Ege Journal of
Fisheries and Aquatic Sciences

www.egejfas.org

E-ISSN 2418-3140

EgeJFAS

Su Ürünleri Dergisi

Volume 40 Number 4 2023

Ege University Faculty of Fisheries

Authors Guidelines

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

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

GENERAL INFORMATION

Aim & Scope

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

The journal does not charge any submission and publication fees.

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

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

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

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

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

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

Language

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

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

Editorial Policy and Referee Process

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

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

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

Article Types

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

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

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

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

Reports

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

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

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

MANUSCRIPT SUBMISSION

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

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

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

While starting

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

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

To follow the status of the article;

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.

After you submit your article via the online system, you will be able to follow the status of your article and you will be automatically notified by e-mail when there is any action.

Similarity Report

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

Although a similarity report is requested for all submitted articles, a second check will be made with the plagiarism detection software.

Cover Letter

When submitting a manuscript, Cover Letter should be uploaded under the subheading "Cover Letter". Cover letter should be prepared separately from the manuscript file.

Ethics in Publishing

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

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

PREPARATION OF MANUSCRIPTS

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

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

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

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

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

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

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

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

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

First Page

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

Author Names and Affiliation

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

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

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

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

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

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.

Conclusions

This should briefly state the major findings of the study.

Acknowledgements and Funding

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

Examples:

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

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

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

Financial support acknowledgwment should be written like the example given:

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

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

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

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

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

Authorship Contributions

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

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

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

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

Full name/s: Conceptualization, Methodology, Software

Full name: Data curation, Writing- Original draft preparation

Full name/s: Visualization, Investigation

Full name/s: Supervision

Full name/s: Software, Validation

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

Full name/s: Writing- Reviewing and Editing

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

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

Changes to Authorship

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

Conflict of Interest Statement

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

If the authors have financial or personal relationships with any institution/organization or person that may adversely affect their work, they should declare within a separate file by selecting the 'conflict of interest' subheading as the file type when submitting the manuscript. Conflict of interest statement should also be attached to the article after the Acknowledgements section of the article.

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

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

If there is no conflict of interest; "The authors declare that there is no known financial or personal conflict that may affect the research (article)" or "The authors declare that there are no conflicts of interest or competing interests".

Ethics Approval

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

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

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

Examples:

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

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

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

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

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

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

Retrospective Ethics Approval

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

Data Availability

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

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

Examples:

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

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

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

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

For example:

Kocataş, A. (1978)

Kocataş, A., & Ergen, Z. (1972).

Kocataş, A., & Geldiay, R. (1972)

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

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

Journal Articles

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

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

Books

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

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

Chapter in books

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

E-books and chapter in e-books

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

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

Proceedings

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

Websites

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

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

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

Thesis

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

Tables and Figures

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.

Tables and figures should be included in the article after they are cited in the relevant text.

Tables

Tables should be prepared by table tool in Word, tables in image form will not be accepted.

- Table title should be placed above the table.
- If there are table notes and abbreviations, please place them below the table body.
- Prepare the tables vertically. Avoid large tables.
- Give a short heading for each column.
- Make sure that the data presented in the table does not repeat the results described elsewhere in paper.
- Please avoid using vertical lines, colour or shading in table cells.
- Tables need not to exceed 175 x 227 mm.
- The dimensions of the tables do not exceed 175 x 227 mm.

Figures

Figures, which are recommended for electronic formats such as JPEG, TIFF and minimum 300 dpi should be also arranged in available dimensions. Do not submit files that are too low in resolution and disproportionately large for the content.

Photos can be in color or grayscale. Photographs and all illustrations(figures,maps, etc.) should be in clear format. Graphics should be pasted in editable format and not converted to image format. When it is necessary, the original copies of the figures will be asked from author(s) as separate files, after the reviewing process being concluded. Arial Narrow font should be used in the figüre (graphics, etc). A brief title containing the description of the figure should be given under the figure but explain all symbols and abbreviations used.

Permissions

If authors include figures, tables or text passages published elsewhere, or use of software, questionnaires and scales that require permission in the study, they should obtain permission from the copyright holders. Please note that some publishers do not provide electronic rights for free and Ege University/EgeJFAS (Su Ürünleri Dergisi) will not be able to reimburse or be responsible for any costs incurred in obtaining these permissions. In such cases, materials from other sources should not be used.

Page Charges

No page charges are collected. All authors/readers have free access to all papers.

Plagiarism Detection

In accordance with its publishing policies EgeJFAS requires plagiarism check for each study that has undergone the "Review Process". The iThenticate plagiarism checker software is used for plagiarism detection.

Indexes

EgeJFAS is indexed in TUBITAK ULAKBIM TR Dizin, ESCI (Clarivate Analytics), Zoological Record (Clarivate Analytics), EBSCO, CABI, ProQuest, ASFA

COPYRIGHT NOTICE

In order to publish and disseminate articles in Ege Journal of Fisheries and Aquatic Sciences (Su Ürünleri Dergisi), we need certain publishing rights from authors, which are determined by a publishing agreement between the author and the journal. It is a condition of publication in the journal that the authors grant an exclusive license to the journal.

