.23±0.05^{a}	2.14±0.01^{a}	2.41±0.04^{a}	2.37±0.02^{a}
C15:0	0.28±0.00^{a}	0.26±0.00^{a}	0.28±0.00^{a}	0.28±0.00^{a}	0.27±0.00^{a}
C16:0	15.14±0.09^{a}	12.60±0.16^{b}	14.98±0.01^{a}	10.15±0.05^{c}	7.88±0.88^{d}
C17:0	0.25±0.01^{a}	0.21±0.05^{a}	0.29±0.00^{a}	0.20±0.00^{a}	0.15±0.01^{a}
C18:0	4.10±0.03^{a}	3.01±0.60^{b}	4.19±0.02^{a}	2.72±0.03^{b}	1.89±0.26^{c}
C22:0	0.12±0.00^{a}	0.13±0.00^{a}	0.12±0.00^{a}	0.13±0.00^{a}	0.13±0.00^{a}
C24:0	0.18±0.02^{a}	0.26±0.03^{a}	0.22±0.00^{a}	0.22±0.01^{a}	0.23±0.00^{a}
∑SFA	**22.38±0.03^{a}**	**18.73±0.19^{b}**	**22.25±0.01^{a}**	**16.17±0.02^{c}**	**12.96±0.29^{d}**
C14:1	0.09±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.03±0.00^{a}	0.04±0.00^{a}
C16:1	3.26±0.03^{a}	3.36±0.11^{a}	3.13±0.02^{a}	3.52±0.02^{a}	3.61±0.07^{a}
C17:1	0.44±0.01^{a}	0.45±0.01^{a}	0.43±0.00^{a}	0.48±0.01^{a}	0.49±0.01^{a}
C18:1n-9c	22.54±0.12^{c}	24.11±0.08^{b}	22.70±0.16^{c}	23.97±0.11^{b}	25.21±0.35^{a}
C18:1n-9t	0.17±0.00^{a}	0.18±0.00^{a}	0.17±0.01^{a}	0.18±0.00^{a}	0.19±0.00^{a}
C20:1n-9	2.76±0.00^{a}	2.94±0.10^{a}	2.76±0.00^{a}	3.08±0.00^{a}	3.14±0.06^{a}
C22:1n-9	0.81±0.01^{a}	0.55±0.01^{a}	0.52±0.01^{a}	0.54±0.01^{a}	0.60±0.00^{a}
∑MUFA	**30.07±0.04^{c}**	**31.62±0.05^{b}**	**29.73±0.06^{c}**	**31.81±0.04^{b}**	**33.28±0.13^{a}**
C18:2n-6t	0.82±0.00^{a}	0.07±0.01^{a}	0.09±0.00^{a}	0.07±0.00^{a}	0.06±0.00^{a}
C18:2n-6c	22.43±0.12^{e}	24.68±0.19^{c}	23.68±0.14^{d}	25.65±0.12^{b}	26.44±0.37^{a}
C18:3n-6	0.27±0.02^{a}	0.41±0.07^{a}	0.34±0.01^{a}	0.20±0.02^{a}	0.19±0.00^{a}
C18:3n-3	1.42±0.01^{a}	1.61±0.06^{a}	1.52±0.01^{a}	1.56±0.01^{a}	1.64±0.02^{a}
C20:2	0.95±0.02^{a}	1.05±0.04^{a}	0.99±0.00^{a}	1.00±0.01^{a}	1.03±0.01^{a}
C20:3n-6	0.12±0.01^{a}	0.10±0.03^{a}	0.15±0.00^{a}	0.09±0.01^{a}	0.07±0.01^{a}
C20:3n-3	0.47±0.01^{a}	0.86±0.02^{a}	0.84±0.01^{a}	0.89±0.02^{a}	0.90±0.00^{a}
C20:4n-6	0.19±0.00^{a}	0.21±0.01^{a}	0.37±0.22^{a}	0.22±0.01^{a}	0.24±0.00^{a}
C22:2	0.07±0.01^{a}	0.08±0.01^{a}	0.03±0.01^{a}	0.07±0.01^{a}	0.06±0.01^{a}
C20:5n-3	5.01±0.05^{ab}	4.91±0.17^{b}	4.64±0.03^{b}	5.39±0.04^{a}	5.32±0.05^{a}
C22:6n-3	8.60±0.21^{a}	7.94±0.28^{b}	7.53±0.23^{b}	8.42±0.09^{a}	8.71±0.07^{a}
∑PUFA	**40.34±0.07^{d}**	**41.94±0.09^{c}**	**40.18±0.09^{d}**	**43.56±0.04^{b}**	**44.67±0.11^{a}**
Total n-3	**15.50±0.10^{b}**	**15.32±0.12^{b}**	**14.52±0.11^{c}**	**16.26±0.04^{a}**	**16.57±0.03^{a}**
Total n-6	**23.83±0.05^{d}**	**25.48±0.08^{bc}**	**24.63±0.10^{cd}**	**26.23±0.05^{ab}**	**27.00±0.17^{a}**
n-3/n-6	**0.65±0.01^{a}**	**0.60±0.05^{a}**	**0.59±0.06^{a}**	**0.62±0.01^{a}**	**0.61±0.01^{a}**
EPA/DHA	**0.58±0.01^{a}**	**0.62±0.00^{a}**	**0.62±0.02^{a}**	**0.64±0.01^{a}**	**0.61±0.00^{a}**
Undefined	**7.20**	**7.70**	**7.84**	**8.47**	**9.09**

Lowercase letters indicate statistical differences between groups ($p<0.05$).
SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid, n - omega.

Table 3 reports the results of the fatty acid composition of the 30th day 2nd thawing. Although the ∑SFA value was 22.38% in fresh sea bass, it decreased to 15.53% in thawing at ambient conditions. With regard to the ∑MUFA, the value was 30.07% in fresh sea bass, however it increased to 33.54% in thawing under ambient conditions and was determined as the group with the highest increase. The ∑PUFA was 40.34% in fresh sea bass and it increased to 42.22% in thawing under ambient conditions.

In addition, DHA was determined as 8.60±0.21% in fresh fish, but significant losses occurred in all thawing methods ($p<0.05$). The n-3/n-6 ratio was 0.65 in fresh sea bass, 0.63 in the refrigerator, 0.64 in the microwave, 0.60 in ambient conditions, and 0.57 in water thawed samples.

Table 3. Fatty acid analysis results of sea bass thawed for the 2nd time on the 30th day in different conditions (%)

	Day 30 (2nd Thawing)				
	Fresh	**Refrigerator**	**Microwave**	**Ambient Conditions**	**Water**
C12:0	0.03±0.00a	0.02±0.00a	0.03±0.00a	0.03±0.00a	0.03±0.00a
C13:0	0.01±0.00a	0.01±0.00a	0.02±0.00a	0.01±0.00a	0.07±0.09a
C14:0	2.28±0.03a	2.44±0.25a	2.44±0.14a	2.36±0.02a	2.38±0.07a
C15:0	0.28±0.00a	0.29±0.01a	0.29±0.00a	0.29±0.00a	0.29±0.00a
C16:0	15.14±0.09a	13.92±0.04b	13.09±1.10b	9.81±0.96c	9.81±0.21c
C17:0	0.25±0.01a	0.27±0.03a	0.26±0.07a	0.20±0.01a	0.21±0.01a
C18:0	4.10±0.03a	4.02±0.64a	3.45±1.47b	2.50±0.24c	3.13±0.76b
C22:0	0.12±0.00a	0.12±0.01a	0.12±0.01a	0.14±0.01a	0.13±0.00a
C24:0	0.18±0.02a	0.20±0.00a	0.22±0.02a	0.19±0.00a	0.22±0.02a
∑SFA	**22.38±0.03a**	**21.30±0.22b**	**19.91±0.56c**	**15.53±0.32d**	**16.27±0.25d**
C14:1	0.09±0.00a	0.03±0.01a	0.03±0.00a	0.04±0.00a	0.04±0.00a
C16:1	3.26±0.03a	3.17±0.14a	3.38±0.26a	3.45±0.04a	3.42±0.11a
C17:1	0.44±0.01a	0.43±0.01a	0.47±0.03a	0.47±0.00a	0.47±0.01a
C18:1n-9c	22.54±0.12b	23.09±0.11b	22.71±0.27b	25.76±0.43a	25.20±0.03a
C18:1n-9t	0.17±0.00a	0.17±0.01a	0.18±0.02a	0.19±0.01a	0.18±0.01a
C20:1n-9	2.76±0.00a	2.80±0.15a	2.96±0.19a	3.16±0.04a	3.04±0.06a
C22:1n-9	0.81±0.01a	0.47±0.03a	0.54±0.03a	0.48±0.02a	0.52±0.01a
∑MUFA	**30.07±0.04b**	**30.16±0.07b**	**30.28±0.12b**	**33.54±0.15a**	**32.86±0.04a**
C18:2n-6t	0.82±0.00a	0.09±0.00a	0.08±0.01a	0.07±0.00a	0.08±0.01a
C18:2n-6c	22.43±0.12c	23.45±0.38b	23.69±0.36b	24.88±0.34a	25.24±0.24a
C18:3n-6	0.27±0.02a	0.37±0.16a	0.46±0.05a	0.42±0.01a	0.37±0.11a
C18:3n-3	1.42±0.01a	1.54±0.09a	1.52±0.10a	1.64±0.03a	1.58±0.03a
C20:2	0.95±0.02a	0.96±0.02a	1.03±0.07a	1.08±0.03a	1.01±0.02a
C20:3n-6	0.12±0.01a	0.13±0.01a	0.13±0.03a	0.10±0.01a	0.12±0.02a
C20:3n-3	0.47±0.01a	0.81±0.03a	0.84±0.07a	0.84±0.03a	0.81±0.08a
C20:4n-6	0.19±0.00a	0.20±0.00a	0.21±0.02a	0.22±0.02a	0.21±0.00a
C22:2	0.07±0.01a	0.08±0.00a	0.09±0.02a	0.07±0.00a	0.07±0.00a
C20:5n-3	5.01±0.05b	4.82±0.15b	5.56±0.32a	4.76±0.04b	4.75±0.28b
C22:6n-3	8.60±0.21a	8.04±0.17bc	7.87±0.76c	8.14±0.00b	7.71±0.03c
∑PUFA	**40.34±0.07c**	**40.47±0.12c**	**41.48±0.23b**	**42.22±0.10a**	**41.94±0.10ab**
Total n-3	**15.50±0.10a**	**15.20±0.06a**	**15.78±0.32a**	**15.37±0.02a**	**14.85±0.12b**
Total n-6	**23.83±0.05c**	**24.23±0.16bc**	**24.58±0.15b**	**25.70±0.15a**	**26.01±0.10a**
n-3/n-6	**0.65±0.01a**	**0.63±0.02a**	**0.64±0.04a**	**0.60±0.01b**	**0.57±0.03c**
EPA/DHA	**0.58±0.01c**	**0.60±0.03b**	**0.71±0.11a**	**0.58±0.00c**	**0.62±0.03b**
Undefined	**7.20**	**8.08**	**8.32**	**8.70**	**8.93**

Lowercase letters indicate statistical differences between groups (p<0.05).
SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, EPA - eicosapentaenoic acid, DHA – docosahexaenoic acid, n - omega.

Table 4 shows the results of the fatty acid composition of the 30th day 1st thawing. The ∑SFA was 22.38% in fresh sea bass, but it significantly decreased to 14.38% in thawing in the refrigerator ($p<0.05$). The highest value (22.44%) was obtained in the microwave thawing method, and its difference with the control group was found to be statistically insignificant ($p<0.05$). It was determined that while ∑MUFA was 30.07% in fresh sea bass, it significantly decreased to 28.77% in microwave thawing and increased to 33.18% in ambient conditions ($p<0.05$). The ∑PUFA was 40.34% in fresh sea bass and it increased to 44.70% in refrigerator thawing with the highest value among the groups ($p<0.05$). The n-3/n-6 ratio was 0.65 in fresh sea bass, 0.63 in refrigerator and ambient conditions, and 0.64 in microwave and water thawing ($p>0.05$).

Table 4. Fatty acid analysis results of sea bass thawed for the 1st time on the 30th day in different conditions (%)

			Day 30 (1st Thawing)		
	Fresh	Refrigerator	Microwave	Ambient Conditions	Water
C12:0	0.03±0.00a	0.03±0.00a	0.03±0.00a	0.03±0.00a	0.03±0.00a
C13:0	0.01±0.00a	0.01±0.00a	0.01±0.00a	0.01±0.00a	0.01±0.00a
C14:0	2.28±0.03a	2.51±0.01a	2.27±0.02a	2.38±0.01a	2.37±0.00a
C15:0	0.28±0.00a	0.29±0.00a	0.29±0.00a	0.28±0.00a	0.29±0.00a
C16:0	15.14±0.09a	8.74±0.44c	15.11±0.02a	9.28±1.26bc	10.40±0.36b
C17:0	0.25±0.01a	0.17±0.01a	0.26±0.00a	0.18±0.01a	0.19±0.00a
C18:0	4.10±0.03a	2.28±0.09b	4.16±0.02a	2.24±0.38b	2.50±0.13b
C22:0	0.12±0.00a	0.13±0.00a	0.12±0.00a	0.13±0.00a	0.13±0.00a
C24:0	0.18±0.02a	0.21±0.01a	0.19±0.00a	0.21±0.03a	0.18±0.00a
∑SFA	**22.38±0.03a**	**14.38±0.15c**	**22.44±0.01a**	**14.74±0.42c**	**16.10±0.12b**
C14:1	0.09±0.00a	0.04±0.00a	0.03±0.00a	0.04±0.00a	0.04±0.00a
C16:1	3.26±0.03a	3.60±0.02a	3.20±0.02a	3.52±0.03a	3.46±0.01a
C17:1	0.44±0.01a	0.51±0.00a	0.43±0.01a	0.49±0.00a	0.49±0.00a
C18:1n-9c	22.54±0.12c	23.88±0.18b	21.67±0.10d	25.35±0.68a	24.28±0.15ab
C18:1n-9t	0.17±0.00a	0.19±0.00a	0.17±0.00a	0.19±0.00a	0.18±0.00a
C20:1n-9	2.76±0.00a	3.18±0.02a	2.80±0.01a	3.11±0.04a	3.06±0.01a
C22:1n-9	0.81±0.01a	0.55±0.02a	0.47±0.00a	0.48±0.02a	0.46±0.00a
∑MUFA	**30.07±0.04c**	**31.93±0.06b**	**28.77±0.04d**	**33.18±0.25a**	**31.97±0.06b**
C18:2n-6t	0.82±0.00a	0.07±0.00a	0.09±0.00a	0.07±0.00a	0.07±0.00a
C18:2n-6c	22.43±0.12d	25.92±0.13a	23.30±0.04c	25.22±0.41ab	24.88±0.11b
C18:3n-6	0.27±0.02a	0.40±0.01a	0.47±0.00a	0.41±0.00a	0.40±0.01a
C18:3n-3	1.42±0.01a	1.56±0.01a	1.41±0.01a	1.54±0.06a	1.55±0.01a
C20:2	0.95±0.02a	1.04±0.01a	0.96±0.00a	0.97±0.03a	1.05±0.01a
C20:3n-6	0.12±0.01a	0.10±0.01a	0.13±0.00a	0.08±0.00a	0.09±0.00a
C20:3n-3	0.47±0.01a	0.88±0.01a	0.85±0.01a	0.88±0.02a	0.87±0.01a
C20:4n-6	0.19±0.00a	0.22±0.01a	0.20±0.00a	0.21±0.01a	0.21±0.00a
C22:2	0.07±0.01a	0.07±0.01a	0.09±0.01a	0.07±0.00a	0.07±0.00a
C20:5n-3	5.01±0.05a	5.56±0.02a	5.00±0.04a	5.00±0.03a	5.15±0.01a
C22:6n-3	8.60±0.21a	8.90±0.09a	8.28±0.11a	8.97±0.09a	8.86±0.13a
∑PUFA	**40.34±0.07c**	**44.70±0.04a**	**40.79±0.03c**	**43.41±0.12b**	**43.21±0.05b**
Total n-3	**15.50±0.10b**	**16.89±0.04c**	**15.55±0.05b**	**16.39±0.03c**	**16.43±0.06c**
Total n-6	**23.83±0.05c**	**26.70±0.06a**	**24.19±0.02c**	**25.99±0.18ab**	**25.66±0.05b**
n-3/n-6	**0.65±0.01a**	**0.63±0.01a**	**0.64±0.02a**	**0.63±0.01a**	**0.64±0.01a**
EPA/DHA	**0.58±0.01c**	**0.63±0.00a**	**0.60±0.00b**	**0.56±0.00d**	**0.58±0.01c**
Undefined	**7.20**	**8.98**	**8.00**	**8.67**	**8.71**

Lowercase letters indicate statistical differences between groups (p<0.05).
SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid, n - omega.

DISCUSSION

The lipid content of fish varies widely and not only according to the fish species. Changes in the lipid amount of fish meat can also occur due to seasonal conditions, nutritional characteristics, salt content of the water and various other factors (Çilingir Yeltekin, 2012). For this reason, although it is difficult to specify a general amount of lipid content of fish (Baysal, 2002), it is reported that the total amount of lipid varies between 0.5% and 20% (Koubaa et al., 2012). Fish are generally classified as lean, medium oily and oily on the basis of their lipid content. Less than 5% fat content is considered lean, 5-10% medium oily, and more than 10% oily fish (Ackman, 1989). The quality of fish stems from 15-20% saturated and 80-85% unsaturated fatty acids in its composition. Polyunsaturated fatty acids constitute the majority of these unsaturated fatty acids (Yalçın and Yalçın, 2016). Periago et al. (2005) in their study examining the meat quality of cultured and wild sea bass, reported an initial lipid content for fresh cultured sea bass of 6.66%. Orban et al. (2003) also determined a value of 9.36% in their similar studies. In our study, the lipid content of fresh sea bass was determined as 10.29%. According to the results of the present study, sea bass are generally in the class of medium oily fish when compared with other studies.

Lipid content decreased in the thawing processes performed on the 7th and 30th days after the freezing process compared to the fresh fish on day 0 in our study. In general, there was a decrease in lipid content depending on time. On the 7th day and 30th day 1st thawing, the lowest lipid value was determined at microwave thawed samples. The highest lipid content was in the samples thawed in the refrigerator. Samantaray et al. (2021), applied thawing several times in samples of different carps species. As a result, while the lipid content was 2.23±0.03% and 2.02±0.03%, respectively in the fresh sample of Catla and Rohu species, the value decreased to 1.04±0.06% and 1.49±0.03%, respectively in the 5th thawing. Pourshamsian et al. (2012) applied the frying of cultured sturgeon with different oils, followed by freezing and thawing in the refrigerator and microwave three days later. They reported that the lipid ratios in the samples decreased after the thawing processes performed on the 3rd day, but no significant difference was observed between the two thawing methods.

Thawing is the final stage of the freezing process. In the stage of processing frozen fish or consuming it as fresh fish, thawing under inappropriate conditions causes quality loss in the product (Binici and Kurtkaya, 2014). Air thawing, water thawing, vacuum thawing, electrical thawing and high pressure thawing methods are all methods used for food (Binici and Kurtkaya, 2014; Genç et al., 2015). Multiple freezing and thawing processes are generally used in restaurants, hypermarkets and homes. According to researches in the literature, it is not possible to give valid information for the best thawing method (Baygar et al., 2004). For instance, thawing at room temperature is widely practiced in small businesses, but it is not recommended due to the risk of microorganisms development. Excessive water loss, bad odor and taste may occur in the refrigerator thawing due to the slow thawing and bacterial growth over time. In principle, seafood should be thawed as quickly as possible. Faster and more advantageous thawing methods are possible for large facilities, and microwave thawing is one of them (Turan et al., 2006).

Mol et al. (2004) found that different thawing conditions (water, ambient conditions and refrigerator) did not cause a significant change on the quality of imported mackerel according to the sensory, chemical and microbiological analyzes and mackerels can be consumed by thawing at refrigerator, ambient conditions and refrigerator in a healthy way. Tokur and Kandemir (2008) examined the effects of different thawing methods (microwave, flowing water and ambient conditions) on protein quality of frozen fish, and reported that different thawing processes caused a significant decrease in protein solubility. Turan et al. (2006) evaluated the effects of different thawing methods (microwave, water, ambient temperature, refrigerator) on the quality of frozen trout by sensory, chemical and microbiological methods, and their results showed that the best quality was in microwave and water thawing in terms of both chemical and sensory characteristics. It was concluded that the water thawing method would be more appropriate in large facilities, since microwave thawing method is an expensive and requiring control. For consumers who thaw small quantities of fish, the microwave thawing method is recommended because of taking less time. Ersoy et al. (2008) applied refrigerator, water, ambient conditions and microwave thawing to frozen eel, and they concluded that the water thawing was the most appropriate method by a microbiological point of view. Karami et al. (2022) in their study that investigated the effect of different (microwave, ambient and air refrigerator) thawing processes on quality of mullet (*Liza aurata*) fillets during the 60 day storage, while on the first day protein content were 18.25%, 19.13% and 19.18% in the groups of microwave, ambient air and refrigerator thawing, after 60 days it decreased to 17.37%, 18.89% and 18.95%, respectively. They found the lipid content on the first day as 3.76%, 4% and 4% in the groups of microwave, ambient air and refrigerator thawing, after 60 days it decreased to 3.57%, 3.77%, 3.78%, respectively. The highest decrease was observed in the microwave thawing group in terms of protein and lipid content. Also in the same study maximum decrease in moisture content was in the microwave thawing (77.27% on the first day, 66% on the 60th day). Wang et al. (2022) studied the effects of different thawing methods (water immersion thawing, air thawing and refrigerator thawing) on the biochemical properties of tilapia (*Oreochromis niloticus*) fillets during storage at -18°C for 6 months. According to the thawing and cooking losses refrigerator thawing is suggested to be an effective thawing method similar with our study to minimise quality change during frozen storage. Han and Gökoğlu (2022) studied the effects of different freezing and thawing (on air, in refrigerator and in microwave oven) methods on the quality of giant red shrimp (*Aristaeomorpha foliacea*). Results showed that microwave thawing conditions are not suitable similar with our study for thawing of frozen shrimp as it negatively affects texture and colour and increases cooking loss. Benjakul and Bauer (2001) studied thawing of catfish using different techniques and they determined that the fast thawing process caused a better change in the sensory and textural quality of the meat compared to the slow thawing process. They also reported that deterioration in the structure of meat, lipid oxidation and protein denaturation occured due to the osmotic pressure of water during thawing in water. In thawing methods, temperature fluctuations significantly affect the quality. Frozen foods are mostly thawed before processing or consumption and it can cause chemical, physical and microbiological damage to food (Pourshamsian et al., 2012). Polyunsaturated fatty acids, which are abundant in the structure of fish, are also oxidatively damaged during storage (Nazemroaya et al., 2009).

In our study, according to the fatty acid composition of sea bass thawed at different times with different methods, it was determined that the dominant saturated fatty acids were palmitic acid (C16:0), myristic acid (C14:0) and stearic acid (C18:0) in all groups. The highest palmitic acid was determined in fresh samples with a value of 15.14%. A decrease in palmitic acid was observed with the storage period. In general, the lowest total SFA occurred in the water and ambient conditions thawing on all thawing days. Durmuş and Özoğul (2018) studied the effect of nanoemulsions on the fatty acids of sea bass fillets in cold storage and they reported the total SFA of the control group as 19.21% at the beginning of storage, and an increase in the SFA during the storage period. In addition, the total SFA increased to 24.49% on the 8th day of storage for the control group, and this value was 24.05% at the end of the storage (12th day). In our study, the total SFA was 22.38% at the beginning of the storage, and the total SFA of all thawing methods during the storage were lower than the results of the researcher.

In our study, linoleic acid, EPA and DHA were determined as 22.43%, 5.01% and 8.60%, respectively, in the fresh sample. The total PUFA was determined as 40.34% in the fresh sample and between 40.47-44.70% in the samples thawed in the refrigerator. Durmuş and Özoğul (2018) stated that the highest polyunsaturated fatty acids were linoleic acid, EPA and DHA with 14.52%, 4.21% and 8.09%, respectively, for the control group at the beginning of storage. Kocatepe and Turan (2012) determined that the total polyunsaturated fatty acids (PUFA) in the fatty acid composition of cultured sea bass were 33.2%. Durmuş and Özoğul (2018) reported that the total polyunsaturated fatty acids (PUFA) was 29.25% at the beginning of storage, while there was a decrease in PUFA during the storage period and this value was 20.94% at the end of the storage (12th day). The PUFA values in our study were higher than those reported by the other researchers. Comparing the thawing methods, the highest PUFA and n-3 values were found in the samples thawed in the water (41.94±0.10%-44.67±0.11% and 14.85±0.12%-16.57±0.03%) and ambient conditions (42.22±0.10%-43.56±0.0% and 15.37±0.02%-16.39±0.03%). Lower values were obtained on the 30th day 2nd thawing. Javadian et al. (2013) in a study applying different thawing methods (refrigerator, water, microwave, ambient conditions) to frozen rainbow trout (*Oncorhynchus mykiss*), reported that after freezing/thawing, the contents of polyunsaturated fatty acids (PUFA) and n-3 were significantly reduced compared to the fresh sample. They concluded that thawing in water after freezing is the best method for all rainbow trout. Wu et al. (2021) examined the effects of freezing and thawing processes applied to raw pork meat on protein and lipid oxidation, applying thawing 7 times. As a result, while in the control group, saturated and polyunsaturated fatty acids were 36.27±0.96% and 15.85±0.90%, respectively, the values decreased significantly to 31.77±0.68% and 8.91±1.04% at the 7th thawing. Pourshamsian et al. (2012) evaluated the fatty acid profile of the cultured sturgeon that was fried, freezed and then thawed in refrigerator and microwave after 3 days. They reported that fatty acid profiles was similar to the control group in the refrigerator thawing method than microwave thawing method.

Refrigerator and microwave thawing methods are the most effective methods for thawing animal tissues in large portions. The microwave thawing method is faster and less damaging the tissues (Boonsumrej et al., 2007). The thawing method should preserve the original quality of the food as much as possible. When the studies of various researchers were investigated, results of different thawing methods were obtained. Researchers' results may differ due to fish species, region, season, gender or ovulation period besides thawing method. Thus, it is not possible to give a clear conclusion on the most efficient thawing method.

CONCLUSION

In our study, according to the the lipids analysis, the lowest fat loss was observed in the refrigerator thawing method (9.19±0.18%) and the highest lipid loss (7.46±0.57%) was in the microwave thawed samples at Day 30 (1st thawing). According to the fatty acid profile, due to the lowest loss of unsaturated fatty acids (C18:2 25.92±0.13%, C20:5 5.56±0.02%, C22:6n-3 8.90±0.09%, ∑PUFA 44.70±0.04%) at Day 30 (1st thawing) the most convenient method was determied as refrigerator thawing. As a result, all seafood, including fish, are the products that are highly susceptible to quality loss and deterioration in terms of meat structure and content. Incorrect practices during the storage and thawing of seafood accelerate the losses in all other quality parameters, especially meat quality. Even if the freezing conditions are appropriate, incorrect applications during the thawing of the products can cause undesired quality changes. Thus, the cold chain conditions should not be broken in such processes, the ambient and water temperature should not be high during thawing, the products should not be kept in ambient conditions for a long time. In the case of water thawed samples, the water pressure should not be high to avoid to damage the meat. In addition it should be prevented water penetration inside the meat and excessive freezing and thawing, together with avoid to damage the meat with the effect of heat in applications such as microwaves. As a result of the study, it is recommended to the consumers that frozen foods should be thawed only once, to consume immediately and to freeze and thaw the fish according to their needs.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study. Cansu Metin, Yunus Alparslan and Zerrin Ekşi carried out the laboratory studies; material preparation, lipid and fatty acid analyses. Taçnur Baygar, Yunus Alparslan and Cansu Metin interpreted the results. The writing/editing was carried out by Cansu Metin, Yunus Alparslan and Zerrin Ekşi, Taçnur Baygar and all authors have read and approved the article.

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 this study.

DATA AVAILABILITY

The data supporting the conclusions of this paper are available in the main paper.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Mass mortality report of the critically endangered *Pinna nobilis* (Linnaeus, 1758) in Gökçeada (North Aegean Sea, Turkey)

Gökçeada'da (Kuzey Ege Denizi, Türkiye) nesli kritik şekilde tehlike altında olan *Pinna nobilis* (Linnaeus, 1758)'in toplu ölüm raporu

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Abstract: In August 2020, a total area of 38000 m² around Gökçeada has been scanned to determine the current population status of *Pinna nobilis*. 9 stations (Yıldızkoy, Manastır, Marmaros, Gizli Liman, Laz Koyu, Kapıkaya, Karaçavuş, Karaçavuş shore and Kefalos) have been detected by means of SCUBA and ABC diving techniques. All specimens were found dead (total mass mortality of 100%). The total length range of *P. nobilis* was between 30.8 – 38.3 cm.

