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Authors Guidelines

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

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

GENERAL INFORMATION

Aim & Scope

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

The journal does not charge any submission and publication fees.

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

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

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

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

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

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

Language

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

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

Editorial Policy and Referee Process

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

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

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

Article Types

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

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

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

Review: Reviews may summarize current research areas of broad importance or provide the readers with an insightful introduction to new and groundbreaking areas of research. It should be examined and discussed in-depth and comprehensively written by the author(s) who have expertise in the subject area, not just the literature surveys.

Reports

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

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

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

MANUSCRIPT SUBMISSION

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

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

While starting

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

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To follow the status of the article;

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

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

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

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

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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 inside the manuscript placed properly (not at the end of manuscript). The line number should be given to the whole manuscript.

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

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

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

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

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

First Page

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

Author Names and Affiliation

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

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

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

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

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

Abstract

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

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

Keywords

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

Following pages

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

Introduction

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

Material and Methods

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

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

Results

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

Discussion

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

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This should briefly state the major findings of the study.

Acknowledgements and Funding

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

Examples:

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

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

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

Financial support acknowledgwment should be written like the example given:

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

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

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

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

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

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

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

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

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

Full name/s: Conceptualization, Methodology, Software

Full name: Data curation, Writing- Original draft preparation

Full name/s: Visualization, Investigation

Full name/s: Supervision

Full name/s: Software, Validation

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

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

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

Changes to Authorship

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

Conflict of Interest Statement

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

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

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

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

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

Examples:

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

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

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

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

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

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

Retrospective Ethics Approval

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

Data Availability

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

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

Examples:

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

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

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

Data availability: All relevant data is in the article.

Scientific Style

In writing of systematic /biological papers, international terminology such as "International Codes of Zoological Nomenclature (ICZN), and International Code of Nomenclature for Algae Fungi and Plants (ICNAFP)(Formerly known as the International Code of Botanical Nomenclature - CBN) International Code of Botanical Nomenclature (ICBN)" must be strictly followed. The first mention in the text of any taxon must be followed by its authority including the year. The names of genera and species should be given in italics. Clearly write the full genus name at the first occurrence in the text, and abbreviate it when it occurs again. When

referring to a species, do not use the genus name alone; Be careful when using 'sp' (singular) or 'spp.' (plural).

Equations and units

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

Abbreviations

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

Footnotes

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

References

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

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

In-Text Citation

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

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

For example: Kocataş, 1978, p. 3

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

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

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

One author:

Consider the following examples:

-…….(Kocataş, 1978)

- Kocataş (1978) states…….

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

Two authors:

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

Consider the following examples:

-…….(Geldiay and Ergen, 1972)

- Geldiay and Ergen (1972) states……

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

More than two authors:

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

See below examples:

-Geldiay et al. (1971) state…….

-……(Geldiay et al., 1971).

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

Two or more works by different author:

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

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

Two or more works by the same author:

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

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

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

For Example:

-Geldiay and Ergen, 1972a

-Geldiay and Ergen, 1972a, b

No authors:

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

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

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

No publication date:

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

Example: (Geldiay, n.d.).

Citation to secondary sources:

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

For Example:

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

Personal communication and unpublished results:

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

Example:

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

General use of websites and software:

It should be showed as below.

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

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

In References

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

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

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

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

Hanging indent paragraph style should be used.

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

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

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

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

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

For example:

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

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

For example:

Kocataş, A. (1978)

Kocataş, A., & Ergen, Z. (1972).

Kocataş, A., & Geldiay, R. (1972)

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

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

Journal Articles

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

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

Books

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

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

Chapter in books

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

E-books and chapter in e-books

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

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

Proceedings

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

Websites

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

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

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

Thesis

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

Tables and Figures

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

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The effect of gillnet twine thickness on catching efficiency and selectivity for common carp (*Cyprinus carpio* Linnaeus, 1758) fishery in Marmara Lake

Uzatma ağlarında ip kalınlığının Marmara Gölü'ndeki sazan (*Cyprinus carpio* Linnaeus, 1758) avcılığında av verimi ve seçicilik üzerine etkisi

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Abstract: The effects of multifilament gillnet twine thickness on selectivity and catching efficiency for the common carp fishery were investigated in this study. Sampling was carried out with multifilament gillnets of two different twine thicknesses (with 210d/2 and 210d/3) on 140, 150, 160, 180, 200 mm mesh sizes between June 2015 and December 2016 in Marmara Lake, located in Western Turkey. Higher modal lengths and lower catch per unit effort (CPUE) values and lower sub-minimum landing size (MLS) individuals rate (excluding 180 and 200 mm mesh sizes) were obtained in the thick multifilament material (210d/3) with the same mesh size compared to thin material (210d/2). All mesh sizes in both twine thickness provided modal lengths above the MLS. However, the thin twine material had a higher nominal percentage of undersized fish (8%), greater than the 5% accepted limit for total catches when all mesh sizes are considered together. As a result, the modal lengths and spread values increased and the selectivity and catching efficiency decreased with the thicker twine material.

Keywords: *Cyprinus carpio*, twine thickness, selectivity, catching efficiency, gillnet, Marmara Lake

Öz: Bu çalışma ile sazan avcılığında kullanılan multifilament sade uzatma ağlarının ip kalınlığının, seçicilik ve av verimliliği üzerine etkileri araştırılmıştır. Denemeler, Türkiye'nin batısındaki Marmara Gölü'nde, 140, 150, 160, 180, 200 mm tam göz boyundaki multifilament sade uzatma ağları ile iki farklı ip kalınlığında (210d/2 ve 210d/3), Haziran 2015 ve Aralık 2016 tarihleri arasında gerçekleştirilmiştir. Kalın ipte (210d/3), aynı göz genişliğindeki ince ipe göre (210d/2) daha yüksek model boyları, daha düşük birim çabaya düşen av miktarı (CPUE) değerleri ve daha düşük oranda yasal yakalama boyu (YYB) altında balık (180 ve 200 mm tam göz boyları hariç) elde edilmiştir. Her iki ip kalınlığında tüm ağ göz genişliklerinde YYB üzerinde model boyları sağlanmıştır. Ancak, tüm ağ boyları birlikte değerlendirildiğinde ince ipte YYB altı balık oranı (%8), toplam av için kabul edilen yasal sınırın (%5) üzerinde çıkmıştır. Sonuç olarak, kalın iple model boyları ve yayılım değerleri artmış, seçicilik ve av verimi azalmıştır.

Anahtar kelimeler: *Cyprinus. carpio*, ip kalınlığı, seçicilik, av verimi, uzatma ağı, Marmara Gölü

INTRODUCTION

Common carp (*Cyprinus carpio* Linnaeus, 1758), is the second most caught freshwater fish in Turkey comprising 22% of catches, after Tarek (*Alburnus tarichi* Guldenstaedtii, 1814), an endemic carp species found only in Turkey which comprised 28% of catches between 2008 and 2019. However, there has been a 73% decrease in common carp catches from 11,600 t to 3,100 t in the last 12 years (from 2008 to 2019) (TUIK, 2020). This drastic decline in catches situation emphasizes the need and importance of sustainable freshwater fisheries management in Turkish waters. For sustainable fisheries management, fishing gear should ensure that immature fish are excluded from catches and that only the matured stock is targeted (Armstrong et al., 1990). Thus, studies determining the selectivities of fishing gear are of great importance in advising appropriate fisheries management control (Hamley 1975; Çetinkaya et al., 1995).

There are many factors affecting gillnet selectivity (Yüksel and Aydın, 2012). A limited number of selectivity studies have been conducted on several of these factors in common carp fishing in Turkey such as the effects of mesh size (Balık, 1999; Özyurt and Avşar, 2005; Yalçın, 2006; Cilbiz et al., 2015; Şen, 2016), the color of the material (Balık and Çubuk, 2001a), and hanging ratio (Dartay and Ateşşahin, 2017) on selectivity of the common carp in gillnets.

The gillnet twine thickness and the light condition in the water are considered the most important factors affecting selectivity and catching efficiency of common carp, as they cause the fish to notice the gill net and affect their catchability (Cui et al., 1991; Özdemir and Erdem, 2006). The twine thickness becomes more important in shallow lakes where light transmission is high. There are two studies on the effect of twine thickness on selectivity and efficiency in common carp fishery. In these studies, Aras (2015) studied the selectivity with multi-monofilament nets, and Balık and Çubuk (2004) compared the effect of monofilament and multifilament materials on efficiency. There have been no prior studies on the selectivity of multifilament nets in Turkey for common carp.

Thus, this study aims to determine the effects of twine thickness of multifilament material on selectivity and catching efficiency of gillnets for common carp fishing in Marmara Lake (Figure 1), which is a very shallow lake (approximately 3-4 m deep) located in Western Anatolia, Turkey, which is an important common carp habitat and fishing site. Additionally, the selectivity and catching efficiency of 180 and 200 mm mesh size multifilament gillnets were examined for the first time.

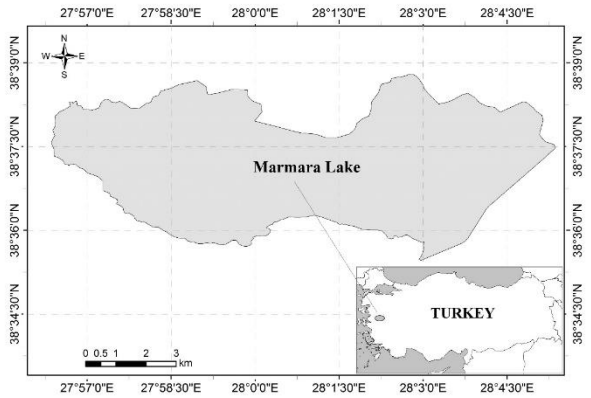

Figure 1. Study area (Marmara Lake)

MATERIAL AND METHODS

This study was carried out monthly between June 2015 and December 2016, excluding March until May (which are closed seasons for fishing), with the help of commercial fishers at different sites in Marmara Lake. In each operation, multifilament gillnets with the same characteristics as the ones used in commercial fisheries: thin twine thickness (210d/2) and thick twine thickness (210d/3), with 140, 150, 160, 180, 200 mm mesh sizes were used (Figure 2). As in commercial fishing, a passive fixed method was used to set the nets in the afternoon and retrieve them the following morning. The soak times averaged 16 hours.

After net retrieval, the caught fish were then sorted according to gillnet mesh size and twine thickness type, and then identified to species level according to Geldiay and Balık (2009). Total length was measured to the nearest 1 mm and weight was assigned to the nearest gram by a digital scale. The minimum, maximum and mean values of carp lengths and weights were calculated for each net group. The weight ratios of common carp below the minimum landing size (40 cm) were calculated for each gear type to determine the proportion of juveniles. The catch per unit effort (CPUE) was calculated as kg/1000 m using the following equation: $CPUE = \Sigma(Y/L)/n$

Y is the catch in weight (kg) of a given species in one operation, L is the length of nets standardized as 1000 m and n is the number of operation (Hyvärinen and Salojärvi, 1991; Balık and Çubuk, 2001b).

Indirect estimation using the SELECT method was used to determine selectivity (Millar, 1992 and 1995; Millar and Holst 1997; Millar and Fryer, 1999), where the expected catch proportions are fitted to the observed catch proportions using maximum likelihoods, under the assumption that catches fall under the Poisson distribution (Feller 1968; Millar and Fryer 1999).

The SELECT method is defined by the following equation;

$$n_{lj} \approx \text{Pois}\left(p_j \lambda_l r_j(l)\right) \quad \textbf{(1)}$$

where n_{lj} is the number of fish of length l caught in mesh size j, p_j is the fishing intensity, λ_l reflects the abundance of the length class l, $r_j(l)$ denotes the retention probability of length l fish in the j'th mesh size.

The Poisson distribution of the number of fish of size l caught by fishing gear with j mesh size is defined as $p_j(l)\lambda_l r_j(l)$ the selectivity curve for j mesh size. The log-likelihood of n_{lj} is:

$$\sum_l \sum_j \{n_l \log[p_j \lambda_l r_j(l)] - p_j \lambda_l r_j(l)\} \quad \textbf{(2)}$$

The selectivity parameters of nets were estimated using GILLNET software (Constat 1998) which is based on the comparison of fish caught with different nets, calculated by the parameters of five different models: Normal location, normal scale, log-normal, gamma, and bi-modal. These models are from Millar (1992); Millar and Holst (1997); and Millar and Fryer (1999):

Normal location: $$\exp\left(-\frac{(l-k.m_j)^2}{2\sigma^2}\right) \quad \textbf{(3)}$$

Normal scale: $$\exp\left(-\frac{(l-k_1.m_j)^2}{2k_2^2.m_j^2}\right) \quad \textbf{(4)}$$

Log-normal: $$\frac{m_j}{l.m_1}\exp\left(\mu - \frac{\sigma^2}{2} - \frac{\left(\log(l)-\mu-\log\left(\frac{m_j}{m_1}\right)\right)^2}{2\sigma^2}\right) \quad \textbf{(5)}$$

Gamma: $$\left(\frac{l}{(\alpha-1).k.m_j}\right)^{\alpha-1}\exp\left(\alpha - 1 - \frac{l}{k.m_j}\right) \quad \textbf{(6)}$$

Bi-modal: $$\exp\left(-\frac{(l-k_1.m_j)^2}{2k_2^2.m_j^2}\right) + c.\exp\left(-\frac{(l-k_3.m_j)^2}{2k_4^2.m_j^2}\right) \quad \textbf{(7)}$$

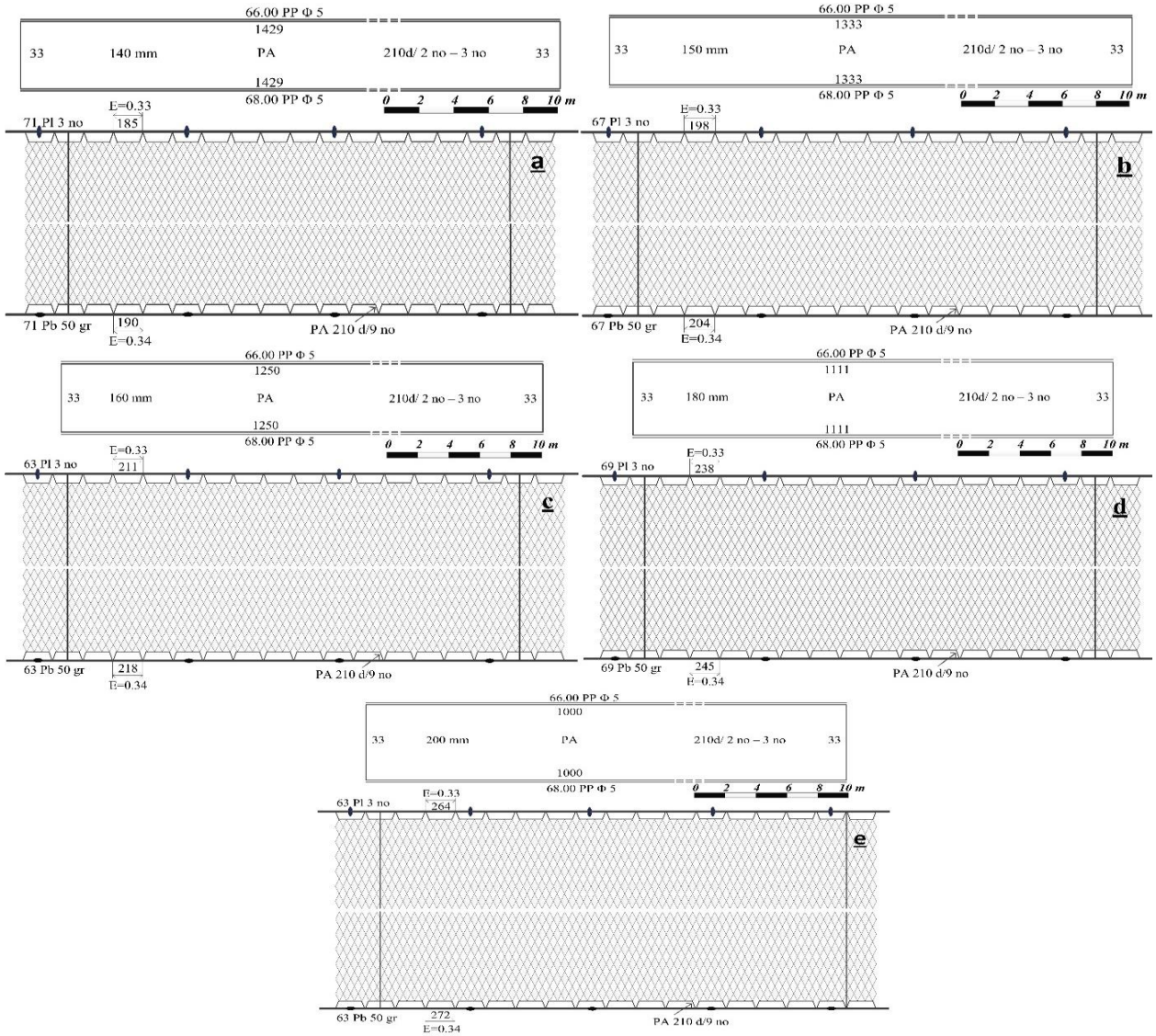

Figure 2. Scaled (top) and detailed (below) technical plans of gillnets of 210d/2 and 210d/3 (a:140 mm, b:150 mm, c:160 mm, d:180 mm, e:200 mm)

The best-suited model was then chosen according to the standard deviance values of the models, and the selectivity curves were plotted according to the parameters of the model with the lowest deviance value (Millar and Holst, 1997; Park et al., 2004). According to this model, optimum modal lengths and spread values were determined and deviance residuals were plotted using the deviance values.

The IBM SPSS (Version 22) program was used in statistical evaluations. The Kolmogorov–Smirnov (K-S) test was used to compare the catch size-frequency distributions. The normality of data was tested (Shapiro-Wilk test) and, whenever necessary, the log-transformation log (x+1) was used. To compare the mean length and CPUE, either the t-test or Mann-Whitney U test was used, depending on if the data were normally distributed. All collected data were pooled together for each mesh size and gear type before the K-S, t-test and Mann-Whitney U test were performed.

RESULTS

From a total of 48 catch operations, a total of 440 fish from four species totaling 970.7 kg were caught. The target species, common carp was the most captured species in both twine thicknesses net groups (390 n; 89% of the number of total fish, and 942.5 kg; 97% of the total catch weight). Also, 40 individuals of pike perch (*Stizostedion lucioperca* Linnaeus, 1758), six individuals of gibel carp (*Carassius gibelio* Bloch, 1782), four individuals of mirror carp (*Cyprinus carpio* L., 1758 var. *specularis)* were caught. In addition to fish, ten crayfish (*Astacus leptodactylus* Eschscholtz, 1823) were caught. According to the net groups, the common carp distributions based on twine thicknesses are presented in Table 1.

Table 1. Total length and weight values of common carp in the 210d/2 and 210d/3 (n: number of fish caught, min: minimum, max: maximum, se: standard error)

	Mesh size (mm)	n	Total weight (kg)	Total length (cm)			Weight (kg)		
				Min.	Max.	Mean±SE	Min.	Max.	Mean±SE
Thin 210d/2	140	81	114.1	23	66.5	44.9 ± 0.66	0.17	4.7	1.41 ± 0.06
	150	85	130	32.2	81.5	45.9 ± 0.75	0.5	7.8	1.53 ± 0.11
	160	27	84.3	32	82	58.6 ± 2.55	0.51	8.1	3.25 ± 0.41
	180	22	116.6	27.5	88.2	67.8 ± 3.5	0.36	10.1	5.3 ± 0.59
	200	10	82.4	66	105.2	80.1 ± 4.08	3.85	18.2	8.24 ± 1.46
	Total	225	527.4						
Thick 210d/3	140	61	107.5	32	91	48.1 ± 1.03	0.61	10.7	1.92 ± 0.17
	150	53	100.1	31	93	48.4 ± 1.31	0.5	11.6	1.89 ± 0.22
	160	26	80.3	44.2	90.1	57.9 ± 2.60	1.3	10.7	3.09 ± 0.53
	180	16	63.5	28	81.1	61.9 ± 4.26	0.88	7.1	3.97 ± 0.56
	200	9	63.7	67.7	82.5	76.7 ± 1.79	5.85	10.3	7.08 ± 0.45
	Total	165	415.1						

A total of 225 individuals (527.4 kg) were caught in the thin 210d/2 gillnets, and 165 individuals (415.1 kg) were caught from the thicker 210d/3 gillnets. Mean lengths and weights for 140, 150, 160, 180 and 200 mm mesh sizes in the 210d/2 and 210d/3 are presented in Table 1. According to increasing mesh size; mean lengths and weights of the carp increased linearly for both twine thicknesses of gillnets, except for the 140 and 150 mm mesh sizes of the 210d/3.

Length-frequency distributions of common carp are provided in Figure 3, and are combined here for 210d/2 and 210d/3. For both net types, the length distribution ranged from 23-105.2 cm (Figure 3).

The ratios of common carp landed below the MLS (40 cm) for 140, 150, 160, 180, 200 mm mesh sizes (210d/2) were 12.3, 8.2, 3.7, 9.1 and 0%, respectively (Figure 4). For 210d/3, the percentages of undersized common carp were 6.6, 5.7, 0, 12.5 and 0%, respectively. When all mesh sizes are considered together, twenty of the fish (8%) caught in the 210d/2 were under the MLS size and nine carp (4.5%) caught in the 210d/3 were below the MLS size as presented in Figure 4.

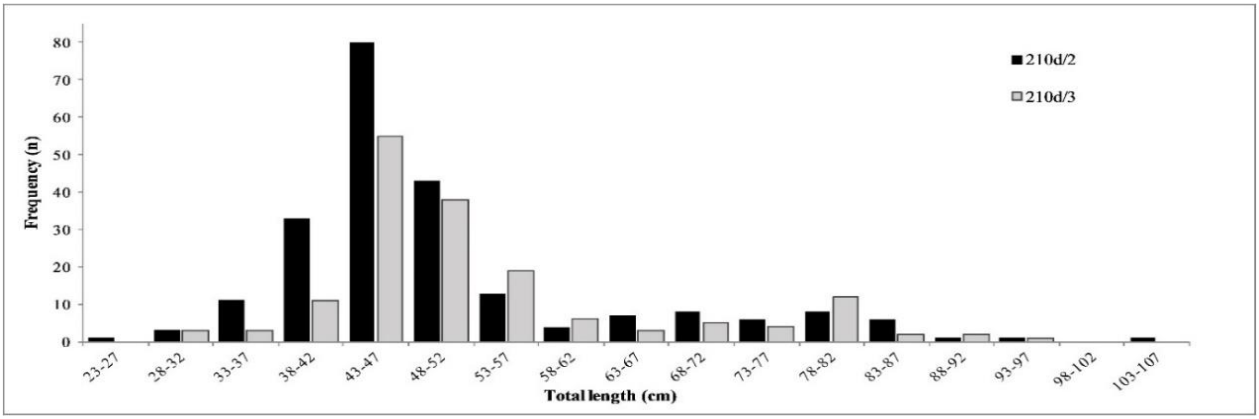

Figure 3. Comparison of total length-frequency distributions of carp caught in 210d/2 (black bars) and 210d/3 (grey bars)

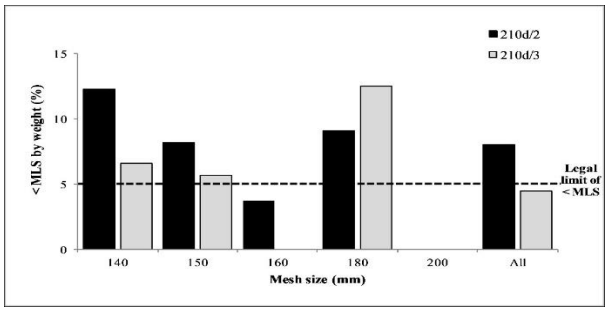

Figure 4. Comparison of the ratios of undersized common carp caught in 210d/2 (black bars) and 210d/3 (grey bars)

The highest CPUE values were determined as 13.27 kg/1000 m in 150 mm mesh size for 210d/2 and as 10.97 kg/1000 m in 140 mm mesh size for 210d/3. The CPUE value decreased with increasing mesh size above 140 mm in the 210d/3 (Table 2).

A comparison of mean total lengths (L), length frequencies and CPUE values of common carp in 210d/2 and 210d/3 are provided in Table 2. Although there were no statistical difference, proportionally higher CPUE values were obtained at 210d/2 in all mesh sizes (Table 2). When all mesh sizes are considered together, the CPUE value of the thinner 210d/2 gillnets was 1.27 times more efficient than the thicker 210d/3 gillnets.

Table 2. Comparison of mean total lengths (L), length frequencies and catch per unit effort (CPUE) values of common carp in gillnets with 2 different twine thicknesses (210d/2 and 210d/3)

Mesh size (mm)	L (mean±se) 210d/2	210d/3	Mann-Whitney U test	t-test	K-S test	CPUE (kg/1000 m) 210d/2	210d/3	Ratio (210d/2: 210d/3)	Mann-Whitney U test
140	44.93±0.66	48.11±1.03	P<0.05 (P=0.004)		P<0.05 (P=0.010)	11.66	10.97	1.06	P>0.05 (P=0.198)
150	45.9±0.75	48.42±1.31	P<0.05 (P=0.032)		P>0.05 (P=0.097)	13.27	10.22	1.30	P>0.05 (P=0.443)
160	58.59±2.55	57.93±2.6	P>0.05 (P=0.742)		P>0.05 (P=0.607)	8.6	8.2	1.05	P>0.05 (P=0.816)
180	67.8±3.5	61.86±4.26		t=1.084 df=36 P>0.05 (P=0.286)	P>0.05 (P=0.496)	11.91	6.48	1.84	P>0.05 (P=0.075)
200	80.11±4.08	76.76±1.79		t=0.752 df=12.287 P>0.05 (P=0.466)	P>0.05 (P=0.435)	8.41	6.51	1.29	P>0.05 (P=0.974)

The mean lengths were higher in 210d/3 with 140 and 150 mm mesh sizes ($p<0.05$). A significant difference also was found between length frequencies for only 140 mm according to the Kolmogorov-Smirnov test ($p<0.05$). Alternatively, the 210d/2 showed higher mean lengths in the 160, 180 and 200 mm mesh sizes, although these were not significant (Table 2).

The selectivity parameters for common carp calculated in the SELECT method are presented in Table 3. By comparing the deviances of the five models in the SELECT method, the normal location model due to its lowest deviance value was the most appropriate model for both sets of twine thicknesses.

Table 3. Selectivity parameter values for 210d/2 and 210d/3 (α, k, μ, σ, k_1, k_2, k_3, k_4: Selectivity constants of models)

Twine thicknesses	Model	Equal fishing powers parameters	Model Deviance	p-value	Fishing power α mesh-size parameters	Model Deviance	p-value	Degree of Freedom (df)
	Normal Location	**(k; σ) (4.0373,10.3334)**	**186.45**	**0.6385**	**(k; σ) (4.1308, 10.3988)**	**185.86**	**0.6500**	**194**
	Normal Scale	(k_1; k_2) (4.1162, 0.5713)	189.83	0.5712	(k_1; k_2) (4.1956, 0.5640)	189.97	0.5684	194
210d/2	Gamma	(k; α) (0.1008, 40.5836)	188.21	0.6037	(k; α) (0.1008, 41.5836)	188.21	0.6037	194
	Log Normal	(μ; σ) (4.0371, 0.1692)	190.87	0.5501	(μ; σ) (4.0657, 0.1692)	190.97	0.5501	194
	Bi-modal	No Fit				No Fit		
	Normal Location	**(k; σ) (4.2120, 12.2172)**	**165.23**	**0.9024**	**(k; σ) (4.3374, 12.3421)**	**165.25**	**0.9023**	**190**
	Normal Scale	(k_1; k_2) (4.3691, 0.6804)	170.59	0.8406	(k_1; k_2) (4.4743, 0.6691)	170.82	0,838	190
210d/3	Gamma	(k; α) (0.1360, 32.0356)	165.86	0.8963	(k; α) (0.1360, 33.0356)	165.86	0.8963	190
	Log Normal	(μ; σ) (4.1029, 0.1930)	166.65	0.8880	(μ; σ) (4.1402, 0.1930)	166.54	0.8880	190
	Bi-modal	No Fit				No Fit		

Table 4. Modal lengths and spread values for 210d/2 and 210d/3

Twine	Mesh size	Modal length	Spread value
	140	56.59	
	150	60.56	
210d/2	160	64.59	10.33
	180	72.67	
	200	80.74	
	140	58.97	
	150	63.18	
210d/3	160	67.39	12.22
	180	75.82	
	200	84.24	

The modal lengths and spread values calculated according to the normal location model for 140, 150, 160, 180, and 200 mm mesh sizes are presented in Table 4.

The modal lengths increased with mesh size in both twine thicknesses nets. The fitted selectivity curves with the corresponding deviance residual plots are presented in Figure 5. 210d/3 has higher values in modal lengths of the same mesh sizes compared to 201d/2. In addition, 210d/3 were higher in spread value (12.22 cm) compared with 210d/2 (10.33 cm) (Table 4 and Figure 5)

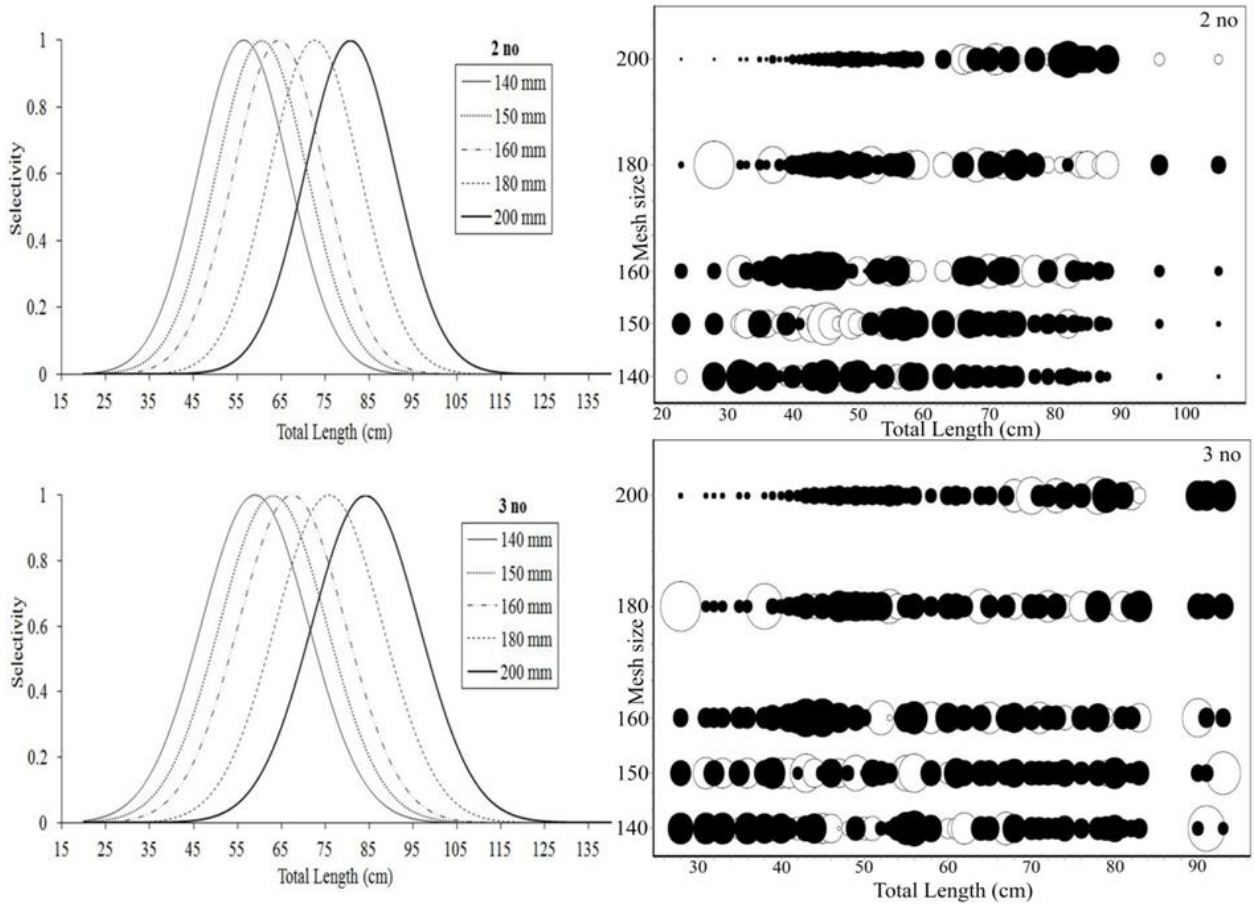

Figure 5. Selectivity curves of 210d/2 and 210d/3, and deviance residual plots (● positive residual, ○ negative residual)

DISCUSSION

Higher modal lengths were found using the thicker multifilament material (210d/3) compared to thinner material (210d/2) in this study. Similarly, Aras (2015) reported that thicker twine material had higher modal lengths than thin twine material for multi-monofilament gillnets in carp fishing. Different results were reported on the effect of twine thickness for other fish species. For example, Hansen (1974), Holst et al. (2002), and Ayaz et al. (2011) found that thinner twines have higher modal lengths than thicker ones, while Yokota et al. (2001) found thicker twine materials to have higher modal lengths than thinner materials. On the other hand, according to Hovgard (1996), Gray et al. (2005), and Turunen (1996) there was no difference found for modal lengths relating to net twine thickness. This variety of results may be resultant of different gear materials (mono vs. multifilament), or from different target species (Ayaz et al., 2011). In terms of selectivity in this study, it was determined that thicker multifilament material (210d/3) were less selective due to their higher spread values.

Regarding other selectivity studies on carp, generally other materials such as monofilament and multi monofilament, and mesh sizes less than 140 mm were considered (Table 5). This study demonstrated model lengths of overlapping mesh size (140 mm) on both thin and thick nets higher than in another study by Aydın et al. (2016), from the same lake, which may be attributable to the modeling used by Aydın et al. (2016). The selectivity results in this study were lower for 140 mm in 210d/2 and higher for 150 and 160 mm in 210d/3 nets than the study conducted in Demirköprü Dam Lake by Şen (2016). While our study was carried out in the shallow (4-5 m) lake, Demirköprü Dam Lake is a deeper lake with a depth of 50 m, thus, it is likely that the nutritional differences of the lakes may affect the overall condition, girth, and thus selectivity of the fish. Since the selectivity of 180 and 200 mm mesh size multifilament gillnets are presented here for the first time, this cannot be compared with other studies (Table 5).

Table 5. Selectivity studies conducted on common carp (*: Bar length, ^: These mesh sizes were not used and their modal lengths were determined by modelling, n: Number of fish caught, E: Hanging ratio)

Author	Study area	Method	n	Mesh size (mm)	Material	Model length (cm)
Özyurt and Avşar (2005)	Seyhan Dam Lake	Holt (1963)	294	28*	Monofilament gillnets	17.55
				32*		20.06
				40*		24.44
				45*		27.5
Carol and Garcia-Berthou (2007)	Different Reservoirs in Catalonia	SELECT	116	29	Monofilament gillnets	10.89
				38		14.27
				51		19.15
				64		24.03
				84.5		31.73
				101.5		38.12
				135.5		50.89
				177.5		66.66
				201.5		75.67
				253		95.01
Cilbiz et al. (2015)	Manyas Lake	SELECT	208	100	Monofilament trammel nets	39.05
				110		42.95
				120		46.85
				130		50.76
				140		54.66
Aras (2015)	Keban Dam Lake	SELECT	219	40*	0.12 mm Multi-monofilament gillnets	26.88
				45*		30.24
				50*		33.6
				55*		36.96
				60*		40.32
			232	40*	0.18 mm Multi-monofilament gillnets	27.2
				45*		30.6
				50*		34
				55*		37.4
				60*		40.8
Aydın et al. (2016)	Marmara Lake	SELECT	40	40	Multifilament gillnets	12.98
				60		19.47
				80		25.96
				100		32.45
				110^		35.7
				120^		38.94
				130^		42.19
				140^		45.43
			79	40	Multifilament trammel nets	12.4
				60		18.6
				80		24.8
				100		31
				110^		34.1
				120^		37.2
				130^		40.3
				140^		43.4
Şen (2016)	Demirköprü Dam Lake	SELECT	239	65*	Multifilament gillnets (210d/2)	53.29
				70*		57.39
				75*	(210d/3)	61.49
				80*	(210d/3)	65.59
Dartay and Ateşşahin (2017)	Keban Dam Lake	Holt (1963)	142	45*	Multi-monofilament gillnets (E=0.5)	29.72
				50*		33.02
				55*		36.32
				60*		46.62
			116	45*	Multifilament gillnets (E=0.5)	29.33
				50*		32.58
				55*		35.84
				60*		37.10
			117	45*	Multi-monofilament gillnets (E=0.67)	30.47
				50*		33.86
				55*		37.24
				60*		40.63
			103	45*	Multifilament gillnets (E=0.67)	28.61
				50*		31.79
				55*		34.96
				60*		38.64

Table 5. Continued

Author	Study area	Method	n	Mesh size (mm)	Material	Model length (cm)
Present study	Marmara Lake	SELECT	225	140	Multifilament	56.59
				150	gillnets (210d/2)	60.56
				160		64.59
				180		72.67
				200		80.74
			165	140	Multifilament	58.97
				150	gillnets (210d/3)	63.18
				160		67.39
				180		75.82
				200		84.24

One of the basic principles guiding sustainable fisheries is to allow a stock to reproduce at least once before they are caught to replenish their population. Using this principle, the modal lengths of fishing gears should ideally be higher than the lengths of first maturity (Lm_{50}) and minimum landing size (MLS). Lm_{50} value for common carp in Marmara Lake has not been reported yet in the literature. However, the MLS for common carp under the current national fisheries legislation is 40 cm for all inland water areas (Anonymous, 2020). When the MLS are examined, both thin (210d/2) and thick (210d/3) material gillnets provided modal lengths over the MLS in this study. On the other hand, fishers are not allowed to land undersized specimens in amounts exceeding 5% of total catch weight (Anonymous, 2020). This study showed that the undersized fish ratio in thick nets (4.5%) was lower than the legal limit (5%), while the ratio of undersized fish in thin nets (8%) was higher, when all mesh sizes are considered together. A lower rate of undersized fish (excluding 180 and 200 mm mesh sizes) was found with thicker twine material of the same mesh size. However, the undersized ratios in three mesh sizes (140, 150, and 180 mm) were not below the legal limits, which should be emphasized for improved technical measures pertaining to the sustainability of common carp in Turkey.

Higher CPUE values were obtained in thin nets than thick nets of the same mesh sizes, but this was not significant. Also, interestingly, this study found total CPUE values of the thinner 210d/2 gillnets to be 1.27 times more efficient than the thicker 210d/3 nets. Hamley (1975) and Jensen (1995) also reported thinner nets to have higher catchabilities than thicker ones owing to lower visibility and higher flexibility, as long as the twine is not too thin that it can be easily torn by larger fish.

CONCLUSION

In this study, the thicker net material resulted in higher modal lengths and spread values and lower catching efficiency and selectivity. All twine thicknesses and mesh sizes in this study provided modal lengths above MLS. Thus, these gear types are sufficient for common carp fisheries in Turkey. However, to achieve improved sustainability in the rapidly declining carp fisheries, we recommend the use of thick nets (210d/3) for 140 and 150 mm mesh sizes, and thinner nets (210d/2) for the larger mesh sizes for the common carp gillnet fishery in Marmara Lake.

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AUTHORSHIP CONTRIBUTIONS

Hakkı Dereli: Conceptualization, methodology, formal analysis, writing - original draft preparation, writing-review and editing, software, visualization, project administration. Turhan Kebapçıoğlu: Investigation, writing-review and editing. Yusuf Şen: Investigation, formal analysis, writing-review and editing, visualization. Zeki Serkan Ölçek: Investigation. Ezgi Dinçtürk: Investigation. Aylin Ulman: 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 availability: For questions regarding datasets, the corresponding author should be contacted.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Diagnosis of bacterial fish diseases and classification of serotypes with slide agglutination method

Lam aglütinasyon metodu ile bakteriyel balık hastalıklarının teşhisi ve serotiplerin sınıflandırılması

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Abstract: Bacterial fish pathogens cause significant losses in rainbow trout farms. In fish farms, bacterial pathogens cause threatening diseases which has made it necessary to develop rapid methods for disease diagnosis. Serological techniques which are applied with a small amount of antiserum and sample, are preferred for the rapid diagnosis of fish diseases. In this study, formalin-killed antigens prepared from reference strains of *Lactococcus garvieae*, *Yersinia ruckeri*, and *Vibrio (Listonella) anguillarum* were injected intravenously in consecutive doses to New Zealand rabbits. One week after the last injection, the sera separated to use in the slide agglutination tests. A total of 42 strains were studied, including *Y. ruckeri* (18 isolates), *V. anguillarum* (14 isolates), and *L. garvieae* (7 isolates) and 3 references (ATCC 43305, ATCC 29473, ATCC 49156) strains. Serotype O1 determined the predominant serotype (86%) in *V. anguillarum* and *Y. ruckeri* (84%) strains examined by the slide agglutination method. *L. garvieae* strains did not react against Japanese antisera but positively reacted against Turkish *L. garvieae* antisera.

