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Authors Guidelines

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

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

GENERAL INFORMATION

Aim & Scope

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

The journal does not charge any submission and publication fees.

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

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

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

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

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

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

Language

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

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

Editorial Policy and Referee Process

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

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

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

Article Types

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

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

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

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

Reports

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

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

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

MANUSCRIPT SUBMISSION

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

Before you start submitting an article, please ensure that the article complies with the journal guidelines (instructions) and that you are ready to upload all requested documents (Article File, Similarity Report, Cover Letter, Copyright Release Form, Ethics Committee Approval (if necessary). Please note that submissions that do not contain the required documents/statements will be returned incomplete.

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

While starting

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

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

To follow the status of the article;

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To verify the authenticity of the submitted article, a similarity report should be obtained by using the services of plagiarism detection software (Crossref Similarity Check, iThenticate: Plagiarism Detection Software). This report should be uploaded as a separate file named "similarity report".

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

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

PREPARATION OF MANUSCRIPTS

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

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

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

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

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

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

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

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

First Page

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

Author Names and Affiliation

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

Present the authors' affiliation addresses should be indicated at the author's name with superscript numbers immediately after the author's name. The full postal address of each

affiliation at the time of research should be listed in order: Department, institution, city with postcode, and country name.

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

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

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.

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

Conclusions

This should briefly state the major findings of the study.

Acknowledgements and Funding

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

Examples:

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

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

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

Financial support acknowledgwment should be written like the example given:

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

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

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

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

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

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

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

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

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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.

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

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

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Ethics Approval

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.

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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. https://doi.org/10.1038/s41598-020-59867-7

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

For example:

Kocataş, A. (1978)

Kocataş, A., & Ergen, Z. (1972).

Kocataş, A., & Geldiay, R. (1972)

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

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

Journal Articles

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

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

Books

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

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

Chapter in books

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

E-books and chapter in e-books

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

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

Proceedings

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

Websites

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

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

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

Thesis

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

Tables and Figures

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Molecular investigation of nematodes isolated from three economical fish species taken from Çanakkale (Türkiye) fish market

Çanakkale (Türkiye) balık pazarından alınan üç ekonomik balık türünden izole edilen nematodların moleküler incelenmesi

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Abstract: This study was based on the molecular identification of endoparasites sampled from three economically valuable fish species caught from the waters of the Canakkale (Türkiye). Subjected samples in the study were from chub mackerel (*Scomber japonicus* Houttuyn, 1782), anchovy (*Engraulis encrasicolus* Linnaeus, 1758), and bogue (*Boops boops* Linnaeus, 1758) without gender discrimination. The nematode parasites obtained from the samples were sent to molecular diagnostic laboratories in alcohol and the results were interpreted. As a result of the study, *Anisakis typica* (Diesing, 1860) Baylis, 1920 and *Anisakis pegreffii* Campana-Rouget and Biocca, 1955 were isolated from the chub mackerel fish and *Anisakis pegreffii* species were also isolated from anchovy and bogue. This parasite was the most isolated nematode parasite among all nematodes.

Keywords: *Anisakis pegreffii*, *Anisakis typica*, phylogenetic tree, molecular, Çanakkale, Aegean Sea

Öz: Bu çalışma, Çanakkale (Türkiye) sularından yakalanan ekonomik açıdan değerli üç balık türünden örneklenen endoparazitlerin moleküler tanımlanmasına dayanmaktadır. Çalışmada cinsiyet ayrımı yapılmaksızın kolyoz (*Scomber japonicus* Houttuyn, 1782), hamsi (*Engraulis encrasicolus* Linnaeus, 1758) ve kupes (*Boops boops* Linnaeus, 1758) balıklarından örnekler alınmıştır. Örneklerden elde edilen nematod parazitler alkol içerisinde moleküler tanı laboratuvarlarına gönderilmiş ve sonuçlar yorumlanmıştır. Çalışma sonucunda kolyoz balıklarından *Anisakis typica* (Diesing, 1860) Baylis, 1920 ve *Anisakis pegreffii* Campana-Rouget ve Biocca, 1955, hamsi ve kupes balıklarından ise *Anisakis pegreffii* türleri izole edilmiştir. Bu parazitin tüm nematodlar arasında en fazla izole edilen nematod parazit olduğu görülmüştür.

Anahtar kelimeler: *Anisakis pegreffii*, *Anisakis typica*, filogenetik ağaç, moleküler, Çanakkale, Ege Denizi

INTRODUCTION

Seafood, which is one of the most valuable food sources, especially fish, contains all the amino acids required for the protection and development of tissues in the human body, being rich in unsaturated fatty acids (omega 9, omega 6, and omega 3) and being easily digestible, vitamins and minerals necessary for humans. It is one of the most valuable food sources that can meet this need with its rich content, high cholesterol, and blood pressure, cardiovascular diseases, diabetes for adults, and protection of the human body against some types of cancer (Ozkan and Koca, 2006; Turan et al., 2006).

Fish production has shown a rapid increase in both aquaculture and hunting due to the increase in demand in recent years. In addition to the increased production amount with the increase in the need, it also brought the existence of serious disease factors. While the diseases observed in the aquaculture area can be easily eliminated, the diseases cannot be prevented in the hunting area. Because the organisms that cause these diseases are widely spread in nature. Some commercially important fish species, which are not yet produced, are under the threat of disease factors that can be found free and need a living thing to cling to at another stage of their lives. So much so that disease agents with this complex life cycle can also use humans and other mammals as a destination. In this context, anisakids, which are included in the life cycle of fish, have been declared by the European Food Safety Authority (2010) as the "biological hazard" of the highest importance in seafood products (Biohaz, 2023). Various negative factors reduce the quality and nutritional value of fish meat. Among these factors, various diseases caused by viruses and bacteria as well as parasites were reported previously (Ozkan and Koca, 2006). Although fish parasites rarely cause problems in the natural environment, it is known that they reduce the nutritional value of fish in aquaculture, negatively affect their growth, reproduction, and nutrition, cause serious disease outbreaks, and thus adversely affect the fishing industry (Quiazon, 2015).

The genera *Anisakis* (Dujardin, 1845), *Pseudoterranova*, and *Hysterothylacium* are nematode parasites of the family Anisakidea (Ángeles-Hernández et al., 2020). *Anisakis simplex* (Rudolphi, 1809) Dujardin, 1845*, Anisakis pegreffii,* and *Pseudoterranova decipiens,* (Krabbe, 1878) Gibson, 1983 are of main public health interest. Anisakiasis is a zoonotic disease caused by the consumption of raw or undercooked seafood containing larval nematodes of the *Anisakis*, *Pseudoterranova*, and *Contracaecum* genera in the Anisakidae family. People catch this infection by consuming some seafood raw or undercooked. Consumption of fish after being caught, frozen without being cleaned, and stored for a certain period, caused an increase in anisakiasis events. On the other hand, if the fish are cleaned as soon as they are caught, the risk of infection decreases when the parasites in the digestive system are removed before they pass into the muscles (Sarımehmetoğlu and Doğanay, 1999; Hochberg and Hamer, 2010; FAO/WHO, 2012; Ludovisi et al., 2017). Anisakiasis is clinically acute or chronic in the human gastrointestinal tract. In addition, allergies occur in individuals sensitive to this parasite. Seafood has a very important place in microorganisms transmitted to humans by food. It is necessary to know the disease factors transmitted by seafood, which has become an important source of human food, and to fight effectively. Although parasites are very common, because seafood is consumed and cooked due to Turkish cultural habits, infections due to such parasites are very rare. Due to the increase in consumption of popular and traditional foods specific to Europe and the Far East in Türkiye in recent years, society needs to be informed about these issues (Kurşun and Erol, 2008; Oktener, 2004).

Species belonging to the genus *Contracaecum*, and especially species belonging to the genera *Anisakis* and *Pseudoterranova*, have been associated with anisakiasis/anisakidosis, both in terms of stomach problems and as fish-borne pathogens producing allergic reactions. Although other nematodes such as *Hysterothylacium* species of the Rhaphidascarididae family are not thought to be pathogenic, data have been reported in studies to cause allergic reactions in humans. Since the *Hysterothylacium* parasite seriously inhibits the growth rate and health of the fish it uses as a host, it can cause death by lowering the immune systems of the fish. A group of these parasitic creatures called zoonosis includes individuals belonging to the Anisakidae family (Audícana et al., 2002; Mattiucci et al., 2017; Li et al., 2016).

Anisakid nematode larvae are the most isolated parasites from aquatic organisms. Nematode parasites are creatures with a complex life cycle. They use different intermediate hosts throughout their lives. Parasitic nematodes use different intermediate hosts throughout their lives - fish and invertebrates. Their final hosts are usually humans or other mammals. These creatures, which pass to humans in larval three and larval four stages, are called zoonotic parasites. It is transmitted to humans by consuming raw or undercooked seafood. Species identification is difficult when nematodes are in larval form. The naming of species may not go beyond genus size in microscopic examinations in terms of morphology. Molecular diagnostic techniques are used for definitive diagnosis. Sometimes, even molecular diagnostic methods may not provide an easy and accurate diagnosis for the identification of the living thing. While some subspecies will cause the living thing to diverge with a single gene, it may create the idea that it shows the gene sequence of the same parasite (Balbuena et al., 2000; Chaia et. al., 2005).

Humans are infected with two species of *Anisakis*, *A. simplex* stricto, and *A. pegreffii*. It has also been confirmed by molecular techniques that these two species cause human infections. Improving taxonomic definitions by using molecular diagnostic methods as well as traditional methods for specific identification will help to understand the biological, ecological, and epidemiological aspects of nematodes by shedding light on their life cycles and geographical distributions.

MATERIALS AND METHODS

Fish were sampled monthly from the Canakkale Fish Market for 12 months between September 2019 and 2020. In the research, a total of 1337 fish, and nematode parasites were collected.

As a result of the observations made, generally, free-moving nematode parasite individuals were found buried on the internal organ surfaces of the fish. All parasites in each fish were carefully collected and taken into 99.9% absolute ethanol for microscopic examinations and molecular determinations.

Molecular diagnosis of nematodes isolated from fish species carried out with manufacturer procedure (Pekmezci, 2021; Mattiuci et al., 2011). EurX GeneMATRIX Tissue & Bacterial DNA isolation kit (Poland) used for DNA isolation from fish species. Spectrophotometric measurement was performed on Thermo Scientific Nanodrop 2000 (USA) device to control the amount and purity of DNA obtained after DNA isolation. Targeted genes for species identification in molecular diagnosis of nematodes, primers of the COII region (211F: 5'-TTT TCT AGT TAT ATA GAT TGR TTY AT-3')- (210R: 5'- CAC CAA CTC TTA AAA TTA TC-3') (PCR optimization was done a company) was replicated in a kyratec thermocycler device. In the study, the PCR mix was adjusted to be 35 µl (Table 1). The following PCR cycle was then performed (Table 2).

Table 1. PCR mixes for the COII gene region

Compound	Stock Concentration	Reaction Concentration
PCR Buffer	10X	1X
MgCl2	25mM	1,5 mM
dNTP mix	20 mM	0,2 mM
F. Primer	10 µM	0,3 µM
R. Primer	10 µM	0,3 µM
Taq DNA Polymerase	5U/ µl	2 U
DNA template	3 µl	
Makeup to 35 µl with PCR-grade water.		

Table 2. The following PCR cycle was then performed

Stage	Definition	Temperature	Duration	
1	initial denaturation	95°C	5 min.	40 cycle
2	denaturation	95°C	45 sec.	
3	annealing	54°C	45 sec.	
4	extension	72°C	60 sec.	
5	final extension	72°C	5 min.	
	stop	4°C	∞	

The amplification products obtained by PCR were run on a 1.5% agarose gel prepared with 1x TAE buffer at a 100-volt current for 90 minutes by electrophoresis. PCR products showing single bands in the gel were purified according to the procedures for sequencing using the MAGBIO "HighPrep™ PCR Clean-up System" (AC-60005) purification kit. Sequence analysis was performed at the Macrogen Netherlands laboratory with the ABI 3730XL Sanger sequencing device (Applied Biosystems, Foster City, CA). The nematode species were detected using the NCBI blast program.

The results of the sequence analysis of the COII gene region were used in MolBio Tools program, which can perform online restriction analysis, and in silico restriction, cuts were performed with HaeIII, HhaI, and HinfI enzymes. The nematode *A. pegreffii* with the code NC_034329.1 retrieved from the NCBI genome database was used as a reference.

RESULTS

This study was carried out to determine the nematode species isolated from some fish with high economic value taken from the Canakkale (Türkiye) fish market of the isolated 137 parasites. The figures showed that the most common parasitism was in chub mackerel, and it was the slightest common fish species with 2 parasites in the bogue included in the study. The COII gene region was used for the molecular characterization of nematodes isolated from fish samples. After PCR amplification of the COII gene region, DNA sequence analysis was performed with the ABI 3730XL Sanger-sequencing device (Applied Biosystems, Foster City, CA). The obtained COII gene sequences were analyzed using BLAST tool on NCBI database and the isolated nematode samples were identified. As a result of the sequence analysis, nematodes (99.5%-100%) isolated from anchovy determined as *A. pegreffii*, those isolated from Bogue (99.50-99.83%) determined as *A. pegreffii* and the nematodes isolated from chub mackerel were defined as *A. pegreffii and A. typica* (99.67%-100%). The COII gene sequences of identified nematodes were submitted on the GenBank platform and accession numbers for sequences were attained and accession numbers were shown in Figure 1.

The determining phylogenetic relationship of identified nematodes, all the COII sequences were aligned using MEGA 11 software and ClustalW v1.6 alignment tool with default parameters. The Neighbour-joining tree was constructed by using the Bootstrap method with 1000 replicates. A bootstrap value of above %60 was given in branches (Figure 1).

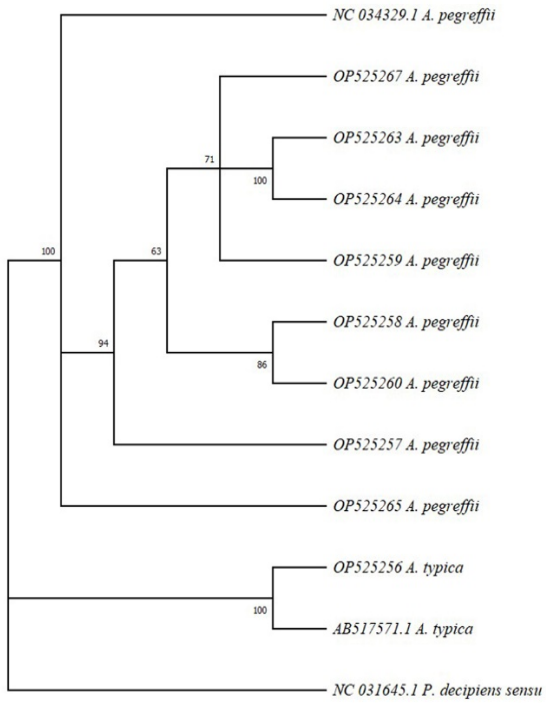

Figure 1. Phylogenetic tree for nematodes

All *A. pegreffii* species showed two restriction profiles because of *in-silico* restriction cuts. While 11 *A. pegreffii* in the first profile did not have cleavage sites for HaeIII and HhaI enzymes, it was found to have two cleavage sites for HhaI enzyme (302-205-88).

It was determined that the nematode parasite *A. pegreffii* from Bogue 3 similarly did not have a cleavage site for the enzyme HaeIII and HhaI but had a single cleavage site for the enzyme HinfI (305-301) (Table 3). *A. pegreffii* nematode with the code NC_034329.1 obtained from the NCBI genome database was used as a reference and it was seen that it had the same restriction profile as 11 species as a result of *in-silico* analysis. As a result of in-silico restriction analysis of the COII gene region of the nematode parasite from Spanish mackerel 1, it was seen that there was no cut-off region for the HhaI enzyme, but it had two cut-off regions for HaeIII and a single cut-off region for HinfI.

As a result of DNA sequence analysis, the difference of *A. pegreffii* nematode from Bogue 3 from other nematode species is due to the difference in a single nucleotide, 5'-AANTC-3', while it should be 5'-G^ANTC-3' in the sequence corresponding to the restriction cut site for the HinfI enzyme. is thought to be possible.

The nematodes that are the subject of the study; It was isolated from the gastrointestinal systems of fish, body cavities with gonads and pyloric caeca, and the liver. No parasite formation was observed on edible meat during the study.

Table 3. Restriction profile of detected nematodes

Rest Profile	Host	Identified Species	Seq Length (bp)	HaeIII	HhaI	HinfI
P-1	Anchovy 3	*A. pegreffii*	595	-	-	302-205-88
	Chub Mackerel 3	*A. pegreffii*	600	-	-	306-206-88
	Bogue 1	*A. pegreffii*	604	-	-	304-212-88
	NC_034329.1	*A. pegreffii -Ref*	697	-	-	333-249-88-27
P-2	Bogue 3	*A. pegreffii*	606	-	-	305-301
P-3	Chub Mackerel 1	*A. typica*	576	359-135-82	-	306-270
	KF356652.1	*A.typica-Ref*	600	356-162-82	-	303-209-88

DISCUSSION

Considering the zoonotic characteristics of Anisakid species, food safety and public health need to determine the presence and occurrence of Anisakid nematode larvae, especially in different fish species used for human consumption. In addition, the European Food Safety Authority (EFSA, 2010) recommends that it is important for public health to regularly collect data on the microhabitats, infection rates, densities, seasonal and geographical distributions, and life cycles of parasites in fish caught from the natural environment. The data of the species is due diligence in the fish caught from the Northern Aegean Sea coasts of our country and includes basic data of the species.

The study was carried out in the Canakkale region of the North Aegean Sea. In the study, 137 nematode larvae were collected. From the isolated parasites, 30 were selected by visual separation under the microscope and the molecular diagnosis was made. The remaining parasites were identified by the traditional method. A molecular examination of these larvae revealed that most of them were *A. pegreffii*, and one isolate was *A. typica*. The parasites found were living freely in the internal organs and body cavities of the fish. While it was observed that the most infected species was chub mackerel, two nematode isolations were observed from the bogue examined in the study. Obtained nematodes were identified by molecular analysis. The molecular technique used is sequencing by sequence analysis. In a study conducted in the Aegean Sea, it was determined that the nematodes obtained were also individuals belonging to the Anisakidae family. The PCR-RLFP technique was used in the study, and it was defined as *A. pegreffii* and *A. pegreffii* hybridized with *A. simplex* (Chaligiannisa et al., 2012).

Klimpe et al. (2011) investigated the deep-water life cycle of *Anisakis paggiae,* in the Irminder sea and their distribution among Kogiid Whales. In the study, they stated that the whale is a typical final host and stated that the *A. paggiae* species was transported to other seas by migratory whales. In addition, nematodes were isolated from the stomach, pyloric caeca, intestine, gonads, and body cavity of the creature, and their identification was made using PCR amplification and sequencing of ITS-1, 5.8S, and ITS-2. In our study, nematodes were collected from the specified regions, and sequencing with the sequencing method was used to identify the samples.

DNA, ITS-1 analysis in anisakid nematodes isolated from the muscles of Codfish in a study from the Norwegian Seas: amplification, NC5 (forward) 5 GTA GGT GAA CCT GCG GAA GGA TCA TT 3 and NC13R (reverse) 5 GCT GCG TTC TTC ATC was constructed using GAT 3 primers. As a result of the study, *Anisakis simplex*, *Pseudoterranova decipiens*, *Pseudoterranova krabbei*, and their hybrid formations were identified. In the sampling areas named FAO ILA1 and ILA2, the prevalence of infection with Anisakidae nematodes was high, 88% in FAO IIa1 and 75% in FAO IIa2, but the parasite fauna composition was different. While the prevalence of infection with FAO IIa1' *Pseudoterranova* sp. was 14% lower, this rate was found to be 39% in FAO IIa2. A reverse trend was observed for the higher prevalence of *Anisakis* infection in FAO IIa1 (88%) than in FAO IIa2 (55%). The intensity of infection with Anisakidae parasites differed in both areas: *Pseudoterranova* spp. Up to 8 parasites per infected fish (abundance 0.44) in FAO IIa1 and up to 53 parasites per fish (abundance 4.34) in FAO IIa2; *Anisakis* spp. They stated that there are up to 30 parasites per fish (abundance 4.46) and up to 25 parasites per fish (abundance 2.32), respectively (Nadolna-Altyn et al., 2022).

Bannai and Jori (2022) conducted a study on the infection of anisakid nematodes, which they isolated from two marine fish species they caught from the northwest of the Arabian Gulf. The study contains various information about the structure of the anisakid population. The nematode parasites isolated from the peritoneum and viscera of *Epinephelus diacanthus* and *E. coioides* fish were subjected to molecular analysis by amplifying the internally transcribed ITS and ITS-1 spacers of nuclear rDNA (rDNA) by PCR using NC13R DNA products, NC5/NC2 and SS1/ primer sets. As a result of evolutionary analyzes based on percent identity in the GenBank database in MEGA X., nematodes isolated from anisakid nematodes, especially *Hysterothylacium* spp. showed that it belonged to nine different taxa. They noted that the presence of individuals of the same species in a host might cause these genetic variations at the species level. We also saw in our study that most nematodes isolated from four different fish individuals

belonged to the *A. pegreffii* species, and one of them belonged to the *Anisakis typica* species. This showed us how wide a distribution network the individuals belonging to the anisakidae family are and that they can form an advanced population of very different fish individuals.

Liu et al. (2022) aimed to summarize the prevalence of anisakid infection in fish in their study in eastern China. They conducted a systematic review and meta-analysis using five bibliographic databases (PubMed, CNKI, ScienceDirect, WanFang, and VIP Chinese Journal Databases). They reported that anisakid nematodes are common in a wide variety of fish species, and the prevalence of anisakid nematodes in fish in China is 45.5%. They found that fresh fish had the highest prevalence rate (58.1%). The highest prevalence rate was observed in East China (55.3%), and fish from the East China Sea showed the highest prevalence of anisakid nematodes (76.8%). In these studies, they stated that molecular techniques were used as well as traditional methods for species determination. In our study, the presence of nematode parasites embedded in the visceral surfaces of the fish bought fresh from the fish market or freely circulating in the body cavity were detected in some fish species, especially from the Canakkale fish market and which can be bought and consumed by people from all income levels. It was observed that the annual nematode rate was 4.26%. It has been determined that the most nematode fish in the Canakkale Strait are chub mackerel. The highest prevalence was calculated in March with 47 (10.68%).

To minimize the negative effects of parasite species, it is necessary to consider issues such as which type of parasite is sheltered in which fish, the seasonal prevalence of parasites, and the effects of length and weight factors on housing. It is more important to investigate parasites in fish with high economic value and used for feeding purposes. Because when enough information about parasites is obtained, it will be easier to eliminate the environments that create them. Thus, because of combating very dangerous ones, the desired efficiency will be achieved. At the same time, increasing the studies on fish parasites will enable the introduction of new species to the parasite fauna of our country and the reaffirmation of existing species.

CONCLUSION

The most fundamental issue with fish and one of the most economically significant issues with aquaculture products is "parasitic diseases" which are secondary disease agents. When parasites are transmitted to fish, they have a negative impact on the health of the fish and cause issues with commercial marketing. Due to their zoonotic characteristics, they not only cause illnesses and economic losses in fish but also major health issues in humans.

As a result, in the data obtained from the study, it was determined that *A. pergeffii* is the most common anisakid nematode in the Canakkale region. The incidence of this species, which can cause infection in humans if not consumed carefully, in economic species has shown that fish should be cleaned by complying with hygienic conditions, and consumption as raw or undercooked is the most important factor in transmission to humans. Since fish is not consumed raw in Türkiye, no parasitic diseases related to seafood have been observed in humans.

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AUTHOR CONTRIBUTION

All authors took part in designing the research, collecting, and writing the manuscript. All authors took part in a part of the article.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

This study numbered HADYEK-2019-1900050960 was evaluated at the meeting of the Animal Experiments Local Ethics Committee dated 04.04.2019 and numbered 2019/03, and it was decided that ethics committee (HADYEK) permission was not required for this research.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Growth parameters with traditional and artificial neural networks methods of big-scale sand smelt (*Atherina boyeri* Risso, 1810)

Gümüş balığının (*Atherina boyeri* Risso, 1810) geleneksel ve yapay sinir ağları yöntemleriyle büyüme parametreleri

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Abstract: In this study, the growth parameters of big-scale sand smelt (*Atherina boyeri* Risso, 1810) in İznik Lake has been determined with traditional (length weight relationships (LWRs), von Bertalanffy (VB), condition factor (CF)) and modern approaches (Artificial Neural Networks - ANNs). A total of 635 specimens (44.84% female and 55.16% male) were collected from the local fisherman during the hunting season between April 2018 to April 2019. Mean fork length (FL) (mm, min-max), mean W (g, min-max) and mean CF (value, min-max) were estimated as 67.31 mm (40.10 - 97.77 mm), 2.57g (0.53 - 7.50 g), and 0.790 (0.170-1.520) for all individuals. The length-weight relationships were determined $W=0.00001437L^{2.8602}$ for female, $W=0.00001570L^{2.8266}$ for male and $W=0.00001328L^{2.8717}$ for all individuals. The von Bertalanffy equations were determined $L_t=136.218\ [1-e^{(-0.240(t+0.51))}]$ for female, $L_t=155.042\ [1-e^{(-0.185(t+0.73))}]$ for male, and $L_t=146.916\ [1-e^{(-0.205(t+0.64))}]$ for all individuals. The values in training (MSE (Mean Squared Error) $4.52559e^{-5}$, R (correlation coefficients) $9.09347e^{-1}$), verification (MSE $4.86111e^{-5}$, R $9.00931e^{-1}$) and test data (MSE $3.391999e^{-5}$, R $9.43465e^{-1}$) were found in calculations made with ANNs. It was determined that ANNs could be an alternative for evaluating growth estimation.

Keywords: Artificial Neural Networks, big-scale sand smelt, growth parameters, length weight relationships, von Bertalanffy

Öz: Bu çalışmada İznik Gölü'nde yakalanan gümüş balığının (*Atherina boyeri* Risso, 1810) büyüme parametreleri geleneksel (boy ağırlık ilişkileri (LWR), von Bertalanffy (VB) ve kondisyon faktörü (CF)) modern yaklaşımlarla (yapay sinir ağları) incelenmiştir. Av sezonunda bölge balıkçılarından Nisan 2018-Nisan 2019 tarihleri arasında toplam 635 adet (%44,84 dişi ve %55,16 erkek) birey toplanmıştır. Tüm populasyon için ortalama çatal boy (FL) 67,31 mm (40,10 - 97,77 mm), ortalama ağırlık (W) 2,57 g (0,53 g - 7,50 g ve ortalama CF 0,790 (0,170-1,520) olarak bulunmuştur. LWR dişi popülasyon için $W=0,00001437L^{2.8602}$, erkek popülasyon için $W=0,00001570L^{2.8266}$ ve tüm popülasyon için $W=0,00001328L^{2.8717}$ olarak bulunmuştur. Von Bertalanffy dişi, erkek ve tüm popülasyonda sırasıyla $L_t=136,218\ [1-e^{(-0,240(t+0,51))}]$, $L_t=155,042\ [1-e^{(-0,185(t+0,73))}]$ ve $L_t=146,916\ [1-e^{(-0,205(t+0,64))}]$ olarak tespit edilmiştir. Yapay sinir ağları (YSA) ile yapılan hesaplamalarda eğitim, doğrulama ve test verilerindeki MSE (Ortalama Kare Hata) ve R (korelasyon katsayısı) değerleri sırasıyla $4.52559e^{-5}$ $9,09347e^{-1}$, $4,86111e^{-5}$ $9,00931e^{-1}$ ve $3,391999e^{-5}$ $9,43465e^{-1}$ olarak bulunmuştur. YSA'ların büyüme tahmininin değerlendirilmesinde bir alternatif olabileceği belirlenmiştir.

Anahtar kelimeler: Yapay sinir ağları, gümüş balığı, büyüme parametreleri, boy ağırlık ilişkileri, von Bertalanffy

INTRODUCTION

The distribution area of *Atherina boyeri* includes the Mediterranean, Black Sea, the Atlantic and Marmara Sea from south of Spain to Morocco and Madeira (Leonardos and Sinis, 2000; Leonardos 2001). The species have been reported exotic from Türkiye inland waters from İznik Lake (Çetinkaya et al., 2011), İznik Lake (Özeren, 2004), Sapanca Lake (Geldiay and Balık, 1996), İznik Lake (Gaygusuz, 2006), İznik Lake (Özeren, 2009), Mogan Lake (Benzer, 2016), Hirfanlı Dam Lake (Gençoğlu and Ekmekçi, 2016), Hirfanlı Dam Lake (Benzer and Benzer, 2017) and Yamula Dam Lake (Benzer, 2020). The *Atherina boyeri* is considered to be an invasive exotic fish species in Turkish inland waters (Küçük et al., 2017).

Length-weight relationship (LWR) is significant in fish biology (Sayfullin and Shakirova, 2014). LWR parameters (*a* and *b* value) allow us to estimate the weight of the fish from the length, calculate the condition index, and compare the morphology and life processes of populations in different habitats (Petrakis and Stergiou, 1995).

Artificial Neural Networks (ANNs) are computer systems developed to derive new information through learning, which is one of the features of the human brain. ANNs is a mathematical modeling of the learning process inspired by the human brain (Bon and Hui, 2017). Since ANNs have a nonlinear structure, they perform better than traditional methods in terms of performance criteria. They can detect nonlinear relationships without any hypothesis (Hyun et al., 2005; Türeli Bilen et al.; 2011; Benzer and Benzer, 2016; Benzer and Benzer, 2017; Özcan and Serdar, 2018; Özcan and Serdar, 2019; Özcan,

2019; Benzer, 2020; Benzer and Benzer, 2020a; Benzer and Benzer, 2020b).

In this article, besides the traditional approaches, it is aimed to determine the ecology, age and growth patterns of atherinid species with modern approaches. These features aim to make a useful contribution to the definition of Atherinides as well as the commonly used traditional taxonomy.

MATERIALS AND METHODS

İznik Lake, which is the largest lake in the Marmara Region and the fifth largest in Türkiye, has an area of 313 km^2. Located in the southeast of the Marmara Region, within the borders of Bursa province, between 40°23'-40°30' north latitudes and 29°20'- 29°42' east longitudes, Lake İznik is a tectonic freshwater lake (Nümann, 1958; Figure 1).