Keywords: *Pinna nobilis,* length, habitat, density, mass mortality, Aegean Sea

Öz: *Pinna nobilis*'in mevcut populasyon durumunu ortaya koymak için, 2020 Ağustos ayında Gökçeada kıyılarında, 38000 m²'lik bir alan incelenmiştir. 9 istasyon (Yıldızkoy, Manastır, Marmaros, Gizli Liman, Laz Koyu, Kapıkaya, Karaçavuş, Karaçavuş altı ve Kefalos), SCUBA ve ABC dalış teknikleri kullanılarak taranmıştır. Çalışma süresince bulunan bütün bireyler ölüdür (%100 kütlesel ölüm görülmüştür). *P. nobilis* 'in toplam boy aralığı 30,8 – 38,3 cm arasında ölçülmüştür.

Anahtar kelimeler: *Pinna nobilis*, boy, habitat, yoğunluk, kütlesel ölüm, Ege Denizi

INTRODUCTION

The island, as called, "Imbros", until 1970's and after "Gökçeada", is a district of Çanakkale and is the largest island of Turkey. It is located in the north of Aegean Sea and the entrance of Saros Bay. The total shoreline is 91 km. Tourism, agriculture, stock breeding, and fisheries are the main subsistence on the island. Specifically, harpoon fishery for swordfish along the coasts is peculiar to Gökçeada (Altın et al., 2016). The marine ecosystem along the coasts of Gökçeada is also quite rich in biodiversity (Öztürk and Pazarkaya, 2014; Acarli et al., 2020a). *Pinna nobilis* (Linnaeus, 1758), an endemic bivalvia species of the Mediterranean, lives in *Posidonia oceanica* or *Cymodocea nodosa* meadows, embedded with a part of umbo and attached with byssus, on a sandy, sandy-muddy, gravel substratum (Tebble, 1966; Zavodnik et al., 1991; Hendriks et al., 2011). The hard shells are home to many benthic organisms (Acarli et al., 2010). *P. nobilis* filter-feed on organic and inorganic materials in the water column, this helps to enhance water quality in the area (Vicente et al., 2002; Natalotto et al., 2015; Acarli, 2021). Individuals with a length of 30 cm were reported to filter more than 2500 L of water per day, depending on their physiological energy (Hernandis Caballero, 2021). However, their population is affected by overfishing, environmental pollution, habitat deterioration, and tourism. Thus, *P. nobilis* has been taken under conservation by the European Council's regulations in 1992. Since 2016, the population is under the thread of a parasite *Haplosporidium pinnae* which caused many mass mortalities recorded along the Mediterranean (Vázquez-Luis et al., 2017). Greece, Italy, Croatia, Turkey, Tunisia, France and Morocco are the Mediterranean countries that have reported mass mortality (Acarli et al., 2020a; Čižmek et al., 2020; Šarić et al., 2020; Öndes et al., 2020; Zotou et al., 2020; Betti et al., 2021; Acarli et al., 2022a). International Union for Conservation of Nature (IUCN) has changed the status of *P. nobilis* as Critically Endangered Species (Kersting et al., 2019).

Although there have been many studies conducted along Gökçeada coasts, such as the fish fauna from shallow waters, species composition, fish egg and larvae (Altın and Ayyıldız, 2018; Kocabaş and Acarli 2019; Acarli et al., 2020b; Daban et al., 2020); only one study on the status of *P. nobilis* population in Gökçeada has been found (Çanak et al., 2006). In this context, the current study aimed to determine the present status of the *P. nobilis* population in Gökçeada, which is located in the northernmost part of the Aegean Sea.

MATERIAL AND METHODS

This study was carried out in August 2020 at 9 stations around Gökçeada (Figure 1). Gökçeada is located at the northernmost part of the Aegean Sea. The coordinates of 9 stations are; Yıldızkoy (40˚14.110' N - 25˚54.230' E), Manastır (40˚11.591' N - 25˚58.696' E), Marmaros (40˚11.620' N - 25˚45.289' E), Gizli Liman (40˚7.432' N - 25˚40.269' E), Laz Koyu (40˚5.975' N - 25˚47.050' E), Kapıkaya (40˚6.288' N - 25˚48.542' E), Karaçavuş (40˚6.944' N - 25˚53.322' E), Karaçavuş shores (40˚7.228' N - 25˚55.285' E) and Kefalos (40˚9.497' N - 25˚59.952' E).

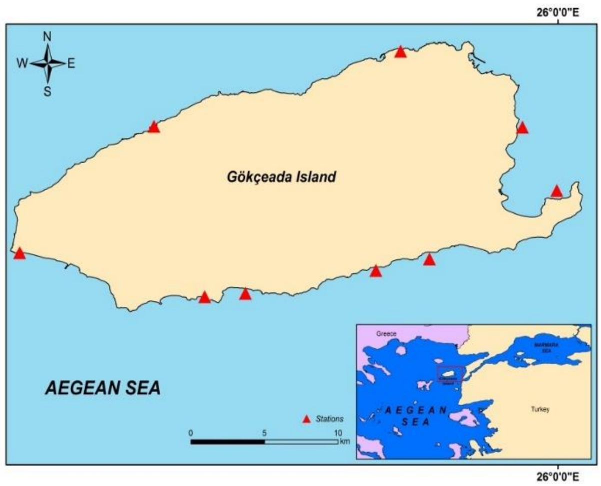

Figure 1. Study area

Depth and temperature values at the stations were measured by the dive computer, Oceanic Geo 2. Current status of *P. nobilis* population was observed by means of SCUBA and ABC dives. Transect method of visual census technique was used to detect the *P. nobilis* individuals. In each station, two divers, with 10 m distance in between, scanned rectangular areas perpendicular to the shore. The rectangles were, minimum 100 m and maximum 300 m in length, and 2-10 m in width. The properties of the scanned area, habitat structure (sandy, seagrass meadow, gravel etc.) and the number and status of *P. nobilis* specimens were recorded on underwater slates. Photographs were taken by an underwater camera (Nikon Coolpix).

During the observation, if the specimen was fully over the sediment, total length of the shell was measured, or if it was buried in the sediment, only the width of the shell was measured and recorded on slates. The length of these buried specimens was later calculated by the equation (1) revealed by Acarli et al. (2018) which was determined with the previously obtained raw data.

$$a=0.8061b+28.61;\ (r^2=0.717) \qquad (1)$$

In this equation *a* is the calculated total length and *b* is the measured width of the shell.

RESULTS

Habitat structure, depth range, underwater visibility, surveyed area, no of individuals per 100 m^2, and mortality ratio of 9 stations are given in (Table 1). A total of 8 *P. nobilis* individuals were found in 5 stations (Figure 2) with a total length range of 30.8-38.3 cm. Only two shells were found buried and they were in Yıldızkoy, all the other individuals (6) were encountered lying over the sediment. The highest number of individuals (3) was in Kefalos Station. There were no dead or alive individuals encountered in stations Marmaros, Gizli Liman, Laz Koyu and Kapıkaya. Mortality rate was 100% for all the stations (Figure 3).

Table 1. Information about stations' characteristics

Stations	N	Length of *P. nobilis*	Habitat Structure	Depth Range (m)	Underwater visibility (m)	Surveyed area (m^2)	Density of Dead Shells (ind/100m^2)	Mortality rate (%)
Yıldızkoy*	2	36.0-36.40	Gravel 10%, Sandy 10%, *P. oceanica* 80%	6-12	9	3000	0.06	100
Manastır	1	38.3	Sandy 10%, *P. oceanica* 90%	8-9	10	3750	0.02	100
Marmaros	-	-	Rocky 30%, *P. oceanica* 70%	7-11	12	4000		-
Gizli Liman	-	-	Sandy 30%, Rocky 20%, *P. oceanica* 50%	7	10	5250		-
Laz Koyu	-		Sandy 30%, Rocky 20%, *P. oceanica* 50%	8	7	6500		-
Kapıkaya	-		Sandy 80%, Rocky 20%	6	5	5000		-
Karaçavuş	1	30.8	Sandy 10%, *P. oceanica* 90%	4	1.5	1000	0.1	100
Karaçavuş Shore	1	35	*P. oceanica* 100%	3.5-4	15	4000	0.02	100
Kefalos	3	36.0-37.0 37.9	Gravel 10%, Sandy 10%, *P. oceanica* 80%	11	12	5500	0.05	100

*Gökçeada Marine Park

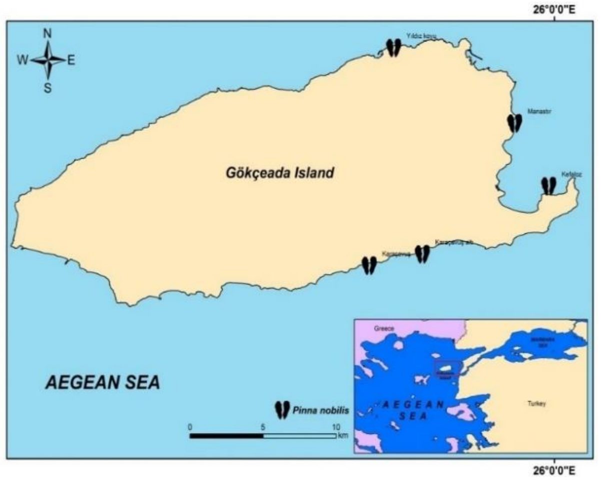

Figure 2. Stations where *P.nobilis* is observed

Habitat type along the study area was generally *P. oceanica* meadow, sand, gravel, and rock. *P. nobilis* individuals were mostly seen among *P. oceanica* meadows. Underwater visibility (horizontally) was lowest in Karaçavuş station (1.5 m) and highest in Karaçavuş shore station (15 m). The number of *P. nobilis* individuals per 100 m^2 was calculated to vary between 0.02-0.1. Water temperature varied between 24°C and 27°C throughout the study.

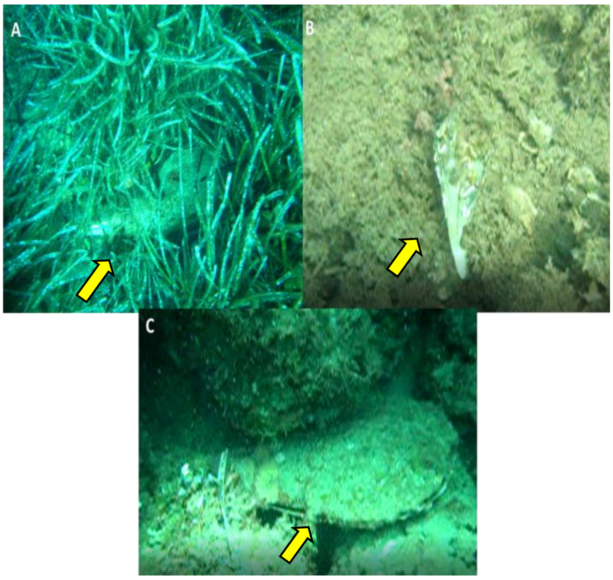

Figure 3. *P. nobilis* images from the stations Kefalos (A), Karaçavuş Shore (B) and Manastır (C)

DISCUSSION

Recently, researchers agreed on the main reason for the mass mortality of *P. nobilis*, is the parasite *H. pinnae* (Caballero 2021; Künili et al. 2021; IUCN 2021). Environmental factors, especially, temperature (over 13.5^{o}C) and salinity (36.5-39.7 PSU) have also been declared as triggers for the mass mortality incidents (Cabanellas-Reboredo et al., 2019). However, mass mortality has been observed even in the Sea of Marmara, which is much colder and less saline than the Mediterranean Sea (Çınar et al., 2021a; Acarli et al., 2022a). Acarli et al., (2022a) indicated that low temperature and salinity conditions may help to prevent the spread of the disease, but they may not be adequate to inhibit the arising of it. In this study, sea temperature values have been recorded between 24-27^{o}C. The fouling and boring organisms found inside the shells give rise to think that they died a while, nearly 3 years ago, before the mass mortality incidents occurred. The fact that not a single living individual was encountered in this region shows how badly the disease effects the population around Gökçeada.

In the course of current study, the sea floor was scanned from 0.5 m depths to 30 m and individuals were encountered between 4-12 m depths. Two local divers also declared that there had been a dense population they used to encounter along the shores of Kefalos at 5-15 m depths (S. Konya and V. Günel, personal communication August 15, 2020). According to Albayrak (2000), 1 individual with a length of 40.62 cm and a width of 16.11 cm had been found in Aydıncık-Kefalos bay, at 3 m depth, on a fine sandy substratum. On the other hand, Efe (2019), (without giving a location) reported 1 individual of 27 cm long and 12 cm wide, weighting 65.1 g. Çanak et al. (2006) indicated that the highest number of individuals were found from 5 to 10 m depths. These researchers measured the unburied length of *P. nobilis* from 8.5 cm to 44.3 and the width from 6 cm to 29.3 cm between 4.6 m to 27 m depths. In the current study, regarding the population distribution, the number of individuals per 100 m^2 was found to be between 0.02 and 0.1, and 100% mass mortality was recorded. Çanak et al. (2006) encountered 37 living and 5 dead individuals between Kaleköy and Yelkenkaya, where the population density of *P. nobilis* was reported as 0.022 ind./100 m^2. According to the other studies; Acarli et al. (2022b) reported 4.9-27.0 ind./100 m^2, Öndes et al. (2020) reported 1.2-13.6 ind./ 100 m^2, Acarli et al. (2021a) reported 0.6-8.2 ind./100 m^2 and finally Acarli et al. (2021b) recorded 10-112 ind./100 m^2.

P. nobilis appears to prefer environments with ideal hydrodynamic characteristics, such as low current velocities and turbulences, low wave action, and a sandy-muddy or muddy substratum, mostly among meadows of the sea grasses (Rabaoui et al., 2008). Even the habitat type of the coast in Gökçeada is mostly pebbles and rocks, dead *P. nobilis* was found on sandy substratum and among sea grass meadows during this study. On the other hand, high tourist activity has been observed on sandy coast of Gökçeada. Consequently, intense deaths seen all over the Mediterranean were encountered on the shores of Gökçeada and the *P. nobilis* population seems to disappear totally.

In case, *P. nobilis* populations with low densities, as in Gökçeada, face any harm from various sources, their ability to survive and sustain is more likely to be inadequate than many other bivalve species, due to *P. nobilis*' low reproduction rate.

According to the studies that have been conducted along the Turkish coasts (Öndes et al. 2019; Öndes et al. 2020; Özalp and Kersting 2020; Çınar et al. 2021a, 2021b; Acarli et al., 2020a, 2021a, 2021b, 2022b), the density of *P. nobilis* was found to be between 0.3-112.0 ind./100m^2. Depending on the current study and the study by Çanak et al. (2006), the density values were 0.020 ind./100 m^2 and 0.022 ind./100 m^2 respectively, which represented the lowest values among the all the studies those have been conducted up to now.

P. nobilis contributes to the ecology in a variety of ways. Large amount of organic matter is filtered from the water column which helps to improve the area's water quality and also many benthic organisms live on the hard shells. It is critical to implement an immediate conservation and management plan aimed at protecting and restoring *P. nobilis* stocks. To detect and monitor the remaining living *P. nobilis* individuals and areas with healthy populations that have not yet been exposed to parasites and even to declare those areas as Marine Protected Areas, is vital for the marine ecosystem. Also collecting and culturing the spat of the population is crucial for the revival and sustainability of damaged populations as Gökçeada's.

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AUTHORSHIP CONTRIBUTIONS

Deniz Acarli, Sefa Acarli and M. İdil Öz designed the study. Deniz Acarli and M. İdil Öz carried out underwater observations. Sefa Acarli supervised the study concept. Writing the first draft, data management, and statistical analyses were conducted by M. İdil Öz and Sefa Acarli. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST STATEMENT

The authors declare that there is no known financial or personal conflict that may affect the research.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

The datasets analysed during the current study are available from the corresponding author on reasonable request.

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Mollusc fauna of Kemer Bay (Sea of Marmara)

Kemer Koyu Mollusca faunası (Marmara Denizi)

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Abstract: In the present study, mollusc species distributed in the Kemer Bay (Sea of Marmara) were determined seasonally (November, February, May, and August) in the years 2010 and 2011. The benthic samples were collected from different biotopes in 16 stations at depth ranging between 0.2 and 5 m by using a quadrate sampling gear. As a result of the evaluation of collected benthic materials, a total of 66 mollusc species belonging to 3 classes (Polyplacophora, Gastropoda, and Bivalvia) were identified. When the number of species and individuals are compared based on the seasons, maximum number of species (46 species) and individuals (168825 ind. m^{-2}) were counted in autumn and spring, respectively. *Mytilus galloprovincialis* (Lamarck, 1819) and *Mytilaster lineatus* (Gmelin, 1791) were the most abundant species in all the seasons. Whereas, *Bittium reticulatum* (da Costa, 1778), *Rissoa membranacea* (Montagu, 1803), and *Tricolia pullus pullus* (Linnaeus, 1758) were characterized as species having the highest frequency index values in all seasons.

Keywords: Mollusca, Kemer Bay, Sea of Marmara, distribution

Öz: Bu çalışmada Marmara Denizi, Kemer Koyu'nda dağılım gösteren mollusk türleri 2010 ve 2011 yıllarında mevsimsel olarak (Kasım, Şubat, Mayıs ve Ağustos) belirlenmiştir. Bentik numuneler kuadrat örnekleme aleti kullanılarak 16 istasyondan 0,2 ve 5 m derinlikler arasında farklı biyotoplardan örneklenmiştir. Alınan bentik materyalin değerlendirilmesi sonucunda 3 sınıfa ait (Polyplacophora, Gastropoda ve Bivalvia) toplam 66 mollusk türü tanımlanmıştır. Mevsimlere göre tür ve birey sayıları karşılaştırıldığında, maksimum tür sayısı (46 tür) ve birey sayısı (168825 birey m^{-2}) sırasıyla sonbahar ve ilkbahar mevsimlerinde sayılmıştır. *Mytilus galloprovincialis* (Lamarck, 1819) ve *Mytilaster lineatus* (Gmelin, 1791) tüm mevsimlerde en baskın türlerdir. Buna karşın, *Bittium reticulatum* (da Costa, 1778), *Rissoa membranacea* (Montagu, 1803) ve *Tricolia pullus pullus* (Linnaeus, 1758) tüm mevsimlerde en yüksek frekans indeks değeri ile karakterize edilen türler olmuştur.

Anahtar kelimeler: Molluska, Kemer Koyu, Marmara Denizi, dağılım

INTRODUCTION

The Kemer Bay is located in the Sea of Marmara near Dardanelles entrance and the preliminary data on the benthic fauna of the region can be encountered by Colombo (1885) who investigated the benthic invertebrate species of Çanakkale. In the following years, some remarkable studies on the subject have been carried out by Sturany (1895) and Ostroumoff (1896). Afterwards, many studies have been accomplished on the mollusc fauna of the region (Marion, 1898; Kaneva-Abadjieva, 1959; Albayrak and Balkıs, 1996a, b; Türkmen and Demirsoy, 2009; Doğan et al., 2016; Çulha and Şahin, 2018; Bitlis, 2019).

Since a new thermal power plant with a large port was constructed in Kemer Bay at the sampling time of the present study, it is aimed to be one of the fundamental studies in order to compare the results of the studies that will be carried out in the area during the coming years. Because, it is known fact that industrialization negatively affects the marine biodiversity by increasing the pollution (Bat et al., 2011; Şahin, 2016). It is inevitable that the settlement of many species of different taxonomic groups in the area as ship traffic in the region would be increased with the newly constructed port. The mean goal of the present study is to determine mollusc species distributed in the Kemer Bay and evaluate statistically the obtained values for their ecological and distributional characteristics.

MATERIAL AND METHODS

Benthic materials were collected seasonally (November, February, May, and August) in the years 2010 and 2011 of the Kemer Bay within the framework of a project supported by TUBITAK (Project Number: 2209). The materials were obtained in 16 sampling sites at depths between 0.2 and 5 m by scraping a quadrate sampling gear (20 x 20 cm) (Figure 1). The collected material was sifted with 0.5 mm mesh size and fixed in seawater-formalin solution (4%) at the field. In laboratory, the material was sorted into taxonomic groups under a stereomicroscope and preserved in 70% ethanol. Later on, mollusc individuals were identified to species level and counted. Some of the ecological features of the sampling sites are presented in Table 1.

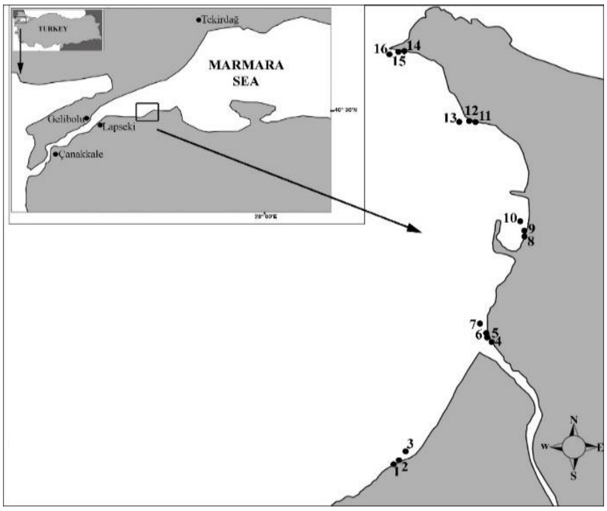

Figure 1. Map of the studied area with the location of sampling sites

Table 1. The coordinates, depth range, and biotopes of the sampling sites

	Coordinates		Depth (m)	Biotope
	Latitude	Longitude		
1	40° 24' 48.17"N	27° 03' 43.49"E	0.2	*M. galloprovincialis*
2	40° 24' 48.26"N	27° 03' 43.40"E	1	*Z. marina*+sand
3	40° 24' 48.50"N	27° 03' 43.33"E	5	*Z. marina*+sand
4	40° 25' 03.59"N	27° 03' 56.04"E	0.2	*M. galloprovincialis*
5	40° 25' 03.54"N	27° 03' 55.01"E	1	*M. galloprovincialis*
6	40° 25' 03.95"N	27° 03' 55.04"E	1	*Z. marina*+sand
7	40 °25' 05.73"N	27° 03' 53.08"E	5	*Z. marina*+grave
8	40° 25' 16.86"N	27° 03' 59.35"E	0.2	Muddy sand
9	40° 25' 17.09"N	27° 03' 58.54"E	1	*Z. marina*+sand
10	40° 25' 17.30"N	27° 03' 58.15"E	5	*Z. marina*+sand
11	40° 25' 29.48"N	27° 03' 54.46"E	0.2	*M. galloprovincialis*
12	40° 25' 29.33"N	27° 03' 54.27"E	1	*Z. marina*+shell
13	40° 25' 29.18"N	27° 03' 53.93"E	5	*Z. marina*+sand
14	40° 25' 39.20"N	27° 03' 36.58"E	0.2	*M. galloprovincialis*
15	40° 25' 39.06"N	27° 03' 36.05"E	1	*M. galloprovincialis*
16	40° 25' 38.82"N	27° 03' 35.80"E	5	*Z. marina*

In the field, the salinity (‰), temperature, (^{0}C), conductivity (µS/cm), and dissolved oxygen (mg/l) concentrations were measured using an oxygen meter (YSI 55) and multiple water analysis probe a SCT meter (YSI 650). pH was analyzed by a pH meter (Orion brand) in the laboratory (Parsons et al., 1984).

Bellan Santini's dominance index (D) (Bellan-Santini, 1969), Soyer's frequency index (F) (Soyer, 1970), Pielou's evenness index (J') (Pielou, 1975), Shannon-Weaver's diversity index ($\log_2$ base) (H') (Shannon and Weaver, 1949) were calculated for each sampling site for each season (winter, spring, summer, and autumn). Pearson's correlation analysis with a significance level of $p < 0.05$ was applied between the community parameters (number of species and individuals, evenness, and diversity indices) and environmental parameters. The Bray-Curtis similarity index based on cluster analysis was used to group the sampling sites. SIMPER analysis was implemented the species assemblages to identify the species most contributed to the similarity and dissimilarity of species assemblages. Statistical analyses were carried out by using the softwares PRIMER 6 and STATISTICA 7. The systematic classification is given according to WORMS (World Register of Marine Species, June 2022). The identified species were deposited at ESFM (Museum of Faculty of Fisheries, Ege University, İzmir, Turkey).

RESULTS

Of the studied benthic material collected of Kemer Bay, a total of 66 mollusc species belonging to 28 families and the 3 classes (Polyplacophora, Gastropoda, and Bivalvia) were determined (Table 2). The class Gastropoda includes the maximum number of species (36 species) followed by Bivalvia (28 species) and Polyplacophora (2 species). Among the families, Rissoidae (9 species) was the most dominant taxon in terms of the number of species, followed by the families Pyramidellidae (8 species) and Veneridae (8 species).

When the community parameters are compared based on the seasons, the maximum number of species (46 species) was determined in autumn and the maximum number of specimens (168825 ind. m^{-2}) was found in spring. The minimum number of species and specimens (25 species and 46225 ind. m^{-2}) were counted in summer. The most abundant species were *Mytilus galloprovincialis* (Lamarck, 1819) 75% and 54% in summer and spring, respectively and *Mytilaster lineatus* (Gmelin, 1791) in autumn (45%) and winter (44%) (Table 3).

According to the frequency index values, *Tricolia pullus pullus* (Linnaeus, 1758) had the highest frequency values in autumn (75%), *Bittium reticulatum* (da Costa, 1778) in winter (69%) and spring (60%), and *Rissoa membranacea* (Montagu, 1803) in summer (69%) (Table 3).

Table 2. List of the species identified within the present study and their total number of specimens belonging to every taxa according to the stations

	Stations															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
POLYPLACOPHORA																
Lepidochitona cinerea (Linnaéus, 1767)	4	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Acanthochitona fascicularis (Linnaéus, 1767)	1	-	-	-	-	-	-	2	1	-	-	1	2	-	-	-
GASTROPODA																
Patella caerulea Linnaéus, 1758	6	-	-	-	-	-	-	1	-	-	1	-	-	-	-	-
Diodora gibberula (Lamarck, 1822)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-
Gibbula ardens (Salis Marschlins, 1793)	-	-	-	-	1	1	-	2	-	-	-	1	-	-	-	-
Phorcus mutabilis (Philippi, 1851)	-	-	-	-	-	-	-	21	-	-	-	-	-	-	-	-
Phorcus richardi (Payraudeau, 1826)	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Phorcus turbinatus (Born, 1778)	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Steromphala adansonii (Payraudeau, 1826)	1	-	-	-	1	2	-	4	7	5	2	2	2	2	13	9
Tricolia pullus pullus (Linnaéus, 1758)	15	9	21	-	20	3	4	5	13	16	1	42	15	-	6	3
Bittium reticulatum (Da Costa, 1778)	-	224	459	-	67	118	249	611	435	531	-	949	672	-	-	2
Cerithium vulgatum Bruguière, 1792	-	-	2	-	-	-	4	-	-	1	-	2	-	-	-	-
Epitonium turtonis (Turton, 1819)	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	1
Alvania mamillata Risso, 1826	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	2
Alvania lactea (Michaud, 1830)	-	-	-	-	-	-	-	-	-	1	-	6	-	-	-	-
Alvania discors (Brown, 1818)	-	-	-	-	-	-	-	-	-	-	-	4	1	-	-	-
Pusillina radiata (Philippi, 1836)	-	1	9	-	-	-	8	-	-	1	2	-	-	-	-	-
Pusillina philippi (Aradas & Maggiore, 1844)	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
Pusillina lineolata (Michaud, 1830)	-	3	2	-	1	-	-	-	-	-	3	-	-	-	-	-
Pusillina inconspicua (Alder, 1844)	-	-	-	-	2	2	-	-	-	-	-	1	-	-	-	-
Rissoa splendida Eichwald, 1830	-	37	64	-	1	9	15	43	27	13	5	178	130	-	4	71
Rissoa membranacea (Adams, 1800)	-	3	4	-	2	2	3	7	6	17	5	57	28	-	1	3
Ecrobia ventrosa (Montagu 1803)	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Ocenebra edwardsii (Payraudeau, 1826)	6	-	-	-	-	-	-	-	-	1	-	-	-	1	-	-
Tritia reticulata (Linnaéus, 1758)	-	2	16	-	-	6	15	2	9	16	-	8	18	-	-	1
Tritia neritea (Linnaéus, 1758)	-	10	1	-	-	23	14	1	2	-	-	-	1	-	1	-
Mangelia costulata Risso, 1826	-	2	1	-	-	-	9	-	1	1	-	7	5	-	-	1
Mangelia brusinae van Aartsen & Fehr-de Wal, 1978	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Mangelia vauquelini (Payraudeau, 1826)	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Parthenina terebellum (Philippi, 1844)	-	-	8	-	-	1	4	1	-	8	-	9	10	-	-	-
Parthenina interstincta (Adams J., 1797)	-	2	4	-	2	-	9	-	35	4	-	17	7	-	-	-
Brachystomia scalaris MacGillivray, 1843	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Odostomia plicata (Montagu, 1803)	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
Megastomia conoidea (Brocchi, 1814)	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-
Pyrgiscus rufus (Philippi, 1836)	-	-	3	-	-	-	4	-	3	-	-	-	1	-	10	-
Turbonilla pusilla (Philippi, 1844)	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Turbonilla acuta (Donovan, 1804)	-	-	-	-	-	-	-	-	-	-	-	3	1	-	-	-
Ebala pointeli (de Folin, 1868)	-	-	1	-	-	-	-	-	-	1	-	1	1	-	-	-

Table 2. Continued

	Stations															
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
BIVALVIA																
Modiolula phaseolina (Philippi, 1844)	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Modiolus barbatus (Linnaéus, 1758)	-	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-
Musculus subpictus (Cantraine, 1835)	-	-	5	-	25	-	-	-	-	-	-	-	-	1	-	-
Mytilus galloprovincialis Lamarck, 1819	336	-	-	169	155	-	-	-	110	-	1007	-	17	3534	432	-
Mytilaster lineatus (Gmelin, 1791)	2147	224	1092	350	1191	-	-	2	3	-	97	26	5	63	-	-
Pinna nobilis Linnaéus, 1758	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-
Flexopecten glaber (Linnaéus, 1758)	-	-	3	-	-	-	1	-	-	-	-	-	-	-	-	-
Anomia ephippium Linnaéus, 1758	-	-	1	-	1	-	-	1	-	-	-	-	-	-	-	-
Ostrea edulis Linnaéus, 1758	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	1
Ctena decussata (Costa O.G., 1829)	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
Lucinella divaricata (Linnaéus, 1758)	-	1	1	-	-	-	3	-	-	-	-	2	2	-	-	-
Loripinus fragilis (Philippi, 1836)	-	-	4	-	-	-	8	-	-	2	-	4	-	-	-	-
Cerastoderma glaucum (Bruguière, 1789)	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
Parvicardium pinnulatum (Conrad, 1831)	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Parvicardium exiguum (Gmelin, 1791)	-	-	-	-	-	-	5	-	5	1	-	1	9	-	-	-
Spisula subtruncata (da Costa, 1778)	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	4
Macomangulus tenuis (da Costa, 1778)	-	-	-	-	-	1	-	-	-	-	-	-	1	-	-	2
Moerella donacina (Linnaéus, 1758)	-	-	-	-	-	-	4	-	-	-	-	2	-	-	-	-
Donax trunculus Linnaéus, 1758	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abra alba (W. Wood, 1802)	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-
Chamelea gallina (Linnaéus, 1758)	-	5	3	-	-	10	4	1	2	4	-	8	2	-	-	-
Gouldia minima (Montagu, 1803)	-	-	1	-	-	-	13	-	-	-	-	10	1	-	-	-
Pitar rudis (Poli, 1795)	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
Polititapes aureus (Gmelin, 1791)	-	-	-	-	-	-	-	-	2	-	-	1	-	-	-	-
Polititapes rhomboides (Pennant, 1777)	-	3	-	-	-	-	-	-	-	-	-	3	2	-	-	-
Ruditapes decussatus (Linnaéus, 1758)	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
Timoclea ovata (Pennant, 1777)	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-
Thracia phaseolina (Lamarck, 1818)	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	2

Table 3. The number of species (S), the number of individuals (N), frequency and dominance values based on seasons

Seasons	S	N (ind/m^{-2})	Frequency index (%)	Dominance (%)
Autumn	46	96625	*S. adansonii* (50%), *B. reticulatum* (56%), *R. splendida* (56%), *T. pullus pullus* (75%)	*M. lineatus* (45%)
Winter	37	110725	*T. pullus pullus* (50%), *T. reticulata* (50%), *B. reticulatum* (69%)	*M. lineatus* (44%)
Spring	34	168825	*B. reticulatum* (60%)	*M. galloprovincialis* (54%)
Summer	25	46225	*R. splendida* (62%), *R. membranacea* (69%)	*M. galloprovincialis* (75%)

Seasonal variations in the number of species and specimens, evenness, and diversity indices at all stations are showen in Table 4. The highest number of species was found at station 12 in spring (19 species) and in autumn (16 species). Station 3 showed the highest species number in winter and station 13 in summer. The largest number of individuals were recorded at station 14 (73575 ind. m^{-2}) in spring and at station 1 (30275 ind. m^{-2}) in autumn.