Keywords: Bacterial fish pathogens, diagnosis, serological characterization

Öz: Bakteriyel balık patojenleri gökkuşağı alabalığı yetiştiriciliği yapan balık çiftliklerinde ciddi kayıplara sebep olmaktadır. Su ürünleri yetiştiriciliğinde hastalık etkeni bakteriyel patojenler, oluşan hastalıkların teşhisi için hızlı teşhis yöntemlerinin gelişmesine yol açmıştır. Hastalıklar yetiştiricilik tesislerinde görüldükten sonra balık patojenlerinin hızlı teşhis edilmesi, hastalıkların tedavi edilerek oluşacak ekonomik kayıpların önüne geçilmesi için önemlidir. Balık hastalıklarının hızlı teşhisinde az miktarda antiserum ve örnek ile uygulanabilen serolojik teknikler tercih edilmektedir. Bu çalışmada *Lactococcus garvieae*, *Vibrio (Listonella) anguillarum* ve *Yersinia ruckeri*'nin referans suşlarından antijenler kullanılmak üzere formalin ile inaktive edilerek, Yeni Zelanda tavşanlarına ardışık dozlarda intravenöz enjeksiyonlar gerçekleştirilmiştir. Son enjeksiyon yapıldıktan bir hafta sonra antiserum elde edilmiştir. 18 *Y. ruckeri*, 14 *V. anguillarum* ve 7 *L. garvieae* ve referans (ATCC 43305, ATCC 29473, ATCC 49156) suşlar dahil olmak üzere toplamda 42 suş ile çalışma yapılmıştır. Lam aglütinasyon metodu ile incelenen *V. anguillarum* suşlarında baskın olarak (%86) serotip O1, *Y. ruckeri* suşlarında (%84) serotip O1 tespit edilmiştir. *L. garvieae* suşlarının Japon KG- antiserumu ile aglütinasyon vermediği, ancak Türk KG- antiserumu ile pozitif reaksiyon oluşturduğu tespit edilmiştir.

Anahtar kelimeler: Bakteriyel balık hastalıkları, teşhis, serolojik karakterizasyon

INTRODUCTION

The risk of disease in fish increases as a result of adverse changes in the interaction between pathogen, host and environment (Toranzo, 2005). The rod-like or spherical cocci Gram-negative and Gram-positive bacterial species can cause disease outbreaks in aquaculture (Austin and Newaj-Fyzul, 2017). Infectious bacterial pathogens have been reported in the majority of the taxonomic groups. However, in the extensive production, only a few bacterial species are responsible for significant economic losses worldwide. (Toranzo et al., 2009). In addition, an extensive antigenic variation has been reported with bacterial pathogens associated with fish diseases (Leblanc et al. 1981; Nakai et al., 1981; Kitao et al., 1983; Stevenson and Airdrie, 1984; Nomura and Aoki, 1985; Sorensen and Larsen, 1986; Toranzo et al., 1987). In the rainbow trout farms, major bacterial pathogens that cause disease are *Pseudomonas fluorescens, Flavobacterium psychrophilum, Flavobacterium columnaris, Listonella anguillarum, Aeromonas hydrophila, Yersinia ruckeri* and *Lactococcus garvieae* (Toranzo, 2004).

The rapid diagnosis of diseases by serological methods has increased the accuracy in the diagnosis and reduced the time required for diagnosis from days to hours (Austin and Newaj-Fyzul, 2017). Since the identification of *Aeromonoas salmonicida* with a simple slide agglutination test by Rabb et al. (1964), the procedure was improved and applied to numerous bacterial fish pathogens. (Eurell et al., 1979, Toranzo et al., 1987; Romalde et al., 1995). Several

monoclonal and polyclonal antibodies against fish pathogens are available commercially, and the selected antibody used in the tests are critical for immunoserological diagnosis. Monoclonal antibodies (mAbs) detect only one epitope on a single target antigen and comprise a homogenous cloned immunoglobulin with high specificity, whereas polyclonal antibodies contain heterogeneous mixed immunoglobulin molecules that can recognize multiple epitopes on a single antigen are superior for the detection of pathogens (Austin and Newaj-Fyzul, 2017).

Vibriosis caused by *V. anguillarum* is probably one of the oldest recognized bacterial fish diseases and is pathogenic to many fish and shellfish (Larsen, 1990; Hickey and Lee, 2017; Hansen et al., 2020). So far, *V. anguillarum* has been divided into 23 O serogroups, (Pacha and Kiehn, 1969; Sorensen and Larsen, 1986; Kitao et al., 1983; Kitao et al., 1984 Grisez and Ollevier, 1995; Pedersen et al., 1999) however between these serotypes O1 and O2 associated with the most isolated and virulent serotypes (Toranzo et al., 2017). *Y. ruckeri,* the causative agent of Enteric Redmouth Diseases has two commonly used serological schemes for the classification. Davies divided *Y. ruckeri* into five serotypes named O1, O2, O5, O6, O7 (Davies, 1990) and Ormsby et al. (2016) extended this scheme with serotype O8. Romalde et al. (1993) described four serotypes subdivided into subgroups O1 (a, b), O2 (a, b, c), O3, and O4. In the serological tests, *L. garvieae* strains have been divided into two serotypes named KG^- and KG^+ that can be differentiated by an agglutination test (Kitao, 1982; Yoshida et al., 1997; Romalde and Toranzo, 2002). In addition, the KG^- strain produces a capsule on its cell surface, which is pathogenic to fish (Yoshida et al., 1997) however, isolates might have result with losing capsule due to subculturing (Morita et al., 2011)

In this study, the major bacterial fish pathogens (*V. anguillarum, Y. ruckeri, L. garvieae*) isolated from different rainbow trout farms between 2014-2021 in the South Aegean region of Turkey were tested for serological diagnosis and classification of serotypes. Proper and rapid diagnosis for the diseases leads to appropriate treatment and avoid indiscriminate use of chemotherapeutics in the fish farm. However, it is essential to study characteristics of bacterial strains and develop better control and treatment strategies in order to prevent economic losses besides the serological classification of the serotypes would contribute to vaccine studies.

MATERIAL AND METHODS

Bacterial strains

Total of 42 strains, including three reference strains (ATCC 43305, ATCC 29473, ATCC 49156) received from Izmir Katip Celebi University Fish Diseases and Biotechnology Laboratory for determination of serological characteristics. In the slide agglutination tests *V. anguillarum* (15 isolates), *Y. ruckeri* (19 isolates), and *L. garvieae* (8 isolates) were examined. *V. anguillarum, Y. ruckeri, L. garvieae* isolates (except ATCC 49156, ATCC 43305 and ATCC 29473) were isolated from rainbow trout in the cases of Vibriosis, Yersiniosis, and Lactococcosis occurred between 2014-2021 in the Southern Aegean Region of Turkey. *V. anguillarum* and *Y. ruckeri* isolates were subcultured on TSA (Tryptic Soy Agar) and incubated at 21ºC, *L. garvieae* strains were subcultured to TSA and incubated at 30ºC to check purity by morphological characteristics and biochemical analysis.

Preparation of thermostable somatic "O" antigens

For agglutination tests, heat-stable somatic O antigens of *V. anguillarum* and *Y. ruckeri* were prepared as described by Davies (1990) and Toranzo et al. (1987). These suspensions are used in the slide agglutination tests as somatic antigens.

Antigens for immunization

Antigens were prepared as described by Toranzo et al. (1987). Reference strains of *V. anguillarum* O1 (ATCC 43305) were streaked on TCBS, *Y. ruckeri* O1 (ATCC 29473) on Waltman-shotts medium to incubated at 21ºC for 24-48h. *L. garvieae* KG- (ATCC 49156), biochemically and molecularly identified (GenBank: MT876413) *L. garvieae* (C3) steaked on TSA and incubated at 30ºC for 24-48 hours. Bacteria inoculated into TSB for grown overnight and killed by adding 2% (v/v) formalin into the culture. Formalin-killed cells were centrifugated and washed twice with 0.3% (v/v) formalin. Formalin-killed cells resuspended with 0.85% saline for centrifugation and density were adequate to 10^9 cells/ml, the density of a McFarland standard No.3.

Obtention of antisera

Antisera is produced from New Zealand rabbits according to Toranzo et al. (1987). Rabbits were injected intravenously with saline washed suspensions (the density of McFarland No.3) of formalin-killed cells. Injections were given to the rabbits on day 1 (0.25 ml), 2 (0.50 ml), 3 (1.0 ml), 4 (2.0 ml) and 11 (1.0 ml), respectively.

One week after the last injection, rabbits bled from the ear vein (Figure 1). Blood was allowed to clot at room temperature for one hour and left at 4ºC overnight. The serum is separated and stored at -20ºC until agglutination assays (Davies, 1990). In addition, blood was collected from non-immunized rabbits to obtain the serum and used in the slide agglutination tests for controls.

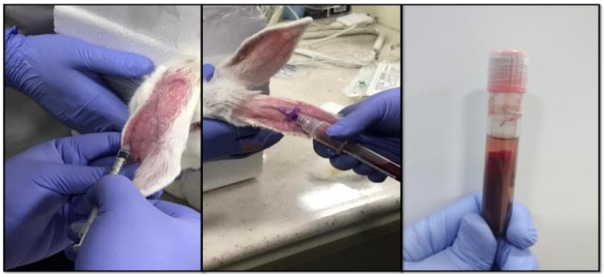

Figure 1. One week after the last injection, blood collection from rabbits and the obtained antiserum

Slide agglutination tests

Serological identification of bacterial strains performed on a black background by slide agglutination method using a loopful of whole-cell antigens against undiluted antisera. Serological classification of *V. anguillarum* and *Y. ruckeri* strains was tested by using heat-stable O antigens against representative antisera.

In the slide agglutination tests intensity of reactions (Figure 2) was determined as; no reaction (-), weak agglutination (+) after 5 minutes considered as a negative result, and a distinct and immediately occurring moderate (++), strong (+++) very strong (++++) agglutination considered as a positive result.

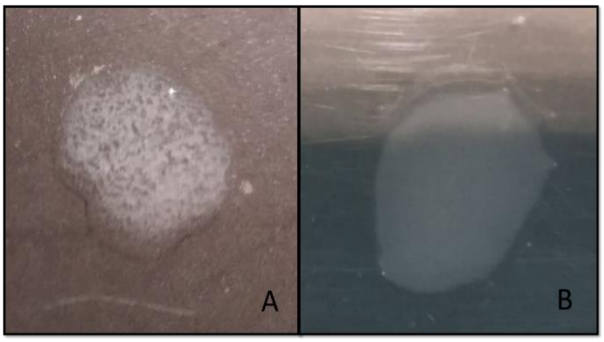

Figure 2. Formation of antigen-antibody clumps visible with naked eye in the slide agglutination test. A: Positive reaction, B: Negative reaction

RESULTS

Agglutination reactions

Whole-cell of 15 *V. anguillarum* isolates showed positive reaction against representative antisera in the tests, however strains V12 and V13 considered as negative when tests performed with somatic O antigens other strains (86%) belonged to serotype O1. Whole-cell and thermostable somatic antigens of *Y. ruckeri* strains did not show any difference against representative antisera and majority (%84) of the strains belonged to serotype O1. *L. garvieae* strains show serological differences based on the geographical origin of the isolate. In addition, in their groups, the biochemical properties of the strains (except ATCC 49156, ATCC 43305, ATCC 29483) were the same for *L. garvieae* isolates, *Y. ruckeri* isolates besides *V. anguillarum* where strain V12 and V13 did not ferment arabinose.

The results obtained from whole-cell and thermostable somatic O antigens of *V. anguillarum* isolates in the slide agglutination tests are presented in Table 1.

Table 2 shows the agglutination reactions of whole-cell and thermostable somatic antigens of *Y. ruckeri* strains against representative antisera.

Table 1. Slide agglutination test results of *V. anguillarum* strains

Whole-cell antigens	*V. anguillarum* O1 (ATCC 43305) antisera	O antigens	*V. anguillarum* O1 (ATCC 43305) antisera
ATCC 43305	++++	ATCC 43305	++++
V3	+++	V3	+++
V12	++	V12	+
V13	++	V13	+
V17	+++	V17	+++
V20	++++	V20	++++
V22	+++	V22	+++
V24	++++	V24	++++
V29	++++	V29	++++
V31	+++	V31	+++
V32	++++	V32	++++
V34	++++	V34	++++
V35	++++	V35	++++
V37	++++	V37	++++
SVA	++++	SVA	++++

Intensity of the reaction: No reaction; -, weak; +, moderate; ++, strong; +++, very strong; ++++

Table 2. Slide agglutination test results of *Y. ruckeri* strains

Whole-cell antigens	*Y. ruckeri* O1 (ATCC 29473) antisera	O antigens	*Y. ruckeri* O1 (ATCC 29473) antisera
ATCC 29473	++++	ATCC 29473	++++
YR5	++++	YR5	++++
YR241118	++++	YR241118	++++
Y1	++++	Y1	++++
Y3	++++	Y3	++++
Y6	++++	Y6	++++
Y12	+++	Y12	+++
Y31	+++	Y31	+++
Y32	-	Y32	-
Y33	++++	Y33	++++
Y34	-	Y34	-
Y35	-	Y35	-
Y36	+++	Y36	+++
Y37	+++	Y37	+++
C26	++++	C26	++++
C27	++++	C27	++++
C29	++++	C39	++++
S31	++++	S31	++++
KYB	++++	KYB	++++

Intensity of the reaction: No reaction; -, weak; +, moderate; ++, strong; +++, very strong; ++++

Table 3 shows the agglutination reactions of *L. garvieae* strains against Japanese KG$^-$ (ATCC 49156) and Turkish KG$^-$ (C3) antisera. In the slide agglutination tests, 7 strains isolated from rainbow trout in the Southern Aegean Region of Turkey did not react with Japanese KG$^-$ *L. garvieae* antisera. However, in the tests against Turkish KG$^-$ *L. garvieae* antisera, 6 strains show very strong agglutination (++++), and 1 strain reacted as strong agglutination (+++).

ATCC 49156 showed no reaction (-) with Turkish KG- *L. garvieae* antisera but gave very strong agglutination (++++) with its own antisera (Japanese KG- antisera).

Table 3. Slide agglutination results of *L. garvieae* strains

Antigen	*L. garvieae* ATCC 49156 (Japanese KG-) antisera	*L. garvieae* C3 (Turkish KG-) antisera
ATCC 49156	++++	-
C3	-	++++
C12	-	+++
ELG	-	++++
SLG	-	++++
BLG	-	++++
LGSO1	-	++++
LGSO4	-	++++

Intensity of the reaction: No reaction; -, weak; +, moderate; ++, strong; +++, very strong; ++++

DISCUSSION

It is critical to apply appropriate control strategies against the causative agents of fish diseases. Different serological procedures have been used to diagnose fish pathogens. It is known that long-term intensive vaccination can cause a consistent selective pressure resulting with the appearance of a distinct serotype (Bachrach et al., 2001). Antigenic variations have been reported for bacterial fish pathogens associated with fish diseases. Bacterial fish pathogens from different sources of isolation or origins can be identified in slide agglutination tests (Toranzo et al., 1987; Kang et al., 2004; Balta et al., 2010; Ürkü and Timur, 2014; Balta et al., 2016). Rapid and preliminary screening of the majority for bacterial pathogens is applicable with whole-cell antigens against representative antisera. However, it is necessary to use thermostable somatic O antigens for serogroups. (Toranzo et al., 1987; Davies, 1990; Romalde et al., 2003; Ormsby et al., 2016).

V. anguillarum affects salmonid and non-salmonid fish worldwide and, this pathogen has been divided into 23 O serotypes however, serotypes that cause mortalities in fish reported for only serotype O1, serotype O2 and less extent serotype O3 (Toranzo et al., 2017). In the present study, antisera was raised from rabbit against the reference strain of *V. anguillarum* O1. In this study when whole cells were utilized agglutination was observed for all strains against O1 antisera, however, V12 and V13 coded strains show moderate agglutination. When heat stable O-antigens were used in the agglutination experiments, strains V12 and V13 were considered negative and were untypable. The biochemical properties of *V. anguillarum* strains were the same except fermentation of arabinose. All the *V. anguillarum* O1 isolates fermented arabinose, whereas untypable isolates (V12 and V13) were unable to ferment. In their review, Toranzo and Barja (1990) reported that strains of *V. anguillarum* serotype O1 fermented arabinose, but strains of serotype O2 could not. Likewise, Larsen and Olsen (1991) stated that *V. anguillarum* strains of serotype O1 were arabinose-positive (97%), whereas strains of serotype O2 were arabinose variable (37%).

Sorensen and Larsen (1986) reported 270 *V. anguillarum* strains were isolated from diseased fish (157 from rainbow trout; 64 from cod; 40 from eels; 9 from plaice). Agglutination assays against representative antisera revealed serotype O1 was the dominant serotype isolated from cultured fish and serotype O2 from wild fish. Tanrıkul (2007) isolated *V. anguillarum* from diseased fish in eight different rainbow trout farms in the South Aegean region and reported the *V. anguillarum* isolates belonged to serotype O1. Avsever and Un (2015) observed serological characterization of 51 *V. anguillarum* strains isolated from 6 different fish farms located in the Aegean Region of Turkey. In the slide agglutination tests against serotype O1, O2, and O3 antisera, the authors stated that 42 strains belonged to serotype O1 and 9 strains to serotype O2. Balta and Dengiz Balta (2017) observed diseased rainbow trout farms between 1999-2014 and isolated 32 *V. anguillarum* strains from 12 different farms located in the Black Sea Region of Turkey. To understand the diversity of the strains, the authors performed slide agglutination tests and reported *V. anguillarum* strains belonged to serotype O1. In accordance with the previous studies, the present study has demonstrated similar results within the diversity of *V. anguillarum* strains.

Wide diversity has been reported in *Y. ruckeri* isolates able to cause infection in rainbow trout and Atlantic salmon. *Y. ruckeri* outbreaks are associated with rainbow trout dominantly represented by serotype O1, whereas the predominant serotype associated with Atlantic salmon is associated with serotype O2, O5, and O8 worldwide. (Ormsby and Davies, 2021). The findings of this study indicate that serotype O1 was responsible for the majority of the ERM cases, except Y32, Y34, and Y35 did not agglutinate with serotype O1 antisera and were untypable. Davies (1990) observed serological characterization of 131 *Y. ruckeri* strains including 127 *Y. ruckeri* and 4 reference strains. Heat-stable O antigens of each 131 isolates were reacted against five antisera (O1, O2, O5, O6, O7) in the slide agglutination tests. Serotypes of the strains were 105 serotype O1, 11 serotype O2, 5 serotype O5, 4 serotype O6, 5 serotype O7, and 1 isolate was untypable. Romalde et al. (1993) demonstrated serological characterization of 53 *Y. ruckeri* strains isolated in Spain. Slide agglutination tests were performed against antisera raised for each serotype (O1 [a, b], O2 [a, b, c], O3, O4) from rabbits, and all the Spain strains positively reacted with serotype O1 antisera. Wheeler et al. (2009) stated serological diversity of 160 *Y. ruckeri* strain isolated from different countries. The serological tests revealed the serotypes; 128 strain determined serotype O1, 17 serotype O2, 11 serotype O5, 2 serotype O6, and 2 serotype O7. Bastardo et al. (2011) pointed out

serological characteristics of 11 *Y. ruckeri* strains isolated from diseased *Salmo salar* in Chile. Serological examinations of the isolates show the majority of the isolates were serotype O1 (9 strain O1b, one strain O1a) and 1 isolate belonged to serotype O2b. Altun et al. (2013) demonstrated the serological characterization of 15 *Y. ruckeri* strains isolated from diseased rainbow trout. Serological assays revealed the majority (11) of the isolates belonged to serotype O1 and, 4 strains were serotype O2. Our findings are consistent with the other researchers, in which serotype O1 is the predominant serotype.

Serotypes of *L.garvieae* have been reported with absence (serotype KG+) or existence (KG-) of capsular material (Romalde and Toranzo, 2002). In addition, it has been reported that the biochemical and genetic characteristics of these two serotypes are very similar to each other. (Eldar et al., 1996). In the fish farms, capsulated isolates of *L. garvieae* are stated as highly virulent, while non-capsulated isolates are hardly able to establish an infection to rainbow trout (Barnes et al., 2002). *L. garvieae* KG+ antigens were detected around the cell surface and not in the cell capsules, whereas KG- antigens were over the capsule material (Oyama et al., 2002). In this study, antisera were raised from rabbits for Japanese and Turkish *L. garvieae* KG^- strains to use in the slide agglutination tests. The present results indicate that Turkish *L. garvieae* strains did not agglutinate with Japanese *L. garvieae* antisera and, the Japanese strain did not agglutinate with Turkish *L. garvieae* antisera. In contrast, antisera raised against Turkish *L. garvieae* agglutinated with *L. garvieae* strains isolated from diseased rainbow trout in the Aegean Region of Turkey. Likewise, in the serological tests against representative KG^- and KG^+ antisera, *Enterococcus seriolicida* strains isolated from diseased yellowtail in Japanese by Yoshida et al. (1996) showed that only KG^- strains agglutinated with KG^- antisera, whereas KG^+ strains reacted positively with both KG^+ and KG^- antisera. Barnes and Ellis (2004) compared serological characteristics of *L. garvieae* KG^-, KG^+ strains isolated from Europe (Italy, United Kingdom, Spain) and Japanese. In the agglutination tests, authors reported European KG- strains did not agglutinate with Japanese KG^- and KG^+ antisera, and Japanese KG- strains did not agglutinate with European KG^- antiserum and KG^+ antiserum. In addition, both European and Japanese KG^+ strains positively reacted to Japanese and European KG^+ antisera. Correlatively, Çağırgan (2004) stated 20 different *L. garvieae* strains were isolated from diseased rainbow trout in Turkey. Serological tests performed with KG^- antisera (Spain strain) and reported *L. garvieae* strains isolated from rainbow trout in Turkey reacted positively to representative antisera. Oinaka et al. (2015) reported an *L. garvieae* strain isolated from yellowtail in Japan between 2012-2013. In the slide agglutination tests, these strains did not agglutinate with reference KG^- and KG^+ antisera. In the current study, our findings revealed *L. garvieae* strains isolated from diseased rainbow trout in Southern Aegean Region showed no-reaction against Japanese *L. garvieae* antisera in the slide agglutination tests however, the strains showed a positive reaction against KG^- antisera produced from the Turkish strain. In accordance with the results of Çağırgan (2004) and Barnes and Ellis (2004) and this study, we suggest Turkish *L. garvieae* strains can be included European KG- serotype.

CONCLUSION

Diseases caused by *V. anguillarum, Y. ruckeri*, and *L. garvieae* is responsible for significant economic losses with a high mortality rate in rainbow trout. In this study, bacterial fish pathogens were diagnosed with the slide agglutination method, and serotypes of the strains were determined. The slide agglutination method allows the rapid diagnosis of pathogens causing fish diseases and the prevention of economic losses within the appropriate treatment.

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AUTHORSHIP CONTRIBUTIONS

Tevfik Tansel Tanrıkul, Kaan Kumaş: Fiction, literature, methodology, data analysis, manuscript writing. Tevfik Tansel Tanrıkul: Performing the experiment with rabbits, supervision. Kaan Kumaş: Preparation of antigens. All authors approved the final draft.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

This study was conducted with the approval of Animal Experiments Local Ethics Committee of Ege University (Date: 24.03.2021, No: 2021-027).

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Age, growth and maturity of false scad *Caranx rhonchus* in the Central Aegean Sea (Teleostei: Carangidae)

Orta Ege Denizi'nde kral balığı *Caranx rhonchus*'un yaş, büyüme ve eşeysel olgunluğu (Teleostei: Carangidae)

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Abstract: This study investigates the age, growth and maturity of false scad (*Caranx rhonchus* Geoffroy Saint-Hilaire, 1817) in the İzmir Bay, the Central Aegean Sea. The total length of 392 fish varied between 12.8-36.0 cm and their weights were measured as 23.51-455.44 g. Sex ratio was 1:0.8 (F:M). Sagittal otoliths were used for age determination. Fish were between I-V age groups in the bay. The length-weight relationships were calculated as $W= 0.0145*L^{2.8911}$ for females; $W= 0.0168*L^{2.8425}$ for males, and $W= 0.0132*L^{2.9259}$ for all fish. The von Bertalanffy growth parameters were found as L_∞= 40.86 cm, K= 0.2181 years^{-1}, t_o= -1.0584 years for females; L_∞= 39.54 cm, K= 0.2156 years^{-1}, t_o= -0.963 years for males; and L_∞= 41.12 cm, K=0.2061 years^{-1}, t_o= -1.0548 years for all fish, respectively. The growth performance index values (Φ) were 2.532 for females, 2.555 for males, and 2.542 for all fish. The length at first maturity was calculated as 18.53 cm for females, and 18.95 cm for males.

Keywords: Length-weight relationship, length at first maturity, age-length relationship, İzmir Bay

Öz: Bu çalışmada, Orta Ege Denizi, İzmir Körfezi'ndeki kral balığı (*Caranx rhonchus* Geoffroy Saint-Hilaire, 1817)'nın yaş, büyüme ve eşeysel olgunluğu araştırılmıştır. 392 balığın total boyları 12,8-36,0 cm arasında değişmekte olup ağırlıkları 23,51-455,44 g olarak ölçülmüştür. Cinsiyet oranı (D:E) 1:0,8'dir. Yaş tayininde sagittal otolitler kullanılmıştır. Balıklar körfezde I-V yaş grupları arasında bulunmuştur. Boy-ağırlık ilişkisi sırasıyla, dişiler için $W= 0,0145*L^{2.8911}$, erkekler için $W= 0,0168*L^{2.8425}$ ve tüm balıklar için $W= 0,0132*L^{2.9259}$ olarak hesaplanmıştır. Von Bertalanffy büyüme parametreleri sırasıyla, dişiler için L_∞=40,86 cm, K= 0,2181 yıl^{-1}, t_o= -1,0584 yıl; erkekler için L_∞= 39,54 cm, K= 0,2156 yıl^{-1}, t_o=-0,963 yıl ve tüm balıklar için L_∞= 41,12 cm, K= 0,2061 yıl^{-1}, t_o=-1,0548 olarak bulunmuştur. Büyüme performans indeks değerleri (Φ) dişilerde 2,532, erkeklerde 2,555, ve tüm balıklarda 2,542'dir. İlk eşeysel olgunluk boyu dişilerde 18,53 cm ve erkeklerde 18,95 cm olarak hesaplanmıştır.

Anahtar kelimeler: Boy-ağırlık ilişkisi, ilk eşeysel olgunluk boyu, yaş-boy ilişkisi, İzmir Körfezi

INTRODUCTION

The *Carangidae* family is represented by 148 species belonging to 30 genera in the world (Fricke et al., 2021). In Turkish waters, 16 species belonging to 11 genera were reported for this family (Bilecenoğlu et al., 2014). The *Carangidae* family has economically important fish species and one of them is *Caranx rhonchus* in the study area.

Variability in size has important implications for many aspects of fisheries and stock assessment which include the modeling of growth, estimation of growth parameters, estimation of age frequencies from length frequencies, size-based demographic modeling, sampling, length frequency analysis, and size-based stock assessment methods (Erzini, 1994). Knowledge of the relationship between length and weight of a fish species in a given geographic region is useful for the estimation of length-at-age, between-regions and life-history comparisons, and it is a practical index of the condition of fish (Petrakis and Stergiou, 1995).

From this perspective, scarce studies were done on *Caranx rhonchus* in the Mediterranean. A study of traditional and experimental floating fish aggregating devices (T/E-FADs) was conducted in the Gulf of Castellammare (NW Sicily). Together with results from catches and visual observations, they reported that *Caranx rhonchus* is one of the most common and abundant species in their study area (D'Anna et al., 1999). Bektas and Belduz (2009), were reported PCR-based identification and discrimination of *Caranx rhonchus* based on nuclear and mtDNA sequences. Ketata Khitouni et al., (2010), have studied variations of the chemical composition of five coastal catch fish species of the Gulf of Gabès (Tunisia) and one of them was *C. rhonchus*. Kožul and Antolović (2013),

reported a short communication of the occurrence of the false scad, in the Adriatic Sea. Raya and Sabatés (2015), have conducted a study about the diversity and distribution of early life stages of carangid fishes in the northwestern Mediterranean. Sley et al., (2008), have studied the diet composition and food habits of *Caranx rhonchus* from the Gulf of Gabes. Sley et al., (2013), have reported a comparative study between parameters of reproduction of *C. crysos* and *C. rhonchus*. Sley et al., (2015) have reported the annual reproductive cycle, spawning periodicity and sexual maturity of false scad from the South-Eastern Mediterranean. Sley et al., (2016) have reported morphometric and meristic characteristics of the species from the Gulf of Gabes. A study on the relationships between fish and jellyfish in the northwestern Mediterranean also includes *Caranx rhonchus* (Tilves et al., 2018). The length-length and length-weight relationships of the *C. rhonchus* were included in research covering some other fish species in the North Aegean Sea (Karachle and Stergiou, 2008) but the age and growth properties have not been studied yet.

Considering the scarcity of biological information about *Caranx rhonchus,* the purpose of the study is to contribute information about the age, growth, and maturity of *C. rhonchus* from İzmir Bay in the Central Aegean Sea.

MATERIAL AND METHODS

A total of 392 *C. rhonchus* were collected seasonally from commercial fishermen between September 2017 and July 2018 in the İzmir Bay, the Central Aegean Sea. Samples were collected from the fishing port on the same fishing day and transported to the laboratory as fresh. Specimens were measured to the nearest 0.1 cm (total length, TL), weighed to the nearest 0.01 g (total weight, W), and dissected in the laboratory. The sex and maturity stages were determined by macroscopic and microscopic examination of the gonads. The sex ratio was calculated for the entire study period, and its significance was tested by Chi-square (χ^2) test (Nikolsky and Birkett, 1963).

Sagittal otoliths were removed, cleaned and stored dry in U–plates. Before age determinations, otoliths were cleaned in 4% NaOH. Otoliths were transferred in an alcohol series (from 30% to 70%) for approximately ten minutes making them more transparent. In this way, opaque and hyaline zone discrimination is facilitated. Age was read from whole otoliths immersed in glycerin (25%) and alcohol (75%) mixture. They were viewed with Olympus SZ–61 models stereo binocular microscope, under reflected light against a black background (Figure 1). To minimize reading errors, the number of opaque rings outside the nucleus was independently evaluated by two readers. 9 otoliths were excluded from evaluation because the readings did not coincide. The allometric growth equation, $W=a*L^b$, was used to examine length-weight relations (Bagenal, 1978), where W is the total weight (g), L is the total length (cm), and a and b are the regression constants. The von Bertalanffy growth parameters were used to describe the growth of fish species (Sparre et al., 1989); $L_t=L_\infty(1-e^{K(t-to)})$ where L_t is the fish length at age t; L_∞ represent the asymptotic length, K is a brody growth coefficient and t_o is the theoretical age when the fish length is zero. To compare the calculated growth parameters with other studies, growth performance index values (Φ value) were calculated as $\Phi=log_{10}K+2*log_{10}L_\infty$ (Pauly and Munro, 1984) where K and L_∞ are the von Bertalanffy growth parameters. The hypothesis of isometric growth was tested by Student's t-test (Ricker, 1975).

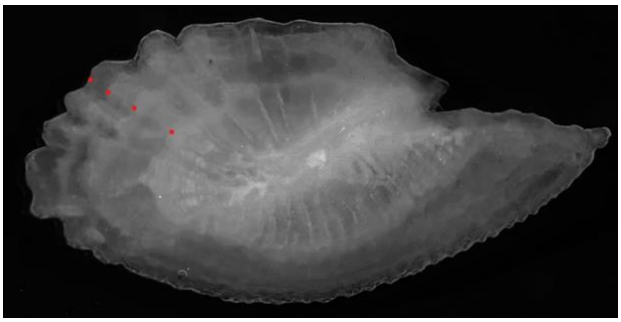

Figure 1. Right sagittal otolith of a 4-years old *C. rhoncus* (27.8 cm TL, 197.2 g) from İzmir Bay

Gonad stages were classified on the modified empirical scale of Holden and Raitt (1974) in both sexes; 1=immature, 2=maturing, 3=ovipositing, and 4=postoviposition, and seems more discriminate and less subject to interpretative error than other scales. To estimate the mean lengths at 50% maturity, a logistic function was fitted to the proportion of the mature individuals by size class using nonlinear regression. The function used was $P=1/1+e^{-r(L-L_{50})}$ where P is the proportion of mature fish per size class, L is the average total length corresponding to the proportion (P), r is the slope of the curve, and L_{50} (length at first maturity) is the total length at 50% maturity (King, 2013).

RESULTS

In the current study, a total of 392 fish were examined. 184 of them were females (46.94%), 149 were males (38.01%), and 59 (15.05%) were unidentified because they were immature or their gonads were not distinguishable. The female to male ratio was calculated as 1:0.8 and found that it was not statistically significant ($\chi^2_{cal}=3.67$, $\chi^2_{th}=3.84$).

Total length distribution for females, males, and all fish was shown in Figure 2. As a result of measurements, females ranged with an average of 20.18±0.53 cm, between 13.3 and 32.2 cm. Males 20.47±0.6 cm ranged between 12.8 and 36.0 cm. All fish ranged with an average of 20.59±0.38 cm, between 12.8 and 36.0 cm. The length-weight relationships were calculated as $W=0.0145*L^{2.8911}$ for females; $W=0.0168*L^{2.8425}$ for males, and $W=0.0132*L^{2.9259}$ for all fish. The von Bertalanffy growth parameters were calculated as $L_\infty=40.86$ cm, $K=0.2181$ years^{-1}, $t_o=-1.0584$ years for females. It was $L_\infty=39.54$ cm, $K=0.2156$ years^{-1}, $t_o=-0.963$ years for males, and $L_\infty=41.12$ cm, $K=0.2061$ years^{-1}, $t_o=-1.0548$ years for all fish. The growth performance index values (Φ) were 2.532 for females, 2.555

for males, and 2.542 for all fish. The female, male, and all fish showed negative allometric growth throughout the year (Table 1). Fish that have negative allometry, get weight more slowly than the length.

Gonads' distinguishes were not clear in small fish and likewise in large fish at the end of the spawning season. The sample size increased when the consideration of individuals whose gender could not be determined. Thus, the b value calculated for all fish may have been found to be higher than the b value calculated for different genders.

Table 1. Length-weight relationship parameters of *C. rhonchus* in the İzmir Bay (*a*, intercept; *b*, the regression coefficient (slope); n, number of specimens studied; SE(*b*), standard error of the slope; R^2, coefficient of determination; GT, growth type; A(–), negative allometric)

Sex	a	b	n	SE (b)	R^2	*t*-test*	GT
Females	0.0145	2.8911	184	0.0424	0.965	2.563	A(–)
Males	0.0168	2.8425	149	0.0433	0.967	3.630	A(–)
All fish	0.0132	2.9259	392	0.0267	0.968	2.772	A(–)

$t_{(183)}$=1.66, p<0.05; $t_{(148)}$=1.65, p<0.05; $t_{(391)}$=1.66, p<0.05

A total of 383 otoliths were used for the age determination. 183 were female, 144 were male, and 56 were unidentified (Table 2). Age groups of *C. rhonchus* ranged from I to V in the İzmir Bay for all fish. The most observed age group was 2 for all fish (45.4%). The mean length of 2 years old females was 19.7±0.54 cm males was 20.1±0.53 cm. For the 2 years of age groups, there was no statistically significant difference between females and males ($t_{(1)}$ = -3.2196, p= 0.19).

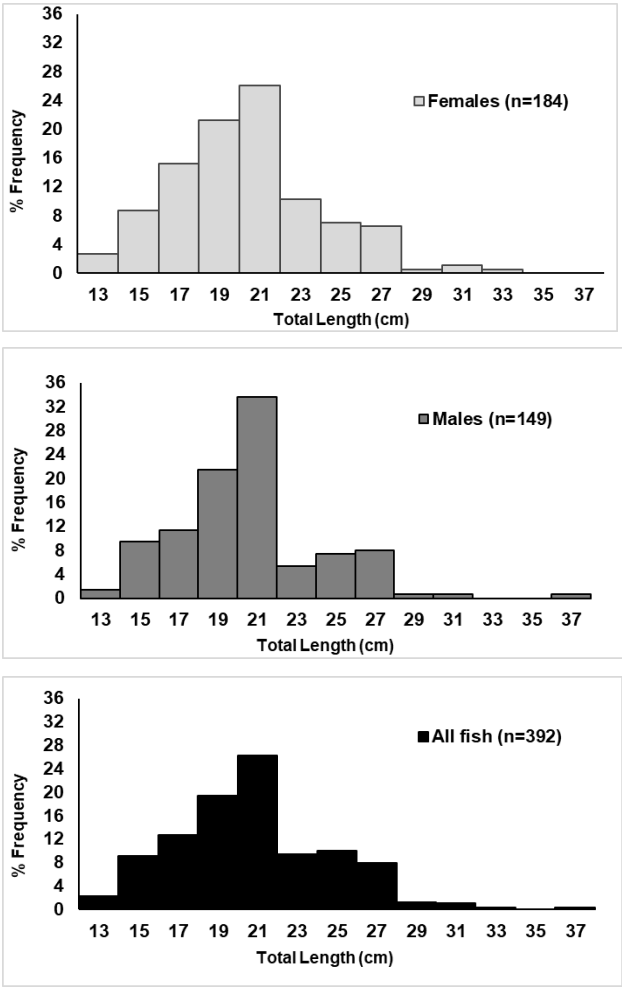

Figure 2. Length distribution of *Caranx rhonchus* in the İzmir Bay

Table 2. The age-length key of *C. rhonchus* from the İzmir Bay (TL, Total length; T, Total; n, the number of specimens; n%, number of percentages; SD, Standard deviation)

	Female						Male						All Fish					
TL (cm)	I	II	III	IV	V	T	I	II	III	IV	V	T	I	II	III	IV	V	T
12							1					1	1					1
13	5					5	1					1	8					8
14	5	2				7	4	1				5	13	3				16
15	6	3				9	6	3				9	12	8				20
16	4	7				11	4	4				8	8	11				19
17	7	9	1			17	5	2				7	13	13	1			27
18	6	9	3			18	3	8	3			14	9	18	6			33
19	7	8	6			21	1	11	4			16	8	20	12			40
20	1	24	8			33	3	19	8			30	4	44	16			64
21	2	6	6			14	2	15	3			20	5	23	10			38
22	2	8	5			15	2	1	1			4	4	11	8			23
23		1	3			4		3	1			4		9	5			14
24		3	3	1		7		4	3	1		8		9	10	3		22
25		2	4			6			3			3		2	15			17
26		1	7	1		9			6			6		1	18	1		20
27			2	1		3		1	5			6		2	8	1		11
28									0						1			1

Table 2. Continued

	Female						Male						All Fish					
TL (cm)	**I**	**II**	**III**	**IV**	**V**	**T**	**I**	**II**	**III**	**IV**	**V**	**T**	**I**	**II**	**III**	**IV**	**V**	**T**
29				1		1			1			1			3	1		4
30			1	1		2			0	1		1			1	3		4
31																		
32				1		1										1		1
36											1						1	
n	45	83	49	6		183	32	72	38	2		144	85	174	114	10		383
%n	24.6	45.4	26.8	3.3			22.2	50.0	26.4	1.4			22.2	45.4	29.8	2.6		
Min	13.3	14.0	17.4	24.8			12.8	15.1	18.6	24.7			12.8	14.0	17.4	24.0		
Max	22.2	26.0	30.2	32.2			22.5	27.1	29.0	30.6			22.5	27.1	30.2	32.2		
Mean	17.3	19.7	22.6	28.5			17.3	20.1	23.3	28			17.1	20.1	23.4	28.0		
SD	2.4	2.5	3	2.5			2.5	2.3	3.2	3			2.5	2.6	3.1	2.8		

Utilizing the total length values of the specimens in all age groups, the von Bertalanffy growth equations' parameters were computed as indicated in Table 3.

Table 3. The von Bertalanffy growth parameters for *C. rhonchus* from the İzmir Bay (n, the number of specimens; *K*, The Brody growth coefficient; t_0, theoretical age of fish before hatching; L_∞, asymptotic length; Φ, growth performance index)

Sex	n	K	t_0	L_∞	Φ
Females	183	0.2156	-0.9632	39.538	2.555
Males	144	0.2181	-1.0584	40.863	2.532
All fish	383	0.2061	-1.0548	41.119	2.542

The data displayed in Figure 3 present the percentage of matured individuals in all fish in a given size class. The length at first maturity was estimated as 18.95 cm and 18.53 cm for males and females respectively.

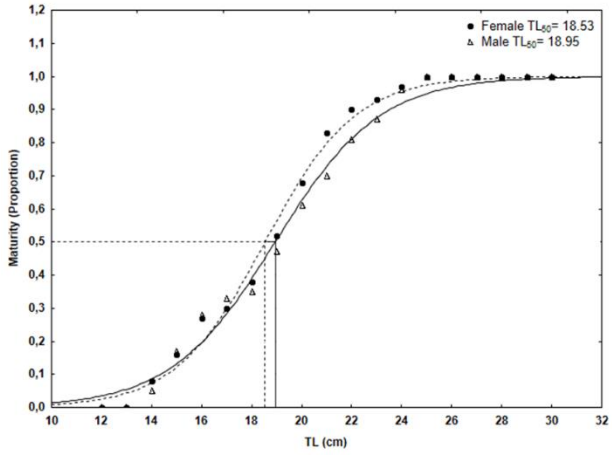

Figure 3. Length at first maturity of female and male *C. rhonchus* from the İzmir Bay

DISCUSSION

Studies on *Caranx rhonchus* have been reported from West Africa and the Mediterranean. It is possible to consider these regions as independent ocean and marine habitats. The effect of the up-welling current on the west African coast should be considered in this context. An evaluation of the current study is presented with a few other studies.

Sley et al., (2015) reported the sex ratio of *C. rhonchus* was 1.04:1 (F: M) in the Gulf of Gabès. They have examined 1313 individuals of whom 668 were females and 645 were males. Females ranged from 3.1 cm to 29.5 cm (TL), and males ranged from 3.0 cm to 30.6 cm (TL) in their study. Although the range was differing, similar results were found in the current study (F: M= 1:0.8). This situation could be evaluated as the similarity of conditions in the Mediterranean. Another group of results was reported from the coasts of the Senegal region. The numerical proportion of the sexes was in favor of females on the coasts of Senegal and Mauritania, albeit the ratio is not specified. (Camarena Luhrs, 1986; Lawal and Milnikov, 1988). The difference between the Senegal-Mauritania region and the Mediterranean region might be due to the occurrence of various conditions in the ocean and marine environment. Besides, the difference in fish size distribution for these two regions should also be taken into account.