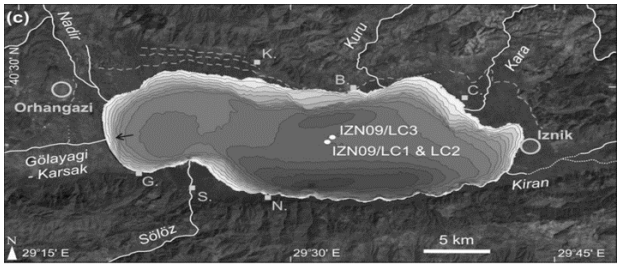

Figure 1.İznik Lake, Bursa, Türkiye

Fish samples (635 individuals) were obtained from fishermen during the hunting season, so ethical approval was not required. The specimens were fixed within 4% formaldehyde solution in polyethylene jars and transferred to the Laboratory. Samples were weighed with a digital scale of ± 0.1 g accuracy and lengths measured with an electronic caliper with an interval of 0.01 mm. Sexes were determined by examination of the gonads macroscopically., Age determination was performed from scales (Lagler, 1966) which taken from the section between dorsal fin and lateral line of each fish, and examined with a binocular microscope and a projection device for all *Atherina boyeri* individuals.

The general regression equation: W = a × L^b was used to calculate the length-weight relationship, where W weight (g) of fish, L length (mm) of fish, a and b are the parameters of the equation (Bagenal and Tesch, 1978).

Growth was calculated using the von Bertalanffy Equations (VB) growth equation (Sparre and Venema, 1992) and growth performance index (φ') (Munro and Pauly, 1983).

$$L_t = L_\infty\left[1 - e^{-K(t-t_0)}\right]$$

$$\varphi' = Log\ K + 2.Log\ L_\infty$$

L_t is the FL (cm) at age t; L_∞ is asymptotic theoretical maximum fork length, K is the Brody growth coefficient, t is the age, t_0 is the age at zero length and same parameters are available for W.

One-sample t test was used in the analysis of the data revealing whether there is a difference between our φ' value and φ' of the studies in the literature. SPSS 23 program was used in the analysis of the data. Confidence interval was 95% (significance level 0.05 p<0.05).

The Condition Factor (CF) was calculated for all individual fish for *A. boyeri,* by using the conventional formula described by (Worthington and Ricardo, 1936):

$$CF = \mathrm{W}\ \frac{100}{L^3}$$

where CF is the condition factor, W (g) is the weight and L_∞ (cm) is the length.

Each neuron in the hidden or output layer combines and modifies the entries in the previous layer, creating a total connection. The neuron output (y_j) is calculated by equation (Hekayati and Rahimpour, 2017).

$$y_j = f\left(\sum_{i=1}^{M} w_{ij}x_{ij} + b_{ij}\right)$$

The equation y_j gives w_{jj} x_{jj} weight evaluations and bias relation value in the j^{th} neuron and b_j. In the calculation of ANNs, error functions used in both training and test data were calculated with the Mean Squared Error (MSE) (Masoud, 2014).

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(Y_i - \hat{Y}_i)$$

Metrics are used to monitor and measure the performance of a model (during training and testing). The mean absolute percentage error (MAPE) is error measure, low result is measure that show high performance that is inversely proportional to performance (Wang and Xu, 2004). Performance metric is a part of ANNs which can be mathematically written as follows:

$$MAPE = \frac{100}{n}\sum_{j}^{n}\frac{|e_j|}{|A_j|}$$

Linear activation function and Levenberg-Marquardt backpropagation algorithm (*trainlm*) are used in the configuration of ANNs. The learning rate is 0.01, the number of iterations is 1000, 15% of the data is used as verification data, 15% is used as test data, and 70% is used as training data. The transfer function mainly uses a sigmoid or a logistic function, which gives values in the range of (0,1).

RESULTS

The sex of the species was 44.84 % females and 55.16 % males (sex ratio 1:1.23). The age of *A. boyeri* caught from İznik Lake ranged between I to IV years and among the different age groups, II and III year classes were dominant in the population. The sample size of the 1, 2, 3 and 4 age groups (female + male) was determined as 3 + 3, 143 + 281, 82 + 80, 30 + 13,

respectively. Mean fork length (FL) (mm, min-max), mean *W* (g, min-max) and mean CF (value, min-max) were founded 67.31 (40.10- 97.77), 2.57 (0.53 - 7.50), and 0.790 (0.170-1.520) for all individuals.

Length-weight relationships were calculated using the data of all fish samples (Table 1). The *b* value for females was higher than for male's individual. The relationship between the age and length is given in Figure 2 as a von Bertalanffy growth.

Table 1. LWRs growth equations and parameters of *A. boyeri* population in İznik Lake

Sex (Individual number)	LWRs growth equations	R^2	L_∞	t_o	K	φ'
Female (258)	W=0.00001437 $L^{2.8602}$	0.947	136.258	-0.51	0.240	3.74
Male (377)	W=0.00001570 $L^{2.8266}$	0.942	155.042	-0.73	0.185	3.73
Female + Male (635)	W=0.00001328 $L^{2.8717}$	0.941	146.919	-0.64	0.205	3.82

R^2: correlation coefficients, L_∞: asymptotic theoretical maximum fork length, t_o: age at zero length, K: Brody growth coefficient, φ':growth performance index

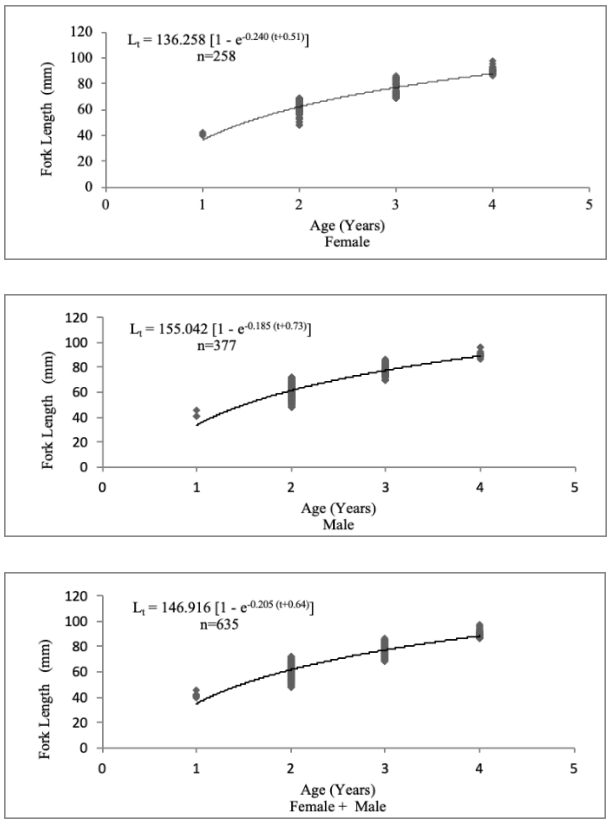

Figure 2.von Bertalanffy growth equations of *A. boyeri* in İznik Lake

The values in training (MSE (Mean Squared Error) $4.52559e^{-5}$, R (correlation coefficients) $9.09347e^{1}$), verification (MSE $4.86111e^{-5}$, R $9.00931e^{-1}$) and test data (MSE $3.391999e^{-5}$, R $9.43465e^{-1}$) were found in calculations made with ANNs. It is seen that it was gathered around the normalization line when the distribution of the data was examined (Figure 3).

After determining that the data distribution is appropriate, ANNs model to be used with coding written with MATLAB application was established (Figure 4). It is used as age, sex and fork length as input data; weight data is also used as output data. The hidden layer was set up as 10. ANNs result relationships can be seen in Figure 5. The mean FL and W values for 1, 2, 3 and 4 years and the mean values obtained from the ANN model, LWR and VB equations are presented in Table 2.

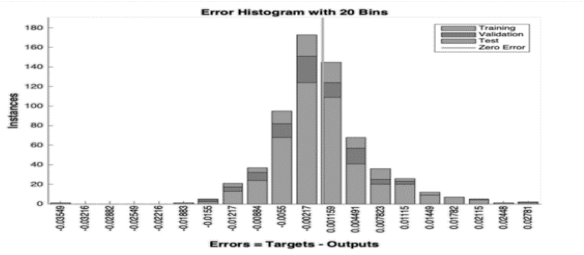

Figure 3. Distribution of data from İznik Lake

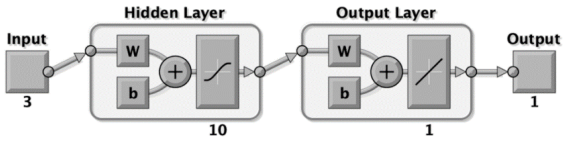

Figure 4. Artificial Neural Networks Model

Table 2.İznik Lake data and calculated values for ANNs, LWR and VB (Mean Fork Length (FL): mm; Mean Weight (*W*): g)

Age	İznik	ANNs	MAPE	LWR	MAPE	VB	MAPE
	FL W	FL W	FL W	FL W	FL W	FL W	FL W
1	41.950 0.669	42.120 0.671	0.405 0.299	43.395 0.607	3.445 9.267	40.947 0.512	2.390 23.467
2	61.652 1.896	61.820 1.905	0.273 0.475	62.370 1.883	1.165 0.685	61.403 1.530	0.403 19.303
3	76.981 3.628	77.130 3.733	0.194 2.894	78.184 3.872	1.562 6.725	77.253 2.958	0.353 18.467
4	90.241 5.537	90.310 5.486	0.077 0.921	90.585 5.477	0.381 1.083	90.165 4.612	0.084 16.705

FL: Fork length, W: Weight

DISCUSSION

Çetinkaya et al. (2011) (2.5:1), Özeren (2009) (1.7:1) and Gençoğlu and Ekmekçi (2016) (1.14:1) reported higher ratios than female male sex ratio of *A. boyeri* in İznik Lake (1:1.46). Although the gender ratio in most species is close to 1, it can vary from species to species, differ from one population to another, and may vary from year to year in the same population (Clarke, 1983).

The slope (*b*) values of the LWR in all gender is found as a 2.8717. Bigger *b* values for *A. boyeri* were reported by Bartulovic et al. (2006), Pombo et al. (2005), Gaygusuz (2006), Özeren (2009), Patimar et al. (2009), Çetinkaya et al. (2011), Lorenzoni et al. (2015), Gençoğlu and Ekmekçi (2016), Boudinar et al. (2016), İlhan and İlhan (2018), but it differed

from those found by Benzer (2016, 2020), Benzer and Benzer (2017, 2019) (Table 3). The fact that the b value of the individual in our study was b<3 is considered to be a factor, either that the large samples are elongated by changing their body shape or that the small individual are well fed (Kuriakose, 2017).

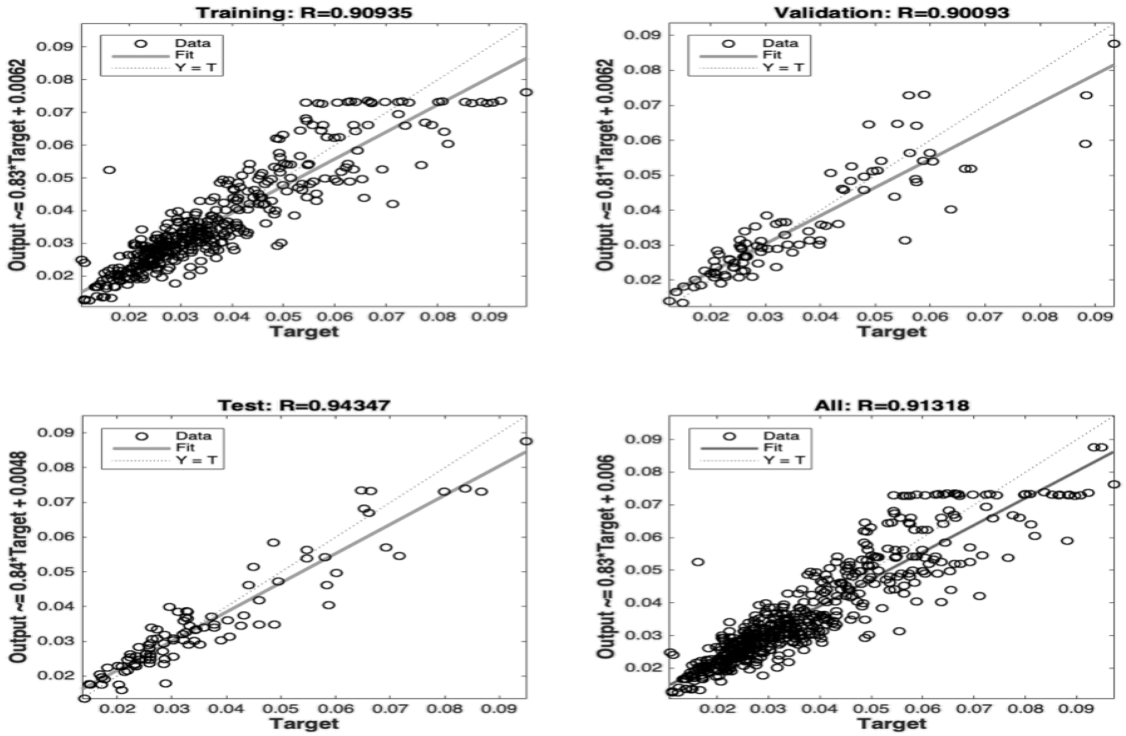

Figure 5. Regression curves by Artificial Neural Networks of *A. boyeri* in Iznik Lake

In all L_∞ value in the von Bertalanffy equations examined, L_∞ value of Pombo et al. (2005), Bartulovic et al. (2006), Gaygusuz (2006), Özeren (2009), Lorenzoni et al. (2015), Boudinar et al. (2016) were smaller İznik Lake. Patimar et al. (2009) and Çetinkaya et al. (2011) reported higher L_∞ value than İznik Lake (Table 4). Although the (L_∞) values obtained are similar to other studies, the difference in the studies can be explained by changes in environmental conditions and temperature. For example, L_∞ will increase when the temperature decreases (Kyritsi and Kokkinakis, 2020).

In all growth performance index examined, φ' value of Pombo et al. (2005), Lorenzoni et al. (2015), Gençoğlu and Ekmekçi (2016), Bartulovic et al. (2006), Boudinar et al. (2016), were smaller İznik Lake. Gaygusuz (2006), Özeren (2009), Patimar et al. (2009) and Çetinkaya et al. (2011) reported higher φ' value than İznik Lake (Table 4). The φ' value determined in this study was tested with the phi-prime index values in all studies and was found to represent the value in the 95% confidence interval (3.1526 -3.9703).

Although there was no enough study about condition factor value of *A.boyeri* in the literature; Çetinkaya et al. (2011) (0.780 for male and 0.822 for female) and Benzer and Benzer (2020c) reported similar condition value (0.804 for all gender) with *A. boyeri* in İznik Lake (0.790 for all gender).

The results obtained with ANNs gave better MAPE results compared to LWR and VB results (Table 2). The MAPE value of the model considered in the estimate should be less than 20%. The performance that gives the smallest value is determined as the highest model (Gilliland, 2010). According to these results, ANNs method has been found to give better predictive results in all cases. The obtained results are examined, it is determined that the best results are given by ANNs, VB and LWR respectively.

A.boyeri is generally distributed among the stagnant water systems of the Mediterranean coast, the Black Sea, the Azov Sea and the Caspian Sea (Kottelat and Freyhof, 2007). Although their natural distribution areas are marine and transition water systems, they have entered freshwater resources and are described as alien species in these habitats. It is an economically important species due to the export of *A.boyeri* in Türkiye. *A.boyeri* is an invasive species. It is important for economic, and their proliferation increases rapidly. *A.boyeri* needs to be followed in many ecosystems for all the reasons.

For years, many scientists have studied the superiority of artificial intelligence (AI) in solving regression problems over conventional classification and statistical models.Benzer and Benzer, (2016) studied the performance of an artificial neural network model, as one of the benchmark modeling techniques in Machine Learning studies, and a statistical linear regression model in fish growth in Mogan Lake. Elsewhere, Benzer et al. (2022) classified the fish age by taking into account the biological characteristics of the fish.

Table 3. Comparison of *A. boyeri* length-weight parameters in different locations

Study Area	*n*	*a*	*b*	R^2	GT	Reference
Ria de Aveiro	2503	3.3×10^{-3}	3.35	-	A+	Pombo et al. (2005)
Mala Neratva River	1200	3.4×10^{-3}	3.24	-	A+	Bartulavic et al. (2006)
İznik Lake	1136	3.2×10^{-3}*	3.336	0.991	A+	Gaygusuz (2006)
İznik Lake	922	4.0×10^{-3}*	3.20	0.978	A+	Özeren (2009)
Gomishan Wetland	2256	5.3×10^{-3}* 5.0×10^{-3}*	3.06m 3.0630f	-	A+	Patimar et al. (2009)
İznik Lake	237	8.0×10^{-3} 7.45×10^{-3}	2.98m 3.05f	0.993 0.996	A− A+	Çetinkaya et al. (2011)
Trasimeno Lake	3998	-2.326 -2.366	3.139m 3.168f	0.956 0.968	A+ A+	Lorenzoni et al. (2015)
Hirfanlı Dam Lake	674	3×10^{-6}*	3.16	-	A+	Gençoğlu and Ekmekçi (2016)
Mellah Lagoon	1402	4.6×10^{-3}	3.179	0.944	A+	Boudinar et al. (2016)
Mogan Lake	488	1.374×10^{-3}*	2.81	0.964	A−	Benzer (2016)
Hirfanlı Dam Lake	1449	1.3×10^{-2}* 1.7×10^{-2}* 1.39×10^{-2}*	2.77m 2.62f 2.74	0.971 0.977 0.973	A− A− A−	Benzer and Benzer (2017)
Marmara Lake	185	5.9×10^{-4}	3.118	0.920	A+	İlhan and İlhan (2018)
Süreyyabey Dam Lake	394	1.2×10^{-3} 6.7×10^{-3} 6.4×10^{-3}	2.67m 2.95f 3.00	0.983 0.969 0.970	A− A− I	Benzer and Benzer (2019)
Yamula Dam Lake	594 516	9.7×10^{-3} 10.7×10^{-2}	2.8690 2.8169	0.950 0.934	A− A−	Benzer (2020)
İznik Lake	635	1.570×10^{-5}* 1.437×10^{-5}* 1.328×10^{-5}*	2.8266m 2.8602f 2.8717	0.942 0.947 0.941	A− A− A−	This Study

n:number, *a*: regression intercept, *b*: regression slope, R^2: coefficient of determination, GT: shape of growth; I: Isometric, A: Allometric, m: male, f: female, *mm

Table 4. Comparison of *A. boyeri* growth parameters in different locations

	n	t_m	L_∞	*K*	t_0	φ'	Reference
Ria de Aveiro	2503	3	11.6	0.099	-3.797	2.59	Pombo et al. (2005)
Mala Neratva River	1200	-	13.503	-	-	-	Bartulavic et al. (2006)
İznik Lake	1136	4	128.83*	0.305	-0.089	3.93	Gaygusuz (2006)
İznik Lake	922	4	141.11*	0.270	-0.490	3.98	Özeren (2009)
Gomishan Wetland	2256	4	155.17* 162.77*	0.280 0.270	-0.738 -0.727	4.21 4.26	Patimar et al. (2009)
İznik Lake	237	4	15.6	0.238	-0.199	4.06	Çetinkaya et al. (2011)
Trasimeno Lake	3998	4	10.031	0.180	-0.443	1.65	Lorenzoni et al. (2015)
Hirfanlı Dam Lake	674	4	151.02m* 156.78f*	0.148 0.197	-0.148 -0.197	3.52 3.88	Gençoğlu and Ekmekçi (2016)
Mellah Lagoon	1402	3	9.49m 11.67f	0.316 0.179	-0.928 -1.514	3.35 3.14	Boudinar et al. (2016)
İznik Lake	635	4	136.258 f* 155.042 m* 146.919*	0.510 0.730 0.640	-0.240 -0.185 -0.205	3.74 3.73 3.82	This Study

n:number, t_m: maximum age of the sample, L_∞: asymptotic theoretical maximum fork length, *K*: brody growth coefficient, t_0: age at zero length, φ': growth performance index, m: male, f: female, *mm.

Using artificial intelligence studies for long-term population monitoring with fewer samples in a certain ecosystem is considered advantageous for ecosystem protection and rapid calculation. It will be useful to evaluate it together with traditional methods.

CONCLUSION

As a result, it was found that the use of ANNs as a prediction tool provides high performance in *A.boyeri* individuals in Iznik Lake. The distribution areas of these invasive species should be determined to prevent irreparable losses in fish biological diversity in the world. Necessary studies should be carried out to determine the invasive fish action plans on native fish species in habitats such as fisheries, disease, hybridization, food, and habitat competition. It is found that the population of *A.boyeri* reflects the expected and previously observed features of the species in natural waters, but it is necessary to activate the national law of fishing,

exploiting and protecting aquatic resources to continuation the fish populations in the freshwater as an economic resource.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed equally to the idea, data collections, design and writing of the manuscript.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Relationships between environmental variables and abundance of Cnidaria and Echinodermata in Çardak Lagoon, Çanakkale Strait

Çanakkale Boğazı, Çardak Lagünü'ndeki Cnidaria ve Echinodermata gruplarının bolluğu ile çevresel değişkenler arasındaki ilişkiler

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Abstract: This study is on the relationships between the abundances, of cnidarians and echinoderms found in Çardak Lagoon and environmental variables. The benthos samples were collected in three replicates in October 2018, February, April, and June 2019, using a metal framed quadrat system of 100 x 100 cm and 393 cm³ sediment core by a SCUBA diver. Sediment material was collected at 7 sampling points of Çardak Lagoon in Çanakkale Strait. A total of 259 individuals belonging to phylum, Cnidaria and Echinodermata were found in the study area. Cnidarian, *Actinia* sp. and echinoderm, *Asterina gibbosa* were the most dominant species. The highest positive correlation value was between Cnidaria species number and organic matter (OM%) content in the sediment. The highest negative correlation value was between Echinodermata species number and NO_2+NO_3 in lagoon system.

Keywords: Macrozoobenthos, lagoon biodiversity, environmental variables, Çardak Lagoon, Çanakkale Strait

Öz: Bu çalışma, Çardak Lagünü'nde bulunan Cnidaria ve Echinodermata gruplarının bollukları ile çevresel değişkenler arasındaki ilişkiyi araştırmaktadır. Benthos örnekleri, bir SCUBA dalgıcı tarafından 100 x 100 cm'lik metal çerçeveli bir kuadrat ve 393 cm³'lük kor kullanılarak Ekim 2018, Şubat, Nisan ve Haziran 2019'da üç tekrarlı olacak şekilde toplandı. Sediment materyali, Çanakkale Boğazı'ndaki Çardak Lagünü'nün 7 örnekleme noktasından toplanmıştır. Çalışma alanında Cnidaria ve Echinodermata şubelerine ait toplam 259 birey tespit edilmiştir. Knidaria türü *Actinia* sp. ve ekinoderm, *Asterina gibbosa* en baskın türlerdir. En yüksek pozitif korelasyon değeri (r=0.94; p<0.01), Cnidaria tür sayısı ile sedimandaki organik madde (%OM) içeriği arasında olmuştur. Lagün sistemde en yüksek negatif korelasyon değeri (r=-0.921; p<0.01) Echinodermata grubuna ait tür sayısı ile NO_2+NO_3 arasında bulunmuştur.

Anahtar kelimeler: Makrozoobentoz, lagün biyoçeşitliliği, çevresel değişkenler, Çardak Lagünü, Çanakkale Boğazı

INTRODUCTION

Coastal lagoons are complex systems that are highly affected by biological and physical changes. Due to the low influx of seawater (Ranasinghe and Pattiaratchi,1999), lagoons are often characterized by strong fluctuations in environmental variables that change the structure and distribution pattern of organisms. The diversity of the macrozoobenthos is very important in determining the quality of the biodiversity of a lagoon. Macrozoobenthos is an important component of lagoon systems, significantly altering the physical structure of the abiotic or biotic formations that make up the habitat and directly or indirectly affecting the availability of resources for other species (Lu, 2005). Macrozoobenthic assemblages are an important food source for organisms in the upper group of the trophic chain (Dauer, 1993). In addition, benthic macrofauna protects the water and contributes to improving water quality by mineralizing and recycling organic material, as well as decomposing organic material. (Sarker et al., 2016). Benthic communities are used as indicators in environmental impact studies (Warwick, 1993) to determine the types and levels of pollutants and to assess the environmental quality of coastal systems (Ponti and Abbiati, 2004; Boutoumit et al., 2021; Hisli et al., 2022).

Cnidarians and echinoderms are important components of the macrozoobenthos and indicators for ecological quality (Boutoumit et al., 2021). The phylum, Echinodermata is one of the major components commonly found in marine benthos and plays a variety of important ecological roles (Menge, 1992). Echinoderms, being sensitive to environmental changes, are important for ecological studies as indicator organisms for detecting changes in the food chain (Özbek, 2013). Echinoderms are associated with commercially important benthic resources and are the most commonly affected epibiotic community, accounting for a large proportion of bycatch in coastal or open-water fisheries (Escolar, 2010). Cnidarians are common in marine environments and they are represented by several species in estuaries, lagoons and/or freshwater. Benthic Cnidaria in particular is an important group of the benthos of various marine habitats, where they often form characteristic assemblages (Boero, 1984). They are

known as indicator species in the marine environment because their colonies are sessile and respond rapidly and variably to stress (Mergner, 1977; Gili and Hughes, 1995).

Although many studies on the biological status and aquaculture of Çardak Lagoon were carried out (Alparslan et al., 1999; Alparslan et al., 2004; Calıskan et al., 2011; Vural et al., 2015; Vural and Acarlı, 2021a, b; Vural, 2022), there is no detailed study on the macrozoobenthic structure of the lagoon. Knowledge of species composition of an ecosystem is one of the most fundamental aspects of biodiversity studies (Nascimento et al., 2019; Ahuatzin-Hernández et al., 2020). Providing this information enriches the value of an ecosystem and enables better management of natural resources. Therefore, the aim of this study is to determine the relationship between cnidaria and echinoderm species distributed in Çardak Lagoon, which has ecological and economical importance, with environmental variables.

MATERIALS AND METHODS

Study area

The study site is Çardak Lagoon has a 180-ha saltwater area with an average depth of 1,5 m (GTHB, 1997) in the northeast of Çanakkale Strait is one of the most important wetlands in the Strait. The lagoon formed between the coastal arrow and the land area has a length of 7,5 km and an area of 1.3 km^2 (Calıskan et al., 2011). The lagoon is under the influence of the sea waters due to its deep and constantly open passage. In the lagoon area, sampling was carried out at 7 stations whose coordinates are given below (Figure 1):

St1; 40˚22´906˝ N, 26˚43´103˝ E,
St2; 40˚23´053˝ N, 26˚43´264˝ E,
St3; 40 ˚23´203˝ N, 26˚43´491˝ E,
St4; 40˚23´345˝ N, 26˚43´399˝ N,
St5; 40˚23´278˝ N, 26˚42´988˝ E,
St6; 40˚23´236 ˝ N, 26˚42´800˝ E,
St7; 40˚22´931˝ N, 26˚42´768˝ E)

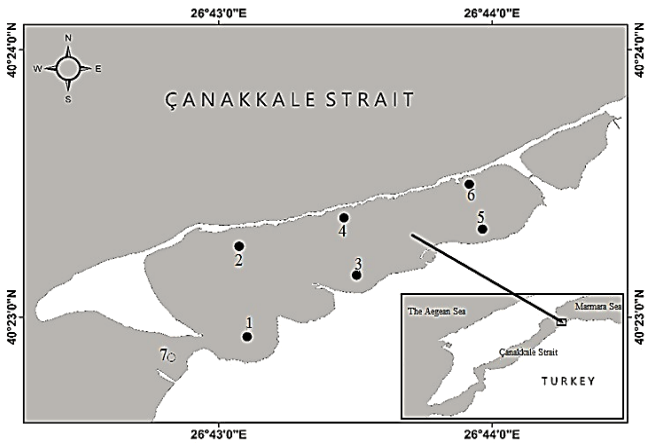

Figure 1. Sampling stations in Çardak Lagoon

Sampling

The benthos samples were collected in three replicates in October 2018, February, April, and June 2019, using a metal framed quadrat system of 100 x 100 cm and 393 cm^3 sediment core by a SCUBA diver. The deepest part of the lagoon area is roughly 2 m and there is a dense accumulation of photophilic algae and organic matter on the bottoms of the land side of lagoon. This has led to an increase in the turbidity of the underwater. Therefore, the samplings carried out at 1.5 and 1,8 m depths were performed by a scuba diver. Sediment material was collected at 7 sampling points of Çardak Lagoon, Çanakkale Strait. The samplings were performed from sandy and mud (silt+clay) bottoms between 1 and 1,8 m depths in the Çardak Lagoon (Figure 1). Sediment material collected in quadrate samples was fixed in 4% neutralized formaldehyde in 1 and 5L plastic jars. Sediment samples collected with quadrate and core were sieved and washed under laboratory conditions using pressurized water through a triple sieve system with a 0.5 mm mesh size. After the material of all faunal species remaining on the sieves had been separated at the macro and micro levels, it was fixed in groups in 70% ethyl alcohol in 50 cm³ glass tubes. The definitions are based on the reference studies by Mortensen (1927), Belloc (1948) and Tortonese and Demir (1960). The number of species (S), total abundance (N), and dominance index (DI) (Bellan Santini, 1969) were calculated for each sampling station and season. The dominance index was estimated by d = m / M x 100, where m = individual number of a species in the stations and M = total individual numbers of all species.

The physicochemical variables of the lagoon water (salinity, temperature, dissolved oxygen, pH) were measured in situ with the YSI 600 QS Multiprobe System (Yellow Springs Instruments). Amounts of nutrients such as NO^{-}_{2}, NO^{-}_{3}, NH^{+}_{4}, PO^{-}_{4}-P and SiO^{-}_{2}, were measured with a Jasco Brand UV Spectrophotometer using chemical and biological analysis techniques (Strickland and Parsons, 1972). Measurements were made at different wavelengths of 543 nm, 410 nm, 640 nm, 850 nm and 810 nm for NO^{-}_{2}, NO^{-}_{3}, NH^{+}_{4}, PO^{-}_{4}-P, SiO^{-}_{2}, respectively. Total suspended solids (TSS) determination was performed gravimetrically after the water samples were filtered through GF/C filters (Clesceri et al., 1998). Chemical oxygen demand (COD) analysis was conducted using the open reflux method and standard methods for the examination of water and wastewater according to Eaton and Franson (2005). For the spectrophotometric determination of anionic surfactants, a standard procedure was conducted using methylene blue (APHA, 1992). Acrylic material was used for the organic matter and the particle size analyses in the soft bottoms of the lagoon. A total of 28 core samples for both analyses were obtained from soft sediments at each sampling point in each sampling period. Particle size analyses of the sediment were performed according to Allen (1997). Organic matter (OM%) content was determined as the difference between the dry weight (80 ◦C, 24 h) of the sediment and the residue left after combustion at 450°C for 2 h (Parker, 1983).

Data analysis

Relationships between abundance and environmental factors were analyzed using MDS (Multidimensional Scaling Analysis). Correlations between environmental variables and the number of species and the number of individuals were determined using Pearson correlation. Pearson correlation was performed using PAST and MDS analysis in SPSS 25.