The maximum diversity index values were calculated at station 7 (3.0) in autumn and at station 10 (2.7) in summer, and similarly, the highest evenness index values were counted at sampling sites 4 (1.0) in spring, at station 10 and 5 (0.9) in summer, and at station 7 and 16 (0.8) in autumn (Table 4).

Table 4. Temporal variation in community parameters at the sampling sites according to the seasons

Stations	Number of species				Number of specimens (ind. m^{-2})			
	A	W	Sp	Su	A	W	Sp	Su
1	7	6	5	1	**30275**	20025	9500	3350
2	9	3	7	6	8575	450	3175	1000
3	12	**14**	7	6	3225	10875	26675	1875
4	4	3	2	1	6850	1675	3250	1325
5	1	9	2	6	1300	**29250**	5775	400
6	4	8	4	5	875	2225	1000	375
7	13	11	5	5	2525	5125	1425	400
8	13	6	7	5	5300	8000	4100	450
9	14	9	3	1	3625	7925	2300	2750
10	8	10	7	9	3025	9450	2675	525
11	8	1	4	1	3200	2625	10850	11400
12	**16**	11	**19**	9	10675	7475	13500	2150
13	13	11	8	**10**	8425	4125	8025	2775
14	4	2	2	1	2750	825	**73575**	**12875**
15	5	1	2	5	5600	250	3000	2850
16	6	7	0	4	400	425	0	1725
Stations	**Diversity index**				**Evenness index**			
	A	W	Sp	Su	A	W	Sp	Su
1	0.5	0.5	0.9	0.0	0.2	0.2	0.4	0.0
2	1.2	1.3	1.0	1.2	0.4	0.8	0.3	0.4
3	1.7	1.4	0.5	1.0	0.5	0.4	0.2	0.4
4	0.7	1.1	1.0	0.0	0.4	0.7	**1.0**	0.0
5	0.0	0.8	0.8	2.2	0.0	0.3	0.8	**0.9**
6	1.2	1.3	1.3	1.7	0.6	0.4	0.6	0.7
7	**3.0**	1.0	1.4	1.6	**0.8**	0.3	0.6	0.7
8	1.6	0.2	0.6	1.4	0.4	0.1	0.2	0.6
9	2.1	1.0	0.3	0.0	0.5	0.3	0.2	0.0
10	1.3	0.7	0.9	**2.7**	0.4	0.2	0.3	**0.9**
11	1.4	0.0	0.1	0.0	0.5	0.0	0.1	0.0
12	1.0	1.6	**1.6**	1.2	0.3	0.5	0.4	0.4
13	1.1	1.7	0.9	1.5	0.3	0.5	0.3	0.4
14	1.1	0.2	0.0	0.0	0.6	0.2	0.0	0.0
15	0.6	0.0	0.1	0.3	0.3	0.0	0.1	0.1
16	2.1	**2.3**	0.0	0.6	**0.8**	**0.8**	0.0	0.3

The relationships between the community and environmental parameters of the mollusc fauna in each season were revealed with Pearson's correlation analysis. Two negative correlation values were counted to be significant between the number of individuals and salinity (r=-0.72) and conductivity (r=-0.54) in autumn. The number of species was negatively correlated with pH values (r=-0.68) and negative correlation was found between the evenness index values and conductivity (r=-0.52) in winter. In spring, the evenness index values were correlated with pH (r= 0.77) and salinity (r=-0.58). The number of individuals was correlated with pH (r=0.62), and conductivity (r=-0.69) in summer, similar to autumn (Table 5).

Table 5. Relationships between the environmental and community parameters in each season. **S**: Number of species. **N**: Number of individuals. **H'**: Shannon-Weaver's diversity index values. **J'**: Pielou's evenness index values. Bold values are statistically significant ($p < 0.05$)

	Autumn					Winter			
	S	N	J'	H'		S	N	J'	H'
Salinity	0.20	**-0.72**	0.18	0.18	**Salinity**	0.40	0.12	-0.20	0.10
Temperature	0.07	0.28	-0.12	-0.01	**Temperature**	-0.05	0.36	-0.13	-0.23
O_2	0.21	0.28	-0.21	-0.36	**O_2**	-0.14	0.29	-0.32	-0.45
pHins	0.43	0.04	-0.11	0.03	**pHins**	**-0.68**	-0.33	-0.11	-0.30
Conductivity	0.32	**-0.54**	0.15	0.24	**Conductivity**	0.23	0.26	**-0.52**	-0.34
	Spring					Summer			
	S	N	J'	H'		S	N	J'	H'
Salinity	0.26	0.11	**-0.58**	-0.10	**Salinity**	-0.10	0.03	-0.01	-0.11
Temperature	-0.17	-0.21	0.29	-0.05	**Temperature**	-0.16	0.24	-0.39	-0.33
O_2	-0.42	0.43	0.33	-0.11	**O_2**	-0.35	-0.10	-0.17	-0.26
pHins	-0.19	-0.33	**0.77**	0.45	**pHins**	0.02	**0.62**	-0.41	-0.38
Conductivity	0.12	-0.25	-0.19	-0.11	**Conductivity**	0.28	**-0.69**	0.27	0.24

According to the Bray-Curtis similarity index values, the stations were clustered in four groups (A, B, C and D) with values higher than 40% (Figure 2). The group A is generally includes the sites with *M. galloprovincialis* biotopes. The most contributing species to the similarity within the group A were *Mytilus galloprovincialis* and *Mytilaster lineatus* (Table 6). The group B comprises the summer stations with average similarity of 53% and *R. splendida*, and *R. membranacea* were the most contributing species to the similarity in the group B. The stations consisting the group C included *Z. marina* biotopes. The similarity of the stations forming the group D showed an average similarity 47%. Within the group *T. pullus pullus*, and *B. reticulatum* were the most contributing mollusc species to the similarity. SIMPER analysis revealed the similarities/dissimilarities in the groups and the most contributing species.

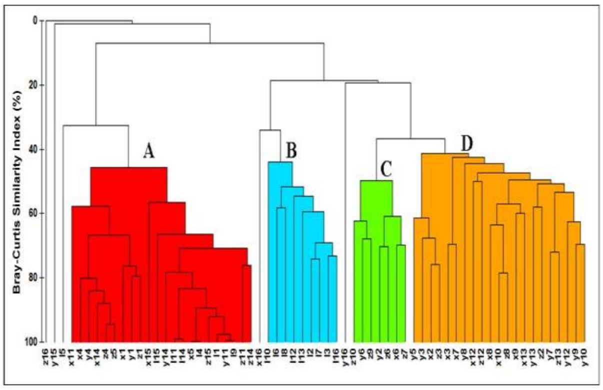

Figure 2. Bray-Curtis similarity analysis of the temporal samples (each association has a similarity higher than 40%). X: Autumn, Y: Winter, Z: Spring, I: Summer

Table 6. The most contributing species to the formation of assemblages in the stations among the seasons

	SIMILARITY				DISSIMILARITY					
Groups	**A**	**B**	**C**	**D**	**A-B**	**B-C**	**A-C**	**A-D**	**C-D**	**B-D**
Similarity/Dissimilarity (%)	**60**	**53**	**56**	**47**	**96**	**91**	**98**	91	**63**	**77**
M. galloprovincialis	87				30		30	18		
M. lineatus	11				11		11			
B. reticulatum			67	53		24	23	21		24
T. pullus pullus				12						
T. neritea			19						9	
R. splendida		56			20	21				10
R. membranacea		29				10				

DISCUSSION

In the present work, a total of 66 mollusc species belonging to three classes were identified in the benthic material sampled in Kemer Bay. The present study is the first exhaustive work on mollusc species in the region and may contribute to the monitoring of the ecological changes that may occur in the region in the following years.

The results of the study are comparable with the results of some studies on mollusc species performed in the Sea of Marmara. The findings in the study by Bitlis (2019) are compatible with those found herein. Bitlis (2019), which in a study on *Cystoseira barbata* facies in the Sea of Marmara, indicated that the class Gastropoda was the richest taxon regarding the species number, followed by the classes Bivalvia and Polyplacophora and among the families Rissoidae, and Pyramidellidae were the richest ones in terms of the number of species. In the same study was also reported that some species such as *R. splendida*, *M. lineatus*, *S. adansonii*, and *B. reticulatum* were the most dominant and frequent species taxa in the region.

In the study by Çınar et al. (2020), who investigated the macroinvertebrates associated with *M. galloprovincialis* in the Sea of Marmara, were obtained similar results presented herein. They found *B. reticulatum*, *M. lineatus, T. pullus pullus*, and *S. adansonii* were found to be the species with high dominance values. Moreover, *M. lineatus* was indicated as a species with constant distribution value of frequency index 77% in the area.

In the present study, an important positive correlation is found between the values of evenness index and pH in the summer season, which result compatible to those reported by Bitlis (2019) who found a negative correlation between the evenness index values and pH in October.

Regarding the SIMPER analysis performed in the present study, *Mytilus galloprovincialis*, *M. lineatus*, *B. reticulatum* and *R. splendida* are of interest as the species the most contributing to the groupings of the stations. A similar result was also indicated by Çınar et al. (2020). *M. lineatus* was mentioned among the species responsible for the groupings of the stations in the PCoA graph by Çınar et al. (2020).

As a result, the ancient city of Parion is also located behind the port of Kemer in the area and Kemer village was evaluated in the category of ancient cities in 2016. Therefore, the Kemer Bay should be protected against human and industrial impacts. In this context, the construction of the thermal power plant (İÇDAŞ) launched in 2011, will probably have some impact on the ecological properties of the area. After the power plant is put into operation, it would be beneficial for the area to be monitored the impact of the pressure of this increasing industrialization, global warming, and mucilage on marine benthic diversity including mollusc species every year or every two years.

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AUTHOR CONTRIBUTIONS

Banu Bitlis: Designing of the study, sorting into taxonomic groups, identification of mollusc species, data analysis, writing-original draft preparation, submission, writing-review and editing, software, visualization. Bilal Öztürk: Designing of the study, identification of mollusc species, checking-original draft preparation. Yusuf Şen: Designing of the study data collection, sorting into taxonomic groups, checking-original draft preparation.

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 this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Growth performance, molting frequency and carapace coloration of marbled crab (*Pachygrapsus marmoratus*) in different salinity levels

Mermer yengecin (*Pachygrapsus marmoratus*) farklı tuzluluk seviyelerinde büyüme performansı, kabuk değiştirme frekansı ve karapas renklenmesi

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Abstract: This study was conducted to evaluate the parameters on growth performance, molting frequency and carapace coloration of marbled crab (*Pachygrapsus marmoratus* Fabricius, 1787). Crabs were collected from Urla, İzmir. The experiment was performed in 10 L plastic containers filled with 6 L of seawater at four different salinity levels (5‰, 15‰, 25‰ and 35‰). Ten crabs with an initial mean weight of 0.78±0.03 g were placed in each container with three replicates. Crabs were fed once a day with a commercial diet (46% protein and 18% lipid) for 12 weeks. At the end of the study, the final mean weight (FMW) of the 25‰ group was significantly higher than the 5‰ and 15‰ groups (P<0.05). Specific growth rates (SGR) of the 15‰ and the 35‰ groups and feed conversion ratio (FCR) of the 25‰ group were significantly higher than the 5‰ group (P<0.05). The mean molting frequency (MMF) of the 25‰ group was significantly higher than the 5‰ group (P<0.05). Final lightness (*L**) of the 5‰ and 15‰ were significantly lower than their initials (P<0.05). Final redness (*a**) of the 25‰ group was the highest among the experimental groups (P<0.05). Final yellowness (*b**) of the 25‰ group was significantly higher than the 5‰ and 15‰ groups (P<0.05). According to the results, it is recommended to keep the salinity at 25‰ under marble crab rearing conditions. Further studies are needed to reveal the potential properties of this species in marine aquariums.

Keywords: Crab feeding, survival, growth, pigmentation, container

Öz: Bu çalışma, mermer yengeçlerin (*Pachygrapsus marmoratus* Fabricius, 1787) büyüme performansı, kabuk değiştirme frekansı ve karapas rengine ilişkin bilgileri değerlendirmek için yürütülmüştür. Yengeçler İzmir, Urla'dan toplanmıştır. Deney, dört farklı tuzluluk seviyesinde (‰5, ‰15, ‰25 ve ‰35) 6 L deniz suyu ile doldurulmuş 10 L'lik plastik kaplarda gerçekleştirilmiştir. On yengeç (başlangıç ortalama ağırlıkları 0,78±0,03 g) her bir kaba üç tekrarlı olarak rastgele yerleştirilmiştir. Yengeçler, 12 hafta boyunca ticari bir yemle (%46 protein ve %18 yağ) günde bir kez doymaya yakın beslenmişlerdir. Deneme sonunda ‰25 grubunun son ortalama ağırlığı, ‰5 ve ‰15 gruplarından anlamlı derecede yüksektir (P<0,05). ‰15 ve ‰35 gruplarının spesifik büyüme oranları ve ‰25 grubunun yem dönüşüm oranı ‰5 grubundan önemli ölçüde yüksektir (P<0,05). ‰25 grubunun ortalama kabuk değiştirme frekansı, ‰5 grubundan önemli ölçüde yüksek bulunmuştur (P<0,05). ‰5 ve ‰15 gruplarının son parlaklığı (*L**) ilk değerlerine göre önemli ölçüde düşmüştür (P<0,05). ‰25 grubunun son kırmızılığı (*a**) deney grupları arasında en yüksektir ve bu grubun son sarılığının (*b**), ‰5 ve ‰15 gruplarından belirgin şekilde daha yüksek olduğu ortaya konmuştur (P<0,05). Bulgulara göre, mermer yengecinin büyütme koşullarında tuzluluğun ‰25'te tutulması tavsiye edilmektedir. Bu türün deniz akvaryumlarındaki potansiyel özelliklerini ortaya koyabilmek için daha fazla çalışmaya ihtiyaç vardır.

Anahtar kelimeler: Yengeç besleme, yaşama oranı, büyüme, pigmentasyon, konteyner

INTRODUCTION

Aquarium enthusiasts have more interested in marine aquariums with the enhancements in aquarium technology. Approximately 1500 fish species have been considered pets in the marine aquarium sector and during the last decade, about 20-24 million fish have been sold annually (Türkmen et al., 2011). Moreover, not only the popularity of fish but also decapod species such as shrimps, lobsters and crabs have become more attention in this sector (Karadal, 2018). Among them, the crabs are preferred in marine aquariums due to their strange appearances. Brachyuran and Anomuran crabs were amongst the most commonly traded decapod species in the saltwater aquarium sector (Penha-Lopes et al., 2005).

The feeding behavior of an organism is very important in terms of determining its living conditions, nutritional status and reproductive activities (Hughes, 1993). Crabs are generally classified as omnivores or detritivores based on their diet. For this reason, they have a key role in the food chain of many ecosystems in nature (Penha-Lopes et al., 2009). Brachyuran crabs' diet mainly consists of limpets, barnacles, mussels and algae (Cannicci et al., 2002). However, artificial feeds can also be used to keep these animals in artificial environments such as aquariums. Hence, it is very important to examine the interactions with the basic water parameters (temperature, salinity, pH, etc.) in the experimental environment where the crabs will be fed with formulated feeds (Cadman and

Weinstein, 1988). Pedapoli and Ramadu (2014) stated the water quality parameters directly impact feed utilization, growth performance, health and survival of crab species. Since the variability of these abiotic parameters can adversely affect aquaculture activities, the importance of studies conducted under controlled conditions has increased. For instance, salinity is one of the most important abiotic parameters affecting larval development, growth, survival, distribution and food stress of crab species (Nurdiani and Zeng, 2007; Bianchini et al., 2008). The juvenile and adult crabs have a wide tolerance of salinity requirements, while their larvae can be vulnerable to varying salinity conditions (Diele and Simith, 2006). While the influences of salinity on various crab species in rearing conditions have been documented by previous studies (i.e., Anger et al., 1990; Anger, 1991; Bas and Spivak, 2000; Jantrarotai et al., 2002; Nurdiani and Zeng, 2007; Ikhwanuddin et al., 2012; Long et al., 2019), only the survival rate and restricted growth of the marbled crab (*P. marmoratus*) at five different salinities have been investigated (Karadal, 2018). In the previous study (Karadal, 2018), the crabs were starved for 140 days in the dry area and five salinities (0‰, 5‰, 15‰, 25‰ and 35‰). No crabs survived for the first 2 weeks in the dry area and with 0‰ salinity. Hence, dry and 0‰ salinity groups were eliminated in this study and according to these results, it was considered to conduct the study at four different salinity levels.

Since the culture of the species traded in the marine aquarium sector is not common, approximately 95% of them are collected from nature (Monticini, 2010; Domínguez and Botella, 2014). Therefore, it is important to consider potential alternatives to these in order to reduce the destruction of the natural populations of popular species. Marbled crab (*Pachygrapsus marmoratus*) is a brachyuran decapod found in the intertidal rocky regions of the Black Sea and Mediterranean (Flores and Paula, 2002; Aydın et al., 2014; Deli et al., 2015). Karadal and Öndes (2018) suggested that there are 35 crab species at the cost of Turkey that can be considered suitable decapods in marine aquariums. Although there are some studies on the natural diets and feeding habits of marbled crab (Cannicci et al., 2002; Cannicci et al., 2007; Silva et al., 2009), no comprehensive feeding study has been found in laboratory conditions. In this study, growth performance, molting frequency and carapace coloration of marbled crab at four different salinity levels were investigated. With this study, the responses of marble crab in terms of growth, molting and coloration parameters at different salinities under laboratory conditions will be recorded and it will be evaluated whether they are suitable for keeping in marine aquariums.

MATERIAL AND METHODS

Collection of crabs

Marbled crabs (*Pachygrapsus marmoratus*) were caught from the rocky shores by hand in Urla, İzmir (38° 22' 17'' N, 26° 45' 44'' E) in June 2018. Crabs, which had same molt stage (hard carapace) and did not have limb loss, were selected for the experiment. A total of 120 crabs were transferred to plastic containers in the Education & Research Unit of Faculty of Fisheries, İzmir Kâtip Çelebi University, İzmir, Turkey.

Rearing systems

The experiment was performed in 10 L plastic containers filled with 6 L of marine water with different salinity levels. Ten crabs (initial mean weights of 0.78±0.03 g) were randomly placed in each container with three replicates (Figure 1). Each container contained PVC pipes with a diameter of 4 cm as shelter and a stone placed in the container. The water temperature was maintained at 18.4±0.3ºC. Salinity, temperature, dissolved oxygen and pH (Hach HQ40D Portable Multi Meter) were checked in each container daily. Dissolved oxygen was 9.27±0.04 ppm, pH was 7.65±0.06 and photoperiod was held at 12:12 (light/dark). The water in all containers was changed at a ratio of 20% once a week.

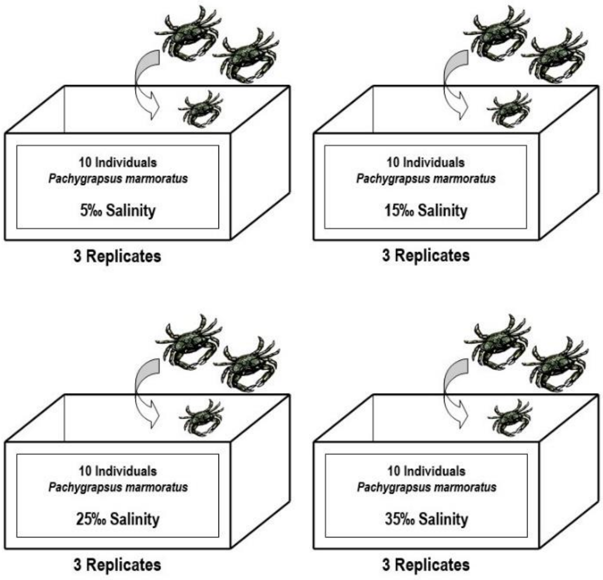

Figure 1. Design of the experimental study

Experimental design and feeding

Four different salinity levels (5‰, 15‰, 25‰ and 35‰) were used to determine the growth performance, molting frequency and carapace coloration of marbled crab. Animals were kept and fed (with the same feed used in the trial) in the rearing system for two weeks for acclimation. In the trial, crabs were fed once a day with 1 mm commercial gilthead seabream diet (46% protein and 18% lipid) and uneaten food was removed after 1.5 h from feeding by siphoning and weighed. At the beginning of the experiment, the same weight of commercial feeds was weighed for each plastic container and was stocked in small boxes and the crabs were fed with these feeds during the trial. The feed intake (FI) of the crabs was recorded by weighing the feeds in these boxes during the biweekly measurements. The experiment was carried out in 12 plastic containers in three replications for 12 weeks.

Evaluation of growth performance

Weight (g) and length (cm) measurements were carried out biweekly during the experiment. All animals were dried with a paper towel to remove the water before the measurement process. Crab weights were individually recorded with an electronic compact scale (SF-400D, precision of ±0.01 g). Carapace length (CL; length of the carapace along the midline) and carapace width (CW; width of the frontal region of the carapace) were measured with the digital caliper. Dead animals were recorded during the experiment, and their survival rates were determined at the end of the trial. Growth performance such as feed conversion ratio (FCR), specific growth rate (SGR) and survival rate (SR) was calculated according to the following formulas:

FI = Average of the total feed given to each experimental group during the study

FCR = Feed intake / Weight gain (where Weight gain was calculated as Final mean weight - Initial mean weight),

SGR (%/day) = 100 x ([Ln Final crab weight] - [Ln Initial crab weight]) / Experimental days,

SR (%) = 100 x (Number of total crab - Number of dead crab) / Number of total crab.

Monitoring of molting frequency

The crabs were checked daily and shells in the containers were recorded for determining the numbers of molts in the experimental groups. According to the recorded data, the mean molting frequency, n: the number of molted individuals; (MMF) = $[(n_1 \times 1) + (n_2 \times 2) + (n_3 \times 3) + \ldots\ldots + (n_k \times k)]$ / total number of crab (Chen and Chen, 2003).

Carapace coloration measurement

Color measurements were taken from all crabs at the beginning and end of the experiment in order to obtain coloration data. Measurements were taken from the carapace region of the crabs on a flat surface with a colorimeter (Color Muse, Variable Inc., Tennessee, USA) (Dang et al., 2021). The measurements were achieved on top surface (4 mm) of carapace of each crab. The CIELAB color space parameters (CIE, 1976) were recorded as lightness (where 100% stands white and 0% stands black), redness (where +a* stands for red and −a* stands for green) and yellowness (where +b* stands for yellow and −b* stands for blue).

Statistical analysis

The Shapiro-Wilk W and Levene tests were subjected to verify normality and homogeneity of variance before further analysis was undertaken, respectively. One-way analysis of variance (ANOVA) was performed for the analysis of the data of growth performance, molting frequency and carapace coloration. Differences between the experimental groups were ranked Tukey's multiple range test. All means were presented with standard errors (±SE). For statistical assessment of the study data, a statistical software (Statgraphics Centurion XVI, Statpoint Technologies Inc., The Plains, VA) was used (Zar, 1999). Differences were considered significant at the 95% confidence interval.

RESULTS

The growth performance of marbled crab reared in different salinity levels is given in Table 1. Final mean weight (FMW) of the 25‰ group was significantly higher than the 5‰ and 15‰ groups (P<0.05). Final mean carapace length of the 5‰ groups was lower than 25‰ group (P<0.05). The highest feed intake was obtained in the 25‰ group (P<0.05). Specific growth rates (SGR) of the 25‰ and the 35‰ groups were significantly higher than the 5‰ group (P<0.05). Feed conversion ratio (FCR) of the 25‰ group was statistically higher than the 5‰ group (P<0.05).

Mean weights (MW) and mean carapace lengths (MCL) during 12 weeks and final survival rates (SR) of marbled crab fed on different salinities are shown in Figures 2, 3 and 4. The MW and MCL of the 5‰ group were lower than the others. The SR of the 15‰ (93.33%) and 25‰ (90%) groups were significantly higher than the 5‰ (63.33%) group (P<0.05).

Table 1. Growth performance of marbled crab reared in saltwater with different salinity levels for 12 weeks

	5‰	15‰	25‰	35‰
Initial mean weight (g)	0.79±0.01	0.85±0.03	0.77±0.02	0.72±0.02
Final mean weight (g)	1.79±0.06^{a}	2.25±0.09^{bc}	2.54±0.14^{d}	2.41±0.08^{cd}
Initial mean carapace length (cm)	1.08±0.03	1.14±0.02	1.08±0.02	1.05±0.01
Final mean carapace length (cm)	1.49±0.04^{a}	1.63±0.07^{ab}	1.74±0.06^{b}	1.68±0.05^{ab}
Feed intake (g)	0.75±0.03^{a}	1.21±0.08^{b}	1.64±0.11^{d}	1.47±0.09^{c}
Specific growth rate (%/day)	0.97±0.12^{a}	1.16±0.08^{ab}	1.42±0.08^{b}	1.44±0.10^{b}
Feed conversion ratio	0.75±0.07^{a}	0.86±0.11^{ab}	0.93±0.05^{b}	0.87±0.09^{ab}

Different letters in the same line indicate statistically significant differences (P<0.05) among the groups.