The length-weight relationship parameters of *C. rhoncus* reported by different authors are as in Table 4. Torres et al., (2012) reported that the total length was in the range of 28.3–37.0 cm in the Gulf of Cadiz, and larger than the current study (12.8-36.0 cm). The reason for this could be, that the fishing depth they use was up to 800 meters, and the mesh size they use was 20 mm. Also, the fishes were sampled only during the spring and autumn (Torres et al., 2012).

Table 4. Length-weight relationships of *C. rhonchus* in different localities as reported by various authors (n, number of specimens; *a*, intercept; *b*, the regression coefficient; R^2, coefficient of determination; SE, standard error)

Author	Research Area	Length	n	Length Range	a	b	R^2	SE(b)
Karachle and Stergiou (2008)	North Aegean	TL	16	18.0-19.8	0.0099	2.997	0.79	0.412
Overko (1979)	Cap Blanc Cap Verde	FL	-	-	0.0200	2.880	-	-
Boely et al. (1973)	Coast of Senegal and Mauritania	TL	-	-	0.0065	3.097	-	-
Fréon et al. (1979)	Senegal	FL	1956	-	0.0124	3.055	0.99	0.0136
Santos et al. (2002)	Coast of southern Portugal	TL	100	15.7-37.6	0.1222	2.241	0.88	0.084
Torres et al. (2012)	Gulf of Cadiz-Spain	TL	32	28.3-37.0	0.0328	2.610	0.92	-
Maxim (1995)	Mauritania	-	-	-	0.0350	2.731	-	-
Do Chi (1994)	Senegal	FL	-	7.0-38.0	0.0123	3.061	-	-
This study	İzmir Bay	TL	392	12.8-36.0	0.0132	2.9259	0.97	0.0267

The length-weight relationship shows negative allometric growth for both sexes (Table 1). The regression coefficients of this species vary between 2.241–3.097 in studies conducted in different regions. These values were reported as 3 from the coast of Senegal (Table 4). This result indicates that this species' growth isometric in that region. Santos et al., (2002) reported the mean total length for *C. rhonchus* was 30.7±2.6 cm, the regression coefficient was 2.241 and the growth type was negative allometric. Although the length range was similar to the current study, the low regression coefficient may be due to regional differences or the low value of the coefficient of determination. The coefficients of determinations that were calculated for the current study are close to one (Table 1).

The mean total lengths of age groups were compared with other studies in Table 5. Overko (1979) reported similar age groups in the Cap Verde/ Cap Blanc region. Boely et al., (1973) reported larger age groups on the coast of Senegal and Mauritania. Evaluating these differences was difficult because the methodologies of these two studies were not clearly explained. We suggest that the species could not reach larger sizes due to the scarcity of prey in the İzmir Bay compared to the upwelling areas. On the other hand, there is a possibility that smaller individuals may be caught due to the local fisheries pressure in İzmir Bay. Besides, the fishing method could be different and the selectivity of the fishing gear might be low so the smaller individuals could be caught. Differences in mean length values may be caused by due to the regional biotic (abundance of prey, predation, etc.) and abiotic (temperature, salinity, etc.) factors, as well as the differences in the methodology of age determination.

Table 5. Comparison of the mean length (cm) of the age groups of *C. rhonchus* populations in various seas

Author	Research Area	Length Type	Age Groups									
			I	II	III	IV	V	VI	VII	VIII	IX	X
Overko (1979)	Cap Blanc/ Cap Verde	FL	12.2	17.9	22.3	26.0	29.7	-	-	-	-	-
Boely et al. (1973)	Coast of Senegal and Mauritania	TL	16.0	24.2	29.2	33.7	36.8	39.0	40.6	41.8	42.7	43.4
This study	İzmir Bay	TL	17.1	20.1	23.4	28.0	-	-	-	-	-	-

Table 6 shows the von Bertalanffy growth parameters of *C. rhonchus* from different areas. The L_∞ values were found lower in the current study, compared to others. The growth performance index (Φ) calculated from K and L_∞ was found as 2.542. All these differences might be related to the relatively smaller size of the mean length of the sampled fish. In addition, it is thought that all these differences may be due to methodological differences.

Table 6. The von Bertalanffy growth parameters of *C. rhonchus* in different areas (L_∞, asymptotic length; *K*, The Brody growth coefficient; t_0, theoretical age of fish before hatching; *Φ*, growth performance index)

Author	Research Area	Length Type	L_∞	K	t_0	Φ
Overko (1979)	Cap Blanc /Cap Verde	FL	48.57	0.160	0.800	2.577
Boely et al. (1973)	Coast of Senegal and Mauritania	TL	45.30	0.303	0.515	2.794
Maxim (1995)	Mauritania	-	55.69	0.136	-1.295	2.625
This study	İzmir Bay	TL	41.12	0.206	-1.055	2.542

Overko (1979) and Camarena Luhrs (1986) stated that the first maturity of false scad was 19.7 cm and 20.0 to 21.0 cm FL in the Eastern Atlantic and on the Senegal coast, respectively. Lawal and Milnikov (1988) and Chavance et al., (1991) reported 19.0 cm (FL) as the first maturity length of false scad for the Mauritanian coast. Fischer et al., (1980) reported the first maturity length of *C. rhonchus* 22.0 cm FL in the Mediterranean. In Tunisian waters, the length of the first

maturity was reported between 16.4 cm (TL) and 20 cm (SL) (Ben Salem and Ktari, 1980, 1994; Ould Mohamed Abdallahi, 1999; Sley et al., 2013, 2015). In this study, the first maturity length of the false scad in the Central Aegean Sea was estimated as 18.95 cm for males and 18.53 cm for females (TL). As stated by Olsen et al., (2004), genetic effects, environmental factors such as temperature, salinity, and food availability, and differences in sampling procedure such as sampling gear and location may affect the sexual maturity length.

In conclusion, the current research investigates the age, growth and maturity of *C. rhonchus* for the first time in the Central Aegean Sea. We suggest that the minimum fishing length should be at least 19 cm (TL) for the sustainability of *C. rhonchus* in İzmir Bay. The literature review showed a need for more detailed and up-to-date information on the biology of this species. We hope the results of this research will contribute to management strategies and regulations needed for the stocks of the *Caranx rhonchus*.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study. Material preparation and research were carried out by Burak ALTAY and Dilek İLHAN. The article was written and edited by Burak ALTAY and all authors have read and approved the article.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

The material used in the article was obtained from commercial fishermen and the research was conducted according to the National Ethics Committee for Animal Experiments (HADMEK, HADYEK) guidelines.

DATA AVAILABILITY

The datasets created and/or analyzed during the current study will be provided by the responsible author upon the request of the editor or reviewers.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Benthic marine litter in the Marmara Sea, Turkey

Marmara Denizi'nde bentik deniz çöpü

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Abstract: This study presents the first data on benthic marine litter in the Marmara Sea, Turkey. To obtain the data, bottom trawl surveys were conducted at 34 sites between May 2017 and February 2018. The litter items were sampled and sorted following the MEDITS' relevant instructions. 660 pieces of litter, weighing 434.9 kg, were sampled. The litter density was found to range between 27.5 n/km^2 and 661.2 n/km^2, averaging 73.9 n/km^2, and the obtained items' weights ranged between 0.03 kg/km^2 and 1597.8 kg/km^2, averaging 48.7 kg/km^2. The plastic group L1 constituted 71.7% of the trawled litter. The highest mean litter density was detected in the Northeastern Marmara Sea in the spring and summer of 2018. The mean benthic litter density was found to be higher than the nearby areas. It was concluded that more effort should be invested in reducing marine pollution.

Keywords: MEDITS, benthic litter, plastic waste, marine pollution, Marmara Sea

Öz: Bu çalışma, Marmara Denizi'ndeki bentik deniz çöpüne ilişkin ilk verileri ortaya koymaktadır. Bu amaçla Mayıs 2017-Şubat 2018 tarihleri arasında 34 istasyonda dip trolü araştırması yapılmıştır. Çöplerin kategorize edilmesi ve sınıflandırılması MEDIT standardına göre belirlenmiştir. Toplam 660 adet ve 434,9 kg çöp tespit edilmiştir. Çöp yoğunluğu 27,5 adet/km^2 - 661,2 adet/km^2 arasında değişirken, ortalama 73,9 adet/km^2 tespit edilmiştir, ağırlık değerleri 0,03 kg/km^2 - 1597,8 kg/km^2 arasında değişmiş ve ortalama 48,7 kg/km^2 belirlenmiştir. Plastik (L1) grubu toplam çöp bolluğunun %71,7'sini oluşturmuştur. 2018 yılında, ilkbahar ve yaz mevsimlerinde ve Marmara Denizi'nin kuzeydoğu kesiminde daha yüksek çöp yoğunluğu tespit edilmiştir. Deniz kirliliğini azaltmak için daha etkin mücadele gerektiği görülmüştür.

Anahtar kelimeler: MEDITS, bentik çöp, plastik atık, deniz kirliliği, Marmara Denizi

INTRODUCTION

Marine litter is defined by UNEP as any persistent, manufactured or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment. It may be indirectly introduced into marine environments by rivers, sewage, storm water, waves, or winds, but it is mainly anthropogenic (UNEP, 2016). Marine litter has been discussed in the last 60 years. Derraik (2002) states that plastics are the essential pollutants among all other known components. Plastic production is estimated to amount to 368 million tonnes (Plastics Europe, 2020) and the increase rate corresponds to 4% a year. Besides, the plastic production of Turkey was recorded to be 9.8 million tonnes in 2020 (PAGEV, 2020). The most pollutant plastic polymers are arranged as polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) (Godoy et al., 2020). Marine species get entrangled in plastic debris such as plastic food wrappers, bottles, and ghost fishing nets, which leads to lethal consequences, e.g., injuries or death due to entanglement. A notable portion of this debris covers sea bottom and prevents gas exchange between the substrate and the overlying water column (Corcoran, 2015) and primarily affect sessile living organisms, such as corals, algae, etc. Besides, aquatic species (fish, crustaceans, cephalopods, etc.) accidentally consume plastic debris as prey. Besides, microplastics formed after decomposition causes damage, especially in the early developmental stages of marine animals. (Ribeiro et al., 2019).

Benthic marine debris is determined with bottom trawling in many areas of the world. The most general understanding is that marine bottom pollution has been increasing in recent years, and plastics are the most common pollutants materials. Benthic marine litter density was found to be 102 n/h on the Malta Shelf (Misfud et al., 2013), 4424 n/h in Spain (Sanchez et al., 2013), 79.6 n/h in the Central Mediterranean Sea (Garofalo et al., 2020), 72-437 n/h in the Echinades Gulf (Koutsodendris et al., 2008), 0-2145 n/h in the Adriatic (Fortibuoni et al., 2019), and 125-594 n/h in Algeria (Mankou-Haddadi et al., 2021). However, the amounts and temporal variations of litter have not yet been known in many great geographical areas. Unless the main problem is known, it is very difficult to take local measures, which results from the lack of scientific research in the relevant local areas.

The Marmara Sea is regarded as a special area that connects the Mediterranean and the Black Sea via the Dardanelles and Bosphorus Straits. In addition to being one of the most important maritime traffic areas globally, it is also home to a metropolis, i.e., Istanbul. In 2018, the number of vessels that passed through the Bosphorus and Dardanelles Straits was 41.103 and 43.999, respectively (TUIK, 2019). Besides, Istanbul's population in 2018 was reported to be 15.7 million by TUIK (2019). In addition, 3000 registered fishing vessels with varied capacities fish in the Marmara Sea (Anonymous, 2018). A considerable number of unlicenced fishing vessels operate in the Marmara Sea. Besides, the industrial facilities in Turkey are mainly located in the Marmara Region (48%), a great majority (31%) of which are stationed in the city of İstanbul (Plastics Europe, 2020). These facilities discharge their waste into the Marmara Sea through a liquid waste process called deep-sea discharge. Maryam and Büyükgüngör (2019) state that only nine of 77 wastewater treatment plants around the Marmara Sea are capable of advanced biological treatment. Most waste is discharged as coarse and fine particulates after physical treatment only (Burak et al., 2021). Besides, İstanbul's Golden Horn (Haliç) estuary, which is one of the most delicate areas in the world, easily becomes polluted and is in constant need of improvement in water quality. Furthermore, the pollutants accumulated in the estuary flow into the Marmara Sea. Additionally, Orhon et al. (2021) argue that the Black Sea Current in the Bosphorus Strait discharges highly polluted water bodies from the Black Sea into the Marmara Sea. Thus, the nutrient load of the Marmara Sea exceeds the capacity of the Marmara's marine ecosystem (Okuş et al.,2002; Taş et al., 2016; Çardak et al., 2015) and results in environmental disasters such as mucilage (Savun-Hekimoğlu and Gazioğlu, 2021).

Another problem that causes inceased marine pollution in the Marmara Sea and Turkey is the plastic imports from the developed countries (Gündoğdu and Walker, 2021). By the end of 2020, Turkey's annual plastic waste import reached 772,831 tonnes (PAGEV, 2021). Gündoğdu and Walker (2021) note that while Turkey's rate of recycling its own waste is very low (<1%), the mismanagement of high amount of imported plastic waste can pose serious environmental problems, particularly increased pollution.

Previous research on marine litter has been mostly conducted in the Northeast Levantine Coasts of Turkey (Güven et al., 2013; Eryaşar et al., 2014; Aydın et al., 2016; Gündoğdu et al., 2017; Olguner et al., 2018, Gündoğdu and Çevik, 2019; Mutlu et al., 2020; Büyükdeveci and Gündoğdu, 2021). Some published data are also available about the neighboring seas. Topçu and Öztürk (2010) have studied the Western Black Sea and Gönülal et al. (2016) have researched the vicinity of the Gökçeada Island, Northeastern Aegean Sea. In most of these studies, benthic marine litter abundance is determined with a swept area of bottom trawl sampling. Aydın et al. (2016) and Artüz et al. (2021) focus on coastal macrolitter around beaches. All in all, no previous studies were found to have researched benthic marine litter abundance in the Marmara Sea.

This research is the first to investigate benthic pollution in the Marmara Sea and to present data on the abundance and spatial and temporal variations of the benthic marine litter in it. Besides, the study also intended to reveal the associated pollution sources to gain a deeper insight into the cause and effect relationships influential in the emergence of seabed pollution in the research area.

MATERIAL AND METHODS

This study is a part of a research project entitled "Determination of the population status and the stock estimation of economically valuable demersal fish in the Marmara Sea". The litter samples were obtained by 246 bottom trawl hauls at 34 sites in the Marmara Sea between March 2017 and December 2018. The surveys conducted in March, July, October and December were tagged with Spring, Summer, Autumn and Winter, respectively (Table 1).

The sampling strategy and technical properties of the trawl nets (polyethylene codend with 200 mesh length with a mesh opening 44 mm; equipped with polyamide cover with 250 mesh length with a mesh opening 20 mm; 200 kg and 1*2 m steel doors) were determined based on "MEDITS International bottom trawl survey in the Mediterranean – Instructional Manual". The sampling sites were characterized by varying depths (10-50, 50-100, 100-200, and >200 m) and a great diversity of geographical features. The bottom trawl hauls were conducted with the commercial trawl vessel Yalçınoğlu at three nautical miles for 30 m.

The marine litter items were counted and weighed to the nearest 0.5 g. The litter items were sorted following the instructions by the MEDITS. They were grouped into eight different categories: plastic, metal, rubber, glass, textile, wood, paper, and others. The swept area method was used to calculate the abundance of litter on the seabed in the number of items per unit area (km^2) and the total weight and item number (n) of items per unit area (km^2). Catch per Unit Effort (CPUE: kg/km^2) was calculated by dividing the catchweight (Cw) by the swept area (a) for each species and each haul (Sparre and Venema, 1992).

CPUE: Cw/a

The swept area (a) or the 'effective path swept' for each hauling was estimated thus:

$a=D.h.X_2$

where h (m) refers to the length of the head rope and D to the distance covered. X is the fraction of head rope length, with 0.5 as the best compromise (Pauly, 1980).

The non-parametric Kruskal-Wallis test was used to test differences between categories of marine debris and depth stratum and between the categories of marine debris and seasons. Besides, The Mann-Whitney U test was applied to see between-group differences. The statistics were conducted with PAST v. 2.17c.

Table 1. Coordinates and depths of sampling sites in the Marmara Sea

Site	Tow Beginning Coordinate		Tow Ending Coordinate		Tow Beginning Depth	Tow Ending Depth
	Latitude (N)	Longitude (E)	Latitude (N)	Longitude (E)	Depth (m)	Depth (m)
1	40 55 724	28 44 679	40 56 045	28 46 286	78.33	78.28
2	40 56 953	28 34 991	40 56 784	28 36 783	48.98	52.68
3	40 55 142	28 34 970	40 54 925	28 36 745	77.13	74.85
4	41 01 180	28 26 091	40 01 098	28 26 227	38.4	37.19
5	40 58 102	28 22 312	40 37 839	28 24 234	71.46	72.57
6	41 00 894	28 05 947	41 01 510	28 06 992	29.44	29.93
7	40 57 614	28 02 645	40 57 765	28 04 497	78.66	79.03
8	40 58 583	27 46 273	40 58 192	27 48 136	41.8	42.19
9	40 53 377	27 29 329	40 54 162	27 30 739	70.02	69.23
10	40 39 249	27 15 660	40 40 156	27 17 338	41.86	43.66
10-A	40 39 826	27 24 210	40 40 622	27 25 500	143.9	159.61
11	40 36 835	27 15 725	40 37 449	27 17 332	79.8	80.86
12	40 35 096	27 04 729	40 35 727	27 06 410	38.55	35.48
13	40 28 529	27 14 960	40 28 994	27 13 236	53.5	55.01
14	40 31 627	27 11 006	40 32 457	27 09 492	64.53	65.31
15	40 21 595	27 25 062	40 22 415	27 23 617	24.07	23.48
16	40 26 953	27 27 998	40 27 467	27 25 159	52.25	54.54
17	40 20 730	27 35 946	40 20 614	27 34 093	28.08	29.55
18	40 26 263	27 35 990	40 26 293	27 34 132	42.55	42.49
19	40 33 362	27 40 876	40 32 531	27 39 394	59.68	61.48
20	40 39 328	27 50 478	40 39 333	27 50 457	83.77	83.38
21	40 33 357	27 51 834	40 33 631	27 46 610	59.46	62.49
23	40 37 582	28 12 254	40 37 491	28 14 654	81.18	80.83
24	40 24 418	28 12 361	40 24 407	28 12 713	33.14	33.85
25	40 30 429	28 11 398	40 30 73	28 13 73	51.62	50.48
26	40 38 618	28 22 448	40 38 791	28 20 597	99.44	102.07
27	40 24 988	28 26 746	40 24 834	28 24 969	30.34	26.18
28	40 30 851	28 24 857	40 31 758	28 23 390	46.96	46.22
29	40 30 652	28 40 400	40 28 784	28 41 372	37.88	37.91
30	40 26 149	28 40 994	40 25 788	28 42 880	57.88	58.28
31	40 24 881	28 49 816	40 24 939	28 47 867	63.62	63.24
32	40 41 004	29 18 997	40 40 460	29 17 739	60.08	73.27
33	40 49 001	29 14 539	40 50 317	29 13 643	60.24	59.49
34	40 50 143	29 03 633	40 49 616	29 05 508	91.22	90.06

RESULTS

Marine litter was found at 32 of 34 sites. 246 trawl hauls conducted in the Marmara Sea yielded a total of 660 pieces of litter, amounting to 434.9 kg. The litter density ranged between 27.5 n/km^2 and 661.2 n/km^2, averaging 73.9 n/km^2, and the weight values between 0.03 kg/km^2 and 1597.8 kg/km^2, corresponding to 48.7 kg/km^2 on average. The mean abundance and weight values of the litter categories are given in Table 2. The mean CPUE values of the litter groups varied

according to numerical abundance and weight. The analyses of the numerical abundance (n/km^2) showed that the plastic group (L1) constituted 71.7% of the total litter abundance. The metal (L3) and textile materials (L5) represented 11.4% and 6.6%, respectively. The rubber, glass, wood, and paper litter groups had rather low numerical litter abundance.

Table 2. The marine litter biomass of the Marmara Sea by main categories and subcategories per the instructions by MEDITS

Mean Abundance	n/km^2	n%	kg/km^2	W%
L1 Plastic (including PVC, polypropylene, polyethylene)	**53**	**71.7**	**8**	**16.4**
L1a. Bags	26.3	35.6	2.6	5.3
L1b. Bottles	6.2	8.4	0.9	1.8
L1c. Food wrappers	15.3	20.7	2.6	5.3
L1d. Sheets (table cover, etc.)	0	0	0	0
L1e. Hard plastic objects (crates, containers, tubes, ashtrays, lids, etc.)	1.9	2.6	1.5	3
L1f. Fishing nets	0.8	1.1	0.1	0.2
L1g. Fishing lines	0	0	0	0
L1h. Other fishing related (pots, floats, etc.)	0	0	0	0
L1i. Synthetic ropes/strapping bands	0	0	0	0
L1j. Others	2.5	3.4	0.3	0.6
L2 Rubber	**1.2**	**1.7**	**15.5**	**31.8**
L2a. Tyres	1	1.4	15	30.8
L2b. Others (gloves, floats, boots/shoes, olskins, sanitaries)	0.2	0.3	0.5	1
L3 Metal	**8.4**	**11.4**	**6.5**	**13.4**
L3a. Beverage cans	5.5	7.4	0.6	1.2
L3b. Other food cans/wrappers	0.8	1.1	0.3	0.6
L3c. Middle-size containers (of paint, oil, chemicals)	1.3	1.8	1.6	3.2
L3d. Large metalic objects (barrels, pieces of machinery, electric appliances)	0.7	0.9	3.5	7.1
L3e. Cables	0	0	0	0
L3f. Fishing-related gears (hooks, spears, etc.)	0.1	0.1	0.5	1
L3g. War remnants	0	0	0	0
L4 Glass/Ceramic/Concrete	**1.3**	**1.7**	**0.5**	**1**
L4a. Bottles	1.2	1.6	0.5	1
L4b. Pieces of glass	0.1	0.1	0	0
L4c. Ceramic jars	0	0	0	0
L4d. Large objects (ceramic basins, etc.)	0	0	0	0
L5 Cloth (textile)/Natural fibres	**4.9**	**6.6**	**9.7**	**19.9**
L5a. Clothing (clothes, shoes, etc.)	2.8	3.8	1	2
L5b. Large pieces (carpets, mattresses, etc.)	1.3	1.8	8.5	17.4
L5c. Natural ropes	0.1	0.1	0.1	0.2
L5d. Sanitary products (diapers, cotton buds, etc.)	0.7	0.9	0.1	0.2
L6 Processed wood (palettes, crates, etc.)	**2.6**	**3.5**	**7.8**	**16**
L7 Paper and cardboard	**1**	**1.4**	**0**	**0**
L8 Others	**1.5**	**2**	**0.8**	**1.6**
L9 Unspecified	**0**	**0**	**0**	**0**

Whereas the highest CPUE in weight (kg/km^2) (15.5%) was detected in the rubber group (L2). The textile and wood items were the other abundant litter groups (9.7% and 7.8%, respectively). The subgroups plastic bags, plastic food wrappers, plastic bottles, and metal beverage cans had the highest numerical CPUE values, corresponding to 26.3 n/km^2, 15.3 n/km^2, 6.2 n/km^2, and 5.5 n/km^2, respectively. Although the numerical CPUE values were found to be lower, the CPUE in weight (kg/km^2) was higher in the subcategories rubber tires, large textile pieces, and wood items and calculated to be 15 kg/km^2, 8.5 kg/km^2, and 7.8 kg/km^2, respectively (Table 2, Figure 1) .

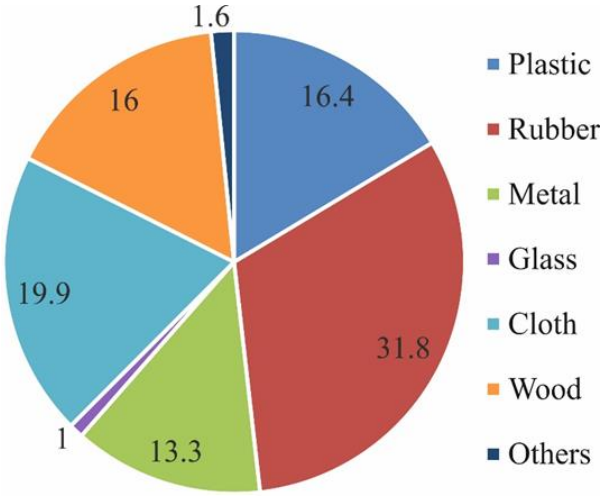

Figure 1. Percentages of marine litter biomass (kg/km^2) in the Marmara Sea by main categories (MEDITS)

The seasonal variations of the mean CPUE values are shown in Table 3. In 2018, the CPUE values were observed to be higher than the ones in 2017. The mean CPUE values were 56.2 n/km^2 in 2017 and 93.8 n/km^2 in 2018. The highest CPUEs in weight (kg/km^2) were detected in Spring 2017 and Spring 2018. The analysis of the mean CPUE values revealed no statistically significant between-season variations (F: 0.5906; df: 3; p>0.05).

Table 3. The seasonal variations in mean density values of marine litter in the Marmara Sea

Season	2017		2018	
	n/km^2	kg/km^2	n/km^2	kg/km^2
Spring	40.91	67.00	192.84	141.23
Summer	31.72	37.46	66.65	23.67
Autumn	109.36	17.13	49.77	27.7
Winter	41.77	9.82	56.3	73.62
Mean	56.16	33.21	93.81	66.07

According to the non-parametric Kruskal-Wallis analyses, the mean CPUE values showed statistically significant variations between the locations. The Mann-Whitney U test was performed to understand the interregional differences. The mean CPUE values showed no statistically significant variations between the Northeastern and Northwestern Marmara Sea and between the Northeastern and Southeastern Marmara Sea (p>0.05). The spatial variations in the mean CPUE values recorded in both the north and south parts and the west and east parts were statistically different. Among the 34 sites, the highest CPUE values were found at the sites 4 and 34. The mean CPUE at these locations was calculated to be higher than 300 n/km^2. The sites 1, 2, 31, and 32 too offered relatively higher CPUE – higher than 200 n/km^2. In the Western Marmara Sea, the highest litter was found at the sites 10, 11, and 12 located around the Hoşköy-Mürefte region (Figure 2).

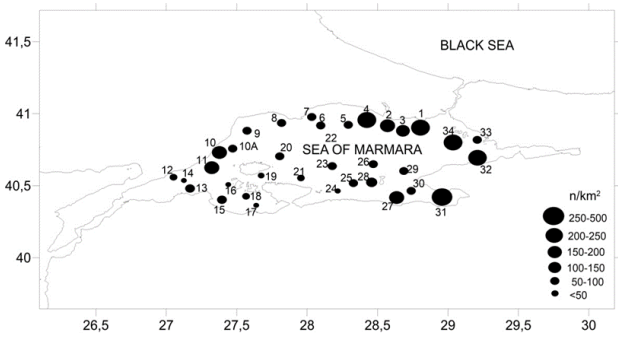

Figure. 2. The spatial variations of marine litter in the Marmara Sea

The mean CPUE values showed no statistically significant variations in terms of the depth strata. The mean CPUE values were observed to be 64.7 n/km^2, 71.7 n/km^2, and 45.9 n/km^2 at the depths of 23-50 m, 50-100 m, and >100 m, respectively.

DISCUSSION

Marine litter causes a great variety of issues. The accumulation of marine debris in coastal areas may create unpleasant sights for visiting tourists. Still, marine organisms are most exposed to and adversely affected by pollution. There is too much evidence for this phenomenon and it cannot be ignored. In addition, other living organisms, including birds, mammals, and invertebrates, too suffer injuries and suffocation from physical entanglement in marine litter items. In the related literature, 660 species are reported to have been physically affected by marine litter so far (Derraik, 2002). Although the degree of species-specific impact of marine litter is well-known, how pollutants affect communities and populations is still unclear. Therefore, the body of information required by managing authorities remains incomplete. For example, it is reported that 70% of plastic litter collapses in the demersal habitat. However, there is almost no research on how this dense plastic accumulation affects the bottom environment or damages the primary production and nutritional cycle (Barnes et al., 2009). In one of the rare studies, Gündoğdu et al. (2017) have found 17 different fouling species of six phyla (Annelida, Arthropoda, Bryozoa, Chordata, Cnidaria, and Mollusca) on plastics. They have revealed the negative impacts of plastics on the bottom environment. Light is vital for the phytobenthos, which plays a significant role in primary production. Due to the collapsed litter, the phytobenthos may be a severely affected group. Katsanevakis et al. (2007) and Akoumianaki et al. (2008) have investigated the nutrient exchange between sediment and water. The authors state that many creatures were adversely affected by anoxic conditions of the benthos arising from the collapsed litter items. Further, the biochemical process of decomposition of organic matter may also be adversely affected, and ammonium and nitrate levels can increase. Green et al. (2015) have identified that the community structure and abundance of species hinged on sediment have decreased in less than nine weeks.

As the results of our study suggest, almost all of the previous studies on the marine environment remark that the most abundant marine litter is plastic items. Plastic products have become an integral part of everyday life in many countries. Undoubtedly, plastics make life easier. It is preferred in every walk of life due to its cheapness, lightness, and flexibility. It has been identified that the annual plastic production of Turkey is approximately 10 million tonnes. Besides, Turkey is in sixth place globally in terms of plastic production (PAGEV, 2019). Due to the high worldwide demand, it is not surprising that the most abundant pollutant is plastic.

This study, conducted at 34 sites located in the Marmara Sea, offers the first comprehensive data on seabed pollution. The results showed key indicators through the spatial distribution of marine litter. There is no doubt that the Marmara Sea is a particular geographic area defined as a semi-closed basin. With the aid of the Bosphorus and the Dardanelles Straits, the Marmara Sea interconnects the Aegean Sea and the Black Sea, which drastically changes the aquatic characteristics of the respective bodies of water. Hence, it creates a system in which current intensity and direction are more effective than in the other seas. However, there are some factors such as human population density, dense industrial areas, marine traffic, anchor areas, fisheries activities, and river systems prevalent in all the seas with which marine pollution is directly associated. Considering the potential pollutants, the Northeastern and Eastern Marmara Sea contain almost all the sources together. According to the General Directorate of Population and Citizenship of Turkey, the Marmara region with a population of 24.5 million people accommodates 30% of Turkey's population and the metropolis Istanbul, holding 15 million people, is inhabited by 18% of Turkey's population (PAGEV, 2019). Besides, the highest number of industrial facilities are stationed in the cities such as İstanbul, Kocaeli, and Yalova, which are located in the Eastern Marmara Region. Due to high industrial production, these areas have dense marine traffic, commercial ports, and many anchorage areas such as Ambarlı, Pendik, Gebze, and Gemlik. What's more, old vehicle tires are used as a collision mat on the handrails of fishing boats, ferries, etc. In this study, the mean CPUE of rubber tyres (L2a) was calculated to be 1 n/km^2, and all were solely collected in the Eastern Marmara Sea. This result proves the impact of maritime traffic stemming from cargo ships and fishing vessels on marine pollution. In addition, the Kocaeli Dilovası Stream discharges the pollutants of the industrial facilities into the Marmara Sea. Istanbul Water and Sewerage Administration (ISKI) reports that only 25% of industrial wastewater undergoes high-tech biological treatment.

In contrast, the remaining 75% is discharged into the Marmara Sea only after pre-treatment (PAGEV, 2019). Besides, fisheries-related pollution caused by such items as jackboots, fisherman gloves, through-hull fitting, vessel upholstery, etc. was observed to be higher in these areas than in the others. Moreover, it was observed from the physical conditions of the collected plastic debris that the plastic food wrappers were newly introduced in to the site. When all these factors are considered, it can be stated that the high marine litter density at the site 1, 2, 3, 4, 31, 32, 33, and 34, which are located around the east part of the Marmara Sea, was not remarkable (Figure 1). The relatively higher litter density around the sites 10 and 11 (in the northwest part) may have arisen from the current system. Owing to the low population in the Northeastern Marmara Region, the absence of streamflow, and relatively fewer industrial activities, the litter may be carried to the area by currents. Although lower litter density was detected around the Southwestern Marmara Sea, relatively higher litter was found at the sites 13 and 15, which is under the incessant influence of the Gönen Stream. The Gönen Stream may have transported the landfills to the sea. Besides, a great number of fishing vessels operate in this area. Thus, fishing vessels may be contributing to the increasing population density.

Besides, the temporal variations in the litter abundance serve as a warning for the managing authorities. Compared with the rates in 2017, a statistically significant increase was detected in the litter abundance in 2018. A relatively higher litter abundance was observed in the spring and summer of 2018. Possible reasons should be the growing population, increasing recreational tours on the Bosphorus in these two seasons, and higher discharge of the Dilovası Stream with the rain-induced faster-flowing currents in spring. Conversely, commercial legal fishery is prohibited between April and August, when the highest pollution was observed. Thus, it may be argued that fishing can be thought to be a secondary pollution source after tourism and population.

The spatial and temporal variations in the mean litter abundance were compared with those in the other areas. In this study, 246 trawl tows yielded a total of 660 litter items (n), weighing 434.9 kg, at 34 sites in the Marmara Sea. 32 of 34 sites were found to contain benthic litter. The mean litter abundance was calculated to be 136.7 n/km^2 and 90.1 kg/km^2. In two recent studies, Mancini et al. (2021) have recorded the benthic litter density between 312.5 and 2125 n/km^2 around the Northern Tyrrhenian Sea, Italy and Saladié and Bustamante (2021) report the same parameter to occur between 71.5 and 192 n/km^2 around the Gulf of Sant Jordi (Western Mediterranean Sea). Relatively few studies have been conducted on the benthic litter abundance in Northern Turkey. The studies were mostly centred around the Mediterranean coasts of Turkey (Büyükdeveci and Gündoğdu, 2021; Mutlu et al., 2020; Olguner et al., 2018; Gündoğdu et al., 2017; Eryaşar et al., 2014). Erüz et al. (2022) and Topçu and Öztürk (2010) have conducted a research study in the Black Sea and Gönülal et al. (2016) in the vicinity of the Gökçeada (Imbros) Island, the North Aegean Sea. A similar sampling method was used in these

two studies (Table 4). High litter densities are reported in all the studies conducted in Turkish seas. Compared with the density values in the present study, the litter density has been found to be lower in the Antalya Bay, Turkey (Olguner et al., 2018), while higher in the İskenderun Bay, Turkey (Büyükdeveci and Gündoğdu, 2021). However, higher benthic litter density is featured in the studies performed in the Black Sea (Topçu ve Öztürk, 2010; Erüz et al., 2022). Among all the studies conducted in the Turkish waters, the lowest benthic litter density has been recorded around the Gökçeada Island, the Northeastern Aegean Sea Gönülal et al. (2016). This may have stemmed from a relatively lower population and the absence of industrial facilities on the Gökçeada Island. It is known that the coastline of the Northeastern Marmara Sea is among the most polluted areas in Turkey. This is one of the reasons why higher litter density rates are observed in the respective areas. For instance, the litter groups plastics and rubber tyres were determined to be most abundant in terms of count and weight. These results corroborate the data in the previous studies.

Table 4. Spatial and temporal variations of litter abundance in the other areas

Authors	Sampling Year	Study Area	Sampling Type	Density		Major Pollutant
				n/km²	kg/km²	
Topçu and Öztürk (2010)	2007-2008	Western Black Sea	Bottom Trawl	128 - 1320	8 - 217	Plastics
Büyükdeveci and Gündoğdu (2021)	2009-2010	İskenderun Bay, Northeastern Mediterranean	Bottom Trawl	Mean: 450.94	Mean: 90.34	Plastics
Gönülal et al. (2016)	2013-2015	Gökçeada Island	Bottom Trawl	0 - 1.6		Plastics
Olguner et al. (2018)	2014-2015	Antalya Bay, Northeastern Mediterranean	Bottom Trawl	13.3 - 651.1	0.02 - 559	Plastics
Erüz et al. (2022)	2016	Southern Black Sea	Dredge and	460.7	80.68	Plastics
This Study (2022)	2017-2018	Marmara Sea	Bottom Trawl	27.5 - 662.2 (Mean:73.9)	0.03 - 1597.8 (Mean:48.7)	Plastics
Mutlu et al. (2020)	2019	Southeastern Aegean Sea	SCUBA	19	18	Plastics
Mancini et al. (2021)	2020	Northern Tyrrhenian Sea	Bottom Trawl	312.5 - 2125		Plastics
Saladié and Bustamante (2021)		Gulf of Sant Jordi (Western Mediterranean Sea)	Bottom Trawl	71.5 -192 (Mean:130)		Plastics

In recent years, people in Turkey and in the world have gained a better awareness of marine pollution. Some good regulations have been enforced in Turkey, such as automated garbage collectors, paid shopping bags, etc. The European Union (EU) projects such as MARLISCO and Clean Up Med are good practices that aim to motivate people to act more responsibly and make them more aware of the marine pollution-related damages. Besides, some state-funded projects (Zero Waste Blue Project; Regional Waste Management and Marine Litter Action Plan) have been implemented, e.g., to collect ghost fishing nets and garbage collection on the coasts of 28 Turkish provinces. Additionally, non-governmental organizations such as BORABDER, TÜDAV, Mediterranean Conservation Society actively work in this field. Contrary to these, nowadays the Marmara Sea suffers from marine mucilage and/or sea snot, and the mucilage is expanding to cover larger areas day by day. Mucilage is defined as phytoplankton exudation of photosynthetically-derived carbohydrates with a structure consisting of exopolymeric compounds with highly colloidal properties released from marine organisms under stressful conditions (Danovaro et al. 2015). Although environmental pollution is listed as a primary cause, natural factors and hydrological conditions are regarded as important (Mecozzi et al., 2012). Even though it is known that benthic litter and mucilage are not directly related, bottom environments covered with benthic litter and mucilage combined create more problems for benthic creatures. Owing to mucilage-covered benthos, the potential food items of demersal fish disappear. Besides, accumulated mucilage asphyxiates less mobile organisms (e.g., crustaceans, coelenterates, and molluscs) and clogs their siphons and burrow openings (Rinaldi et al. 1995; Pellegrini et al. 2003).

Consequently, it is stated that all stakeholders who contribute to this pollution should be informed of the possible dangers of marine pollution. Water resources protection education should be offered at primary schools. Besides, Turkey should immediately desist from importing plastic items from developed countries. Annex V–Prevention of Pollution by Garbage from Ships–of MARPOL (International Convention for the Prevention of Pollution from Ships) prohibits commercial vessels from disposing of all forms of plastics into the sea. Annex V should be applicable to fishing vessels as well. As Wang et al. (2014) state, a reward system may be implemented to collect and deliver vessels' solid waste in inland and international seas. Coastal tourism areas and facilities, such as beaches and cafes, should be inspected and kept under constant control, and administrative sanctions should be increased.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study. Material preparation and investigation were performed by Ali İşmen, Mukadder Arslan İhsanoğlu, Murat Şirin and İsmail Burak Daban. The writing/editing was carried out by İsmail Burak Daban and Mukadder Arslan İhsanoğlu, 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. We have research permission from the Ministry of Agriculture and Forestry General Directorate of Fisheries and Aquaculture with 67852565-140.03.03-E.121379 number and 11/01/2018 date.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Vitamins A, E, C, β -carotene contents and MDA level of freshwater mussel, (*Unio elongatulus eucirrus* Bourguignat 1860) in the Karakaya Dam Lake

Keban Baraj Gölü tatlı su midyesi (*Unio elongatulus eucirrus* Bourguignat 1860)'nin, A, E, C vitamini, β karatone içeriği ve MDA değerleri

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Abstract: In this study, we aimed to determine the effect of domestic and agricultural discharge on the level of antioxidant vitamins A, E, C, β-carotene and malondialdehyde (MDA, an indicator of oxidative stress) in the muscle tissue of freshwater mussel as known bioindicator (*Unio elongatulus eucirrus*). The freshwater mussels were collected from Arguvan (uncontaminated reference site) and Battalgazi (exposed to discharge site) in the Karakaya Dam Lake. In order to examine the effect of this discharge on freshwater mussel collected from these two different regions, antioxidant vitamins A, E, C, β carotene and Malondialdehyde (MDA) levels were analyzed using High Performance Liquid Chromatography (HPLC). The comparison between groups from the different localities showed that concentrations of the vitamins A, E, C, β-carotene and MDA were found statistically significant according to between the localities ($p<0.05$) The results showed that vitamins levels were higher and MDA levels were lower in the reference site.

Keywords: Karakaya Dam Lake, freshwater mussel, antioxidant vitamins, MDA, HPLC, bioindicator

Öz: Bu çalışmada, biyoindikatör (***Unio elongatulus eucirrus***) olarak bilinen tatlı su midyesinin kas dokusunda evsel ve tarımsal deşarjın antioksidan vitaminler A, E, C, β karoten ve malondialdehit (MDA, oksidatif stresin bir göstergesi) düzeyine etkisini belirlemeyi amaçladık. Tatlı su midye örnekleri Karakaya Baraj Gölü'ndeki Arguvan (kirlenmemiş referans alanı) ve Battalgazi'den (deşarj alanına maruz kalan) toplanmıştır. Bu deşarjın bu iki farklı bölgeden toplanan tatlı su midyesi numuneleri üzerindeki etkisini incelemek için, antioksidan vitaminler A, E, C, β karoten ve Malondialdehit (MDA) seviyeleri Yüksek Performanslı Sıvı Kromatografisi (HPLC) kullanılarak analiz edildi. Bölgeler arası yapılan karşılaştırmada tatlı su midye dokularında tespit edilen antioksidan vitaminler ve MDA değişimleri arasında istatistiki olarak önemli bir farkın olduğu belirlenmiştir ($p<0,05$). Sonuçlar, referans bölgede vitamin seviyelerinin daha yüksek ve MDA seviyesinin daha düşük olduğunu gösterdi.