RESULTS

A total of 8 species and 259 specimens (4 cnidarians, 4 echinoderms) were collected in the Çardak Lagoon between autumn 2018 and summer 2019. (Table 1). A total of 212 Echinodermata individuals and 47 Cnidaria individuals were recorded. *Actinia* sp. from the group of cnidarians (Cnidaria) was the predominant species (22 individuals). Echinoderm *Asterina gibbosa* was the most dominant species in different seasons. Cnidarians, *Actinia* sp. were most abundant in summer (6.67 ind.m^{-2}), while *Asterina gibbosa* was represented with a high number of individuals in winter (19.67 ind.m^{-2}) (Table 1).The distribution of species numbers by stations is shown in Table 2. The total number of individuals per m^2 in the study area is 86.33 (ind.m^{-2}). The species with the highest number of individuals belonging to cnidarians is *Actinia* sp. and has the highest number of individuals at station 3 (4.67 ind.m^{-2}). Looking at the dominance values, the most abundant species is *Asterina gibbosa* from echinoderms with 127 individuals.

Correlations between the environmental variables and the abundances are shown in Table 3. The number of cnidarian species is negatively correlated with temperature and sand content in sediment, while the organic matter in sediment has a strong positive relationship. The environmental variable that affects the echinoderm abundance is NO_2+NO_3 concentration in lagoon water. The relationship was found to be statistically significant in the negative direction.

Correlations between the environmental variables and the abundance are shown in Figure 2 for the MDS (multidimensional scaling analysis). The variables that have the greatest effect on the number of cnidarian individuals are temperature and pH, while the factors that affect the distribution of echinoderm are organic matter and NO_2+NO_3.

Table 1. The number of individuals (average number of individuals per m^2) of Cnidaria and Echinodermata species in Çardak Lagoon (sum of all stations) by seasons (∑: Abundance DI%: Dominance)

Taxa	Autumn-18	Winter-19	Spring-19	Summer-19	∑	DI%
Cnidaria						
Cerianthus membranacea (Spallanzani, 1784)	-	-	0.33	-	0.33	0.39
Edwardsia claparedii (Panceri, 1869)	1	3	2	0.33	6.33	7.34
Edwardsia sp.	0.33	0.67	0.67	-	1.67	1.92
Actinia sp.	-	-	0.67	6.67	7.33	8.46
Echinodermata						
Asterina gibbosa (Pennant, 1777)	4	19.67	13	5.67	42.33	49.03
Amphiura chiajei Forbes, 1843	2.67	16.67	7.67	-	27	31.15
Ophiuroidea (sp.)	-	-	0.33	0.67	1	1.15
Holothuroidea (sp.)	-	0.33	-	-	0.33	0.38
Total	8	40.33	24.67	13.33	86.33	

Table 2. The number of individuals (average number of individuals per m^2) of Cnidaria and Echinodermata species in Çardak Lagoon (sum of all seasons) by stations (∑: Abundance, DI%: Dominance)

Taxa	St1	St2	St3	St4	St5	St6	St7	∑	DI%
Cnidaria									
Cerianthus membranacea (Spallanzani, 1784)	-	-	-	-	-	-	0,33	0.33	0.39
Edwardsia claparedii (Panceri, 1869)	2.33	-	-	-	4	-	-	6.33	7.34
Edwardsia sp.	0.67	-	0.67	-	0.33	-	-	1.67	1.93
Actinia sp.	1.33	0.33	4.67	0.33	0.33	-	0,33	7.33	8.49
Echinodermata									
Asterina gibbosa (Pennant, 1777)	-	18.67	0.33	21	1	1	0,33	42.33	49.03
Amphiura chiajei Forbes, 1843	-	0.67	-	25	1.33	-	-	27	31.27
Ophiuroidea (sp.)	0.67	-	0.33	-	-	-	-	1	1.16
Holothuroidea (sp.)	-	-	-	-	0.33	-	-	0.33	0.39
Total	5	19.67	6	46.33	7.33	1	1	86.33	

Table 3. Correlations between the number of cnidarian and echinoderm species and the environmental variables (TP: Total phosphate, NO_2+NO_3: Nitrite + Nitrate, TN: Total nitrogen, TSS: Total suspension solid, COD: Chemical oxygen demand, AnDet: Anionic detergent, OM: Organic matter)

	Echinodermata (NS)		Cnidaria (NS)	
	Pearson Correlation	Sig. (2-tailed)	Pearson Correlation	Sig. (2-tailed)
Temperature (°C)	0.116	0.804	**-0.871***	0.011
Salinity (‰)	0.093	0.843	-0.002	0.997
DO (mgL^{-1})	-0.135	0.773	-0.143	0.759
pH	0.354	0.436	-0.679	0.094
SiO_2 (µg L^{-1})	0.575	0.177	-0.187	0.688
TP (µg L^{-1})	-0.448	0.313	-0.193	0.679
NO_2+NO_3 (µg L^{-1})	**-0.921****	0.003	0.226	0.625
TN (µg L^{-1})	**-0.773***	0.042	0.650	0.114
TSS (µg L^{-1})	-0.595	0.159	0.191	0.682
COD	-0.530	0.221	0.168	0.718
AnDet (µg L^{-1})	0.125	0.789	0.237	0.609
PO_4 (µg L^{-1})	-0.016	0.973	-0.058	0.902
OM (%)	-0.365	0.421	**0.924****	0.003
Sand (%)	0.511	0.242	**-0.888****	0.008
Gravel (%)	-0.520	0.231	0.669	0.100

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

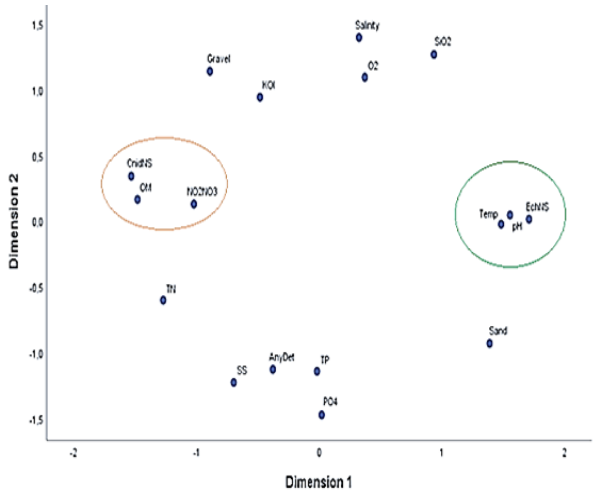

Figure 2. Results of MDS analysis between environmental variables and abundance (stress= 0.11)

DISCUSSION

The biodiversity of such ecosystems is thought to be spatially distributed, e.g. in terms of salinity, temperature and sediment properties (particle size, mud and/or organic matter enrichment) (Fišer, 2004; Karachle and Stergiou, 2008; Politou and Papaconstantinou, 1994). This structure results from organisms having an environmental tolerance to the stresses in lagoon systems (water body dynamics, physiological stress and biotic interactions). Changes in the distribution and abundance of marine invertebrates have been used as a measure of environmental impact (Warwick and Clarke, 1991), and changes in species composition may be of interest for retrospective or prospective monitoring (Philippi et al., 1998).

Mainly, excessive salinity, temperature, nutrient changes, and slowing of water regeneration in the environment affect the structure of benthic macrofauna (Afli et al., 2008). According to many previous studies performed on lagoon systems, the distribution and abundance of macrobenthos are highly influenced by the degree of salinity in water (Lardicci et al., 1997; Gamito, 2008; Specchiulli et al., 2010). Approximately 60% of the species found in this study were collected from seaside stations. Since these sampling points are under the influence of the sea, salinity has a positive effect on echinoderms, while it has a negative effect on the distribution of cnidarians. While the number of species and individuals of macrozoobenthic communities, which are very important in the nutrient cycle of coastal lagoons in Mediterranean Ecosystem (Cataudella et al., 2015), increases in winter-spring, it decreases in the summer-autumn period (Lardicci et al., 1997). Similarly, in this study, it was observed that the number of individuals increased in the winter and spring periods.

Cnidarians play an important role in coastal ecosystems due to their biological and ecological properties and are known to be good bioindicators. Due to their constant presence in the benthic fauna, they are used as bioindicators to evaluate environmental conditions. Their distribution depends on the nutrient abundance of benthic and planktonic communities, biotic factors and various environmental factors such as human activities (Arai, 1992; Bourget et al., 2003). The distribution of some cnidarian species depends on their narrow tolerance to salinity and temperature variation (Russel, 1953; Gusmão et al., 2015; Govindan and Ramanibai, 2017; Yüksel, 2019; Ahuatzin-Hernández et al., 2020). This study also found that the distribution of cnidarians was most strongly related to temperature. It is also known as a potentially invasive group due to its wide distribution in polluted areas, harbors and shipping, and its tolerance to temperature and salinity variations (Megina et al., 2013).

Cnidarian, *Actinia* sp. which is the most dominant species in this study, was previously reported from Turkish coasts. Çınar et al. (2014) reported that *Actinia* sp. was common in Turkish Seas, generally distributed at depths of 0-10 m and preferring hard bottoms as habitat. Recently, Hisli et al. (2022), recorded *Actinia* sp. at a station near the sea within Hersek Lagoon in Sea of Marmara. Conversely, we recorded most *Actinia* sp. samples (19 samples) from the stations (St1, St3 and St5), mainly on the land side. Another cnidarian, *Cerianthus membranaceus*, was recorded from Sea of Marmara and Aegean Sea by Çınar et al. (2014). *Cerianthus membranaceus* is one of most abundant species in Çanakkale Strait (Özalp and Ateş, 2015) that there are Cnidaria species, were abundant at the stations (especially at station 3, where the highest organic matter in the sediment) near the land side of the lagoon area under the influence of light pollution.

Echinoderms comprise a large number of species that dominate the benthos qualitatively and quantitatively and play

a structuring role in the habitat through their circulation. Changes in their populations can affect the entire ecosystem (Saier, 2001; Coteur et al., 2003). It is a key species for marine life as they all live in the benthos. The echinoderm species provide the balance of organic matter in the benthos. It is thought that changes in the food chain occur when they are removed from the environment because the input of organic matter is disturbed. In addition, members of Asteroidea group are known to be predators of some bivalves such as clams and oysters. In this study, *Asterina gibbosa* and *Amphiura chiajei* were the most abundant echinoderm species. These two species have a wide distribution in shallow waters of Turkish Seas (Öztoprak et al, 2014). *Amphiura chiajei* is an infaunal species typically burrowing in soft bottom sediments, especially in muddy sands (Bengil et al., 2012). In the present study, *Asterina gibbosa* and *Amphiura chiajei* are found to be quite shallow (1 m) in Çardak Lagoon and the sediment was quite clean. MDS analysis showed that environmental variables such as pH and temperature have a quite strong effect on echinoderm distribution. In addition, NO_2+NO_3 concentrations in water have statistically significant and negative correlations with echinoderm distribution. Previous studies indicated that sediment structure and especially temperature have an influence on the distribution of echinoderm species. The results found in the study, support those found in similar studies (Aronson, 1992; Hinchey et al., 2006; Rosellon-Druker and Stokesbury, 2019).

CONCLUSION

The relationships between the species diversity, abundance and environmental variables in the Çardak Lagoon are presented herein. We think that the results obtained in this study allow us to have information about the ecological quality condition of the lagoon. 80% of the species found in the study area were found in the clean areas of the lagoon under the influence of the sea. Sand content, OM%, temperature and NO_2+NO_3 were found to be the most effective parameters for the distribution of Cnidaria and Echinoderm species. Our research showed that cnidarians are dominant on the bottoms that are observed to carry the pollution load (with high organic matter content) of the study area. Whereas, echinoderms are abundant on the bottoms of seaside stations where contamination is traced (with low organic matter content).

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AUTHOR CONTRIBUTIONS

Seçil Acar: Designing of the study, identification of the investigated species, writing of the draft, submission, writing-review, and editing. A. Suat Ateş: Designing of the study, sorting the materials into taxonomic groups, and checking the original draft. Ertan Dağlı and Alper Doğan: Sorting the materials into taxonomic groups and checking the original draft.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

DATA AVAILABILITY

For any questions, the corresponding author should be contacted.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Feeding habits of two skate species, *Raja miraletus* Linnaeus, 1758 and *Dipturus oxyrinchus* (Linnaeus, 1758) (Chondrichthyes, Rajidae), around the Gökçeada Island (Northern Aegean Sea)

Gökçeada (Kuzey Ege Denizi) çevresindeki iki vatoz türünün, *Raja miraletus* Linnaeus, 1758 ve *Dipturus oxyrinchus* (Linnaeus, 1758) (Chondrichthyes, Rajidae) beslenme alışkanlıkları

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Abstract: This study investigated the feeding habits of *Raja miraletus* Linnaeus, 1758 and *Dipturus oxyrinchus* (Linnaeus, 1758). For the purpose of this study, the specimens were obtained monthly from the commercial trawlers operating around the Gökçeada Island in the Northern Aegean Sea between February 2019 and February 2020. A total of 29 (24 female and 5 male) brown rays (*Raja miraletus*) and 36 (20 female and 16 male) longnosed skates (*Dipturus oxyrinchus*) were captured. The total lengths of the *R. miraletus* specimens ranged between 24.0 cm and 53.9 cm, while the *D. oxyrinchus* specimens measured between 17.1 cm and 85.0 cm. Total length-weight relationships of *R. miraletus* and *D. oxyrinchus* specimens, respectively; $W=0.0002TL^{3.92}$ ($R^2=0.97$) and $W=0.0007TL^{3.43}$ ($R^2=0.97$) were calculated. The analysis of the stomach content of the specimens showed that crustacea were the primary and the most important prey group for *R. miraletus* (IRI% = 67.09), followed by teleosts (IRI% = 1.00) and cephalopods (IRI% = 0.45). The *D. oxyrinchus* specimens were determined to primarily feed on crustacea (IRI% = 74.3), followed by teleosts (IRI% = 6.50) and nematoda (IRI% = 0.40).

Keywords: Feeding habits, *Raja miraletus*, *Dipturus oxyrinchus*, Gökçeada Island, Northern Aegean Sea

Öz: Bu çalışmada *Raja miraletus* Linnaeus, 1758 ve *Dipturus oxyrinchus*'un (Linnaeus, 1758) beslenme alışkanlıkları incelenmiştir. Bu çalışmanın amacı doğrultusunda, Şubat 2019-Şubat 2020 tarihleri arasında Kuzey Ege'de Gökçeada ve çevresinde faaliyet gösteren ticari trol teknelerinden aylık olarak numuneler alınmıştır. Toplam 29 (24 dişi ve 5 erkek) kahverengi vatoz (*Raja miraletus*) ve 36 (20 dişi ve 16 erkek) sivriburun vatoz (*Dipturus oxyrinchus*) avlandı. *R. miraletus* örneklerinin total boyları 24,0 cm ile 53,9 cm arasında, *D. oxyrinchus* örneklerinin ise 17,1 cm ile 85,0 cm aralığında ölçülmüştür. *R. miraletus* ve *D. oxyrinchus* örneklerinin toplam boy-ağırlık ilişkileri sırasıyla; $W=0,0002TL^{3,92}$ ($R^2=0,97$) ve $W=0,0007TL^{3,43}$ ($R^2=0,97$) olarak hesaplanmıştır. Örneklerin mide içeriği analizi, kabukluların *R. miraletus* (%IRI = 67.09) için birincil ve en önemli av grubu olduğunu, onları kemikli balıklar (%IRI = 1.00) ve kafadan bacaklılar (%IRI = 0,45) takip ettiğini göstermiştir. *D. oxyrinchus* örneklerinin ise başlıca kabuklularla (%IRI = 74,3), ardından kemikli balıklarla (%IRI = 6,50) ve nematoda (%IRI = 0,40) beslendiği belirlenmiştir.

Anahtar kelimeler: Beslenme alışkanlıkları, *Raja miraletus*, *Dipturus oxyrinchus*, Gökçeada, Kuzey Ege Denizi

INTRODUCTION

The Gökçeada Island is in the Northern Aegean Sea. Because found on a significant streamline, the region where the island is located is rich in nutrients brought by the Meriç River, in addition to the nutritious waters flowing from the Black Sea via the Dardanelles. The Gulf of Saros plays a vital role in the fish population around the island. Besides, the fact that pelagic fish are available on the migration routes increases this importance even more (Karakulak, 2002). Fishing activities in the vicinity of the island are mostly performed with bottom trawls, longlines, and gillnets.

The brown ray, *Raja miraletus* Linnaeus, 1758 has a wide distribution, including the northeast of the Atlantic Ocean and the Mediterranean. It is a benthic species, mostly distributed between 50-150 m depths (Bianchi et al., 1999). The maximum length reported to date is 63.0 cm in males and 59.7 cm in females (McEachran et al., 1989). They show oviparous spawning characteristics and usually spawn in spring and summer. An individual lays an average of 40-72 egg capsules per year. Embryos in the egg capsule are fed only with yolk (Dulvy and Reynolds, 1997). It feeds on all kinds of benthic organisms. Although the status of *R. miraletus* is classified as "Least Concern (LC)" by the International Union for Conservation of Nature (IUCN), it is caught as bycatch (IUCN, 2021).

The longnosed skate, *Dipturus oxyrinchus* (Linnaeus, 1758) has a wide distribution including the Eastern Atlantic,

Faroe Islands, Canary Islands and the Mediterranean. It has been reported that *D. oxyrinchus*, a bathy-demersal species, ranges between 70-1230 m depths (Last et al., 2016). This species, which shows oviparous reproduction, reproduces in spring and summer (Stehmann and Bürkel, 1984). They lay their egg capsules on sandy or muddy ground. Embryos in the egg capsule are fed only with yolk (Dulvy and Reynolds, 1997). While the main food of *D. oxyrinchus* is cephalopods and crustaceans, they also feed on all kinds of benthic organisms (Stehmann and Bürkel, 1984; Yığın and İşmen, 2010a). Although the status of *D. oxrinchus* is "Near Threatened (NT)" by the International Union for Conservation of Nature (IUCN), this species is still caught as bycatch by fishermen (IUCN, 2021).

The fact that the majority of skate species typically exhibit low fecundity, slow growth, and late maturity suggests that these species are exclusively susceptible to overfishing and over-exploitation (Walker and Hislop, 1998; Sağlam et al., 2010). Most skate species are often captured as bycatch along with the commercially targeted species in their environment. Skates are benthic and demersal species, primarily feeding on fish and invertebrates (McEachran and Musick, 1975; Ajayi, 1982; Ebert et al., 1991; Ellis et al., 1996; Orlov, 1998; Sağlam et al., 2010).

Investigation on the feeding habits of these fish and the predator-prey relationships are worthwhile to assess the role of the species in the ecosystem. Furthermore, data on diet composition are worthwhile for developing trophic models as a tool for understanding the complexity of marine ecosystems (Lopez-Peralta and Arcila, 2002; Stergiou and Karpouzi, 2002). Removal of these fish from ecosystems can result in cascading effects through the trophic levels below, completely restructuring the food web (Frank et al., 2005). Many skate species are overly fished, which gravely affects them due to their life cycle characteristics. For the sustainable management of fish stocks, it is necessary to investigate the biological characteristics of these species. The present study described the food compositions of the poorly investigated Northern Aegean Sea stock of *R. miraletus* and *D. oxyrinchus* to contribute to the sustainability and management of the respective ecosystems.

MATERIALS AND METHODS

Samples were obtained as bycatch from commercial bottom trawler boats using with a mesh size of 44 mm between February 2019 and February 2020. Commercial trawlers operate 12-14 hours a day at depths of 100-400 m at intervals of 3-6 hours (Figure 1).

A total of 29 brown rays (*R. miraletus*) and 36 longnosed skates (*D. oxyrinchus*) were captured, and they were stored in a fish box filled with ice and immediately transported to the laboratory. Each specimen was sexed and their total lengths (TL) were measured to nearest 0.1 cm and weights (TW) to ± 0.1 g. The stomachs of the specimens were removed and weighed and then placed in 4% formaldehyde solution to be used in analysis. The allometric equations (Sparre and Venema, 1992) – i.e., $W = aTL^b$ were operationalized to find the TL-W relationships. $W = aL^b$ where, W is the total weight (expressed in g), L is the total length (expressed in cm), "a" and "b" are the power regression coefficients (i.e., "a" is a coefficient of body form and "b" is an exponent referring to isometric growth when equal to 3 and to allometric growth when significantly different from 3) (Froese, 2006; Loyola Fernández et al., 2017).

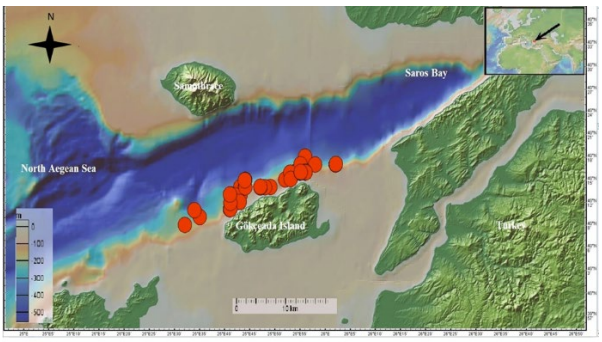

Figure 1. Study area and sampling stations around the Gökçeada Island, Northern Aegean Sea

Pauly's t-test was performed (Pauly, 1984). Pauly's t-test statistic was calculated; Sd_{logTL}= is the standard deviation of the log TL values, Sd_{logW}= is the standard deviation of the log W values, n is the number of specimens used in the computation (eq.1). The value of b is different from b = 3 if calculated t value is greater than the tabled t values for n-2 degrees of freedom (Pauly, 1984; Ahmed et al., 2015).

After each specimen's stomach was excised and left to dry on blotter paper, the stomachs' weights were recorded. The items were categorized, enumerated, and weighed on a precision balance, each of which was identified to the lowest possible taxonomic level. The individuals of each determined group were enumerated. The references availed of to identify the food items are as follows: (Murduchay-Boltouski, 1969; Fischer, 1973; Whitehead et al., 1986a,b; Fischer et al., 1987a,b). Formulas used in dietary component analysis; $N_i\%$ is the percent of prey i, N_i is the total number of prey i, N_p is the total number of prey, $O_i\%$ is the percent frequency of occurrence of prey i, FO_i is the frequency of occurrence of prey i, N_s is the total number of stomachs examined, $W_i\%$ is the percent by weight of prey i, W_i is the total weight of prey i, W_p is the total weight of prey (eq.2,3,4,), (Hyslop, 1980; Demirhan et al., 2007) and the Index of Relative Importance (IRI) (Pinkas et al., 1971) were the parameters factored in for the analyses of their contexts. IRI was used to determine the important food items in the diet of the fish (eq.5,6) (Pinkas et al., 1971; Windell and Bowen, 1978).

$$t=(Sd_{log\,TL} / Sd_{log\,W})(\,|\,b-3\,|\,/(\sqrt{1-r^2}))(\sqrt{n-2}) \quad \text{eq.1}$$

$$O_i\% = (FO_i/N_s)*100 \quad \text{eq.2}$$

$$N_i\% = (N_i/N_p)*100 \quad \text{eq.3}$$

$$W_i\% = (W_i/W_p)*100 \quad \text{eq.4}$$

$$IRI = O_i\% (N_i\% + W_i\%) \quad \text{eq.5}$$

$$(IRI / \sum IRI)*100 \quad \text{eq.6}$$

RESULTS

A total of 29 (24 females and 5 males) *R. miraletus* and 36 (20 females, 16 male) *D. oxyrinchus* specimens which were sampled the commercial bottom trawling season. Since the b value was significantly different from the three, it was determined that both species showed positive allometric growth (P>0.05) (SPSS/21.0) (Table 1).

Table 1. Length–weight relationships for *R. miraletus* and *D. oxyrinchus* from Gökçeada Island

Species	Sex	N	Length (cm)		Weight (g)		Relationship parameters				Growth
			Min.	Max.	Min.	Max.	a	b	95% CI of b	R^2	
Raja miraletus	M	5	28.30	46.60	67.40	493.40	0.0001	4.01	3.73-4.28	0.99	(+)A
	F	24	24.00	53.90	37.70	788.10	0.0002	3.88	3.57-4.19	0.97	(+)A
	C	29	24.00	53.90	37.70	788.10	0.0002	3.92	3.65-4.18	0.97	(+)A
Dipturus oxyrinchus	M	16	17.10	79.80	9.43	2375.00	0.0005	3.48	3.26-3.71	0.99	(+)A
	F	20	17.50	85.00	13.10	3550.00	0.0008	3.41	3.07-3.74	0.96	(+)A
	C	36	17.10	85.00	9.43	3550.00	0.0007	3.43	3.22-3.64	0.97	(+)A

N, sample size; Min., minimum; Max., maximum; a and b, intercept and slope of length–weight relationships; 95% CI of b, confidence intervals of b; R^2, coefficient of determination; (+)A, positive allometric growth type; M, male; F, female; Combined, males and females.

The stomach contents of 21 of 29 *R. miraletus* individuals were examined. In 20 of the examined stomachs, the food content was identified, while one was empty. On the otherhand 8 out of 29 stomachs had suffered damage that cannot be examined. The total number of the preys in the examined stomachs was 58.

The analyses revealed no significant between-sex differences in the indices of relative importance (IRI%; p > 0.05). The analysis of the stomach contents in the *R. miraletus* individuals showed that Crustacea were their primary and most important prey group (IRI% = 67.09), followed by teleosts (IRI% = 1.00) and cephalopods (IRI% = 0.45). The by-percentage numbers (N%), weights (W%), occurrence (O%), and indices of relative importance (IRI%) of the main prey items are presented in Table 2.

Considering the frequency of the prey groups consumed by *R. miraletus* individuals according to size groups, it was determined that crustaceans were consumed in every size group. However, it was observed that the prey groups diversified with the increase in length (>40 cm) (Table 3).

Table 2. Food composition of *R. miraletus*

Prey taxa	Prey weight (g)	n	N%	W%	O%	IRI%
Crustaceans						
Decapoda						
Caridea	13.50	26	44.10	31.80	32.50	56.50
Parapenaeus longirostris	9.50	5	8.47	22.40	10.00	7.06
Brachyura						
unidentified Brachyura	2.34	6	10.20	5.51	7.50	2.69
Isopoda	0.23	3	5.08	0.54	5.00	0.64
Stomatopoda	1.08	1	1.69	2.54	2.50	0.20
Teleost						
Trachurus trachurus	6.67	1	1.69	15.70	2.50	1.00
Cephalopoda						
Loligo sp.	2.63	1	1.69	6.19	2.50	0.45
Nematoda	0.02	2	3.39	0.05	2.50	0.20
Unidentified	6.49	14	23.70	15.30	35.00	31.30

Percentage by number (N%), weight (W%), occurrence (O%) and index of relative importance (IRI%).

Table 3. Frequency of prey groups for each size class of *R. miraletus*

N	Size	Crustaceans	Brachyura	Teleost	Isopoda	Cephalopoda	Stomatopoda	Nematoda	Unidentified
6	20	20.70							28.60
6	30	31.00	33.30					100.00	28.60
4	40	48.30	66.70	100.00	100.00	100.00	100.00		35.70
1	50+								7.14

N: Number of fish.

The stomach contents of 34 of 36 *D. oxyrinchus* individuals were examined. Three of the examined stomachs were empty, while stomach contents were determined in 31. On the otherhand 2 out of 36 stomachs had suffered damage that cannot be examined. The total number of the preys in the examined stomachs amounted to 85. The calculated indices of relative importance were not significantly different between the sexes (IRI%; P > 0.05). The analysis of the stomach contents in the *D. oxyrinchus* individuals showed that crustacea were their primary and most important food group (IRI%= 74.3), followed by teleosts (IRI% = 6.50) and Nematoda (IRI% = 0.40). The by-percentage numbers (N%), weights (W%), occurrence (O%), and indices of relative importance (IRI%) of the main prey categories are presented in Table 4. Considering

the distribution frequency of the food groups consumed by *D. oxyrinchus* according to size groups, it was observed that it mostly fed on crustacea and teleostei (Table 5). Seasonal and sexual variations of the percentage of relative importance (IRI%) of *R. miraletus* and *D. oxyrinchus* were presented in Table 6.

Table 4. Food composition of *D. oxyrinchus*

Prey taxa	Prey weight (g)	n	N%	W%	O%	IRI%
Crustaceans						
Decapoda						
Caridea	42.50	51	60.00	43.40	38.30	71.70
Parapenaeus longirostris	11.00	4	4.70	11.20	8.50	2.50
Plesionika sp.	0.96	1	1.18	0.98	2.10	0.10
Teleost	23.20	7	8.20	23.60	10.60	6.10
Trachurus trachurus	8.09	2	2.35	8.26	2.10	0.40
Nematoda	0.80	4	4.70	0.80	4.30	0.40
Unidentified	11.50	16	18.80	11.70	34.00	18.80

Percentage by number (N%), weight (W%), occurrence (O%) and index of relative importance (IRI%).

Table 5. Frequency of prey groups for each size class of *D. oxyrinchus*

N	Size class (cm)	Crustaceans	Teleost	Nematoda	Unidentified
8	20	24.10	16.70		26.70
11	30	29.60	16.70		26.70
7	40	9.30	16.70	100.00	20.00
8	50+	37.00	50.00		26.60

N: Number of fish.

Table 6. Seasonal and sexual variations of the percentage of relative importance (IRI%) of *R. miraletus* and *D. oxyrinchus*

Species	Prey	Season				Sex	
		Spring	Summer	Autumn	Winter	F	M
R. miraletus	Crustaceans	36.31		100.00	74.39	78.99	27.61
	Teleost	15.69				1.24	
	Nematoda				0.31		2.47
	number of prey	10.00		5.00	43.00		
D. oxyrinchus	Crustaceans	18.90	77.70	27.70	94.70	78.7	78.10
	Teleost	11.50	11.70		4.01	6.10	8.38
	Nematoda	69.60		3.51	0.87	0.27	0.41
	number of prey	22.00	9.00	9.00	21.00		

DISCUSSION

In the length-weight relationship of *R. miraletus* and *D. oxyrinchus* specimens, the "a" value was calculated as 0.0002, while the "b" value was observed to vary between 3.43-3.92 (Table 7 and Table 8). In studies conducted in similar species in the Aegean Sea, it was determined that the "b" values of the species showed positive allometric growth (P>0.05) (Filiz and Mater, 2002; Yankova et al., 2011). Moutopoulos and Stergiou (2002) stated that the reasons for these differences in "b" values are based on differences in sample number, region and seasonal parameters. In addition, other important reasons for these differences may be caused by various ecological factors such as temperature, specific spawning and feeding conditions, and biotopic characteristics (Ricker, 1975). The depths and sampling methods of fish species are effective in these differences. It is thought that the difference between the length-weight relationships determined in this study and the studies conducted in other regions is mostly due to the sampling methodology of the species, sample size and sampling season.