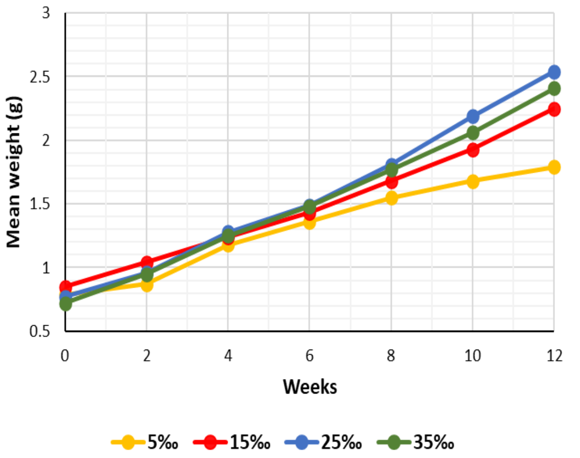

Figure 2. Mean weights of marbled crab during 12 weeks of feeding on different salinities

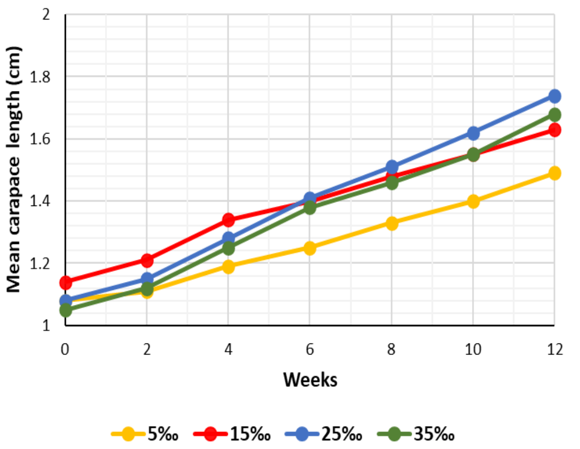

Figure 3. Mean carapace lengths of marbled crab during 12 weeks of feeding on different salinities

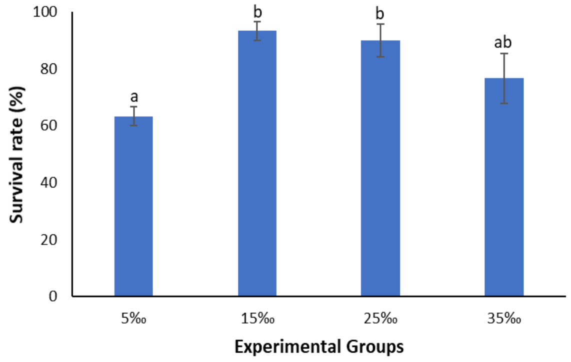

Figure 4. Survival rates of marbled crab fed on different salinities

Molting parameters of marbled crab reared in different salinities are presented in Table 2. Mean molting frequency (MMF) of the 25‰ group and mean molting number (MMN) of the 15‰ and 25‰ groups were significantly higher than the 5‰ group (P<0.05). According to the number of molts, the highest number of individuals in terms of groups was recorded as 8 (2 times) in the 5‰, 12 (3 times) in the 15‰, 11 (4 times) in the 25‰ and 7 (2, 3 and 4 times) in the 35‰ groups.

Carapace coloration parameters of marbled crab in different salinity levels are listed in Table 3. There was no significant difference in the final lightness (*L**) of the experimental salinity groups (P>0.05). Final lightness (*L**) of the 5‰ and 15‰ were significantly lower than their initials (P<0.05). Final redness (*a**) of the 25‰ group was the highest among the experimental groups (P<0.05). No significant difference was noted between the initial and final parameters of the redness (P>0.05). Final yellowness (*b**) of the 25‰ group was markedly higher than the 5‰ and 15‰ groups (P<0.05). Initial yellowness (*b**) of the 25‰ and final yellowness (*b**) of the 5‰ and 15‰ groups were significantly lower than their final and initial parameters, respectively (P<0.05).

Table 2. Molting parameters of marbled crab reared in saltwater with different salinity levels for 12 weeks

		5‰	15‰	25‰	35‰
Crab number (*n*)		19	28	27	23
Number of molting	**1**	4	4	1	2
	2	8	8	5	7
	3	6	12	10	7
	4	1	4	11	7
Mean molting frequency		2.19±0.21[a]	2.58±0.11[ab]	3.16±0.13[b]	2.80±0.18[ab]
Mean number of molting		14.00±2.08[a]	24.00±0.58[b]	28.33±1.20[b]	21.67±3.33[ab]

Different letters in the same line indicate statistically significant differences (P<0.05) among the groups.

Table 3. Carapace coloration of marbled crab reared in saltwater with different salinity levels for 12 weeks

	5‰	15‰	25‰	35‰
Initial lightness (*L)**	42.10±0.21^{B}	40.66±0.33^{B}	41.15±0.16	43.07±0.20
Final lightness (*L)**	36.25±0.54^{A}	33.78±0.39^{A}	40.47±0.25	41.83±0.49
Initial redness (*a)**	8.02±0.15	7.88±0.12	8.18±0.09	7.93±0.11
Final redness (*a)**	7.96±0.13^{a}	8.11±0.07^{a}	8.35±0.17^{b}	8.09±0.05^{a}
Initial yellowness (*b)**	15.67±0.21^{B}	16.13±0.32^{B}	15.37±0.19^{A}	15.88±0.25
Final yellowness (*b)**	10.26±0.18$^{a, A}$	12.37±0.41$^{a, A}$	18.93±0.65$^{b, B}$	15.98±0.32^{ab}

Different lowercase and uppercase letters in the same line and the same column indicate statistically significant differences (P<0.05) among the groups, respectively.

DISCUSSION

The present study showed that marbled crabs (*Pachygrapsus marmoratus*) fed in different salinities survived at 15‰ and 25‰ with higher levels. However, the final mean weight (FMW), feed conversion ratio (FCR) and mean molting frequency (MMF) were enhanced at the 25‰, while specific growth rates (SGR) of the 15‰ and the 35‰ groups were higher. In addition, the final redness (*a**) and final yellowness (*b**) of the crab's carapace were improved at the 25‰ salinity level.

Salinity has important in the growth and nutrition of crab species (Fisher, 1999; Nurdiani and Zeng, 2007; Castejón et al., 2015). Fisher (1999) declared a negative correlation between growth performance and salinity of blue crab (*C. sapidus*). In this study, growth depending on optimum salinity level was determined as 25‰ in marbled crabs and they showed a lower growth rate at the further salinity level (35‰). But, as an important issue on this topic, salinity is considered a vulnerable environmental factor for the larval development of crab species and it is possible to say that previous studies focused on this way (Bas and Spivak, 2000; Baylon and Suzuki, 2007; Nurdiani and Zeng, 2007; Ikhwanuddin et al., 2012). For instance, Jantrarotai et al. (2002) stated that the survival rates of orange mud crab (*Scylla olivacea*) to the stage of megalopa from zoea were recorded as 13.16, 22.19, 8.25 and 7.08 percent at 28‰, 30‰, 32‰ and 34‰, respectively. Larvae advanced to the next stage (megalopa) with higher survival at 30‰ than other salinity levels. Therefore, in crustaceans, the survival rate could have been affected by the feeding due to their cannibalistic behaviors. However, salinity tolerance is higher in larger sizes of crabs. Rahi et al. (2020) recorded that even survival at 0‰ in the orange mud crab (*S. olivacea*). Nevertheless, the authors reported that crabs fed at salinity levels of 10‰ and 20‰ had higher FMWs. In the present study, the FMW of the crabs reared at the 25‰ was markedly higher than that of the 5‰ and 15‰ salinity levels. This can be explained by enhanced growth performance at the optimum salinity level for the species. The SGR and FCR results have proved this situation. Both parameters were enhanced by the optimum salinity level (25‰).

In previous research conducted by Cházaro-Olvera and Peterson (2004), the authors studied the different salinity levels (5‰, 15‰ and 25‰) on two *Callinectes* species (*C. rathbunae* and *C. sapidus*) and they stated no significance in molting period of *C. rathbunae*, while the duration was lessened with the further salinity levels in *C. sapidus*. Though, it can be said that the intermolt duration of blue crab (*C. sapidus*) was affected by salinity. A similar result was reported in a study (salinity levels of 5‰, 10‰, 20‰, 30‰ and 40‰) carried out with mud crab (*Scylla paramamosain*) (Gong et al., 2015) as in the *C. sapidus*. Ruscoe et al. (2004) noted a wide range of salinity tolerance in intermolt duration of the 18.43 mg crablets of mud crab (*S. serrata*) and they advised that these resistant features were considered the species a potential nominee for cultivation. In contrast, an ornamental marine crab species, *Stenorhynchus seticornis*, was reared at 30, 35 and 40‰ salinity levels, but the authors declared the salinity affected the percentage survival of the zoeal stages (Hernández et al., 2012). In the present study, marbled crabs (*P. marmoratus*) have successfully molted even at low salinity levels. For this reason, it is thought that this species can temporarily adapt to extreme salinities in aquarium conditions.

The marbled crab is one of the common intertidal decapods in the Mediterranean Basin (Flores and Paula, 2002), which can be considered a potential aquarium animal due to its high population and its attractive colors of the body (Karadal and Öndes, 2018). The colors of the crab show considerable variation from violet brown to black with a yellowish-brown with marbled form and there is also great patterning in crab species (Ingle, 1997; Caro, 2018). Salinity-related coloration changes in crabs are a study limited. In previous research, McGaw and Naylor (1992) stated a shore crab (*Carcinus maenas*) shows a range of carapace coloration from green to orange through its molting period and orange crabs are less tolerant to salinity decreases over this duration. It is known that the color difference due to this molting period is a natural physiological change, and the difference in salinity tolerance between two colors can be explained in relation to the vulnerable period in the duration of molting. In this study, unlike the previous research, no major color change was observed in the marbled crabs' inter- or during the molting period. However, a statistically significant color change was noted, which did not affect the visual coloration regarding the different salinity levels. It can be said that this statistical color difference is not a natural change and is a physiological response of the animal depending on external influences i.e., water parameters, lightning. Furthermore, Caro (2018) underlined various factors such as starvation or salinity are related to carapace coloration in several crabs. This raises the issue that changes in other water parameters, not only depending on salinity, may be effective on carapace coloration of the marble crabs, and it is recommended to increase studies on carapace coloration in crabs due to external factors.

CONCLUSION

According to the growth, molting and coloration data evaluated in the study, it was revealed that these parameters increased up to 25‰, which was recorded as the optimum salinity level, and started to decrease at the level of 35‰ salinity. In conclusion, it is recommended to maintain the salinity at 25‰ in the rearing conditions of the marbled crab. While growth and molting are significant parameters in carcinoculture, coloration is an important criterion for potentially identifiable species in aquarium fisheries. Therefore, rearing studies that can improve coloration along with growth are of great importance. Further studies are needed to reveal the potential properties of this species in marine aquariums.

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AUTHORSHIP CONTRIBUTION

Onur Karadal: Conceptualization, Investigation, Data curation, Writing- original draft preparation, reviewing and editing.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

The present study was carried out in accordance with animal welfare and the ethics requirements and complied with the guidelines of the EU Directive 2010/63/EU for animal experiments.

DATA AVAILABILITY

All data generated and analyzed during this study are presented in this article.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Distribution of phytoplanktonic species in the sea snot in 2021 in the Marmara Sea

Marmara Denizi'nde 2021 yılında görülen deniz salyası içerisindeki fitoplanktonik türlerin dağılımları

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Abstract: Sea snot, which was seen and reported in the Adriatic and Tyrrhenian Seas in the early 1990s, had been on Turkey's agenda as an environmental massive disaster from the winter months of 2021 until the end of summer in the Sea of Marmara. Due to the magnitude and topicality of the subject, the samples collected from the coastal areas where sea snot is observed in Marmara from January until July were examined. According to the results obtained, 5 classes were determined in sea snot. Species of algae that secrete mucilage, which provides stickiness to the formation, were also been identified in sea snot. These are 1 dinoflagellate, 2 Prymnesiosides, 5 diatoms, and 2 cyanobacteria species. 8 toxic planktonic species were detected in sea snot: 1 causing PSP poisoning and 3 of dinoflagellate causing DSP poisoning; 1 of prymnesiophisid that releases ichthyotoxin to the sea environment, and 3 of diatoms that cause ASP poisoning.

Keywords: Mucilage, sea snot, dinoflagellates, diatoms, Cyanobacteria

Öz: 1990'lı yılların başlarında Adriyatik ve Tiren Denizlerinde görülüp raporlanan deniz salyası, Marmara Denizi'nde 2020 kış aylarından itibaren yaz sonuna kadar bir çevre felaketi olarak Türkiye'nin gündemini oluşturmuştu. Konunun öneminin büyüklüğü ve güncelliği sebebiyle 2021 Ocak ayından itibaren Marmara'da deniz salyası görülen kıyısal bölgelerden Temmuz ayına kadar toplanan örnekler incelenmiştir. Elde edilen sonuçlara göre deniz salyası içerisinde planktonik olarak 5 sınıf, bu sınıflara ait 8 tür tespit edilmiştir. Deniz salyasında bulunan, oluşumun yapışkanlığı sağlayan müsilajı salgılayan türler de oluşumun içerisinde tespit edilmiştir. Bunlar; 1 dinoflagellat, 2 prymnesiosid ve 5 diyatome türüdür. Bunlara ilave olarak 2 tür müsilaj salgısı yapan siyonabakteri görüşmüştür. Deniz salyasında 8 toksik planktonik tür müsilaj içerisinde tespit edilmiştir: PSP zehirlenmesine yol açan 1 ve DSP zehirlenmesine yol açan 3 tür dinoflagellat; denize ihtiyotoksin salan 1 tür prymnesiofisid, ASP zehirlenmesine yol açan 3 tür diyatomdur.

Anahtar kelimeler: Müsilaj, deniz salyası, dinoflagellat, diyatome, siyanobakteri

INTRODUCTION

Climate change affects all living things on earth. In addition to global warming, the other thing that affects vibrancy is pollution. As dramatic examples of these environmental problems on a global scale, we observe algal blooms of several colors seen in certain periods in our seas, Sargassum macroalgae patterns detected from time to time in Dikili, planktonic algae, and sea lettuce overgrowth in the Gulf of Izmir, cyanobacteria-induced breakage, dead fish and jellyfish on our shores. In addition, the sea snot or mucilage phenomenon, which began in the winter of 2020, has increased in the Marmara Sea during the summer since the spring of 2021 and has been added to these negative processes.

In Türkiye, sea snot was first seen on the northeast coast of the Marmara Sea in the autumn of October 2007, around the shores of Izmit and Erdek (Tüfekçi et al., 2010), and reported in the following years. The unseen formation to create a major environmental disaster was reported in January 2008, and the snot structure was recorded to form filamentous structures in the Gulf of Izmit and on the shores of Erdek (Tüfekçi et al., 2010).

Since December 2020 and January 2021, it has been distributed from the Gulf of Izmit to Çanakkale. After 2007 and in 2020, the study of the species *Gonyaulax* of the dinoflagellate was intensely detected and reported in the sea snot body in the winter months (Aktan et al., 2008a). Its cyst formed after the summer months and is never seen until winter. The type of dinoflagellate that is releasing a direct tap into the sea environment *G. fragilis* was also reported as the most dominant species in the environment in the haulage studies in the Adriatic Sea (Pompei et al., 2003; Pistocchi et al., 2005).

Chrysophaeum taylorii I.F. Lewis and H.F. Bryan, 1941 species attracted attention by overgrowth in coastal areas of Florida. This species has been detected in sea snot in the Tyrrhenian Sea. This species was detected in the thick slimy tissue covering the seabed and marine plants, mainly *Posidonia oceanica* (Linnaeus) Delile, 1813, on the eastern coast of Sardinia Island and reported in 2007. It has been reported that *C. taylorii* species forms clusters and covers the seafloor by overgrowth on the seabed on the shores of Bodrum in the Eastern Mediterranean. This species, which is capable of producing slimy mucilage, accumulated at the bottom, and formed clumps in the middle water, but was not seen on the surface (Aktan and Topaloğlu, 2011). In the research conducted on the sea snot content, it was reported that some types of predominant, mucilage-capable dinoflagellate and diatom were not directly responsible for the creation of the sea snot environmental disaster (Negro et al., 2005). Similarly, certain planktonic species detected in the sea snot formations seen in different parts of the world are not directly responsible for the sea snot (Sherr and Sherr, 1987). *G. fragilis* (=*G. hyalina* (Mackenzie et al., 2002), which is densely detected in the sea snot in the Gulf of Tasmania; *Phaeocystis* sp. (Lancelot, 1995), which is seen in the winter sea snot on the shores of the North Sea; In the Adriatic, (Pompei et al., 2003) and the appearance of the *G. fragilis* (Aktan et al., 2008b; Tüfekçi et al., 2010; Balkis et al., 2011; Toklu-Alicli et al., 2020) species seen during the spring period in Marmara Sea are other examples in terms of species that are not responsible for the formation of direct sea snot.

Starting in the 18 st century in the Adriatic, 19th century irregularly the formation of the sea snot continues to be seen in the 21st century. By the early 20th century, it was one of the things that was not studied very much (Innamorati et al., 1995). In 1977, in the North Sea (Lancelot, 1995), mainly on the beaches of France, Belgium, the Netherlands, and Germany; in 1981 in the Gulf of Tasmania in New Zealand (Bradstock and Mackenzie, 1981), 1988 in the Adriatic (Rinaldi et al., 1995; Vollenweider et al., 1995) was observed in Tiren Bay (Melley et al., 1998) in 1991, in the Ligurian Sea (Schiaparelli et al., 2007) in 2003, in Marmara Sea (Aktan et al., 2008a; Tüfekçi et al., 2010; Balkis et al., 2011, 2013; Toklu-Alicli et al., 2020; Balkis-Ozdelice et al., 2021) and in the North Aegean (Nikolaidis et al., 2008) in 2008. In the Adriatic, Tiren Sea, Mediterranean, and Marmara Sea, the frequency of the sea snot is shown in Figure 1.

The aim of this study is to determine the phytoplanktonic species in the sea snot, which became an environmental disaster in the Marmara Sea in 2021, to create a list, to determine the species that directly secrete mucilage and release marine toxins, and to determine their rates.

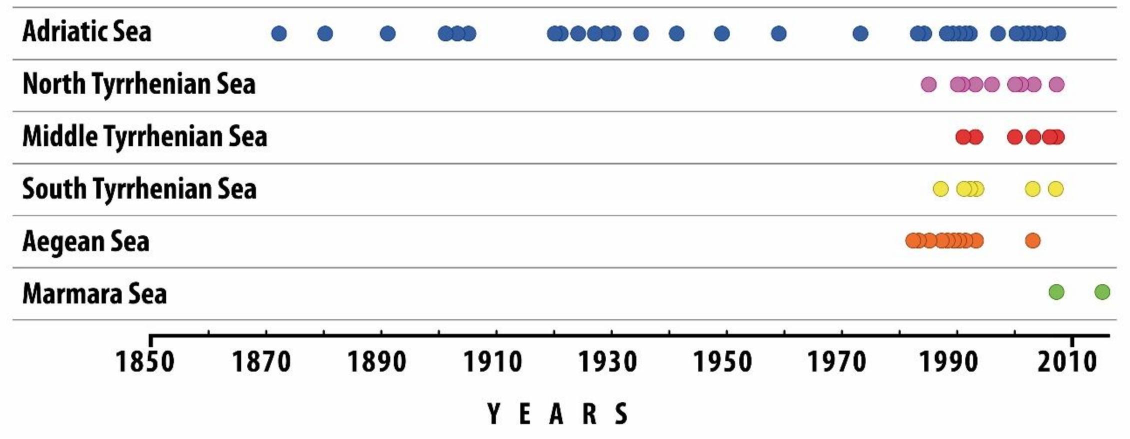

Figure 1. Frequency of sea snot appearance (by changing from Danovaro et.al., 2009)

MATERIAL AND METHODS

At the beginning of 2021, samples were collected monthly from 6 stations between January and July (Figure 2), when the stratification of marine snot began to be observed in the Marmara Sea, and the species list was formed by combining the results of the 7-month examination (Table 2).

Sea snot formation from the sea surface and up to 10 cm below the surface was collected with gloves for protection from pathogens in its content and put in 5-liter containers with seawater. Samples fixed in situ with 4% formaldehyde were brought to the laboratory on ice in an ice storage container within 6 hours at the latest. Samples stored at +4 degrees were examined with Olympus BX-50 and Olympus CX-31 microscopes. For identification of diatoms and dinoflagellates, Anderson et al. (1995), Balech (1988), Cupp (1943), Delgado and Fortuno (1991), Dodge (1982), Hasle and Syaten (1997), Hendey (1964), Koray et al. (2007), Lebour (1930), Marshall (1969), Rampi and Bernhard (1978, 1980), Ricard (1987), Steidinger and Williams (1970), Steidinger and Tangen (1997), Sournia (1968, 1976, 1986), Taylor (1976), Tomas (1997), Trégouboff and Rose (1957), and Wood (1954).

The studies of Anderson et al. (1995) and Landsberg (2002) were also used for HAB types. The current naming of the taxa was checked from the AlgaeBase web page (Guiry and Guiry, 2022).

Figure 2. Stations from which sea snot samples were taken between January and July (Illustrated by Yurga, 2022)

RESULTS

By examining the sea snot samples collected in the Marmara Sea between January and July 2021, a total of 55 taxa and 44 genera belonging to 5 algae classes were determined. These genera are: 14 Dinophyceae, 2 Prymnesiophyceae, 1 Dictyochophyceae, 26 Bacillariophyceae, and 1 Euglenophyceae. In addition to these classes, 2 species from the cyanobacteria class, 1 from the Florideophyceae class, 1 from the Ulvophyceae class, and 1 from the Pelagophyceae class are other species detected in the sea snot structure. The species belonging to the largest class, which was detected with a rate of 59.1% in sea snot, belong to the class Bacillariophyceae, followed by Dinophyceae with a rate of 31.8%. Prymnesiophyceae is 4.5%, and Dictyophyceae is 2.3% (Table 1).

A species list of 55 taxa and 44 genera, belonging to 5 classes detected in sea snot, was created (Table 2).

In the sea snot, 3 non-phytoplanktonic classes were determined as present in the salivary structure. These species, which release the mucilage slime structure to the marine environment, are the species that contribute to the thickening of the sea snot (Table 3).

The distribution of the species detected in sea snot, 19 taxa belonging to the Dinophyceae class. 2 taxa Coccolithophorid *Emiliania huxleyi* (Lohmann) W. Hay & H. P. Mohler, 1967 and *Phaeocystis pouchetii* (Hariot) Lagerheim, 1896 of the Prymnesiophyceae class. *Octactis speculum* (Ehrenberg) F.H.Chang, J.M.Grieve & J.E.Sutherland, 2017 is the only taxa detected in sea snot belonging to the class Dictyochophyceae. 32 taxa of the class Bacillariophyceae were detected in sea snot. *Eutreptiella gymnastica* Throndsen 1969 is the only taxa identified belonging to the class Euglenophyceae.

Table 1. The frequency of the types of classes detected in the period, the frequency of detection, and the percentage of the types secreting toxins in the environment

Class	Frequency of detection				Frequency %		
	G	T	PTM	TX	T	PTM	TX
DINOPHYCEAE	14	19	1	4	31.8	12.5	80.0
PRYMNESIOPHYCEAE	2	2	2	1	4.5	25.0	20.0
DICTYOCHOPHYCEAE	1	1	0	0	2.3	0.0	0.0
BACILLARIOPHYCEAE	26	32	5	0	59.1	62.5	0.0
EUGLENOPHYCEAE	1	1	0	0	2.3	0.0	0.0
Total	**44**	**55**	**8**	**5**	**100**	**100**	**100.0**

G: Genus, T: Species, PTM: Produces the mucilage, TX: Toxic

Table 2. Classes and species detected in sea snot

DINOPHYCEAE	**Phylum**
Alexandrium minutum Halim, 1960 *Cochlodinium polykrikoides* Margalef, 1961 *Dinophysis caudata* W.S.Kent, 1881 *Dinophysis tripos* Gourret, 1883 *Gonyaulax fragilis* (Schütt) Kofoid, 1911 *Gymnodinium simplex* (Lohmann) Kofoid & Swezy, 1921 *Gyrodinium fusiforme* Kofoid & Swezy, 1921 *Noctiluca scintillans* (Macartney) Kofoid & Swezy, 1921 *Oxytoxum scolopax* Stein, 1883 *Phalacroma rotundatum* (Claparéde & Lachmann) Kofoid & J.R.Michener, 1911 *Pronoctiluca pelagica* Fabre-Domergue, 1889 *Prorocentrum micans* Ehrenberg, 1834 *Prorocentrum scutellum* Schröder, 1900 *Protoperidinium bipes* (Paulsen, 1904) Balech, 1974 *Pyrophacus horologium* F.Stein, 1883 *Scrippsiella acuminata* (Ehrenberg) Kretschmann, Elbrächter, Zinssmeister, S.Soehner, Kirsch, Kusber & Gottschling, 2015 *Tripos furca* (Ehrenberg) F.Gómez, 2013 *Tripos fusus* (Ehrenberg) F.Gómez, 2013 *Tripos muelleri* Bory de Saint-Vincent, 1826	Myzozoa
PRYMNESIOPHYCEAE	**Phylum**
Emiliania huxleyi (Lohmann) W.W.Hay & H.P.Mohler, 1967 *Phaeocystis pouchetii* (Hariot) Lagerheim, 1896	Haptophyta
DICTYOCHOPHYCEAE	**Phylum**
Octactis speculum (Ehrenberg) F.H.Chang,J.M.Grieve & J.E.Sutherland, 2017	Ochrophyta
BACILLARIOPHYCEAE	**Phylum**
Asteromphalus flabellatus (Brébisson) Greville, 1859 *Cerataulina pelagica* (Cleve) Hendey, 1937 *Chaetoceros teres* Cleve, 1896 *Coscinodiscus lineatus* Ehrenberg, 1841 *Coscinodiscus radiatus* Ehrenberg, 1840 *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C.Lewin, 1964 *Dactyliosolen fragilissimus* (Bergon) Hasle, 1996 *Ditylum brightwellii* (T.West) Grunow, 1885 *Grammatophora marina* (Lyngbye) Kützing, 1844 *Guinardia flaccida* (Castracane) H.Peragallo, 1892 *Leptocylindrus danicus* Cleve, 1889 *Licmophora abbreviata* C.Agardh, 1831 *Licmophora flabellata* (Grev.) C.Agardh, 1831 *Navicula tripunctata* (O.F.Müller) Bory de Saint-Vincent, 1822 *Nitzschia longissima* (Brébisson) Ralfs, 1861 *Nitzschia sigma* (Kützing) W.Smith, 1853 *Pleurosigma elongatum* W.Smith, 1852 *Proboscia alata* (Brightwell) Sundström, 1986 *Pseudo-nitzschia delicatissima* (Cleve) Heiden, 1928 *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle, 1993 *Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle, 1993 *(Nitzschia pungens)* *Pseudosolenia calcar-avis* (Schultze) B.G.Sundström, 1986 *Rhizosolenia setigera* Brightwell, 1858 *Skeletonema costatum* (Greville) Cleve, 1873 *Stephanopyxis palmeriana* (Greville) Grunow, 1884 *Striatella unipunctata* (Lyngbye) C.Agardh, 1832 *Surirella ovata* Kützing, 1844 *Synedra undulata* (Bailey) W.Smith, 1956 *Thalassionema nitzschioides* (Grunow) Mereschkowsky, 1902 *Thalassiosira pseudonana* Hasle & Heimdal, 1970 *Thalassiosira rotula* Meunier, 1910	Ochrophyta
EUGLENOPHYCEAE	**Phylum**
Eutreptiella gymnastica Throndsen, 1969	Euglenozoa

Table 3. Non-phytoplanktonic classes detected in sea snot and species belonging to these classes

FLORIDEOPHYCEAE	**Phylum**
Ceramium diaphanum (Lightfoot) Roth, 1806	Rhodophyta
ULVOPHYCEAE	**Phylum**
Cladophora laetevirens (Dillwyn) Kützing, 1843	Chlorophyta
PELAGOPHYCEAE	**Phylum**
Chrysoreinhardia giraudii (Derbès & Solier) C.Billard, 2000	Ochrophyta
Nematochrysopsis marina (J.Feldmann) C.Billard, 2000	Ochrophyta

Two taxa belonging to the class Cyanobacteria were detected in sea snot. These are *Leptolyngbya lagerheimii* (Gomont ex Gomont) Anagnostidis and Komárek, 1988, and *Pseudanabaena rutilus-viridis* H.J.Kling, H.D.Laughinghouse and J.Komárek, 2012. Both species are known to secrete the slimy and sticky mucilage into the marine environment, causing thickening of the salivary structure of sea snot.

Planktonic species responsible for secreting mucilage and providing stickiness in sea snot were determined in the sea snot content (Flander-Putrle & Malej (2008). These are *G. fragilis* of the class Dinophyceae; *E. huxleyi* and *P. pouchetii* from the class Prymnesiophyceae; *C. closterium, L. flabellata, S. palmeriana, S. ovata,* and *T. pseudonana* species from Bacillariophyceae class.

Some of the species detected in the examined sea snot samples produce mucilage themselves and secrete mucilage into the marine environment. The number of mucilage-producing species detected in sea snot was 14, and the number of classes belonging to these species was 7. Of these 14 classes, only 3 are planktonic and one is bacterial (Table 4).