Anahtar kelimeler: Karakaya Baraj Gölü, tatlı su midyesi, antioksidan, vitaminler, MDA, HPLC, biyoindikatör

INTRODUCTION

Water pollution is an important part of environmental pollution and contaminants that occur cause damage to the ecological balance. Such pollutants affect the eggs of living resources and young individuals much more, endangering the survival of their generation and increasing the number of less sensitive and less valuable species that can survive in contaminated environments. As a result, biological diversity in the environment decreases and the number of individuals in dominant species increases and natural wealth disappears (Kanar, 2012). The mussels that are fed by filtering the particles in the water can accumulate the substances they take with the water for a long time (Pytharopoulou et al., 2008; Faria et al., 2014). For this reason, mussels have advantages such as being able to make comparisons during the evaluation of the information obtained, collecting information about the pollutants in the same region for long periods and being widely used in order to quickly evaluate the current situation of many pollutants in aquatic environments (Esen, 2006; Ergüden, 2012). *Unio elongatulus eucirrus* is densely distributed in Turkish rivers and freshwater reservoirs. Molluscs, which have an important place in the food chain, form the food of various water birds and aquatic mammals (sable, beaver, etc.), especially fish. In addition to being consumed by humans in some countries, they are also used in the breeding of some animals (fish, chicken, pig). For these reasons, every study on the nutritional value of mollusks gains importance (Ekin et al., 2010). In addition, the mussels are also a major contributor to filtration of pollutants in the aquatic environment and to increase water quality (Aksul et al., 2012).In this context, two different stations were determined on the Karakaya Dam Lake,

Arguvan, which was chosen as a reference, and Battalgazi, exposed to domestic and agricultural discharges. With the presence of toxic chemicals in their shells and tissues, they have a great role in reflecting the environmental quality of water basins and they have great ecological importance (Yalçın, 2006; Aksul et al., 2012; Şahin et al., 2016). Aquatic pollution is a major contributor to oxidative stress in fish and other aquatic creatures resulting from the redox cycling of pollution. Reactive oxygen species (ROS) mediated oxidation of membrane lipids results in the formation of lipid peroxidation products such as malondialdehyde (MDA) and isoprostanes. To cope with the continuous generation of ROS from normal aerobic metabolism, cells and tissues contain a series of cellular antioxidants with both enzymatic (e.g. catalase) and non-enzymatic (e.g. vitamin E, carotenoids) activities. Some non-enzymatic low molecular weight antioxidant compounds are consumed and may be below the normal ranges (Karatepe, 2004; Barım and Karatepe, 2010)

Vitamin C and vitamin E are widely known antioxidants. However, β-carotene, which is the precursor of vitamin A, also has many antioxidant properties. Therefore, vitamins A, C and E are emphasized as antioxidant vitamins. Antioxidant vitamins; It is known that they maintain the oxidant-antioxidant balance by using ways such as stopping and suppressing reactions, scavenging free radicals, repairing tissue damage, increasing antioxidant capacity. The MDA is not a specific or quantitative indicator of fatty acid oxidation, it is very important in that it correlates well with the degree of lipid peroxidation. Therefore, Malondialdehyde (MDA) is the main and most studied product of polyunsaturated fatty acid peroxidation (Karatepe, 2004; İriş and Çınar, 2019; Kızılkaya et al., 2021).

The present study aimed to determine whether non-enzymatic antioxidants (vitamin E, A, C and β-carotene) and MDA could be useful indicators of the aquatic pollution in *U. elongatulus eucirrus* samples collected from two different regions.

MATERIAL AND METHOD

Study area

Karakaya Dam Lake is the third largest dam lake in Turkey after Atatürk and Keban Dam Lakes. It has an extremely important place in terms of both electricity production and aquaculture. Although the Karakaya Dam Lake was put into service about 17 years ago, it is rapidly becoming polluted. The most important reason for this is the discharge of wastewater from the surrounding residential areas, city center and farmland into the streams that meet with the dam lake. In addition to the wastes of small and large-scale industrial establishments, especially large factories, agricultural wastes coming from the lands used as agricultural land around the lake are directly or indirectly discharged into the Karakaya Dam Lake (Özen, 2018). For these reasons, it is planned to monitor the pollution level of the dam, which has such an important socio-economically important place, and this level through mussels known as biomonitors. For this, the Arguvan region on the dam lake, one of which is not exposed to any pollution and selected as the reference region of our study, and our other sampling point was the Battalgazi region which constituted our sampling points (Figure 1).

Figure 1. Location of the study area and sampling the stations

Experimental design

The levels of vitamins A, E, C, β-carotene and MDA were determined in muscle tissues of the freshwater mussels. 47 freshwater mussels were collected only in March and April throughout the year 2015.The samples were collected from two stations Arguvan (uncontaminated, references); Battalgazi (exposed to discharge site); in Karakaya Dame Lake. The mussels were obtained in these stations. Mussel samples were transported to the laboratory in refrigerated tanks as soon as possible. Identification of the collected samples and all morphometric measurements including weight, shell length, shell height and shell width were done and recorded during the study. The tissues of the mussels were stored at -80 ^{o}C for biochemical analysis. Mussel's wet muscle tissue was thoroughly broken down in the homogenizer in order to determine the levels of vitamin A, E, C, β-carotene and MDA.

Determination of lipid soluble vitamins

Lipid soluble vitamins in tissues were assayed according to the method of Catignani and Bieri (1983). Approximately 0.3 grams of the crushed samples were weighed and taken into polyethylene tubes, 3 mL of Ethanol: Sulfuric acid (99: 1) and 1mL of water were added for precipitation of proteins. After thorough mixing with vortex, it was centrifuged at 4500 rpm for 5 minutes. Then 300 µL of n-hexane (0.05% butylated hydroxytoluene) was added to the centrifuged samples. With the addition of hexane, the lipid-soluble vitamins in the medium were extracted into the hexane phase. The tubes were mixed in a vortex and centrifuged again. At the end of the centrifuge, the hexane phase was carefully separated and taken into the glass tube. 300 µL of n-hexane was added to the sample again, mixed and centrifuged, and the n-hexane phase was combined with the hexane phase in the glass tube. The extracted hexane phase was carefully evaporated by using nitrogen gas. The residue from the hexane was dissolved in 100 µl of mobile phase (methanol/acetonitrile/chloroform, 47:42:11, v/v). 50 µL of this solution was taken and injected into HPLC.

Determination of vitamin C and MDA

MDA and vitamin C levels were assayed according to the method of Karatepe, with small modifications (Karatepe, 2004). Briefly, the 0.3 g tissues were weighed and taken into polyethylene tubes. Then the crushed tissues were treated with 1 ml of 0.5 M perchloric acid and 1 ml of water. The cells were scraped from the tubes and centrifuged for 5 min at ambient temperature. The 20 µL supernatant was taken and separated 17.5 % methanol (v/v) in 30 mM monobasic potassium phosphate buffer (pH 3.6) mobile phase.

Instrumentation

The liquid chromatographic system consisted of LC-20AD pumps, DGU-20A5 degasser, SIL 20A autosampler, CTO-10AS VP column oven, SPD-M20A DAD system. These apparatus were connected via a communication module (Model CBM-20A) and controlled by a Shimadzu LC Solution Workstation (Shimadzu, Kyoto, Japan). A Shimadzu Shim-pack vp-ODS column (150 L×4.6) was used.

Statistical analysis

All statistical analyzes in the study were performed using the SPSS (22.0)/PC package program. Data are presented as mean and standard error. The difference between the two means was determined by the Mann-Whitney U test (P<0.05).

RESULTS

Morphometric measurements and their standard deviations in freshwater mussels used in this study were calculated. Results were recorded on average weight, shell length, shell height and shell width. While the average weight of the mussels collected from the Battalgazi region was 51.07 ± 13.69 g, the shell length was 72.76 ± 7.41 mm, the shell height was 36.05 ± 3.68 mm, and the shell width was 29.36 ± 4.20 mm, the average weight of the mussels collected from the Arguvan region was recorded as 86.25 ± 16.19 g, shell length 85.00 ± 6.29 mm, shell height 41.77 ± 4.10 mm and shell width 34.15 ± 3.83 mm. The results of antioxidant vitamins A, E, C and β-carotene and MDA levels which are an indicator of lipid peroxidation analyzed in this study are given in (Table 1). As can be understood from the results, it was determined that the antioxidant vitamins A, E, C and β-carotene levels of the mussel samples collected from both stations were high in the Arguvan region, which was chosen as a reference, while the level of MDA, which was an indicator of oxidative stress, was low. On the other hand, MDA levels increased while antioxidant vitamin levels decreased in the Battalgazi region exposed to pollution. When the data obtained were evaluated statistically, the levels of antioxidant vitamins A, E, β -carotene ($p < 0.001$), vitamin C ($p < 0.01$) and MDA ($p < 0.05$) detected from both stations were found to be significant.

Table 1. Antioxidant vitamins and malondialdehyde levels detected in wet mussel samples (mean ± standard error)

Parameters	Sampling Areas		*P* value
	Battalgazi	Arguvan	
C Vitamin, mg/kg	0.32±0.06	0.58±0.04	0.0011
E Vitamin, mg/kg	7.04±0.74	20.65±2.73	0.0001
A Vitamin, mg/kg	0.15±0.02	0.41±0.05	0.0001
β-Carotene,mg/kg	26.18±4.11	58.73±9.01	0.0009
Malondialdehit,mg/kg	0.058±0.010	0.028±0.005	0.011

(Note: Significantly different; *p<0.05, **p<0.01, ***p<0.001).

DISCUSSION

The aim of this study was to evaluate the usefulness of antioxidant parameters in freshwater bivalve (*U. elongatulus eucirrus*) as biomarkers of exposure to pollutants and to examine their potential relevance in predicting toxicity. Since environmental pollutants toxically affect hemostatic tissues and organs, they also affect mechanisms such as the immune system (immune system). Examining several components that make up an integrated biological system, such as the immune system, can provide a precise and comprehensive measure of an organism's health status. This reflects the degree of pollutant-induced stress and thus may be an early indicator of disease susceptibility (Pipe et al., 1999). This determines the organism's capacity to survive against environmental pollutants (Dyrynda et al., 1998). Freshwater mussels are thought to be sensitive to the genotoxic effects of metals and complex pollutants (Bolognesi et al., 1996; Chandurvelan et al., 2013). Antioxidant defense systems control the damage of reactive oxygen species and free radicals in the body. Since these systems act on different free radicals and in different cells, they complement each other (Diplock, 1998). Cells are protected against oxidative stress caused by free radicals and peroxides through antioxidant defense systems under normal physiological conditions. In general, non-enzymatic antioxidants are more active outside the cell, while enzymatic antioxidants are more active inside the cell (Halliwell and Gutteridge, 2000). Antioxidant enzymes and lipid peroxidation triggered by the effect of pollutants are used as oxidative stress biomarkers (Cossu et al., 2000).

This study is the first comprehensive study to determine the levels of antioxidant vitamins A, E, C and β-carotene and MDA in the muscle tissue of freshwater mussels (*U. elongatulus eucirrus*) collected from Karakaya Dam Lake. As a result of the data of the study, it has been determined that there is a significant difference between the two areas exposed to pollution the reference, and it has been tried to be revealed by monitoring aquatic pollution through selected mussels as bioindicators. It was clearly seen that the levels of antioxidant vitamins were low and the level of MDA was high in the freshwater mussel samples collected from the polluted area. When we look at the studies carried out on mussels in order to

reveal the effect mechanism of pollution on antioxidant vitamins and enzymes, both at home and abroad, we see that the findings are parallel and confirm each other (Box et al., 2007; Vlahogianni et al., 2007; Faria et al., 2014). Ribera et al. (1991) aimed to reveal the relationship between free radical and lipid peroxidation in *Mytilus edulis* mussels. Mussels were exposed to compounds known for their ability to generate free radicals (carbon tetrachloride, CCl4) and reactive oxygen species via the redox cycle (menadione) and investigated the effects on digestive glands, gills and remaining tissues. They stated that lipid peroxidation parameters and free radicals (glutathione, vitamins A, E and C) were more affected by menadione exposure than CCl4. At the end of the study, they revealed the state of change in free radicals. Vitamin A and carotene have a number of biochemical functions, including oxyradical scavenging. These antioxidants levels were decreased in mussels with pollution exposure. Vitamin C is the other most important water-soluble antioxidant, acting either directly or by regenerating reduced tocopherol from tocopheroxy radical. Vitamin C levels were decreased in mussel tissues that were affected by pollution. Vitamin E is the major fat-soluble antioxidant present in the mussel membrane. It reacts directly with oxyradicals and singlet oxygen. The observed decreases in the muscle of mussels. The changes of free radical scavengers in all tissues with exposure to pollution, indicate that the molecules are involved in mechanisms of oxyradical detoxication and lipid peroxidation control *in vivo*. In the studies conducted by us and Ribera et al. (1991), the decrease in antioxidant enzyme levels in mussels exposed to oxidative stress showed similarity in the findings of both studies. Borkovic-Mitic et al. (2013) showed the differences in vitamin E concentrations in mussels that were observed in the samples obtained from the four sites on the Sava River. This may be due to higher levels of heavy metals (Cu, Cd and Mn). Significant negative correlations were established between the concentrations of vitamin E and Cu and Cd, and positive correlations between vitamin E and Mn. From the presented results it can be concluded, that investigated antioxidant enzymes and non-enzymatic components represent suitable biomarkers of metal pollution and that different tissues plays an active role in oxidative stress generation and antioxidant responses and can therefore be used as bioindicators of metal pollution. In our study of monitoring the ecological status of freshwater mussels, non-enzymatic antioxidant vitamin levels were also examined. At the end of the study, the amount of vitamin E in the mussel samples collected from the Battalgazi region, which was exposed to the discharge, was lower than the reference region. The findings of both studies were found to be similar.

CONCLUSION

It is known that antioxidants are widely used in biomonitoring studies. The high levels of these substances in polluted areas are linked to the key role they play in the detoxification of compounds that cause oxidative stress. Determining the toxic effects of pollutants on bivalves will help predict the toxic effects that pollutant levels will have on humans. It is planned to expand the study by considering other heavy metals, organic pollutants, and metallothionein proteins.

AUTHORSHIP CONTRIBUTIONS

Ayşe Gül ŞAHİN: Project administration, resources, funding acquisition, writing- reviewing and editing, Mustafa KARATEPE**:** Methodology, software, validation, writing-reviewing and editing.

CONFLICTS OF INTEREST

There was no financial or personal interest in our article titled "Vitamins A, E, C, β -carotene contents and MDA level of freshwater mussel, (*Unio elongatulus eucirrus* Bourguignat 1860) in the Karakaya Dam Lake".

ETHICS APPROVAL

Samples were provided within the scope of the TAGEM project titled "The effect of heavy metal pollution on biochemical parameters and meat quality in some fish species in Keban, Karakaya, and Atatürk Dam Lakes". Approval was granted by Elazığ Veterinary Control Institute Animal Experiments Local Ethics Committee (Date: 20.09.2016 /No: 16/02).

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Weight and color evaluation of whole and filleted carp by image analysis

Görüntü analizi ile bütün ve fleto sazanların ağırlık ve renk değerlendirmesi

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Abstract: Weight estimation of whole fish and fillets, and skin color of whole fish and fillet meat colors of the male and female scaled and mirror carp (*Cyprinus carpio*) were evaluated by image analysis. After measuring the weight of 10 scaled and 10 mirror carp and their fillets, pictures of both sides of whole fish, and meat side of fillets were taken in a light box. The relationship between weight (W) and view area (V) was calculated by linear ($W = A + BV$), and power ($W = A V^B$) equations. According to the power equation B values, scaled and mirror carps showed positive allometric growth in culture conditions. Statistically, there was no significant difference between the parameters of whole fish left and right sides, as well as whole fish gender. The same was true for right and left fillets, and female and male fish fillets. For both left and right sides scaled and mirror carp had no difference between average L*, a* and b* values (P>0.05). Also, there was no difference between average L*, a* and b*values of male and female of scaled and mirror carp fillets (P>0.05). Image analysis can be used to determine the size, weight, view area and skin and meat color of two carp species and their fillets.

Keywords: Common carp, mirror carp, image analysis, size, color, gender

Öz: Bütün balık ve filetoların ağırlık tahmini, bütün balığın deri rengi ile erkek ve dişi pullu ve aynalı sazanların (*Cyprinus carpio*) fileto et renkleri görüntü analizi ile değerlendirilmiştir. 10 adet pullu ve 10 adet aynalı sazan balığı ve filetolarının ağırlıkları ölçüldükten sonra ışık kutusunda bütün balık için her iki yüzün ve filetoların et yüzünün resimleri çekilmiştir. Ağırlık (W) ve yüzey alanı (V) arasındaki ilişki lineer ($W = A + BV$) ve güç ($W = A V^B$) denklemleriyle hesaplanmıştır. Güç denklemi B değerlerine göre, pullu ve aynalı sazanlar kültür koşullarında pozitif allometrik büyüme göstermiştir. İstatistiksel olarak, bütün balığın sol ve sağ taraf parametreleri ile bütün balık cinsiyeti arasında anlamlı bir fark bulunmamıştır. Aynı durum sağ ve sol filetolar ile dişi ve erkek balık filetoları için de tespit edilmiştir. Bütün pullu ve aynalı sazan için hem sol hem de sağ taraf için ortalama L*, a* ve b* değerleri arasında fark bulunmamıştır (P>0.05). Ayrıca pullu ve aynalı sazan filetolarının erkek ve dişi ortalama L*, a* ve b* değerleri arasında fark tespit edilmemiştir (P>0.05). Görüntü analizi, iki sazan türünün ve bunların filetolarının boyutunu, ağırlığını, görüş alanını ve deri ve et rengini belirlemek için kullanılabilir.

Anahtar kelimeler: Sazan, aynalı sazan, görüntü analizi, boyut, renk, cinsiyet

INTRODUCTION

Aquaculture production reached 82.1 million tons in 2018, up by 3.2 percent from 2017 (FAO, 2020a). Cyprinids are the most cultivated fish group worldwide and their production is increasing. Common carp (*Cyprinus carpio*) is a major cultured fish species especially in Asia and European freshwater aquaculture, due to its fast growth, strong adaptability, good flesh qualities, high nutritional value, good taste, high meat content and cheap price (Ljubojevic et al., 2017; Yang et al., 2020). The global production of common carp peaked at over 4.18 million tons in 2018 (FAO, 2020b).

Carps are used as a whole, gutted, scaled, or fillets (Bauer and Schlott, 2009). Flesh quality is affected by many biological or nonbiological parameters (Lie, 2001). Fillets have been the focus of processing studies (Gela et al., 2003; Kocour et al., 2007). The relationship of morphology and fillet yield has been studied (Cibert et al., 1999). The economic importance of marketing carp as fillet has grown.

Morphological parameters such as the length-weight relationship (LWR) are important to understand growth patterns in fish, and the condition factor is used as an important feature in estimating average weights of whole fish of given length groups (Froese, 2006). LWR is considered as an important biological parameter to generate information about the growth and condition of fish living in both natural and culture conditions (Samsun et al., 2017; Awas et al., 2020). The relationship between the length and weight of the fish is given by the equation $W = AL^B$, with B = 3 as an isometric weight gain. If B is different from 3, the weight gain is negative or positive allometric (B> 3; B <3) (Froese et al., 2014; Khristenko and Otovska, 2017).

Accurate measurement of length, area and weight manually is not easy and may result in measurement errors. With computerized image analysis, length and surface area measurement can be done accurately and easily, leading to rapid weight estimation (Gümüş and Balaban, 2010; Balaban et al., 2010a).

The carp fillet meat color is one of the important parameters in determining its acceptability for consumers (Johnston et al., 2006; Song et al., 2020). Despite its affordability and high nutritional value, carp fillet might be ignored if it has an unattractive look. A more acceptable appearance might improve the market adoption of carp fillets.

The color, size, shape and visual texture of fish can be obtained by computerized image analysis (Gümüş et al., 2011). There are many such studies on color quantification (Balaban et al., 2014; Ünal-Şengör et al., 2019; Gümüş, 2021). However, no studies have been found to determine the size and color quality of carp fillets by the computerized image analysis method.

In many studies, one side of the fish is used in image analysis. Evaluating differences on right and left sides may confirm or deny this practice. Erikson and Misimi (2008) reported that there was no statistical difference between the right and left side colors of Atlantic Salmon. Also, in some fish, the appearance of male and female fish is different. In this study, using image analysis, it was aimed to determine the length-weight, and area-weight relationships of two species of the whole carp (scaled and mirror carp) and right and left fillets from them. In addition, the skin color of whole fish, and meat color of fillets were quantified. The effect of gender on these attributes was evaluated.

MATERIAL AND METHODS

Fish samples and weighing

Scaled carp and mirror carp (*C. carpio*) were obtained from the Fisheries Research, Production and Training Institute, Kepez, Antalya, Turkey in May 2021. The fish were harvested after starving for one day. A total of 20 fish, including 10 scaled and 10 mirror carp, were immediately transferred to Akdeniz University Fisheries Faculty in ice in Styrofoam boxes. Before imaging, the weight of each fish was measured and recorded on an electronic balance (max. 4100 g, 0.1 g precision, Precisa Instruments Ltd./Switzerland). The weights of the fish varied between 246-769 g.

Image acquisition

After weighing each fish, images were taken in a light box described by Gümüş et al. (2021). A Nikon D610 DSLR camera (Nikon Corp., Tokyo, Japan) with a 24-300 mm zoom Nikon lens with a circular polarizing filter was used. Camera settings are given in Table 1. Only polarized images were taken to assure correct colors, and the spoon reflecting the upper LED panel indicated that the polarization was used, since the reflection was black. Size and color references were present in each picture, as described by Gümüş et al. (2021). The dual-image method was used to take the images (Alçiçek and Balaban, 2012). Images of each fish were taken from both the left and right sides. In addition, each fish was classified as male and female.

The fish were then filleted manually without prior bleeding. The skin and all visible pin bones were removed. The fillets were weighed, and images of the meat side of the fillets were taken. Corel PhotoPaint (Corel Corp., Ottawa, Ontario, Canada) was used to clear the bottom-lighted images to isolate the color reference (Figure 1 for whole fish, Figure 2 for fillets).

Image analysis

LensEye-NET (ECS, Gainesville, FL) was used to analyze images. Since the true color of the reference color was known, the whole image was color-corrected to make the color of the reference color match its true color. Then, the size reference of known surface area (9 cm^2) was used to convert pixel-based areas to cm^2 and pixel-based lengths to cm (Figure 3).

$$Fish\ view\ area\ (cm^2) = \frac{\#\ pixels\ of\ fish}{\#\ pixels\ of\ size\ ref}\ x\ 9 \quad \text{(Eqn. 1)}$$

Table 1. The Nikon D610 camera control settings for front-lighting and back-lighting images

Camera settings	Front-lighting	Back-lighting
Exposure mode	manual	manual
Shutter speed	1/2.5 sec	1/20 sec
Aperture	f/9	f/9
Exposure compensation	0 EV	0 EV
ISO sensitivity	200	200
White balance	Preset 1	Preset 1
Image small size (pixels)	3008*2008	3008*2008

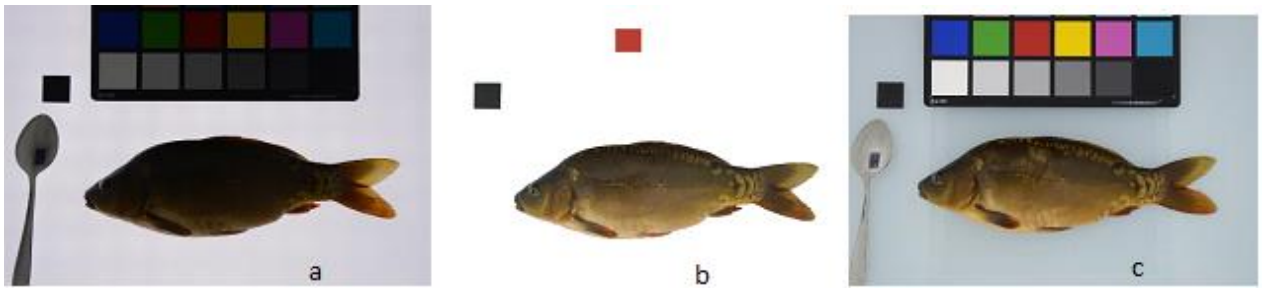

Figure 1. Example of the dual-image method applied to whole scaled and mirror carps. a) backlighted image. b) backlighted image cleaned. c) front lighted image

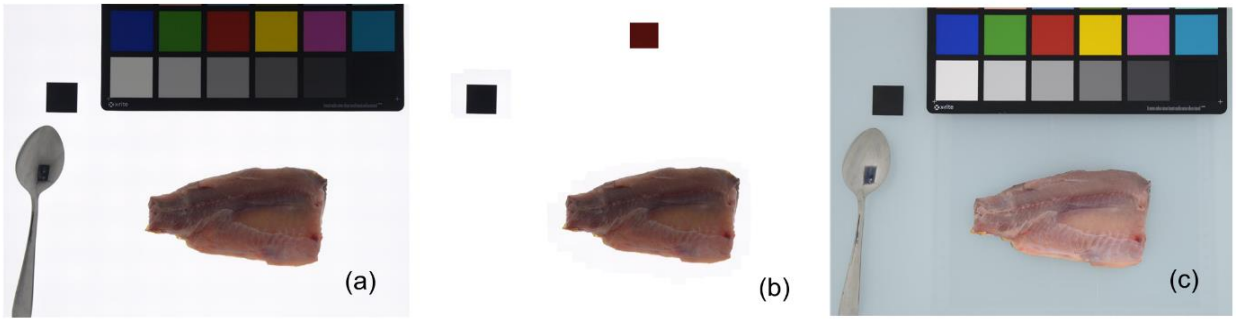

Figure 2. Example of the dual-image method applied to scaled and mirror carp fillets. a) backlighted image. b) backlighted image cleaned. c) front lighted image

Weight-View area relationship

The following equations between the weight and the view area (VWR) were tried (Balaban et al., 2010a):

Linear: $W = A + B\,V$ (Eqn. 2)

Power: $W = A\,V^B$ (Eqn. 3)

In the equations above, W=weight (g), V=view area (cm^2), A, B are coefficients obtained by regression.

Weight-Length relationship

The length of each fish was obtained by fitting the best rectangle (rectangle of minimum surface area that encloses the fish), and the length of the rectangle was taken as the length of the fish.

The following equations between the weight and the length were tried for the length – weight relationship (LWR) (Balaban et al., 2010a):

Linear: $W = A + B\,L$ (Eqn. 4)

Power: $W = AL^B$ (Eqn. 5)

In the equations above, W=weight (g), L=length (cm), A, B are coefficients obtained by regression.

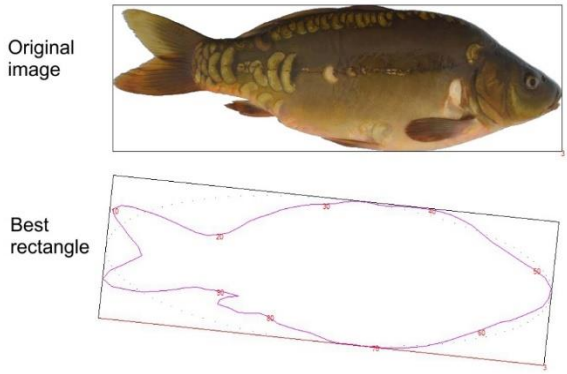

Figure 3. Example of an original fish image, and the best rectangle fitted to it to obtain fish length

Color analysis

The LensEye-NET program obtained the L*, a*, and b* values of each pixel of the fish image. Averages and standard deviations for each object were calculated.

The surface visual texture of each fish was quantified as Texture Change Index (TCI) using the texture primitives method (Balaban, 2008). LensEye-NET program was used for this.

The delta E values of the skin color of whole fish and meat colors between the right and left fillets were calculated based on the equation below:

$$Delta\ E = \sqrt{(L_R{}^* - L_L{}^*)^2 + (a_R{}^* - a_L{}^*)^2 + (b_R{}^* - b_L{}^*)^2} \quad \text{(Eqn.6)}$$

where L*, a* and b* are the CIE color components, the subscripts R and L represent right and left fish/fillets, respectively.

Statistical analysis

The statistical procedures were performed using SPSS v.23 (IBM-SPSS, Armonk, NY, USA). For analysis of variance (ANOVA), differences between the means were subjected to one-way analysis, and Duncan's multiple range test was used to compare the means ($P < .05$). The R^2 values for each fit were also calculated. Results were given as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Using both length and view area of catfish (Gümüş et al., 2021), and commercial Mullidae species (Gümüş, 2021) for weight estimation have been reported. Measuring length may be problematic if the fish can bend easily. Area measurement does not have this disadvantage, and may be more reliable in predicting weight.

Weight - Area relationship of whole fish

The upper part of Table 2 summarizes the calculated parameters of the linear and power fits to the view area vs weight of scaled carp. All R^2 values are higher than 0.94. For female fish, the linear and power fit parameters (A and B) for the left and right sides are not different at 95% confidence. This is also true for the male fish. Therefore, it is not necessary to separate the fish by gender when analyzing VWR or to separate the left and right sides. All scaled carp can be lumped together for VWR analysis, and the side does not matter.

Balaban et al. (2010a) developed the equations to predict the weight of 4 Alaskan salmon species, and Balaban et al. (2010b) did the same for Alaskan pollock. Gümüş and Balaban (2010) developed the equations to predict the weight of whole rainbow trout from different farms using the view area of the fish. Konovalov et al. (2018) estimated the weight of Asian Seabass from its images. When view area (length^2) is correlated to weight (and therefore to volume, length^3) the exponent term of the power equation to correlate view area to weight is expected to be in the vicinity of 1.5. Again, differences in the shape, thickness and morphology of the fish will result in different fitted parameters for different equations.

Table 2. Weight vs right and left side view area relationships for female and male whole scaled and mirror carps

Scaled carp	Area-Weight	Female		Male	
		Linear W = A+BV	Power W = AV^B	Linear W=A+BV	Power W = AV^B
Left	A	-192.19±237.1	0.308± 9.181	-132.24± 162.61	0.401±1.415
	B	3.444± 1.208	1.393± 0.420	3.058± 0.893	1.337±0.511
	R^2	0.94	0.955	0.991	0.984
Right	A	-207.98± 209.6	0.264±7.065	-129.43± 144.52	0.417± 10.542
	B	3.511± 1.064	1.422±0.371	3.018±0.787	1.328±0.454
	R^2	0.955	0.966	0.993	0.988
Mirror carp	**Area-Weight**	**Female**		**Male**	
		Linear W = A+BV	Power W = AV^B	Linear W=A+BV	Power W = AV^B
Left	A	232.629±763.842	23.188± 726.098	-327.687±217.55	0.0253±26.433
	B	1.596±3.222	0.598±1.218	3.858±0.953	1.836±0.607
	R^2	0.694	0.691	0.969	0.946
Right	A	242.203±726.608	25.139±568.351	-333.807±215.78	0.024±26.593
	B	1.552±3.057	0.583±1.160	3.879±0.944	1.848±0.608
	R^2	0.705	0.701	0.970	0.947

W = weight, g, V = view area, cm^2. A and B are parameters.

The lower part of Table 2 presents the same VWR analysis results for the mirror carp. In this case, some R^2 values are much lower (0.691 – 0.970). This is because the shape of the mirror carp is slightly different than that of the scaled carp which has a more uniform shape. Also, the fins and tail may affect the results (Balaban et al., 2010b). Regardless, the left and right fit parameters (A and B) of the female fish are within the 95% confidence interval of each other, therefore there is no need to separate the fish as right or left. However, there is a distinguishable, but not statistically significant difference

between the parameters of the female and male fish. For example, the B value for the power fit of the left sides of female and male fish are 0.598±1.218 and 1.836±0.607, respectively. The difference in values is easily noticeable, but the 95% confidence intervals make them not statistically different.

Weight - Length relationship (WLR) of whole fish

The upper part of Table 3 shows the results of linear and power fits to the length-weight relationship for whole scaled carp, with right and left sides, and female and male fish separated. The R^2 values range from 0.776 to 0.983. The calculated parameters A and B, both for the linear and power fits, are statistically not different for the left or right sides, or for the female and male fish. The 95% confidence intervals are large enough so that the ranges overlap. Therefore, for scaled carp, the LWR can be applied to the combination of female and male fish, either on the left or the right sideThe lowerwer part of Table 3 shows results of linear and power fits to the LWR for whole mirror carp, with right and left sides, and female and male fish separated. The R^2 values range from 0.404 to 0.910. Again, the R^2 values are lower than that of scaled carp. The calculated parameters A and B for mirror carp are not statistically different between the right and left sides, and between the male and female fish. Therefore, the LWR can be calculated for mirror carp by lumping the male and female fish together and using either the right or the left side.

Measuring the length to estimate the weight of fish has been practiced widely. Ak et al. (2009) developed the length-weight relationship of 16 species from Eastern Black Sea. Bengil (2019) developed the same relationship for fish from the Mediterranean Sea. Ergüden et al. (2009) developed the length-weight relationship for trawl-caught fish in Iskenderun Bay.

Table 3. Weight vs right and left side length relationships for male and female whole scaled and mirror carps

Scaled carp	Weight-Length	Female		Male	
		Linear W = A+BL	Power $W = AL^B$	Linear W=A+BL	Power $W = AL^B$
Left	A	-828.03±1030	0.0207± 1210	-689±450	0.0535±75.6
	B	44.9±33.4	2.93±2.07	37.4± 15.2	2.64±1.28
	R^2	0.776	0.794	0.983	0.975
Right	A	-842± 918	0.0308±452	-658± 478	0,068±101
	B	43.1± 29.9	2.82±1.79	36.3±16.1	2.57±1.36
	R^2	0.800	0.827	0.979	0.971
Mirror carp	**Area-Length**	**Female**		**Male**	
		Linear W = A+BL	Power $W = AL^B$	Linear W=A+BL	Power $W = AL^B$
Left	A	124.06±1800	41.5±1770	1240±838	1180±5180
	B	15±55.5	0.773±2.81	56.1±26.4	3.76±2.48
	R^2	0.404	0.411	0.897	0.816
Right	A	161±1570	50.8±5060	1180±762	0.00165±2460
	B	13.8±47.9	0.713±2.45	54.3±23.9	3.66±2.26
	R^2	0.433	0.440	0.908	0.835

W = weight, g, V = view area, cm^2. A and B are parameters.

Fernandes et al. (2020) used machine vision to extract body measurements to predict the weight of Nile tilapia. Gökçe et al. (2010) developed the length-weight relationship of marine fish from Yumurtalık coast. Kalaycı et al. (2007) used trawl-caught 10 fish species, and Samsun et al. (2017) 11 fish species from Middle Black Sea to predict weight from length. Miranda and Romero (2017) developed a device to measure the length of rainbow trout using image processing, and Shafry et al. (2012) from digital images. Özvarol (2014) presented the length-weight relationship of 14 species of fish from the Gulf of Antalya. Sangun et al. (2007) used 39 fish species from Northeastern Mediterranean to develop length-weight relationships. Since the shape, thickness and morphology of the fish are different, it is expected that the parameters for the length-weight relationship will be different.

Weight - Area relationship of fillets

VWR depends on how the fillet was cut: thin, or thick. In the upper part of Table 6, the linear and power fits to the view area – weight relationship for left and right fillets, and for those from female and male scaled carp are presented. The R^2 values ranged from 0.850 to 0.990. The calculated parameters A and B are not statistically different between the right and left sides, and between the male and female fish ($p<0.05$). Therefore, male and female fish can be grouped together, and left and right fillet specification is not needed.

Table 4. Weight vs right and left side view area relationships for male and female scaled and mirror carp fillets

Scaled carp	Area-Weight	Female		Male	
		Linear W = A+BV	Power $W = AV^B$	Linear W=A+BV	Power $W = AV^B$
Left	A	-38.7±81.6	0.112± 36.6	-4.57±60	0.838±26.1
	B	1.23±0.7	1.44±0.758	0.912± 0.569	1.01±0.704
	R^2	0.855	0.874	0.960	0.950
Right	A	-42.9± 86.7	0.105±46.1	-27.3± 41.9	0.245±5.88
	B	1.27± 0.74	1.45±0.805	1.12±0.386	1.27±0.379
	R^2	0.850	0.862	0.987	0.990
Mirror carp	**Area-Weight**	**Female**		**Male**	
		Linear W = A+BV	Power $W = AV^B$	Linear W=A+BV	Power $W = AV^B$
Left	A	-25.7±322	$0.511±1.88\ 10^5$	-47.7±18.1	0.0951±2.25
	B	1.13±2.33	1.12±2.47	1.31±0.144	1.47±0.169
	R^2	0.683	0.657	0.994	0.993
Right	A	10.3±479	$2.18±3.74\ 10^7$	-50.3±24.5	0.113±1.97
	B	0.917±3.6	0.840±3.57	1.32±0.185	1.43±0.14
	R^2	0.376	0.339	0.990	0.995

W = weight, g, V = view area, cm^2. A and B are parameters.

In the lower part of Table 4, the linear and power fits to the view area – weight relationship for left and right fillets, and for those from female and male mirror carp are presented. The R^2 values ranged from 0.339 to 0.995. The calculated parameters A and B are not statistically different between the right and left sides, and between the male and female fish. Therefore, male and female fish can be grouped together, and left and right fillet specification is not needed.

Color

Table 5 displays the average and standard deviation color parameters of whole scaled and mirror carp. The right and left sides, and gender are separated. It can be seen that for the left and right sides of female scaled carp, there are no significant differences between the average L*, a* and b* values. This means that for color evaluation purposes the right and left sides of whole female scaled carp can be used interchangeably. The same is also true for the L*, a* and b* parameters of male scaled carp, left and right sides. Therefore, the side does not make a difference in the color evaluation of male scaled carp. When comparing the color parameters of the right and left sides of female and male mirror carp, there is no significant difference.

Comparing the female and male scaled carp, there is no significant difference between the color parameters for both the left and right sides. The same is true for the mirror carp. However, there are differences between the scaled carp and mirror carp color parameters.

There is not much literature on the comparison of the color from the right and left sides of fish. Balaban et al. (2014) monitored the average skin color of gurnard (*Chelidonichtys kumu*) and snapper (*Pagrus auratus*) for 12 days at 0°C. There was no statistical difference between the L*, a* or b* values over the storage period. This is despite the fading of the red color in both fish, meaning that the change in color was the same on both sides of the fish.

Another means of determining if the color difference is perceptible by human eye is to examine the Delta E value (Eqn 6). It is generally accepted that a Delta E value below 1 is visually not detectable by humans: Delta E = 1 is "just noticeable difference" for the human eye (Abeyta, 2011). Based on this definition, the delta E value between the right and left side of whole fish varied between 1.46 and 2.78. This implies that the color difference between right and left side skin color of whole fish may be detectable by human eye. The reason for this apparent difference in color between the sides is unknown.

Table 5. Average skin colors of whole scaled and mirror carps, right and left sides and gender

Carp	Gender	Color	Left	Right
Scaled	Female	L*	42.148±1.751^{a}	41.467±1.917^{ab}
		a*	3.497±1.220	3.483±1.210
		b*	22.067±2.873	22.147±2.654
	Male	L*	39.603±3.740^{abc}	39.358±3.741^{abc}
		a*	3.260±1.268	3.730±0.222
		b*	20.283±3.123	21.005±2.716
Mirror	Female	L*	37.508±4.306^{bc}	36.383±3.998^{c}
		a*	1.895±0.746	2.900±1.341
		b*	23.363±3.307	22.228±3.157
	Male	L*	37.473±2.324^{bc}	36.828±1.388^{c}
		a*	2.768±1.087	3.318±2.400
		b*	23.082±1.586	23.552±2.887

Means with different letters are significantly different (p< .05)

The visual texture of skin surface

One immediately noticeable difference in the appearance between the scaled carp and mirror carp is visual texture of skin. Visual texture is the concept of how "rough, uneven, variable" the surface looks. One method to quantify the visual texture is the "Texture Change Index (TCI)" based on texture primitives (Balaban, 2008). The rougher the appearance of the surface the higher the TCI value. In Table 6, The TCI values of scaled carp are much higher (3.2 times) than those of the mirror carp. Evaluating the standard deviations of the TCI values, there is no statistically significant difference between the left and right sides of a given fish, and between the female and male fish. However, the difference in the fish species is significant. Literature on the skin-side or meat side of fish regarding visual texture, including TCI analysis, is scarce. Since "objective" image analysis-based visual texture measurement must be correlated with human sensory panel results, and since there is not much standardization on how to conduct visual texture by sensory panels, this area needs more research and standardization (Balaban and Alçiçek, 2016). Then, many computerized methods, including TCI, can be used to reliably quantify the visual texture of fish in particular, and foods in general.

Table 6. Skin TCI values of whole scaled and mirror carps, right and left sides and gender

Carp	Gender	Left TCI value	Right TCI value
Scaled	Female	16.38±0.78^{a}	15.94±1.13^{a}
	Male	16.88±1.46^{a}	16.23±1.87^{a}
Mirror	Female	5.15±1.22^{b}	4.41±1.46^{b}
	Male	4.68±0.91^{b}	4.55±0.72^{b}

Means with different letters are significantly different (p< .05)

One method to visualize the TCI method is to depict the texture primitives as equivalent circles (Figure 4). In mirror carp, the visual texture is relatively smooth, so the texture primitives are large and less numerous. In scaled carp, however, there are many small texture primitives, indicating that the appearance of the surface is rougher.