As law regulations, the corresponding author should complete and sign a copyright release form on behalf of all co-authors during the submission of the article for publication in this journal.

The manuscript will not go through review without submission of this form. Authors can download the form beforehand and submit it at the same time as the manuscript.

Authors publishing in this journal accept the conditions described below.

Authors retain copyright and grant the journal right of first publication with the work simultaneously licensed under a Creative Commons Attribution Non-Commercial License that allows others to share the work with an acknowledgement of the work's authorship and initial publication in this journal.

Authors are able to enter into separate, additional contractual arrangements for the non-exclusive distribution of the journal's published version of the work (e.g., post it to an institutional repository or publish it in a book), with an acknowledgement of its initial publication in this journal.

Authors are permitted and encouraged to post their work online (e.g., in institutional repositories or on their website) prior to and during the submission process, as it can lead to productive exchanges, as well as earlier and greater citation of published work (See The Effect of Open Access).

In the meanwhile, authors should be aware that they are responsible for the plagiarism check of their manuscript (Similarity Report) (using iThenticate or similar programmes) and including the plagiarism report during the manuscript uploading (as "Similarity Report") is strongly advised.

Ege Journal of Fisheries and Aquatic Sciences are in no way liable for any lawsuit derived from plagiarism, as it is author´s responsibility to submit original work. However, the journal is in full right of rejecting manuscripts if detecting plagiarism of any sort.

If the study requires an ethics committee document, it should not be forgotten that the "Ethics Committee Approval" must be uploaded.

Submission checklist

Please read carefuly the Submission Checklist before submitting your article to the journal.

Important Note

Authors are highly recommended to read carefully EgeJFAS Ethical Principles and Publication Policy and Author Guidlines before submitting their manuscripts.

Editor-in-Chief/Editors reserve the right to reject manuscripts that do not comply with the explanations detailed above. The author/s will be responsible for incorrect/incomplete statements or failure to comply with the above-mentioned explanations.

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

E- ISSN 2148-3140

Su Ürünleri Dergisi
Ege Journal of Fisheries and Aquatic Sciences

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

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

Yayımlanma Tarihi **Publishing Date**
15 Aralık December 15th, 2023

İletişim **Contact**
Ege Üniversitesi Su Ürünleri Fakültesi, 35100, Bornova, İzmir Ege University Faculty of Fisheries, 35100, Bornova, İzmir, Türkiye
Tel: +90 232 311 3096 Fax: +90 232 388 3685 http://www.egejfas.org info@egejfas.org

Su Ürünleri Dergisi

Ege Journal of Fisheries and Aquatic Sciences

Volume 40 Number 4 **ISSN 1300-1590 / E-ISSN 2418-3140**

İÇİNDEKİLER CONTENTS

ARAŞTIRMA MAKALELERİ RESEARCH ARTICLES

KISA MAKALE SHORT COMMUNICATION

Published by

Ege University Faculty of Fisheries, İzmir, Türkiye

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 235-243 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.01

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Fish diversity and community structure of a wetland system of the western Mediterranean Basin of Türkiye: Lake Koca (Dalaman)

Türkiye'nin Batı Akdeniz Havzası'ndaki bir sulak alan sisteminin balık çeşitliliği ve topluluk yapısı: Koca Göl (Dalaman)

Nehir Kaymak[1*] ● Yılmaz Emre[2] ● Nesrin Emre[3] ● Şenol Akın[4]

[1]Department of Biology, Faculty of Science, Akdeniz University, 07058, Antalya, Türkiye https://orcid.org/0000-0002-9970-4467
[2]Department of Biology, Faculty of Science, Akdeniz University, 07058, Antalya, Türkiye https://orcid.org/0000-0003-4793-2262
[3]Department of Mathematics and Science Education, Faculty of Education, Akdeniz University, 07058, Antalya, Türkiye https://orcid.org/0000-0001-9047-1823
[4]Faculty of Science and Letters, Department of Biology, Yozgat Bozok University, 66900, Yozgat, Türkiye https://orcid.org/0000-0002-9047-0430

*Corresponding author: nehirbozkurt@hotmail.com

Received date: 04.07.2023 Accepted date: 23.09.2023

How to cite this paper:
Kaymak, N., Emre, Y., Emre, N., & Akın, Ş. (2023). Fish diversity and community structure of a wetland system of the western Mediterranean Basin of Türkiye: Lake Koca (Dalaman). *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 235-243. https://doi.org/10.12714/egejfas.40.4.01

Abstract: Lake Koca (Dalaman), a wetland in the western Mediterranean basin of Türkiye, is an extraordinary lake with salty, fresh, and sulfurous waters and is home to high biodiversity. The present study examines the spatial and seasonal variation in fish communities and assesses the influence of environmental parameters on the community structure of Lake Koca. A total of 1.530 specimens were captured, representing seven families and 11 species (2 non-native, 1 introduced, and 8 native species). Total fish abundance and richness were higher at the littoral than at the limnetic zone, but no seasonal variation was observed. Non-native fish species, *Coptodon zillii*, was the most abundant in the littoral zone, followed by species of Mugilidae. The abundance of the two endemic fish species (*Capoeta aydinensis* and *Ladigesocypris irideus*) was relatively low in both habitats. Shannon-Wiener diversity index and evenness did not vary seasonally and spatially. Fish abundance and richness were significantly and positively correlated with chlorophyll-a and macrophyte coverage while negatively correlated with depth. Spearmen's Correlation analysis revealed that native fish species show a relationship with the chemical parameters of the water, while *C. zillii* showed a distribution related to depth and macrophyte density. Both anthropogenic activities and the presence of non-native fish may affect the distribution and abundance of endemic fishes.