Table 4. Mucilage-producing classes and strains identified in mucilage

Class	**Mucilage producing species**
Dinophyceae	1
Prymnesiophyceae	2
Bacillariophyceae	5
Florideophyceae	1
Ulvophyceae	1
Pelagophyceae	2
Cyanobacteria	2
Total	**14**

8 toxic species, which cause the death of living things by releasing toxic chemicals into the marine environment, have been detected in the formation of sea snot. 4 toxic species belonging to Dinophyceae class were determined. These are: *Alexandrium minutum* Halim, 1960, which causes paralytic shellfish poisoning (PSP), *Dinophysis caudata* Saville-Kent, 1881, which causes diarrheal shellfish poisoning (DSP) *Dinophysis tripos* Gourret, 1883, and *Phalacroma rotundatum* (Claparéde and Lachmann) Kofoid and J. Michener, 1911.

Phaeocystis pouchetii (Hariot) Lagerheim, 1896, of the class Prymnesiophyceae, is a normally harmless species. However, when fish larvae and zooplankton become abundant, they release ichthyotoxin into the environment (Danovaro et al., 2008).

It has been detected that 3 species that secrete domoic acid biotoxin belonging to the Bacillariophyceae class cause Amnesic shellfish poisoning (ASP). These are: *Pseudo-nitzschia delicatissima* (Cleve) Heiden 1928, *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle 1993 and *Pseudo-nitzschia pungens* (Grunow ex Cleve) Hasle 1993.

In addition to these; *Cochlodinium polykrikoides* Margalef, 1961 is another non-armored dinoflagellate species that is detected as a cyst, although it is inside the sea snot. Even though these are not alive in the environment, their cysts are ichthyotoxic (Tang and Gobler, 2009, 2012).

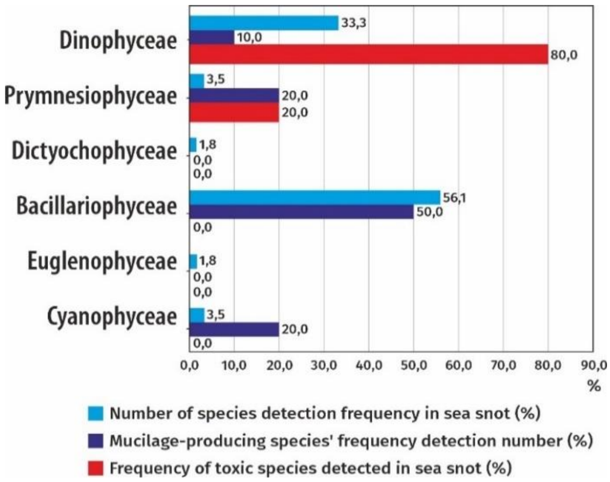

Figure 3. Frequency of occurrence of 5 planktonic groups and species of cyanobacteria detected in sea snot, distribution of species capable of producing mucilage, and toxic species in the sea snot

The frequency of occurrence of the species of 5 planktonic groups detected in the sea snot the mucilage producers of these species and the species detected in the sea snot that have the feature of releasing toxic toxins to the sea (Figure 3). The most dominant class and mucilage-producing species in sea snot belong to the class Bacillariophyceae. The toxin-releasing classes found in sea snot are the species belonging to the Dinophyceae and Bacillariophyceae classes. The most toxic species in sea snot was Dinophyceae (80%). The incidence of cyanobacterial species, which are capable of producing mucilage and detected in sea snot, was calculated as 3.5%. In terms of mucilage-producing species in sea snot, the incidence rate of cyanobacteria was determined as 20%. (Figure 3).

DISCUSSION

Mass accumulations of mucilage aggregates, which are not commonly seen in the oceans, and large, several meters in size, light and dark green cloud clusters in the water column can only be seen periodically in heavily polluted seas such as the Adriatic and Marmara Seas (Aktan et al., 2008a; Najdek et al., 2005). An example of a major oil spill in the Gulf of Mexico is the triggering of sea snot formation in an area where sea snot has never been seen before as a result of excessive pollution in the sea. The major oil spill on the BP-operated platform in the Gulf of Mexico on April 20, 2010, is an example of a major marine pollution disaster. The leak was fixed only after 18 months, on 19 September. It is estimated that approximately 780 thousand cubic meters of oil leaked into the sea during this period. This pollution is the largest oil spill in history. After the disaster, most of the native species in the region came to the point of extinction. Due to the great stress on the marine ecosystem, the formation of sea snot in square kilometers has been observed temporarily in the region (Passow et al., 2012).

Some of the cyanobacteria species detected in sea snot are capable of producing mucilage themselves (Durai et al., 2015). Some of these cyanobacterial species can release toxic biotoxins into the sea. *Lyngbya majuscula* Harvey ex Gomont, 1892, which covers the deep, stones, sea plants, and macroalgae on the seabed in coastal areas, is one of the dermatotoxic alkaloids that cause the formation of inflammatory wounds on the skin (Yüksel, 2021). In addition, it is stated that lipopolysaccharides, which are structural elements of bacterial species such as *Escherichia coli* and *Vibrio harveyi* (Danovaro et al., 2009) detected in sea snot, may cause irritation and blisters in case of contact with the skin (Durai et al., 2015).

In this study, 8 HAB-forming species, four of which belong to the Dinophyceae class, were detected in sea snot and were reported in a study conducted in the Sea of Marmara (Taş et al., 2016).

In another study conducted in 2021 on sea snot, 83 phytoplankton species were identified and the dominant class was Bacillariophyceae. The most concentrated species of this class in terms of its concentration per liter was determined as *S. costatum* (11200 l^{-1}) (Ergul et al., 2021).

The distribution of phytoplanktonic species in the sea snot of the Sea of Marmara was made by Balkis-Özdelice et al., 2021 for the first time. 47 species were reported in the study. Three new records were given for these species *and P. pouchetii* was responsible for the mucilage on the surface, and *C. taylorii* and *N. marina* were responsible for the benthic mucilage. When this study was compared with the researchers, 41 common species were determined (74.5%). Researchers also referred to the abundance situation in the environment and stated that coccolithophorids also play an active role in this formation. *C. laetevirens*, belonging to the class Ulvophyceae, was noticed and reported on the Southwest coast of Istanbul in the early summer of 2010 as it is over-proliferated and formed a sea snot-like structure on its own (Taş et al., 2016; Balkıs et al., 2013; Balkis-Ozdelice et al. 2021). This species was detected in sea snot during our examination of samples in the Sea of Marmara. *P. puchetti* from Prymnesiophyceae, *C. giraudii* and *N. marina* from Pelagophyceae have been actively detected and reported in sea snot in the Sea of Marmara were also found responsible for the benthic mucilage (Balkis-Ozdelice et al., 2021).

The first official step in our country in the fight against sea snot is the Marmara Sea Action Plan Coordination Meeting held in Kocaeli on June 6, 2021. In the declaration prepared at the end of the meeting, the Sea of Marmara was declared a protection zone. Monitoring action plans have been initiated regarding the inspection of industrial facilities that discharge wastewater into flowing streams and the installation of biofilters, the problems that may occur in the strait and the difficulties experienced by the fishing industry, and the collection of ghost nets, and sea snot. At the Marmara Municipalities Union (MBB), Marmara Sea Action Plan Sea Snot Science and Technical Board meeting held on April 19, 2022, the situation of sea snot after it is collected from the sea surface and an action plan of 22 items planned to be made urgently were created. Within the scope of the Action Plan for the Protection of the Marmara Sea, carried out at TÜBİTAK Marmara Research Center (MAM) on June 29, 2021, all aspects of combating sea snot and the process were discussed. Turkish Academy of Sciences (TÜBA), Marmara Marine Ecology; and prepared a 249-page study called Marmara Marine Ecology; Sea Snot Formation, Interactions and Suggestions for Solutions. In the study, solution suggestions are given for the problem, formation, causes, and prevention of sea snot in Marmara (Öztürk and Şeker, 2021).

CONCLUSION

Sea snot formation, which was intensely observed for the first time in the Adriatic in 1988 (Vollenweider et al., 1995; Rinaldi et al., 1995) and in the Tyrrhenian Sea in 1991 (Melley et al., 1998), was observed in the Marmara Sea in 2007 (Aktan et al., 2008a; Tüfekçi et al., 2010; Balkis et al., 2011) and in 2008 in the North Aegean (Nikolaidis et al., 2008). The formation, which was observed in Marmara and North Aegean in the following years, continued its existence until the winter months of 2021. In the elapsed time, the physico-chemical parameters in the places where sea snot is seen, and the species in the snot formation have been determined and reported. Action plans were created for the phenomenon thought to be caused by global warming and pollution, online evaluation meetings were held at universities during the pandemic process and final declarations were prepared. This phenomenon, which closely affects tourism, fisheries, and marine life, should be followed seriously.

The sea snot environmental disaster is nothing but a remarkable warning for our understanding of Marmara. All living things in our seas, whether they have commercial value or not, are indispensable for a balanced and healthy ecosystem. In addition to events that affect the whole world such as global warming, pollution in the sea affects all living things in the sea. Thanks to canals such as the Suez opened by the shortening of commercial sea routes (Ben-Tuvia, 1973), already endangered species are forced to compete with invasive species. The Marmara Sea is perhaps the most valuable of our seas, where fish are fished, as an ecosystem. Bluefish are also added to the anchovy and sardines, which have decreased due to intense overfishing in the Marmara and Black Seas. This fish, which can barely reach the size of a chinekop, will soon be considered among the endangered species in Marmara (Artüz, 2021).

In conclusion, sea snot is an environmental disaster and wreaking havoc caused by both global warming and human-induced marine pollution. It occurs in heavily polluted seas where there is no food and oxygen left in the environment and is a process initiated by bacteria. The process initiated by the enzymes of the bacteria is a rich stinking viscous slimy coenose, rich in nutrients and oxygen in an oxygen-free and nutrient-free environment, but containing toxic species, and colonies of bacteria and viruses that are harmful to the environment and human health. However, when we control and reduce pollution, we will be able to leave clean seas for future generations.

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CONFLICT OF INTEREST STATEMENT

The author declare that there is no known financial or personal conflict that may affect the research article.

ETHICS APPROVAL

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

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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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Türkiye su ürünleri endüstrisinin rekabet gücünün değerlendirilmesi

Evaluation of competitiveness power of fishing and aquaculture industry in Turkey

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Öz: Türkiye su ürünleri sektöründe girdi ve ürün piyasaları, tüketim karakteristikleri, üretim, pazarlama ve dağıtım konuları ile birlikte arz ve değer zinciri hakkında önemli düzeyde veri ve bilgi eksikliği vardır. Söz konusu veri eksikliğini azaltmak için bu araştırmada Türkiye su ürünleri endüstrisinin rekabet gücünün belirlenmesi ve değerlendirilmesi amaçlanmıştır. Araştırma verileri Türkiye İstatistik Kurumu (TÜİK), Dünya Ticaret Örgütü (DTÖ) ve Birleşmiş Milletler Gıda ve Tarım Örgütünden (FAO) elde edilmiştir. Su ürünleri üretimi ve ticaretiyle ilgili değişkenlere ait 1976-2020 yıllarını kapsayan zaman serisi verileri kullanılmıştır. Türkiye su ürünleri sektörünün rekabet gücü Balassa indeksi, toplam faktör verimliliği ve maliyetler dikkate alınarak ortaya konulmuştur. Araştırma sonuçları, Türkiye'de su ürünleri işletmelerinin küçük ölçekli olduğunu, teknolojik imkanlarının yetersiz kaldığını ve finansman sorunları bulunduğunu göstermiştir. Türkiye su ürünleri endüstrisinin rekabet gücünü gösteren açıklanmış karşılaştırmalı üstünlük (RCA) değeri dünyaya karşı 0.65, AB ülkelerine karşı ise 1.86'dır. Türkiye'nin dünyada rekabet gücü düşük düzeydedir ancak AB ülkelerine karşı mukayeseli üstünlüğü vardır. Türkiye su ürünlerinde arz zinciri vardır, fakat değer zinciri tam olarak oluşturulamamıştır. Türkiye'nin su ürünleri sektöründe arzu edilen yere gelinebilmesi için su ürünleri üretim ve verimliliğinin artırılması, üretim aşamasından itibaren piyasa ile kuvvetli iletişim ve etkileşimde olunması, avcı ve yetiştiriciler ile birlikte arz zincirindeki aktörlerin fiyat eğilimleri, tüketici istek ve ihtiyaçları konusunda farkındalık sahibi olması, üretim ve dağıtımda modern teknolojilerin kullanılması ve yayım/eğitim faaliyetlerinin etkin bir şekilde gerçekleştirilmesi önemlidir. Türkiye su ürünleri sektöründe ihracat imkânları ve rekabet gücünün artırılması için kalite standartlarının uluslararası düzeyde olması sağlanmalıdır.

Anahtar kelimeler: Su ürünleri sektörü, rekabet gücü, Balassa index, toplam faktör verimliliği, veri zarflama yöntemi, Malmquist verimlilik indeksi, Türkiye

Abstract: There has been a significant lack of data on product and input markets, aquaculture consumption characteristics, production, distribution and marketing issues, and supply and value chain in Turkish fishing and aquaculture sectors. Therefore, the study examined the competitiveness of the Turkish aquaculture industry. Time series data belonging the time period of 1976-2020 gathered from Food and Agriculture Organization (FAO), World Trade Organization and Turkish Statistical Institute (TURKSTAT) were used in the study. In the research, the competitiveness of the Turkish fishing and aquaculture sector has been explored by using the Balassa index, total factor productivity and production costs. The research results show that the fisheries in Turkey are small-scale, technological level are insufficient and there are financial problems. Based on the revealed comparative advantage (RCA) values of the world and EU, which are 0.65 and 1.86, respectively, Turkey 's has competitive power over EU countries, while the reverse is the case for the world. Although fisheries supply chain has been established in Turkey, the value chain has not yet been fully established and activated. The study suggests increasing the efficiency in aquaculture production, having a strong interaction with the market during the production phase, having a high awareness of the price trends and consumer needs throughout the supply chain, using modern production and distribution technologies, and developing effective extension/education activities to reach the desired point in the Turkish aquaculture sector. Quality standards should be at the international level to increase export opportunities and competitiveness of Turkish aquaculture sector.

Keywords: Fishing and aquaculture, competitiveness, Balassa index, total factor productivity, data envelopment analysis, Malmquist index, Turkey

GİRİŞ

Dünya nüfusunun ihtiyacı olan gıdanın dengeli ve yeterli bir şekilde karşılanabilmesi için küreselleşmiş gıda endüstrilerinden biri olan, ayrıca sanayi sektörlerine hammadde sağlayan, istihdam olanaklarına ve yüksek ihracat potansiyeline sahip olan su ürünleri sektörüne verilen değer her geçen gün artmaktadır. Geçmişte avlanan balıkların nihai tüketiciye ulaştırılmasını basit arz zinciri ile sağlayan su ürünleri sektörü, günümüzde avcılıkla birlikte su ürünleri yetiştiriciliğinin de yapıldığı çok taraflı ve çok karmaşık bir endüstri haline dönüşmüştür. Küresel ticarette yüksek ve istikrarlı getirisi olan su ürünleri endüstrisi su ürünleri üretme potansiyeline sahip ülkeler için sürdürülebilir tarımsal kalkınmanın ve ekonomik büyümenin önemli bir unsuru olmuştur. Giderek artan öneminden dolayı Birleşmiş Milletler

Gıda ve Tarım Örgütünün 2030 gündeminde ekonomik, sosyal ve çevresel açıdan sürdürülebilir tarımsal kalkınmayı sağlamak amacıyla su ürünleri sektörünün geliştirilmesine vurgu yapılmıştır. Günümüze kadar yaşanan gelişmeler su ürünleri endüstrisinde değer zincirinin oluşmasına ve değer zinciri boyunca uluslararası düzeyde rekabetin artmasına sebep olmuştur. Su ürünleri endüstrisinde artan rekabet, ulusları sahip olunan kaynakları daha verimli ve etkin kullanmaya ve değer zincirini sağlıklı bir şekilde geliştirmeye zorlamıştır. Bu sebeple iklim değişikliği, su kaynaklarındaki kirliliğin artması ve bilinçsiz kaynak kullanımı gibi etkenlerin var olduğu bir ortamda su ürünleri sektörünün sürdürülebilirliğini sağlamak ve ülkelerinin rekabet gücünü artırmak politika yapıcıların gündeminde öncelikli konular arasında yer almaya başlamıştır.

Dünyada yaşanan bu gelişmeler, üç tarafı denizlerle çevrili olan, çok sayıda iç su kaynağı bulunan, neredeyse toplam tarım alanları kadar su ürünleri üretim alanına (25 milyon ha) sahip olan ve ekolojik özellikleri nedeni ile avlanma ve yetiştirme için oldukça uygun bir ortamı olan Türkiye'de de yaşanmıştır. Başlangıçta neredeyse tamamen avcılığa dayalı bir su ürünleri sektörü söz konusu iken, günümüzde yetiştiricilik ile avcılığın payı birbirine yaklaşmış ve sektör çok taraflı ve daha rekabetçi bir yapıya dönüşmüştür. Türkiye su ürünleri sektörünün kendine uluslararası rekabet ortamında hak ettiği seviyede yer bulabilmesi için su ürünleri değer zincirinin tam olarak olgunlaşması ve rekabet gücünün sürekli değerlendirilerek uygun bir gelişim stratejisinin izlenmesi gerekmektedir. Rekabet gücünün ölçülmesi konusu her geçen gün önemini artırmakla beraber, günümüze kadar rekabet gücünün ulusal düzeyde, sektörler arasında, sektör düzeyinde ve sektör içinde değerlendirildiği çalışmalar yapılmıştır. Ulusal düzeyde rekabet gücünü ölçen daha önce yapılmış çok sayıda yerli ve yabancı çalışma bulunmaktadır (Adamkiewicz, 2019; Altay ve Gacaner, 2003; Arezki vd., 2020; Aybudak, 2020; Bandura, 2005; Beckley, 2018; Cho ve Moon, 2000; Esmaeili, 2014; Freudenberg, 2003; Kaitila ve Widgrén, 1999; Kara ve Erkan, 2011; Lovrinčević vd., 2008; Moon vd., 1998; Pilinkienė, 2014; Porter, 1990, 2000; Riaz ve Jansen, 2012; Stanovnik ve Kovačič, 2000; Zinnes vd., 2001). Su ürünleri sektörü dışındaki sektörlerde, rekabet gücünün sektör düzeyinde ölçüldüğü çalışmalar oldukça fazla sayıdadır. (Akmermer ve Ayyıldız, 2010; Arslan ve Tatlıdil, 2012; Canan ve Ceyhan, 2016; Collignon ve Esposito, 2017; Dağdeviren ve Yüksel, 2010; Erkan, 2013; Halkos, 2019; Havrila ve Gunawardana, 2003; Keskingöz, 2018; Marczak ve Beissinger, 2018; Saraçoğlu ve Nezir, 2000; Wijnands vd., 2015).

Su ürünleri sektöründe rekabet gücünün ölçüldüğü çalışma sayısı ise diğer sektörlere nazaran daha sınırlı düzeydedir. Sınırlı sayıdaki çalışmalardan bazıları sadece belirli balık türüne odaklanmış ve seçilmiş göstergeleri veya indeksleri kullanarak rekabet gücünü değerlendirmiştir. Ndong vd. (2007), Tilapia endüstrisinin mevcut durumunu ortaya koymuş ve ihracat açısından rekabet gücünü ele almışlardır. Benzer şekilde, Tveteras ve Bjorndal, (2001) somon yetiştiriciliği endüstrisinin ve Hidaka ve Torii, (2005) tuna yetiştiriciliğinde rekabet gücünü ortaya koymuşlardır. Su ürünlerinde rekabet gücünün analiz edildiği diğer çalışmalarda ise su ürünleri sektörünün bütünü için değerlendirilme yapılmıştır (Akmermer ve Ayyıldız, 2010; Aydın vd., 2014; Bashimov ve Aydın, 2016; Bashimov, 2017; Bashimov ve Aydın, 2018; Candemir ve Dağtekin, 2020; Demir ve Aksoy, 2021; Hossain, 2006; Kuşat ve Kuşat, 2019). Ancak sektöre ilişkin mikro ve makro düzeyde yeterli veri bulunmaması sebebi ile su ürünleri sektörünün rekabet gücünün ticaret, verimlilik ve maliyet fiyat göstergeleri bir arada kullanılarak ölçüldüğü çalışmalar oldukça sınırlıdır.

Türkiye'de su ürünleri sektörü ile ilgili olarak daha önce yapılan akademik çalışmalar sahip olunan kaynakların yönetilmesi için gerekli verileri ve rekabet düzeyini ileriye taşımak için gerekli stratejileri geliştirmeyi sağlayacak düzeyde değildir. Su ürünleri sektöründe girdi ve ürün piyasaları, tüketim karakteristikleri, üretim, pazarlama ve dağıtım konuları ile rekabet gücü hakkında önemli düzeyde veri eksikliği vardır. Söz konusu veri eksikliğini azaltmak için bu araştırmada Türkiye su ürünleri endüstrisinin rekabet gücünün belirlenmesi ve değerlendirilmesi amaçlanmıştır.

MATERYAL VE YÖNTEM

Araştırma verileri daha önce yapılmış çalışmalardan ve su ürünleri üretimi ve ticaretiyle ilgili Türkiye İstatistik Kurumu (TÜİK), Dünya Ticaret Örgütü (DTÖ) ve Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO) tarafından yayınlanmış olan ve 1976-2020 yıllarını kapsayan zaman serisi verilerinden elde edilmiştir.

Rekabet gücü ticaret, verimlilik, vemaliyet-fiyat göstergeleri ayrı ayrı veya bir arada kullanılarak ölçülmektedir. Türkiye su ürünleri sektörünün rekabet gücünü ortaya koymak için bu çalışmada ticaret göstergesi olarak Balassa indeksi, verimlilik göstergesi olarak toplam faktör verimliliği ve maliyet-fiyat göstergesi olarak yurtiçi/yurtdışı fiyatlar ile maliyetler dikkate alınmıştır.

Uluslararası ticarette karşılaştırmalı üstünlüğü belirleyebilmek için geliştirilen ticaret göstergeleri arasında en önemlilerinden biri Balassa'nın 1965 yılında ortaya attığı Açıklanmış Karşılaştırmalı Üstünlükler (revealed comparative advantages - RCA) teoremidir. Balassa, ülkelerin ticarete konu olan hangi ürünlerinde karşılaştırmalı olarak üstünlük sahibi olduklarını gerçekleşen ihracat değerlerinden yola çıkarak belirlemektedir. Buna göre, incelenen ülkedeki hangi ürün veya ürün grubunun toplam ihracat içindeki payı daha büyük ise, ülke o alanda karşılaştırmalı üstünlüğe sahiptir. Balassa'nın bu yaklaşımı, ülkeler arasında var olan karşılaştırmalı üstünlüğün nedenlerini detaylı incelemeden, aralarında avantaj farkı bulunup bulunmadığını göstermeyi amaçlar. RCA indeksi aşağıda yer aldığı gibi formüle edilmektedir (Balassa, 1965)

$$RCA_{ij} = (X_{ij}/X_i)/\,(X_{wj}/X_w)$$

Eşitlikte RCA açıklanmış karşılaştırmalı üstünlük değerini, X ihracat değerini, i ülkeyi, j sektörü ve w dünyayı ifade etmektedir. RCA indeksi sıfırdan sonsuza kadar devam eden bir değer almaktadır. RCA indeks değerinin 1'den büyük olması incelenen ülkenin ilgili sektör içerisinde karşılaştırmalı bir üstünlüğü olduğunu göstermektedir. Elde edilen indeks değeri 1'den küçük ise o ülkenin ilgili sektörde üstünlüğünden söz edilemez, aksine karşılaştırmalı olarak dezavantaja sahip olduğu değerlendirilir (Balassa, 1965).

Araştırmada RCA değeri hesaplanması için Türkiye'nin, Avrupa Birliği'nin ve dünya genelinin ihracat değerlerinin gelecekteki değerleri tahmin edilmiştir. İhracat değerine ait zaman serisi verilerinde mevsimsel etki olmayıp, zaman içinde yapısal değişiklikler göstermeyen trend (deterministik trend) söz konusu olduğundan zaman serisi analizi için trend analizi tercih edilmiştir. Trend analizi gerçekleştirilirken alternatif matematik formlar (doğrusal, quadratik, lojistik vb.) denenmiş ve doğruluk ölçümleri (MAP, MAPE, RMSE) kullanılarak en iyi sonucu doğrusal trend analizinin verdiği tespit edilmiştir. Aşağıda verilen doğrusal trend modeliyle uzun dönem eğilimi ortaya konulmuştur.

$$Y = a + bt + e$$

Eşitlikte Y gerçek zaman serisi değerini, t zamanı, a sabit terimi, b uzun dönemde ihracat değerinde meydana gelen değişimi yansıtan trend doğrusunun eğimini ve e tesadüfi hata terimini ifade etmektedir.

Araştırmada incelenen Türkiye su ürünleri sektörünün toplam faktör verimliliğindeki (TFV) değişim, 1996-2018 yıllarını kapsayan panel veriler kullanılarak ortaya konulmuştur. Rakip ülkelere ait panel veriler bulunmadığı için TFV diğer ülkeler için hesaplanmamıştır. Toplam faktör verimliliği Türkiye'nin 7., 8., 9. ve 10. kalkınma planı dönemleri için Malmquist Toplam Faktör Verimliliği İndeksi (TFV) kullanılarak ortaya konulmuştur. Malmquist, (1953) tarafından geliştirilen bu endeks, toplam faktör verimliliğindeki değişimi her bir veri noktasının ortak teknolojiye nispi uzaklık oranlarını hesaplamaktadır. Toplam faktör verimliliği, etkinlikte ve teknolojide meydana gelen değişim olmak üzere iki temel bileşenden oluşmaktadır. Etkinlikteki değişim, incelenen birimin teknik etkinlik skorlarında ilgili dönemde meydana gelen değişimi göstermektedir. Teknolojideki değişim ise, incelenen birimin teknoloji transferi ve inovasyon ile teknoloji seviyesinde meydana gelen iyileşmeyi ifade etmektedir (Coelli, 1998).

Malmquist TFV endeksi tek girdi ve tek çıktı var sayımı altında aşağıda yer alan eşitlik ile ifade edilmektedir (Färe vd., 1997)

$$m(Y_s, X_s, X_s, X_t) = \frac{d^t(Y_t, X_t)}{d^s(Y_s, X_s)} \sqrt{\frac{d^s(Y_t, X_t)}{d^t(Y_t, X_t)} x \frac{d^s(Y_s, X_s)}{d^t(Y_s, X_s)}}$$

Eşitlikte d^s (Y_t, X_t) t döneminde yapılan gözlemin s dönemindeki teknolojisinden olan uzaklığını, m(.) TFV endeksini ifade etmektedir. Bu değerin 1 değerinden büyük olması TFV'de artış olduğunu, 1 değerinden küçük olması ise azalış olduğunu göstermektedir. Eşitliğin sağında bulunan ilk terim teknik etkinlikte meydana gelen değişimi ifade ederken, ikinci terim teknolojik değişmeyi ifade etmektedir.

Araştırmada 1 çıktı, 4 girdiden oluşan model kullanılmıştır. Modelde tek çıktı olarak su ürünleri üretim değerine (TL) ve 4 girdi olarak ise sabit masraflara (TL), değişken masraflara (TL), personel sayısına (kişi) ve gemi sayısına (adet) yer verilmiştir. TFV endekslerinin hesaplanmasında Fare ve arkadaşlarının (1997) geliştirdiği matematiksel programlama modelleri kullanılmıştır.

[d^t,y_t,x_t]^(-1)= 〖max〗 _(φ,λ) φ St 〖-φy〗 _it+Y_t λ≥0, x_it-X_t λ≥0, λ≥0

[d^s,y_s,x_s]^(-1)= 〖max〗 _(φ,λ) φ St 〖-φy〗 _is+Y_s λ≥0, x_is-X_s λ≥0, λ≥0

[d^t,y_s,x_s]^(-1)= 〖max〗 _(φ,λ) φ St 〖-φy〗 _is+Y_t λ≥0,x_is-X_t λ≥0,λ≥0

[d^s,y_t,x_t]^(-1)= 〖max〗 _(φ,λ) φ St 〖-φy〗 _it+Y_s λ≥0, x_it-X_s λ≥0,λ≥0

BULGULAR VE TARTIŞMA

Dünya ve Türkiye su ürünleri endüstrisi

Su ürünleri sektörü dünyada 328 milyar dolar dış ticaret hacmine sahiptir. Bu hacmin %51'ini ihracat, %49'unu ithalat kaynaklıdır. İthalatçı ülkeler paylarına göre sırasıyla Amerika Birleşik Devletleri, Japonya, Çin, İspanya ve Fransa; ihracatçı ülkeler paylarına göre Çin, Norveç, Vietnam, Hindistan ve Amerika Birleşik Devletleri'dir. Dünya genelinde son 10 yıl içerisinde %12,3 artış gösteren su ürünlerinin üretim değeri en fazla artan ülkeler Bangladeş ve Nijerya'dır. Kanada'nın ise üretim değeri azalmıştır (FAO, 2018).

Türkiye'deki su ürünleri dış ticaret hacmi dünya ticaret hacminin binde 4'ü kadar olan 1,4 milyar dolar değerindedir. Dış ticaret hacminin %68'i ihracat, %32'si ise ithalat değerinden oluşmaktadır (FAO, 2019). Su ürünleri ihracatının en fazla yapıldığı ülkeler Hollanda, İtalya, İngiltere ve Almanya iken, en çok ithalat yapılan ülkeler sıralaması Norveç, Fas, İspanya, İzlanda ve Çin'den oluşmaktadır (TÜİK, 2020). Son 10 yılda Türkiye'de su ürünlerinin üretim değeri %7,5 yükselmiştir.