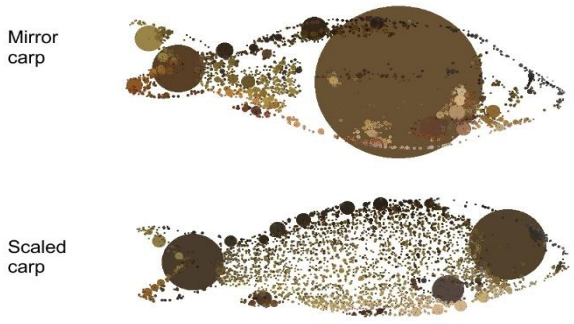

Figure 4. Example of texture circles for mirror scaled and mirror carps. Each circle represents a texture primitive. The more primitives, the higher the visual texture, and the TCI value (Balaban, 2008).

Fillets

Table 7 presents the average color parameters of the meat side of fillets of female and male fish, as well as the right and left side colors. It can be seen that based on the standard deviations, there is no significant difference between the L*, a* and b* values of the left and right fillets from the same fish. Also, there is no significant difference between the female and male fillets of the same species. Finally, there is no significant difference between the scaled carp fillet colors and the mirror carp colors.

Table 7. Average meat colors of scaled and mirror carp fillets, right and left sides and gender

Carp	Gender	Color	Left	Right
Scaled	Female	L*	65.765±2.818	66.032±2.288
		a*	10.381±1.493	9.700±1.250
		b*	11.760±2.043	10.774±2.260
	Male	L*	65.025±3.527	65.115±4.177
		a*	10.095±2.248	9.962±1.896
		b*	9.850±2.244	9.562±1.961
Mirror	Female	L*	65.510±1.271	65.477±2.036
		a*	10.765±0.567	10.600±1.298
		b*	12.222±1.975	12.260±1.884
	Male	L*	64.853±1.960	65.508±1.982
		a*	11.220±1.729	10.750±1.578
		b*	12.81±3.052	12.478±2.813

Finally, the Delta E values represent color differences between the right and left sides of the fillet from the same fish hover between 1.02 and 1.67. This suggests that these color differences are barely detectable by the human eye.

CONCLUSION

The results of this study confirm the use of one side of the carp species used for image analysis, be it for length, view area, or skin color evaluation. In addition, there was no statistically significant difference between male and female results, in evaluating weight using length or view area. Therefore, for these species of carp, males and females can be grouped together for this type of analysis. In many studies, the B value in the power equation in determining weight from view area is close to 1.5. In whole mirror carp, this value ranged between 0.583±1.160 to 1.848±0.608. The 95% confidence intervals of these values include 1.5, and the wide variability may be due to the low number of samples. For scaled carp, the B value of the power equation ranged from 1.33±0.5 to 1.42±0.37. Again, these confidence intervals include the value of 1.5. Using the same reasoning, the B value of the power fit to weight vs length relationship is expected to be 3. For whole scaled carp, the B value ranged from 2.57±1.36 to 2.93±2.07. For mirror carp, this range was 0.713±2.45 to 3.76±2.48. Considering their 95% confidence interval, these ranges include the number 3. As before, the mirror carp had more variability.

For carp fillets, the prediction of weight using view area by power equation resulted in B values for scaled carp in the range 1.01±0.704 to 1.45±0.805, and for mirror carp in the range 0.840±3.57 to 1.47±0.169. Considering their confidence intervals, these ranges include the theoretical value of 1.5. Again, the wide variability of mirror carp values is observed. In some fish, the appearance of male and female fish is different. In this study, using image analysis, for the length-weight, and area-weight relationships of two species of whole carp and the fillets from them, there was no significant difference between female and male fish. This also applied to the skin color of whole fish, and the meat color of fillets: there was no significant color difference between right and left sides, and between female and male fish. There was a very significant difference between the visual texture of whole scaled carp skin and whole mirror carp skin.

Image analysis can easily and rapidly estimate the weight of whole fish from its length or view area. It can also quantify the color parameters of the skin and fillet meat, and the visual texture of skin.

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AUTHORSHIP CONTRIBUTIONS

Bahar Gümüş: Conceptualization, formal analysis, data analysis, investigation, project administration, resources, writing-original draft. Erkan Gümüş: Conceptualization, formal analysis, data analysis, investigation, project administration, software, writing-original draft, writing-review & editing, visualization. Murat O. Balaban: Conceptualization, data curation, formal analysis, methodology, resources, software, supervision, visualization, writing-original draft, writing-review & editing.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Invasive *Coptodon* (Perciformes: Cichlidae) in southwest Turkey: Species identification using sequence data

Türkiye'nin güneybatısındaki istilacı Coptodon (Perciformes: Cichlidae): Dizi verileri kullanılarak tür tanımlaması

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Abstract: Nonnative cichlids (*Coptodon zillii*) have established populations in the Köyceğiz and Koca Lakes, located on the west coasts of Mediterranean Turkey. Conflicting species names in these lakes have been reported for many years. We studied samples from current populations of *Coptodon* in these lakes and the Pecenek canal concerning existing GenBank data. We estimated the possible ancestral population using sequence data in the mitochondrial D-loop segment. Inter and intra-population morphological variations of *Coptodon* were examined using 25 morphological and six meristic characters. Haplotype analysis revealed three unique haplotypes in three populations of *Coptodon*, indicating poor genetic diversity. Both maximum likelihood and Bayesian trees showed that these three haplotypes constitute a distinct subclade within the *Coptodon zillii* clade. This phylogenetic pattern indicates that populations of both lakes were founded by a single invasion of *C. zillii* and belong to a single species. Consistent with phylogenetic data, the populations of both lakes do not exhibit significant phenotypic divergence, though the Pecenek population is slightly divergent. Intra-population morphological variability may be due to phenotypic plasticity in response to habitat heterogeneity within the lakes.

Keywords: *Coptodon zillii*, invasive fish, mitochondrial control region, phylogeny, morphometric variation

Öz: Egzotik bir tür olarak tilapyalar (*Coptodon zillii*) Akdeniz'in batı kıyılarında bulunan Köyceğiz ve Koca Göllerinde populasyonlar oluşturmuşlardır. Bu göllerde bu familyaya ait farklı tür isimleri uzun yıllardır rapor edilmektedir. Bu çalışmada mevcut GenBank verileri referans alınarak her iki gölde ve Peçenek kanalında mevcut populasyonlar oluşturan *Coptodon* örnekleri incelenmiştir. Mitokondriyal D-loop segmentinin dizi verilerini kullanarak olası atasal populasyon tahmin edilmiştir. Ayrıca populasyonlar arası ve populasyon içi morfolojik varyasyonu, 25 morfolojik ve altı meristik karakter kullanılarak incelenmiştir. Haplotip analizi sonuçları, üç *Coptodon* populasyonunda, zayıf bir genetik çeşitliliği göstermiş ve üç benzersiz haplotip ortaya çıkarmıştır. Hem maksimum olabilirlik hem de Bayesian ağaçları, bu üç haplotipin *Coptodon zillii* kladında ayrı bir alt klad oluşturduğunu göstermiştir. Bu filogenetik model, her iki gölün populasyonlarının da *C. zillii* türüne ait olduğunu ve bölgede tek bir türün alanı istila ettiğini ortaya koymuştur. Filogenetik verilerle tutarlı olarak, her iki gölün populasyonları arasında, Peçenek populasyonu biraz farklı olsa da, önemli fenotipik varyasyon göstermediği belirlenmiştir. Populasyon içi morfolojik farklılıklar, göllerdeki habitat heterojenliğine tepki olarak fenotipik esneklikten kaynaklandığı ileri sürülebilir.

Anahtar kelimeler: *Coptodon zillii*, istilacı balık, mitokondriyal kontrol bölgesi, filogeni, morfometrik varyasyon

INTRODUCTION

Turkey harbors one of the most diverse freshwater fish fauna in the Mediterranean Basin (Ekmekçi et al., 2013). A total of 384 fish species belonging to 20 orders and 34 families have been reported in Turkish inland waters, of which 208 (54.2%) were reported as endemic and 15 (3.9%) as introduced (Çiçek et al., 2020, 2022). The available information about how and when these fish species entered and their distribution routes are pretty limited (Innal and Erk'akan 2006).

Ecosystems are threatened by global change (Linders et al., 2019). The introduction of fish species constitutes one of the most critical threats to aquatic biodiversity and ecosystem sustainability. Genus *Coptodon* and *Oreochromis* (Cichlidae, Tilapinae) are known as invasive fishes introduced to freshwaters of Turkey for aquaculture (Altun et al., 2006) and almost all over the world as well (Et et al., 2017). The General Directorate of State Hydraulic Works (DSI) started the introduction process of these fish in the 1970s. DSI brought different species of cichlid (*Oreochromis niloticus* and *Coptodon zillii* from Syria, *Coptodon rendalli* and *Sarotherodon galilaeus* from Scotland, and *Oreochromis aureus* from Israel) from other countries to research centers/institutions in the Çukurova and Hatay region, later from here to other provinces

of Turkey. After these introductions, four genera (*Coptodon, Oreochromis, Hemichromis,* and *Sarotherodon*) and five species (*C. zillii, C. rendalli, O. niloticus, O. aureus, O. mossambicus, S. galilaeus,* and *H. letourneuxi*) managed to establish populations in Turkey (Keskin et al., 2018; Innal and Sungur, 2019; Çiçek, 2021).

The biodiversity of the Köyceğiz-Dalaman river basin, declared a Special Environmental Protection Area, contributes significantly to individual and social welfare. Unfortunately, this unique ecosystem is also under the influence of introduced fish, especially species of the Cichlidae, and these invasive populations increase in size year by year (observation of local fishermen). While there is no report on the fish fauna of Koca Lake, conflicting cichlid species have been reported for the Köyceğiz Lake. For example, Çalışkan and Yerli (1999) identified *Oreochromis mossambicus* as the only species in Köyceğiz Lake, while Akın et al. (2005) reported the existence of 3 species: *Coptodon zilli, Oreochromis aureus,* and *Oreochromis nilotica*. Hereafter, studies in this lake reported only a single genus and species, *Coptodon zillii*, adhering only to morphology without any genetic research (Balık et al., 2005; Yılmaz et al., 2006; Tarkan et al., 2015; Çoban, 2018). A distinctive character (dark tilapia spot on the dorsal fin) distinguishes the genera *Coptodon* and *Oreochromis* (except for some species) from each other. However, it isn't easy to determine red-breasted tilapia, *C. rendalli,* from red-bellied tilapia, *C. zillii* (Froese and Pauly, 2019).

The morphology-based cichlid taxonomy has been revised using molecular phylogenetic data (Dunz et al., 2013), and this has returned to standard practice for the invasive species of *Coptodon* such as *C. zillii* and *C. rendalli* (Nagl et al., 2001; Szitenberg et al., 2012; Gu et al. 2016; Kide et al., 2016; Colihueque et al., 2019). Developing functional conservation approaches and strategies requires the identification of the invasive cichlid fish species that have established dense populations in the Köyceğiz and Koca Lake systems. The present study has two main goals. The first is to identify the species of *Coptodon* in these lakes and estimate the source population using sequences of the mitochondrial D-loop by reference to existing sequences of the genus in GenBank. The second is to reveal the morphological diversity of the determined species within and between lakes in the context of metric and meristic morphological characters. As no sequence-based studies have been conducted to identify the invasive members of *Coptodon* from Turkey, the present study is the first on this subject in Turkey.

MATERIAL AND METHODS

Study area and sampling

The research was carried out in two coastal lake systems (Koca Lake, Köyceğiz Lake, and the Peçenek Drainage Canal connected to the second lake) in the southwest of Anatolia (Figure 1, Table 1). The length of the Köyceğiz Lake is approximately 12-13 km, and its width is 5-6 km. It is connected to the Mediterranean Sea by a natural canal. The lake's surface area is 5500 ha, the average depth is 2.5 m, and the maximum depth is 60 m (Ayaz et al., 2013). Koca Lake is located within the borders of Kapukargın Village, about 6 km away from the Dalaman district of Mugla (Figure 1, Table 1).

Figure 1. Location of study sites (arrows pointed to study sites).

The lake is also located 35 km southeast of Köyceğiz Lake, and they are not hydrologically linked. Depth varies between 1 and 20 m, and the lake's surface area is 260 ha (Ayaz et al., 2013). The northwest part of Koca Lake is shallow and covered mainly by submerged plants and reeds. The lake water is rapidly warming up in the spring, is highly productive with abundant vegetation, and provides suitable breeding habitat for cichlid fish (Emre, Y., unpublished report).

Table 1. Study sites with coordinates, and number (N), weight and standard length of each fish.

Study sites	N	Coordinate	Weight (g)		Standard length (mm)	
			Min-Max	Mean	Min-Max	Mean
Koca Lake	96	36°54'49.24"K - 28°41'29.34"D	5.48 -152.11	48.82	57.99-166.00	108.04
Köyceğiz Lake	64	36°41'38.96"K - 28°49'12.95"D	6.96 - 216.86	69.95	61.29-184.50	112.87
Pecenek Channel	10	36°51'18.32"K - 28°41'1.96"D	13.97-108.70	31.61	79.61-108.26	90.82

The fish communities of Köyceğiz Lake are dominated numerically by cyprinids (*Vimba vimba*, *Capoeta aydinensis,* etc.), while cichlids (*Coptodon* sp.) and mugilids (*Mugil cephalus, Lisa ramada*, etc.) for Koca Lake (Emre, Y., unpublished report). The Pecenek drainage canal was created around Köyceğiz Lake for agricultural irrigation and water drainage (Figure 1, Table 1). Its water was very turbid due to heavy domestic waste (sewer and garbage) and the mud on the ground. Fish were caught using fyke (15m x 1.7cm) and gill nets (30m x 1.5 cm) in October 2019 and June 2020, representing different micro-habitats from the densely vegetated littoral and open-water pelagic habitats.

Morphological studies

Morphological identification of *Coptodon* individuals was made using standard identification keys (Teugels and Thys van den Audenaerde, 2003; Gu et al., 2016; Kide et al., 2016). Then, twenty-nine morphometric and six meristic characters were measured per specimen according to Boussou et al. (2010); Kide et al. (2016) (for the character list, see Table 2). All measurements were taken to the nearest 0.1 mm with a digital caliper. To minimize any variation resulting from allometric growth, data was standardized according to the following formula (Elliott et al., 1995):

$$Madj = M(Ls / Lo)b$$

where M: actual measurement, Madj: size adjusted measurement, Lo: standard length of fish, Ls: overall mean of standard length for all fish from all samples in each analysis. Parameter b was estimated for each character from the observed data as the slope of the regression of log M on log Lo, using all samples. This transformation best reflects shape variation among groups independently of size factors. Therefore, each specimen's total length, standard length, fork length, and weight were excluded from the final analysis. On the other hand, meristic characters are not standardized as they do not show a significant correlation with the body size of fish individuals (Turan et al., 2006).

It was observed that some of the morphological characters (KYY, KFU, IOM, PDM, DYTU, AYTU, PYU1, POKU, and SDPG; see Table 2 for abbreviations) exhibited normal distribution (ND). However, the rest of the characters did not show ND. A one-way analysis of variance (ANOVA) was used to look for differences between populations based on morphological traits. The Tukey Posthoc test was used for data with ND, and Kruskal-Walsh and Dunn were used for data with no ND.

Principal component analysis (PCA, variant-covariant matrix) was used to test the contribution of twenty-five morphological characters to the configuration of variance. A discriminant analysis (DA) was performed, which linearly combined a selection of body size measurements to produce a mathematical function that could categorize individuals into groups. Wilks' lambda (λ) was used to detect morphological variation between the three populations. Past 4.04 and R programs were used for all analyses.

Molecular studies

DNA extraction and amplification

We preserved 50 mg of muscle tissue in 95% ethanol for each fish. Genomic DNA was extracted using the PureLink Genomic DNA Extraction Kit (Invitrogen, Thermo Fischer Scientific) with validated modifications to the protocol. We studied the mitochondrial control region (D – loop), as in other studies of cichlid populations (Szitenberg et al., 2012). A fragment of the app. 472 bp was amplified using primers Ormt-449UP (5'-CTAACTCCCAAAGCTAGGATTCT-3') and Ormt-917LP (5'-CTTATGCAAGCGTCGATGAAA-3') (Nagl et al., 2001). PCR amplicons with adequate amplification were sequenced via an intake service from Macrogen Europe (Macrogen Inc.), and sequences were deduced from the obtained AB1 files for computer-based analysis.

Sequence data analysis

Consensus sequences were formed by aligning the sequences produced with forward and reverse primers using the Sequencher v.4.01 (Gene codes Corp.). The unique haplotypes and their frequencies among 93 samples were detected by DNASP v.5 (Librado and Rozas 2009). The characteristics of the matrix, such as the nucleotide composition, the number of variables, indels, and parsimony-informative sites were calculated in MEGA v.X (Kumar et al., 2018). Then, a second matrix was established by combining the obtained unique haplotypes with sequences of *Coptodon* species downloaded from GenBank (Table 2). One sequence per *Pelmatolapia mariae*, *Oreochromis niloticus*, *Oreochromis sp*., and *Cyprichromis leptosoma* were selected as outgroups (see Table 2 for accession numbers). This matrix's multiple sequence alignment was done using MAFFT v.7 (Katoh et al., 2019) with an auto-alignment strategy, and a data matrix was created. The unique haplotypes it contains and the sequence characteristics were determined with the DnaSP v.5 and MEGA v.X, respectively. This second matrix was used in phylogenetic analyzes.

Before the phylogenetic analysis, the substitution model of the data set was estimated using PartitionFinder v.1.1.1 (Lanfear et al., 2012). Phylogenetic relationships among haplotypes were estimated using the maximum likelihood (ML) and Bayesian (BI) phylogenetic algorithms. The ML phylogenetic analysis was conducted using RAxML v.8.0.9 (Stamatakis 2006) implemented in to Geneious v.9.0.5 with a 1000 non-parametric bootstrap (Felsenstein 1985) and GTR+I+G substitution model suggested by PartitionFinder. BI analysis was conducted using MRBAYES v.3.2.2 (Ronquist et al., 2012) with two independent runs, four Markov chains, for 10 million generations, sampling every 1000th generation using the GTR+I+G model proposed by PartitionFinder. The first 25% of trees were discarded as burn-in, and a majority-rule consensus tree was generated from the remaining trees. BI analysis was monitored by TRACER v.1.7 (Rambaut et al., 2018), and trees were visualized using FIGTREE v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

Table 2. *Coptodon* samples were used in phylogenetic analyses, including GenBank accession number (AN), species, location, and outgroups (shown in bold).

AN	Species/ Locality	AN	Species/ Locality	AN	Species/ Locality
KU180527.1	*C. zillii Chinese*	KU180510.1	*C. zillii Chinese*	KU180531.1	*C. zillii Chinese*
KU180619.1	*C. zillii Chinese*	KY587518.1	*C. zillii Japan*	KU180564.1	*C. zillii Chinese*
KU180516.1	*C. zillii Chinese*	KY587521.1	*C. zillii Japan*	KU180618.1	*C. zillii Chinese*
KU180608.1	*C. zillii Chinese*	KY465487.1	*C. zillii Egypt*	KU180620.1	*C. zillii Chinese*
KU180515.1	*C. zillii Chinese*	KY465486.1	*C. zillii Egypt*	KU180605.1	*C. zillii Chinese*
KU180595.1	*C. zillii Chinese*	KY465488.1	*C. zillii Egypt*	KY587519.1	*C. zillii Japan*
KU180558.1	*C. zillii Chinese*	KY465484.1	*C. zillii Egypt*	KY587522.1	*C. zillii Japan*
KU180606.1	*C. zillii Chinese*	KY465482.1	*C. zillii Egypt*	KY587517.1	*C. zillii Japan*
KU180629.1	*C. zillii Chinese*	FJ613474.1	*C. zillii Egypt*	KY587516.1	*C. zillii Japan*
KU180512.1	*C. zillii Chinese*	KY465489.1	*C. zillii Egypt*	KY587523.1	*C. zillii Japan*
KU180520.1	*C. zillii Chinese*	KY465485.1	*C. zillii Egypt*	KY587520.1	*C. zillii Japan*
KU180596.1	*C. zillii Chinese*	KY465483.1	*C. zillii Egypt*	KX523912.1	*C. deckerti*
KU180519.1	*C. zillii Chinese*	EU163723.1	*C. zillii Israel*	MH644435.1	*C. rendalli Tanzania*
KU180522.1	*C. zillii Chinese*	KU180602.1	*C. zillii Chinese*	AF296503.1	*C. rendalli Africa-Egypt*
KU180607.1	*C. zillii Chinese*	KU180599.1	*C. zillii Chinese*	AF296505.1	*C. rendalli Africa-Egypt*
KU180523.1	*C. zillii Chinese*	KU180601.1	*C. zillii Chinese*	AF296504.1	*C. rendalli Africa-Egypt*
KU180592.1	*C. zillii Chinese*	KU180627.1	*C. zillii Chinese*	AF296498.1	*C. bemini*
KU180600.1	*C. zillii Chinese*	KU180559.1	*C. zillii Chinese*	AF296.500.1	*C. discolor*
KU180616.1	*C. zillii Chinese*	FJ613477.1	*C. zillii Israel*	AF296499.1	*C. guineensis*
KU180628.1	*C. zillii Chinese*	EU163719.1	*C. zillii Israel*	AF296506.1	*Tilapia ruweti*
KU180528.1	*C. zillii Chinese*	FJ613479.1	*C. zillii Israel*	AF296497.1	*Pelmatolapia mariae*
KU180617.1	*C. zillii Chinese*	EU163717.1	*C. zillii Israel*	MG728003.1	*Oreochromis niloticus*
KU180604.1	*C. zillii Chinese*	EU163718.1	*C. zillii Israel*	AF296491.1	*Oreochromis sp.*
				AY740331.1	*Cyprichromis leptosoma*

RESULTS

Morphological results

In total, 170 individuals of *Coptodon* from Koca and Köycağiz lakes and the Pecenek drainage canal were examined for morphometric and meristic analysis. The univariate analysis results revealed individuals of the Koca Lake population had a significantly bigger head, larger mouth and split eyes, smaller pharyngeal bone length and width, eye diameter, and pelvic fin length than those of the other two sites (Table 3 and 4). Individuals had larger body height and weight from populations of Köyceğiz and Koca Lake than those of the Peçenek canal (Table 3 and 4).

There were significant differences in only two meristic characters (dorsal-fin rays and scales along the lower lateral line) between the populations of Koca and Köyceğiz Lake ($F=13.51$; $P<0.001$, $F=21.89$; $P<0.001$, respectively).

Table 3. Mean and standard deviations (SD) of the transformed morphometric measurements, and mode, minimum and maximum value of meristic characters of each character of each population.

Morphometric Trait(mm)	**Code**	**Koca Lake**		**Köyceğiz Lake**		**Pecenek**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Body Height	VY	44.14	2.69	46.19	3.71	40.60	2.52
Body Width	VG	17.92	1.87	17.81	1.54	15.25	1.92
Caudal Peduncle Depth	KSD	14.45	1.40	14.24	1.30	14.38	0.47
Caudal Fin Length	KYY	34.09	6.27	36.60	5.94	35.49	3.10
Head Length	KFU	35.20	1.41	34.50	1.31	34.40	0.81
Head Depth	KFD	23.22	1.23	23.59	0.91	24.07	0.69
Interorbital Distance	İOM	11.63	0.68	11.23	0.62	11.26	0.38
Diameter of eye	GC	8.21	0.60	8.52	0.48	9.26	0.19
Snout Length	BU	6.82	0.89	7.03	0.49	6.91	0.21
Mouth Width	AG	8.43	0.91	8.01	0.45	8.01	0.43
Mouth Depth	AD	9.76	1.31	9.02	0.45	9.11	0.42
Predorsal Distance	PDM	35.14	1.96	32.39	1.71	32.53	0.73
Preanal Distance	PAM	75.73	7.33	76.62	2.39	74.97	1.70
Prepectoral Distance	PPM1	39.05	2.15	38.53	2.02	37.73	1.22
Prepelvic Distance	PPM2	35.90	2.01	34.25	1.29	34.59	1.59
Base length of dorsal fin	DYTU	60.75	1.90	61.24	2.18	61.58	1.30
Base length of anal fin	AYTU	18.18	1.05	18.02	0.93	18.55	0.76
Length of pectoral fin	PYU1	30.84	2.35	31.90	1.94	30.66	1.47
Length of pelvic fin	PYU2	34.32	2.83	36.34	1.75	36.48	1.80
Caudal peduncle height	KSP	16.44	1.27	16.26	0.77	16.43	0.60
Length of the first spine of the dorsal fin	EUDY	28.29	3.51	27.46	2.40	27.29	2.51
Length of the third spine of the anal fin	ATIU	23.11	2.80	23.49	1.49	23.29	1.63
Preorbital distance	POKU	12.72	1.26	12.30	0.80	12.35	0.45
Width of pharyngeal bone	SDPG	13.39	0.88	13.86	0.60	13.55	0.67
Length of pharyngeal bone	FKU	10.51	0.93	11.23	0.57	11.25	0.54

Meristic Traits (mm)	**Code**	**Koca Lake**			**Köyceğiz Lake**			**Pecenek**		
		Mode	**Min**	**Max**	**Mode**	**Min**	**Max**	**Mode**	**Min**	**Max**
Dorsal-fin rays	SYIS	11,0	9,0	15,0	12,0	10,0	13,0	12,0	11,0	13,0
Dorsal-fin spines	SYDS	15,0	14,0	16,0	15,0	15,0	16,0	15,0	15,0	16,0
Anal-fin rays	AYIS	8,0	7,0	9,0	9,0	8,0	10,0	9,0	8,0	9,0
Anal-fin spines	AYDS	3,0	2,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Scales along the lower lateral line	YCPS	12,0	9,0	14,0	13,0	11,0	14,0	12,0	11,0	13,0
Gill rakers on the first ceratobranchial gill arch	1SYDS	11,0	9,0	13,0	11,0	11,0	12,0	11,0	11,0	11,0

The results for the DA showed that the scatter plot for DF1 and DF2 generated a clear separation only between the populations of Pecenek and the two lakes. At the same time, there was a partial overlap between the two lake populations (Figure 2). The percentages of morphological differences among the three populations indicated highly significant differences (Wilks' lambda = 0.018; F= 264.44; P < 0.001). The first DF accounted for 81.20%, and the second accounted for 18.08% of the between-group variability, explaining 100% of the total between groups variability. The characters of primary importance in distinguishing between the groups were body height (0.49), interorbital (-0.43), predorsal (-0.52) and

preorbital distance (-0.60), and length of pharyngeal bone (0.46) variables for the first canonical variable, and body height (-0.61), body width (-0.50) and eye diameter (0.68) variables for the second one. Each individual could be classified correctly into the three populations with an accuracy of 95.9%. Additionally, it was seen that only 2 variables (dorsal-fin rays (0.63) and scales along the lower lateral line (0.75) out of 6 meristic characters were important in the formation of DF1, which explained 96.6% of the total variance (Wilks' lambda = 0.77; F= 43.13; P < 0.01) (Figure 2).

Table 4. Summary of Kruskal-Wallis (Chi2) and ANOVA (F) results for 25 morphological characters of *C. zillii* from three site. Significance levels; * P < 0.05; ** P < 0.01; *** P < 0.001.

Trait	Chi2	*P*	Population	
			Dunn's Post hoc test	
VY	27.87	<0.001	Koca Lake - Köyceğiz Lake - Peçenek	***
VG	13.88	<0.001	Koca Lake- Peçenek. Köyceğiz Lake - Peçenek	*
KSD	2.28	0.32		
KFD	7.39	<0.05	Koca Lake - Peçenek	*
GC	30.52	<0.001	Koca Lake - Köyceğiz Lake - Peçenek	***
BU	0.85	0.65		
AG	17.21	<0.001	Koca Lake - Köyceğiz Lake - Peçenek	***
AD	17.18	<0.002	Koca Lake - Köyceğiz Lake	***
PAM	4.95	0.08		
PPM1	5.69	0.08		
PPM2	37.54	<0.001	Koca Lake - Köyceğiz Lake. Koca Lake- Peçenek	***
PYU2	28.29	<0.001	Koca Lake - Köyceğiz Lake. Koca Lake- Peçenek	***
KSP	5.61	0.06		
EUDY	2.76	0.25		
ATIU	0.54	0.76		
FKU	25.99	<0.001	Koca Lake - Köyceğiz Lake. Koca Lake- Peçenek	
Trait	**F**	***P***	**Population**	
			Tukey Post hoc test	
KYY	3.39	0.04	Koca Lake - Köyceğiz Lake	*
KFU	5.96	<0.001	Koca Lake - Köyceğiz Lake	***
İOM	8.06	<0.001	Koca Lake - Köyceğiz Lake	***
PDM	46.86	<0.001	Koca Lake - Köyceğiz Lake. Koca Lake- Peçenek	***
DYTU	1.64	0.20		
AYTU	1.38	0.25		
PYU1	1.38	0.25		
POKU	3.03	0.06		
SDPG	6.99	0.00	Koca Lake - Köyceğiz Lake	***

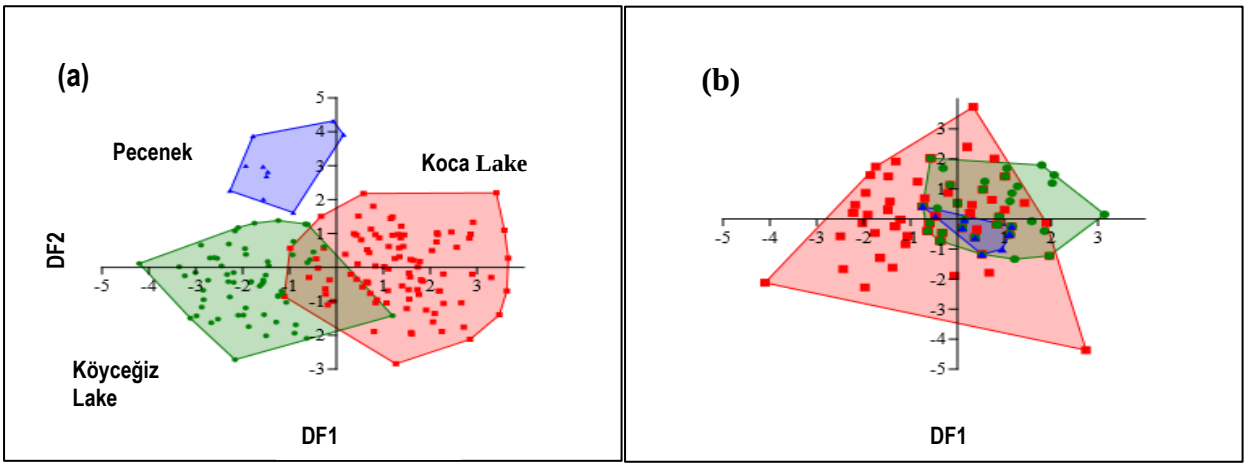

Figure 2. Scatter plot of the DF1 and DF2 axes of the DA of (a) morphometric; (b) meristic characters of *Coptodon zillii* collected at three sites.

Intra-population morphological differences were explained by one to five functions in the discriminant analyses (Figure 3). Plots of DF1 and DF2 for the individuals from the populations illustrate a noticeable variation in the morphological traits between microhabitats (DF2 scores) and sampling years (DF1 scores) in the Koca Lake population (Wilk's Lambda = 0.007; F= 442.4; P<0.000). While individuals from the littoral habitat in the Koca Lake population generally had longer pelvic fins, those from the pelagic habitat had a larger head, nose, mouth, eyes, and pharynx bone. Individuals caught in 2019 have a larger mouth and the dorsal fins located further back than those in 2020. The morphological variation within the population of Köycegiz Lake appeared both in the micro-habitat and sampling years (Wilk's Lambda = 0.016; F= 200.9; P<0.000). In 2020, the caudal peduncle was high, and in 2019, the snout length was high. It was determined that the lengths of the pelvic and pectoral fins and the distance between the anal and caudal fins of pelagic individuals were longer than those living in the littoral habitat (Figure 3).

There was no significant intra-population meristic variation in the two lakes (Wilk's Lambda = 0.99; F= 1.69; P=0.90).

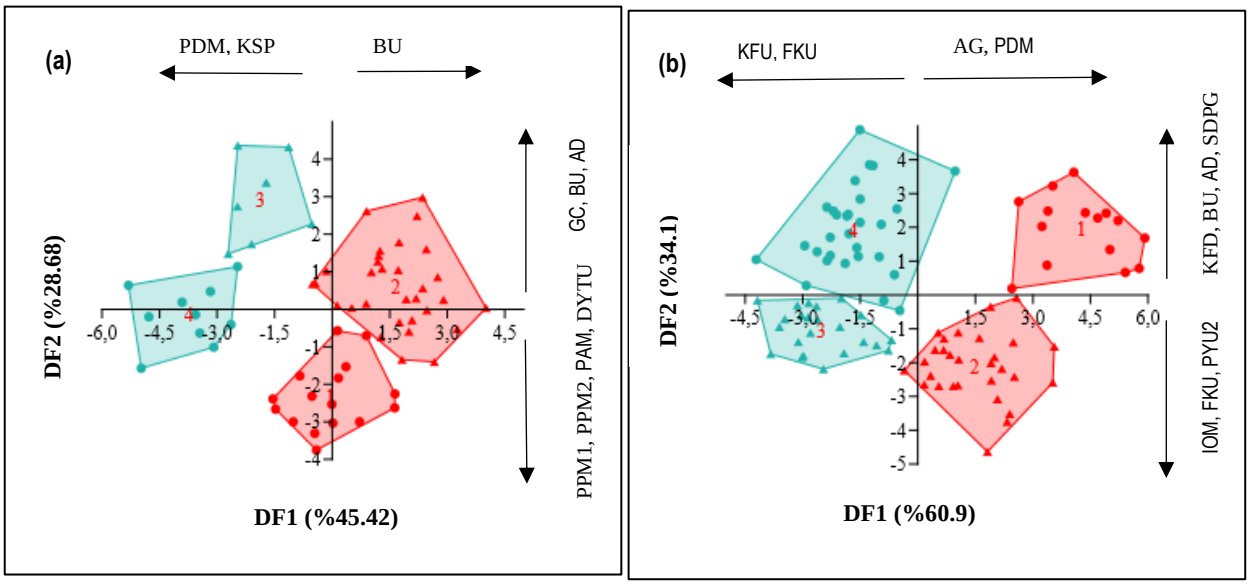

Figure 3. Plot of discriminant function one and two for the different individuals (●: pelagic habitat (1-4), ▲ : littoral habitat (2-3); red color represent October 2019, blue represents June 2020) for each of the two populations of *C. zillii*: (a) Köycegiz Lake; (b) Koca Lake

Molecular results

Three unique haplotypes were recognized among the D-loop sequences of a total of 93 *C. zillii* samples. The sequences have been deposited in the GenBank database and were assigned H1-ON337141, H2-ON337142, H3-ON337143. Haplotypes H2-H3 were obtained from Köyceğiz and Koca lakes, and H1 only from Pecenek Canal. The most common haplotype was H3 (73 individuals; 78.5%), then H2 (18 individuals; 19.35%), and last was H1 (2 individuals; 2.15%). We have not calculated genetic diversity parameters since only three unique haplotypes were detected. A data matrix was established using these three unique haplotypes plus 70 sequences of *Coptodon* species downloaded from GenBank (Table 2). After alignment and trimming, the final length of the sequences was 472, of which 270 were constant and 200 variables. In total, 73 unique haplotypes were detected, 68 representing *Coptodon* members as ingroup and four representing the outgroup.

The BF and ML trees produced from this matrix differed in topology. ML tree supported the monophyly of *Coptodon* haplotypes with a bootstrap support value of 70 (Figure 4a), but a haplotype of *Tilapia ruweti* was nested in the *Coptodon* haploclade. The single haplotypes of *Coptodon bemini* branch off at the base of the *Coptodon* haploclade, leading to all others, and the monophyly of this later haploclade was supported with 84% bootstrap values. The later haploclade consists of three subhaploclades: (i) *C. rendalli* (1 Tanzanian + 2 Egyptian haplotypes), (ii) *T. ruweti* + *C. deckerti,* and (iii) the clade including one haplotype per *C. discolor* and *C. guineensis* plus 60 haplotypes belonging to *C. zillii. Coptodon discolor* + *C. guineensis* constitute a sister clade to the clade, including haplotypes of *C. zillii* and the *C. zillii* haploclade received 85 bootstrap support. Relationships between haplotypes of *C. zillii* are mainly unresolved, but the three haplotypes obtained from Koca and Köyceğiz lakes plus Pecenek formed an internal clade within the *C. zillii* lineage (Figure 4a).

The BI tree supported the monophyly of *Coptodon zillii* when a haplotype from Egypt was omitted (Figure 4b). BI tree occurs with a basal trichotomy, and one haplotype per *Coptodon deckerti* and *Tilapia ruweti* constitute independent branches, while all others as a single haploclade supported by 0.99 posterior probability. The last clade includes two sister haploclades, which we defined as C1 and C2 (Figure 4b). The C1 consists of all outgroup haplotypes plus one haplotype of *C. zillii* and three haplotypes of *C. rendalli*.

Coptodon discolor + *C. guinensis* branch off basally, leading to the C2_2 haploclade including only haplotypes of *C. zillii,* which is supported by 1.0 posterior probability support. The C2_2 haploclade has 25 branches in polytomy, and three haplotypes obtained from Mugla make up one of these 25 branches.

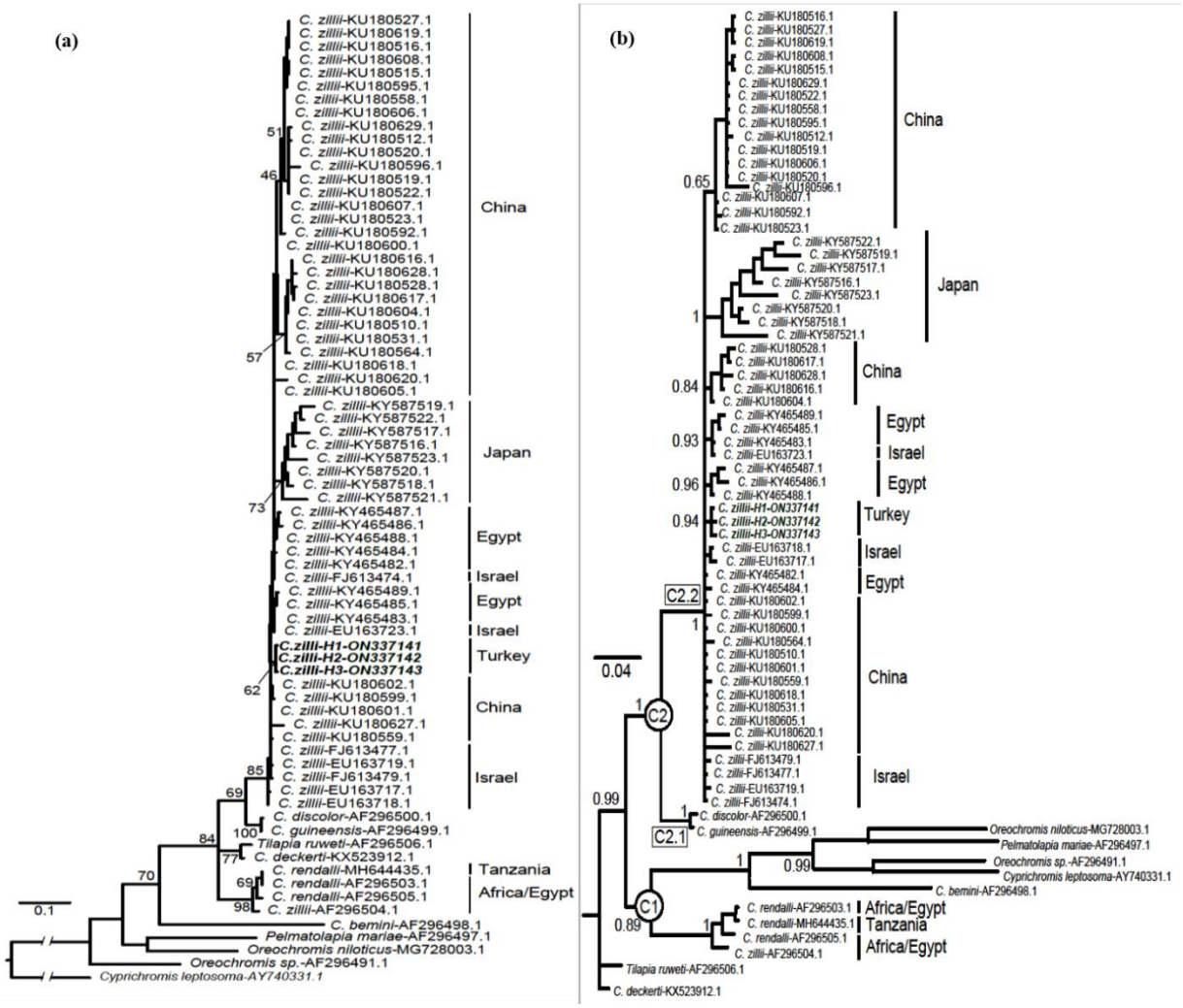

Figure 4. Phylogenetic tree from (a) ML and (b) BI analysis with D-loop dataset

DISCUSSION

Three unique haplotypes detected from the study area constitute a haploclade within *C. zillii*. These results suggest that a single species, *C. zillii*, occurs in Koca and Köyceğiz lakes and the Pecenek drainage canal.

However, this three-haplotype clade constitutes an independent branch within the *C. zillii* haploclade with no relationship to any other geographically specific haplotypes. Therefore, it was not possible to determine the origins of the populations that invaded these systems. As these three haplotypes differed by a single base position from each other and formed a monophyletic haploclade, we concluded that the population in all systems was established by a single introduction. Further, the population's genetic diversity was low for the same reasons (Freeman and Herron 2007).