The obtained results showed that *R. miraletus* and *D. oxyrinchus* feed on benthic prey items, with observed differences in the indices of relative importance (IRI%) among the prey groups, crustaceans being the most important diet of these skates. This finding is substantiated by many research studies where crustaceans are reported to be the main food item for skates (Demirhan et al., 2005; Sağlam and Başçınar, 2008; Follesa et al., 2010; Karachle and Stergiou, 2010; Sağlam et al., 2010; Yığın and İşmen, 2010a, b; Šantić et al., 2012; Kadri et al., 2014a, b; Eronat and Özaydın, 2015; Mulas et al., 2015; Yemişken et al., 2017; Biton-Porsmoguer, 2020; Cabbar and Yığın, 2021). According to the total IRI, the other prey groups (Brachyura, Teleosts, Cephalopoda, Isopoda, Stomatopoda, and Nematoda) were of less importance (Rosecchi and Nouaze, 1987).

D. oxyrinchus exhibits large ontogenetic changes in feeding behaviour, and their early life stages are characterized by a benthopelagic diet that changes during growth (Mulas et al., 2015). *Parapenaeus longirostris* was detected to be their favourite prey group. In consideration of the IRI% values between the sexes, there were no significant differences in the dietary compositions of the male and female longnosed skates (P = 0.66). The frequency of occurrence (F%) with size showed that crustacea were always present in their diet (Yığın and

İşmen, 2010a). In this study, it was determined that crustaceans constituted the most important food group of *D. oxyrinchus*. The most prominent species among the crustaceans was *Parapenaeus longirostris*.

Table 7. Length-weight relationships parameters of *Raja miraletus* from different regions

Region	Sex	N	Length (cm)		Weight (g)		Relationship parameters			Reference
			Min.	Max.	Min.	Max.	a	b	R^2	
Balearic Islands, Western Mediterranean	C	28	16.60	41.00	-	-	0.002	3.25	0.99	Merella et al., 1997
North Aegean Sea	C	13	30.00	56.50	100	1001	0.0001	4.02	0.93	Filiz and Mater, 2002
Aegean Sea	C	16	25.60	49.30	-	-	0.003	3.29	0.94	Moutopoulos and Stergiou, 2002
Eastern Adriatic Sea	C	339	13.40	50.00	10.40	633	0.005	2.98	0.95	Pallaoro et al., 2005
Saros Bay, North Aegean Sea	C	30	6.50	30.50	6.00	350	0.009	3.22	0.97	İşmen et al., 2007
İzmir Bay, Aegean Sea	C	12	39.00	53.50	-	-	0.006	2.95	0.96	Özaydın et al., 2007
Aegean Sea	C	10	23.90	45.10	-	-	0.035	2.83	0.98	İlkyaz et al., 2008
Saros Bay, North Aegean Sea	C	52	10.50	53.50	5.82	1010	0.002	3.27	0.95	Yığın and İşmen, 2009
İskenderun Bay	C	22	24.00	54.00	58.00	998	0.002	3.26	0.95	Başusta et al., 2012
South Aegean Sea	C	62	26.70	49.30	-	-	0.001	3.44	0.97	Bilge et al., 2014
Lebanese marine waters, eastern Mediterranean	C	30	25.00	44.00	72.00	490	0.002	3.34	0.91	Lteif et al., 2016
Marmara Sea	C	9	22.40	54.60	41.70	830	0.002	3.18	0.95	Karadurmuş, 2022
Gökçeada Island North Aegean Sea	C	29	24.00	53.90	37.70	788	0.0002	3.92	0.97	In This Study

C, both males and females; N, sample size; Min., Minimum; Max., Maximum; a and b, intercept and slope of total length–weight relationships; R^2, coefficient of determination.

Table 8. Length-weight relationships parameters of *Dipturus oxyrinchus* from different regions

Region	Sex	N	Length (cm)		Weight (g)		Relationship parameters			Reference
			Min.	M	Min.	Max.	a	b	R^2	
North Aegean Sea	C	8	17.90	62	10.40	851.00	0.001	3.40	0.99	Filiz and Bilge, 2004
Saros Bay, North Aegean Sea	C	1	10.00	63	9.00	4056.00	0.004	3.29	0.99	İşmen et al., 2007
Saros Bay, North Aegean Sea	C	1	14.90	10	8.00	4074.00	0.001	3.35	0.99	Yığın and İşmen, 2009
İzmir Bay and Sığacık Bay	C	8	18.10	46	14.80	285.00	0.031	3.13	0.99	Eronat and Özaydın, 2014
Gulf of Gabes, Tunisia, Central	C	5	16.50	10	30.00	5300.00	-	3.01	-	Kadri et al., 2014a
Northeastern Mediterranean	C	2	8.00	93	8.50	3828.00	0.002	3.19	0.97	Başusta and Özel, 2017
Syrian, Eastern Mediterranean	C	2	34.10	10	-	-	0.001	3.35	0.96	Alkusairy and Saad, 2017
Aegean Sea	C	6	17.10	34	10.70	112.80	0.001	3.37	0.99	Soykan and Kınacıgil, 2021
Northeastern Mediterranean Sea	C	2	12.20	93	8.34	3828.00	0.002	3.19	0.97	Başusta and Özel, 2022
Gökçeada Island, Northern Aegean Sea	C	3	17.10	85	9.43	3550.00	0.001	3.43	0.99	In This Study

C, both males and females; N, sample size; Min., Minimum; Max., Maximum; a and b, intercept, and slope of total length–weight relationships; R^2, coefficient of determination.

Considering the foods consumed by *R. miraletus* according to size groups, Šantić et al. (2013) stated that specimens smaller than 25.0 cm mostly feed on amphipoda and mysidacea, while specimens larger than 25.0 cm feed on decapoda, teleost and cephalopoda in the Adriatic Sea. Kadri et al. (2014c) determined that *R. miraletus* specimens smaller than 38 cm fed on mysidacea and amphipoda, while specimens larger than 38 cm fed on decapoda, cephalopoda and isopoda, mostly teleost in the Gulf of Gabes. In this study, when we look at the food groups consumed by *R. miraletus* specimens according to size groups, it was observed that crustaceans were consumed in every size group, and food groups diversified (crustaceans, brachyura, teleost, isopoda, cephalopoda, stomatopoda, nematoda) with an increase in length (>40cm).

Although most of the diet of *D. oxyrinchus* in the Sardinian Sea consists of crustaceans at all life stages, their importance decreases during growth and consumption of cephalopods and teleosts increases (Mulas et al., 2015). It is thought that these differences in the studies may differ according to the number of specimens sampled, the number of specimens in length groups and habitats.

All in all, because skates are opportunistic predators, the results may vary across research studies performed in different research areas. Particular species – e.g., crustaceans – were found to be the major prey items in the dietary compositions of the *R. miraletus* and *D. oxyrinchus* specimens in the Northern Aegean Sea.

Despite the known high biodiversity of the respective research field (Daban et al., 2022), the lower prey biodiversity in *R. miraletus* and *D. oxyrinchus* requires attending to a critical research question: How strongly can it compete for food? This question calls for further research to develop a better understanding of the food overlap across all living competitors. Furthermore, very little is known about such subject matters as early life biology, habitat use, post-fishing survival rates, selectivity of trawls, and stock size. More comprehensive research is mandatory to present more applicable data for fisheries management authorities.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed equally to the idea, data collections, design and writing of the manuscript.

CONFLICTS OF INTEREST

The authors declares that there is no conflict of interest in this manuscript.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Growth and feeding features of the four-spotted megrim *Lepidorhombus boscii* (Risso,1810) in the Aegean Sea, Türkiye

Cangidez (*Lepidorhombus boscii* (Risso, 1810))'in Ege Denizi'ndeki büyüme ve beslenme özellikleri

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Abstract: The growth and feeding features of *Lepidorhombus boscii* (Risso, 1810) (four-spotted megrim) obtained from the Aegean Sea between May 2009 and April 2010 were determined. During the study, 1864 specimens were examined, and the female/male ratio was 1:0.41. The total length range and age distribution for all specimens were 6.3-34.8 cm and I-XIV, respectively. The Von-Bertalanffy growth equations were calculated by using the mean total lengths of each age group. The results were as follows: (L∞= 43.24 cm, K=0.088, t0= -2.897) for females, (L∞= 28.53 cm; K= 0,.25; t0= - 0.829) for males, and (L∞= 42.9 cm, K=0.097, t0= -2.070) for all specimens. The stomach contents of the *L. boscii* species were also examined; a total of 9 main groups (Amphipoda, Mysidacea, Natantia, Anomura, Brachyura, Stomatopoda, Cephalopoda, Teleostei, Decapod Crustacea) were determined.

Keywords: Aegean Sea, feeding features, growth, *Lepidorhombus boscii*

Öz: Mayıs 2009-Nisan 2010 tarihleri arasında Ege Denizi'nden temin edilen *Lepidorhombus boscii*'nin (Risso, 1810) (Cangidez) büyüme ve beslenme özellikleri belirlenmiştir. Çalışma süresince, 1864 birey incelenmiş ve dişi erkek oranı 1:0.41 olarak bulunmuştur. Tüm örnekler için total boy aralığı ve yaş dağılımı sırasıyla, 6,3-34,8 cm ve I-XIV 'dir. Von-Bertalanffy büyüme denklemleri, her yaş grubunun ortalama Total Boy uzunlukları kullanılarak hesaplmıştır. Sonuçlar sırasıyla, dişiler için (L∞= 43,24 cm, K=0,088, t0= -2,897), erkekler için (L∞= 28,53 cm; K= 0,225; t0= - 0,829) ve tüm bireyler için (L∞= 42,9 cm, K=0,097, t0= -2,070) dir. *L. boscii* türüne ait mide içerikleri incelendiğinde toplam 9 ana besin grubu (Amphipoda, Mysidacea, Natantia, Anomura, Brachyura, Stomatopoda, Cephalopoda, Teleostei, Decapod Crustacea) tespit edilmiştir.

Anahtar kelimeler: Ege Denizi, beslenme özellikleri, büyüme, *Lepidorhombus boscii*

INTRODUCTION

Lepidorhombus boscii is a flatfish species that is distributed across the Northeast of the Atlantic Ocean and the Mediterranean basin except The Black Sea region and it inhabits sandy-muddy bottoms that are about 50-800 m in depths (Norman, 1934; Froese and Pauly, 2015). They are most likely to be inhabited at depths between 100 and 450 m (Sanchez et al., 1998). They are usually caught with *Lepidorhombus whiffagonis* Walbaum 1792 (common megrim) which is another specimen in the same genus and both of them become economically important in European fisheries (Crego-Prieto et al., 2012). Especially lengths of 20 cm and above individuals are of commercial interest on the Mediterranean seas (Vassilopoulou, 2000; Ulutürk et al., 2012; Cengiz et al., 2013).

There are many studies on four spotted megrim because of a common component of benthic fauna (Castilho et al., 1993) and also economic value (Crego-Prieto et al., 2012). These studies mainly related to age and growth (Castilho et al., 1993; Santos, 1994; Landa, 1999; Robson et al., 2000; Landa et al., 2002; Teixeira et al., 2010; Landa and Fontela, 2016; Landa and Hernandez, 2020) in the Atlantic and (Bello and Rizzi, 1987; Vassilopoulou and Ondarias, 1999; Bostancı and Polat, 2008; Cengiz et al., 2013) in the Mediterranean Sea. Despite the importance of benthic fauna, information on the feeding habits of these specimens is limited. These were in the Portuguese waters (Teixeira et al., 2010) and the Mediterranean Sea (Morte et al., 1999; Vassilopoulou, 2006).

The main aim of our study is to determine the growth parameters and feeding habits of the four-spotted megrim in the central Aegean Sea; furthermore, it is thought to contribute to limited studies for the four-spotted megrim in the Aegean Seas.

MATERIALS AND METHODS

This study was carried out between May 2009 to April 2010 and fish specimens were obtained from bottom trawl ships targeting *Parapenaeus longirostris* (Lucas, 1846) from Karaburun and Kusadası in the central Aegean Sea. Three main areas (Karaburun; Sığacık Bay and Kuşadası Bay) have been identified for our sampling (Figure 1).

Fish lengths were measured to the nearest 1 mm and

weights weighed to the nearest 0.01 g. Otolith for age determination was suspended in a 10% solution of NaOH and cleared with pure water then passed through five stages of ethanol series (90%, 70%, 50%, 30%, 10%). Otoliths were read using a black background filled with 50% water and 50% glycerin under reflected light. The stomachs were fixed in 4% formalin after the specimens were dissected.

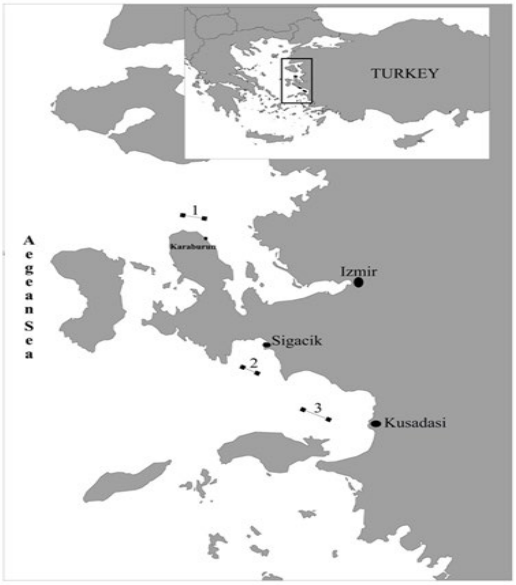

Figure 1. Sampling locations of *L. boscii* in the Aegean Sea

The form of the exponential equation "$W = a * L^b$" was used to indicate the relationship between individuals' weight and length parameters (Ricker, 1979). The calculation of using The Von Bertalanffy growth "$Lt = L\infty [1-e-k(t - to)]$" equation is used to calculate the growth of *L. boscii* specimens. In the equation: Lt is the fish's total length (cm) at age t, L∞ is the asymptotic fish length (cm), t is the fish age(year), to(year) is the hypothetical time at which the fish length is zero, k is the growth coefficient (year $^{-1}$) (Sparre and Venema, 1998). The Gulland and Holt plotting method was used to estimate the parameters of the von Bertalanffy growth function (Avşar, 2005).

The stomach fullness index was estimated by using the equation FI= Fs/(Fs+Es) (Sartor and Ranieri, 1996). In this formula; FI: Stomach Fullness Index; Fs: Number of the full stomach; Es: Number of Empty Stomachs.

Food items were identified to the lowest taxonomic level possible. Their number was recorded and weights were determined with the nearest 0.0001 g. The diet of *L. boscii* specimens was determined by four indices as follows: (i) frequency of occurrence (F%), based on the number of stomachs in which a food item was found; (ii) weight percentage (W%), based on weights of each prey item expressed as the percentage of the total weight of stomach contents; (iii) numerical abundance (N%), based on the number of each prey item in all non-empty stomachs; (iv) the index of relative importance IRI=[N%+W%]* F%*100 (Pinkas et al., 1971) and The IRI value was expressed as a percentage [IRI%= (IRI/∑IRI) x 100] (Cortes,1997). Food items were grouped into preference categories using the method recommended by Morato-Gomes et al. (1998) and Sever et al. (2008). The categories were defined as follows:

Main important prey (MIP): IRI≥ 30*(0.15*∑%F),

Secondary prey (SP): 30*(0.15*∑%F)>IRI>10*(0.05*∑%F),

Occasional prey (OP): IRI≤10*(0.05*∑%F)

RESULTS

A total of 1864 individuals were obtained during the observation. The number of females was 1283(68.83%), the male was 521 (27.95%) and undetermined was 60 (3.22%). The sex ratio for *L. boscii* specimens was 1:0.41. Significant differences were observed between the sexes (χ^2=160.83, p≤0.05).

Maximum and minimum total lengths of female and male *L. boscii* specimens were 34.8 and 11.1 cm, 26.7 and 6.3 cm respectively. Specimens between 15.0-17.9 cm were abundant in both sexes (Figure 2).

The mean total lengths value was significant differences between female and male individuals (df: 1802, p<0.0001). Length-weight relationships were calculated separately as female, male, and all individuals. The t-test results of b values results showed positive allometric growth for all individuals and female specimens, and isometric growth for male specimens (Table 1).

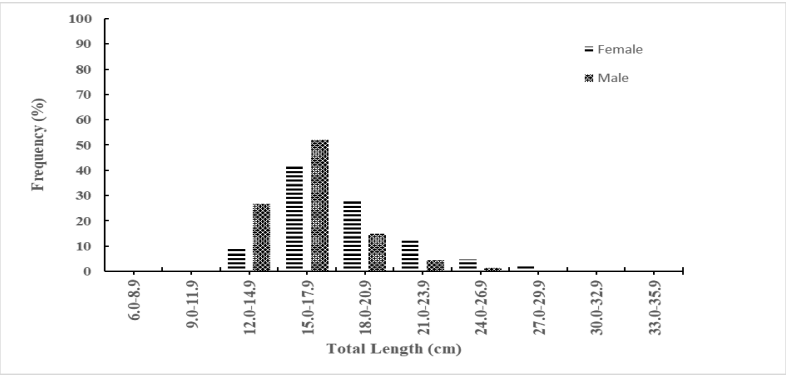

Figure 2. Length distribution of *L. boscii* specimens

The age distribution was observed between I to XIV. Dominant age groups in female, male, and all specimens were II (26.57%), III (26.83%), and II (26.24%) years respectively. (Table 2). Significant differences were observed in age and sex composition between female and male specimens (χ^2=59.38, p≤0.05). Total length values corresponding to the age group of female and male megrim individuals were evaluated separately and obtained from the sampling results (Table 3). The Von Bertalanffy growth equations were calculated as L_∞=43.24 cm, k=0.08 $year^{-1}$, t_0=-2.897 year for females, L_∞=28.53 cm, k=0.11 $year^{-1}$, t_0=-0.829 for males and L_∞= 42,9 cm, K=0.097, t0= -2.070 for all individuals. A total of 1864 stomach samples were examined, of which 1313 were found to be empty and 521 were full. Stomach fullness index values were found for females, males, and all individuals 0.31, 0.27, and 0.30 respectively. A total of 9 prey groups were identified from the stomach contents of *L. boscii* specimens. According to the IRI% value to the overall feeding composition, Natantia (IRI%: 70.15) was the first place and Mysidacea (IRI%: 23.42), Anomura (IRI%: 3.09), Teleostei (IRI%: 1.72) and Brachyura (IRI%: 1.30) followed them respectively. According to preference categories, Natantia and Mysidacea specimens were found main important prey (MIP), and Decapod Crustacea, Anomura, and Brachyura specimens were secondary prey (SP). Other groups of diet were found as occasional prey (OP) (Table 4).

Table 1. Length-Weight relationship values of *L. boscii* individuals (♀: Female Specimens; ♂: Male Specimens; ?: Undetermined Specimens)

Sex	N	Lmax.-Lmin.(cm)	Wmax.-Wmin.(g)	a	W=a*L^{b}b±%95CI	r	Growth
♀+♂+?	1864	34.8-6.3	393.57-1.24	0.0053	3.13±0.027	0.982	Allometric(+)
♀	1283	34.8-11.1	393.57-8.64	0.0053	3.14±0.32	0.982	Allometric(+)
♂	521	26.7-6.3	152.35-2.00	0.0071	3.03±0.058	0.975	İsometric

Table 2. Age and sex composition of *L. boscii* specimens

Age	♀+♂		♀		♂		♀:♂
	N	N%	N	N%	N	N%	
I	8	1.72	4	1.14	4	3.48	1:1.00
II	122	26.24	93	26.57	29	25.22	1:0.31
III	81	17.42	49	14.00	32	27.83	1:0.65
IV	70	15.05	47	13.43	23	20.00	1:0.49
V	61	13.12	47	13.43	14	12.17	1:0.30
VI	40	8.60	35	10.00	5	4.35	1:0.14
VI	28	6.02	25	7.14	3	2.61	1:0.12
VIII	22	4.73	21	6.00	1	0.87	1:0.05
IV	14	3.01	12	3.43	2	1.74	1:0.17
X	7	1.51	5	1.43	2	1.74	1:0.40
XI	2	0.43	2	0.57	-	-	-
XII	4	0.86	4	1.14	-	-	-
XIII	4	0.86	4	1.14	-	-	-
XIV	2	0.43	2	0.57	-	-	-
Total	**465**	**100**	**350**	**100**	**115**	**100**	**1:0.33**

Table 3. Total length values corresponding to the age group of *L. boscii* specimens

Sex	Age	N	Lmax (cm)	Lmin (cm)	Lmean (cm)
♀	I	4	13.00	11.50	12.55
	II	93	16.90	13.20	15.50
	III	49	18.40	17.00	17.61
	IV	47	20.40	18.50	19.38
	V	48	23.90	20.50	21.41
	VI	34	24.40	22.50	23.51
	VII	25	25.90	24.50	24.99
	VIII	21	27.90	26.00	26.71
	IV	12	28.90	28.00	28.34
	X	5	29.90	29.20	29.58
	XI	2	30.30	30.20	30.25
	XII	4	31.50	31.00	31.30
	XIII	4	32.70	32.00	32.33
	XIV	2	34.80	33.50	34.15
♂	I	4	13.00	6.30	9.63
	II	29	15.60	13.40	14.46
	III	32	17.80	14.20	16.20
	IV	23	19.70	16.20	18.30
	V	14	22.00	18.10	20.58
	VI	5	22.60	22.00	22.30
	VII	3	23.50	23.20	23.30
	VIII	1	24.20	24.20	24.20
	IV	2	25.50	25.20	25.35
	X	2	26.70	26.30	26.50

Table 4. Dietary groups for *Lepidorhombus boscii*, concerning the frequency of occurrence (F%), weight percentage (W%), (IRI: Percent of the Index of Relative Importance, PC: Preference categories)

Prey Groups	N	N%	F%	W%	IRI%	PC
Amphipoda	14	0.83	2.54	0.01	0.0004	OP
Mysidacea	843	49.82	152.99	10.79	23.42	MIP
Natantia	522	30.85	94.74	52.20	70.15	MIP
Anomura	129	7.62	23.41	9.31	3.09	SP
Brachyura	95	5.61	17.24	5.31	1.30	SP
Stomatopoda	10	0.59	1.81	0.71	0.02	OP
Cephalopoda	12	0.71	2.18	3.95	0.12	OP
Teleostei	47	2.78	8.53	14.24	1.72	OP
Decapod Crustacea	20	1.18	3.63	3.49	0.18	SP

DISCUSSION

Sexes of flatfish specimens show differences in growth and also female individuals reach a larger size than males (Rljnsdorp and Ibelings, 1989; Vassilopoulou and Ondarias, 1999). Differences in growth between male and female individuals have been found in other studies for *L.boscii* specimens (Bello and Rizzi, 1987; Castilho et al., 1993; Vassilopoulou and Ondarias, 1999; Robson et al., 2000; Teixeira et al., 2010; Cengiz et al., 2013; Landa and Hernandez, 2020). Growth between sexes was determined as statistically different in this study. Growth studies of *L.boscii* specimens have focused on two different areas (Northeast Atlantic and Mediterranean Sea) (Table 5). According to these studies, the Maximum Total length of *L.boscii* specimen was reported in the North Aegean Sea at 48 cm (Sartor et al., 2002).

In this study the growth of megrim, in females was allometric (P<0.005, tcal:8.53, t0.05(1282):1.96), and in males was isometric (p>0.005, tcal:1.00, t0.005(520): 1.96). These growth results have found some studies on length-weight relationships for *L. boscii* by other authors (Table 6). As a result age determination from *L.boscii* specimens ranged between I to XIV. Mean lengths of age groups were observed in this study and other *L. boscii* studies were given in Table 7. Differences in age group and mean lengths related to age depend on various factors such as food ability; latitudinal differences, fishing efforts, and temperature (Nash and Geffen, 2005). It was observed that female individuals reached older ages than males. This difference in the growth of *L. boscii* specimens was found in other studies (Castilho et al., 1993; Santos, 1994; Vassilopoulou and Ondrias, 1999; Robson et al., 2000; Landa et al., 2002; Cengiz et al., 2013; Landa and Fontela, 2016; Landa and Hernandez, 2020).

Table 5. A comparison of *L. boscii* specimen's maximum and minimum length values

Area	Locality	N	Length	Max. (cm)	Min. (cm)	References
Atlantic	Portuguese Coasts	578	TL	39.1 (♀)	10.2 (♂+♀)	Castilho et al. (1993)
	The West Coast of Ireland	150	TL	41.9 (♀)	24.2 (♂)	Robson et al. (2000)
	Portuguese Coasts	302	TL	34.6	14,9	Teixeira et al. (2010)
Mediterranean Sea	North Aegean Sea	15323	TL	29.5 (♀)	5 (♂)	Vassilopoulou and Ondarias (1999)
	Morroco-Spain-France Coasts	3427	TL	36	3	Sartor et al. (2002)
	Sardinia- Tyrrhenian Sea	6163	TL	38	4	Sartor et al. (2002)
	Adriatic Sea	900	TL	35	7	Sartor et al. (2002)
	Ionian Sea	1628	TL	41	2	Sartor et al. (2002)
	North Aegean Sea	4080	TL	48	2	Sartor et al. (2002)
	Saros Bay North Aegean Sea	788	TL	40.8	10.9	Cengiz et al. (2013)
	Aegean Sea	1864	TL	34.8 (♀)	6.3 (♂)	This study

Table 6. Comparison of the Length-Weight Relationship values between *L. boscii*'s studies

Area	Locality	Sex	N	a	b	r	Growth Types	References
Atlantic	Portuguese Coasts	♀	250	$2.01x10^{-6}$	3.25	0.98	Allometric	Castilho et al. (1993)
		♂	315	$4.11x10^{-6}$	3.11	0.98	Allometric	
	Portuguese Coasts	♀	306	0.0025	3.36	-	-	Santos (1994)
		♂	351	0.0045	3.16	-	-	
	Ireland Coasts	♀+♂+?	150	0.0062	3.37	0.96	Allometric (+)	Robson et al. (2000)
Mediterranean Sea	South Adriatic Coasts	♀+♂+?	237	0.00333	3.25	0.99	-	Bello and Rizzi (1987)
		♀	109	0.00323	3.26	0.99	-	
		♂	128	0.00344	3.24	0.99	-	
	Saros Bay	♀+♂+?	521	0.00316	3.29	0.99	-	İşmen et al. (2007)
	Saros Bay	♀	553	0.0032	3.31	0.99	Allometric (+)	Cengiz et al. (2013)
		♂	235	0.0069	3.04	0.98	Isometric	
	Aegean Sea (Karaburun-Kusadası)	♀+♂+?	1864	0.0053	3.13	0.98	Allometric (+)	This study
		♀	1283	0.0053	3.14	0.98	Allometric (+)	
		♂	521	0.0071	3.03	0.97	Isometric	

Table 7. Lengths of age groups for *L. boscii* specimens were stated by other studies (mean or range of TL in cm as given in the related studies)

Area	Locality	Sex	N	0+	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	References
Atlantic	Portuguese Coasts	♂	327	-	11	13.3	16.4	18.7	22	24.4	25.2	27.1	31.8	-	-	32.5	-	-	Castilho et al. (1993)
		♀	249	-	13.2	17.2	20.2	23.3	27	28.7	30.2	32.5	35.1	37.1	37.2	37.6	-	-	
	Northern Coasts of Spain	♂	1895	-	5-10	10-15	15-17	17-20	20-22	22-23	23-24	24-25	-	-	-	-	-	-	Landa et al. (2002)
		♀	2977	-	5-9	12-15	15-18	20-24	25-27	27-29	30-32	32-33	33-34	35-36	-	-	-	-	
	Cantabrian Sea & Galician waters	♀+♂	11741	7.4	14.3	18.8	22	24.3	26.6	28.6	30.5	32.4	33.8	-	-	-	-	-	Landa and Fontela (2016)
	Porcupine Bank	♀+♂	1334	4.6	11.9	15.9	19.2	22.4	24.1	27.2	29.4	32.0	34.3	37.3	39.4	-	-	-	Landa and Hernandez (2020)
Mediterranean Sea	South Adriatic Coasts	♂	82	-	9.81	14.13	16.45	18.22	19.6	22.3	-	-	-	-	-	-	-	-	Bello and Rizzi (1987)
		♀	55	-	-	14.93	18.12	20.45	25.1	-	-	-	-	-	-	-	-	-	
	North Aegean Sea	♂	923	5.1-11.0	7.1-16.0	10.1-17.0	12.1-19.0	15.1-21.0	17.1-22.0	20.1-22.0	-	-	-	-	-	-	-	-	Vassilopoulou and Ondarias (1999)
		♀	1241	5.1-12.0	8.1-15.0	12.1-19.0	15.1-21.0	16.1-23.0	18.1-25.0	20.1-27.0	23.1-26.0	23.1-28.0	-	-	-	-	-	-	
	North Aegean Sea (Saros Bay)	♂	140	-	12.5	14.3	17.1	19.5	20.8	24	25	27.8	29.9	-	-	-	-	-	Cengiz et al. (2013)
		♀	282	-	12.6	15.8	19.4	22.6	23.6	26.8	28.9	31.7	32.7	34.6	36.4	37.2	38.2	-	
	Aegean Sea (Karaburun-Kuşadası)	♂	115	-	9.63	14.46	16.2	18.3	20.6	22.3	23.3	24.2	25.35	26.5	-	-	-	-	This Study
		♀	350	-	12.55	15.5	17.61	19.38	21.4	23.51	24.99	26.71	28.34	29.58	30.25	31.3	32.3	34	

When we compare growth parameters between other *L. boscii* studies and our results, there are some differences between Atlantic and Mediterranean studies. *L. boscii*'s L∞ values were determined higher in the studies of the Atlantic than in the Mediterranean. Differences in studies may be attributed to many factors (sample sizes, geographical differences, aging methodology, water temperature, etc.) (Cengiz et al., 2013; Landa and Hernandez, 2020) and the different methodologies to find age estimation and length frequency analysis (Landa and Fontela, 2016). Obtained results of growth parameters are given in Table 8.

Table 8. Some of the studies on growth parameters of *L. boscii* specimens [The methodology of age estimation: Direct age estimation (DAE), Back-calculation (BC), and Direct age estimation & back-calculation (DB)]

Area	Locality	N	Sex	Methodology	L∞(cm)	k (year^{-1})	t_0(year)	References
Atlantic	Portuguese Coasts	250 328	♀ ♂	DAE DAE	44.0 37.5	0.14 0.14	-1.52 -1.93	Castilho et al. (1993)
	Portuguese Coasts	227 217	♀ ♂	DAE DAE	39.77 34.79	0.157 0.198	-1.858 -1.436	Santos (1994)
	The West Coast of Ireland	150	♀+♂	DAE	34.39	0.27	-1.997	Robson et al. (2000)
	Porcupine Bank (ICES div. 7/b/c/k)	1334	♀+♂	DAE BC DB	56.46 43.74 50.09	0.11 0.17 0.13	-0.09 -0.13 -0.30	Landa and Hernandez (2020)
Mediterranean Sea	South Adriatic Coasts	55 82	♀ ♂	DAE DAE	28.5 27.6	0.26 0.21	-0.85 -1.27	Bello and Rizzi (1987)
	North Aegean Sea	1241 923	♀ ♂	DAE DAE	30.5 25.53	0.179 0.219	-1.103 -1.087	Vassilopoulou and Ondarias (1999)
	North Aegean Sea (Saros Bay)	553 235	♀ ♂	DAE DAE	49.8 39.1	0.09 0.11	-2.15 -2.59	Cengiz et al. (2013)
	Aegean Sea (Karaburun-Kusadası)	1283 521	♀ ♂	DAE DAE	43.2 28.5	0.09 0.23	-2.9 -0.83	This study

A total of 1864 four spotted megrim's stomach contents were examined during the study. The number of empty stomachs was 1313 (70%) and the number of full stomachs was 511 (30%). In the cost of Spain in the West Mediterranean Sea, it was determined that 81% of the examined specimens had full stomachs (Morte et al., 1999). The difference between the two studies may be thought that the stomachs of *L boscii* individuals in the study on the coast of Spain were fixed immediately to stop digestive activity thus this may be caused the number of full stomachs to be high. In our sample stomachs could be fixed after coming to the laboratory. For this reason, it is thought that some half-digested or easily digestible foods continue to be digested even after death, causing empty stomachs to count to be high.