Dünyada 40 milyon civarında balıkçı su ürünleri üretimini yaklaşık 4,6 milyon adet gemi ile gerçekleştirmektedir. Gemilerin %61'i motorlu ve %81'i 12 metreden daha küçüktür. Motorlu gemilerin %2'si 24 metreden uzundur (FAO, 2018). Dünyada avlanma ve yetiştirme yöntemleri ile toplamda yaklaşık 214 milyon ton su ürünleri elde edilmektedir. Gerçekleştirilen üretimin %83'ünü su bitkileri haricindeki su ürünleri oluşturmaktadır. 1950 yılından günümüze kadar artış eğilimdeki toplam su ürünleri üretimi, üretilen su ürünü türü ve üretimde kullanılan yöntem çevresel, ekonomik ve politik nedenlere bağlı olarak değişmiştir. Özellikle dünyada avlanma ile elde edilen ürün miktarının artış hızına kıyasla yetiştiricilik yöntemiyle elde edilen ürün miktarı daha hızlı artmıştır. Son 30 yılda avlanan su ürünleri miktarı yıllık 90 milyon ton civarında dengelenmiştir. Su ürünleri yetiştiriciliğinin toplam üretim içindeki payı bu süre içinde sürekli artmıştır. Günümüzde dünya toplam su ürünleri üretimi içinde yetiştiriciliğin payı %56, avcılığın payı ise %44'tür. Yetiştiricilik ile yapılan 120 milyon tonluk üretimin %44'ü iç sularda %56'sı ise denizlerde gerçekleştirilmektedir. Avlanan 94 milyon ton ürünün %87'si denizden, %13'ü iç sulardan elde edilmektedir (FAO, 2019).

Türkiye'de su ürünleri üretimi toplam 30.878 kişi ve 18.483 adet gemi ile yapılmaktadır. Gemilerin çoğu 12 metreden küçük (%90), %1'i ise 30 metreden daha büyüktür (Tarım ve Orman Bakanlığı, 2021). Türkiye 2019 yılında gerçekleştirdiği 835 bin tonluk üretimiyle dünya su ürünleri yetiştiriciliği içerisinde yaklaşık binde 4 oranında bir paya sahiptir. 2000 yılından bugüne kadar yetiştirilen su ürünleri miktarında yaklaşık 6 katlık artış ile üretim miktarı 372 bin ton olmuştur. Böylece toplam su ürünleri üretiminde yetiştiriciliğin payı %45'e ulaşmıştır. Avlanan su ürünleri ise toplam üretimin %55'ini oluşturmaktadır (FAO, 2019). Denizlerde yaşanan kirlilik ve diğer nedenlerle 2020 yılında toplam üretim miktarında azalma meydana gelmiştir. Yetiştiricilik ile elde edilen su ürünleri miktarı 2020 yılında avlanan su ürünleri miktarını aşmış, toplam üretimdeki payını %55'e çıkarmıştır (TÜİK, 2020). Yetiştiricilik ile üretilen su ürünlerinin %69'u denizlerde, %31'i ise iç sularda gerçekleştirilmektedir. Avlanan su ürünlerinin %7'si iç su, %93'ü deniz kaynaklıdır. Türkiye'de denizlerde avlanan balıkların yarısından fazlası hamsidir. En çok avlanan diğer deniz balıkları ise sırasıyla çaça (%12), sardalya (%9), istavrit (%5), mezgit (%3)'tir. Türkiye'de yetiştirilen balıkların %40'ı alabalık %36'sı levrek ve %22'si çipuradır (FAO, 2019).

Dünyada yaklaşık sayısı 21 milyon olan su ürünleri yetiştiricilerinin çok büyük bölümü Asya kıtasında yer almaktadır (FAO, 2018). Türkiye'de ise 2139 tesiste su ürünleri yetiştiriciliği yapılmaktadır. Bu tesislerin %20'si denizlerde, %80'i iç sulardadır. Toplam proje kapasitesi yıllık yaklaşık 306 bin ton olan deniz kaynaklı tesislerin üretim ölçeği iç sulardan daha büyüktür. Bu tesislerden üretim kapasitesi bin tonun üzerinde olanların oranı %22'dir. Türkiye'de deniz kaynaklı su ürünleri yetiştiriciliğinde en çok paya sahip olan il %37 ile İzmir'dir. Daha sonra Aydın, Mersin, Trabzon, Ordu ve Samsun illeri gelmektedir. %66'sının üretim kapasitesi 50 tonun altında olan iç su kaynaklı tesislerin toplam proje kapasitesi yıllık yaklaşık 211 bin tondur (Tarım ve Orman Bakanlığı, 2021). Türkiye'de kapasite kullanım oranı %85 olan su ürünleri yetiştiriciliği yapan işletmeler içinde bu oran tam kapasite ile çalışan Mersin'de en yüksek, Trabzon'da en düşüktür. İç sularda yapılan yetiştiricilikte ise en büyük paya sahip iller Elâzığ (%17) ve Muğla (%16)'dır. Diğer önemli iller ise Burdur, Tokat, Samsun, Kayseri, Denizli, Isparta, Kahramanmaraş ve Gaziantep'tir (Aydoğan vd., 2019).

Su ürünleri arz ve değer zinciri

Gelişmiş ülkelerde su ürünleri için etkin çalışan bir arz ve değer zinciri olmasına rağmen, gelişmekte olan ülkelerde tam tersi durum söz konusudur. Türkiye'de ve gelişmekte olan birçok ülkede maliyet odaklı, balıkçı ve nakliyecilerin kontrol ettiği bir yapı vardır. Pazarlama kanalı boyunca kalite kontrolü yetersiz, soğuk zincir imkânları kısıtlı ve nakliyede uzun bekleme süreleri söz konusudur (De Silva, 2011; Schuurhuizen vd., 2006).

Türkiye su ürünlerinde arz zincirini oluşturmuş ve işleyen bir pazarlama sistemine sahiptir. Ancak Türkiye için etkin ve oturmuş bir değer zincirinden bahsetmek mümkün değildir. Türkiye su ürünleri arz zincirinin temel aktörleri üreticiler, kooperatifler, toptancılar, aracılar, tüccarlar, işleyiciler ve perakendecilerdir.

Türkiye'de su ürünleri üretimi ve karakteristik özellikleri

Arz zincirinin üretim boyutunda avcılık ve yetiştiricilik yapan işletmeler yer almaktadır. Türkiye'de su ürünleri üretiminde küçük ölçekli işletmeler hâkimdir ve kullanılan hasat teknolojileri gelenekseldir. Dolayısıyla, verimlilik düşüktür ve su ürünleri piyasaları iyi izlenememektedir. Finansman sorunu yaşayan balıkçılar, teknolojik imkanlar açısından yeterli düzeyde değildir. Bu nedenle faaliyetlerini etkin yürütememektedirler. Küçük ölçekli balıkçılar ortalama 16 metre uzunluğunda olan gemileri ile bir günde yaklaşık 10 saat operasyonda bulunmaktadırlar. Küçük ölçekli balıkçıların denizdeki avlanma süreleri yılda 186 gündür. Türkiye balıkçılarının %82'si kendi gemilerine sahiptir. Gemilerin dörtte üçü ahşaptır. Balıkçıların %66'sı tek, %34'ü ise birden fazla gemiye sahiptir. Balıkçılık kooperatifine üyelik yüzdesi %84'tür (Hasdemir vd., 2018). Türkiye'de büyük ölçekli ticari balıkçıların %51'i yalnızca trol ile, %34'ü hem algarna trol hem ile avlanırken, sadece algarna ile avlanan balıkçıların oranı %15'tir (Ceyhan ve Gene, 2014). Türkiye'de faaliyet gösteren tipik bir balıkçının ortalama yaşı 40'tır. Eğitim düzeyi ilkokul, balıkçılık deneyimi ise yaklaşık 20'yıldır. Trol işletmecileri avcılıklarını yaklaşık 21 metre uzunluğunda, 484 beygir güç üretebilen ve 19 yıllık bir gemi ile yılda 129 gün sürdürmektedir. Günde ₺2140 masraf ile her biri yaklaşık 1,5 saat süren 5 operasyon yapabilen ortalama 5 tayfalı bir trol gemisi, 60 ton ağırlığındaki hedef avın yanında 3,2 ton ağırlığında ıskarta ürün elde etmektedir. Bu masrafın %43'ü sabit %57'si değişken masraftır. Değişken masrafların %57'sini ve toplam masrafın %37'sini oluşturan yakıt masrafı deniz balıkçılığının en yüksek masraf kalemidir. Toplam masrafların %21'i amortisman, %6'sı gemi tamir bakımı, %7'si ise kumanya masrafları ile birlikte tayfa ücretleridir. Türkiye'de tipik bir trol işletmecisi kullandıkları sermaye karşılığında yıllık %43,2 oranında getiri elde etmektedir. Tipik bir algarna işletmecisi ise 11 metrelik, 170 beygir güç üretebilen, yaklaşık 13 yıllık gemisi ile yılda 117 gün denizlerde avlanma faaliyetini sürdürmektedir. Sahip olduğu 2 tayfası ile günlük yaklaşık olarak her biri 30 dakika süren 20 operasyon yapan bir algarna, 134 ton hedef av ile birlikte 3,4 tonluk ıskarta ürün elde etmektedir. Günde ₺1025,5 masraf yapan algarna'nın toplam masraflarının %87'si değişken masraftır. Yakıt en önemli masraf unsuru olup, değişken masraf içindeki payı %68'dir. Toplam masrafların %24'ü amortisman, %9'u gemi tamir bakımı, %16'sı ise kumanya masrafları ile birlikte tayfa ücretleridir. Algarna işletmecisinin toplam sermaye getirisi yıllık %39,5'dir (BENTHIS, 2016) (Tablo 1).

Türkiye'de alabalık yetiştiriciliği yapan işletmeler toplam yatırdıkları sermayelerinin %12'si oranında net gelir kazanmaktadırlar. Türkiye'de alabalık yem dönüşüm oranı ortalama 1,12 kg'dır. Bu oranın en yüksek olduğu il 1,35 ile Ordu iken, en düşük 0,90 değeri Adana ve Burdur illerindedir.

Çipura ve levrek yetiştiriciliği yapan işletmelerin net gelirleri yatırdıkları sermayelerinin %26'sı kadardır. Çipura ve levrek üretiminde ekonomik rantabilite değeri %54 ile Mersin'de en yüksektir. Türkiye'de levrek yetiştiriciliğinde ortalama yem dönüşüm oranı 1,93, çipura yetiştiriciliğinde ise 1,88'dir. Levrek üretiminde Samsun (1,8), çipura üretiminde ise Mersin (1,5) en iyi yem dönüşüm oranına sahipken, İzmir her iki üründe de en kötü değere sahiptir. Her üç ürünün satış fiyatları, birim üretim maliyetlerinin üzerindedir (Aydoğan vd., 2019; Ekmekçi ve Gül, 2017; Terzi, 2018).

Tablo 1. Ticari ölçekteki trol ve algarnaya ait bazı teknik ve ekonomik göstergeler
Table 1. Some technical and economic characteristics of commercial trawling and seine fishing

İşletmecinin karakteristik özellikleri (yıl)	Trol*	Algarna*
Yaş	42,1	40,8
Eğitim süresi	6,3	6,4
Deneyim	23,1	21,9
Gemilerin karakteristik özellikleri		
Uzunluk (metre)	20,5	10,9
Yaş (yıl)	19,3	13,3
Tonaj (groston)	42,0	36,1
Motorunun beygir gücü (ortalama)	483,5	169,5
Sezonda deniz geçirilen gün sayısı	129,0	117,0
Günlük operasyon adeti	5,0	20,0
Bir operasyonun süresi (dakika)	90,0	30,0
Tayfa adeti (kişi)	5,0	2,0
Ekonomik göstergeler		
Barbun ve mezgit (kg/sezon)	59358,2	-
Salyangoz (kg/sezon)	-	133614,0
Iskarta balık miktarı (kg/sezon)	3289,5	3393,0
Değişken masraflar (₺/gün)	1863,7	579,7
Sabit masraflar (₺/gün)	992,8	445,8
Toplam masraflar (₺/gün)	2139,9	1025,5
Sezonluk üretim değeri (₺) **	820329,8	534456,0
Sezonluk toplam masraflar (₺)	368481,6	119983,9
Sezonluk net gelir (₺)	451848,2	414472,1
Sermaye getirisi (%)	43,1	39,6

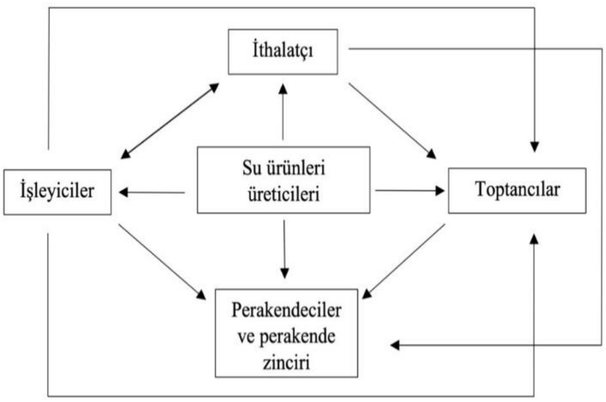
*(BENTHIS, 2016; Ceyhan ve Gene, 2014)
**Avlanan balıkların fiyatlarının ağırlıklı ortalaması 13,8 TL/kg, salyangoz fiyatı 4 TL/kg

Türkiye'de su ürünleri dağıtımı, pazarlaması ve tüketimi

Arz zincirinde yer alan dağıtım aşaması, nakliye ile lojistik faaliyetlerini kapsamaktadır. Türkiye'de tedarik için var olan sistem geleneksel yapıda ve komisyonculuğa dayalı olarak sürdürülmektedir. Nakliye ve dağıtım alt yapısında yaşanan gelişmelere rağmen, istenilen alt yapıya henüz kavuşulamamıştır. Buz üretimi ve soğuk hava deposu ihtiyacı tam olarak karşılanamamaktadır. Su ürünlerinin etkin bir şekilde işlenememesi ve sınıflandırma eksikliği sektörde atık miktarının artmasına neden olmaktadır.

Üretilen veya avlanan su ürünlerinin tüketicilere ulaştırılmasında yer alan pazarlama kanalları ve sürdürülen pazarlama faaliyetleri hem iç pazarda hem dış pazarda ülkelere göre farklılıklar göstermektedir. Endüstriyel amaçlı balıkçılık yapan işletmelerin ilgi alanına yüksek ekonomik değeri bulunan türler girmektedir. Üretim ölçekleri oldukça büyük ama sayıları az olan bu balıkçılar bazı ülkelerde değer zincirindeki diğer aktörlerle dikey bir entegrasyon sağlayarak piyasayı tamamen yönlendirebilmektedir. Meksika'da bu düzeyde olan 5 adet işletme kendilerine ait olan işleme tesisleri, ihracatçıları, oluşturdukları markaları ve sahip oldukları pazarlama ağları ile su ürünleri piyasasına hâkimdir (De Silva, 2011). Kooperatifleşerek örgütlenen bazı ülkelerdeki balıkçılar hem iç piyasada hem de dış piyasada organizasyonu sağlamaktadır. Yine Meksika'da yaklaşık 5000 karides avcılığı yapan işletme 140 birim kooperatif oluşturarak örgütlenmiştir (Huntington ve Hasan, 2009). Genel olarak dünyada üretimi yapılan su ürünleri aşağıda belirtilen şekilde tüketicilere ulaşmaktadır (Şekil 1) (De Silva, 2011).

Türkiye'de avlanan miktarın belirsiz olması pazarlama faaliyetlerini olumsuz etkilemektedir. Özellikle pazarlama etkinliğini düşüren en büyük neden taze balığı tüketicilere sunan perakendecilerin iyi organize olamamalarıdır.



Şekil 1. Dünyada su ürünleri pazarlama kanalları
Figure 1. Aquaculture and fisheries marketing channels worldwide

Türkiye'de su ürünleri sektöründe yer alan temizleme, dondurma, konserve yapımı, tuzlama işlemi ve paketleme için gerekli üniteleri içeren entegre ve modern tesis sayısı yetersizdir. Türkiye'de 234 adet işletme su ürünlerini işlemekte ve pazarlamaktadır. AB'ye ürün satabilecek yeterlilik belgesi olan işletmelerin oranı %92'dir. Ortalama olarak yıllık 150 bin ton su ürününün işlendiği tesislerin %82'si avlanma alanlarına ve tüketicilerin yoğun olduğu merkezlere yakın olan Marmara, Ege ve Batı Karadeniz bölgelerinde kurulmuştur (Yılmaz, 2015).

Pazarlama kanalında yer alan diğer önemli aktör balık unu ve yağı fabrikalarıdır. Bu fabrikaların hammadde olarak kullandığı balık türleri ülkelere göre değişmektedir. Güney Amerika'da hamsi, Avrupa'da mezgit, çamuka ve çaça yaygın olarak tercih edilmektedir (Tarım ve Orman Bakanlığı, 2005). Dünya genelinde üretimi yapılan balık ununun su ürünleri yetiştiriciliğinde (%63), domuz yetiştiriciliğinde (%25), kanatlı hayvanlar ile evcil hayvan beslenmesinde (%12) kullanılmaktadır. Üretilen balık yağının çoğunluğu su ürünleri yetiştiriciliğinde (%81) olmak üzere, bir kısmı insanların tüketiminde (%13) ve endüstriyel amaçlı işlemede (%6) kullanılmaktadır. Balık unu somon ve alabalık yetiştiriciliğinde (%27), deniz balıkları yetiştiriciliğinde (%26), kabuklu hayvan üretiminde (%26), tatlı su çipurası yetiştiriciliğinde (%6) ve diğer türlerin üretiminde (%25) yoğun olarak kullanılmaktadır. Balık yağı ise somon ve alabalık yetiştiriciliğinde (%68), deniz balıkları yetiştiriciliğinde (%19) ve diğer türlerin yetiştirilmesinde (%13) kullanılmaktadır (Ceyhan ve Emir, 2015).

Türkiye'de bulunan 12 adet balık unu ve yağı işleme tesisi günlük ortalama 829 ton işleme kapasitesine sahiptir. Birim üretim (1 kg balık yağı+1,5 kg balık unu) maliyeti ₺10,6 olan bu ürünlerden, balık ununun kg fiyatı ortalama $1,4, balık yağının ise $2,0 civarındadır. Balık unu ve yağını üreten tesisler 1 ton hamsi için 2019 yılı fiyatlarıyla ₺1282 işleme masrafı yapmakta ve ₺1463 gelir elde etmektedirler. Türkiye'de yıllık 128 bin tonu hamsi ve 87 bin tonu çaça olmak üzere toplam 215 ton balık bu tesislerde işlenmektedir. Balık unu ve yağı üretiminde Türkiye net ithalatçı konumundadır. Türkiye'nin her yıl yıllık yaklaşık 128 milyon dolar dış ticaret açığı söz konusudur. Yetiştiricilik sektörü geliştikçe bu dış ticaret açığında artış olması öngörülmektedir (Ceyhan ve Emir, 2015).

Pazarlama kanallarında yer alan su ürünleri kooperatifleri Türkiye'de ilk olarak 1942 yılında örgütlenmiş, 1971 yılında çıkarılan Su ürünleri Kanunu ile kooperatifleşme hızı artmıştır. Günümüzde 30.845 balıkçının ortak olduğu 552 adet su ürünleri kooperatifi vardır. 16 adet bölge birliği ve bağlı olarak faaliyet gösteren 233 birim kooperatif ile üst örgütlenme konusunda da ilerleme kaydedilmiştir. Üst örgütlenme Marmara ve Karadeniz Bölgesinde en iyi durumdadır (Dogan, 2017).

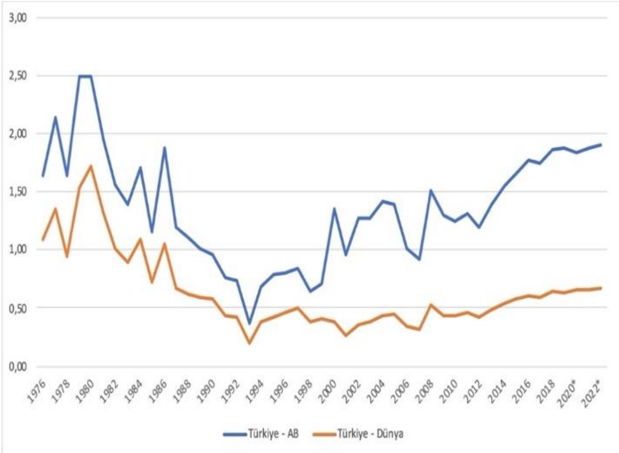
Su ürünlerindeki pazarlama sistemi Türkiye açısından değerlendirildiğinde, en temel sorun doğru tür, kaliteli ve güvenli su ürünleri arzıdır (Ceyhan, 2019). Yerli ve ithal edilen su ürünleri birlikte pazarlanmaktadır. Büyük marketlerin bu pazardaki rolü gün geçtikçe artmaktadır. Oteller ve restoranlar gibi kurumsal kanallarla bazı gıda tedarikçileri de sektörde rol almaktadırlar. Ayrıca ulusal ve uluslararası standartlarla birlikte yasal düzenlemelerinde sistem içerisindeki önemi ve üstlendiği görev artmaktadır. Gelişmiş ülkelerdeki pazarlama sistemlerine kıyasla Türkiye'deki sistem içerisindeki firmaların ölçeği ve dolayısıyla da sistemin genel ölçeği küçüktür. İşlenmiş üründen ziyade taze balık tüketimi tercihi daha yaygındır. Türkiye su ürünleri sektöründe büyük marketlerin artan payı küçük ölçekli satıcıların ve toptancıların ticaretinde bir tehdit unsurudur. Gelişmiş ülkelere kıyasla Türkiye su ürünleri sektöründe düşük düzeyde ve dar kapsamlı standartlar yer almaktadır.

Geçmişte daha çok taze tüketimi tercih edilen su ürünleri, günümüzde işleme teknolojisinin ve muhafaza imkanlarının artmasıyla tüketicilere farklı şekillerde sunulmaktadır. Su ürünlerinin insanların tüketimine doğrudan sunulan miktarı toplam üretim miktarının %88'idir. Dünyada üretilen ve %88'i doğrudan insan tüketimine sunulan su ürünlerinin %45'i taze, %34'ü ise dondurulmuş, %11'i konservelerle ve %10'luk kısmı ise kurutularak pazarlanmaktadır. Gelişmekte olan ülkelerde taze su ürünlerini tüketimi daha çok tercih edilirken, gelişmiş ülkelerde işlenmiş ve dondurulmuş su ürünleri daha fazla tüketilmektedir. Dünyada su ürünleri tüketimi son 60 yılda %1,6 olan nüfus artış hızından daha hızlı bir oranda %3,2 civarında artış göstermiştir. Su ürünleri tüketiminin yıllık artış oranı diğer tüm etlik hayvanlardan daha fazla artmıştır. Tüketilen su ürünleri miktarı 1961'de kişi başına 9 kg civarındayken bugün 2 katından fazla artarak yaklaşık 21 kg değerini ulaşmıştır. Kişi başına düşen yıllık su ürünleri tüketimi sırasıyla 142 kg ile Maldiv Adaları, 90 kg ile İzlanda, 65 ile Çin, 57 kg ile Portekiz, 52 kg ile Japonya ve 43 kg ile İspanya'da en fazladır (Lockyer ve Stanner, 2016). Kişi başına düşen ortalama 6,3 kg su ürünleri tüketim miktarı ile çok az tüketen ülkeler grubunda yer alan Türkiye ve hem dünya ortalamasının (21 kg) hem de Avrupa ortalamasının (25 kg) oldukça altındadır. Türkiye'deki su ürünlerinin %75'i taze olarak, %4'ü dondurulmuş ve %2'si işlenmiş olarak toplamda %79'luk kısmı yurt içi tüketicilere pazarlanmaktadır. %14,4'ü balık unu ve yağı üretimine ayrılmaktadır. Balık tüketimi lojistik masrafları, tüketici tercih ve alışkanlıkları nedeniyle ülkenin her bölgesinde farklılık göstermektedir. En fazla su ürünleri tüketimi kişi başına yıllık 25 kg ile Karadeniz Bölgesinde, en az tüketim ise kişi başına yıllık 0,5 kg ile Doğu ve Güneydoğu Anadolu Bölgesinde 0,5 kilogramdır. Uygun fiyatlı hamsi, istavrit ve sardalya sezonunda tüketimi daha çok tercih edilirken, yüksek fiyatlı kalkan, çipura ve levrek ise daha yüksek gelirli kesim tarafından ve turizm merkezlerinde daha fazla tüketilmektedir.

Türkiye'de su ürünleri talebinin fiyat elastikiyeti -0,515 ve gelir elastikiyeti 1,5'dir (Yılmaz, 2015). Tüketicilerin su ürünleri fiyat değişimlerine hassasiyetleri düşük iken, tükettikleri su ürünleri miktarı gelir değişimlerinden daha fazla etkilenmektedir. Bu bulgu dünyanın farklı yerlerinde daha önce yapılmış araştırmaların sonuçlarıyla uyumludur (Lee and Chang, 2014; Sonoda vd., 2012).

Su ürünleri sektörünün rekabet gücü

Araştırma sonuçları ticaret göstergeleri açısından Türkiye su ürünleri sektörünün Avrupa Birliği karşısında rekabet üstünlüğüne sahip olduğunu göstermiştir. Türkiye'nin AB'ye karışı rekabet gücü zaman zaman durağanlaşmış olsa da genellikle her dönemde bir artış eğilimi söz konusudur. AB karşısında rekabet üstünlüğü olan Türkiye, dünyanın tamamını kapsayan ticaret incelendiğinde, üstünlüğünü kaybetmektedir. Türkiye su ürünleri sektöründe olumlu gelişmeler olmakla birlikte, Türkiye'nin dünyadaki rekabet gücünü ortaya koymak için hesaplanan Balassa indeksi birden küçüktür. 1976 yılından itibaren yaklaşık 10 yıl rekabet üstünlüğü bulunan Türkiye, uluslararası su ürünleri ticaretinde daha hızlı gelişen ülkeler nedeniyle bu üstünlüğü kaybetmiş ve Balassa indeks değerinde 2000'li yıllara kadar azalma eğilimi görülmüştür Daha sonra özellikle su ürünleri yetiştiriciliğinin hızlı gelişimi, saklama koşulları ve işlenerek katma değer kazandırılması gibi nedenlerin etkisiyle indeks değerinde günümüze kadar artış eğilimi ortaya çıkmıştır (Şekil 2). Türkiye su ürünleri sektörünün rekabet gücünü dünya karşısında bir miktar yükseltmiştir (Tablo 2).



Şekil 2. Türkiye su ürünleri sektörü rekabet gücü (1976-2022 RCA değerleri)
Figure 2. Competitive power of Turkish aquaculture (RCA values from 1976 to 2022)

Tablo 2. Türkiye su ürünleri sektörünün AB ve Dünya karşısında rekabet gücü
Table 2. Comparative competitive power of Turkey, EU and World in terms of aquaculture and fisheries

Yıl	RCA (Türkiye–AB)	RCA (Türkiye–Dünya)
1976-2018 dönemi ortalaması	1,32	0,64
2015	1,66	0,57
2016	1,76	0,61
2017	1,74	0,59
2018	1,86	0,65
2019*	1,87	0,63
2020*	1,84	0,66
2021*	1,88	0,65
2022*	1,90	0,67

*Tahmin değerleridir

Türkiye su ürünleri sektörünün rekabet gücü verimlilik göstergeleri açısından sadece balık avcılığı için değerlendirilebilmiştir. Balık yetiştiriciliğine ilişkin zaman serisi verilerine ulaşmak mümkün olmadığından balık yetiştiriciliğinde rekabet gücü verimlilik göstergeleri açısından ortaya konulamamıştır. Türkiye'de balık avcılığında TFV 2001-2013 yılları arasında (8 ve 9. Kalkınma planları) arttığı, sonra yatay bir seyir izlediği görülmüştür. Türkiye balık avcılığında TFV, yedinci kalkınma planı döneminde %11 oranında azalmıştır. Balık avcılığında yaşanan toplam faktör verimliliği azalışının sebebi teknolojik yetersizliktir. Sekizinci kalkınma planı döneminde balık avcılığında yaşanan teknolojik ilerlemelerin etkisiyle TFV bu kalkınma döneminde %2 artmıştır. Türkiye balık avcılığı TFV açısından en büyük artışı yine teknolojik değişimin etkisiyle dokuzuncu kalkınma planı dönenimde yaklaşık %19'la göstermiştir. Türkiye balık avcılığında TFV onuncu kalkınma planı döneminde %4 civarında azalmıştır. Bu düşüşün nedeni balık avcılığında kullanılan teknolojilerin ortaya çıkardığı riskler ve örgütlenme ile ilgili yetersizlikler olarak gösterilebilir (Tablo 3).