In this study, we also aimed to reveal the inter-and intra-population morphological differences of *C. zillii* in the context

of metric and meristic morphological characters. Although Wilk's Lambda test showed that the morphometric and meristic differences observed between populations were statistically significant, this difference was not supported by the discriminant and PC analysis results. The observed similarity of morphometric traits based on DA between the populations of Köyceğiz Lake, Koca Lake, and Pecenek suggest individuals belonging to the same source population were recently introduced in both lakes. Thus, the results of morphological analyses are consistent with genetic results.

Although the populations exhibited similar morphological characteristics, fish from the Pecenek population had the greatest eye diameter, thus distinguishable from the populations of the other two lakes (Figure 2). Jawad et al. (2018) presented evidence supporting this phenomenon for *C. zillii* and *O. aureus*. Variation in eye size can result from differences in water transparency (as in the Pecenek canal) or differences in the size of available food (Solem et al., 2006). These results suggest that the large eyes of nonnative fish such as *C. zillii* make them superior predators or competitors, even in anthropogenically modified systems (Moran et al., 2018).

Morphometry of lake can also predict the likelihood of habitat coupling between littoral and pelagic zones by a mobile fish (Chavarie et al., 2015). Overall, we obtained different morphological patterns in both lakes about their area and time of capture. When we examined micro-habitats, it was seen that the *C. zillii* from the pelagic habitat of the Koca Lake typically exhibited morphological differences in head traits and conspecifics from Köyceğiz Lake in terms of fin traits. Lakes can represent a rich source of environmental gradients (e.g., size, depth, temperature, light, amount of vegetation cover, salinity, types of predators, and competitors) associated with different prey species and habitat characteristics that have the potential to promote ecological segregation (Chavarie et al., 2015). This study's morphological pattern observed in head and fin morphology suggests related to feeding and swimming traits based on the heterogeneity of habitat and season. This might be due to phenotypic plasticity, not genetic differences.

CONCLUSION

In the light of these data, the following conclusions were reached: (i) The Koca and Köyceğiz lakes, and Pecenek drainage canal in Muğla Province – Turkey, were invaded at once by a single founder population of *C. zillii*, (ii) this population contains a poor genetic diversity, due to recent foundation, (iii) determining origin population requires richer genetic data and (iv) there is no significant inter-lake morphological difference, but there is a significant intra-lake difference, possibly due to local ecological condition.

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AUTHORSHIP CONTRIBUTIONS

Dilara Sarıbaş: Analyzed the collected sampling and wrote the first draft of the manuscript. Nehir Kaymak: Corrected the draft and built the final version of the manuscript. Özgül Yahyaoğlu: Provided assistance and guidance throughout the molecular analysis. Battal Çıplak: Contributed to the interpretation of molecular data and reviewed the manuscript. All authors have read and approved the manuscript.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

Approval was granted by the Ethics Committee of Burdur Mehmet Akif Ersoy University (Date: 19.07.2018 / Approval Number: 93773921).

DATA AVAILABILITY

For questions regarding datasets, the corresponding author should be contacted

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Hayalet yengeç *Ocypode cursor* (Linnaeus, 1758) yuvalarının insan etkileri altında morfolojik değişimleri

Variation in burrow morphology of ghost crab *Ocypode cursor* (Linnaeus, 1758) under human influence

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Öz: Hayalet yengeçler, kumsallarda insan etkilerinin tespiti için sıklıkla kullanılan biyoindikatör türlerdendir. Hayalet yengeçler, insan etkileri altında popülasyon dinamiklerinde yuvalama davranışlarında değişiklikler gösterirler, fakat bu değişikliklerin nasıl ve ne şekilde oldukları Türkiye'nin Akdeniz kıyılarında yeterince bilinmemektedir. Bu nedenle, insan baskısı açısından fark gösteren iki farklı alanda *Ocypode cursor*'ın yuva morfolojileri incelenmiştir. Çalışma neticesinde insan etkilerinin yoğun olduğu bölgelerde yaşayan yengeçlerin, düşük insan etkileri altında yaşayan yengeçlere kıyasla daha küçük, daha dik ve daha basit yuvalar oluşturdukları ortaya çıkmıştır. Bununla birlikte, çalışma alanları arasında yengeç yuvalarının derinliği ve hazneye sahip olma sıklıkları açısından bir fark görülmemiştir. Ek olarak, her iki çalışma bölgesinde de büyük yengeçlerin kumsalın suya daha uzak üst bölgelerine, küçük yengeçlerin ise denize yakın bölgelerine yuvaladıkları ve bu tabakalaşmanın *O. cursor* için genel bir davranış olduğu anlaşılmıştır. Çalışma neticesinde, *O. cursor*'ın insan etkileri altındayken popülasyon dinamiklerinin yanı sıra yuva morfolojilerinde ve özelliklerinde de değişiklikler yaptıkları ve hayalet yengeçleri insan etkilerinin tespiti için kullanacak olan çalışmaların bu yuva özelliklerini de göz önüne almaları gerektiği sonucuna ulaşılmıştır.

Anahtar kelimeler: *Ocypode cursor*, yuvalama davranışı, insan etkileri, kumsal, Türkiye

Abstract: Ghost crabs are common bioindicator species for human disturbance on sandy shores. Ghost crabs often alter their population dynamics under human disturbance. Ghost crabs, further, alter their burrowing behavior under human influence, however, these changes are not well known on the Turkish coast of the Mediterranean Sea. Therefore, burrowing morphology of *Ocypode cursor* at two sites that differ in the degree of human disturbance was compared. Ghost crabs created smaller, steeper and simpler burrows at the site under higher human disturbance compared to the crabs living at the sites with lower human influence. Further, there was no difference in the ghost crab burrow depth and the frequency of existence of chambers between sites. Moreover, the results of this study revealed that larger crabs preferred higher parts of the beach at both sites, suggesting that this is a common behavior for the populations of *O. cursor*. Consequently, the results of this study emphasized that *O. cursor* alter their burrowing morphology and characteristics under human disturbance besides their population demographics; suggesting that studies that focus on the use of ghost crabs for human disturbance should include burrow morphology in their assessments.

Keywords: *Ocypode cursor*, burrowing behavior, human impact, sandy beach, Turkey

GİRİŞ

İnsan etkilerinin ekosistemlerde neden oldukları değişimlerin ve zararların en kolay tespit edilme yöntemlerinden biri biyoindikatör türler üzerinden gerçekleştirilen gözlemlerdir (Siddig vd., 2016). İnsan etkilerinin varlığında, biyoindikatör türlerin popülasyon dinamiklerinde değişimlere gittiği bilinmektedir. Bu değişimler çoğunlukla popülasyon yoğunluğunda ve ortalama birey boylarındaki düşüşler şeklinde kendilerini gösterirler (Carignan ve Villard, 2002; Solomon vd., 2003). Popülasyon dinamiklerindeki değişimlerin yanı sıra, bazı biyokontrol türler insan etkilerinin mevcut olduğu ekosistemlerde var olma veya yok olma, fizyolojik ve davranışsal değişimler de gösterirler (Spellerberg, 2005).

Hayalet yengeçler çok yaygın şekilde kumsallarda insan etkilerinin neden olduğu bozulmaların tespitinde kullanılırlar (Schlacher vd., 2016; Gül ve Griffen, 2018). Diğer biyoindikatör türlerle benzer olarak, hayalet yengeçler insan etkilerinin mevcut olduğu kumsallarda popülasyon yoğunluklarında ve ortalama birey boylarında düşüşler (Schlacher vd., 2016; Gül ve Griffen, 2018), kıskaçlarında küçülmeler (Gül ve Griffen, 2020a), yuvalama davranışlarında ve yuvalarının morfolojilerinde farklılıklar (Schlacher ve Lucrezi, 2010; Gül ve Griffen, 2018), beslenme alışkanlıklarında farklılıklar (Morrow vd., 2014; Tewfik vd., 2016; Gül ve Griffen, 2020b) ve dolayısıyla fizyolojik kalitelerinde düşüşler şeklinde değişimler gösterirler (Gül ve Griffen, 2020b).

Ocypode cursor (L, 1758) Akdeniz'de bulunan tek hayalet yengeç türü olarak ortaya çıkmaktadır (Sakai ve Türkay, 2013). Diğer hayalet yengeç türleriyle benzer şekilde insan etkilerinin mevcut olduğu kumsallarda bu etkilerin tespitinde biyoindikatör tür olarak kullanılabilir (Bal vd., 2021). Hayalet yengeçler, kumsal bitkileri ve omurgasızlarının tamamının dahil olduğu çok çeşitli canlılar üzerinden beslenebilirler (Lucrezi ve Schlacher, 2014). Ek olarak, kaplumbağa yumurta ve yavruları (Lucrezi ve Schlacher, 2014) ve insanların kumsallara bıraktığı besin atıkları üzerinden de beslenebilirler (Fisher ve Tevesz, 1979; Strachan vd., 1999). Önceki çalışmalar, *O. cursor*'un kaplumbağa yuvaları üzerindeki yıkıcı etkilerinin diğer hayalet yengeçlerle kıyaslandığında nispeten düşük fakat yine de önemli olduğunu göstermiştir (Strachan vd., 1999). Diğer taraftan, hayalet yengeçler kıyısal bölgelerde yaşayan bazı memelilerin besinleri arasında yer alırlar (Lucrezi ve Schlacher, 2014), fakat Türkiye kumsallarında *O. cursor* için rapor edilmiş bir predatör bilinmemektedir. Sahip oldukları bu karmaşık av-avcı ilişkileri nedeniyle trofik seviyeler arasında bir geçiş sağlaması da bu türü önemli bir hale getirir (Lucrezi ve Schlacher, 2014; Tiralongo vd., 2020). Hayalet yengeçler dünyanın çok sınırlı bölgelerinde av hayvanı olarak görülürler (Lucrezi ve Schlacher, 2014) fakat Türkiye'de herhangi bir avcılık baskısından söz edilemez.

Hayalet yengeçler, kumsallar üzerinde kendilerine özgü kısa süreli yuvalar oluşturur ve aktif olmadıkları zamanları (gündüzleri ve kış ayları boyunca) bu yuvaların içerisinde geçirirler (Türeli vd., 2009; Lucrezi ve Schlacher, 2014). Hayalet yengeçlerin insan etkilerine verdikleri yanıtlardan biri yuvalarının şekil, büyüklük ve derinliklerinde meydana getirdikleri değişikliklerdir (Schlacher ve Lucrezi, 2010; Gül ve Griffen, 2018). Hayalet yengeçler, insan etkileri altında genellikle daha küçük, daha derin ve daha dik yuvalar oluştururlar (Schlacher ve Lucrezi, 2010; Gül ve Griffen, 2018). Fakat, *O. cursor* türünün Antalya kıyılarında insan etkilerine cevap olarak yuva özelliklerinde herhangi bir değişikliğe gidip gitmedikleri üzerine elde edilmiş bilgiler eksiktir. Bu nedenle, araştırma hayalet yengeçlerin insan etkileri açısından birbirinden farklı özellikler gösteren aynı kumsalın iki farklı bölgesindeki hayalet yengeç yuvalarının özelliklerinin belirlenmesini amaçlamıştır.

MATERYAL VE METOT

Çalışma alanları

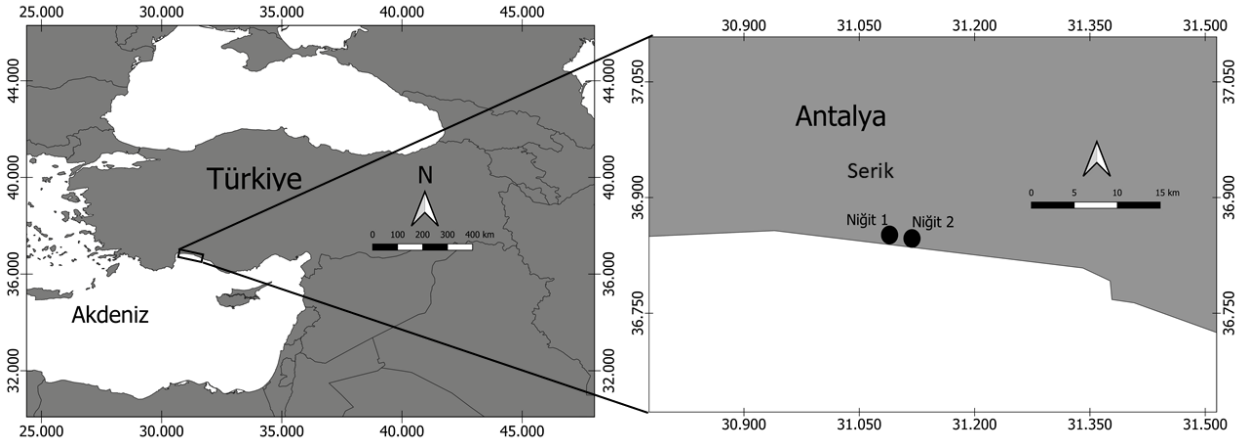
Türkiye Cumhuriyeti Hükümeti Covid-19 virüsünün neden olduğu salgından ötürü turizmin de dahil olduğu pek çok faaliyetleri Mart 2020 itibariyle kısıtlamıştır. Aynı yılın Nisan ayının son günlerinde düşen hasta sayıları neticesinde 1 Haziran 2020 itibariyle turizmin gerek yerli gerekse de yabancı turistlerin erişimine açılacağı duyurulmuştur (Anadolu Ajansı, 2020). Nitekim, bu açılma bu çalışmanın yürütülebilmesini mümkün kılmıştır.

Çalışmamızın amacına uygun olarak Antalya ilinin Serik ilçesi sınırları içerisinde Boğazkent bölgesinde bulunan Niğit kumsalı çalışma bölgesi olarak seçilmiştir (Şekil 1). Niğit plajı, yaklaşık 6 km uzunluğunda ve ortalama yaklaşık 45 m genişliğinde bir plajdır. Plajın her iki sonunda bulunan küçük akarsular, plajı diğer kıyı şeridinden ayırmaktadır. Bu kumsalın seçilmesinin nedeni, kumsalın bir bölgesinin insan ve araç etkisi altındayken (Niğit 2), diğer bölgesinin girişe uzak kalması ve doğrudan bir girişi olmaması nedeniyle nispeten daha az insan ve araç varlığına sahip olmasıdır (Niğit 1). İnsan etkilerinin iki alanda karşılaştırılabilmesi için kum üzerinde doğrudan insan ve araç sayımı yapılmıştır. Hafta içi bir gün 13:00 ile 15:00 saatleri arasında yapılan sayımlar neticesinde Niğit 1 bölgesinde ziyaretçi sayısı 6 iken, Niğit 2 bölgesinde ziyaretçi sayısı 47 ve araç sayısı 6 olarak tespit edilmiştir. Sayımın yapıldığı zaman aralığında Niğit 1 bölgesinde araç gözlenmemiştir. Kumsalın sahip olduğu bu özellikler insan etkilerinin hayalet yengeçlerin yuva özelliklerine tesirlerinin belirlenmesi açısından kolaylık sağlamaktadır. Ayrıca kumsalların jeomorfolojik özelliklerinin hayalet yengeçlerin pek çok davranışına etki ettikleri bilinmektedir (Schlacher ve Lucrezi, 2010; Gül ve Griffen, 2018). Bu yan yana iki bölgenin seçilmesi farklı jeomorfolojik karakterde olmadıklarından, canlı üzerine olası etkilerinin göz ardı edilmesine olanak sağlamaktadır. Ek olarak, çalışma aynı plajın iki farklı bölgesinde yürütüldüğünden gel-gitten etkilenme ve eğim gibi özelliklerin bölgeler arasında farklılık göstermemesi beklenir.

Örneklemeler

Hayalet yengeçlerin yuvalarında insan etkisine bağlı olarak herhangi bir değişiklik olup olmadığının anlaşılması için her bir kumsalda 20 m uzunluğunda ve 20 m genişliğinde birer transekt örneklemesi yapılmıştır. Transektler, alt ucu deniz suyunun ulaştığı en üst seviyeye değecek şekilde yerleştirilmiştir. Kumsal üzerinde deniz suyuna 20 m'den daha uzak bölgelerde yuva gözlenmediğinden transekt genişliği buna uygun olarak seçilmiştir. Çalışma sırasında tekrarlı örnekleme yapılmamıştır. Hayalet yengeçlerin yuvaları sık sık boş olduğundan dolu (aktif) yuvaların tespiti için öncelikle transektler belirlenmiş ve içerisindeki yuvaların tamamı kumla kaplanmıştır. 24 saat sonra transektler aynı bölgeye kurularak ağzı açık olan yuvalar aktif yuva olarak değerlendirilmiştir (Pombo ve Turra, 2019). Tüm örneklemeler bu aktif yuvalar üzerinden yürütülmüştür. Aktif yuvaların ağız açıklıkları (en geniş mesafeden) yengeç büyüklüğünün göstergesi olarak (Türeli vd., 2009) ve denize olan uzaklıkları (suyun ulaştığı en yüksek seviye) ölçülmüştür.

Hayalet yengeçler güneş batınca yuvalarından çıktıkları için, güneşin batması beklenmiş ve güneş battıktan sonra yuvalara alçı ve su karışımı (2:1 oranında, Chan vd., 2006) dökülmüştür. Yaklaşık bir saat sonra (alçı ve su karışımı kuruduktan sonra) yuvaların etrafındaki kum kazılmış ve yuva kalıpları çıkarılmıştır. Bunu takiben yuvaların eriştiği en derin nokta yuva derinliği olarak ölçülmüştür. Elde edilen yuva kalıpları daha ileri analizler için laboratuvara götürülmüştür. Laboratuvara getirilen kalıpların şekli ve hazneli (chamber) olup olmadığı gözle belirlenmiş ve bunu takiben ağırlıkları (0,01 g hassasiyetle) ve kazılma açıları ölçülmüştür.



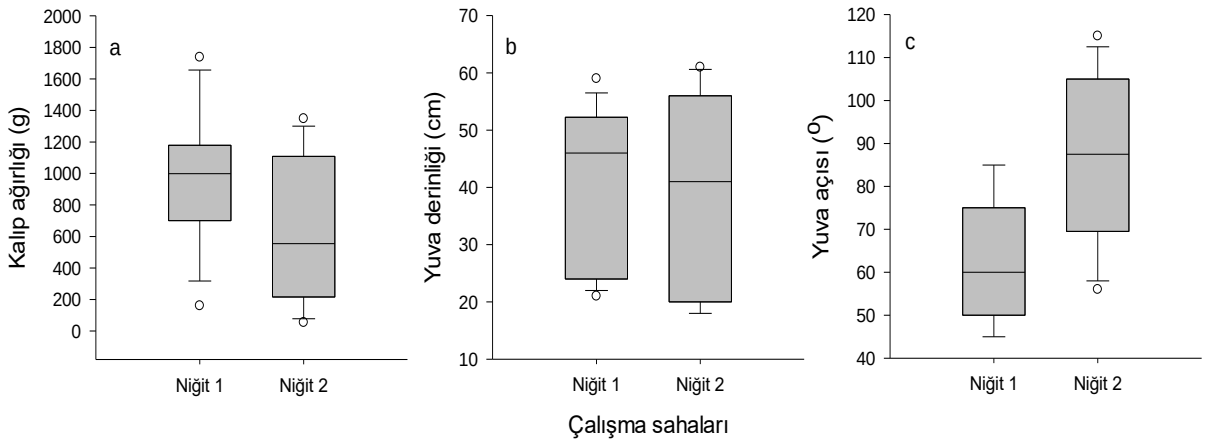
Şekil 1. Türkiye'nin Akdeniz kıyılarında bulunan çalışma alanı
Figure 1. Study site on the Turkish coast of the Mediterranean Sea

İstatistiksel analizler

Hayalet yengeçlerin insan etkilerine göre farklılık gösteren iki kumsaldaki yuvalarının ağırlıkları hacimlerinin göstergesi olarak kullanılmıştır (Schlacher ve Lucrezi, 2010). Hayalet yengeçlerin yuva hacim ve derinlikleri, yuva sahibi yengecin büyüklüğüyle doğru orantılıdır (Chan vd., 2006). Bu nedenle, yengeçlerin yuva ağız açıklıkları ile yuvaların hacim büyüklükleri arasında basit regresyon analizleri kurulmuş ve asıl veriyle tahmin edilen verileri arasındaki fark (residuals) yengeç yuvalarının büyüklüklerinden arındırılmış veri olarak kullanılmıştır (Packard ve Boardman, 1999). Yengeçlerin farklı kumsallardaki yuva hacimleri, yuva derinlikleri, yuva oluşturma açıları ve denize mesafeleri arasındaki farklar t-testinden faydalanılarak ortaya konulmuştur. T-testi parametrik bir test olduğundan, eldeki verilerin normal dağılım ve homojen varyans gibi özellikleri sağlayıp sağlamadıkları sırasıyla Shapiro- Wilk testi ve Levene testi ile belirlenmiştir. Çalışma alanları arasındaki yuvaların benzer şekilli olup olmadıkları ve haznelerinin mevcudiyetleri arasındaki farklar Ki-kare testi ile anlaşılmıştır. Son olarak yuva büyüklükleri ile denize mesafe arasında herhangi bir ilişki olup olmadığı Genellenmiş Doğrusal Modelleme (GLM) yöntemi ile belirlenmiştir. Tüm istatistiksel analizler R programı ile yürütülmüştür (v 3.6.2).

BULGULAR

Çalışma sırasında toplam 25 hayalet yengeç yuvası (14 tane Niğit 1 ve 11 tane Niğit 2 olmak üzere) incelenmiştir. Hayalet yengeçler, insan faaliyetlerinin daha az olduğu bölgelerde (ort.±S.S., 975,72±409,58 g) insan faaliyetlerinin yüksek olduğu bölgelere oranla (642,08±448,67 g) daha büyük yuvalar oluşturmuşlardır (t-testi, t=2,686, p=0,0319, Şekil 2a). Diğer taraftan, hayalet yengeçlerin yuva derinlikleri açısından Niğit 1 (41,28±13,14 cm) ve Niğit 2 (38,45±16,36 cm) alanları arasında anlamlı bir fark bulunamamıştır (t-testi, 0,822, p=0,419, Şekil 2b). Ek olarak, Niğit 2 bölgesindeki yengeçlerin (86,5±18,57°) Niğit 1 bölgesindeki yengeçlerden (63,63±14,67°) daha dik yuvalar oluşturdukları anlaşılmıştır (t-testi, t=-3,437, p=0,0022, Şekil 2c).



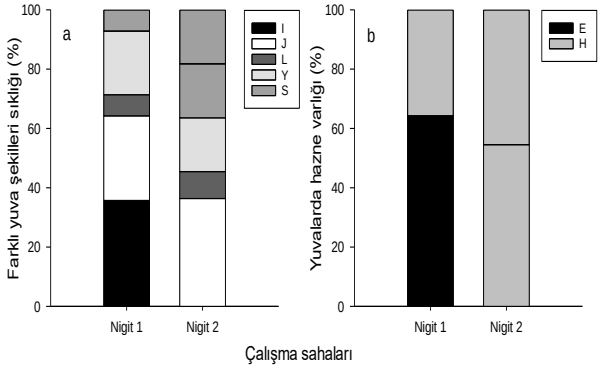
Şekil 2. *O. cursor* yuvalarının araştırma sahaları arasında hacim (a), derinlik (b) ve açıları (c) bakımından farklılıkları

Figure 2. Variations in volume (a), depth (b) and inclination angle (c) of the *O. cursor* burrows between the study sites

O. cursor'un yuvaları kolaylık sağlaması açısından alfabedeki harflere olan benzerlikleri üzerinden gruplara ayrılmışlardır. Yuvalar, I, J, L, Y, S şekilli olarak sınıflandırılmışlardır (Şekil 3).

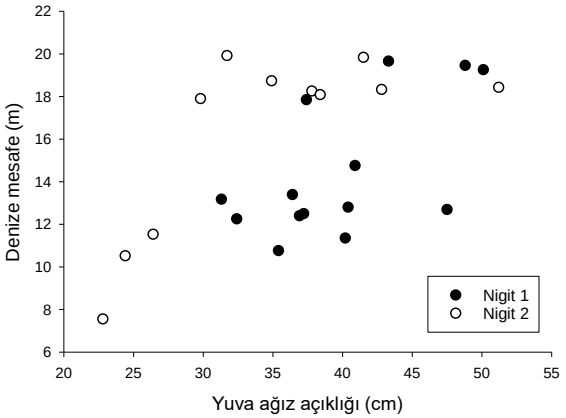
Şekil 3. Çeşitli şekillerdeki hayalet yengeç yuva kalıpları
Figure 3. Ghost crab burrow casts with various shapes

Her iki çalışma alanındaki yuvaların şekilleri birbirleriyle benzerlikler göstermiş, fakat bu şekillerin sıklıkları araştırma alanları arasında farklılık göstermiştir (Ki-kare testi, X^2= 11,453, p=0,0253, Şekil 4a). Ek olarak, çalışma alanları arasında yuvaların hazneye sahip olup olmama açısından bir fark bulunamamıştır (Ki-kare testi, X^2 = 0,676, p = 0,0934, Şekil 4b).



Şekil 4. Yuva şekillerinin (a) ve hazne varlığının (b) araştırma alanları arasındaki farklılıkları (Şekil içerisinde E ve H sırasıyla evet ve hayır anlamındadır)
Figure 4. Variations in burrow shapes (a) and the existence of chambers (b) between the study sites (E and H on the graph stand for yes and no, respectively)

Araştırma alanlarımız içerisinde yengeç büyüklükleri (daha büyük yuva ağız açıklığı) ile denize olan mesafe arasında doğrusal bir ilişki tespit edilmiştir (GLM, t = 3,049, p = 0,0056). Bu ilişki gerek Niğit 1 (GLM, t = 2,681, p = 0,02) ve gerekse de Niğit 2 (GLM, t = 3,256, p = 0,0099) sahalarında farklılık göstermeyip, her iki alanda da daha büyük yengeçlerin kumsallarda daha yukarılara yuva kazdığı anlaşılmıştır (Şekil 5).



Şekil 5. Yuva ağzı büyüklükleri ile denize olan mesafeleri arasındaki ilişki
Figure 5. The relationship between the burrow opening diameter and the distance to the highest water mark

TARTIŞMA

Çalışma neticesinde, hayalet yengeçlerin (*O. cursor*) Türkiye'nin Akdeniz kıyılarında insan etkileri altında olduklarında daha küçük, daha dik ve daha basit şekilli yuvalar oluşturdukları anlaşılmıştır. Diğer taraftan, insan etkisinin az veya çok olduğu bölgelerdeki yengeçler arasında yuva derinlikleri ve hazne varlıkları açısından fark yoktur.

Yuva oluşturma hayalet yengeçlerde yüksek miktarda enerji harcanmasına neden olan davranışlardandır (Gül ve Griffen, 2019a). Ek olarak, yuva oluşturmak için gereken enerji miktarı farklı seviyelerdeki insan etkilerine maruz kalan yengeçlerde de farklılık göstermektedir (Gül ve Griffen, 2019a). Hayalet yengeçler, insan etkilerinin yoğun olduğu kumsallarda daha az kaliteli besin bulabilir ve böylece yeterince enerji depolayamazlar (Morrow vd., 2014; Tewfik vd., 2016; Gül ve Griffen, 2020b). Bu nedenle, yengeçler insan etkilerinin yoğun olduğu kumsallarda yuvalama davranışları için ihtiyaç duydukları enerji miktarlarını aşağı çekebilmek için aynı yuvayı daha uzun süre kullanmak (Gül ve Griffen, 2019a; Pombo ve Turra, 2019; Costa vd., 2021), insan etkilerine daha az maruz kalacakları kumsalların daha üst bölgelerinde yuvalarını oluşturmak ve daha küçük hacimli yuvalar kazmak gibi davranışlar geliştirmişlerdir (Schlacher ve Lucrezi, 2010; Gül ve Griffen, 2018). Nitekim bu çalışmada elde edilen bulgular daha önceki çalışmaların bulgularıyla benzerlikler göstermektedirler. *O. cursor*, diğer hayalet yengeç türleriyle benzer şekilde insan etkilerinin daha yüksek olduğu kumsallarda daha küçük yuvalar oluşturmaktadırlar ve tahminen bunu bir enerji koruma davranışı olarak sergilerler.

Hayalet yengeçler, hayatlarının farklı evrelerinde farklı şekilli yuvalar tercih ederler. Örneğin üreme dönemlerinde

çoğunlukla S şekilli yuvalar kazarlar (Hartnoll, 1969). Diğer taraftan, hayalet yengeçler çoğunlukla I, J, L veya Y şekilli yuvaları tercih ederler (Türeli vd., 2009; Lucrezi ve Schlacher, 2014). Nitekim, insan aktivitelerinin daha yoğun olduğu Niğit 2 bölgesindeki yengeçlerin daha basit yuvalar oluşturdukları anlaşılmıştır. Dahası, her iki bölgede S şekilli yuvalara rastlanmış ve bu her iki çalışma alanındaki yengeçlerin bir kısmının üreme döneminde oldukları ve her iki çalışma alanındaki yengeçlerin benzer zamanlarda üreme özellikleri gösterdikleri şeklinde yorumlanmıştır. Ek olarak, hayalet yengeçlerin yuvalarında hazne varlığı bazı hayalet yengeç yuvalarında rapor edilmiş ve hazne varlığının yuva sahibi yengecin üreme döneminde olduğu şeklinde yorumlanmıştır (Lucrezi ve Schlacher, 2014). Nitekim, çalışmamızda iki çalışma alanı arasında benzer oranlarda yuvalarda bu hazneler görülmüştür. Bu da her iki popülasyonun benzer oranlarda (%50) üreme dönemine erişmiş yengece sahip olduğu şeklinde yorumlanmıştır.

Hayalet yengeçler, solunum yapabilmek için optimum sıcaklık ve optimum nem miktarına bir arada ihtiyaç duyarlar (Wolcott, 1976). Özellikle yaz aylarında hava ve kum sıcaklıkları yükselince hayalet yengeçlerin yüksek sıcaklıkların üstesinden gelebilmek için daha yüksek nem miktarına ihtiyaç duyarlar (Gül ve Griffen, 2018). Yengeç yuvalarındaki nem miktarı da yuva derinlikleri ile doğrudan ilişkilidir çünkü kumdaki nemin miktarı altındaki su tabakasına yakınlıkla doğrudan ilişkilidir. Bu nedenle, insan etkilerinin yoğun olduğu bölgelerde özellikle yüzey kum sıklığının yüksek olması ve bu yüzden yüzeydeki nemin alt tabakaya ulaşamaması nedeniyle hayalet yengeçler daha derin yuvalar oluştururlar (Schlacher ve Lucrezi, 2010; Gül ve Griffen, 2018). Fakat bu durum kumsalın jeomorfolojisiyle de doğrudan ilişki içerisindedir (Schlacher ve Lucrezi, 2010; Gül ve Griffen, 2018). Çalışmamız neticesinde, çalışma alanları arasında yuva derinlikleri açısından fark bulunamamıştır. Bu durum, her iki çalışma alanının benzer jeomorfolojilere sahip olması ve kum altı su tabakasının her iki çalışma alanında da benzer derinlikte olmasının bir sonucu olarak yorumlanabilir. Ek olarak, insan etkilerinin yoğun olduğu bölgelerde yuvaların daha dik olması bu bölgelerdeki kumun sıklığına bağlı olarak düşük su tutma kapasitesinin bir sonucu olarak yorumlanabilir. Nitekim, bu dik yuva açısı daha az enerji harcayarak ihtiyaç duyulan neme ulaşmayı sağlar, çünkü daha dik yuvalar oluşturulduğunda daha az kumun kazılması gerekir (Gül ve Griffen, 2019a).

Hayalet yengeçlerin bir alanı kendileri için korudukları ve bu alana özellikle de kendisinden küçük yengeçleri yaklaştırmadıkları pek çok çalışmada vurgulanmıştır (Türeli vd., 2009; Lucrezi ve Schlacher, 2014; Costa vd., 2021). Özellikle insan etkilerinin mevcut olduğu kumsallarda daha büyük yengeçler üst bölgelere daha küçükler ise denize yakın alt bölgelere yuvalarlar. Böylece kumsal üzerinde yengeç büyüklüklerine bağlı olarak bir tabakalaşma oluşur. Diğer taraftan, insan etkilerinin az olduğu kumsallarda ise yengeçler daha homojen bir dağılım gösterirler (Gül ve Griffen, 2018). Nitekim, son yıllarda yapılmış olan bir çalışma, kumsaldan büyük yengeçler çeşitli sebeplerle çıkarılınca küçük yengeçlerin kumsalın üst taraflarına kaydıklarını ortaya koymuştur (Gül ve Griffen, 2019b). Bu durumun muhtemel sebebi olarak, kumsalın denize yakın taraflarında gerek dalga etkileri ve gerekse de insan etkilerinin daha yoğun olması sebebiyle yengeçlerin buraları tercih etmemesi durumu olarak yorumlanmıştır (Schlacher ve Lucrezi, 2010; Gül ve Griffen, 2018), ve muhtemelen büyük yengeçler küçükler karşısındaki rekabet ve belki de kanibalizm etkisini buradaki tabakalaşma için kullanmaktadırlar (Gül ve Griffen, 2019b). Çalışmamız neticesinde *O. cursor* türünün yengeç büyüklükleri ile yuvaladıkları bölgenin suya mesafesi arasında doğrusal bir ilişki tespit edilmiştir.

SONUÇ

Hayalet yengeçler dünyanın pek çok kumsalında biyoindikatör tür olarak kullanılırlar (Schlacher vd., 2016) ve *O. cursor* da bu kullanımın bir istisnası değildir (Bal vd., 2021). Nitekim, pek çok çalışma hayalet yengeçlerin popülasyonlarının yoğunluklarına ve ortalama birey boylarına odaklanmıştır. Bu çalışma neticesinde, *O. cursor*'ın, diğer hayalet yengeçlerle benzer şekilde, popülasyon dinamiklerindeki değişimlerinin yanı sıra insan etkilerinin mevcut olduğu kumsallarda yuva özelliklerini de değiştirdiği anlaşılmıştır. Nitekim, *O. cursor* insan etkisinin daha yoğun olduğu kumsallarda daha küçük, daha dik ve daha basit şekilli yuvalar oluşturmaktadır. Sonuç olarak, *O. cursor*'un biyoindikatör olarak kullanıldığı çalışmalarda, yuva özelliklerinin de çalışmaya dahil edilmesi gerektiği anlaşılmıştır.

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Yazar, araştırmasını (makale) etkileyebilecek bilinen herhangi bir mali veya kişisel çatışma olmadığını beyan eder.

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Bu makale, insan ve hayvan ile ilgili bir çalışma içermemektedir.

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

KAYNAKÇA

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Yemlere propolis ilavesinin çipura (*Sparus aurata* L. 1758)'nın yağ asidi profili ve kan parametreleri üzerine etkisi

The influence of the dietary propolis on the fatty acid profile and the hematological parameters of seabream (*Sparus aurata* L. 1758)

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Öz: Bu çalışma, çipura (*Sparus aurata*) diyetlerinde yem katkı maddesi olarak artan oranlarda propolis ilavesinin hematolojik parametreler ve balık eti yağ asitleri profili üzerine etkilerini araştırmak amacıyla yapılmıştır. Ortalama 12,04±0,01 g ağırlığındaki çipuralar, 10 hafta boyunca 0 (kontrol), 1,25, 2,5, 5, 10, 20 g/kg propolis (kısaca P1,25; P2,5; P5; P10 ve P20) ilaveli diyetlerle beslenmiştir. Deneme, 400 L'lik 18 polyester tanka 50'şer balık eklenerek üç tekerrürlü olarak yürütülmüştür. Deneme grupları %45 protein, %17 yağ içeren yemle günde iki kez (09:00 ve 17:00) doyana kadar elle beslenmiştir. Propolis takviyesi, balık eti doymuş yağ asitleri (SFA) ve tekli doymamış yağ asitleri (MUFA) üzerine herhangi bir etki etmemiştir, bununla birlikte 10 g/kg propolis ilavesinden itibaren çoklu doymamış yağ asidi (PUFA) içerikleri önemli düzeyde artmıştır. Dolayısı ile yemlere propolis ilavesi, balık eti toplam yağ asidi kompozisyonunu etkilemiştir ($p < 0,05$). Sonuç olarak, propolis takviyesi, çipura eti yağ asidi kompozisyonunun kalitesini iyileştirmiştir. Denemenin sonunda balıklardan alınan kan örneklerinde RBC (kırmızı kan hücrelerindeki eritrosit sayısı), HGB (kanda bulunan hemoglobin sayısı) ve HCT (kanda bulunan eritrosit ve hemoglobin sayısı) gibi hematolojik parametreler P20 grubunda diğer gruplardan daha yüksek bulunmuştur. Deneme grupları arasında anlamlı fark bulunmamıştır ($p > 0,05$). Yemlerine propolis ilavesi çipura yavrularının hematolojik parametreleri üzerine herhangi bir etki etmemiştir. Elde edilen sonuçlar propolisin, çipura yemlerinde kullanılabilme potansiyeline sahip olduğunu göstermiştir.

Anahtar kelimeler: *Sparus aurata*, propolis, besin bileşenleri, yağ asidi profili, hematolojik parametreler

Abstract: This study was carried out to investigate the effects of increasing levels of propolis addition as a feed additive in seabream (*Sparus aurata*) diets on the hematological parameters and the fish fatty acids profile. Seabream with an average weight of 12.04 ± 0.01 g were fed 0 (control), by 1.25, 2.5, 5, 10, 20 g/kg (abbreviated with P1.25; P2.5; P5; P10 and P20 respectively) propolis supplemented diets for 10 weeks. The experiment was conducted in three replications by adding 50 fish to 18 polyester tanks of 400 L. The trial groups were fed twice daily (09:00 and 17:00) by hand to satiation with feed containing 45% protein and 17% lipid. Although the propolis supplementation had no effect on seabream saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA), the polyunsaturated fatty acid (PUFA) contents increased significantly after the addition of 10 g/kg propolis. Therefore, the addition of the propolis to feeds significantly affected the total fatty acid composition of fish ($p < 0.05$). As a result, the propolis supplement improved the quality of seabream fatty acid composition. At the end of the experiment, hematological parameters such as RBC (erythrocyte count in red blood cells), HGB (hemoglobin count in blood) and HCT (erythrocyte and hemoglobin count in blood) in blood samples taken from fish were found to be higher in P20 group than the other groups. There was no significant difference between the experimental groups ($p > 0.05$). The addition of the propolis to their feed did not have any effect on the hematological parameters of seabream. The results obtained showed that the propolis has the potential to use in seabream feeds.

Keywords: *Sparus aurata*, propolis, proximate composition, fatty acid profile, hematological parameters

GİRİŞ

Kültür balığı işletmelerinde yüksek stok yoğunluğunda yetiştiricilik yapılmaktadır. Balıkların, yetiştirildiği koşullarla bağlantılı olarak çeşitli akut ve kronik stres etkenlerine maruz kaldığı bilinmektedir. Bu durum çoğunlukla büyümede azalma, bağışıklık sisteminin baskılanması, çeşitli patojenlere karşı hassasiyetin artmasına neden olarak hem balık fizyolojisini hem de davranışlarını ters etkilemektedir (Segvic-Bubic vd., 2013). Son yıllarda arı ürünlerinin (polen, arı sütü ve propolis) sayısız işlevsel, biyolojik ve farmasötik yararlı etkileri nedeniyle araştırılmasına yeniden ilgi duyulmuştur (Seven vd., 2014). Propolis, bitkilerin farklı kısımlarından reçine toplanması sonucu ortaya çıkan ve güçlü antioksidan aktiviteye sahip bir arı ürünüdür (de la Cruz-Cervantes vd., 2018; Touzani vd., 2018; Farag vd., 2021). Propolis'te bulunan ana kimyasal

sınıflar flavonoidler, fenolikler ve çeşitli aromatik bileşiklerdir. Bununla birlikte, propolis birçok B kompleksi vitamini, önemli mineralleri ve eser elementleri içerir. Gerek kanatlı hayvanlar gerekse balıklarda propolisin büyüme destekleyici ve immunostimulant olarak etkileri belirlenmiştir (Soltani vd., 2017; Keleştemur ve Seven 2013; Šegvić-Bubić vd., 2013; Bae vd., 2012; Meurer vd., 2009; Seven vd., 2008; Cuesta vd., 2005). Ayrıca arı ürünlerinin serbest radikallerin neden olduğu lipid peroksidasyonunu önleyen bir antioksidan etkiye sahip olduğu belirtilmektedir (Seven vd., 2014). Propolisin içeriğinde bulunan tüm bileşikler önemli biyolojik aktivitelere sahiptir. Kafeik asit, ferulik asit ve kafeik asit fenil ester gibi antioksidan bileşikler bunlardan birkaçıdır (Ahn vd., 2007). İlaveten gliserol eter propoliste en yaygın bulunan eter lipidlerindendir ve hücre kültürlerinde oksidatif strese karşı bazı koruyucu etkileri gösterilmiştir. Bu antioksidan aktivite çeşitli reaktif oksijen türleri tarfından hedeflenen enol eter çift bağından gelir (Soltani vd., 2017). Bu özellikler dikkate alınarak yürütülen çalışmalarda yemlere propolis ilavesinin kanatlı ve memelilerde kas dokusu ve yağ asidi profilini, özellikle PUFA kalitesini arttırabileceği belirtilmiştir (Arslan ve Seven, 2017; da Silva vd., 2019; İtavo vd., 2019; Seven vd., 2014) ancak bu konuda balıklarla ilgili herhangi bir çalışma tespit edilememiştir.