Other diet studies of *L.boscii* stated that the food of specimens is composed of living creatures that mostly live buried in the benthic zone (Morte et al., 1999). *L.boscii* like other Scolphthalmid fish species, has a large and muscular esophagus, stomach, and intestine. This advanced nutrition system makes it easy to swallow and digest great food such as shrimp, fish, and crustaceans (De Groot, 1971). The stomach contents of the individuals in our study were obtained when viewed based on IRI value %, shrimp specimens of Natantia were obtained as the first group with a rate of 70.15 %. The obtained results seem to support the above studies.

The study of the feeding regime of four spotted megrim specimens in Portugal waters has been found to mainly Decapoda and Teleostei individuals (Teixeira et al., 2010). In our study, the Mysidacea group of individuals was found an important prey group in the diet of *L. boscii* specimens. This difference is thought to be due to the sampling of more small-sized than those studied in Portugal waters and these small individuals generally preferred the Mysidacea group.

In a study on the Western Mediterranean coast of Spain, are mainly Mysidacea and Natantia groups of individuals in *L. boscii* specimens (Morte et al., 1999). In another study about the feeding regime of four spotted megrim specimens on the North Aegean sea coast of Greece, mainly feeding groups of specimens are Mysidacea and Natantia group of individuals (Vassilopoulou, 2006). The similarities between our results and studies in Spain and Greece are observed.

CONCLUSION

International waters in the rest of Kusadasi – Karaburun (located in the central zone of the Aegean Sea) defined as the study area is an important fishing area for the Aegean region. It is being carried out in this region of intensive trawl fisheries. While Deep-water rose shrimp [*Parapenaeus longirostris* (Lucas, 1847)] and European hake [*Merluccius merluccius* (Linnaeus, 1758)] were identified as target species, Four-spotted Megrim (*L. boscii*) individuals are also considered to be economic in recent years. It is believed that the presented

results improve our knowledge of the feeding features and growth parameters of *L. boscii* specimens in the Aegean sea stocks.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study. Material preparation and investigation were performed by Efe Ulutürk and Murat Kaya. The writing was carried out by Efe Uluturk 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

All relevant data is inside the article

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Two different methods of sperm collection in European catfish (*Silurus glanis* Linnaeus, 1758)

Avrupa yayın balıklarında *(Silurus glanis* Linnaeus, 1758) iki farklı sperm toplama yöntemi

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Abstract: The European catfish (*Silurus glanis* L., 1758) is an important species for the aquaculture sector and the production of quality broodstock in artificial fertilization methods as well. One of the crucial steps determining the success of the reproduction of catfish is to obtain sufficient and good-quality sperm. The aim of this study is to compare two different methods used to obtain sperm from European catfish. The first method is surgery extraction of testicular sperm from taking testicles (CS) and the second method collection of sperm by stripping (SS). The fertilization rate was calculated as a percent for CS and SS groups and the highest fertilization rate was found in the SS group (81.87 ± 17.38%). According to the findings in the present study, it was concluded that it is not necessary to kill male European catfish to get sperm for fertilization.

Keywords: European catfish, fertilization, reproduction, sperm collection method

Öz: Avrupa yayın balığı (*Silurus glanis* L., 1758) su ürünleri sektörü ve yapay üretim yöntemlerinde kaliteli anaç üretimi için önemli bir türdür. Yayın balığı üreme başarısını belirleyen en önemli adımlardan biri yeterli ve kaliteli sperm elde etmektir. Bu çalışmanın amacı, Avrupa yayın balığından sperm elde etmek için kullanılan iki farklı yöntemi karşılaştırmaktır. İlk yöntem, testislerden cerrahi olarak sperm elde edilmesi (CS) ve ikinci yöntem ise sağım yoluyla sperm toplanmasıdır (SS). Döllenme oranı CS ve SS grupları arasında yüzde olarak hesaplanmış olup döllenme oranı en yüksek (%81.87±17.38) SS grubunda elde edilmiştir. Mevcut çalışmadaki bulgulara göre, döllenme için sperm elde etme yönteminde erkek Avrupa yayın balığını öldürmenin gerekli olmadığı sonucuna varılmıştır.

Anahtar kelimeler: Avrupa yayın balığı, döllenme, üreme, sperm toplama metodu

INTRODUCTION

The European catfish (*Silurus glanis* L., 1758) lives as a natural species in the basins of the Aral Sea, Caspian Sea, Black Sea, and Baltic Sea (Copp et al., 2009) and is a valuable fish caught in European waters (Froese and Pauly, 2019). However, European catfish is a commercial target species in the areas where it naturally occurs, thus sustaining and protecting the natural stocks is crucial (Zibiene and Zibas, 2019). European catfish has been listed as a protected species under the Berne Convention and entered into the International Union for Conservation of Nature Red List for species as of 2008 (Linhart et al., 2020).

European catfish is an important species in the fishing and aquaculture industry with its high growth abilities (Linhart et al., 2002; Ulikowski et al., 2003; Alp et al., 2011). In recent years, European catfish, that economic importance continues to increase, has become very attractive to humans for its delicious meat (Paschos et al., 2004) which rapidly makes, it a preferred fish for culture in most European countries. The maximum aquaculture production of European catfish was reported by Uzbekistan with 700 tons in 2019 and by Poland, with 127 tons in Europe (FAO, 2022).

Research on the production of *S. glanis* dates back to 1970 (Çelikkale, 1988; Horvath et al., 1992; Alpbaz and Hoşsucu, 1996; Linhart et al., 2002; Brzuska, 2001). Some authors have studied European catfish biology (Alp et al., 2004), bio-ecology (Akyurt, 1988), age-length and age-weight relationships (Saylar, 1993; Yılmaz et al., 2007), nutrition (Bora and Gül, 2004), growth characteristics (Uysal et al., 2009), and controlled production (Saygı and Güleç, 2019). While this species is naturally found in almost all inland waters in Turkey, its culture study still remains uncommon (Çelikkale, 1994; Geldiay and Balık, 1988; Alpaz, 2005; Saygı and Güleç, 2019). However, the cultivation and production of this species in Turkey are known to slow increase in a limited number of small-scale enterprises. European catfish production was 8.0 tons in 2017, and it was 84.0 tons in 2021 in Turkey (TÜİK, 2022).

Advancements in artificial culture techniques in Asia and Europe have led to progress in European catfish production. Despite the implementation of this reproductive method prior to 2000, significant production issues remain, with limited countries utilizing the technique, mainly in France and Central Europe (Kouril et al., 1996; Krasznai et al., 1980). In European

catfish production, the most important problem is the difficulties in obtaining sperm. Therefore, there is a need to develop different methods of sperm collection to ensure the development of catfish culture (Szabó et al., 2015; Idahor et al., 2018).

In this study, considering the difficulties in obtaining sperm from European catfish males, the effect of two different sperm collection methods on fertilization and hatching rate was investigated.

MATERIALS AND METHODS

Broodstock characteristics

This experiment carried out in this study was performed in compliance with the national legislation for fish welfare and approved by the institutional ethics committee. The suitable 7 male and 7 female European catfish broodstocks (6-9 years old) were selected for experiments and stocked in soil ponds within the Mediterranean Fisheries Research, Production, and Training Institute, Antalya. The fish were anesthetized using 2-phenoxyethanol at a concentration of 0.5 ml/l before each manipulation (hormone injection, gamete collection, and surgical intervention) (Neiffer and Stamper, 2009). The collection of egg and sperm was applied according to Linhart et al. (2004). Both female and male broodstock of European catfish were stimulated by intramuscular injection of carp pituitary hormone at a dose of 5 mg/kg^{-1} before gamete collection. The eggs were stripped into plastic bowls 24 hours after hormonal injection.

Experimental design

Sperm collection in male European catfish was carried out in two methods. In the first method, the laparotomy method which is surgical was used to remove the surgical testicular tissue (CS method). For the surgical intervention was used a surgery kit of sterilized surgical instruments. The surgical method of testicular sperm operation included separate stitches of the peritoneum and skin to create a supplemental anastomosis between inner organs and prevent infections. The abdomens of the three male individuals were cut approximately 5 cm. The intervention followed the division of skin from the peritoneum, peritoneum incision, taking of testicular tissue (Figure 1), and suturing of the injury creating intermittent eight-shaped stitches on the peritoneum. The operation time lasted 10-12 minutes. The testis was carefully removed so that other organs were not damaged. After testis removal, the testicle particles were tightened using a 200 µm net.

Figure 1. Surgical removal of the testis in European catfish (Photos by Merve Tınkır)

In the second method used in the study, sperm were collected from four male European catfish by hand stripping method (SS method) using a catheter (Figure 2).

Figure 2. Getting the sperm of the European catfish through stripping (vacuum method) (Photos by Merve Tınkır)

Fertilization procedure

European catfish is generally contaminated by urine during sperm collection, and contaminated sperm is activated (Linhart et al., 2004). Therefore, sperm samples taken by stripping different methods were kept in an immobilization solution (30 mM TRIS-HCl and 200 mM NaCl adjusted to pH 7) in order to sperm remain immotile. The sperm was added to an immobilizing solution at a volume ratio of approximately 1:1 and kept on ice.

Immediately after the sperm samples were added to the egg, the activation solution was added (Horvath and Tamas, 1976; Linhart et al., 2004). 300 g of eggs taken from each female were fertilized at 22 °C with 1 ml of sperm samples which were activated using 150 ml of hatchery water. After one minute of fertilization, 0.5 g/l of tannic acid for 20 seconds was used to remove the stickiness of the eggs. Fertilized eggs

treated with tannic acid are washed with hatchery water and transferred to plastic boxes filled with chlorine-free tap water. Plastic boxes measuring 18.0 cm × 18.0 cm × 6.0 cm filled with chlorine-free water were used. Each group (total 8 boxes) was replicated four times. In a laboratory, these plastic boxes (with fertilized eggs) were kept at 22 °C.

The eggs in every plastic box were counted and recorded on the first day. Dechlorinated water was gently changed in the plastic boxes 48 hours after post-fertilization, non-developing embryos were controlled counted, and removed. Also, the final total number of eggs was recorded. The malformed larvae and hatched larvae were manually counted immediately after hatching. It has completed an incubation period of approximately 4 days at 22 °C. The fertilization rate for each Petri dish was determined by dividing the number of eye-stage embryos at 48 hours post-fertilization by the initial number of eggs. The hatching rate was calculated by dividing the total number of hatched larvae by the initial number of eggs, while the malformation rate was calculated by dividing the number of malformed larvae by the initial number of eggs. The embryo development process was observed under a stereomicroscope (Leica Microsystems). The experimental data were analyzed using (SPSS 20.0) and expressed as mean ± SD, followed by paired sample t-test.

RESULTS

The result of the first group, testicular sperm (CS) >14 ml of fresh sperm was collected from each individual male during sampling. In the second group, stripping sperm (SS) >11.5 ml of sperm was collected from each individual during the sampling. For fertilization, over 1 kg of eggs was taken from each female during the stripping process (Table 1).

Table 1. Male and female catfish numbers, amount of sperm and eggs

Number of broodstocks female	Number of egg (g/total)	Number of broodstocks male	Amount of sperm (ml)
1	1390	1 ^{s}	13.5
2	1275	2^{c}	16.4
3	1380	3^{c}	14.0
4	1450	4 ^{s}	12.8
5	1225	5^{c}	14.5
6	1600	6 ^{s}	12.0
7	1720	7 ^{s}	11.5

^{c} surgical method testicular amount of sperm, ^{s} stripping method amount of sperm

Fertilization and hatching rates of 81.87 ±17.38% and 71.26 ±13.48%, were attained through insemination using the stripping method (SS). The surgical obtained of testicular sperm (CS) has found a fertilization rate of 66.18±11.39% and a hatching rate of 50.23±11.80%. The malformation rate post-hatching in the larvae was found to be similar between the two methods. With the addition of malformations larvae, the total larvae hatching rate was determined to be 76.10±12.39% in the stripping group and 53.83±11.28% in the surgical group respectively. Statistical analysis showed a significant difference in fertilization, hatching, and total hatching between the two different sperm collection methods ($p<0.05$) (Figure 3).

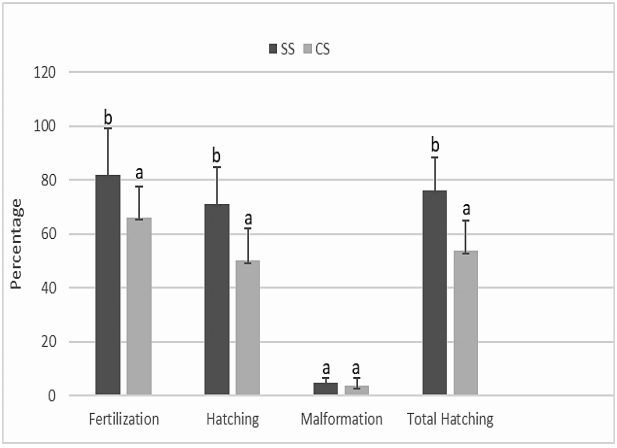

Figure 3. Fertilization (%), hatching (%), malformation (%), total hatching (%) rate of European catfish

The results were presented as the mean ± SD. Different superscripts indicate statistical significance differences (p <0.05).

DISCUSSION

In some locations where animal conservation regulations have not been improved, injuries from offensiveness have been decreased by suturing the fish's mouth later a bore is made in the jowl throughout sperm collection (Horvath et al., 1992). This method is likely to prevent breathing and is against EU legislation. As previously stated in many European and Asian countries, the killing of males and the acquisition of gametes in the production of European catfish is against Bern's convention to preserve and maintain genetic diversity. With the exception of the Czech Republic and France, stripping of male European catfish is still not widely practiced (Linhart et al., 2000).

One reason why male European catfish is killed during production is due to insufficient sperm production during spermiation. Two methods are used for obtaining sperm from male European catfish. The surgical method involves the use of non-lethal surgical techniques for complete (Sanap et al., 2018) or partial (Diyaware et al., 2010) resection of testicular tissue in male catfish through laparotomy. However, laparotomy can cause post-operative diseases, necessitating continuous monitoring (Romanova et al., 2017). While gonadectomized fish can be sold, they cannot be used for breeding, and they may die after a period of time. The non-surgical method involves obtaining sperm through stripping. This method involves obtaining sperm through vacuum aspiration without killing the male individual. This practice is more advantageous and allows the individual to continue reproductive activity for years. However, contamination of sperm with urine during stripping is inevitable in some fish species due to the close proximity of the sperm canal and ureter or the presence of a single urogenital pore through which

both sperm and urine are released. Sperm collection through stripping cannot prevent contamination with urine, which causes the loss of sperm motility within two minutes (Linhart et al., 1987). Spermatozoa suddenly activate during the sperm-collecting procedure when urine is mixed with the sperm. This early activation of spermatozoa is caused by urine contamination and has a negative impact on artificial reproduction (Linhart et al., 1987; Linhart et al., 2004). Similar corruption of sperm quality, likely related to infection with urine, has been reported in pikeperch (Křišťan et al., 2014) and European catfish (Legendre et al., 1996). Sperm motility can be preserved through the use of an immobilizing solution (Linhart et al., 2004). Sperm can be directly aspirated into an immobilizing solution through vacuum pumping into the papillae of male broodstock, minimizing bacterial contamination and other microflora (Linhart et al., 2020; Tinkir et al., 2021).

It has been observed that when spermatozoa are activated due to urine infection, a small amount of energy content is depleted a few seconds before exposure to the immobilizing solution (IS) (Alavi et al., 2019). The motility parameters of sperm undergo rapid changes due to the high energy consumption after activation, just before being affected by IS. The relationship between ATP production and motility for European catfish has been investigated by Billard et al. (1997). Within the first 5 to 10 seconds of activation, catfish sperm loses 50% of its ATP (Boryshpolets et al., 2009). However, by allowing the sperm to develop in the IS or seminal plasma, this lost energy can be restored within a matter of minutes, whether through artificial or natural means. Also, this situation has also been reported for carp (*Cyprinus carpio* L., 1758) and sturgeon (*Acipenser ruthenus* L., 1758) (Linhart et al., 2008; Xin et al., 2020).

The quality of gametes is a major factor in the success of fertilization, embryo quality, and larval growth performance (Linhart et al., 2020). Various abnormalities and embryonic malformations have been linked to sperm from males that have been exposed to pesticides or other toxic compounds (Labbé et al., 2017). However, no correlation was found between sperm aging and embryonic malformations (Linhart et al., 2020). There is no significant correlation between the rate of larval malformation and the method of sperm collection. However, sperm collection methods affect the rate of fertilization and hatching (Linhart et al., 2020). A study comparing the use of sperm from male African catfish (*Clarias gariepinus*) broodstock found that fertilization percentages were 71.5% and 81% for sperm obtained by stripping and from testicular tissue (fifty percent of testicular tissue), respectively (Adebayo et al., 2012). In northern pike, the mean hatching rates were found 79 ± 6% and 78 ± 6% for eggs fertilized with stripped sperm and testicular sperm, respectively (Kristan et al., 2020). The mean concentration of stripped sperm was found to be lower than that of testicular sperm in northern pike (Hulak et al., 2008). In European catfish, the fertilization rate was found to be higher when using sperm obtained through stripping (85.1%) compared to sperm collected from the testicular tissue of killed fish (65.6%) (Linhart et al., 1987). A study comparing fertilization rates of sperm obtained through stripping and from the testicular sperm of rainbow trout male broodstock found an average of 81% and 39.2%, respectively (Geffen and Evans, 2000). The results in the present study are in accordance with the other studies above, while it was discovered that the fertilization rate achieved through sperm obtained via stripping was 81.87%, whereas the fertilization rate obtained from sperm in testicular tissue was 66.18%. The success of fertilization in European catfish, the quality of the embryos, and the subsequent performance of the offspring are largely dependent on the quality of the gametes and the method of obtaining sperm.

CONCLUSION

According to the results of the current study, it can be concluded that it's not essential to kill male European catfish to obtain sperm by vacuum method for fertilizing female eggs. Consequently, successful fertilization was achieved at a rate of 81.87% through the use of stripping (vacuum method) in this investigation. This practice is more advantageous and allows the individual to survive and continue their reproductive activity the next reproduction season.

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AUTHORSHIP CONTRIBUTIONS

Merve Tınkır and Devrim Memiş: Conceptualization, methodology. Merve Tınkır: Data curation, writing- original draft preparation. Merve Tınkır: Visualization, investigation. Devrim Memiş: Supervision, project administration, resources, funding acquisition. Merve Tınkır, Adil Yılayaz, and Devrim Memiş: Writing-reviewing and editing.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

In order for the experimental design to be carried out on the basis of an ethic, the approval numbered 09/2020 was obtained by the local ethics committee for animal experiments at the Mediterranean Fisheries Research, Production, and Training Institute.

DATA AVAILABILITY

All relevant data are inside the article.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Investigation of the structure and hardness properties of *Anodonta anatina* mussel shells

Anodonta anatina midye kabuklarının yapısı ve sertlik özelliklerinin araştırılması

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Abstract: In this study, the shell structure of the freshwater mussel *Anodonta anatina* (Linnaeus, 1758) which has a widespread population in Gölbaşı Lake (Hatay) and is not economically exploited, was microscopically examined at a morphological level. It was determined that the shells of *Anodonta anatina*, which are not under significant fishing pressure, are mostly found discarded along the shores of the lake. This mussel species is important as a composite biological material with multifunctional roles in freshwater ecology. Considering the potential use of freshwater mussel shells as a biological material, an assessment of the shell structure, physical properties, mechanical strength, shell microstructure, and morphological characteristics of *A. anatina* was conducted. When cross-sections of the shell taken from the umbo, middle periostracum, and the region close to the pallial edge were examined in the dorsal-ventral direction, it was determined that the periostracum layer in the umbo region had a more prismatic and polygonal structure. The interior of the shell was found to consist of a shiny nacreous layer. In nacreous shell sections, it was observed that the nacreous layer contained more distinct layers near the pallial edge. Vickers microhardness tests were performed on individual shells, and it was found that the hardness value of the inner layer was the highest (625.5 ±172.7 HV), while the outer layer had a lower hardness value (531.5 ±110.7 HV). Based on XRF data, it was shown that the seashell powder is mainly composed of calcium oxide (98.8% wt., CaO) as a biological material.

Keywords: Biocomposite, bivalve, microhardness, morphological properties, nacre

Öz: Bu çalışmada, Gölbaşı Gölü'nde (Hatay) yaygın bir popülasyonu bulunan ve ekonomik olarak değerlendirilmeyen tatlı su midyesi *Anodonta anatina*'nın (Linnaeus, 1758) kabuk yapısı, morfolojik olarak mikro düzeyde incelenmiştir. Midye avcılığı bakımından üzerinde av baskısı olmayan *A. anatina'nın* kabukları çoğunlukla gölün kıyısında âtıl olarak bulunduğu tespit edilmiştir. Bu midye türü, tatlı su ekolojisi açısından, çok işlevli rollerle ilişkili kompozit bir biyolojik malzeme olma yönünde önem taşımaktadır. Biyolojik bir materyal olarak tatlı su midye kabuğunun olası kullanımı göz önüne alındığında, model tür olarak *A. anatina*'nın kabuk yapısı, fiziksel özellikleri, mekanik dayanımları, kabuk mikro yapıları ve morfolojik özelliklerinin değerlendirmesi yapılmıştır. Kabuğun dorsal-ventral yönde umbo, orta periostrakum ve pallial kenara yakın bölgeden alınan enine kesitleri incelendiğinde, umbo bölgesindeki periostrakum tabakanın daha prizmatik ve poligonal olduğu tespit edilmiştir. Kabuğun iç kısımlarının parlak sedef tabakasından oluştuğu ve sedefli kabuk kesitlerinde, sedefli tabakanın pallial kenara yakın bölgede daha belirgin sedef katmanları içerdiği belirlenmiştir. Midye kabukları üzerinde, Vickers mikrosertlik testleri yapılmış, iç tabakanın sertlik değerinin en yüksek (625,5 ±172,7 HV) ve dış tabakanın sertlik değerinin iç tabakaya göre daha düşük (531,5 ±110,7 HV) olduğu belirlenmiştir. *A. anatina* kabuk tozlarının kimyasal bileşimi, X-ışını floresans spektroskopisi ile kalsinasyon işleminden sonra analiz edilmiştir. XRF verilerine dayanarak, biyolojik bir malzeme olan kabuk tozlarının ana bileşiminin kalsiyum oksitten (CaO, ağırlıkça %98,8) oluştuğu belirlenmiştir.

Anahtar kelimeler: Biyokompozit, çift kabuklu yumuşakça, mikrosertlik, morfolojik özellikler, sedef

INTRODUCTION

Anodonta anatina (Linnaeus, 1758) is an enigmatic nominal taxon with a limited distribution in the Orontes River drainage in Turkey and Syria (Graf and Cummings, 2007; Lopes-Lima et al., 2021). It was first identified from the former Amik Lake (historically Lake of Antioch), (Linnaeus, 1758) a large but shallow freshwater lake in the Lower Orontes River basin, Hatay Province, Turkey (Graf and Cummings, 2007; Tomilova et al., 2020). The reproductive cycle and glochidia of *A. anatina* have been carefully examined in the literature (Şereflişan et al., 2009a, b). The shell of a freshwater mussel at the larval stage contains an organic matrix and chitin, primarily composed of calcium carbonate (Schönitzer and Weiss, 2007; Weiss and Schönitzer, 2006; Lopes-Lima et al., 2008). In mollusk shells, hard, protective, and solid inorganic mineral formation mechanisms are often referred to as biomineralization. The most common biominerals in mollusk shells are calcium carbonate and calcium phosphate (Istin and Kirschner, 1968; Lowenstam and Weiner, 1989; Pratoomchat et al., 2002; Wegner, 2005; Ziegler et al., 2006). Mollusks produce a highly ordered organomineral composite skeleton to protect and support their soft tissues (Carter, 1991).

Biomaterials in engineering have become increasingly popular due to their unique properties and potential for sustainable development. One such biomaterial is the mineral composition of mollusk shells, which typically consist of calcium carbonate ($CaCO_3$) in the form of calcite and/or

aragonite. These two forms of $CaCO_3$ have distinct morphological and microstructural characteristics, which have been attributed to several different factors. Three estimates have been proposed regarding the orientation of these structures and crystals, including the specific proteins present in the organic matrix of the shell, cellular remodeling, and physicochemical conditions in the microenvironment of biomineralization (Zhang et al., 2019). Understanding the mechanisms behind the formation of these mineral structures is critical for their use in various engineering applications. For example, mimicking the structure and properties of mollusk shells could lead to the development of stronger and more durable materials for use in the biomedical industry, material science, marine science, and other industries. Additionally, the renewable and biodegradable nature of these biomaterials makes them an attractive alternative to traditional, non-renewable materials that are harmful to the environment (Barthelat et al., 2009; Dhanaraj and Suresh, 2018; Lemos et al., 2006).

In this experimental study, the researchers aimed to investigate various properties of the shell of *A. anatina*, a type of mollusk, that could potentially make it a valuable resource for different fields of biomaterial. Specifically, they examined the microstructure, chemical composition, and microhardness of the shell in order to gain a better understanding of its potential uses. Although the properties of mollusk shells have been discussed in previous marine sciences literature, this study marks the first time, to the authors' knowledge, that the structural shell properties of *A. anatina* have been evaluated in such detail. By conducting this study, the researchers hope to provide valuable insights into the potential applications of this particular type of mollusk shell and to inspire further research into the unique properties of natural materials like this.

MATERIALS AND METHODS

Preparation of freshwater mussel shell samples

A. anatina shells were collected from Gölbaşı Lake between April and May of 2022. Lake Gölbaşı is located in Turkey's Eastern Mediterranean region, 50 kilometers north of Antakya. The mesotrophic nature of Gölbaşı Lake was determined through a study conducted using the Brachionus/Trichocerca index (Türkmen et al., 2006). The lake has a surface area of approximately 12,000 decares, with 4,000 decares consisting of wet-reed fields. During summer, the maximum depth of the lake reaches 4 meters, while in winter, it can reach up to 6 meters (Şereflişan, 2003). Four stations were designated in the lake for mussel collection. Among the mussels collected, mature individuals whose shells were not damaged were preferred. The location and coordinate information of the collection stations was provided in Figure 1. *A. anatina* was found to inhabit the muddy substratum of the lake. The collection was performed manually by SCUBA diving at depths ranging from 0 to 3 meters. After collection and identification (Graf and Cummings, 2007; Kinzelbach, 1989), the soft tissues were carefully removed using a scalpel. The remaining shells were cleaned under running water, disinfected with ethanol, and then sun-dried for three days. In addition, any fresh remains adhering to the shells were carefully removed before using them in the experiments. The shells were measured for total length to the nearest 0.01mm using a digital vernier caliper and weighed to the nearest 0.01g. The shell length of the mussels is the distance from the anterior edge of the valve to the posterior edge, the shell width is the distance from the dorsal edge of the valve to the ventral edge, and the shell height was measured as the umbo height between the two valves of the mussel held horizontally (Figure 2).

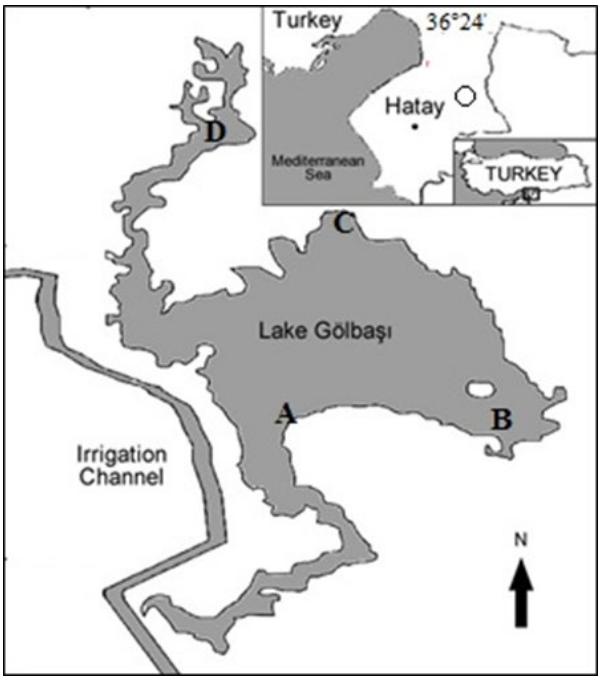

Figure 1. Collection area of *A. anatina* and coordinates of four stations determined in the study area. A: 36°30'15" N - 36°29'14" E, B: 36°30'10" N - 36°29'43" E, C: 36°30'43" N - 36°29'20" E, D: 36°30'55" N - 36°28'53" N

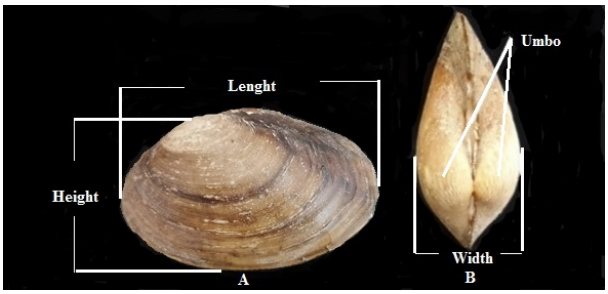

Figure 2. Schematic image of body length, height (A) and width (B) measurement of *A. anatina*

In order to achieve the characterization of the shells, 4 shells of *A. anatina* were randomly selected from the collections. The mean length, height, width, live weight, and total shell weight of the mussels were determined as 10.08±0.22 cm, 5.98±0.19 cm, 3.02±0.15 cm, 58.29±0.23 g, and 34.15±0.50 g respectively. Selected areas were marked and prepared for the experiments related to shell microstructure and mechanical characterization (Lee et al.,

2008; Liang et al., 2016). All experiments were performed in triplicate.