Tablo 3. Türkiye'de balık avcılığında toplam faktör verimliliğindeki değişim
Table 3. Total factor productivity change in Turkish fisheries sector

Değerler	7. Plan Dönemi (1996-2000)	8. Plan Dönemi (2001-2005)	9. Plan Dönemi (2007-2013)	10. Plan Dönemi (2014-2018)
Etkinlik değişimi	1,00	1,00	1,00	1,00
Teknoloji değişimi	0,89	1,02	1,19	0,96
TFV	0,89	1,02	1,19	0,96

Türkiye su ürünleri sektörünün rekabet gücü maliyet-fiyat göstergeleri açısından sadece balık yetiştiriciliği için değerlendirilebilmiştir. Balık avcılığına ilişkin karşılaştırılabilir verilere ulaşmak mümkün olmadığından balık avcılığın için rekabet gücü maliyet-fiyat göstergeleri açısından ortaya konulamamıştır. Türkiye'de yetiştiriciliği yapılan ve dünyayla rekabet durumunu ortaya koymak için maliyet ve fiyat açısından kıyaslanabilecek levrek ve çipuraya ilişkin veriler Tablo 4'te verilmiştir. Yapılan değerlendirmeler, levrek ve çipura için Türkiye'nin dünya ortalamasına göre daha ucuza üretip daha uygun fiyatla piyasaya bu balıkları arz edebilen bir konumda olduğunu göstermiştir (Tablo 4).

Tablo 4. Türkiye ve Dünyada çiftliklerde yetiştirilen levrek ve çipura maliyeti ile satış fiyatları
Table 4. Comparative cost and sales prices of sea bass and sea bream in Turkey and World

	Türkiye*		Dünya*	
	Maliyet	Satış fiyatı	Maliyet	Satış fiyatı
Levrek ($/kg)	4,0	7,5	6,0	16,5
Çipura ($/kg)	4,3	7,2	5,8	16,0

*2020 yılı fiyatlarıyla ifade edilmiştir. Karşılaştırmalı analizde $1=₺7,5 olarak esas alınmıştır.

Sonuç ve Öneriler

Türkiye su ürünleri sektöründe son yıllarda önemli gelişmeler yaşanmıştır. Geleneksel yapı değişerek su ürünleri endüstrisine dönüşmüştür. Türkiye'de su ürünleri arz zinciri oluşmuş olmasına rağmen, etkin bir değer zinciri oluşturulamamıştır. Dünyada rekabet gücü çok iyi olmayan Türkiye su ürünleri sektörü, AB ülkeleri karşısında karşılaştırmalı mukayeseli üstünlüğe sahiptir. Özellikle son yıllarda su ürünleri sektörünün rekabet gücündeki yükseliş, sektöre moral vermekte ve gelecekteki beklentileri olumlu kılmaktadır. Ancak toplam faktör verimliliğindeki düşüş özellikle teknolojiden kaynaklı ortaya çıkan risklerin iyi yönetilemediğini, piyasa izleme mekanizmalarında aksaklıklar olduğunu ve etkin çalışan bir pazarlama sisteminin eksikliğini göstermektedir. Toplam faktör verimliliğindeki azalışın bir diğer nedeni de değer zincirinde yer alan aktörlerin hem üretim hem hizmet etkinliklerinin yeterli düzeyde olmamasıdır.

Su ürünleri yetiştiriciliğinde önemli bir girdi olan balık unu ve balık yağı üretiminin yetersizliği, gelecekte olası su ürünleri üretim artışının en önemli unsuru olacak bu sektörün dışa bağımlılık riskini yükseltmektedir. Bu temel girdinin imalatının hammaddesi olan av balıklarının insan tüketimine sunulması veya balık unu ve yağına dönüştürülmesi seçenekleri arasında doğru kararın verilmesi gerekir. Bu kararın fırsat maliyeti hesabına dayanarak verilmesi büyük önem taşımaktadır.

Su ürünleri sektörünün daha da gelişerek arzu edilen seviyelere getirilebilmesi için verimlilik artırılmalı, piyasa aktörleri arasında kuvvetli bir iletişim kurulmalı, tüketici ihtiyaçları ile fiyat eğilimleri konusunda farkındalık düzeyi yükseltilmeli, üretim teknolojilerinde güncel gelişmeler takip edilmeli ve yayım faaliyetleri etkili bir biçimde yapılmalıdır. Avlanma konusunda yaşanan bazı yapısal sorunlar vardır. Geleneksel yapı ve avlanması hedeflenen canlı populasyonun doğal koşullarının karmaşık olması bu sorunların başında gelmektedir. Dolayısı ile ıskarta, kayıp ve atık gibi nedenlerle önemli miktarlarda ürün ekonomik olarak değerlendirilememektedir. Çözüm olarak özellikle avlanan ve yetiştiricilik yapan su ürünleri işletmelerinin ölçek problemini ortadan kaldırmak amaçlı bir takım yapısal düzenlemeler yapılmalıdır. Buna ilave olarak, uluslararası avlanma sahalarında avlanma imkanlarının değerlendirilmesi Türkiye su ürünleri üretimini olumlu yönde etkileyebilecektir.

Türkiye su ürünleri sektöründe rekabet gücünün sürdürülebilir bir şekilde arttırılması için arz ve değer zincirindeki tüm aktörlerin ölçeğinin artırılması için gereken yapısal önlemler alınmalıdır. Türkiye'de yeterli veri olmayan taze balık satıcıları düzeyinde, kaliteli üretimi teşvik edecek yaygın bir organizasyon yapısı ortaya çıkarılmalıdır. Ayrıca yerel bazda tüketicilerin işlenmiş su ürünleri tercihlerinin teşvik edilmesi yönünde işleme tesislerinin etkinleştirilmesi ve uygun pazarlama yöntemlerinin belirlenmesi gereklidir. Üretilen su ürünlerine katma değer kazandıracak tesisleşme ve altyapı imkanları artırılmalı, havaalanı ve limanlarda soğutma imkanları geliştirilmeli, uluslararası düzeydeki kalite standartlarının benimsenmesi ve uygulanması sağlanmalı ve böylece de ihracat imkanları ile rekabet gücü artırılmalıdır.

Kalıcı ve sürdürülebilir bir gelişim sağlanabilmesi için gelecek eğilimlerini bilmek ve ulusal piyasalar yanında uluslararası piyasaları da anlamak önemlidir. Türkiye su ürünleri sektöründe bu açıdan piyasaların izlenmesi ve erken uyarı kabiliyetine sahip istemlerin kurulması kaçınılmaz bir ihtiyaçtır. Etkin izlenen bir su ürünleri piyasası politika yapıcıların politika oluşturma süreçlerine katkı sağlayabilecek, kaynakların etkin dağıtımına imkân verecek, uygulanan politikaların sonuçlarını analiz edebilecek fırsatları sağlayacaktır. Oluşturulacak bu izleme sistemi bütün ilgili piyasalarla uyumlu olmalı, en küçük yerel birimden ulusal boyuta kadar etkin çalışmalı ve hem sektör hem de kategori özelinde gerekli erken uyarıları oluşturabilmeli, gelecekle ilgili beklentileri tahmin edebilmeli, tüketici tercihlerini dikkate alabilmeli ve politika yapıcılardan, üreticilere ve işleyicilere kadar referanslar oluşturabilme yeteneğine sahip olmalıdır.

Gelişmelerin oldukça hızlı olduğu bugünlerde zamanın etkin kullanımı açısından, su ürünleri ile ilgili biyolojik boyutta yapılan çalışmalar ile ekonomik boyutta yapılan çalışmaların bir araya getirilmesi ile ortaya çıkacak olan biyoekonomi yönelimli çalışmalar sektörün sürdürülebilir gelişimine büyük faydalar sağlayacaktır.

YAZARLIK KATKISI

Çağatay Yıldırım, Vedat Ceyhan: Kavramsallaştırma, metodoloji, yazılım. Çağatay Yıldırım, Hatice Türkten, Vedat Ceyhan: Veri küratörlüğü, yazma- orijinal taslak hazırlama. Çağatay Yıldırım, Hatice Türkten: Görselleştirme, araştırma. Vedat Ceyhan: Gözetim ve denetim. Çağatay Yıldırım, Hatice Türkten: Yazılım, doğrulama. Çağatay Yıldırım: Proje yönetimi, kaynaklar, finansman edinme. Çağatay Yıldırım, Hatice Türkten, Vedat Ceyhan: Yazma-gözden geçirme ve düzenleme.

ÇIKAR/REKABET ÇATIŞMASI BEYANI

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

VERİ KULLANILABİLİRLİĞİ

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

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Balık silajı üretimi için basit bir yöntem: İnokulum olarak yoğurt kullanımı

A simple method for fish silage production: Using yoghurt as inoculum

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Öz: Su ürünleri atıklarının değerlendirilmesi hem çevre kirliliğinin önlenmesi hem de bu atıklara ekonomik olarak değer kazandırılması açısından önemli bir konudur. Balık atıklarının değerli bir yem kaynağına ve bitki gübresine dönüşümünü sağlayabilecek en pratik yöntem silaj yapımıdır. Bu çalışmada, laktik asit bakteri kültürü (*Streptococcus thermophilus*), asitleştirici kimyasallar (formik asit) ve fermente bir gıda olan yoğurdun direkt ilavesi ile hazırlanan balık silajlarının olgunlaşma süreleri ve besin bileşenleri karşılaştırılmıştır. Araştırmada balık silajı üretiminde sardalya balığı (*Sardina pilchardus*) atıkları (baş, deri, kılçık, iç organları vs) kullanılmıştır. Tüm balık silajlarının maksimum 10 gün içerisinde olgunlaşma evresini tamamladıkları gözlenmiştir. Asit, bakteri ve yoğurt ile hazırlanan grupların nem, kül, protein ve yağ içerikleri sırasıyla % 63-66; % 5,6-5,7; % 11,3-11,8 ve %12.18-13.65 aralığında olduğu belirlenmiştir. Araştırma sonucunda direkt saf bakteri kültürü ilavesi veya asit ilavesi ile yapılan silajlara alternatif olarak yoğurt ilavesi ile yapılan balık silajlarının hem olgunlaşma süresi hem de besin madde bileşenleri açısından beklentileri karşıladığı görülmüştür.

Anahtar kelimeler: Balık atıkları, balık silajı, fermentasyon, yoğurt, *Streptococcus thermophilus*

Abstract: The evaluation of seafood processing wastes is an important issue in terms of both preventing environmental pollution and adding value to these wastes economically. The most practical method that can convert seafood processing waste into a valuable feed source and plant fertilizer is to make silage. In this study, the ripening times and nutritional components of fish silages prepared with direct addition of lactic acid bacteria culture (*Streptococcus thermophilus*), acidifying chemicals (formic acid) and yogurt, which is a fermented food, were compared. In the research, sardine fish (*Sardina pilchardus*) wastes (head, skin, bones, internal organs, etc.) were used in fish silage production. It was observed that all fish silages completed the ripening phase within a maximum of 10 days. It was determined that the moisture, ash, protein and lipid contents of the groups prepared with acid, bacteria and yoghurt ranged between 63-66%, 5.6-5.7%, 11.3-11.8% and 12.18-13.65%, respectively. As a result of the research, it was observed that fish silages made with the addition of yoghurt as an alternative to silages made with direct addition of pure bacterial culture or acid addition met the expectations in terms of both ripening time and nutrient components.

Keywords: Fish waste, fish silage, fermentation, yoghurt, *Streptococcus thermophilus*

GİRİŞ

Mesleki balıkçılık faaliyetlerindeki ıskarta türler ve su ürünleri işleme atıklarının ekonomik olarak değerlendirilebilmesi, balıkçılık sektöründe çözülmeye çalışılan önemli bir sorun olarak görülmektedir. Bu durum, ekonomik bir sorun olmanın ötesinde önemli bir çevre problemi olarak da değerlendirilmektedir. Bu atıklar un haline getirilerek değerlendirilebilir ancak bu yöntem ekonomik olarak önemli bir alt yapı gerektirmektedir. Bu durum, büyük miktarlarda oluşan balık atıklarının kontrollü fermantasyon gibi ucuz alternatif biyoteknolojik araçlarla değerlendirilmesi üzerine araştırmaların artmasına yol açmıştır. Bu biyoteknolojik yöntem ile büyük ekipmanlara ihtiyaç duymadan asitleştirici mikroorganizma ve karbon kaynağı kullanılarak, mikrobiyal açıdan güvenli son ürünler elde edilebilmektedir. Laktik asit fermantasyonunu temel alan yöntemler, su ürünlerinden biyomoleküllerin geri kazanımını da sağlayan, çevre dostu yöntemler olarak bilinmektedir (Rai vd., 2012).

Laktik asit bakterileri, çeşitli tatlı su ve deniz balıklarında veya balıkların iç organlarında bulunur. Laktik asit bakterileri fermantasyon gibi gıda koruma tekniklerinde en yaygın olarak kullanılan mikroorganizmalardır. Laktik asit bakterileri tarafından üretilen laktik asit, fermente ürünlerde asidik koşullar sağladığı ve gıdalarda bozulma ve zehirlenme etmeni olan bakteriler üzerine öldürücü etkiye yol açtığı için gıda korumada kullanışlı bir bileşik olmaktadır (Özyurt vd., 2016). Ülkemizde su ürünlerinden silaj yapımı yaygın olmamakla beraber genellikle asit yöntem ile yapılmakta, laktik asit bakterilerince silaj kurulması ile ilgili genel bir bilgiye rastlanmamaktadır (Özyurt, 2016). Ancak balık silajları, düşük maliyetle elde edilebilir ve hayvan beslemede kullanımı yüksek bir potansiyele sahiptir (Vidotti vd., 2003; Goddard ve Perret, 2005). Bununla birlikte bitki tarımında yaygın bir şekilde kullanılabildiği ve bunların ticari gübrelerle benzer verimlilikte oldukları bilinmektedir (Wyatt ve McGourty, 1990).

Balık silajı tüm balık veya parçalarının asit, enzim veya laktik asit üreten bakterilerin ilavesi ve balığın sindirim sisteminde bulunan enzimlerin yardımları sonucu oluşan sıvı kıvamlı bir üründür (Özyurt, 2016). Geleneksel yöntemlerle balık silajı hazırlanmasında organik veya inorganik asitler kullanılır. Bununla birlikte son yıllarda yapılan çalışmalarda laktik asit bakterileri ile hazırlanan fermentatif ürünlerin vücuda yararlı peptidlerin üretimini sağladığı, lezzeti geliştirdiği, protein ve vitamin varlığını arttırdığı ve çevre dostu olduğu yönünde yapılan bildirimler dikkat çekicidir (Kuley vd., 2020). Laktik asit bakterilerinin birçok fermente üründe dominant mikroorganizma olduğu bildirilmiştir (Ostergaard vd., 1998; Shirai vd., 2001; Llanes vd., 2011; Vidotti vd., 2011). Bu mikroorganizmalar organik asit, diasetil, hidrojen peroksit ve bakteriyosin gibi çeşitli bileşikler üretmektedir (Özyurt vd., 2020). Bu bileşikler gıdanın tadı ve tekstüründe istenilen etkisinin yanı sıra, gıdada mevcut diğer istenmeyen mikroorganizmaların inhibisyonunda önemli etkilere sahiptirler (Jini vd., 2011). Kıyılmış balık veya balık atıklarına tahıl ürünleri ve laktik asit bakterilerinin ilavesi ile hazırlanan silajlarda, fermantasyon oluşumu sonucunda pH 4.5'in altına düşmekte ve sonuç olarak düşük pH'dan dolayı balıkta bozulma etmeni ve patojen bakterilerin gelişimi elimine edilmektedir (Özyurt vd., 2017; Özyurt vd., 2018a). Depolama süresince azot bileşiklerinin yıkımı gerçekleşmekte ve uçucu bazik azot bileşikleri, aminoasit ve peptitlerde artışlar gözlenmektedir (Espe ve Lied, 1999). Proteolitik aktivite başlıca doku proteazından (katepsin) ve kısmen mide-bağırsak proteazından kaynaklanmaktadır (Shahidi vd., 2019). Fermente balık silajı yapımında laktik asit kültürlerinin seçimi büyük bir önem arz etmektedir. Pratik şartlarda saf laktik asit bakteri kültürünü edinip bu işlemi yapmak kolay ulaşılabilir değildir. Bu nedenle iyi bir laktik asit bakterisi kaynağı olan yoğurt bu amaçla kullanılabilir.

Ülkemizde ve birçok ülkede yaygın olarak kullanılan yoğurt, *Streptococcus thermophilus* ve *Lactobacillus bulgaricus* gibi laktik asit bakterilerinin sütte bulunan laktozu fermente etmesiyle oluşan bir süt ürünüdür. İçerdiği besin maddeleri açısından ideal bir gıda maddesi olan yoğurt biyolojik değeri yüksek ve hazmı kolay bir gıda maddesidir. Kolesterolü düşürücü, immün sistemi uyarıcı ve kanser oluşumunu önleyici özellikleri de öne sürülen yoğurt yüzyıllardan beri sevilerek tüketilmekte ve sofralarımızda yer almaktadır. Llanes ve Toledo (2011) Atlantik somonu (*Salmo salar,* Linnaeus, 1758) beslemede su ürünleri işleme atıklarının (kafa, yüzgeç, deri ve iç organlar) %2 sülfirik asit, %1 formik asit karışımıyla asit silajı eldesine karşın, %3 yoğurt ile hazırlanan fermente silajlarının besin madde bileşenleri ve sindirilebilirliklerini araştırmışlardır. Araştırmacılar hazırlanan silajların kimyasal kompozisyonlarının ham materyalden farklılıklar gösterdiğini, ancak bunların besinsel değerlendirmelerinde önemli bir farklılık gözlenmediğini, bu nedenle her iki silaj tipinin Atlantik somonu için hazırlanan rasyonların formülasyonunda alternatif bir protein kaynağı olduğunu belirtmişlerdir. Bu konuda elde edilen bilgiler, küçük işletme ölçeğinde ve ev şartlarında bulunan biyolojik atıkların yararlı hale getirilerek geri dönüşümlerinin sağlanabilmesi ve böylece elde edilen ürünlerin hayvan ve bitkiler için yararlı bir gıda kaynağına dönüştürülebilmesi için önemlidir. Bu amaçla bu araştırmada, su ürünleri atıklarından silaj üretiminde direkt laktik asit bakteri kültürü ilavesi, asitlendirici kimyasal madde ilavesi ve bunların yerine fermente bir gıda olan ve kolay ulaşılabilen yoğurt ilavesi ile balık silajlarının hazırlanması hedeflenmiş ve hazırlanan silajlar olgunlaşma süreleri ve besin madde bileşenleri yönünden değerlendirilmiştir.

MATERYAL VE METOT

Araştırmada deneme materyali olarak kullanılan sardalya balığı (*Sardina pilchardus,* Walbaum, 1792) bölgedeki (İskenderun Körfezi, Kuzeydoğu Akdeniz) balıkçılardan temin edilmiştir. *Streptococcus thermophilus* Çukurova Üniversite'sinin bakteri kültür stokundan temin edilmiştir. Silaj hazırlanmasında sardalya balıklarının baş, deri, kılçık ve iç organları kullanılmış ve bunlar bir kıyma makinesi (3 mm çaplı delik plakalı) yardımıyla kıyılarak homojen hale getirilmiştir. Daha sonra bu karışım her biri 3 kg olacak şekilde üç eşit parçaya bölünmüştür.

Birinci bölüme asit silaj yapmak için asit (%3 formik asit), ikinci bölüme fermente silaj yapmak için *Streptococcus thermophilus* (%3) ve üçüncü bölüme ev yapımı fermente yoğurt (%5) ilave edilmiştir. Bakteri silajların fermentasyonda kullanabilmeleri için %15 oranında bir karbonhidrat kaynağı (melas) ilave edilmiştir (Özyurt vd., 2016). Hazırlanan silajlar 500 ml'lik plastik kovalarda ağızları hava almayacak şekilde muhafaza edilerek olgunlaşıncaya kadar her gün karıştırılmış ve pH değerleri ölçülmüştür. pH ölçümleri dijital bir pH metre (WTW 315i, Germany) ile belirlenmiştir. Tüm gruplar 28 ^{o}C'ye ayarlanmış oda sıcaklığında bekletilmiştir. Deneme grupları her bir muamele için en az üç paralel olacak şekilde kurulmuştur.

Hazırlanan balık silajları olgunlaştıktan sonra genel besin madde bileşenlerini belirleyebilmek amacıyla protein, yağ, nem ve ham kül değerleri belirlenmiştir. Silaj örneklerinin kuru madde ve ham kül tayini için yaklaşık 3,5 - 4 g tartım yapılmış ve darası alınmış porselen kaplara konmuştur. Porselen kaplardaki örnekler kurutma dolabında 103^{o}C'de 4 - 5 saat süreyle (sabit bir ağırlığa kadar) kurutulmuştur (AOAC, 1990). Bu işlem her bir muamele gurubuna ait örneklerden en az 6 paralel olacak şekilde yapılmıştır. Ham kül tayini AOAC (935.47, 1998) metoduna göre yapılmıştır. Örnekler, yakma fırınına yerleştirilerek 550^{o}C'de, 5 saat süreyle (sabit bir ağırlığa ve açık gri bir renk oluşumuna kadar) yakılmıştır.

Kjeldahl metoduna (AOAC 981.10, 1998) göre yapılan toplam ham protein oranı yaş yakma, distilasyon ve titrasyon aşamaları takip edilerek en az üç paralel olacak şekilde gerçekleştirilmiştir. Kjeldahl tüpleri içerisindeki 1 g homojenize edilmiş örnek üzerine, 2 adet kjeldahl tablet (Merck, TP826558) ve 20 ml H_2SO_4 eklenerek yakma ünitesinde örnekler yeşil-sarı renk alana kadar 2-3 saat yakılmıştır. %40'lık NaOH ile distilasyon işlemi sonrasında 0,1 M HCl ile renk değişimi olana kadar titre edilmiş ve sarf edilen HCl miktarı belirlenerek protein miktarı hesaplanmıştır. Lipit analizi Bligh ve Dyer (1959) metoduna göre yapılmıştır. Silaj örneklerinden yaklaşık 3 g alınarak, 1:2 oranında methanol kloroform karışımı eklenmiştir. Örneklerin alkol ile homojenizasyonunda Ultra-turaks (T25 basic IKA-WERKE), yağ ekstraksiyonu ise bir rotary evaporatör (Herdolph WB 2000, Germany) kullanılarak sağlanmıştır.

Elde edilen veriler tek yönlü varyans analizine (one-way ANOVA) tabi tutulmuştur. Çoklu karşılaştırma testi olarak da Duncan multiple testi kullanılmıştır. Önem seviyesi $p<0,05$ olarak alınmıştır.

BULGULAR VE TARTIŞMA

Asit, bakteri ve yoğurt ile sardalya balığı atıklarından hazırlanan balık silajlarının pH değişimleri aşağıda görülmektedir (Tablo 1). Başlangıçta 6.87 olan pH değeri asit ilave edilen grupta hızlıca düşerek denemenin ilk haftasından sonra pH 4 seviyelerine gelmiştir. Bakteri ilave edilen grupta ise bu düşüş biraz daha yavaş seyretmiş ve denemenin ilk haftasından sonra pH 4,5 seviyelerine gelmiştir. pH düşüşü yoğurt ilave edilen grupta daha yavaş ilerlediği görülse de, saf bakteri kültürünün ekildiği "bakteri" grubuna benzer bir seyir izlemiş ve denemenin 10. gününden sonra pH 4,5 seviyesinin altına inmiştir. Dolayısı ile tüm silaj grupları maksimum on gün içerisinde olgunlaşma evresini tamamlamışlardır. Bu dönemde yapılan gözlemlere göre başlangıçta katı parçalar (pul, kılçık vb.) içeren balık atığının gün geçtikçe asit ve bakterilerin etkisiyle daha akışkan, homojen kıvamlı bir ürüne dönüştüğü gözlemlenmiştir (Şekil 1). Bununla birlikte 28°C sıcaklıkta saklanan tüm gruplarda Güllü vd. (2015) belirttiği gibi kokunun rahatsız etmeyen kabul edilebilir hoş malt kokuda olduğu belirlenmiştir. Silaj depolama süresince yumuşamakta ve silajda malt kokusu gelişmektedir (FAO, 1995). Bu durum bize balık atığı içerisinde bozucu bakterilerin gelişemediği, ortam şartları kendi lehine olan laktik asit bakterilerinin geliştiğini göstermektedir. Laktik asit bakterileri gelişip çoğaldıkça ortamda laktik asit birikimi olmuş ve pH değerleri düşmüştür. Balık veya atıkları yalnızca küçük miktarlarda şeker (glikojen) içermektedirler. Fermantasyonun gerçekleşebilmesi yani laktik asit bakterilerinin gelişmeleri için ortamda şeker gibi bir enerji kaynağının olması gerekmektedir. Yapılan bu araştırmada, bu nedenle bakteri silaj gruplarına melas ilavesi yapılmıştır. Şeker kaynağının varlığında bakteri gruplarının pH değerini başarılı bir şekilde düşürdüğü görülmektedir. Bakteri silajlardaki pH düşüşü şekerin açık bir şekilde kullanıldığını, pH'ın düşmesini sağlayan organik asitlerin üretildiğini ve bozucu mikroorganizmaların gelişimlerinin önlendiğini göstermiştir (Özyurt vd., 2016).

Tablo 1. Asit, bakteri ve yoğurt ile hazırlanan balık silajlarının pH değişimi
Table 1. pH change of fish silages prepared with acid, bacteria and yoghurt

Günler	Asit	Bakteri	Yoğurt
0	6,87±0,00	6,87±0,00	6,87±0,00
1	4,02±0,01^a	6,58±0,00^c	6,43±0,01^b
2	4,19±0,02^a	5,25±0,01^b	5,28±0,05^b
3	4,20±0,01^a	5,02±0,01^b	5,12±0,03^c
4	4,40±0,02^a	4,97±0,01^b	5,12±0,01^c
5	4,13±0,02^a	4,71±0,02^b	4,80±0,00^c
6	4,06±0,05^a	4,51±0,08^b	4,64±0,07^b
7	4,10±0,03^a	4,43±0,06^b	4,61±0,01^c
8	4,05±0,02^a	4,38±0,07^b	4,55±0,00^c
9	4,08±0,00^a	4,30±0,04^b	4,54±0,01^c
10	4,07±0,02^a	4,28±0,05^b	4,50±0,01^c
11	4,00±0,01^a	4,23±0,05^b	4,43±0,03^c
12	4,07±0,02^a	4,29±0,04^b	4,46±0,04^c
13	4,08±0,02^a	4,26±0,04^b	4,45±0,07^c
14	3,90±0,00^a	4,20±0,07^b	4,31±0,00^c
15	3,86±0,06^a	4,16±0,04^b	4,30±0,01^c
16	3,86±0,04^a	4,13±0,00^b	4,27±0,00^c
17	3,86±0,02^a	4,15±0,00^b	4,28±0,01^c
18	3,87±0,01^a	4,17±0,02^b	4,31±0,04^c
19	3,85±0,04^a	4,16±0,01^b	4,24±0,05^b

Aynı satırda yer alan farklı harfler (a-c) istatistiksel olarak farklılıkları göstermektedir ($P < 0,05$)
Ortalama değer ±standart sapma, n=3


Şekil 1. Olgunlaşma öncesi ve olgunlaşma sonrasında balık silajından örnek görüntü
Figure 1. Sample image of fish silage before and after ripening

Araştırmada olgunlaşmış balık silajlarının nem, ham kül, protein ve yağ oranlarının belirlenmesiyle hem hayvan yemi hem de bitki gübresi olarak kullanılabilirliği incelenmiştir. Asit, bakteri ve yoğurt ile hazırlanan grupların nem içeriği sırasıyla % 66,93; % 63,18 ve % 64,48 olarak bulunmuştur (Tablo 2). Elde edilen bu verilere göre incelenen üç grup içerisinde en yüksek nem içeriğinin formik asit ile hazırlanan grupta olduğu görülmüştür (P<0.05). Benzer şekilde, Palkar vd. (2017) balık marketlerinden topladıkları balık atıklarından silaj üretimi üzerine yaptıkları araştırmada %3,5 formik asit ilavesi ile hazırladıkları silajlarda nem içeriğini %75,32 olarak bulurken, fermantasyon kaynağı olarak lor peyniri kullandıkları biyolojik silajlarda nem içeriğini %73,62 bulmuşlardır.

Tablo 2. Araştırma gruplarının besin madde bileşenleri (%)
Table 2. Proximate compositions of the research groups (%)

	Nem	Ham Kül	Protein	Yağ
Asit	66,93±0,41[a]	5,64±0,47[a]	11,83±0,44[a]	12,18±0,27[a]
Bakteri	63,98±0,38[b]	5,75±0,05[a]	11,63±0,48[a]	13,65±0,25[b]
Yoğurt	63,81±0,61[b]	5,68±0,17[a]	11,30±0,41[a]	13,21±0,43[b]

Aynı sütunda yer alan farklı harfler (a-b) istatistiksel olarak farklılıkları göstermektedir (P < 0,05)
Ortalama değer ±standart sapma, n=3

Araştırma gruplarının ham kül içerikleri asit gurubunda % 5,64, bakteri gurubunda % 5,75 ve yoğurt gurubunda % 5,68 olarak bulunmuştur (Tablo 2). Elde edilen bu verilere göre incelenen üç grubun ham kül değerlerinin benzer oranlarda olduğu gözlenmiştir. Ham kül bir gıda maddesi içerisindeki toplam inorganik maddeyi ifade etmektedir. Balık silajlarında belirlenen bu ham kül değerlerinin balık atıkları içerisinde bulunan kafa, pul, kılçık ve benzeri kısımlardan kaynaklanmaktadır. Elde edilen bu verilerin tüm bir balık için yapılan ham kül analizlerinde elde edilen verilere benzer olduğu görülmektedir.