Diğer yandan eksojen ajanların eritrosit sayısı, HGB miktarı, hematokrit değeri ve toplam lökositler gibi hematolojik parametreleri değiştirebileceği belirtilmektedir (Talas ve Gülhan, 2009). Propolisin canlı organizmalar üzerindeki etkilerini anlamak için, fizyolojik, hematolojik ve biyokimyasal parametrelerin incelenebileceği bildirilmektedir (Talas ve Gülhan 2009). Çeşitli çalışmalarda gösterildiği gibi, propolis antimikrobiyal, anti-inflamatory, antioksidan, antifungal, antiprotozoan ve antiviral ajanlar gibi birkaç biyolojik ve farmakolojik özelliklere sahiptir (Bankova vd., 2000). Bu özellikler göz önüne alındığında diyetlere eklenmesi onu tüketen canlı için yararlı olabilir, ancak normal diyet içinde gerekenden daha yüksek miktarlara maruz kaldıklarında propolisin insanlara ve hayvanlara zarar verebileceği düşünülmektedir. (Talas ve Gülhan 2009). Son yıllarda birkaç balık türünde propolisin biyokimyasal ve hematolojik parametreler üzerine etkileri konusunda yürütülmüş çeşitli çalışmalar bulunmaktadır (Hassaan vd., 2019; Acar 2018; Dotta vd., 2015; Keleştemur vd., 2012; Talas ve Gülhan, 2009; Yonar ve Silici, 2010). Tüm bu veriler gözönünde bulundurulduğunda, bu çalışmada çipura yemlerine farklı oranlarda propolis ilavesinin balık kas dokusu yağ asitleri profili ve hematolojik parametreler üzerine etkisinin incelenmesi amaçlanmıştır.

MATERYAL VE METOT

Deneme düzeni ve balıklar

Çalışma Akdeniz Su Ürünleri Araştırma, Üretme ve Eğitim Enstitüsü Müdürlüğü Beymelek Araştırma (AKSAM) biriminde yürütülmüştür. Deneme balıkları AKSAM kuluçkahane biriminden temin edilmiştir. Deneme başlamadan önce balıklar 2 hafta boyunca 400 L'lik tanklarda karantinaya alınarak adaptasyona bırakılmıştır. Adaptasyon sürecinde %45 ham protein içeren ticari yemle beslenmiştir. Deneme ünitesinin bulunduğu ortamda otomatik zamanlayıcılar kullanılarak 12 saat aydınlık 12 saat karanlık fotoperiyot rejimi uygulanmıştır. Denemede ortalama sıcaklığı 28,61 ± 0,12 ^{o}C, çözünmüş oksijen konsantrasyonu 7,09 ± 0,18 mg/L, pH'sı 7,66 ± 0,01, tuzluluğu ‰38,98 ± 0,03 olan filtre edilmiş deniz suyu kullanılmıştır. Su sıcaklığının ölçümü dijital termometrelerle, pH WTW 315i pH metre ile, oksijen WTW 315i oksijen ölçer ile yapılmıştır. Su kalitesi parametreleri günlük olarak izlenmiştir.

10 haftalık denemede 18 adet 400 litrelik polyester deneme tankları kullanılmıştır. Denemenin başında ve her 30 günlük periyotta 0,2 ml/L dozundaki (Fazio vd., 2013) fenoksiethanol ile bayıltılan balıkların tartımları ve ölçümleri yapılmıştır. Araştırma Akdeniz Üniversitesi Hayvan Deneyleri Etik Kurulu tarafından onaylanmıştır (Protokol No: B.30.2. AKD.0.05.07.00/58). Ortalama canlı ağırlığı 12,04 ± 0,01g, ortalama uzunluğu 9,37 ± 0,02 cm olan toplam 900 adet çipura tesadüf parselleri deneme planına göre, her tanka 50 adet olacak şekilde 3 tekerrürlü 6 grup halinde stoklanmıştır. Deneme süresince, çipura yavruları 09:00-17:00 saatlerinde propolis ilaveli yemlerle doyana kadar elle beslenmiştir.

Yem materyali

Ticari çipura yemine artan oranlarda propolis ilavesi ile elde edilen deneme yemlerinin besin madde içeriği ve yağ asidi profili analizleri Akdeniz Su Ürünleri Araştırma Üretim ve Eğitim Enstitüsü Kepez Yerleşkesi laboratuvarlarında yapılmıştır, sonuçları Tablo 1'de verilmiştir.

Çamlı Yem Besicilik Sanayii ve Ticaret A.Ş.'den temin edilen çipura yemi yem kırma makinesiyle toz haline getirilmiştir. Toz propolis ekstraktı, sırasıyla 1,25, 2,5, 5, 10 ve 20 g/ kg toz yeme eklenmiştir. Kontrol grubu yemlerine propolis ilave edilmemiştir. Eklenen toz propolis ekstraktı Karmas LEO-6 Batch mixer (0,15 kW) yem karıştırıcıyla homojen bir şekilde karıştırıldıktan sonra, 1/3 oranında su eklenerek hamur kıvamına getirilmiş, yem makinesi ile tekrar 2 ve 3 mm yemlere dönüştürülmüştür. Yemler oda sıcaklığında 24 saat kurutulduktan sonra kullanılıncaya kadar 4 ^{o}C' de muhafaza edilmiştir.

Tablo 1. Deneme yemlerinin besin bileşimi (% kuru ağırlık) ve yağ asidi profili
Table 1. The proximate composition (dry weight %) and the fatty acid profile of the experimental diets

	Deneme yemleri					
	Kontrol	**P1,25**	**P2,5**	**P5**	**P10**	**P20**
Kuru madde (%)	90,28	90,10	90,36	90,47	90,46	90,43
Ham protein (%)	45,51	45,31	45,23	45,18	45,24	45,12
Ham yağ (%)	17,13	17,12	17,13	17,15	17,18	17,24
Ham kül (%)	9,93	9,99	10,03	10,01	10,04	10,11
Ham selüloz (%)	2,95	2,94	2,94	2,95	2,97	2,98
Azotsuz öz madde (%)	24,48	24,65	24,66	24,71	24,58	24,56
Yağ asitleri						
C14:0	2,62±0,04	2,59±0,06	2,58±0,04	2,59±0,04	2,60±0,08	2,65±0,05
C15:0	0,26±0,02	0,26±0,01	0,25±0,03	0,26±0,01	0,25±0,02	0,27±0,02
C16:0	12,83±0,08	12,85±0,07	12,87±0,06	12,90±0,08	12,99±0,07	13,13±0,04
C17:0	0,27±0,01	0,28±0,01	0,30±0,01	0,32±0,01	0,33±0,01	0,35±0,01
C18:0	12,80±1,48	12,98±1,48	13,34±1,29	14,52±0,48	14,80±0,94	15,67±0,51
C20:0	0,57±0,02	0,55±0,02	0,54±0,02	0,53±0,02	0,60±0,02	0,61±0,04
C22:0	0,22±0,00	0,20±0,01	0,23±0,03	0,23±0,02	0,24±0,02	0,27±0,03
∑SFA	**29,55±1,37**	**29,67±1,29**	**30,09±1,16**	**31,34±0,36**	**31,79±0,79**	**32,96±0,46**
C16:1	2,64±0,06	2,62±0,05	2,65±0,06	2,65±0,05	2,69±0,06	2,70±0,03
C17:1	0,09±0,01	0,10±0,02	0,08±0,01	0,10±0,01	0,11±0,01	0,13±0,02
C18:1n9	30,50±0,42	31,02±0,06	30,89±0,30	30,87±0,08	31,08±0,09	31,09±0,12
C20:1	0,15±0,01	0,14±0,01	0,16±0,01	0,17±0,01	0,18±0,01	0,18±0,01
C22:1n9	0,37±0,01	0,37±0,01	0,35±0,02	0,38±0,02	0,39±0,01	0,41±0,02
C24:1	0,38±0,01	0,38±0,01	0,37±0,02	0,37±0,02	0,41±0,01	0,41±0,01
∑MUFA	**34,11±0,50**	**34,62±0,30**	**34,50±0,12**	**34,52±0,08**	**34,83±0,04**	**34,90±0,12**
C18:2n6	11,63±0,19	11,64±0,30	11,76±0,17	11,67±0,16	11,70±0,25	11,73±0,25
C18:3n3	3,61±0,11	3,63±0,10	3,61±0,05	3,62±0,10	3,68±0,10	3,70±0,11
C18:4n3	1,01±0,03	0,98±0,02	1,01±0,01	1,02±0,02	1,02±0,02	1,07±0,02
C20:2n6	0,66±0,01	0,66±0,03	0,63±0,04	0,69±0,02	0,70±0,03	0,74±0,04
C22:2n6	0,06±0,01	0,06±0,01	0,06±0,01	0,08±0,02	0,04±0,01	0,08±0,02
C20:5n3	4,12±0,07	4,15±0,09	4,08±0,07	4,17±0,03	4,08±0,03	4,12±0,03
C22:4n6	0,19±0,01	0,15±0,01	0,17±0,01	0,21±0,01	0,23±0,01	0,25±0,01
C22:5n3	1,11±0,04	1,07±0,03	1,10±0,04	1,09±0,01	1,09±0,03	1,14±0,03
C22:6n3	5,41±0,06	5,36±0,06	5,37±0,05	5,38±0,05	5,39±0,02	5,42±0,02
∑PUFA	**27,77±0,51**	**27,68±0,59**	**27,76±0,51**	**27,89±0,31**	**27,94±0,37**	**28,22±0,15**
TOPLAM	**91,43±0,36**	**92,28±0,34**	**92,37±0,28**	**93,27±0,66**	**94,70±0,23**	**94,59±0,97**
PUFA/SFA	0,94±0,06	0,94±0,01	0,93±0,06	0,89±0,02	0,88±0,04	0,86±0,02
∑n6	12,53±0,21	12,50±0,37	12,55±0,20	12,54±0,21	12,54±0,21	12,55±0,20
∑n3	15,25±0,30	15,18±0,32	15,15±0,25	15,26±0,13	15,29±0,19	15,44±0,01
n6/n3	0,82±0,00	0,83±0,01	0,83±0,00	0,82±0,01	0,82±0,01	0,82±0,02
DHA/EPA	1,31±0,01	1,29±0,01	1,32±0,02	1,29±0,01	1,33±0,01	1,32±0,01
Diğerleri	**8,57±0,36**	**8,03±0,57**	**7,65±0,53**	**6,25±0,02**	**5,44±0,46**	**3,92±0,34**

Propolis

Toz propolis ekstraktı Antalya-Yavuzbal firmasından temin edilmiştir. Denemede kullanılan propolis Antalya çevresindeki kovanlardan toplanmıştır. Denemede kullanılan propolisin besin bileşenleri ve yağ asidi profili analizi Akdeniz Su Ürünleri Araştırma Üretim ve Eğitim Enstitüsü Kepez Yerleşkesi laboratuvarlarında yapılmıştır, sonuçlar Tablo 2' de verilmiştir.

Tablo 2. Propolisin besin bileşimi (% kuru ağırlık) ve yağ asidi profili
Table 2. The proximate composition (dry weight %) and the fatty acid profile of the propolis

Parametreler	Propolis (%)
Nem	10,18±0,11
Ham protein	3,40±0,04
Ham yağ	18,80±0,19
Ham kül	15,38±0,13
Azotsuz öz madde	62,41±0,33
Yağ Asitleri	
C16:0	7,52±0,08
C17:0	0,24±0,03
C18:0	76,55±1,74
∑SFA	**84,31±1,12**
C18:1n9	4,11±0,07
C22:1n9	0,43±0,03
∑MUFA	**4,54±0,29**
C18:2n6 (LA)	0,64±0,03
C18:3n (LNA)	0,54±0,03
C20:5n3 (EPA)	0,32±0,02
∑PUFA	**1,50±0,04**
TOPLAM	**90,35±1,41**
PUFA/SFA	0,02±0,01
∑n6	0,64±0,03
∑n3	0,86±0,01
n6/n3	0,74±0,03
DHA/EPA	0,00±0,00
Diğerleri	9,65±1,41

Kimyasal analiz yöntemleri

Deneme sonunda her tanktan 5 balık alınarak 400 ppm (Fazio vd., 2013) fenoksiethanol uygulanmış, öldürülen balıklar tüm vücut besin kompozisyonu ve yağ asidi analizleri için -20 ^{o}C'de analize kadar muhafaza edilmiştir.

Denemede kullanılan balık yemi ve balık eti besin kompozisyonu AOAC (2000)'e göre belirlenmiştir. Yem ve balık numuneleri, kuru maddeyi belirlemek için 105 °C' de 24 saat fırında kurutulmuştur. Yaklaşık 2 g civarında tartılan örnek, daha önceden kül fırınında yakılmış desikatörde soğutulmuş krozelere koyulduktan sonra 600 °C'de 2 saat yakılmıştır. Daha sonra desikatöre alınarak, oda sıcaklığına kadar soğutulmuştur. Ham protein miktarı (NX6,25) Dumas metodu kullanılarak Dumas Azot Analiz cihazı (Velp NDA 701-Monza, Brianza-İtalya) ile tespit edilmiştir. Ham yağ analizi için yaklaşık 1 g kurutulmuş örnek tartılıp, XT4 filter bag içerisine koyulmuş ve ağzı yapıştırılmıştır. 3 saat 105 °C' ye ayarlanmış etüvde bekletilmiştir. Daha sonra tartım alınmıştır. Filter bag'ler yağ tayin cihazına yerleştirilmiştir (ANKOM XT15) ve dietil eterle 30 dakika ekstraksiyona devam edilmiştir. Ekstraksiyon işlemi bitince 30 dakika 105 °C ye ayarlanmış etüvde bekletilmiş ve tekrar tartım alınmıştır. Tartımlar arası farktan ham yağ miktarı hesaplanmıştır.

Yağ asitleri analizi

Yağ ekstraksiyon işlemi Bligh ve Dyer (1959) metoduna göre yapılmıştır. Yağ asitleri metil esterleri, Ichihara vd., (1996) tarafından tanımlanmış metoda göre n-hexane ve methanol içerisinde 2 M KOH kullanılarak transmetilasyon ile hazırlanmıştır. Eksrakte edilmiş 10 mg'lık yağ örneği üzerine 4 ml, 2 M'lık KOH ve 2 ml n-hekzan ilave edilmiştir. Ardından, oda sıcaklığında 2 dakika vortekste karıştırılmış ve 4000 rpm' de 10 dakika süreyle santrifüj edilmiş sonra hekzan tabakası GC'de analiz için alınmıştır.

Yağ asitleri analizi bir adet alev iyon detektörü ve silika kılcal kolon (30 mx 0,32 mm, ID x 0,25 µm film) ile donatılmış Thermo Focus GC cihazında yapılmıştır. Enjektör ve dedektör sıcaklıkları sırası ile 220 ºC ve 280 ºC'ye ayarlanmıştır. Bu esnada fırın sıcaklığı 5 dakika 140 ºC'de tutulmuştur. Sonrasında her dakikada 4 ºC arttırılarak 200 ºC'ye, 200 ºC'den 220 ºC'ye de her dakika 1 ºC arttırılarak getirilmiştir. Numune ölçüsü 5 µl ve taşıyıcı gaz da 16 ps'de kontrol edilmiştir. Ayıraç 1:40 oranında kullanılmıştır. Yağ asitleri FAME karışımının (SUPELCO) gelme zamanlarına bağlı olarak karşılaştırılmasıyla tanımlanmıştır. Sonuçlar % alan olarak ifade edilmiştir.

Deneme balıklarından kan örneği alınması

Örnek toplama ve analiz

Deneme sonunda, balıklar 24 saat aç bırakılmış ve her bir tanktan rastgele 5 balık örneği alınmıştır. 0,3 ml/L'lik bir dozda 2-fenoksietanol ile anestezi yapıldıktan sonra (Fazio vd., 2013), heparinize tek kullanımlık şırıngalar kullanılarak kaudal venden kanları alınmıştır. Kan örnekleri antikoagülan ajan olarak EDTA (1,26 mg / 0,6 ml) içeren mikro tüplere (Miniplast 0,6 ml, LP Italiana Spa, Milano) ayrılmış ve hematolojik oto analizör (MS4, Melet Schloesing laboratories, Pontoise, Cedex – Fransa) ile analiz edilmiştir. Balıkların kuyruk bölgesinden alınan kan örnekleri analizlerinde hemogram için; beyaz kan hücrelerinde bulunan lökositlerin sayısı (WBC), kırmızı kan hücrelerindeki eritrosit sayısı (RBC), kanda bulunan hemoglobin sayısı (HGB), kanda bulunan eritrosit ve hemoglobin sayısı (HCT), eritrositlerin sahip olduğu ortalama büyüklük (MCV), eritrositlerde bulunan hemoglobin sayısı (MCH), ortalama korpusküler hemoglobin konsantrasyonu (MCHC) ve platelets trombosit (PLT) değerleri belirlenmiştir.

İstatistiksel analizler

Denemede elde edilen verilerin değerlendirilmesinde varyans analizi (ANOVA) ve grup ortalamalarının karşılaştırılmasında Duncan testi kullanılmıştır. Bu amaçla SPSS 14,0 paket programlarından yararlanılmış, istatistiki karşılaştırmalarda p <0,05 önem seviyesi seçilmiştir.

BULGULAR

Vücut kompozisyonu ve yağ asidi profili

Çalışma sonunda çipura balık eti besin kompozisyonu incelendiğinde, 10 g/kg propolis ilavesi protein oranını arttırırken kül oranını düşürmüş, kuru madde ve yağ oranları üzerine etki etmemiştir (Tablo 3).

Propolis ilaveli yemlerle beslenen çipura balık eti doymuş yağ asitleri (SFA) ve tekli doymamış yağ asitleri (MUFA) gruplar arasında anlamlı farklılık göstermemiştir, bununla birlikte Tablo 4.'de görüldüğü gibi 10 g/kg ve üzeri propolis ilavesi çoklu doymamış yağ asidi (PUFA) ve toplam yağ asidi içeriklerini önemli düzeyde artmıştır (p < 0,05).

Hematolojik profil

Deneme sonunda balıklardan alınan kan örnekleri incelendiğinde WBC, RBC, HGB, HCT değerlerinin en yüksek P20 grubunda olduğu, gruplar arasında anlamlı farklılık bulunmadığı tespit edilmiştir (p >0,05), MCV, MCH, MCHC, PLT değerleri için de gruplar arasında anlamlı farklılık bulunmadığı tespit edilmiştir (Tablo 5).

Tablo 3. Artan oranlarda propolis ilaveli yemlerle beslenen çipura *S. aurata* 'nın vücut kompozisyonu
Table 3. The proximate composition (wet weight %) of the whole body of the seabream *S. aurata* fed diets with the increasing level of the propolis in the feed

	Kontrol	P1,25	P2,5	P5	P10	P20
Kuru madde	37,57 ± 1,49	37,18 ± 0,68	37,74 ± 0,48	36,47 ± 0,87	37,99 ± 1,17	38,21 ± 0,90
Ham protein	16,66 ± 0,59^{ab}	16,87 ± 0,57^{ab}	15,82 ± 0,66^{b}	17,86 ± 0,42^{ab}	19,09 ± 1,55^{a}	17,54 ± 0,77^{ab}
Ham yağ	17,41 ± 1,65	16,32 ± 0,51	15,79 ± 0,38	14,47 ± 0,60	16,62 ± 0,96	16,58 ± 0,66
Ham kül	3,98 ± 0,10^{ab}	4,08 ± 0,09^{ab}	3,90 ± 0,07^{ab}	4,20 ± 0,21^{a}	3,74 ± 0,07^{b}	3,86 ± 0,09^{ab}

Her bir parametre için (n=5) ortalama değerler ve standart hata (±SH) gösterilmiştir.
Aynı satırda farklı küçük harf olan ortalamalar arasındaki farklılık önemlidir (p <0,05).

Tablo 4. Artan oranlarda propolis ilaveli yemlerle beslenen çipura *S. aurata*'nın yağ asidi profili
Table 4. The fatty acid profile of the seabream *S. aurata* fed with the increasing levels of the propolis in the feed

Yağ asitleri	Kontrol	P1,25	P2,50	P5,0	P10	P20
C14:0	1,83±0,23	2,21±0,11	2,06±0,17	1,97±0,07	1,98±0,15	2,12±0,08
C15:0	0,23±0,01	0,25±0,01	0,23±0,02	0,23±0,01	0,24±0,01	0,27±0,01
C16:0	16,53±0,87	15,88±0,32	16,23±0,70	16,45±0,40	16,63±0,51	15,53±0,19
C17:0	0,21±0,02	0,20±0,00	0,19±0,01	0,19±0,00	0,19±0,00	0,19±0,01
C18:0	13,19±2,19	7,25±1,29	10,69±2,93	11,22±1,76	11,95±2,83	9,15±1,34
C20:0	0,38±0,01	0,33±0,02	0,37±0,02	0,37±0,03	0,36±0,02	0,37±0,01
C22:0	0,36±0,02^{ab}	0,33±0,01^{abc}	0,32±0,01^{bc}	0,38±0,02^{a}	0,35±0,02^{abc}	0,30±0,01^{c}
∑SFA	**32,73±2,87**	**26,45±1,48**	**30,09±3,46**	**30,81±1,93**	**31,69±3,18**	**27,93±1,46**
C16:1	2,80±0,25	3,44±0,17	3,10±0,33	3,07±0,16	3,05±0,32	3,42±0,14
C17:1	0,14±0,03	0,19±0,02	0,17±0,03	0,17±0,02	0,16±0,01	0,17±0,02
C18:1n9	30,14±2,34	34,46±1,29	31,78±2,25	31,23±1,41	29,93±2,19	32,37±0,82
C20:1	0,17±0,01	0,19±0,01	0,19±0,02	0,17±0,01	0,17±0,01	0,17±0,01
C22:1n9	0,63±0,10	0,50±0,07	0,52±0,08	0,58±0,06	0,57±0,04	0,54±0,04
C24:1	0,29±0,03^{ab}	0,41±0,04^{a}	0,35±0,05^{ab}	0,35±0,02^{ab}	0,27±0,05^{b}	0,37±0,03^{ab}
∑MUFA	**34,18±2,55**	**39,19±1,38**	**36,10±2,86**	**35,57±1,54**	**34,15±2,58**	**37,05±0,94**

Tablo 4. Devamı
Table 4. Continued

Yağ asitleri	Kontrol	P1,25	P2,50	P5,0	P10	P20
C18:2n6	10,66±0,89^{b}	11,73±0,35^{ab}	11,54±0,77^{ab}	12,13±0,52^{ab}	11,30±0,84^{ab}	13,08±0,34^{a}
C18:3n3	2,61±0,40^{b}	3,10±0,20^{ab}	2,83±0,33^{ab}	2,65±0,23^{ab}	3,93±0,92^{ab}	4,29±0,47^{a}
C18:4n3	0,55±0,07	0,69±0,05	0,61±0,10	0,60±0,07	0,61±0,07	0,63±0,03
C20:2n6	0,67±0,04	0,79±0,10	0,66±0,03	0,67±0,01	0,66±0,03	0,69±0,02
C22:2n6	0,06±0,02^{b}	0,09±0,01^{ab}	0,08±0,01^{ab}	0,08±0,01^{ab}	0,07±0,01^{ab}	0,10±0,01^{a}
C20:5n3	3,09±0,21	2,75±0,21	2,78±0,17	2,83±0,12	3,32±0,18	2,91±0,01
C22:4n6	0,23±0,02	0,22±0,02	0,20±0,00	0,21±0,02	0,24±0,01	0,23±0,01
C22:5n3	1,59±0,04	1,62±0,04	1,54±0,06	1,58±0,01	1,65±0,03	1,63±0,02
C22:6n3	7,32±0,99	5,65±0,72	5,94±0,75	6,13±0,52	7,10±0,82	6,05±0,33
∑PUFA	**26,77±0,23^{b}**	**26,64±0,26^{b}**	**26,18±0,46^{b}**	**26,89±0,91^{b}**	**28,86±0,76^{a}**	**29,62±0,23^{a}**
TOPLAM	**93,69±0,15^{ab}**	**92,28±0,34^{b}**	**92,37±0,28^{b}**	**93,27±0,66^{ab}**	**94,70±0,23^{a}**	**94,59±0,97^{a}**
PUFA/SFA	0,83±0,09	1,01±0,05	0,90±0,11	0,88±0,07	0,93±0,11	1,06±0,04
∑n6	11,61±0,92^{b}	12,84±0,40^{ab}	12,48±0,81^{ab}	13,09±0,55^{ab}	12,27±0,86^{ab}	14,10±0,36^{a}
∑n3	15,16±0,69^{ab}	13,80±0,63^{b}	13,70±0,50^{b}	13,80±0,46^{b}	16,00±0,34^{a}	15,51±0,85^{ab}
n6/n3	0,77±0,10	0,94±0,07	0,92±0,09	0,95±0,03	0,74±0,06	0,92±0,08
DHA/EPA	2,35±0,17	2,04±0,11	2,12±0,14	2,16±0,11	2,13±0,13	2,08±0,04
Diğerleri	6,31±0,15^{ab}	7,72±0,34^{a}	7,63±0,28^{a}	6,73±0,66^{ab}	5,30±0,23^{b}	5,41±0,97^{b}

Her bir parametre için (n=5) ortalama değerler ve standart hata (±SH) gösterilmiştir.
Aynı satırda farklı küçük harf olan ortalamalar arasındaki farklılık önemlidir (p <0,05).

Tablo 5. Artan oranlarda propolis ilaveli yemlerle beslenen çipura *S. aurata*'nın hematolojik parametreleri
Table 5. The hematological parameters of the seabream *S. aurata* fed with the increasing levels of the propolis in the feed

	Kontrol	P1,25	P2,5	P5	P10	P20
WBC (x10^{9} L^{-1})	201,90±11,64	173,17±39,21	182,80±14,35	200,27±10,13	190,93±25,61	225,40±9,81
RBC (x10^{12} L^{-1})	3,33±0,46	3,16±0,96	3,05±0,33	3,29±0,26	3,32±0,50	4,23±0,49
HGB (g dL^{-1})	11,03±1,15	10,47±2,99	10,13±0,96	11,03±0,80	10,73±1,48	12,50±1,65
HCT (%)	51,37±7,92	45,57±9,99	44,30±7,25	54,07±6,08	49,37±11,07	63,50±7,60
MCV (fL)	153,83±5,22	151,67±12,54	145,00±15,47	163,57±5,45	145,40±14,33	150,30±8,58
MCH (pg)	33,37±1,13	33,47±0,67	33,23±0,74	33,50±0,29	32,50±0,92	29,43±1,32
MCHC (g dL^{-1})	21,77±1,07	22,33±1,64	23,50±2,46	20,53±0,78	22,83±2,59	19,80±1,94
PLT (x10^{9} L^{-1})	71,67±13,22	68,67,67±12,17	66,00±6,66	67,00±5,03	55,67±5,17	70,00±7,37

Her bir parametre için (n=5) ortalama değerler ve standart hata (±SH) gösterilmiştir.

TARTIŞMA

Yetiştiricilik ortamında stres koşulları altında, adrenal glukokortikoidler kültüre alınan hayvanda kortikal bölgeden salınır. Bu hormonların başlangıçta gerekli enerjiyi artırmak için yağ dokusundan lipidleri harekete geçirdiği bilinmektedir. Önce doymamış yağ asitleri harekete geçirilir (Mumma vd., 2006). Kanatlılarla yapılan çalışmalarda kronik stres altında devam eden kortikosteron salınımının, dokularda lipid peroksidasyonuna neden olduğu rapor edilmiştir (Lin vd., 2004). Morrissey vd. (1994), lipid peroksidasyonunun, vücudun kronik strese (stresin ikinci evresi) adaptasyon döneminde dokuların fosfolipid fraksiyonunda PUFA'nın azalmasına neden olduğunu bildirmişlerdir. Devam eden kortikosteron sekresyonunun, stres koşulları altında yağ sentezi, özellikle SFA sentezi doğrultusunda enerji metabolizmasını değiştirdiği rapor edilmektedir (Szabo vd., 2004).

Nakajima vd. (2009) propolisteki kafeik asidin kasların ve iç organların PUFA oranlarını artırarak yağ asidi profillerini iyileştirebileceğini belirtmiştir. Propolis ilavesinin doza bağlı olarak kanatlı dokularının PUFA içeriğinde bir artışa neden olabileceği daha önceki çalışmalarda bildirilmiştir (Rymer ve Givens 2005). Bu çalışmada propolis ilavesinin balık kas dokusunda yağ asidi profili üzerine etkisi ilk kez incelenmiştir, kontrol grubu kas dokularının SFA oranının, propolis ilaveli gruplardan bir miktar yüksek olduğu ancak gruplar arasında farkın anlamlı olmadığı bulunmuştur ($p > 0,05$). Ayrıca 10 g/kg ve üzeri propolis ilave edilen gruplarda toplam PUFA'nın diğer gruplardan önemli düzeyde daha yüksek olduğu belirlenmiştir ($p < 0,05$). Arı ürünleri çok sayıda fenolik bileşik içerir (Viuda vd., 2008). Önceki çalışmalar, fenollerin ve polifenollerin indirgeyici ajanlar, hidrojen donörleri ve tekli oksijen söndürücüler olarak hareket eden redoks özelliklerine sahip olduğu göstermiştir (Caldwell, 2003). Guo vd. (2008), arı ürünlerinin doymamış yağ asitlerinin peroksidasyonuna karşı güçlü antioksidatif aktiviteye sahip olduğunu bildirmiştir. Tüm bu faktörler PUFA oranlarını arttırarak kasların yağ asidi profillerini iyileştirmiş olabilir.

Çalışma sonunda çipura balık eti besin kompozisyonu incelendiğinde, 10 g/kg propolis ilavesinin protein oranını arttırırken kül oranını düşürdüğü, tespit edilmiştir. Deng vd. (2011) gökkuşağı alabalığı yemlerinde propolis kullanımının balık eti besin kompozisyonunu etkilemediğini belirtmişlerdir. Bae vd. (2012) yılan balığı yemlerine propolis ilavesinin balık eti besin kompozisyonunu etkilediğini, 0,5 g/kg propolis ilavesinin balık etinde ham protein ve yağ değerini arttırırken, kül oranını düşürdüğünü, propolis oranı arttıkça protein ve yağda düşme, kül oranında ise yükselme meydana geldiğini, bu sonuçların propolis içerisindeki flavonoidlerin besin metabolizması, absorbsiyon ve besin alımını arttırmasından oluşmuş olabileceğini belirtmişlerdir. Ancak kesin etki mekanizması henüz belirlenememiştir.

Hematolojik ve biyokimyasal özellikler çevre ve insan kaynaklı stres faktörlerinin etkilerini gösteren önemli parametrelerdir (Keleştemur vd., 2012). İlaveten immunostimulantların vücutta oluşturduğu fizyolojik değişiklikleri ortaya koyması bakımından da önemlidir (Yonar ve Silici 2010). Hematokrit düzeyi balık sağlığı için genel bir indikatördür ve immunostimulanlardan kaynaklanan anormaliteleri açıklamaya yardım etmektedir. Balıklarda bir çok immunostimulant karakterdeki madde ile yapılan çalışmada lökosit düzeyinde önemli farklılıkların olduğu görülmüştür. Talas ve Gülhan (2009) alabalıklarda propolis konsantrasyonun artmasıyla hematokrit değerinin düştüğünü saptamışlardır. Yonar ve Silici (2010) ise immunostimulant karakterdeki propolisin alabalıkların hematokrit değerini istatistiksel olarak anlamlı olmasa da arttırdığını rapor etmişlerdir. İlaveten eritrosit ve lökosit sayısında farkın önemli olduğunu belirtmişlerdir. Cuesta vd. (2005) propolisin çipurada, Talas ve Gülhan (2009) alabalıkta lökosit sayısını arttırdığını saptamışlardır. Bizim çalışmamızda 20 g/kg propolis ilavesi lökosit sayısında bir miktar artışa yol açmış ancak farklılık önemli düzeyde olmamıştır ($p > 0,05$).

Eritrosit indeksleri (MCV, MHC, MCHC), hematokrit, eritrosit ve hemoglobin yoğunluğu ile ilişkili olup eritrositlerin büyüklüğü veya çapı ile hemoglobin miktarını belirtir. Eritrosit indeksleri anemi tiplerinin ayırıcı tanısında yardımcı olur (Yonar ve Silici 2010). Talas ve Gülhan (2009) propolisin balıklarda makrositik anemiye neden olduğunu belirtmişlerdir. Bu araştırmada ise propolisin eritrosit indeksleri üzerinde herhangi bir etkisi olmadığı tespit edilmiştir. Sonuç olarak incelenen parametreler çerçevesinde, çalışmamızda propolisin balıkların kan profili üzerine olumsuz herhangi bir etki göstermediği tespit edilmiştir.

SONUÇ

Deneme sonuçlarından elde edilen veriler; propolisin çipura balık eti besin kompozisyonu ve yağ asitleri üzerine olumsuz bir etkisi olmadığını, aksine PUFA ve toplam yağ asidi miktarını arttırabileceğini, ilaveten çipurada hematolojik parametreleri olumsuz etkilemediğini göstermiştir. Propolisin elde edildiği coğrafi bölge, bitki örtüsü, hasat edildiği mevsim gibi faktörlerin kimyasal özellikleri üzerine etkili olduğu bilinmektedir. Uygulanan doz, balık türü, balık büyüklüğü gibi değişkenlerin de sonuçlar üzerine etkili olduğu ileri sürülmektedir. Bütün bunlar gözönüne alındığında farklı propolis örneklerinin, farklı balık türlerine etkisinin belirlenmesi için gelecekte yeni çalışmalara ihtiyaç vardır.

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SHORT COMMUNICATION KISA MAKALE

Maximum length record and some biological characters of devil firefish *Pterois miles* Bennett, 1828 for Aegean Sea, Turkey

Kırmızı aslan balığı *Pterois miles* Bennett, 1828'in Ege Denizi'nin Türkiye kıyısı için maksimum boy kaydı ve bazı biyolojik özellikleri

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Abstract: Present work reports the maximum size record of *Pterois miles* for Turkish Seas with some additional biological information. The greatest individual of *P. miles* was caught off Çökertme Bight, Bodrum (Southern Aegean Sea) at 10 m depth with a spear gun on 15 July 2021. Captured individual of devil firefish was 34.0 cm in total length and 707.55 g in total weight. Specimen was male with a gonad weight of 13.6 g and it was determined to be 6 years old. A prey fish *Chromis chromis* were detected in the stomach in definable visible condition. Total length and weight of the mentioned individual are the greatest for the Turkish Seas among the reported studies up to date.

Keywords: Devil firefish, Scorpaenidae, maximum size, age, feeding, Southern Aegean Sea

Öz: Bu çalışma, bazı ek biyolojik bilgilerle birlikte Türkiye Denizleri için *Pterois miles* in maksimum boy kaydını sunmaktadır. Örnek 15 Temmuz 2021'de Bodrum (Güney Ege Denizi) Çökertme Koyu açıklarında 10 m derinlikte zıpkınla yakalanmıştır. Yakalanan kırmızı aslan balığının toplam boyu 34.0 cm ve toplam ağırlığı 707.55 olarak tespit edilmiştir. Örneğin 13.6 g gonad ağırlığında ve 6 yaşında bir erkek birey olduğu belirlenmiştir. Mide içeriğinde bir av balığı olan *Chromis chromis* tanımlanabilir bir durumda tespit edilmiştir. Söz konusu bireyin toplam boy ve ağırlığı, bugüne kadar bildirilen çalışmalar arasında Türkiye Denizleri için en büyük olanıdır.

Anahtar kelimeler: Aslan balığı, Scorpaenidae, maksimum boy, yaş, beslenme, Güney Ege Denizi

INTRODUCTION

The devil firefish (*Pterois miles*) is a marine and reef associated fish, which inhabits shallow waters on rocky or sandy bottoms down to 85 m depth (Froese and Pauly, 2022). It naturally lives in the Indian Ocean (Persian Gulf), Atlantic Ocean, South Africa and occurred in the Red Sea (Frose and Pauly, 2022). The devil lionfish is reported to be one of the most successful invasive alien species in the history of aquatic invasions (Rocha et al., 2015). Environmental tolerances, reproductive output, predation defense, diet composition, and feeding behaviour are the main factors of its wide distribution (Cote´ et al., 2013). *Pterois miles* together with its congeneric *Pterois volitans* have been exploited for marine aquarium trade worldwide and both are consumed by local people as a part of their tradition. One of the most important biological characteristics of the fish is highly venomous fin spines which may cause human death. Despite of its interesting features scientific studies focusing on the biology and the fisheries of the species are limited.

The records of the devil firefish were given from the Mediterranean Sea at Haifa Bay in 1991 (Golani and Sonin, 1992), Lebanon coast (Bariche et al., 2013), İskenderun Bay in Turkey (Turan et al., 2014), Rhodes in Greece (Crocetta et al., 2015), Tunisia (Azzurro et al., 2017; Karachle et al., 2017), Italy (Azzurro et al., 2017) and Libya (Al Mabruk and Rizgala, 2019). Invasiveness of the species for Turkish seas has emphasized in comprehensive studies (Filiz et al., 2017; Tarkan et al., 2021; Vilizzi et al., 2021). Several papers reported a westerly migration of the species from the Southern coast of Turkey (Bilge et al., 2016; Yağlıoğlu and Ayaş, 2016; Turan et al., 2017; Özgül, 2020) and first occurrence reports for the Aegean Sea were from Fethiye Bay and Dalyan coast in 2015 (Turan and Öztürk, 2015). Distribution of the species along the Aegean Sea has expanded towards the northeast and the northernmost presence of the species was reported from Kokar Bay-İzmir (Özgül, 2020).

Despite its invasiveness and occurrences in Turkish seas, scientific studies focusing on the biology and the fisheries of the species are limited. Especially, in the absence of basic data, the maximum observed length is useful for the rapid evaluation of growth rates (Froese and Binohlan, 2000). This paper documents the maximum size record of *P. miles* for Turkish Aegean Sea waters with some additional biological information and is considered to make a contribution to biology of the species.

MATERIAL AND METHODS

On 15 July 2021, a male specimen of *P. miles* was captured by the second author of this paper from Çökertme Bight-Bodrum (Figure 1) by a speargun at depth of 10 m on rocky bottoms. Fish was taken to the laboratory in the ice box and metric measurements with meristic counts were performed when the fish was fresh. Identification of the specimen was based on Schultz (1986) and Golani et al. (2006). Total length (TL) of the individual was measured to the nearest mm and weighted to the nearest 0.1g. Sex determination was based on macroscopically investigation of the gonads and gonad weight (GW) was measured to the nearest 0.01 g. Age estimation was based on otolith examination. For this purpose, sagittal otoliths were removed, prepared for age readings and observed by a stereoscopic microscope. Three readers were involved in the age determination and winter rings (black ones) were counted during the age readings. One opaque and 1 transparent rings together were considered to be the indicator of one-year growth.

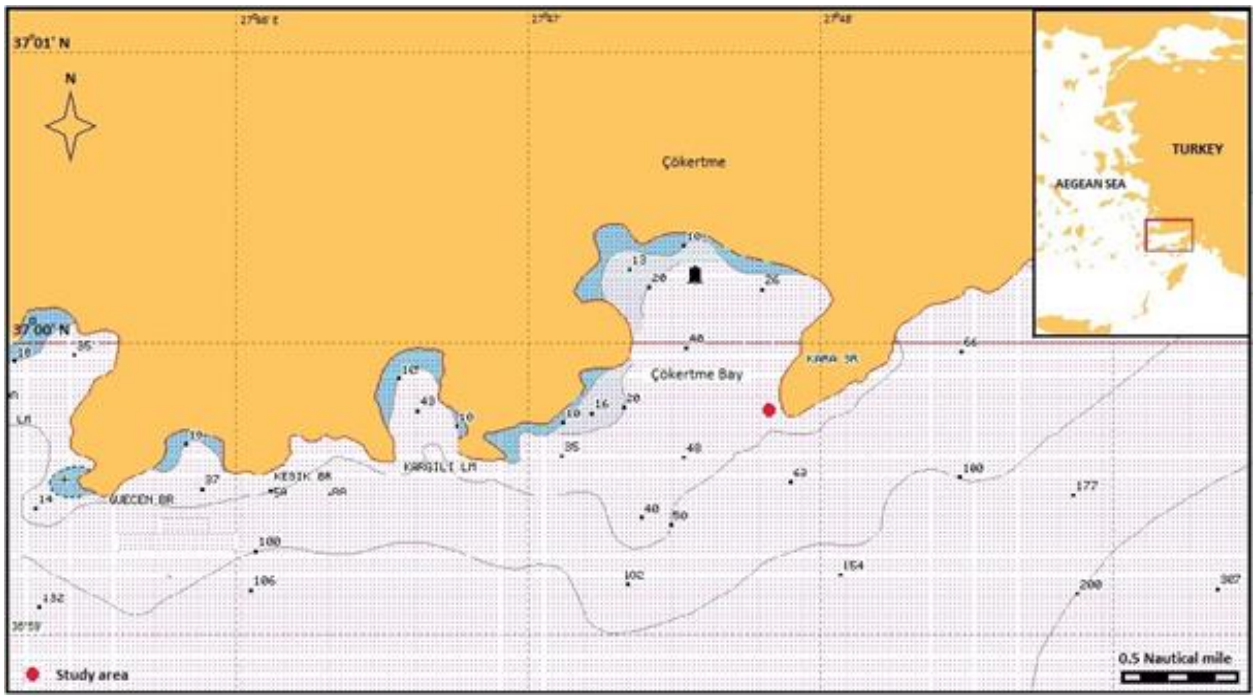

Figure 1. Sampling location (Red spot on the map indicates the sampling point)

RESULTS

Captured individual of devil firefish was 34.0 cm in total length (TL) and 707.55 g in total weight (TW) (Figure. 2). Some morphometric characters for *P. miles* are given in Table 1. Sex of the specimen was male (Figure 3) with a gonad weight of 13.6 g during the III. maturity stage (mature) which was creamy white, soft and occupying 3/4 of the body cavity. Age of the specimen was determined to be 6 (Figure 4).

During the spearfishing trial, crowded schools of *Chromis chromis* (damselfish) were observed in the area and one individual was detected in the stomach of the captured specimen with a good visible condition (Figure 5) during the stomach observation.