Vickers microhardness (HV) experiments

In order to investigate the Vickers microhardness of shells, the shells with dimensions of 65mm × 15mm × 1mm were cut from the umbo to toward the posterior end of the body using a precision cut-off machine (Brilliant 220–ATM GmbH) with diamond disc and cooling water under a fixed cutting condition of n = 4000 rpm and f_z =5 mm/z. The cut shells were sliced transversely again. To achieve homogeneity in the loading direction and to facilitate the microhardness test, the specimens were fixed with epoxy resin (Epoxy Cure, Buehler). After grinding off the epoxy resin on the surface of the sample, it was carefully polished using abrasive papers to measure the microhardness of the outer layer. The same modified procedure was applied to the inner layer of the shells (Liang et al., 2016). The Vickers microhardness test was performed on a Shimadzu HMV-2000 model 3212 at 0.1 kg-f load and 15 s dwell time according to ASTM C1327-08. Thirty-two indents were made per region (outer/inner surface) for each mussel shell, and the data are presented as mean ±standard deviation (SD). The distribution of the Vickers microhardness values for the outer layer and inner layer was characterized and discussed (Özer and Öksüz, 2019; Yang et al., 2011).

Shell microstructure analysis

Light microscopy (LM) investigations were conducted using a Nikon ECLIPSE L150 instrument to observe the microstructures of the freshwater mussel shells. Freshwater mussel shells selected for morphological analysis were gently broken into pieces, and smooth surfaces were chosen to capture images of the inner, outer, and cross-section surfaces. Each shell fragment was embedded separately in epoxy resin and marked on the surface of the surrounding epoxy resin block. Subsequently, resin blocks were carefully grinded with emery paper from 400# to 1500# and then ultra-polished using aluminum oxide (5µm) and polycrystalline diamond paste (0.25µm). To access the successive inner layers of shells, a dilute chemical etching agent, ethylene diaminetetraacetic acid (EDTA) was used at room temperature for 5 minutes. The samples were cleaned in an ultrasonic bath with anhydrous ethanol for 30 minutes at 35°C and then sun-dried for microstructural observations (Meng et al., 2018; Yang et al., 2011).

X-ray fluorescence analysis (XRF)

Energy dispersive X-ray fluorescence spectroscopy (XRF, Thermo Scientific™ Niton™ XL3t, USA) was used to evaluate the chemical composition of the shell powders. Freshly prepared dry shells from four different, undamaged, mature, and healthy specimens were ground into powder using a mortar and pestle for XRF analysis. The crushed mussel shells were then calcined at 900°C for 3 hours, with a heating rate of 5°C/min, to determine the amount of calcium oxide (CaO) present (Gao et al., 2019; Moustafa et al., 2015).

Statistical analyzes

The collected data were presented as means ± standard deviations of the mean SD based on at least thirty-two independent measurements. Data were statistically analyzed using a Bivariate Correlate test was used to determine the degree of correlation between the variable of external shell hardness and internal shell variables of hardness. The strength of the correlation was measured by using a correlation coefficient (Pearson's Correlation r) at a statistical significance level of 0.05 using SPSS 20.0 (Newyork, USA) software.

RESULTS

Microstructure observation and characterization

The optical microscope photographs of *A. anatina* mussel shells showed three distinct layers in the transverse section: the outer periostracum layer, the prismatic layer, and the inner nacreous layer. The optical microscope photographs presented in Figure 3 of the *A. anatina* mussel shells show that the outermost layer, known as the periostracum, consist of a proteic sclerous double layer that cover the external surface of the shells (Checa, 2000). Below the periostracum, there is a layer without a definite shape, which is comprised of calcite ($CaCO_3$). Next, comes the aragonite sheet nacreous layer, which makes up nearly the entire thickness of the shell, and finally, the aragonite prismatic layer (Chakraborty et al., 2020). Polishing and chemical acid etching revealed the inner vacuolar periostracum, which was frequently continuous with the following inner mineralized layers. Optical microscope photos of the inner vesicular layers of the shells periostracum revealed prominent vacuoles (Carter, 1991; Nakamura-Filho et al., 2014; Vaughn, 2017). The microstructure described can be readily observed in Figure 4 and Figure 5 through the optical microscope photographs, displaying distinct features and characteristics.

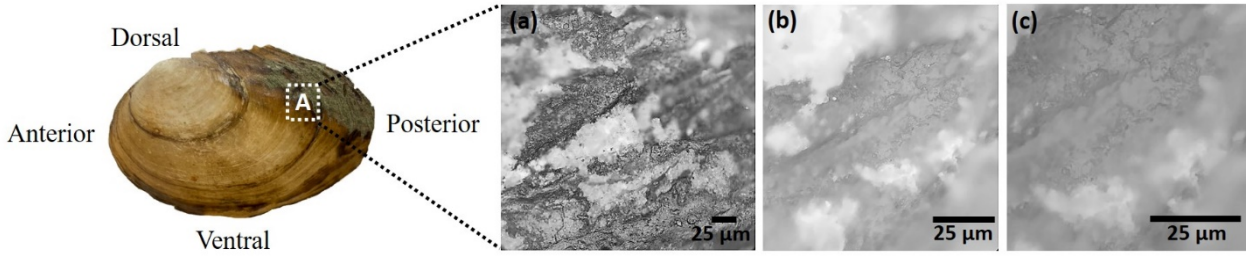

Figure 3. General and microstructural photographs of the periostracum of *A. anatina* at various magnifications in the dorsal-ventral direction. (A) Middle periostracum, (a) 20x, (b) 50x, (c) 100x

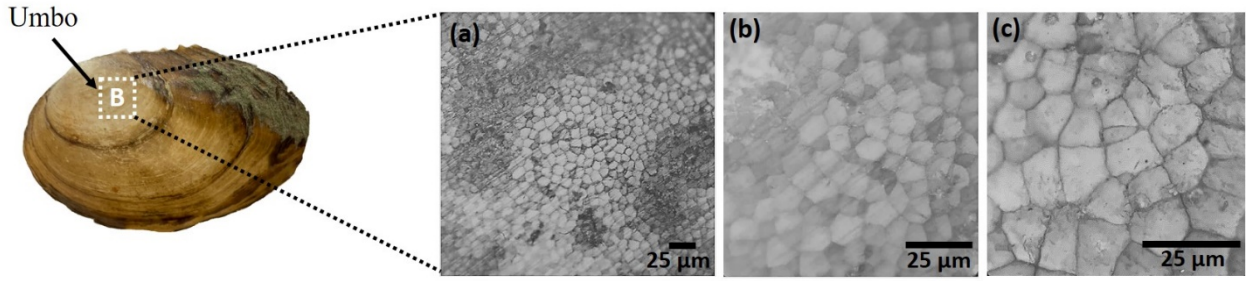

Figure 4. General and microstructural photographs of the periostracum of *A. anatina* at various magnifications in the dorsal-ventral direction. (B) Umbo, (a) 20x, (b) 50x, (c) 100x

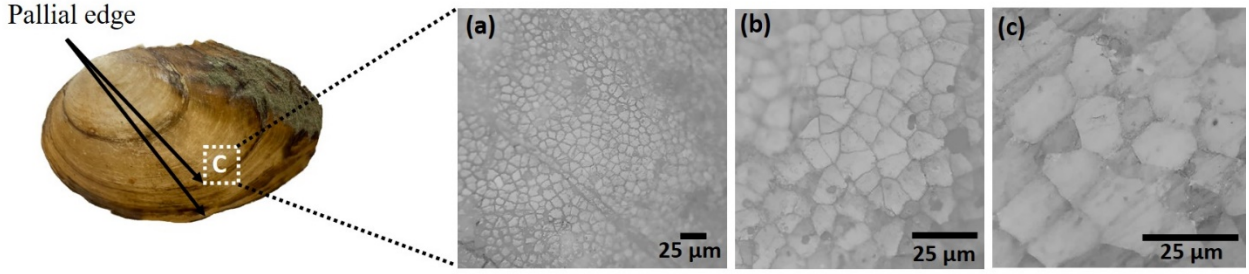

Figure 5. General and microstructural photographs of the periostracum of *A. anatina* at various magnifications in the dorsal-ventral direction. (C) Pallial edge, (a) 20x, (b) 50x, (c) 100x

The optical microscope examination of *A. anatina* mussel shells reveals that all the samples studied exhibit well-preserved nacreous layers. Figure 6 provides microstructural representations of the successive shell layers, with a focus on the mineralized internal layers. It shows that the inner part of the *A. anatina* shell structure is primarily composed of the polymorph aragonite, arranged microstructurally in superimposed sheets. This aragonite sheet nacreous layer covers almost the entire thickness of the shell and is made up of brittle $CaCO_3$ platelets stacked in layers and held together by organic biopolymers (Chakraborty et al., 2020; De Paula and Silveira, 2009).

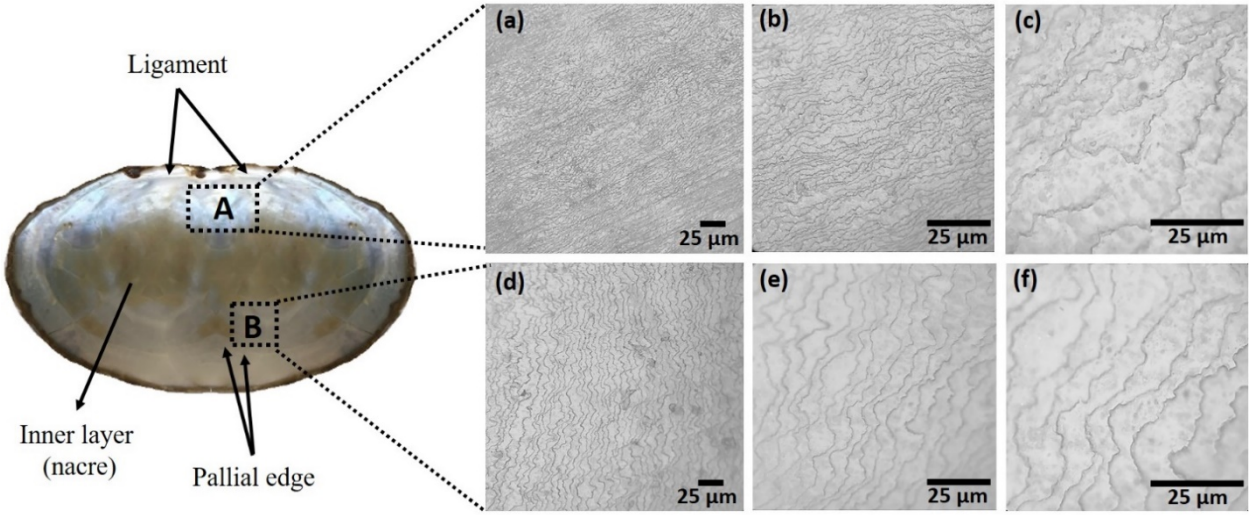

Figure 6. General and microstructural photographs of the inner layer (nacre) of *A. anatina* at various magnifications in the dorsal-ventral direction. (A) Ligament edge, (B) Pallial edge. (a-d) 20x, (b-e) 50x, (c-f) 100x

The shell of the *A. anatina* freshwater mussel contains two prismatic layers, as observed from the cross-section views in Figure 7. The prismatic layer is composed of various columnar crystals and an organic matrix, and a fiber prismatic structure with a common structural nodule is located beneath the periostracum layer. The cloud layer, located below the periostracum layer, is composed mainly of the calcite phase above the aragonite nacreous sheet layer (Carter et al., 2012).

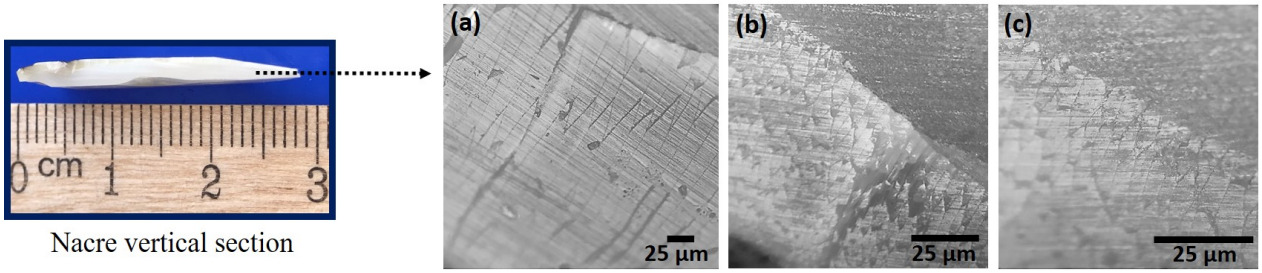

Figure 7. Cross-sectional photographs of the *A. anatina* at various magnifications (a) 20x, (b) 50x, (c) 100x

Microhardness of *A. anatina* shells

Figure 8 presents the relationship between the average microhardness and their corresponding standard deviation of different shell structures at different applied distances (µm). It shows that the Vickers microhardness for the inner and outer surfaces exhibited opposite trends. The experimental studies revealed that the external shell of *A. anatina* has a comparatively lower microhardness than the internal shell from the umbo to the posterior end regions. The mean microhardness values of the external and internal shell samples of *A. anatina* were 531.5 ±110.7 HV and 625.5 ±172.7 HV, respectively.

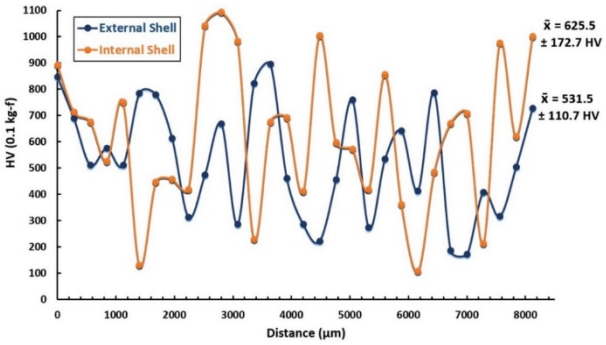

Figure 8. The Vickers microhardness results of the *A. anatina* shell samples

In this study, we conducted an investigation into the relationship between the microhardness values of the external shell and internal shell. To analyze this relationship, we utilized the Bivariate Correlate test, a statistical tool commonly employed to examine the association between two variables (Table 1). The strength of the relationship was quantified using the Pearson correlation coefficient, which yielded a value of r = -0.121. Additionally, we determined the two-tailed significance value, denoted as P, which was calculated to be P = 0.511. To assess the statistical significance of the obtained results, we compared the significance level of 0.025, which represents the predetermined threshold for accepting or rejecting the hypotheses. Upon careful evaluation, we observed that the calculated significance value (P=0.511>0.025) exceeded the significance level (0.025). Moreover, the negative value of the Pearson correlation coefficient (r=-0.121<0) indicated a negative association between the microhardness values of the external and internal shells. Considering these findings, we conclude that there is no significant linear relationship between the microhardness values of the external shell and internal shell in the context of our study.

Table 1. Correlation factors of microhardness values

Correlations		External Shell HV	Internal Shell HV
External Shell HV	Pearson Correlation	1	-,121
	Sig. (2-tailed)		,511
	N	32	32
Internal Shell HV	Pearson Correlation	-,121	1
	Sig. (2-tailed)	,511	
	N	32	32

X-Ray Fluorescence Analysis (XRF)

The elemental/mineral composition of shell powders from the freshwater mussel *A. anatina* was qualitatively determined using XRF analysis (Tomilova et al., 2020). The XRF examination of shell powders revealed that the concentration of calcium oxide (CaO) was 98.8 wt.% after calcination at 900°C for 3 hours as shown in Table 2. In Table 2, as for the other oxides, it predominantly comprised 0.628 wt.% SiO_2, 0.174 wt.% K_2O, 0.132 wt.% MgO, and 0.069 wt.% SrO_2 and the remainder were present in negligible levels (SO_3, Al_2O_3, P_2O_5, Fe_2O_3, and CuO were <0.05 wt.%).

Table 2. XRF analysis of the *A. anatina* shells (wt.%: weight)

Shell composition of *A. anatina*		Results
CaO	[wt.%]	98.8±0.435
SiO_2	[wt.%]	0.628±0.035
K_2O	[wt.%]	0.174±0.031
MgO	[wt.%]	0.132±0.028
SrO	[wt.%]	0.069±0.009
SO_3	[wt.%]	0.047±0.002
Al_2O_3	[wt.%]	0.028±0.003
P_2O_5	[wt.%]	0.08±0.011
Fe_2O_3	[wt.%]	0.032±0.010
CuO	[wt.%]	0.01±0.003

DISCUSSION

The shell microstructure of *A. anatina* has been studied to understand their unique shell structure in this experimental study. The periostracum layer of *A. anatina* serves as a protective layer, shielding the inner layers of the shell from external factors such as erosion, corrosion, and predation. It is the first layer secreted by the folds of the mantle and is made of proteins (Piwoni-Piórewicz et al., 2017). The presence of prominent vacuoles in the inner vesicular layers of the periostracum suggests that this layer is also involved in ion

regulation and deposition (Salas et al., 2011). The layer below the periostracum, comprised of calcite, is not well-defined, but it likely plays a role in providing a transition zone between the organic periostracum and the mineralized nacreous layer (Chakraborty et al., 2020). The aragonite sheet nacreous layer, which covers almost the entire thickness of the shell, is responsible for providing the strength and toughness required for the shell to resist mechanical damage (Marin, 2012). The preservation of the nacreous layers observed in all the samples studied highlights the robustness of the shell structure of *A. anatina*. The microstructure of the mineralized internal layers, particularly the aragonite sheet nacreous layer, demonstrates the intricate organization of the shells components. The presence of organic biopolymers plays a significant role in the stability of the aragonite platelets and the overall structural integrity of the shell (Xu and Li, 2011). The specific zones in the layers of the *A. anatina* freshwater mussel shell observed from the cross-section views in Figure 7 provide valuable information regarding the composition and structure of the shell. The prismatic layer, which is mainly composed of columnar crystals and an organic matrix, and the fiber prismatic structure with a common structural nodule located beneath the periostracum layer are noteworthy features. The amorphous and supersaturated cloudy structure in the cloud layer below the periostracum layer promotes shell biomineralization (Carter et al., 2012). Additionally, the overlap between the $CaCO_3$ layer and the contiguous nacre layer, as well as the stacking of the aragonite tablets in parallel, elongated rows in the nacre layer, were observed from the cross-section photos (Frenzel and Harper, 2011). These observations provide valuable insight into the biomineralization process and the structure of the *A. anatina* freshwater mussel shell.

Hardness is a crucial mechanical property of all crustaceans as it helps to protect the live organism within (Lee et al., 2008). Our experimental studies revealed that the external shell of *A. anatina* has a comparatively lower microhardness than the internal shell from the umbo to the posterior end regions. Based on the microhardness values obtained in our study, it can be concluded that there is no statistically significant linear relationship between the microhardness values of the external shell and internal shell (Table 1). When the load was applied perpendicularly to the internal section, the nacreous structure (internal zone, 625.5 ±172.7 HV) exhibited higher hardness values than the prismatic structure (external zone, 531.5 ±110.7 HV) as observed by Leung and Sinha (2009) and Lv et al., (2015). The difference in microhardness values is believed to be due to the internal structure of the shell, where $CaCO_3$ crystals form a harder texture through biomineralization (Marie et al., 2012).

The high concentration of CaO in mussel shells, as revealed by the XRF analysis, makes them an attractive natural source of this important mineral for biomedical applications. The purity of the CaO found in mussel shells is also notable, as the concentrations of other oxides are minimal, which suggests that the shells can be used as a highly pure and natural source of CaO for various applications. These findings could potentially lead to new developments in the use of mussel shells as a sustainable and cost-effective source of highly pure CaO, with implications for a wide range of fields.

CONCLUSION

The microstructures, resistance and material components of shells are crucial for the survival of freshwater mussels. This experimental study investigated the microstructure, phase components and mechanical properties, including microhardness, of *A. anatina's* shell. The shells microstructure had a uniform surface morphology, and the periostracum and ostracum layers were mainly composed of $CaCO_3$ with a calcite density on the crystal basis. Consequently, this surface was more prone to wear than the nacre layer, which contained quadrangular and pentagonal nacre plates. No prismatic nacre crystals were found when examining the periostracum layer. The inner surface of *A. anatina* had a glossy, pearlescent appearance clear nacre plates. A sharp distinction was observed between the periostracum layer and the nacre layer upon examining the cross-sectional surface in the vertical direction. Microhardness measurements were taken from the inner layer to the outer layer in both the periostracum and nacre layers of the shell. The highest microhardness value (625.5 ± 172.7 HV) was found in the inner layer, decreasing to approximately 531.5 ± 110.7 HV as the test moved towards the outer layer. The XRF analysis revealed that mussel shells have a high concentration of CaO, with minimal concentrations of other oxides, making them an attractive and natural source of this mineral. These findings could potentially lead to new developments in using mussel shells as a sustainable and cost-effective source of highly pure CaO, with implications for a wide range of fields. The microstructural information and subsequent analysis of shell microhardness provided an important experimental basis for developing models on *A. anatina.*

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AUTHORSHIP CONTRIBUTIONS

Kerim Emre Öksüz: Conceptualization, methodology, formal analysis, writing - original draft preparation, writing-review and editing, software and visualization. Hülya Şereflişan: Investigation, sample collection and preparation, writing-review 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

Data supporting the findings of the present study are available from the corresponding author upon reasonable request.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

By-catch ratios from the bogue (*Boops boops*) gillnet fishery around the sea-cage fish farms in Güllük Bay (Aegean Sea)

Güllük Körfezi'nde (Ege Denizi) balık çiftlikleri etrafında yapılan kupes (*Boops boops)* uzatma ağı avcılığından elde edilen hedef-dışı av oranları

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Öz: Bu çalışma, Güllük Körfezi'ndeki (Ege Denizi) balık çiftlikleri çevresinde küçük ölçekli balıkçı tekneleri tarafından uzatma ağlarıyla hedeflenen *Boops boops* avcılığı sırasındaki hedef dışı avı ortaya koymak ve birim çaba başına hedef dışı av (BPUE) oranlarını belirlemeyi amaçlamaktadır. Çalışma, Kasım-Nisan arasındaki iki sezon boyunca (2017-2018 / 2018-2019), Göltürkbükü, Gündoğan, Yalıkavak ve Torba limanlarında rıhtım örneklemeleri yoluyla gerçekleştirilmiştir. Toplam 13 kupes avcısı tekneden 147 balıkçılık operasyonu rastgele izlenmiştir. Kupes uzatma ağı ile avcılıkta omurgasızlar dâhil 30 familyaya ait 48 türden toplam 18163 kg av yakalanmıştır. Hedef balık olan *Boops boops* en bol (%91,9) bulunan tür olup, bunun %8,1'si hedef dışı av olarak kaydedilmiştir. Hedef dışı avda en çok yakalanan türler sırasıyla *Diplodus annularis* (Linnaeus, 1758), *Scomber colias* (Gmelin, 1789), *Trachurus trachurus* (Linnaeus, 1758), *Pagellus acarne* (Risso, 1827), *Scomber scombrus* (Linnaeus, 1758)'tur. 2018 yılı ortalama BPUE 4,77 ± 0,85 kg.1000 m^{-1}, 2019 yılı BPUE ise 3,20 ± 0,58 kg.1000 m^{-1} olarak hesaplanmıştır. Kasım ayı BPUE değerleri açısından en yüksek ortalamayı vermektedir. Ortalama BPUE 0 ile 19,1 kg.1000 m^{-1} arasında (ortalama: 7,97 ± 2,83 kg.1000 m^{-1}) değişmiştir. İstatistiki olarak aylar arasında BPUE değerleri bakımından önemli bir fark vardır (Kruskal-Wallis test, $p < 0,05$).

Anahtar kelimeler: Kupes, küçük ölçekli balıkçılık, hedef-dışı av, Güllük Körfezi

Abstract: This study aims to reveal the by-catch rates for gillnet fishery, targeted *Boops boops* by small-scale fisheries around fish farms in Güllük Bay (Aegean Sea), and to determine the by-catch per unit effort (BPUE) rates. The study was carried out through deck sampling at Göltürkbükü, Gündoğan, Yalıkavak, and Torba fishing ports during two seasons between November and April (2017-2018 / 2018-2019). A total of 147 fishing operations from 13 boats were randomly monitored. In gillnet fishing for bogue, a total of 18163 kg of the catch was caught from 48 species belonging to 30 families, including invertebrates. The target *Boops boops* was the most common species with a rate of 91.9%, of which 8.1% was determined as a by-catch. The most caught by-catch species are *Diplodus annularis* (Linnaeus, 1758), *Scomber colias* (Gmelin, 1789), *Trachurus trachurus* (Linnaeus, 1758), *Pagellus acarne* (Risso, 1827), *Scomber scombrus* (Linnaeus, 1758). While the average BPUE was 4.77 ± 0.85 kg.1000 m^{-1} in 2018, it was 3.20 ± 0.58 kg.1000 m^{-1} in 2019. November gives the highest average in terms of BPUE values. The average BPUE ranged from 0 to 19.1 kg.1000 m^{-1} (mean: 7.97 ± 2.83 kg.1000 m^{-1}). Statistically, there is a significant difference in BPUE values between the months (Kruskal-Wallis test, $p < 0.05$).

Keywords: Bogue, small-scale fisheries, non-target catch, Güllük Bay

GİRİŞ

Yüzen yapılar veya diğer objeler genellikle balık cezbetme düzenekleri [Fish Aggregating Devices (FAD)] olarak bilinirler. Denizlerde yüzen balık kafesleri ise aynı zamanda mega FAD'ler olarak hizmet etmektedir (Dempster ve Taquet, 2004; Sanchez-Jerez vd., 2007). Pelajik balıklar, kıyıdaki balık çiftliklerine güçlü bir şekilde çekilir ve oldukça yoğun balık kümelenmeleri ortaya çıkabilir. Balık çiftliklerinin yakınında en bol bulunan balık familyaları genellikle Sparidler, Carangidler, Clupeidler ve Mugilidlerdir. Bu kümelenmeler yerel ihtiyofaunayı ve balıkçılığı önemli ölçüde etkileyebilir (Sanchez-Jerez vd., 2007). Bu nedenle, çoğunlukla pelajik olan ve ticari açıdan önemli balık türleri kafesler civarında yoğunlaştığından, yerel balıkçıların ilgisini balık çiftliklerine çekmektedir (Fernandez-Jover vd., 2008).

Bu doğal balıklar hem profesyonel hem de amatör yerel balıkçılar için kolay bir hedef stok haline gelirler. Denizdeki balık kafesleri çevresinde amatör olta balıkçılığı son on yılda popüler bir balıkçılık etkinliği haline gelmiştir (kişisel gözlem). Diğer taraftan İzmir'de ilk balık çiftliğinin 1985 yılında kurulmasından sonra, Ege Denizi'nde çiftliklerin bulunduğu koy ve körfezlerde balık miktarının hızla arttığını gözlemleyen kıyı balıkçıları bu çiftliklerin 200 m'lik koruma sahası (Anonim, 2016) dışında geleneksel balıkçılıklarını yapmaya başlamışlardır. Aslında, Ege Denizi'nde bu sahalarda günümüzde küçük ölçekli balıkçılar, balık yetiştiricileri ve amatör balıkçılar olmak üzere üç ilgi grubu vardır. Bununla beraber, bu gruplar arasında bazı çatışmaların da olduğu saptanmıştır (Akyol vd., 2019). Ayrıca Akyol ve Ertosluk

(2010), çiftlik personelinin yabani balıkları yakalamak için kendi deniz kafeslerinin etrafında özel bir tuzak, olta ve uzatma ağı kullandığını belirlemişlerdir.

Genel olarak, Akdeniz'deki balık çiftlikleri çevresinde en bol bulunan balık kupestir (*Boops boops* Linnaeus, 1758) ve yerel küçük ölçekli balıkçılığın (KÖB) hedefidirler (Dempster vd., 2002; Valle vd., 2007; Fernandez-Jover vd., 2008; Akyol ve Ertosluk, 2010; Akyol vd., 2020). Ege Denizi'nde ağ kafesler civarında toplanan doğal balıklar arasında kupesin bolluğu %80,5 olarak bildirilmiştir (Akyol vd., 2020). Bu kafes civarında toplanan balıklar, kayıp pelet yemler nedeniyle kondisyonlarını arttırmakta, yağlanmakta ve irileşmektedirler (Izquierdo-Gomez vd., 2015). Bu tip iri balıklar KÖB'ın dikkatini ağ kafeslere doğru çevirmekte olup, bu bölgelerde balıkçılık artmaktadır (Arechavala-Lopez vd., 2011). Türkiye'de sade uzatma ağlarıyla yapılan bu avcılıkta av miktarları, türler, hedef dışı av miktarları bilinmemektedir.

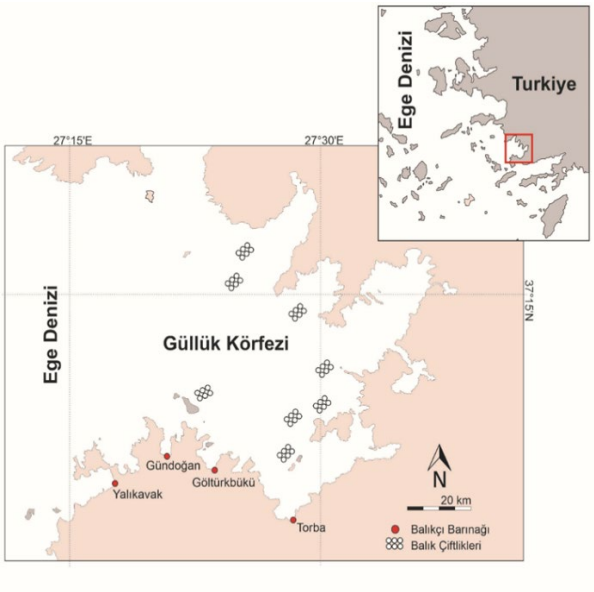
Sürdürülebilir balıkçılık yönetimi açısından irdelendiğinde hedef dışı av ve ıskarta önemli yer teşkil eden sorunlardır ve bu konularda genel anlamda veri eksikliği bulunmaktadır (Saila, 1983; Alverson vd., 1994). Bu yönden incelendiğinde, KÖB açısından av yeri dolayısıyla görece yeni bir faaliyet olarak adlandırılabilecek olan balık çiftlikleri etrafında uzatma ağlarıyla kupes avcılığı üzerine kurgulanan bu ilk çalışmada av aracının teknik planı, yakalanan hedef dışı avın kompozisyonu ve birim çaba başına hedef dışı av (BPUE) oranlarını belirlemek amaçlanmıştır.