Beyaz balıktan yapılan silajlar yapıldıkları balığa benzer olarak ortalama %75-80 su, %10-18 protein, %4-5 kül ve %0,5-2 yağ içermektedir. Yağ içeriği yüksek türlerden elde edilen silajlarda ise genel olarak nem içeriği %62-75, % 4-7, %13-16 ve yağ %5-20 olduğu belirtilmektedir (Özyurt, 2016). Benzer şekilde bu araştırmada da elde edilen protein ve yağ değerlerinin bu sınırlar içerisinde olduğu görülmektedir. Sardalya atıklarından hazırlanan silajların protein oranları asit grupta %11,83 bakteri grupta % 11,63 ve yoğurt grubunda %11.30 olarak belirlenmiştir. Gruplar arasında istatistiksel olarak önemli bir fark gözlenmemiştir (P<0.05). Benzer şekilde Özyurt vd. (2016) %3 *Streptococcus thermophilus* ve %3 formik asit ile hazırladıkları balık silajlarında (*Equulites klunzingeri,* Steindachner, 1898) protein içeriklerini sırasıyla %15,6 ve %16,4 olduğunu ve gruplar arasında istatistiksel olarak önemli bir fark olmadığını belirtmişlerdir.

Yağ içeriklerine bakıldığında, asit grubun yağ içeriğinin %12,18, bakteri grubunun yağ içeriğinin %13,65 ve yoğurt grubunun yağ içeriğinin %13,21 olduğu bulunmuştur.

Fermantasyon için saf bakteri kültürü ve yoğurt kullanılan grupların yağ içeriğinin asit ile hazırlanan balık silajı yağ içeriğine göre istatistiksel olarak daha yüksek olduğu görülmüştür (P<0.05). Fermente silajların lipit içeriğinin, asidik silajdan daha yüksek olmasının nedeni, muhtemelen fermantasyon sırasında kompleks lipitlerin alkolde daha çözünür olan bileşiklere parçalanmasından kaynaklandığı düşünülmektedir (Russell vd., 1993). Birçok araştırmacı balık silajlarından yağın geri kazanımının hem silaj raf ömrünü uzatacağını hem de elde edilen yağların hem insan hem de hayvan beslenmesinde değerlendirilebileceğini belirtmiş ve bunun üzerine araştırmalar yapmışlardır (Goosen vd., 2014; Özyurt vd., 2018b; Özyurt vd., 2019). Özyurt vd. (2018b) özellikle laktik asit bakteri suşları ile üretilen silajdan balık yağının geri kazanılmasının, su ürünleri işleme atıklarının değerlendirilmesi ve katma değerli ürün elde edilmesi için umut verici bir yöntem olduğunu belirtmişlerdir. Bu çalışmaya göre hayvan yemi ve insan diyetleri için bir gıda katkı maddesi ve tamamlayıcı olarak kullanmak için büyük bir potansiyele sahip olan balık silajı yağlarının üretiminde, yağ içeriği oranlarına da bakıldığında fermente silajların tercih edilmesinin daha uygun olacağı görülmektedir.

Tablo 3. Besin madde bileşenleri bildirilen bazı asit ve fermente balık silajları

Table 3. Proximate compositions of some acidic and fermented fish silages

Silaj ham maddesi	Silaj türü	Nem (%)	Kül (%)	Protein (%)	Yağ (%)	Kaynak
Iskarta balık (Kısa gövdeli uskumru, *Rostreliger brachysoma*)	Asit silaj (formik asit)	72,36	3,50	7,66	5,32	Hasan, B, (2003)
	Fermente silaj (*L. pentosus*)	69,12	3,92	14,39	3,97	
	Fermente silaj (*L. plantarum*)	68,92	3,83	13,89	4,06	
Iskarta balık (Eksi balığı, *Equulites klunzingeri*)	Asit silaj (formik asit)	76,9	5,2	16,4	2,1	Özyurt vd. (2016)
	Asit silaj (Formik asit ve sülfirik asit)	74,2	6,3	15,8	2,0	
	Fermente silaj (*L. plantarum*)	76,2	5,5	14,1	1,9	
	Fermente silaj (*Streptococcus thermophiles*)	75,00	5,5	15,6	2,0	
Iskarta balık, fileto atıkları ve iç organları (*Lutjanus spp., Haemulon plumieri, Epinephelus spp.*)	Formik asit	74,3	5,52	15,27	7,45	Gallardo vd. (2012)
Balık işleme atıkları (kafa, iç organlar, deri vs, *Bagre panamensis*, *Peprilus snyderi*, *Sphyraena ensis*, *Trachynotus ovatus*, *Argyrosomus regius*, *Diplodus vulgaris*)	Fermente silaj (*Lactobacillus sp. B2*)	65,5	6,21	13,77	4,97	Ramírez vd. (2013)
Tatlısu balığı temizleme atıkları (kafa, iç organlar, yüzgeç vs)	Asit silaj (Formik ve hidroklorik asit)	74,5	1,13	9,74	9,99	Tanuja vd. (2014)
Tilapia işleme atıkları (*Oreochromis spp.*, kafa, iç organlar, pullar, kemik, deri vs)	Asit silaj (formik asit)	80	3,6	9,7	7,6	Madage vd. (2015)
Balık işleme atıkları (*Otholitus sp.*, kafa, iç organlar, yüzgeç vs)	Fermente silaj (*L. plantarum*)	71,73	7,67	12,51	5,24	Pagarkar vd. (2006)
	Asit silaj (sülfirik asit)	68,04	8,26	15,82	5,47	
	Asit silaj (organik asit karışımı, formik ve propiyonik asit)	67,18	8,50	15,54	5,99	
Tüm sardalya (*Sardina pilchardus*)	Fermente silaj (*L. plantarum*)	58,4	2,1	13,2	12,0	Davies vd. (2020)
	Asit silaj (formik asit)	63.5	2,8	13,3	14,3	
Balık işleme atıkları (belirtilmemiş)	Asit silaj (formik ve propiyonik asit)	80,83	3,3	15,30	5,78	Raj vd. (2018)
Tuna iç organları (*K. pelamis*, bağırsaklar, mide, karaciğer, pankreas, yüzme kesesi, gonadlar vs)	Asit silaj (Asetik asit)	77,3	2,76	15,18	2,54	Banze vd. (2017)
Alabalık iç organları *(Oncorhynchus mykiss*)	Asit silaj (Laktik asit)	68,48	1,72	12,61	19,18	Raeesi vd. (2021)
	Asit silaj (Formik asit ve propiyonik asit)	69,37	1,90	12,79	19,53	
	Fermente silaj (*L. plantarum*)	59,33	2,10	9,40	29,94	

Tablo 3'de asit ve fermente yöntemlerle hazırlanmış bazı balık silajlarında belirlenen nem, kuru madde, protein ve yağ içerikleri görülmektedir. Bu araştırmalarda %59-80 oranlarında nem, %1,9-8,5 oranlarında kuru madde, %7,6-16,4 protein ve %1,9-29,9 oranlarında yağ içeriği belirtilmiştir. Elbette çalışılan bu balık silajlarının besin madde bileşenlerindeki farklılıklar yapıldıkları ham balık materyaline ve kullanıldıkları vücut bölgelerine göre farklılıkları yansıtmış durumdadır. Yapılan bu araştırmada da incelenen asit, bakteri ve yoğurt ilavesi ile hazırlanan balık silajlarının diğer araştırmacıların belirttikleri değer aralığında olduğu görülmekle birlikte, özellikle yoğurt ilavesi ile yapılan balık silajlarının hem olgunlaşma süresi hem de besin madde bileşenleri açısından beklentileri karşıladığı görülmüştür. Tüm bu veriler değerlendirildiğinde elde edilen üç balık silajı grubunun da yüksek protein ve yağ içeriği ile değerli bir besin kaynağı olduğu belirlenmiştir.

SONUÇ

Balık, besleyici bir gıda olarak her zaman önemli sayılmış, genel diyet önerileri ve hayvan rasyonları içerisinde yerini daima korumuştur. Bu durum, esas olarak yüksek protein içeriklerinin yanında, omega-3 serisi yağ asitleri olarak bilinen çoklu doymamış yağ asitlerinin ana kaynağı olmalarından ileri gelmiştir. Su ürünleri işleme sanayi atıklarının değerlendirilmesi hem çevre kirliliğinin önlenmesi hem de bu atıklara ekonomik olarak değer kazandırılması açısından önemli bir konudur. Güllü vd. (2015) balık atıklarından ürettikleri silajların maliyetinin 0,72 TL/kg olduğu ve bu silajların yemde balık unu yerine kullanımının %21 oranında yem maliyetini azaltabileceğini belirtmiştir. Üretilecek silajların, düşük maliyetle elde edilebilme ve hayvan beslemede kullanılabilme potansiyeline sahiptir olduğu açıktır. Bununla birlikte ticari gübrelere alternatif veya destek olacak şekilde bitki tarımında kullanılabildikleri bilinmektedir (Karim vd., 2015; FAO,1995). Balık silajlarında diğer su ürünlerinde olduğu gibi kimyasal, duyusal, fiziksel ve özellikle mikrobiyolojik değerlendirmelerin yapılması önemlidir. Yapılan birçok çalışmada asit veya fermente hazırlanan silajlarda bakteri yüklerinin silajların olgunlaşmasıyla birlikte önemli ölçüde azaldığı kaydedilmiştir (Zahar vd., 2002; Delgado vd., 2008; Ramasubburayan vd., 2013; Tanuja vd., 2014). Benzer şekilde Özyurt vd. (2020) ve Kuley vd. (2020) ıskarta ve su ürünleri işleme sanayi atıklarından hazırladıkları silajlarda toplam bakteri sayısı azalırken, toplam laktik asit bakteri sayısında önemli artışlar olduğu, maya ve küfe rastlanmadığı ve depolama sürecinde koliform bakterilere rastlanmadığını rapor etmiştir. Fermente ürünlerde fermantasyon işlemi sonunda koliform gibi indikatör mikroorganizmaların eleminasyonu asidifikasyon ve/veya laktik asit baterileri tarafından üretilen bazı inhibitör bileşiklerden kaynaklandığı düşünülmektedir (Ndaw vd., 2008). Bu nedenle, özellikle, küçük ölçekli üretici için dikkat edilmesi gereken en önemli parametre pH değerinin takip edilmesidir. Balık silajları, depolamada eğer asitliğine dikkat edilirse aylar veya bir kaç yıl boyunca bozulmadan saklanabilmektedir (Larsen, 2015; FAO, 1995). Yapılan bu çalışma ile de birlikte, küçük işletme ölçeğinde ve ev şartlarında bulunan bu biyolojik atıkların bitki ve hayvanlar için çok yararlı bir besin kaynağına başarılı bir şekilde dönüştürülebileceği saptanmıştır.

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Yazarlar, araştırmalarını (makale) etkileyebilecek bilinen herhangi bir mali veya kişisel çatışma olmadığını beyan etmektedir.

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Bu makalede, insan ve hayvan ile ilgili etik kurul onayına gerek olan bir çalışma içermemektedir.

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SHORT COMMUNICATION **KISA MAKALE**

The invasive silver-cheeked toadfish, *Lagocephalus sceleratus* (Gmelin, 1789) appeared in the Sea of Marmara after more than a decade: A call for awareness

İstilacı benekli balon balığı, *Lagocephalus sceleratus* (Gmelin, 1789) on yıldan fazla bir süre sonra Marmara Denizi'nde görüldü: Bir farkındalık çağrısı

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Abstract: This study presents a new locality record of poisonous pufferfish, *Lagocephalus sceleratus* (Gmelin, 1789), one of the 18 worst invader fish species identified globally by IUCN, from the Sea of Marmara caught by a commercial purse seine fishing vessel while fishing anchovy on 22 February 2021. Previously, this invasive species was first recorded at the Gelibolu peninsula in the Sea of Marmara between September 2007 and October 2008. *Lagocephalus sceleratus* recorded in this study is larger in size with 142 mm total length than previous records (126 mm in 2007; 95 mm in 2008) for the Sea of Marmara. The presently reported record represents the first documented occurrence of this species in the Erdek Bay of the Sea of Marmara and the third documented record for the entire Sea of Marmara.

Keywords: Invasive alien species (IAS), lessepsian migration, range expansion, pufferfish, Tetraodontidae, Tetrodotoxin

Öz: Bu çalışma, Marmara Denizi'nde 22 Şubat 2021'de ticari gırgır balıkçı teknesi tarafından hamsi avı sırasında avlanan ve IUCN tarafından küresel olarak en tehlikeli 18 istilacı balık türünden biri olarak tanımlanan balon balığının *Lagocephalus sceleratus* (Gmelin, 1789)'un yeni bir lokalite kaydını sunmaktadır. Bu istilacı tür Marmara Denizi'nde ilk olarak Eylül 2007 ile Ekim 2008 arasında Gelibolu yarımadasında kaydedilmiştir. Bu çalışmada kaydedilen birey, 142 mm toplam boya sahip olup, Marmara Denizi için verilen önceki kayıtlardan (2007'de 126 mm; 2008'de 95 mm) daha büyüktür. Halihazırda rapor edilen kayıt, bu türün Marmara Denizi'nin Erdek Körfezi'ndeki varlığını ilk kez ortaya koymakta olup, tüm Marmara Denizi için belgelenmiş üçüncü kaydını temsil etmektedir.

Anahtar kelimeler: İstilacı yabancı türler (IAS), lesepsiyan göçü, menzil genişlemesi, balon balığı, Tetraodontidae, Tetrodotoksin

INTRODUCTION

The Suez Canal has cut the sailing distance between the edges of Europe and Asia by 43% and thus considerably reduced the cost of delivering goods between these continents since 1869 (Fletcher, 1958; Savitzky, 2015). This man-made sea-level waterway connects the Mediterranean Sea to the Indian Ocean through the Red Sea and currently handles up to 12% of global trade (Feyrer, 2021). On the other hand, the Suez Canal is considered a wormhole for bringing in marine alien invaders from the Red Sea into the Mediterranean Sea (MedECC, 2020). More than 600 invasive species, among them above 120, have flowed into the Mediterranean Sea, establishing populations in their new environment, potentially threatening the region's fisheries, endemic biodiversity, tourism, and human health (Fitt, 2020; Golani et al., 2020; Kovacic et al., 2021). The other concern is the permanent colonies of poisonous species that have neurotoxins such as tetrodotoxin in their skin, ovaries, liver, and reproductive organs that can kill an adult at a dosage of as little as 2 mg (Noguchi and Ebesu, 2001). Consequently, such poisonous invaded species have posed severe problems, especially for fishermen unfamiliar with these newly established poisonous species. A fishmonger living in the eastern province of Van Türkiye died last year after eating Pufferfish *Lagocephalus sceleratus* (Gmelin,1789), which he likened to the rockfish (Hürriyet Daily News, 2021).

Up to 13 different Pufferfishes and Porcupinefishes (*viz*., *L. sceleratus*, *L. lagocephalus*, *Ephippion guttiferum, L. guentheri*, *L. suezensis*, *Sphoeroides pachygaster*, *Sphoeroides spengleri*, *Sphoeroides marmoratus*, *Tylerius* spinosissimus, *Torquigener flavimaculosus* belong to the family Tetraodontidae, and *Chilomycterus reticulatus*, *Cyclichthys spilostylus*, *Diodon hystrix* belong to the family Diodontidae) have introduced their populations in the Mediterranean and adjacent seas (Ulman et al., 2021). Tetraodontidae species are known to contain the neurotoxin "tetrodotoxin" (Noguchi and Ebesu, 2001; Ulman et al., 2021).

The silver-cheeked toadfish, *L. sceleratus*, was reported for the first time from the Aegean Sea, Gökova Bay, in February 2003 (Filiz and Er, 2004). Later, the species of Pufferfish was also recorded in the Çanakkale Strait (Sea of Marmara) between 2007 and 2008 (Tuncer et al., 2008; Irmak and Altinagac, 2015).

In this paper, we presented a new locality record of this poisonous puffer fish species, which should be accounted as the first documented record for the Erdek Bay in the Sea of Marmara and the third documented record for the entire Sea of Marmara. The morphometric and meristic properties of the obtained *L. sceleratus* were provided in this paper.

MATERIALS AND METHODS

One male *L. sceleratus* specimen was caught in the Sea of Marmara (Erdek Bay; 40°26'28"N; 27°40'47"E) by a commercial purse seine fishing vessel (usually fishing between 110-130 m depth) while fishing anchovy on 22 February 2021 (Figure 1).

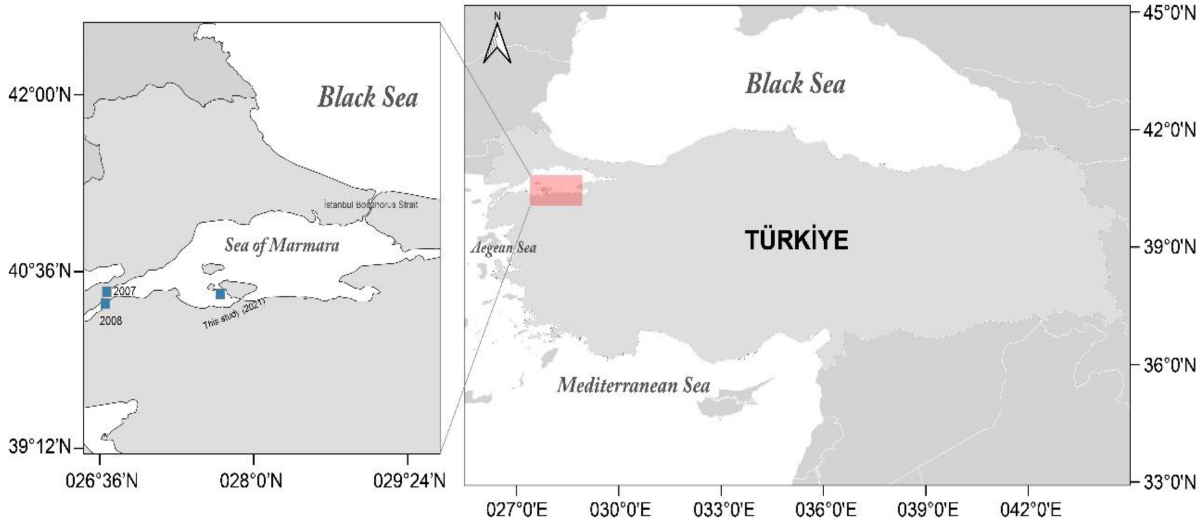

Figure 1. Map showing the area in the Sea of Marmara from where the current specimen of the poisonous Pufferfish, *Lagocephalus sceleratus,* was recorded on 22 February 2021.

The specimen was purchased from the fisherman and brought to the laboratory for a detailed examination. The identification keys provided by Akyol et al. (2005), Irmak and Altinagac (2015), and TUDAV (2022) were used to identify the obtained specimen. Body measurements such as weight (g) and various metric measurements such as total, fork and standard lengths, orbital diameter, head length, body depth, predorsal length, caudal peduncle length, and caudal peduncle depth were recorded. The meristic characteristics were also counted for dorsal fin rays, pectoral fin rays, caudal fin rays, and anal fin rays

RESULTS

The total length of obtained *L. sceleratus* was 142 mm with a body weight of 32.88 g. The morphometric and meristic data are provided in Table 1.

The dorsal of *L. sceleratus* was covered with dark spots with a dominant green-bluish colour. The dorsal fin was yellowish, while soft anal rays were whitish. The ventral part of the body was silver from the nose to the caudal. The eyes were big and wide in diameter (Figure 2).

Figure 2. The silver-cheeked toadfish pufferfish, *Lagocephalus sceleratus*, individual was caught in Erdek Bay of the Sea of Marmara by a commercial purse seine fishing vessel while fishing anchovy on 22 February 2021 (photo: Habib BAL).

Table 1. Morphometric and meristic of the poisonous Pufferfish, *Lagocephalus sceleratus* specimens captured in February 2021 Erdek Bay, together with previous records, from the Sea of Marmara.

Measurements	This study	Irmak and Altinagac (2015)	Tuncer et al. (2008)
Total length (L_T), mm	142	95.03	126
Fork length (L_F), mm	134	91.02	
Standard length (L_S), mm	123	82.24	111
Body depth, mm (%L_T)	36.6 (25.7)		
Head length, mm (%L_T)	17.4 (12.2)		
Orbital diameter, mm (%L_H)	12.2 (68.9)		
Predorsal length, mm (%L_T)	52.7 (37.1)	70.93 (86.24;%L_s)	
Caudal peduncle length, mm (%L_T)	31.8 (22.3)		
Caudal peduncle depth	4.23		
Dorsal fin rays	12	12	12
Pectoral fin rays	18	18	17
Caudal fin rays	20		
Anal fin rays	10	10	12

DISCUSSION

Lagocephalus sceleratus has been subsequently documented in the Adriatic Sea, the central Mediterranean Sea, the Italian coasts of the Ionian Sea, and the Levantine Sea after its first occurrence in the Gökova Bay, the Aegean Sea in February 2003 (Figure 3, Irmak and Altinagac, 2015; Akyol and Ünal, 2017; Ulman et al., 2021).

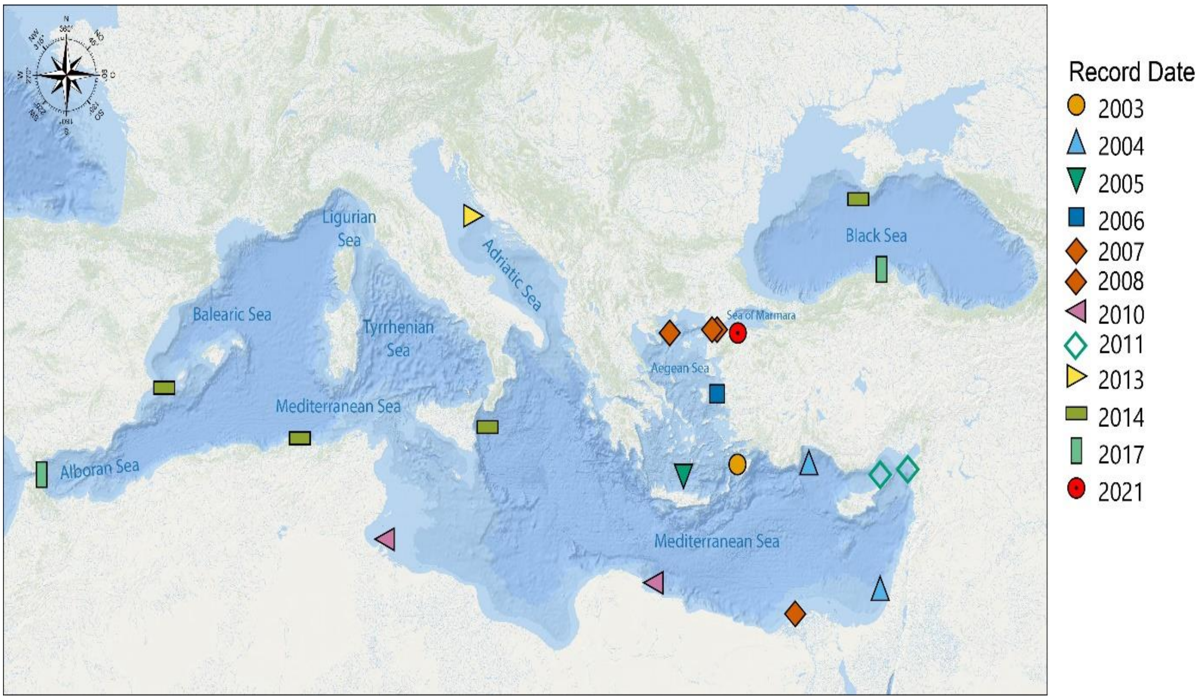

Figure 3. Range expansion of the poisonous Pufferfish, *Lagocephalus sceleratus* in the Mediterranean and the Black marine waters (consult Akyol and Ünal, (2017) for details)

The maximum length record for Turkish territorial waters was determined as 6 kg with 755 mm total length caught in Fethiye (Ulman et al., 2021), which is well below the maximum value of 8 kg with 12000 mm total length reported from the Eastern Mediterranean (Zakynthos, Greece) by Ulman et al. (2022). For the Sea of Marmara, this study documented the maximum size *L. sceleratus* compared to previous records of 126 mm in 2007 (Tuncer et al., 2008) and 95 mm in 2008 (Irmak and Altinagac, 2015). Both of these records were obtained on the off coast of Çanakkale Strait in the Sea of Marmara (Tuncer et al., 2008; Irmak and Altinagac, 2015). The current documentation has provided the third record for the entire Marmara Sea, constituting an important observation as it was observations in the inner parts of the Sea of Marmara (Figure 2).

Pufferfishes are spreading rapidly in the Mediterranean Sea and the Aegean Sea, threatening native fish species in the area. These destructive invasive species negatively affect biodiversity and alter the food chain. The diet composition of such fish species is quite diverse, which consisting of 54% shrimp (Penaeidae), 17% crab (Portunidae), 14% fish, 4% squid, and cuttlefish (Cephalopods), and 11% other prey (Aydin, 2011). At a size of 420–430 mm, they reach maturity in their third year of life (El-Ganainy et al., 2006). The results of the previous research, which have been detected in Turkish marine waters, are given in Table 2.

Table 2. Historical records of the poisonous Pufferfish, *Lagocephalus sceleratus,* in the Turkish marine waters

LOCATION	Latitude ° N	Longitude ° E	Depth (m)	Record date	N	Total length (mm)	References
MEDITERRANEAN SEA							
Gökova Bay	37.2	28.3	15	2003–02–17	1	459	Akyol et al. (2005)
Gökova Bay	37.0	28.3	-	2003–01–08	1	-	Filiz and Er (2004)
Kemer, Antalya	37.4	31.1	-	2004–09–18	1	389	Bilecenoglu et al. (2006)
Bodrum,Gökova Bay	37.0	28.6	-	2005–03–10	2	-	Bilecenoglu et al. (2006)
Adrasan, Antalya	-	-	3	2005–05–14	2	200	Bilecenoglu et al. (2006)
Kaş, Antalya	-	-	-	2005–10–03	1	-	Bilecenoglu et al. (2006)
Iskenderun Bay	36.3	36.9	-	2009–01–02	4	388–611	Torcu Koç et al. (2011)
Antalya Bay	36.8	30.7	-	2010–08–20	656	125–650	Aydin (2011)
Mersin Bay	36.4	35.8	2	2010–11–10	2	75–84	Yaglioglu et al. (2011)
Iskenderun Bay	36.6	34.8	3	2010–11–29	2	65–75	Yaglioglu et al. (2011)
Antalya Bay	36.6	31.0	-	2011-2013	100	126–535*	Zengin (2014)
Iskenderun Bay	-	-	50	2012–11–20	77	89–784	Başusta et al. (2013)
AEGEAN SEA							
Hekim Island, Izmir	38.4	26.8	0–12	2006–04–21	1	498	Bilecenoglu et al. (2006)
Asos, Çanakkale	-	-		2008–01–07	1	-	Türker-Çakır et al. (2009)
Saros Bay	40.4	26.3		2017–01–12	1	556	Tunçer and Önal (2014)
SEA OF MARMARA							
Gelibolu coast	40.2	26.4		2007–09–23	1	126	Tuncer et al. (2008)
Gelibolu coast	40.4	26.7		2008–01–10	1	95	Irmak and Altinagac (2015)
Erdek Bay	40.4	27.7		2021–02–22	1	142	This study

*SL: standard length

CONCLUSION

The poisonous pufferfish, *L. sceleratus,* has been blacklisted by the International Union for Conservation of Nature (IUCN) as one of the 18 worst invasive fish species (Otero et al., 2013). The occurrence of this poisonous species in the Sea of Marmara strongly indicates that it can flow into the Black Sea in the future. Though the first Black Sea record of the species was given from the western coast of Sevastopol, where two mature individuals with standard lengths of 59.0 and 50.3 cm were collected on 2 and 7 November 2014, respectively (Boltachev et al., 2014). It has also been observed on the Türkeli shore, Sinop Black Sea, based on a piece of news that appeared in a local Turkish newspaper (Bilecenoğlu and Öztürk, 2018). In addition to our record, this fish was reported again in the Sea of Marmara, and this time it was caught in the district of Gemlik by amateur fishing gear (NTV newspaper, 2021). This second record from the Sea of Marmara, in less than two months, could reaffirm the above statement of its subsequent flow into the Black Sea in the future. However, the specimens reported by Bilecenoğlu and Öztürk (2018) and NTV newspaper (2021) were not examined by an expert and thus certainly require a future confirmation. Considering the recent accident of the death of a fisherman in Türkiye, the local authority should upskill the people using social media and arrange special broadcasting programs on TV and radio to avoid and protect them from the consumption of this poisonous fish species.

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AUTHORSHIP CONTRIBUTIONS

Both authors contributed equally.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

ETHICS COMMITTEE APPROVAL

Ethics Board approval was not required.

DATA AVAILABILITY

All data underlying the results are available as part of the article and no additional source data are required.

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