Figure 2. The maximum sized devil firefish from Çökertme Bay, Southern Aegean Sea

Table 1. Some morphometric characters for *P. miles*

Metric measurements	Value
Total length (TL) (cm)	34
Weight (g)	707.55
Standard length (SL) (cm)	26
Max. Body depth (cm)	11.4
Girth (cm)	29.2
Head length (HL) (cm)	7.9
Pre-orbital length (cm)	4
Eye diameter (ED) (mm)	15.6
Pre-dorsal lenght (cm)	7.5
Pre-anal length (cm)	17.3
Pre-pelvic lenght (cm)	7.3
Pre-pectoral fin length (cm)	7.8
Caudal peduncle depth (CPDe) (cm)	3.8
Interorbital width (cm)	2.1
First dorsal fin length (cm)	10.8
Second dorsal fin length (cm)	5.8
Total Dorsal length (cm)	18.1
Anal fin base length (AL) (cm)	4.3
Gonad weight (GW) (g)	13.6
Liver weight (g)	14.5
Meristic counts	**Number**
Number of dorsal fin spines	13
Number of dorsal fin soft rays	11
Number of anal fin spines (AS)	3
Number of anal fin soft rays (AR)	7
Number of pectoral fin rays	14
Number of pelvic fin spines	1
Number of pelvic fin soft rays (PeR)	5
Number of caudal fin soft rays	14

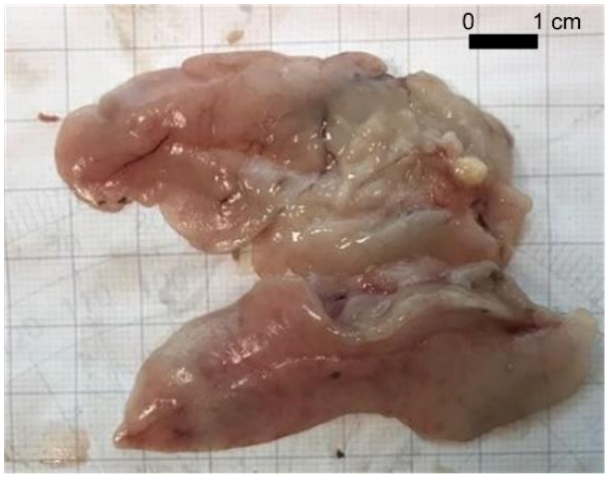

Figure 3. Male gonad of the devil firefish

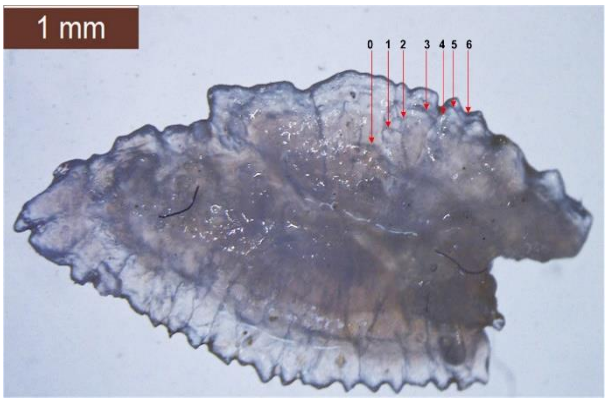

Figure 4. Sagittal otolith of the devil firefish

Figure 5. Stomach content of the devil firefish

DISCUSSION

The length of a fish is the fundamental criteria required to identify reproduction, recruitment, growth and mortality. Therefore, length data when supported with other parameters such as maturity and age, especially for species like *P. miles* which has limited biological data, becomes of crucial importance. The present study proves that invasive devil firefish can grow above the previously reported maximum length values along the Turkish coast and presents the maximum size for Turkish waters. Although our TL value is the highest for Aegean Sea among the reported studies so far, the maximum length record of the species for Mediterranean belonged to Oray et al. (2015) with a TL value of 37.3 cm. The number of scientific studies with regard to biology and other aspects of *P. miles* is still scarce in the Aegean Sea due to its continuous settlement and dispersal to northwards. Therefore, length and weight records for *P. miles* were given for all possible localities instead of only from the Aegean Sea, in order to make a better comparison of these parameters (Table 2).

Table 2. Reported length and weight of *P. miles* in previous studies(L: Length, W: Weight)

Author(s)	Sex	Area	N	Lmax (cm)	Wmax (g)
Oray et al. (2015)	-	Northern Cyprus	1	37.3	-
Özgür et al. (2017)	-	Antalya, Türkiye	8	29.3	398.7
Zannaki et al. (2019)	♂	Rhodes, Greece	21	25.8	352.3
	♀		12	31.5	330.0
Ozgül (2020)	-	İzmir, Türkiye	1	14.4	38.8
Present study	♂	Çökertme Bay, Türkiye	1	34.0	707.5

The number of lionfishes is rapidly increasing in the Mediterranean Sea and also along the Turkish coast including the Aegean Sea. *P. miles* has been reported to be a temperature dependent (Dabruzzi et al., 2017) species and it can not survive below 10 °C (Kimball et al., 2004). However, Özgür et al. (2017), stated that devil lionfish continued feeding in low winter temperature (14.9 °C). Rapid invasion of devil firefish along the Mediterranean coast is related to increase in water temperature in recent years and from this point, *P. miles* is expected to expand its' dispersal in the Aegean Sea. The probable results of the invasion are habitat and ecosystem destruction due to their potential to directly consume or outcompete native species. Although the introduction of the species to the Mediterranean is still scarce (aquarium release, ballast water transfer, Atlantic based dispersal or passage through the Suez Canal) (Yapıcı, 2018), observations of established populations with juvenile individuals in the Mediterranean Sea is a strong proof of colonization (Bariche et al., 2017).

Feeding habit of a fish is one of the most important biological features affecting its growth. We found only one *C. chromis* individual in the stomach parallel to Zannaki et al. (2019) who focused on the feeding habit of *P. miles* by notifying *C. chromis* as prey with a 5% abundance among 51 prey items. Damselfish is reported to be the most abundant fish in the Mediterranean ecosystem which is also a prey source for predator fish and sea birds. It is also found in the diet of Scorpaenidae family (Pinnegar, 2018). High abundance of damselfish in the sampling area may indicate the feeding habit of devil firefish. On the other hand, invasive *Pterois* species were also reported to be preys of dusky grouper (*Ephinephelus marginatus*) and goldblotch grouper (*Ephinephelus costae*) (Turan et al., 2017). The absence of predators (mainly due to over exploitation) that consume devil firefish may also be effective in reaching large size and wide range distribution. Therefore, the preservation of top predators especially by reducing illegal fishing pressure on such species in the Mediterranean and the Aegean Sea can be nominated to be the best administrative tool for controlling the rapid dispersal of the species. Maximum length is an important theoretical parameter in fisheries science (Dulcic and Soldo, 2005). Further comprehensive studies are urgently required on the biology and feeding habits of devil firefish and red lionfish and struggle against them by applying ecosystem friendly capture techniques not only for Turkish coast but also for Mediterranean basin. The information presented here is considered to contribute to fisheries biology of the species and international scientific literature.

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AUTHORSHIP CONTRIBUTIONS

Ozan SOYKAN: Conceptualization, methodology, software, writing and editing. Ali ULAŞ**:** Data curation, visualization, investigation.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

Approval was granted by the Ethics Committee of Ege University (22.04.2020 /No:2020-048). The authors declare that all applicable guidelines for sampling, care, and experimental use of animals in this study have been followed.

DATA AVAILABILITY

The data sets generated during and/or analyzed during the current study will be provided by the corresponding author upon the request of the editor or reviewers. For questions regarding datasets, the corresponding author should be contacted

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CASE REPORT DURUM RAPORU

Lateral line and caudal fin anomalies in common sole (*Solea solea* Linnaeus, 1758) from southern Aegean Sea

Güney Ege Denizi'nden yakalanan dil balığında (*Solea solea* Linnaeus, 1758) yanal çizgi ve kaudal yüzgeç anomalileri

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Abstract: This study presents two anomalies that were found in two wild common sole (*Solea solea* Linnaeus, 1758) specimens. Specimens were captured with 80 mm stretched mesh size trammel net incidentally (in June 2014 and in December 2015) from Güllük Bay, southwest of Turkey where is one of the important common sole fishery areas. Female common sole specimen has lateral line anomaly. According to observations, the lateral line has unordinary shape – labyrinth like, furcate. Other sample, male specimen, has caudal fin anomaly. The specimen has large body size and hence, it was thought that having no caudal fin did not affect its swimming ability besides activities like feeding. The present study contributes to literature about rare anomalies in wild fish stocks.

Keywords: Soleidae, common sole, fish anomaly, small-scale fishery, Güllük Bay

Öz: Bu çalışmada doğal ortamdan yakalanan iki dil balığı (*Solea solea* Linnaeus, 1758) bireyinde bulunan anomalilere yer verilmiştir. Bireyler, önemli dil balığı avlak sahalarından biri olan Türkiye'nin güneybatısında yer alan Güllük Körfezi'nden tesadüfi olarak (Haziran 2014, Aralık 2015) 80 mm ağ göz genişliğine sahip fanyalı uzatma ağları ile yakalanmıştır. Dişi dil balığı bireyinde yanal çizgi anomalisi görülmektedir. İncelemelere göre yanal çizgi sıra dışı bir şekle sahiptir (labirent gibi, çatallı). Diğer erkek birey ise kaudal yüzgeç anomalisine sahiptir. Bu bireyin kaudal yüzgecinin olmaması, büyük bir vücuda sahip olması nedeniyle beslenme gibi aktivitelerinin yanı sıra yüzme yeteneğini etkilenmediğini düşündürmüştür. Bu çalışma literatüre doğal balık stoklarında nadir görülen anomaliler ile ilgili katkı yapmaktadır.

Anahtar kelimeler: Soleidae, dil balığı, balık anomalisi, küçük ölçekli balıkçılık, Güllük Körfezi

INTRODUCTION

Anomalies seen in various fish species, when scientific literature is taken into consideration, affect wild and reared species (Akyol and Şen, 2012; Lagardere et al., 1993). Additionally, fish anomaly researches include different species such as *Solea solea* (Dulcic and Soldo, 2005), *Dicentrarchus labrax* (Costa et al., 2015) and *Grahamina capito* (Jawad et al., 2006). Cases of fish anomalies are reported as deformity in pigmentation (ambicolouration, albinism, and xanthochroism) (Jawad et al., 2006; Tokaç et al., 2013; Golani et al., 2019), malformations of lateral line, scale and ray shape (Costa et al., 2015; Metin et al., 2009), absence of caudal fin (Dulcic and Soldo, 2005), vertebral and caudal skeleton deformities (Gavaia et al., 2002), anomalies in cephalic structures (Lagardére et al., 1993), disorders in skeletal formation (Boglione et al., 2013) and otolith anomalies (Vinagre et al., 2014). The diversity of anomalies seen at fish species necessitates both recording of anomalies and the investigation of underlying reasons.

The aforementioned necessity is not only related to anomaly types but also originates from its problematic nature regarding biological diversity and rearing environments. The diversity of the underlying causes of anomalies extends the scope of the problem. The pollution of sediments due to anthropogenic and industrial activities (Akyol and Şen, 2012), genetic factors (Costa et al., 2015), exposition to unfavourable conditions during embryological stages (Dulcic and Soldo, 2005) have been suggested as the reasons for fish anomalies. Yet, the etiology of most anomalies largely remains unknown, and it is mentioned that a wide range of physical, chemical and biological factors might be the causes of anomalies (Tutman et al., 2000).

Common sole, *Solea solea* (Linnaeus, 1758), is a commercially important flatfish in all around the world (Bolle et al., 2012; Seafish, 2013; Diopere et al., 2014; Saleh et al., 2016) and also in Turkish fisheries (Türkmen, 2003). In Turkey, common sole is one of the most important targeted species for southern Aegean Sea small-scale fishermen. This species is captured mostly with different mesh sized trammel nets in some seasons (Cerim, 2017).

The aim of this study is to present lateral line and caudal fin anomalies of two common sole specimens, caught in the Southern Aegean Sea, Turkey.

MATERIAL AND METHODS

Güllük Bay is an overused area for several sectors like tourism, aquaculture systems, fisheries and sea transport. Besides these exposures, domestic pollution also affects here (Yıldız et al., 2002).

Two common sole individuals, one with lateral line and other one caudal fin anomalies, were captured in June 2014 and December 2015, respectively. Both individuals were obtained by 80 mm full mesh size trammel net in Güllük Bay (Figure 1).

Figure 1. Sampling area (Güllük Bay, southern Aegean Sea of Turkey)

After capture, individuals were stored in ice and were brought to the laboratory for morphological examinations. Lateral line and caudal fin anomalies subsequently photographed with a DSLR camera. Due to having no x-ray machine, it was not possible to obtain the radiographic photograph of the caudal fin anomaly.

RESULTS

Morphometric measures and sexes of two sole specimens are presented in Table 1. According to otolith readings, age of both sole specimens were determined as 3 years. A total of 225 individuals (527.4 kg) were caught in the thin 210d/2 gillnets, and 165 individuals (415.1 kg) were caught from the thicker 210d/3 gillnets. Mean lengths and weights for 140, 150, 160, 180 and 200 mm mesh size in the 210d/2 and 210d/3 are presented in Table 1. According to increasing mesh size; mean lengths and weights of the carp increased linearly for both twine thicknesses of gillnets, expect for the 140 and 150 mm mesh sizes of the 210d/3.

Lateral line anomaly in common sole

Normally, the main lateral line starts from caudal fin and can be seen clearly on the eyed side of the fish. However, one of the common sole specimens, female, had meandering and ramous lateral line shape (Figure 2). These abnormal meanders and disfigurations of lateral line were observed to appear mostly on the dorsal portion of the eyed side.

Caudal fin anomaly in common sole

During examinations it was seen that the caudal fin of the specimen was absent and obviously had not developed (Figure 3). Although the general morphology of specimen was normal, a ventral oriented curve on the distal part of vertebra was evident

Table 1. Morphometric values of specimens

	TL (cm)	SL (cm)	W (g)	Sex
Common sole with lateral line anomaly	20.6	17.5	174.37	Female
Common sole with caudal fin anomaly	19.3	-	158.69	Male

(TL: total length, SL: standard length, W: weight)

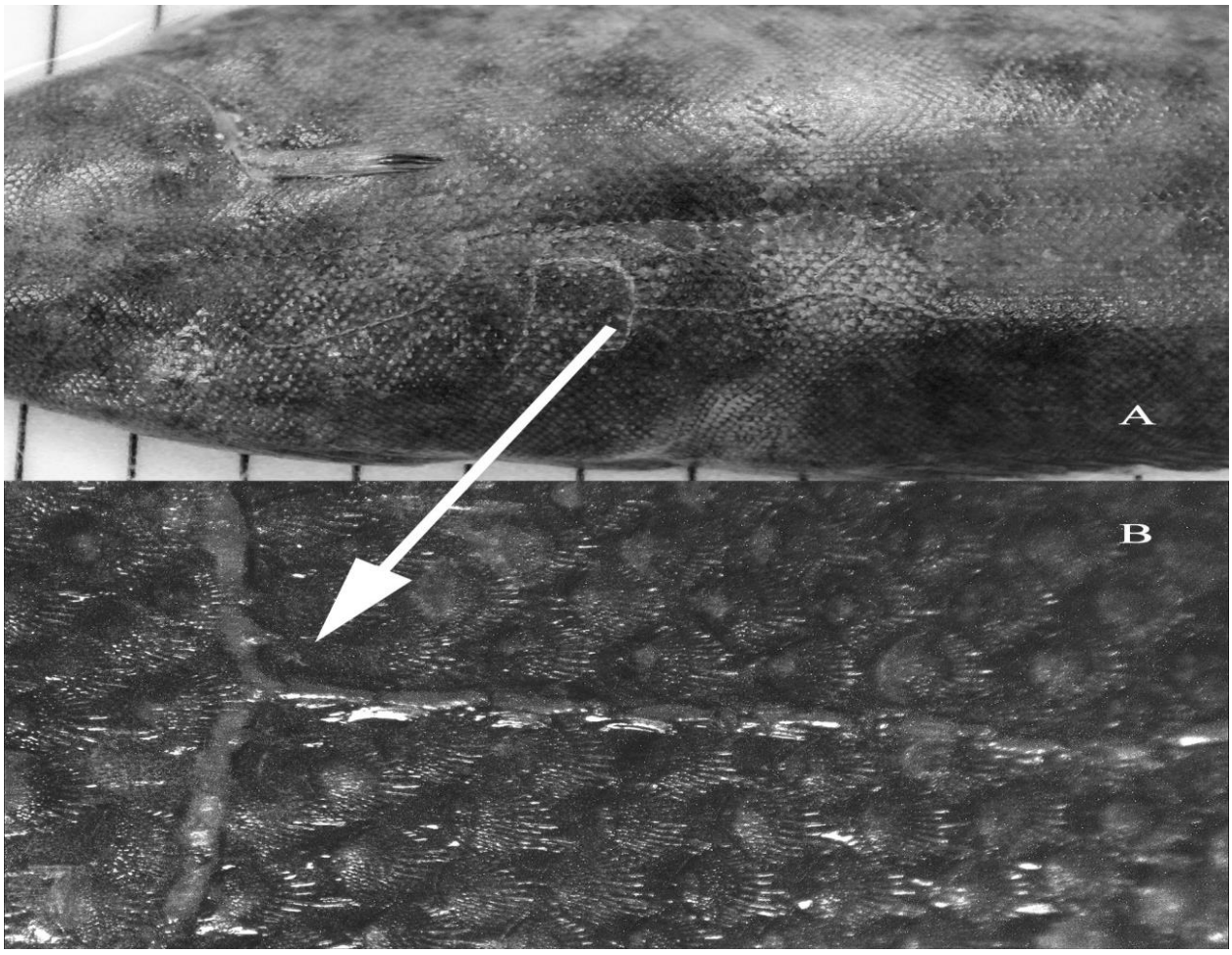

Figure 2. General view of lateral line anomaly for female specimen (A); close-up image of a section of lateral line anomaly (B)

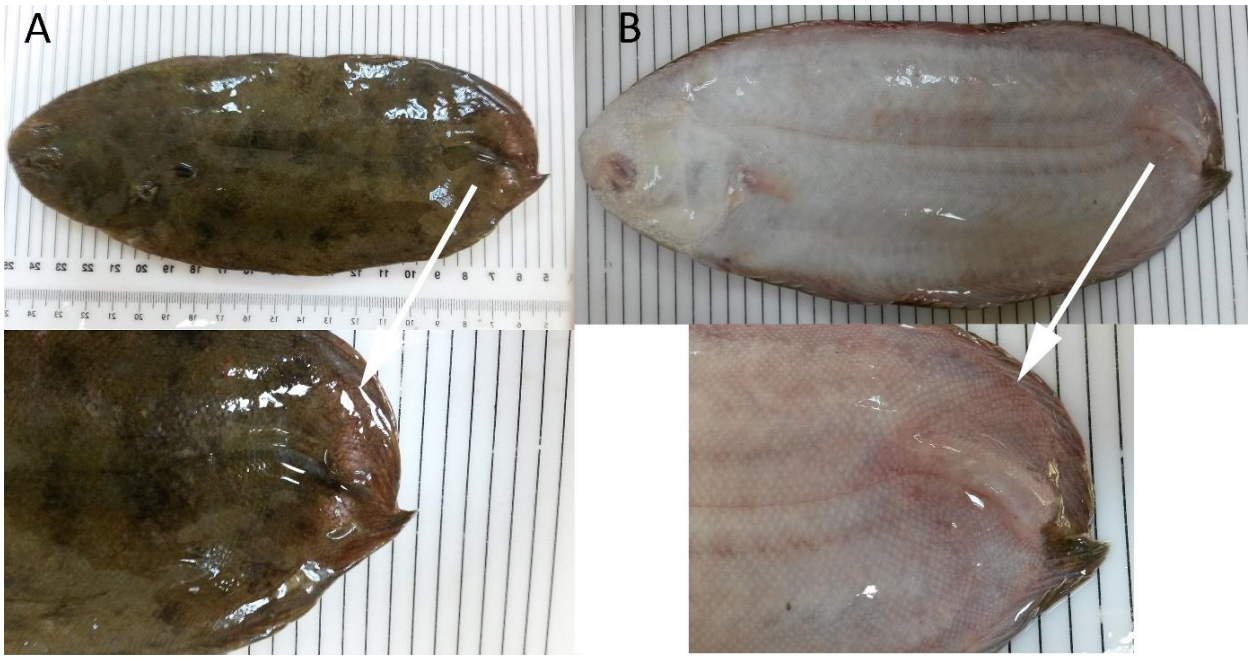

Figure 3. Caudal fin anomaly in the male specimen of common sole; Eyed side (A); Blind side (B)

DISCUSSION

Current study presents two common sole specimens with external morphological anomalies. It is stated that lateral line malformations can be linked to several factors: irregular scalation, mechanical dysfunction in ontogeny, nervous system dysfunction, and environmental factors, genetic mutations (Popovici, 1930; Popov, 1931; Kozikowska, 1960; Whitfield et al., 1996 in Jawad et al., 2006). It is also suggested that the shape of the lateral line could be a useful tool to detect the spine anomalies (especially lordosis) (Andrades et al., 1996).

When literature on the caudal fin anomalies and/or absence were taken into consideration, it was seen that this anomaly can be related to hereditary factors, damage during embryonic phases, injuries caused by predators, diseases, and damage due to environmental factors. Because the caudal fin plays a major role in the life of fish, revealing the underlying factors and causes of these anomalies earns the utmost attention (Tutman et al., 2000). Moreover, it has even been suggested that skeletal deformities in fish could serve as useful bioindicators of pollution (Gavaia et al., 2009).

Skeletal anomalies have a negative impact on animal well-being, biological performance, product quality and product cost. Also, morphological abnormalities in species sold as a whole reduce the consumer's expectation from aquaculture products. Moreover, skeletal abnormalities reduce the biological performance of reared species. The reflection of this situation is mainly seen in the growth rate (Koumoundouros et al. 1997). Skeletal abnormalities such as lordosis also reduce endurance and swimming speed in fish (Başaran et al., 2007).

Considering todays' disasters occurring in aquatic environments, documentation of life traits of aquatic organisms is becoming critical more than ever. Because, malnutrition linked to environmental problems can also be another reason for skeletal development defects (Fjelldal et al., 2021).

Güllük Bay is an overused area for several sectors like tourism, aquaculture systems, fisheries and marine transportation. Besides these exposures, domestic pollution also affects here (Yıldız et al., 2002).

CONCLUSION

Anomalies that were found in this study, may have been affected by aforementioned environmental and human originated pressures. Jawad et al. (2018) mentioned that scale abnormalities can be indicators of polluted environments. In connection with this perspective, minimizing of pollution may prevent or reduce the anomalies in wild populations. However, impacts of genetic factors were also considered as other effects on anomalies besides environmental influences.

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AUTHORSHIP CONTRIBUTIONS

Hasan Cerim: Sampling, visualisation, writing – original draft preparation. Sercan Yapıcı: Conceptualisation, writing, reviewing, editing. Özgen Yılmaz: Conceptualisation, writing – original draft preparation.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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DERLEME REVIEW

Türk mutfak kültüründe midye dolma: Osmanlı İmparatorluğu dönemi

Stuffed mussels in Turkish culinary culture: Ottoman Empire period

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Öz: Toplumların yemek ve beslenme alışkanlıkları, çoğunlukla yaşadıkları coğrafyaya bağlı olarak şekillenmekte ve zaman içinde kültürlerine yerleşmektedir. Türklerin yemek kültürünün ise değişen coğrafya, iklim, yerel ürünler ve yaşanan çeşitli kültür etkileşimleriyle şekillendiği görülmektedir. Midye dolma, Türk mutfak kültürüne Osmanlı İmparatorluğu döneminde giren bir sokak yemeği türüdür. Aslında "dolma", pirinç, bulgur, et, fındık, fıstık ve baharatla doldurulmuş sebze ve meyveye verilen bir isimdir, ancak Türk mutfağında bu yemek hemen her çeşit ana malzemeyle; et tavuk, deniz ürünleri, sebze vb., ile yapılabilmektedir. Midye dolma da pirinç ve baharatlardan oluşan karışımla doldurularak elde edilen, eşsiz bir lezzet, pratik bir üründür. Üretim şekli ve içeriği üreticisine göre az da olsa değişebilen bu ürün, doğduğu yer olan İstanbul ile özdeşleşmiş, Osmanlıdan günümüze hız kesmeden gelmiştir.

Anahtar kelimeler: Midye dolma, kültürlerin etkileşimi, sokak yemeği

Abstract: Food and nutrition habits of societies are mostly shaped depending on the geography they live in and settle in their cultures over time. It is seen that the food culture of the Turks is shaped by the changing geography, climate, local products and various cultural interactions. Stuffed mussels is a type of street food that entered Turkish culinary culture during the Ottoman Empire. In fact, "dolma" is a name given to vegetables and fruits stuffed with rice, bulgur, meat, nuts, peanuts and spices, but in Turkish cuisine, this dish is served with almost all kinds of main ingredients; meat, chicken, seafood, vegetables, etc., can be made. Stuffed mussels is a unique flavor and practical product obtained by stuffing with a mixture of rice and spices. This product, the style of manufacture and content of which may vary slightly depending on the manufacturer, has become identical to Istanbul, its birthplace, and has come from the Ottomans to the present without slowing down.

Keywords: Stuffed mussels, cultural interactions, street food

GİRİŞ

Türk Mutfağı, dünyanın en zengin mutfaklarından biridir. Bunun sebeplerinden biri Türklerin göçebe yaşamla yerleşik yaşamı bir arada sürdürebilmiş olmasıdır. Göçebeliğin getirdiği pratiklik ve yerleşik yaşamın etkisiyle elde edilen farklı uygulamalar, geleneksel ve klasik dönemden çıkarak "sokak kültürü" ve "Osmanlı Saray Mutfağı" oluşumuna katkı vermiştir (Ertaş ve Gezmen, 2013; Güldemir, 2014). Temelde yer alan geleneksel Türk mutfak kültürü İslamiyet'in kabulü ile beraber Arap ve İran mutfaklarıyla etkileşim geçirmiş, imparatorluk bakiyesinde yaşayan Rum, Gürcü, Ermeni, Musevi vb. azınlıkların etkisiyle birlikte Anadolu'da yetişen zengin ürün yelpazesi de Türk mutfağını, çeşit ve lezzet açısından en üst noktaya taşımıştır (Şevket, 1921; Samancı, 2007; Faroghi, 2019).

Bu kültür yolculuğu sırasında, su ürünlerine ait yemekler de Osmanlı döneminde mutfak kültürümüze girmiş ve yerleşmiştir. Balık her durumda kendini kanıtlamış, tebdil-i kıyafetle çıktıkları sokak teftişlerinde kabuklu deniz mahsulleri ile tanışan padişahlar, bu lezzetleri de beğenerek mutfak menülerine dâhil ettirmişlerdir. Bunlardan biri olan Midye dolma, Anadolu insanının damak zevkine uygun tadı ile kolayca kabul görmüş ve özel bir lezzet olarak günümüze kadar gelmiştir.

Midye dolmanın saray mutfağına girişi

Midye dolmanın İstanbul'un fethinden sonra başlayan hikâyesi, aslında İstanbul'un tarihi ile iç içe geçmiş bir süreçtir. Anadolu'daki Ermenilerin İstanbul'a yerleştirildiği bu dönemde, etle beslenmeye alışan Anadolu halkı et daha pahalı olduğundan deniz ürünleri ile beslenmek durumunda kalmışlardır. Özellikle deniz kıyısına yerleştirilen Ermeniler, denizden kolayca topladıkları midyeleri baharatlar eşliğinde pirinçle doldurarak keyifli bir yemek haline getirmişlerdir. Dolayısıyla Türklerin midye dolma ile ilk tanışıklığı, İstanbul'un fethinden sonra saray mutfağı üzerinden gerçekleşmiştir (Bey, 1977; White, 1845). Fatih Sultan Mehmet Han döneminde (1461) saray mutfağında büyük değişiklikler olmuş, deniz ürünlerine ait yemekler de bu dönemde Osmanlı Saray mutfağında yerini almıştır. Ortodoks Ermenileri tarafından

Galata Rıhtımı'nda bolca üretilen ve sokakta satılan midye dolmayı tadan ("haşerat-ı bahriye", "midyayi dolması") Fatih Sultan Mehmet Han, çok beğendiği bu yemeği sarayda özel davetlilere hazırlatmıştır (Eren, 1999; Faroghi, 2019). Midye dolmanın ve diğer deniz ürünlerinin fazla rağbet gördüğü diğer bir dönem ise, II. Bayezid dönemidir. Deniz mahsullerinden istiridye, karides, ıstakoz gibi ürünlerin II. Bayezid döneminde sıkça tüketildiği bildirilmektedir (Yerasimos, 2002). Bununla beraber II. Bayezid Han zamanında midye dolmanın, saray mutfağında dönemsel olarak yasaklandığı da olmuştur. Padişaha karşı mutfakta dönen entrikalar ve suikast girişimleri, 1491-1492 Şahkulu isyanı ve benzeri olaylar, midye dolmanın sarayda yasaklanmasına neden olmuştur (Yerasimos, 2002; Yerasimos, 2010).

Midye dolmanın tekrar sarayda menülere dahil edilmesi ise Yavuz Sultan Selim Han'ın Rodos'u fethi (1520-1521) sırasında gerçekleşmiştir. Yavuz Sultan Selim çok beğendiği midye dolma ile çeşitli su ürünleri yemeklerinin sarayda yapılabilmesi için Rodos'tan özel aşçılar getirtilmesini emretmiştir (Anonim, 1985; Toygar, 2002).

Midye dolmanın Osmanlı Devleti'nde ilk olarak kayıtlara geçmesi ise Kanuni Sultan Süleyman dönemine rast gelmektedir. Bu dönemde Kanuni Sultan Süleyman Han (1539-1540) deniz ürünleri ile çeşitli tariflerin düzenlenerek her dönemde mutfakta menülerde bulunmasını, ileri kuşaklara da aktarılabilsin diye kayıtlara geçirilmesini emretmiştir (Anonim, 1539). Diğer taraftan Edirne ve Topkapı Sarayında düzenlenen şenlikleri konu alan, Sürnamelerde de "Midyayi dolmayı" (midye dolma) dan bahsedilmiştir (Pürad, 2010).

Midye dolmanın halkla buluşması

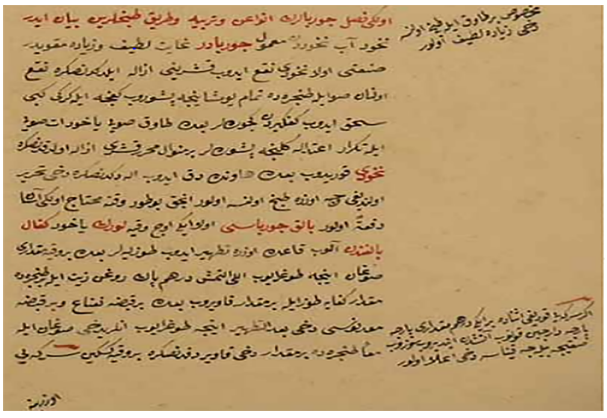
Sultan II. Selim zamanında (1572) ise, saray ve konaklar için aşçılar yetiştirilmesi amacıyla bir heyet kurulmuş bu heyet akademik çalışmalar yaparak, başka birçok tarif gibi midye dolma tarifinin de saray ve konak aşçıları arasında aktarılmasını sağlamıştır. Bu zamana kadar daha çok saray ve konak ahalisi tarafından tanınan ve yenilen bu yemeğin, saray ve konaklar dışında halka tanıtılması ise, ilk olarak III. Murat döneminde gerçekleşmiştir. 1585 yılında III. Murat, Rodos ve Girit'ten getirttiği ekiplere ("gemismena mydia" adıyla) midye dolmayı yaptırıp halka dağıtılması talimatını vermiş, halkın da bu lezzeti öğrenmesini sağlamıştır (Anonim, 1585). Kültür etkileşimlerinin yaşandığı bu durumda, sahip olunan inanç, yaşam tarzı, aynı ürünlerin bölgede yetişmesi vb. sebepler benzer yeme-içme kültürünü oluşmasına imkân sağlamıştır. Osmanlı döneminde pilav, halk tarafından çok beğenilen rağbet edilen bir yemek olduğundan ve ziyafetlerde çoğunlukla pilav ikram edildiğinden, iç pilavla yapılan midye dolma da halk genelinde kolayca kabul görerek zamanla geleneksel bir hal almıştır (Anonim, 1572; Fahriye, 1883; Reyhanlı, 1983).

Midye dolma ile ilgili diğer bir bildirim, XVII. yy. ortalarında IV. Mehmet Han dönemindedir. IV Mehmet İstanbul'da değişik tatların tespiti üzerine, sosyopolitik işlerin düzenlenmesinden sorumlu olan Zuhuri danışmanını görevlendirmiş, Zuhuri danışmanı İstanbul'da yaptığı çalışma ile deniz ürünleri yemeklerini hazırlatıp padişaha sunulmasını sağlamıştır. IV. Mehmet bu lezzetleri tattıktan sonra konu ile ilgili Evliya Çelebi'yi görevlendirilmiş ve bu tatların nasıl oluştuğunun tespiti için talimat vermiştir. Evliya Çelebi Seyahatnamesinde; "Haliç'in kuzeyinden Galata'ya kadar balık ve balık ürünleri stantları görülürdü ve yeni lezzetler ve tatlar için oraya giderdik, midyayi dolmanın tadı buradan çıktı ve saray mutfağına bildirildi." diyerek kitabında bahsetmiştir (Çelebi ve İbn Derviş, 1895).

On sekizinci yüzyıl başlarından itibaren ise İstanbul'un, Avrupa ile artan ticari ve ekonomik bağları nedeniyle, mutfakta kullanılan gıda malzemelerinin niteliği ve mutfak düzeni Avrupa'dan etkilenmiştir. Bu dönemde, diğer padişahlar gibi İstanbul sokaklarında sık sık dolaşan Sultan I. Abdülhamid, tattığı sokak tatlarının bu değişim yüzünden yok olmasını istememiştir (Efendi, 2005). Bu nedenle çok iyi bildiği ve hiçbir zaman vazgeçemediği deniz ürünlerinden 'midyayi dolmayı' saray mutfağında sık sık hazırlatmıştır (Anonim, 1820; Yerasimos, 2002; Yerasimos, 2010).

Sultan II. Mahmut ve sonrasında Sultan Abdülmecid döneminde de Saray mutfağında midye dolmanın yapılmaya devam edildiği, Mehmet Kâmil tarafından kaleme alınan bir eserde ifade edilmektedir. Bu eserde 'dokuzuncu fasıl' bölümünde midye dolmasının tarifi verilmiş ve şöyle anlatılmıştır (Kâmil, 2015):

"San'at-i tabhı: Matlûbü'l-'adet midyeyi su içinde iyice her tarafını kazıyıp birkaç kere elden geçirip tathîrden sonra keskin bıçak ile enli tarafından açıp ancak sivri tarafının bağını koparmayarak hazır edeler. Badehu ince soğan çentip rûgan-ı zeyt te kızarttıkta mikdâr-ı vâfî yıkanmış pirinci fıstık, üzüm ile dahi beraber kavurup birkaç fincan su ile yalancı dolmanın içi gibi bir miktar pişirdikte indirip yenibahar, tarçın ve karanfil ve biber ile iyice karıştırıp işbu hazırlanan midyelerin içine mutedilce koyup ağızlarını kapayalar. Badehu tencere ye dizip üstüne çıkar çıkmaz su koyup üzerine sahan veya küçük tencere kapağıyla bastırdıkta tencerenin kapağını kapayıp suyunu çekince alevli ateşte ba'de't-tabh soğudukta tabaklara dizip tenâvül buyrula (Şekil 1)".



Şekil 1. Melceü't-Tabbâhîn'i el yazması kitabında midye dolma tarif notu

Figure 2. Stuffed mussels recipe note in "Melceü't-Tabbâhîn'i" book

Osmanlı Saray Mutfağının gelişmesi için Avrupa yemek kültürü ile etkileşime tam desteğini veren II. Abdülhamid ise, bu dönemde üstat aşçıları olan Mehmet Kâmil Usta, Abdullah Usta ve Fethi Usta'dan oluşan ekibe çeşitli el yazmaları geliştirmelerini emretmiştir. Mehmet Kâmil 1844 yılında İstanbul'daki aşçıların hep aynı eski yemekleri pişirmeleri ve yeniliklere önem vermemeleri nedeniyle, Ağdiye Risalesi ve Yemek Risalesi adı verilen iki yazma eserden de yararlanarak ve yanı sıra tecrübeli kişileri de dinleyerek Melceü't-Tabbâhîn'i hazırlamıştır. Abdullah Usta 1874 yılından sonra aşçılara yol göstermek amacıyla, dönemin mutfak sanatlarını anlatan bir kitap yazmış, Fethi Usta ise 1889 yılında saray mutfak ziyafetlerini anlatan kitap notları hazırlamıştır. Osmanlı saray arşivlerinde yer alan bu eserlerde deniz ürünlerine ağırlıklı olarak yer verilmiş ve midye dolma tarifleri değişik şekilde atfedilmiştir (Yar, 2008).

Midye dolma ile ilgili bildirimlerin 1888 yılı Bizans Salnamesi'nde (yıllık) de geçtiği, Ermenilerin yemeğe düşkünlükleri ve özellikle midye dolmaları nasıl sanatkarane bir şekilde yaptıkları ve yediklerinden bahsedilmektedir (Anonim, 1892). XIX. Yüzyılda İstanbul'da Türk kadınlarının da mutfaklarında midye dolma yaptıkları anlatılmaktadır (Anonim, 1854; Yerasimos, 2002; Yerasimos, 2007). Midye dolma ile yapılan en çarpıcı bildirim, Reşat Ekrem Koçu (2005) tarafından yazılan 'Eski İstanbul'da Meyhaneler ve Meyhane Köçekleri' adlı kitapta yer almıştır. Koçu (2005) kitabında, bir Ermeni olan Boghos Yeghiazar'ın ağzından zamanın kültür yoğunluğu içinde midye dolma tarifini ve yeme kültürünü okuyucuya detaylarıyla sunmuştur. Kitapta, ayrıca Ermenilerin Erivan'da İstanbul'da pişen Ermeni yemeklerini bilmediklerini, midye dolmasının sadece İstanbul'da yaşayan Ermeniler tarafından büyük yortularda hazırlanıp yenildiği ifade edilmektedir (Koçu, 2005).

Midye dolma İstanbul'dan sonra Anadolu'daki öncelikle tüm kıyı şehirlerinde tanınmış ve yaygın olarak tüketilmeye başlanmıştır (Eren, 1999). Midye Dolma'nın tarihi hakkında bilinmesi gereken en önemli özelliği, o zamanlardan beri genel olarak tablalara dizilerek satılmasıdır. 1960'lı yıllarda Midye dolma hazırlığı ve işletmeciliği, o zamanlar Galata semtinde, İstanbul'a yeni göç etmiş olan Mardinli (Suryanilerin) göçmenlerin hakimiyetine geçmiştir. Günümüzde halen Mardinlilerin midye dolma pazarını büyük ölçüde ellerinde tuttukları bilinmektedir (Dağdeviren, 2012).

Midye dolmanın, farklı toplumlarda farklı şekillerde yapılışı da söz konusudur. Pirinç ve baharatlarla hazırlanan ama bileşimi farklı midye dolmalar olduğu gibi midyenin içinin çeşitli sebze, peynir veya pirinç topları ile doldurulduğu çeşitlemeler de (Hindistan) bulunmaktadır. Bununla birlikte, Türkiye'de dolma yapımında kullanılan pirincin baharatlarla ahengi ve üzerine sıkılan limonla bütünleşmesi, tüketen kişiyi müptelası yapmaktadır (Tavernier, 1985; Samancı, 2007).

SONUÇ

Asıl itibariyle Akdeniz ülkelerinde popüler olan midye, binlerce yıldır yiyecek olarak kullanılan bir deniz ürünüdür. Farklı toplumlarda çok çeşitli şekillerde hazırlanarak tüketilebilen midyeler; tütsülenebilir, kaynatılabilir, buharda pişirilebilir, kavrulabilir, mangalda pişirilebilir, tereyağı veya bitkisel yağda kızartılabilir (White, 1845; Yerasimos, 2002). Ancak yukarıda bahsedilen bu tüketim şekilleri, Türkiye'de midye dolma kadar benimsenmemiş ve yaygın talep görmemiştir. Üretim şekli ve içeriği üreticisine göre az da olsa değişebilen ve bir sokak lezzeti olarak ifade edilen bu ürün, artık sokak satıcılarının ötesinde market ve şarküteri raflarında da görülmeye başlamış, yerini almıştır. Hatta midye dolma yapan restoranlar markalaşıp bayilik vererek ülke geneli ve yurt dışında yaygınlaşmaya başlamıştır.

YAZARLIK KATKISI

Tüm yazarlar çalışma fikrine ve tasarımına katkıda bulunmuştur. Materyal hazırlama ve araştırma İbrahim Ulaş Yüzgeç ve Fatma Çolakoğlu tarafından gerçekleştirilmiştir. Makalenin yazılması ve düzenlenmesi Fatma Çolakoğlu ve Serhat Çolakoğlu tarafından yapılmıştır ve tüm yazarlar makaleyi okumuş ve onaylamıştır.

ÇIKAR/REKABET ÇATIŞMASI BEYANI

Makalede herhangi bir çıkar çatışması bulunmamakta olup, "Yazarlar, makaleyi etkileyebilecek bilinen herhangi bir mali veya kişisel çatışma olmadığını beyan eder."

ETİK ONAY

Bu çalışmada, konu tarihsel bir bakış açısı ile ele alınarak değerlendirildiği, insan ve hayvan ile ilgili bir çalışma içermediği için etik onaya gerektirmemektedir.

VERİ KULLANILABİLİRLİĞİ

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

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