MATERYAL VE YÖNTEM

Bu araştırma, Ege Denizi'nin güneyinde Güllük Körfezi'nde balık çiftlikleri çevresinde balıkçılık yapan kıyı balıkçıları ile rıhtım örneklemeleri yoluyla gerçekleştirilmiştir. Kupes avcılık sezonu Kasım'da başlayıp, Nisan'da balıkların üreme göçü yapması nedeniyle sadece altı ay sürmektedir. 2017-2018 ve 2018-2019 sezonu boyunca Kasım-Nisan ayları arasında Göltürkbükü, Gündoğan, Yalıkavak ve Torba limanlarından toplam 13 kupes avcısı tekneden 147 balıkçılık operasyonuna ait veriler toplanmıştır (Şekil 1). Rıhtım örneklemeleri yapılarak, balıkçıların ağlarından balıklar sayılmış ve tartılmıştır. Her sabah balık avı dönüşünde, rıhtım örneklemeleri ve bazı seyir defteri verilerinden (1) tarih, konum ve derinlik, (2) balıkçı teknesinin uzunluğu, makine gücü (HP) ve ağın teknik özellikleri, ağın toplam uzunluğu gibi uzatma ağı verileri, (3) hedef balık kupes ve diğer hedef-dışı balık yaş ağırlıklarının (kg) alınması şeklinde kayıtlar tutulmuştur. Ağın teknik planı FAO standartlarına göre ölçekli olarak çizilmiştir.

Balıkçının hedeflediği kupes dışındaki tüm av 'hedef-dışı av' olarak kaydedilmiştir. Balıkçılık çabası (f) ve birim çaba başına hedef dışı av (BPUE), de Metrio ve Megalafonou (1988)'ya göre hesaplanmıştır: f = (a'/1000) × g, burada a', balıkçının kullandığı ağın uzunluğu, (a'/1000) günlük olarak denize atılan ağın 1 km'ye standardize edilmiş ağ ünitesi; g, av günlerinin sayısıdır. BPUE, ağın km başına hedef dışı av ağırlığı olarak, BPUE = kg/f formülü ile hesaplanmıştır. Burada kg, hedef dışı avın toplam ağırlığını ifade etmektedir. Her iki yıldaki aylar birleştirilmiştir.

Tüm veri analizi, görselleştirme ve istatistiksel anlamlılık testleri, temel R paketleri yanında tidyverse (Wickham vd., 2019) kütüphanesi de kullanılarak R programlama dilinde (R Core Team, 2022) yapılmıştır. Yıllar arasındaki BPUE ortalamaları arasında istatistiksel önemin belirlenmesinde verilerin normal dağılım göstermemesi nedeniyle, non parametrik testler olan Man Withney-U testi, aylar arasında ise Kruskal Wallis H testi kullanılmıştır. Tüm ortalamalar standart hatalarıyla verilmiştir (± S.E.).

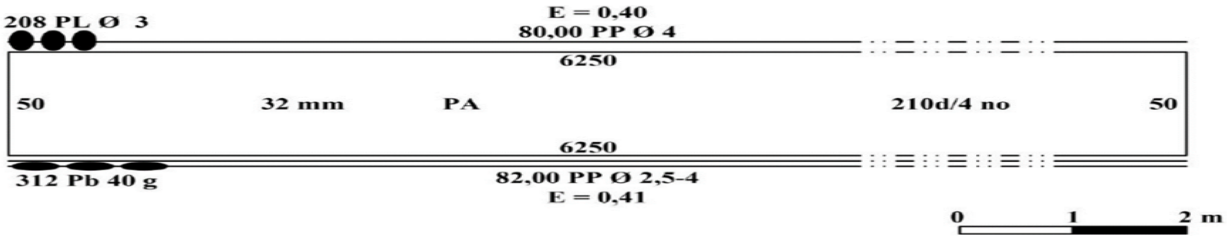


Şekil 1. Çalışma sahası ve örneklemenin yapıldığı balıkçı limanları
Figure 1. Study site and fishing ports sampled

BULGULAR

Örneklenen teknelerin toplam uzunluğu (LOA) ve makine gücü (HP) sırasıyla 8 ila 10 m ve 28 ila 135 HP, uzatma ağlarının uzunluğu ise 500 ila 5000 m arasında değişmektedir (ortalama: 2716 ± 69,6 m). Sade kupes ağları 32 mm göz açıklığında olup donam faktörü E=0,40 'dır. Yani potluğu %60'tır. Donatıldıktan sonraki uzunluğu 80 m olan bir posta ağın mantar yakasında 208 adet 3 no mantar, kurşun yakasında 312 adet 40 g'lık kurşun bulunmaktadır. 2,5 mm polipropilen (PP) malzemeden koşma halatına sahip olan bu ağ; mantar ve kurşun yakalarda 4 mm PP halatlara sahiptir (Şekil 2). Balık çiftlikleri çevresinde yoğun kupes avı sezonu Ocak ve Mayıs ayları arasındadır. Ancak balıkçılar Kasım sonu itibariyle kısmen bu ava başlamaktadır. Uzatma ağları dönek (akşamdan atılıp sabah toplama) yöntemiyle genellikle kafeslerin 200 m sınırına, yaklaşık 40-60 m derinliğe gün batımında atılıp, gün doğumunda kaldırılmaktadır.

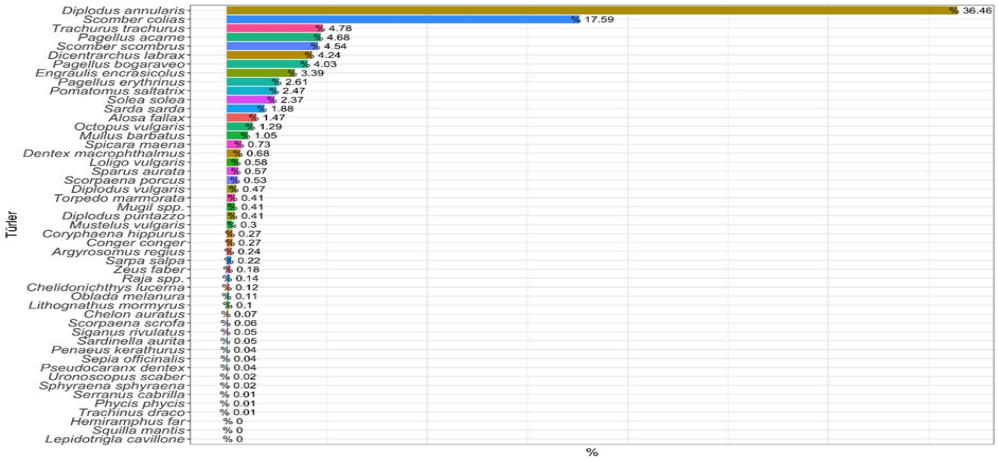
Kupes uzatma ağı balıkçılığında 147 operasyonda toplam 18163 kg balık yakalanmıştır. Biyo-çeşitlilik omurgasızlar dâhil 30 familyaya ait 48 türden oluşmuştur.



Şekil 2. Güllük Körfezi'nde kullanılan sade kupes ağının teknik planı

Figure 2 Technical plan of the bogue gill-net, used in Güllük Bay

Hedef balık olan *B. boops* doğal olarak en bol (%91,9) bulunan türdür. Avın ağırlıkça %8,1 ise hedef dışı av olarak kayıtlara girmiştir. Burada (Şekil 3) %8,1'lik hedef dışı av oranı üzerinden oransal değerlendirmeler yapılmıştır. Hedef dışı avda en çok avlanan ilk beş tür sırasıyla *Diplodus annularis* (L., 1758), *Scomber colias* Gmelin, 1789, *Trachurus trachurus* (L., 1758), *Pagellus acarne* (Risso, 1827) ve *Scomber scombrus* (L., 1758)'tur (Şekil 3).

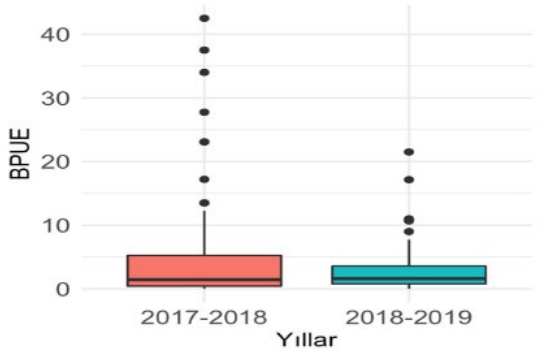


Şekil 3. Güllük Körfezi balık çiftlikleri civarında gerçekleşen kupes avcılığında hedef dışı türler ve yakalanma oranları

Figure 3. Capture rates of non-target species from bogue fishing around fish farms in Güllük Bay

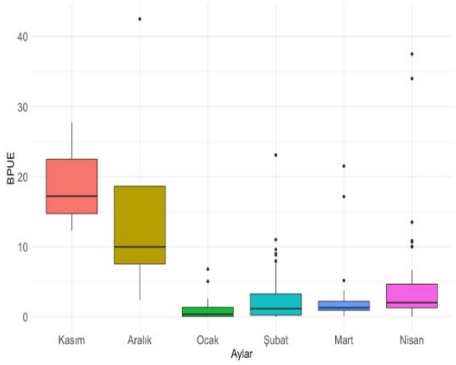
Bölgede yapılan 147 operasyonun 92'si 2018 yılında, 55'i ise 2019 yılında gerçekleşmiştir. Ortalama BPUE 2018 yılında 4,77 ± 0,85 kg.1000 m^{-1} ve 2019 yılında 3,20 ± 0,58 kg.1000 m^{-1} olarak hesaplanmıştır (Şekil 4). İstatistiki olarak yıllar arasında ortalama BPUE değerleri arasında önemli bir fark bulunmamıştır ($p > 0,05$). En yüksek hedef dışı av 42,5 kg ile Aralık ayında gerçekleşmiştir.

Kasım ayı BPUE değerleri açısından en yüksek ortalamayı (19,07 ± 4,57 kg.1000 m^{-1}) vermektedir. Ortalama birim çaba başına düşen hedef-dışı av (BPUE) 0 ile 19,1 kg.1000 m^{-1} arasında (ortalama: 7,97 ± 2,83 kg.1000 m^{-1}) değişmiştir (Şekil 5). İstatistiki olarak aylar arasında ortalama BPUE değerleri bakımından önemli bir fark vardır ($H = 29,811$, $df = 5$, $p < 0,05$).



Şekil 4. Güllük Körfezi balık çiftlikleri civarında gerçekleşen kupes avcılığında yıllara göre birim çaba başına hedef dışı av (BPUE) değerleri

Figure 4. By-catch per unit effort (BPUE) by years from bogue fishing around fish farms in Güllük Bay



Şekil 5. Güllük Körfezi balık çiftlikleri civarında gerçekleşen kupes avcılığında aylara göre birim çaba başına hedef dışı av (BPUE) değerleri

Figure 5. By-catch per unit effort (BPUE) by months from bogue fishing around fish farms in Güllük Bay

TARTIŞMA VE SONUÇ

Ege Denizi'nde balık çiftlikleri etrafındaki kupes uzatma ağı avcılığı son yıllarda önemli bir avcılık yöntemi haline gelmiştir. Çıkan balıklar, yağlı ve kondisyonu yüksek balıklar olduğundan, pazarda rağbet görmektedir. Bununla beraber bu araştırma, bir balık çiftliğinde *B. boops*'u hedef alan ticari ağ balıkçılığından hedef dışı avın tür çeşitliliği ve miktarını ortaya koyan ilk çalışmadır.

Kupes balıkçılığından toplam 48 tür elde edilmiş olup, *B. boops* en baskın (%91,9) türdür. Bilindiği gibi, Akdeniz'de balık çiftlikleri çevresinde en bol bulunan yabani balıklardan biri olan kupes, tüm Akdeniz'deki balık topluluklarının önemli bir bölümünü oluşturmaktadır (Dempster vd., 2002; Valle vd., 2007; Fernandez-Jover vd., 2008; Akyol ve Ertosluk, 2010). Balık çiftlikleri etrafında doğal balık topluluklarının çeşitliliği, bölgeden bölgeye, hatta derinliğe göre değişmektedir. Akyol ve Ertosluk (2010), Ege Denizi'nde İzmir bölgesinde çiftlikler açığa taşınmadan önceki dönemde yaptıkları çalışmada, balık çiftlikleri çevresinde fanyalı uzatma ağı, tuzak, olta, zıpkın kullanarak 34 tür (*B. boops* en boldu) kaydetmiştir. Buna ilaveten, Akyol vd. (2020), Ege Denizi'ndeki balık çiftliklerinde, hızlı görsel sayım yöntemiyle 21 familyaya ait toplam 39 balık türünü gözlemlemiştir. Bu son çalışmada, *B. boops* en bol balık türüydü ve çiftlikle ilişkili balıkların yaklaşık %80,5'ini oluşturmaktaydı. Bunu *A. boyeri* (Risso, 1810), *S. colias*, *Sardinella aurita* (Valenciennes, 1847) ve *Oblada melanura* (L., 1758) izlemiştir (Akyol vd., 2020). Ancak *D. annularis, S. colias, T. trachurus, P. acarne* ve *S. scombrus* bu çalışmada ilk beş hedef dışı balık türü olmuştur. Kupes sade ağlarının gerçekten hedef balıkları ve bazı Scorpaenidler, Uranoscopidler, Triglidler, Soleidler gibi bazı bentik balıkları yakaladığı açıktır. Bu nedenle uzatma ağından çıkan balık çeşitliliğinin sadece pelajik balıkları dikkate alan hızlı görsel sayıma göre çok daha fazla balık çeşitliliğine sahip olması doğaldır.

Balık çiftlikleri etrafında dağılım gösteren ve bu çalışmayla elde edilen türlerin çok yağlı ve büyük oldukları gözlemlenmiştir. Zaten bu yüzden kıyı balıkçıları özellikle bu türleri avlarına dâhil etmek istemektedirler (Arechavala-Lopez vd., 2011). Örneğin, balık çiftlikleri alanından yakalanan toplam uzunluğu 402 mm toplam boy (TL) olan *B. boops* ve 357 mm TL olan *O. melanura* maksimum boyut olarak bildirilmiştir (Akyol vd., 2014; Ceyhan vd., 2018). Izquierdo-Gomez vd. (2015), doğal balıkların kıyı bölgesi boyunca balık çiftliklerinin etrafındaki aşırı yemle beslendiğini, bunun olması halinde, avlanmaya kapalı bir alanda (kiralık alan) yabani balıkların kondisyonunun artabileceğini ve lipit profillerinin değişebileceğini belirtmiştir. Yakın zamanda yapılan bir mide içeriği araştırması, Karadeniz'deki deniz kafesi balık çiftlikleri çevresinde mezgit (*Merlangius merlangus* L., 1758) tarafından tüm mevsimlerde en çok tüketilen gıdanın pelet yem olduğunu göstermiştir (Şensurat-Genç vd., 2019).

Bacher ve Gordoa (2016) İspanya'da bir balıkçı barınağı açıklarındaki çipura çiftliği çevresinde avcılık yapmakta olan KÖB ve amatör balıkçılar dan her biri en az 40 av günü olan beş tekneyi analiz ettikleri araştırmalarında, yakalama oranının 36,1 ila 119,4 kg. gün^{-1} arasında değiştiğini ve av kompozisyonunun *Merluccius merluccius* (L., 1758), *Solea solea* (L., 1758), *Dentex dentex* (L., 1758), *Diplodus sargus* (L., 1758), *Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817), *Mullus barbatus* (L., 1758), *M. surmuletus* (L., 1758), *Pagellus erythrinus* (L., 1758), *Pagrus pagrus* (L., 1758), *Serranus cabrilla* (L., 1758), *Sparus aurata* (L., 1758), *Sphyraena sphyraena* (L., 1758), *Trachurus* spp., bazı Mugilidler ve Scorpaenidler olmak üzere 15 tür civarında olduğunu tespit etmişlerdir. Bu araştırmada hedef tür kupes olmasına karşın Bacher ve Gordoa (2016)'nın çalışmasındaki av kompozisyonu içerisinde *B. boops* yer almamaktadır. Bu husus, balıkçı teknelerinin çiftlik sınırlarının 800 m uzağında örneklenmiş olması ile açıklanabilir. Çünkü bu araştırmada örneklenen balıklar 200 m'lik koruma zonunun bittiği bölgede avlanmıştır. Bu durumda, *B. boops*'un muhtemelen 200 m'lik sınır dâhilinde deniz kafeslerine çok yakın yaşayan, kafese bağımlı balıklardan birisi olduğu düşünülebilir.

Ortalama 19,1± 4,6 kg.1000 m^{-1} ile Kasım ayı en yüksek BPUE'ye sahipken onu 16,2± 8,9 kg.1000 m^{-1} ile Aralık ayı takip etmiştir. Bunun sebebi, asıl kupes avının Ocak ayı itibariyle başlıyor olmasıdır. Yani Kasım-Aralık gibi aylarda kupes fazla av vermeyip, diğer balıklar ağdan oldukça yüksek oranda çıkmakta, Ocak ayından sonra ise kupes ön plana çıkmaktadır. Özellikle Nisan ayında ise en yüksek verime ulaşmaktadır. Zira kupesin ilkbahardaki üreme göçü ile av verimi arasında bir ilişki olduğu balıkçılar tarafından da onaylanmaktadır (T. Bıçak, kişisel görüşme). Daha sonra, yazın balıkçılar bu balıkçılığı terk etmekte ve genellikle turizm ve/veya diğer balıkçılık tekniklerine yönelmektedirler.

Sonuç olarak, balık çiftlikleri etrafında yapılan kupes avcılığının hedef dışı av oranları görece düşüktür. İri boyutlu kupesi hedefleyen görece iri gözlere sahip (32 mm) ağlar ve kullanım tekniği %92 oranında hedef türü yakalıyor olması, hedeflenen türe yönelik av aracının oldukça verimli çalıştığının da bir göstergesidir. Balık çiftlikleri çevresinde 200 m koruma zonunda avlanmak yasa dışıdır. Bu durum Ege Denizi'nde amatör/ticari balıkçılar ile balık çiftlikleri arasında çatışmalara neden olmaktadır (Akyol vd., 2019). Bilindiği gibi, balık çiftliklerinin etrafındaki yabani balıklar, organik pelet yemlerle beslenen ve aynı zamanda suyu iyi filtre eden balıklardır ve suyun arıtılmasına katkıda bulunurlar. Böylece, bentik ortamlardaki çökelmeyi en aza indirgemek için yabani balıkların biyo-filtre işlevinin sürdürülmesi garanti edilmelidir. Ek olarak, ticari ve amatör balıkçılıkla balık çiftlikleri çevresinde ne kadar balık yakalandığı ve bu balıkçılığın yabani balık popülasyonu üzerindeki ekolojik etkisinin boyutu ile ilgili çalışmaların sayısı oldukça sınırlıdır. Bu nedenle, balık çiftlikleri etrafında yer alan balıkların biyo-filtre özellikleri ve bir çeşit üreme biyokitlesi olarak korunmaları ve/veya çok dikkatli bir şekilde, sınırlı bir miktarda avlanmasına yönelik izin verilebilir toplam ava yönelik çalışmaların yapılması gereklidir.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Vurgulu elektrik alanın sardalya (*Sardina pilchardus* Walbaum, 1792) balıklarında tuz transferi üzerine etkisi

Effect of the pulsed electric field method on salt transfer in European sardine (*Sardina pilchardus* Walbaum, 1792)

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Öz: Besleyici değeri ve lezzetiyle tüketiciler tarafından yoğunlukla talep edilen Sardalya (*Sardina pilchardus*) balığının daha uzun raf ömrü ile pazarlanma seçeneklerinden biri salamuraya işlenmesidir. Salamura prosesinde tuz difüzyon hızının düşük olması balıkların depolama kalitesini etkileyen önemli bir faktördür. Son yıllarda vurgulu elektrik alan yöntemi (PEF) kütle transferini hızlandıran yenilikçi bir yöntem olarak değerlendirilmektedir. Bu çalışmada, sardalyaların salamura işlemi öncesi ve salamura işlemi süresince PEF (6,36 kV/cm) uygulanmış ve son üründeki tuz konsantrasyonu incelenmiştir. PEF uygulanmış örneklere göre, kontrol grubunun sahip olduğu tuz konsantrasyonunun (%16,32) daha düşük olduğu bulunmuştur ($p<0,05$). Ancak PEF uygulanmış örnekler arasında tuz konsantrasyonu (%18,17 ve %18,22) açısından önemli bir fark tespit edilmemiştir. Tüm örneklerde tuz absorbsiyonuna bağlı olarak kül oranı artmış ve su aktivitesi değeri azalmıştır. pH değerleri kıyaslandığında, örnekler ve depolama zamanları arasında istatistiksel olarak önemli bir fark gözlenmemiştir. Taramalı elektron mikroskobu (SEM) görüntüleri incelendiğinde ise PEF uygulama süresi arttıkça gözeneklerin sayısının ve çapının arttığı belirlenmiştir. Bu nedenle, PEF uygulamasının sardalya balıklarında tuz difüzyonunu artırmak için bir ön işlem olarak değerlendirilebileceği sonucuna varılmıştır.

Anahtar kelimeler: Sardalya, vurgulu elektrik alan, salamura, tuz difüzyonu

Abstract: One of the marketing options for European sardine (*Sardina pilchardus*), which consumers demand nutritional value and taste, is processing in brine that provides a longer shelf life. However, the slow rate of salt diffusion is the biggest obstacle to be overcome. In this context, the pulsed electric field method (PEF) is considered an innovative method that accelerates mass transfer. This study applied PEF to sardine before and while they were kept in brine. The salt concentration of the final product was found to be higher than the control group, which was not applied PEF ($p<0.05$). However, the difference between the PEF applied samples was insignificant while ash content increased, and water activity value decreased in all samples due to salt absorption. When pH values were compared, no significant difference was observed between samples and storage periods. According to the scanning electron microscope (SEM) images, the number and diameter of the pores increased due to the rise in application time. Therefore, it was concluded that PEF application could be considered a pretreatment to increase salt diffusion in European sardine.

Keywords: European sardine, pulsed electric field, brine, salt diffusion

GİRİŞ

Ülkemizde en çok avlanan deniz balıkları arasında yer alan sardalya, değerli besin kompozisyonu ve lezzet kazandıran duyusal bileşenlerinin yanı sıra düşük fiyatı ile de tüketiciler tarafından tercih edilen su ürünleri arasında yer almaktadır (Wawire vd., 2019; Anonim, 2020; Çöteli, 2021). Ancak, taze sardalyalar bakteriyel gelişim, enzim aktivitesi ve yağ oksidasyonu gibi değişimler sonucunda kolayca bozulabilmektedir. Hem sardalyaların raf ömrünü uzatmak hem de tüketime hazır işlenmiş su ürünlerine karşı artan talebi karşılayabilmek için tuzlama, kurutma, marinasyon, konserve gibi uygulamalar duyusal, fiziksel ve kimyasal dönüşümlere uğratılmaktadır. Uygulaması kolay ve ucuz bir yöntem olan tuzlama işlemi, balığı otolitik dekompozisyondan korumakta ve yağ oksidasyonunu en aza indirgemektedir (Ormancı vd., 2018; Wawire vd., 2019). Salamurada muhafaza ile tuzun dokulara nüfuz etmesi sağlanarak gerçekleştirilen dehidrasyon sonucu hem raf ömrü geliştirilmekte hem de ham maddeye yeni yapısal özellikler kazandırılmaktadır. Tuzun difüzyonu ürüne göre farklı sürelerde görevini tamamlamakta, bu sürenin hızlandırılması sadece salamura ile hazırlanan ürünlerde değil, farklı formülasyona sahip marinatlarda veya kürlenmiş ürünlerde de önem kazanmaktadır. Bu amaçla son yıllarda ultrases, yüksek basınç ve vurgulu elektrik alan (pulsed electric field; PEF) gibi yöntemlerin uygunluğu araştırılmaktadır (McDonnel vd., 2014; Inguglia vd., 2020).

PEF, hem gıdaların muhafazası hem de yapısal değişikliklerin gerçekleştirilmesi için yaygın olarak araştırılan ısıl olmayan, yenilikçi bir yöntemdir. PEF yöntemi ile iki elektrot arasında bulunan ortama çok küçük zaman (µs veya ms) dilimlerinde yüksek voltajlı elektrik akımı uygulanmakta, böylece hücre yapısında geçici veya kalıcı düzenlemeler

gerçekleştirilmektedir. Bu düzenlemeler hücre zarında gözenek oluşumu ile sonuçlandığında elektroporasyon olarak adlandırılmaktadır. Yüksek yoğunluklu elektrik darbeleri (>~18 kV/cm) uygulandığında hücre zarında kalıcı elektroporasyon oluşurken, göreceli olarak düşük elektrik alan gücüne sahip darbeler (~5-15 kV/cm) ile hücre zarında geri dönüşümlü olarak geçirgenlik artmaktadır (Aşık Canbaz vd., 2020). Aynı zamanda enerji tasarrufu sağlama ve çevreci olma gibi avantajları bulunan PEF yöntemi, tek başına veya diğer yöntemlerle birlikte et ve et ürünlerinin kalitesini ve proses etkinliğini geliştirmek üzere kullanılmaktadır (Gómez vd., 2019). Toepfl vd. (2006), domuz etine uyguladıkları 2 kV/cm yoğunluktaki vurgulu elektrik alanın, salamuranın dokuda homojen yayılmasını sağladığı, su tutma kapasitesini artırdığı, pişme esnasında ağırlık kaybını azalttığı ve son ürüne daha yumuşak bir yapı kazandırdığı sonucuna varmışlardır. PEF ile et ve et ürünlerinin tekstürel özelliklerinin iyileştirilmesi membran geçirgenliğinin artması ile gerekli iyon ve enzimlerin salınması mümkün olmaktadır (Bekhit vd., 2014). PEF aynı zamanda, balık atıklarında ekstraksiyon verimini artırmak için bir ön-işlem olarak uygulanabilmektedir. Zhou vd. (2012) ve He vd. (2014), PEF kullanarak balık kılçığından sırasıyla kalsiyum ve kondroitin sülfat ekstrakte ettikleri çalışmalarında, daha kısa sürede daha verimli sonuçlar aldıklarını vurgulamışlardır. PEF ile hücre geçirgenliğinin artması sonucu, marinasyon, kurutma veya kürleme işlemlerinde kullanılan baharat, tuz, antimikrobiyal ajanlar gibi maddelerin, taze etlere nüfuz etmesi sağlanmaktadır. Toepfl vd. (2006), PEF uyguladıktan sonra salamura çözeltisine daldırdıkları domuz etinde, tuz ve nitrat geçişinin arttığını bildirmiştir. McDonnell vd. (2014) ise, PEF uygulamasını domuz etinin tuzlanmasını hızlandıran bir ön işlem olarak değerlendirmiştir. Astráin-Redín vd. (2019), sosislerde bir ön işlem olarak PEF uygulamanın su transferini hızlandırdığını ve kurutma süresini yaklaşık bir hafta kısalttığını vurgulamışlardır.

PEF yönteminin su ürünlerinde kütle transferi üzerine etkisini içeren araştırmalar oldukça kısıtlıdır. Söz konusu bu çalışmada da PEF yönteminin bir ön işlem olarak veya tuzlama işlemine eşlik ederek uygulanmasının, salamura sardalyalarda tuz difüzyonu üzerine etkisi, farklı kalite parametreleri ile desteklenerek araştırılmıştır.

MATERYAL ve METOT

Materyal

Araştırmada kullanılan sardalya balıkları avlanmanın hemen ardından satış yerine ulaştığı gün soğuk zincir korunarak laboratuvara getirilmiştir. Çalışmada kullanılan balıkların ortalama boyları 11,16±0,42 cm ve ortalama ağırlıkları 11,76±0,90 g olarak tespit edilmiştir. Her bir uygulama için toplam 200 g ağırlığında 17 adet sardalya kullanılmıştır. Ayıklama işlemi satış yerinde gerçekleştirilen sardalyalar, aseptik koşullar altında hazırlanmıştır. Akan su altında yıkanan sardalyaların fazla suyu süzme işlemi ile uzaklaştırılmıştır. 11 cm çapı ve 12 cm yüksekliğinde polipropilen (PP) kaplara yerleştirilen sardalyalar beş gruba ayrılmıştır. Birinci grup (KS), %18'lik salamura (1:2) (w/v) içinde 24 saat boyunca +4°C'ta muhafaza edilmiştir. İkinci grup (KP), +4°C'ta 60 dakika PEF uygulandıktan sonra %18'lik salamura (1:2) (w/v) içinde 24 saat boyunca yine +4°C'ta muhafaza edilmiştir. Üçüncü grup (KPS) ise %18'lik salamura (1:2) (w/v) içinde 24 saat boyunca buzdolabı koşullarında muhafaza edilirken PEF uygulanmıştır. KP ve KPS gruplarında farklı uygulama sürelerinin kullanılması, KP grubunda PEF etkinliğinin bir ön işlem olarak değerlendirilmek istenmesinden kaynaklanmaktadır. Ayrıca, SEM analizinde ele alınan sardalyalarda taze balık örnekleri kontrol (K), sadece PEF uygulanan örnekler ise (P) grubu olarak değerlendirilmiştir.

Vurgulu elektrik alan düzeneği ve uygulaması

Buzdolabına entegre edilmiş bir güç kaynağı (Türkoğlu Neon Trafoları, İstanbul, Türkiye) ve uygulama bölmesinden meydan gelen PEF sistemi Süleyman Demirel Üniversitesi Elektrik-Elektronik Mühendisliği Bölümü laboratuvarlarında prototip olarak üretilmiştir (Şekil 1). Darbe jeneratörü cihazın metal gövdesi ile canlı uç çıkışı arasında 70 kV potansiyel farkı vermiş ve bu yüksek gerilim kablolar ile emniyetli biçimde buzdolabı içindeki paralel plakalara aktarılmıştır. Darbe jeneratöründen 0,2 ms genişliğe ve 11,6 ms sıklığa sahip darbeler elde edilmiştir. Güç kaynağına eklenmiş olan doğrultma devresi ile plakalara iletilen darbelerin tek kutuplu (DC) ve üstel azalan şekilde olması sağlanmıştır. Uygulama bölmesinde bulunan 21,5×29×0,2 cm boyutlarındaki paralel plakalar (Cr-Ni) kullanılarak elektrik alan oluşturulmuştur.

Örneklerin maruz kaldığı elektrik alan değeri (kV/m) aşağıdaki eşitlik ile hesaplanmıştır.

E = V/d

E: elektrik alan değeri (kV/cm)

V: voltaj (kV)

d: mesafe (cm)

PEF sistemi ile paralel plakalar arasındaki mesafe değiştirilerek 2,5-7 kV/cm aralığında elektrik alan değerleri elde edilebilmektedir. Bu çalışmada plakalar arasındaki mesafe 11 cm'ye ayarlanarak 6,36 kV/cm elektrik alan değeri uygulanmıştır. CST Microwave Studio (Computer Simulation Technology GmbH, Almanya) programı kullanılarak gerçekleştirilmiş olan denemelerle PP kabın elektrik alan vektörlerini engellemediği görülmüştür. Dolayısıyla, diğer çalışmalardan farklı olarak bu çalışmada elektrotların gıda veya ambalaj ile temas etmediği indirekt bir uygulama gerçekleştirilmiştir.

Tuz tayini

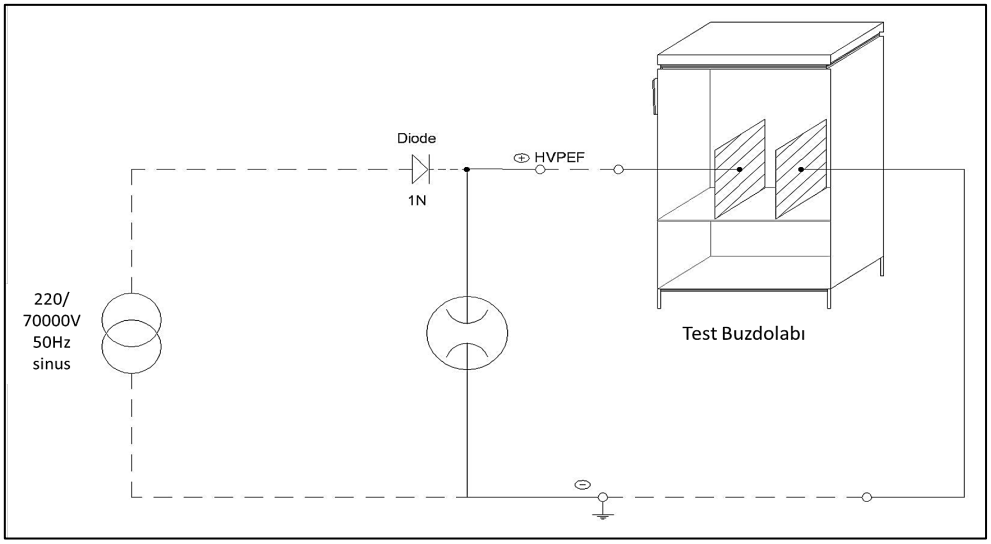
Sardalya balıklarının tuz içerikleri Mohr yöntemi uygulanarak çalışmanın başında ve depolamanın 12. ve 24. saatlerinde tespit edilmiştir. 5 gram homojen örnek K-kromat (%5) ve O,1 N NaOH eşliğinde normalitesi belli $AgNO_3$ çözeltisi ile titre edilmiştir (Dericioğlu vd., 2019). Örneklerin tuz miktarı aşağıda gösterilmiş olan formül ile hesaplanmıştır:

$$Tuz\ miktarı\ (\%) = V.f.0{,}00585.Sf.\frac{100}{m}$$

V: titrasyonda harcanan 0,1 N $AgNO_3$ (ml)

f: 0,1 N $AgNO_3$ çözelti faktörü

S_f: Seyreltme faktörü



Şekil 1. Buzdolabına entegre edilmiş PEF sisteminin şematik gösterimi

Figure 1. Schematic representation of PEF system entegrated into refrigerator

Kül tayini

Sardalya örneklerinin kül miktarının belirlenmesi için sabit ağırlığa getirilen porselen kül kapsüllerine yaklaşık 3-4 g örnek tartılmıştır. Örnekler kül fırınına yerleştirildikten sonra sıcaklık kademeli olarak artırılarak 600 ºC'a getirilmiş ve kül kapsülündeki örnek rengi gri-beyaz olana kadar yakma işlemine devam edilmiştir. Kül kapsüllerinin tartım farkından örnekteki % kül miktarı belirlenmiştir (Anonim, 1990).

Su aktivitesi tayini

Sardalya balıklarının su aktivitesi değeri su aktivitesi cihazı (LabSwift, Novasina, İsviçre) kullanılarak tespit edilmiştir. Homojenize edilmiş (Waring Commercial, A.B.D.) örnekler cihazın ölçüm haznesine yerleştirildikten sonra, cihazın uyarı sesi ile gözlenen değer kaydedilmiştir.

pH tayini

10 g sardalya örneği 90 ml destile su ile homojenize edildikten (Waring Commercial, A.B.D.) sonra pH-metre (Schott Lab 860, Almanya) kullanılarak belirlenmiştir (Anonim, 1990).

Taramalı elektron mikroskobu (SEM) ile görüntüleme

Sardalya balıklarının salamurada beklemesi sonucu ve PEF uygulaması ile yapısında meydana gelen değişim taramalı elektron mikroskobu (SEM) (FEI QUANTA FEG 250, A.B.D.) kullanılarak izlenmiştir. Örnek hazırlama aşamasında Castejón (2012) tarafından önerilen kriyojenik kırma metodu uygulanmıştır.

Depolamanın başında taze balıktan (K), sadece PEF uygulanan sardalya balıklarından (P), depolamanın sonunda KS, KP ve KPS gruplarından alınan örnekler sıvı nitrojene daldırıldıktan sonra dikey kesit alacak şekilde kırılmıştır. Ardından, çift taraflı yapışkan bant kullanılarak SEM numune haznesine yerleştirilerek incelenmiştir.

İstatistik analiz

İki tekerrürlü ve 2 paralelli olarak gerçekleştirilen deneylerin sonucunda uygulamalar arasındaki fark varyans analizi (ANOVA) ve Duncan çoklu karşılaştırma testi kullanılarak %95 güven aralığında belirlenmiştir. İstatistiksel analiz, Statistical Package for Social Science software (SPSS Inc. version 24.0, A.B.D.) kullanılarak gerçekleştirilmiştir.

BULGULAR

Salamurada bekletilmiş sardalya balıklarının tuz ve kül içeriği (%) ile a_w ve pH değerleri Tablo 1'de gösterilmiştir. Örneklerin başlangıç tuz konsantrasyonu %2,75 olarak tespit edilmiş olup depolamanın sonunda bu değer KS, KP ve KPS örnekleri için sırasıyla %16,32, %18,17 ve %18,22 seviyesine ulaşmıştır. Depolama boyunca her üç örnek grubunda da tuz konsantrasyonu artmış olmakla birlikte 12. saatte tuz içeriği açısından örnekler arasında istatistiksel bir fark gözlenmezken, 24. saatin sonunda PEF uygulanmış örnekler, uygulanmamış örneklere göre önemli seviyede ($p<0,05$) yüksek tuz içeriğine ulaşmıştır. PEF tekniğinin salamuraya daldırmadan önce veya salamurada bekletilirken uygulanması tuz difüzyonu açısından aynı etkiyi göstermiştir.

Sardalya balıklarının tuz oranındaki artışa paralel olarak kül oranının arttığı ve su aktivitesi değerinin ise azaldığı gözlenmiştir. Başlangıçta tespit edilen %2,14 oranındaki kül miktarı aynı örnek grubunun 12. ve 24. saatlerinde önemli seviyede artış gösterirken ($p<0,05$), örnekler arasındaki fark anlamsız bulunmuştur. Başlangıçta kaydedilen su aktivitesi değerinin (0,92) ise depolamanın sonunda KS grubunda 0,87, PEF uygulanmış olan örnek gruplarında ise 0,88'e düştüğü gözlenmiştir. Ancak örnek grupları arasındaki fark 12. ve 24. saatlerde önemsiz bulunmuştur. Taze sardalya balıklarının pH değeri 6,68 olarak tespit edilmiş olup depolama sonunda KS ve KP örnekleri için 6,54, KPS örnekleri için ise 6,58 olarak belirlenmiştir. Gözlenen bu düşüş değerlendirildiğinde ise hem grup içi hem de gruplar arasındaki değişimin önemsiz olduğu kaydedilmiştir.

Tablo 1. +4°C'ta 24 saat boyunca depolanan sardalya örneklerinin tuz (%), kül (%), a_w ve pH değerleri

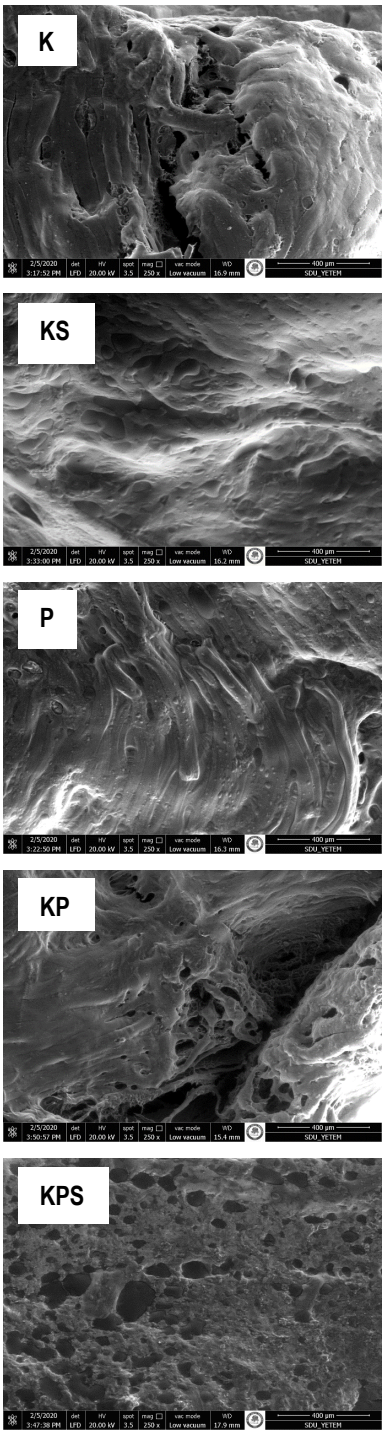
Table 1. Salt (%), ash (%), a_w, and pH values of sardine samples storaged at +4°C for 24 hours

	0 (h)	12 (h)	24 (h)
Tuz (%)			
KS	2,75±0,61^{Ac}	14,52±0,44^{Ab}	16,32±0,76^{Ba}
KP	2,75±0,61^{Ac}	15,22±0,68^{Ab}	18,17±0,24^{Aa}
KPS	2,75±0,61^{Ac}	14,28±0,98^{Ab}	18,22±0,17^{Aa}
Kül (%)			
KS	2,14±0,19^{Ab}	6,60±0,76^{Aa}	7,07±0,96^{Ba}
KP	2,14±0,19^{Ac}	6,06±0,92^{Ab}	7,72±0,97^{Aa}
KPS	2,14±0,19^{Ac}	6,47±0,71^{Ab}	8,48±1,01^{Aa}
a_w			
KS	0,92±0,003^{Aa}	0,88±0,001^{Ab}	0,87±0,006^{Ac}
KP	0,92±0,003^{Aa}	0,89±0,007^{Ab}	0,88±0,004^{Ac}
KPS	0,92±0,003^{Aa}	0,88±0,006^{Abc}	0,88±0,003^{Ac}
pH			
KS	6,68±0,07 ^{Aa}	6,60±0,09 ^{Aa}	6,54±0,07 ^{Aa}
KP	6,68±0,07 ^{Aa}	6,60±0,18 ^{Aa}	6,54±0,08 ^{Aa}
KPS	6,68±0,07 ^{Aa}	6,51±0,12 ^{Aa}	6,58±0,06 ^{Aa}

"KS" PEF uygulanmadan, "KP" 60 dakika PEF uygulandıktan sonra ve "KPS" PEF uygulanarak depolanan örnekleri göstermektedir.
$^{A-B}$: Aynı sütunda farklı harfleri taşıyan örnek ortalamaları arasındaki fark istatistiksel olarak önemlidir ($p<0,05$).
$^{a-c}$: Aynı satırda farklı harfleri taşıyan örnek ortalamaları arasındaki fark istatistiksel olarak önemlidir ($p<0,05$).
"KS"; samples stored without PEF application, "KP"; samples stored after 60 minutes PEF application; "KPS"; samples stored under PEF.
$^{A-B}$: Upper case letters over the indicate a statistically significant difference between sample groups ($p<0.05$).
$^{a-c}$: Lower case letters over the bars indicate a statistically significant difference between storage days ($p<0.05$).

PEF uygulamasının canlı hücre yapılarında sebep olduğu değişiklikler proses etkinliği açısından avantajlı olarak kabul edilmekte ve dokularda meydana gelen dönüşümlerin izlenebilmesi için görüntüleme tekniklerine başvurulmaktadır. Bu çalışmada SEM kullanılarak elde edilen görüntüler Şekil 2'de gösterilmektedir.

Elektroporasyon ile oluşan gözeneklere K ve KS grubunda rastlanmazken, KPS grubunda KP grubuna göre gözeneklerin çapı büyümekte ve sayısı artmaktadır. Depolama boyunca PEF uygulanmış KPS grubu örneklerinde gözlenen fiziki deformasyon diğer örneklere göre daha belirgin görülse de bu değişiklik tuz difüzyonunda önemli bir fark ile sonuçlanmamıştır.



Şekil 2. Sardalya balıklarına ait SEM görüntüleri. K: taze sardalya; KS: salamurada muhafaza edilmiş sardalya; P: PEF uygulanmış taze sardalya; KP: PEF uygulandıktan sonra salamurada muhafaza edilmiş sardalya; KPS: salamurada muhafaza edilirken PEF uygulanmış sardalya

Figure 2. SEM images of sardine samples. K: fresh sardine; KS: brined sardine; P: PEF applied fresh sardine; KP: sardine brined after PEF application of 60 minutes; KPS: sardine brined under PEF

TARTIŞMA

Su ürünlerinin salamurada işlenmesi aşamasında tuzun koruyucu ve dönüştürücü etkisinden faydalanılmaktadır. Dokulara nüfuz eden tuz konsantrasyonu nihai ürün kalitesini, difüzyon hızı da proses verimini etkilemektedir. Bu çalışmada PEF uygulamasının salamurada bekletilen sardalya balıklarının sahip olduğu tuz içeriği üzerinde etkili olduğu tespit edilmiştir. Bu durum PEF uygulamasının, balık kasının mikroyapısında meydana getirdiği mekanik zararlar dolayısıyla kas hücre zarının bütünlüğünün bozulması ile açıklanabilmektedir. Ayrıca hücreler arası boşlukların artması ve yeni kanalların oluşması da kütle transferini kolaylaştırmaktadır (Cropotova vd., 2021). Benzer şekilde, Toepfl vd. (2007) ve McDonnell vd. (2014) PEF yöntemini domuz etinin tuzlanmasını hızlandıran bir ön işlem olarak değerlendirmiştir. McDonnell vd. (2014), farklı enerji (22,6-281, 1 kJ/kg), elektrik alan (120 veya 230 kV/cm), frekans (100 veya 200 Hz) ve darbe sayısı (150 veya 300) değerlerini kıyasladıkları çalışmalarında, elektrik alan uygulanması ile beraber tuz difüzyonunda %13'ten fazla artış sağlandığını bildirmişlerdir. Atlantik somon balığı için PEF (2 kV/cm) yönteminin değerlendirildiği başka bir çalışmada ise, proses süresinin %80'e kadar kısaldığı ve son üründe tuz konsantrasyonunda artışın olduğu aktarılırken, PEF uygulamasının kalite parametrelerini etkilemediği belirtilmiştir (Simpson vd., 2018). Núñez vd. (2020) de salamuraya işledikleri (6% ve 24%) somon filetolarına 6 °C'ta 20 saat boyunca elektrik alan (0-2 V/cm) uygulamışlar, elektroporasyona bağlı olarak artan hücre geçirgenliği ile tuzun somon dokularına kütle transferinin hızlandığını, dolayısıyla somon filetolarının tuz oranının arttığını tespit etmişlerdir. Ayrıca bu artışın elektrik alan değeri, süre ve tuz konsantrasyonu arttıkça daha belirgin olduğunu aktarmışlardır. Benzer şekilde, Cropotova vd. (2021), levrek balıklarına salamuraya işlemeden önce PEF (0,3 kV/cm ve 0,6 kV/cm) uygulanmasının tuz absorbsiyonunu %77'ye kadar hızlandırdığını ve kasta tuzun homojen dağılmasını sağladığını belirtmişlerdir. %5 ve %10 konsantrasyona sahip salamura ile 0,3 kV/cm ve 0,6 kV/cm elektrik alan parametrelerinin kombinasyonlarının kullanıldığı bu çalışmada birinci günde, %10'luk salamurada bekletilen balıkların tuz konsantrasyonunun %5'lik salamurada bekletilenlere nazaran daha yüksek olduğu belirlenirken aynı tuz konsantrasyonuna sahip salamuralar içinde PEF uygulanmış olanların oranı da daha yüksek bulunmuştur.

Bazı çalışmalarda daha düşük elektrik alan yoğunluğu kullanılmasına rağmen son üründe artan tuz konsantrasyonunun aktarılması kullanılan hammadde ile ilişkilendirilmektedir. PEF etkinliği üzerine elektriksel parametrelerin yanı sıra kullanılan ham maddenin özelliklerinin de etkili olduğu bilinmektedir (Dunn, 2001). Balıklar arasında gözlenen yapısal farklılıklar çalışmaların sonuçları arasındaki farklılıkları doğurmakta, bu da PEF uygulaması açısından her balığın ayrı değerlendirilmesi gereğini ortaya çıkarmaktadır.

Su aktivitesi değerlerine bakıldığında, PEF uygulanmış örnekler ile kontrol örnekleri arasında depolama boyunca önemli bir fark gözlenmemiştir. Benzer şekilde Semenoglou vd. (2020), ozmotik dehidrasyon (%40–60'lık gliserol ve %5'lik NaCl çözeltisi) ile deniz levreklerinden gerçekleşen kütle transferi üzerine PEF (1,6 kV/cm, 1500'e varan darbe sayısı, 19,7 kJ/kg) yönteminin etkisini inceledikleri çalışmalarında, su difüzyonunun %50'ye ve çözünen madde difüzyonunun da %66'ya kadar artmasına rağmen, su aktivitesinde dikkate değer bir azalma olmadığını aktarmışlardır. Bu durum, gıdaların sorbsiyon izotermi ile açıklanabilmektedir. Gıdaların yüksek su aktivitesi değerlerinde nem oranında önemli bir azalma gözlense de su aktivitesi değerindeki düşüş önemsiz kalabilmektedir (Rahman, 1995). Ayrıca, Cropotova vd. (2021) de PEF yoğunluğunun veya salamurada bekletme süresinin taze levrek filetolarının su aktivitesi değerini etkilemediği sonucuna varmışlardır.

PEF uygulamasının sardalya balıklarının pH değeri üzerindeki etkisi incelendiğinde depolama sonunda KS ve KP gruplarının pH değerlerinin 6,54'e, KPS grubuna ait pH değerinin ise 6,58'e düştüğü tespit edilmiş olmakla beraber pH değerinde gözlenen düşüş üzerinde PEF uygulamasının etkili olmadığı sonucuna varılmıştır. Benzer şekilde Anggo ve Suharto (2020) da PEF uyguladıkları sazan balıklarının pH değerlerini 6,46-6,62 aralığında olduğunu ve uygulamalar arasında önemli bir farka rastlanmadığını belirtmişlerdir. Diğer taraftan, Wawire vd. (2019), sardalya balıklarının pH değerindeki değişimin tuzlama işlemi sonucunda aminoasitlerin ve peptitlerin daha küçük bileşenlere ayrılması ile ilişkilendirmektedir.

PEF yönteminin elektroporasyona dayanan etki mekanizması kas hücrelerinde gözenekler oluşması ile sonuçlanmaktadır. Bu çalışmada da örneklerin kas hücrelerindeki değişimler mikroskobik bir görüntüleme metodu olan SEM ile incelenmiştir. KP örneğinin SEM görüntüleri incelendiğinde kontrole göre belirgin gözeneklerin olduğu, KPS grubu örneklerinde ise bu gözeneklerin hem sayısının hem genişliğinin arttığı tespit edilmiştir. Ancak, bu değişiklik tuz difüzyonu açısından PEF uygulanmış örnekler arasında önemli bir fark ile sonuçlanmamıştır. Bu durumda, gözeneklerdeki değişimin depolamanın sonuna doğru gerçekleştiği tahmin edilmektedir. Gudmundsson ve Hafsteinsson (2001), morina balıklarına 18,6 kV/cm yoğunluğa kadar PEF uyguladıklarında proteinlerin birincil yapısının etkilenmediğini, ancak somon balıklarında hücreler arası boşluğun artarak balık dokusunun yapısal bütünlüğünü kaybettiğini vurgulamışlardır. Klonowski vd. (2006) de morina balıklarında su tutma kapasitesini artırmak üzere PEF (20-120 darbe sayısı, 1,2 kV/cm veya 2 kV/cm elektrik alan yoğunluğu, 400 µs darbe genişliği) uygulamışlar, morina balıklarında 2 kV/cm'den yüksek elektrik alan yoğunluklarında hücrelerde ve hücreler arası boşluklarda gözeneklerin oluştuğunu, bu nedenle özellikle su ürünlerinin kurutulmasında PEF yönteminin kullanılabileceğini bildirmişlerdir. Anggo ve Suharto (2020) ise sazan balığına PEF (30 kV, 60 kV, 90 kV; frekans 50 Hz; darbe genişliği 0,4 s; darbe sayısı 600) uyguladıkları çalışmalarında voltaj 60 kV olduğunda sazan balığı etinin hücre duvarındaki boşluk ve

gözenek sayısıyla beraber, dokudaki hasarın da az olduğu sonucuna varılmış, voltaj 90 kV değerine yükseltildiğinde ise balık etinin hemen her yerine yayılmış çeşitli boyutlarda birçok sayıda gözenek olduğu tespit etmişlerdir.

SONUÇ

Sardalya balıklarında tuz difüzyonu üzerine PEF yönteminin etkisi incelenmiş ve salamuraya işleme esnasında kütle transferini hızlandırmak üzere kullanılabileceği sonucuna varılmıştır. Aynı zamanda, depolamanın sonunda örnekler arasında tuz konsantrasyonu açısından önemli bir farkın olmaması, PEF yönteminin dokulardaki deformasyonu en aza indirecek bir ön işlem olarak uygulanabileceğini göstermiştir.

Ayrıca, marinasyon, tütsüleme, kurutma gibi işlemlerde de PEF uygulamasının kullanılma potansiyeli araştırmaya değer bir konu olarak öne çıkmaktadır. Ancak, yapısal farklılıklar göz önünde bulundurulduğunda proses veriminin artırılması amacıyla her su ürününün ayrı ayrı değerlendirilmesi gereği doğmaktadır. Buradan yola çıkılarak, sonraki çalışmalarda farklı su ürünlerinde, tekstürel özellikler başta olmak üzere farklı kalite kriterlerinin ve elektriksel parametrelerin üzerinde durulması önerilmektedir.

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Yazarlar bu çalışma için özel bir etik onayın gerekli olmadığını beyan eder.

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İlgili tüm veriler makalenin içindedir.

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SHORT COMMUNICATION KISA MAKALE

New record for the algal flora of Türkiye: *Antithamnionella spirographidis* (Schiffner) E.M.Wollaston, 1968 (Ceramiales, Ceramiaceae)

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Abstract: In this study, the new record of the non-indigenous Rhodophyta *Antithamnionella spirographidis* (Schiffner) Wollaston (1968) has been reported from the Marmara Sea that the connection area between the Mediterranean Sea and the Black Sea. The species, originally described from Trieste (Northern Adriatic Sea), is currently known throughout the Central and Western Mediterranean Sea. Our finding is the easternmost record of this tiny filamentous alga in the Mediterranean Sea.

Keywords: *Antithamnionella spirographidis,* Rhodophyta, Ceramiales, Marmara Sea, Algae

INTRODUCTION

Antithamnionella spirographidis (Schiffner) E.M. Wollaston has reported firstly by Schiffer as *Antithamnion spriographidis* in 1916 based on a specimen that he collected in 1914 the harbour at Trieste (Italy). Wollaston has located the species under the genus *Antithamnionella* (Wollaston, 1968). The species is known for with rapid reproduction and high colonization capacity. Species spreads by fragmentation and the rapid production of new thalli, whereas it is ephemeral. *Anthithamnionella spirographidis* is a fouling organism; however, considering its small size and life circle, the effect is dispensable.

The Sea of Marmara is a semi-enclosed sea connected through the Strait of Istanbul (also known as Bosporus Strait) to the Black Sea and through the Strait of Çanakkale (also known as Dardanelles Strait) to the Aegean Sea. It has a limited size of approximately 250 × 70 km, and the deepest point reaches 1,370m. The Sea of Marmara is characterized by high current movements that originate from hydrostatic pressure differences created by the different water masses of the adjacent seas and explained the existence of a counter-current of Mediterranean water below the surface flow of Black Sea water (Beşiktepe et al., 1994; Guiry, 1996; Sannino et al., 2017). The persistence of these two-layer structures occurs due to salinity differences. Low salinity Black Sea waters (~18‰) flow into the Marmara Sea through the Bosporus Strait and denser, salty Aegean waters (~38.5‰) water flows into the Sea of Marmara through the Dardanelle Strait. A sharp pycnocline separates the two different water bodies at a depth of approximately 25 m (Beşiktepe et al., 1994). Thus, even though the maritime traffic is high, reported alien species are lower respect to Aegean and Levantine, and they are mainly composed of Algal species (Çınar et al., 2005). The aim of this study is to examine algal samples collected during "Diversity and Community Structure of Benthic Invertebrate in the Sea of Marmara and Bosporus Survey" and contribute algal biodiversity information of Turkish waters.

MATERIALS AND METHODS

The identified specimens have found in the Sea of Marmara during "The Diversity and Community Structure of Benthic Invertebrate in the Sea of Marmara and Bosporus Survey" in 2013. The sampling point where the species is found is shown in Figure 1.

Figure 1. Working area; sampling point referred to as red dot represents between 10-20 meter depth range

Materials were collected once between June 6th, 2013 and June 26th, 2014, with three replicates per station at depths of 10, 25, and 50 meters, as part of the Benthic Invertebrate Diversity and Community Structure in the Sea of Marmara and Straits Project 2013-2015. The sampling has done with a box corer. Taken specimens were fixed in 4% formaldehyde water-solution and afterward they examined under Olympus BX53 optical microscope of the Department of Biology, Dokuz Eylül

University. For identification of the species, the studies of Athanasiadis (1996) and Schiffner (1916) works were used.

RESULTS

Growth of indeterminate axis sinusoidal, by slightly oblique division of apical cells successively to one side and then the other, shifting orientation at sites of future indeterminate branches; branching of determinate branches variable; gland cells cut off laterally, covering length of the bearing cell, or proximal to a lateral filament; nuclei and plastids as in Antithamnion (Nägeli, 1847).

Antithamnionella spirograpgidis is a small, delicate, dark red species of alga that can form carpets or fringes of up to 4 cm in height. The thallus is composed of a single row of cells without cortex and has creeping, prostrate axes bearing flexuous, unattached branches which can reach up to 1 cm in length. On the upper part of the algal body, there are side branches opposite to the branchlets, and 3-5 branches on the side branches produce branchlets that are biased to one side.

The opposite branchlets of the lower part of the main axis and side branches are often bent towards the axial plane. On erect branches these may be either opposite or unilateral on the axis. Rhizoids near the base of the algae often grow on the lowest cells of the branchlets. 2-3 whorl branches are found per whorl, and whorl-branchlets are initiated laterally from axial cells several below the apex of branches. In the upper parts of the thallus, 2nd and/or 3rd cells of whorl-branchlets have pitted gland cells, 10-1211 µm in diameter, which partially curve around the parent cell, cut off from their upper side. Details is shown in Figure 2, 3, 4. Basionym type locality, homotypic and heterotypic localities of species have been given as Guiry (2018).

The species has observed in northeast part of the Sea of Marmara with the coordinates 40°57'13"N 28°51'25"E, in 10-meter depth. 22 °C degree water temperature was recorded for the sampling point. 16‰ salinity, total organic carbon 0.06%, 0.10-12.53 µM ammonium nitrogen values were registered up to 10-meter depth.

Figure 2. General view of *Antithamnionella spirographidis*

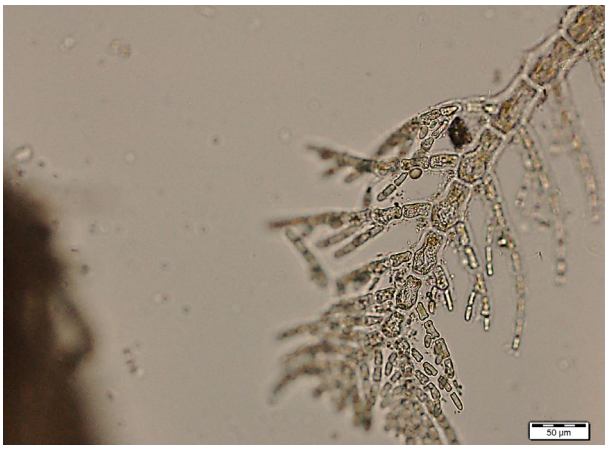

Figure 3. Apical branches of *Antithamnionella spirographidis*

Figure 4. Gland cells of *Antithamnionella spirographidis*

DISCUSSION

The distribution of the *A. spirographidis* is worldwide and it has predicted that naval ropes and worldwide oyster tread caused to the spread of the species (Haydar and Wolff, 2011; Maggs and Stegenga, 1998). This species even though widely seen in Mediterranean waters, reports indicate that it mostly seen in the western and central Mediterranean region (Furnari, 1999; Gallardo et al., 2016; Gómez Garreta et al., 2001), the easternmost place has been reported in Greece (Wollaston, 1968). Bathymetrically it is found in the low intertidal region. *A. spirographidis* is naturally attached on rocks or are epiphytic on various organic bases such as algae, molluscs and polychaetes tubes and also found on artificial substrata such as ropes and marina pontoon bridges. In our research the benthic habitat characterized by sandy and Rhodolith based bottom. Other filamentous algae such as *Polysiphonia* spp, *Ceramium* spp. were observed attached on Rhodoliths. The species was observed on the *Ceramium* spp. and *Laurencia* spp. without showing mass coverage.

As the closest point to the Sea of Marmara, the reference station was found in the Greece, where dominant species *are Cystoseira corniculata* (Turner) Zanardini, 1841, *Cystoseira schiffneri f. latiramosa* (Ercegovic) Giaccone, 1992 and

Hydrolithon farinosum (Lamouroux) Penrose and Chamberlain (1993) sampling depth reported as 0.2- 0.5 m from Malian Gulf where the gulf contact with Aegean Sea (salinity between 34.7 and 38.7‰, during year 50.000 t/year for nitrate and 30.000 t/year for phosphate) (Bacoyannis Chryssovergis, 1995). Temperature tolerance of this species are unknown but the records from the North Sea show it could resist also low temperature as well as Mediterranean temperate waters (Guiry, 1996; Stegenga et al., 1997).

Our samples were taken in June and there were no tetrasporongial individual. Spermatangia, procarps and cytocarps reported in July and December, tetrasporangia in June-July, September December and March in the works of Maggs and Hommersand (1993) while the culture plant gave rise to 1:1 ratio of female and male gametophytes, the carpospores grew into tetrasporephytes; females later also spermatagania or tetrasporocytes (Drew, 1955; L'Hardy-Halos, 1986). In another work of Maggs and Stegenga (1998) plants occur year-round but are most abundant during the second half of the year. Tetrasporangia is formed throughout the year, and gametangia in May to November.

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AUTHORSHIP CONTRIBUTIONS

The author confirm contribution to the paper as follows: study conception and design: Senem Onen Tarantini; investigation, validation, writing- reviewing & editing. All author(s) reviewed the results and approved the final version of the manuscript.

CONFLICT OF INTEREST STATEMENT

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

All relevant data is in the article.

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