Keywords: Fish biodiversity, seasonal variation, assemblage structure, environmental parameters, non-native fish, Lake Koca

Öz: Türkiye'nin Batı Akdeniz havzasında bir sulak alan olan Koca Göl, tuzlu, tatlı ve kükürtlü suları ile sıradışı bir sistem olup, yüksek biyolojik çeşitliliğe ev sahipliği yapmaktadır. Bu çalışma, balık topluluklarındaki mekansal ve mevsimsel değişimi incelemekte ve çevresel parametrelerin Koca Gölü'nün balık toplulukları üzerindeki etkisini değerlendirmektedir. Çalışmada yedi familya ve 11 türü (2 yabancı, 1 taşınmış ve 8 yerli tür) temsil eden toplam 1.530 örnek yakalanmıştır. Toplam balık bolluğu ve zenginliği littoral bölgede limnetik bölgeye göre daha yüksek bulunmuş, ancak mevsimsel bir değişiklik göstermemiştir. Yabancı balık türlerinden, *Coptodon zillii*, littoral bölgede en bol bulunan türdür ve bunu Mugilidae'ye ait türler izlemiştir. İki endemik balık türünün (*Capoeta aydinensis* ve *Ladigesocypris irideus*) bolluğu her iki habitatta da nispeten düşük bulunmuştur. Shannon-Wiener çeşitlilik indeksi ve düzgünlüğü mevsimsel ve mekansal farklılık göstermemiştir. Balık bolluğu ve zenginliği, klorofil-a ve makrofit kapatıcılığı ile anlamlı ve pozitif bir şekilde ilişkiliyken, derinlik ile negatif bir şekilde ilişkilidir. Spearmen Korelasyon analizi, yerli balık türlerinin suyun kimyasal parametreleri ile ilişki olduğunu, *C. zillii*'nin ise derinlik ve makrofit yoğunluğuna bağlı bir dağılım gösterdiğini ortaya koymuştur. Bu sulak alanda hem antropojenik faaliyetler hem de yabancı balıkların varlığı, endemik balıkların dağılımını ve bolluğunu etkilemiş olabilir.

Anahtar kelimeler: Balık biyoçeşitliliği, mevsimsel varyasyon, topluluk yapısı, çevresel parametreler, yabancı balıklar, Koca Göl

INTRODUCTION

Among ecosystems with high biodiversity, wetlands cover only about 1% of the Earth's surface but provide habitat for about 20% of the world's species (Cheng et al., 2012). Species community structures of highly biodiverse wetland lakes exhibit spatial and seasonal variations (Fitzgerald et al., 2017; Jin et al., 2019). Wetland hydrology affects organisms' population and community dynamics by affecting abiotic factors (Winemiller et al., 2000). In summer, when the depth of wetland is low, high temperature, hypoxia, and predation can cause a decrease in fish species diversity and abundance, while an increase in depth can increase fish diversity as it causes habitat stability (Cvetkovic et al., 2010; Grubh and Winemiller, 2018). In addition, most fish species in a wetland system use coastal areas as seasonal or ontogenetic stages of development (Westrelin et al., 2018) for feeding, spawning, nursery, or shelter habitat (Lewin et al., 2004; Matern et al., 2021). The aquatic vegetation in wetlands increases the spatial complexity and heterogeneity of these ecosystems (Marin Avendaño and Aguirre Ramírez, 2017), supporting production, high species diversity (Dustin and Vondracek, 2017), and consequently, food web complexity (Carey et al., 2010; Ziegler et al., 2015).

Lake Koca is a wetland under a special environmental protection zone with 395.03 ha. It is located in the western Mediterranean basin of Türkiye, within the borders of the Dalaman district of Muğla in Türkiye (Ayaz et al., 2013). This area is surrounded by mountains and seas and is connected to the Mediterranean Sea by a natural channel covered with reeds. Lake water is slightly sulfurous due to the hot springs

around the lake (Ayaz et al., 2013). In the past, the lake was fed by floodwaters coming from Tersakan Stream. However, when the direction of the stream was changed due to flood prevention in 1974, it caused the natural seasonal hydrological cycles to change. In addition, the high reinforced concrete bridge built on the canal connecting the Tersakan stream channel and Lake Koca caused the reinforcement to narrow and shallow with reeds, and as a result, migrating fish species such as Mugilidae species and *Anguilla anguilla*, etc. were prevented easy entry and exit between the sea and the lake (Şahin, 2019a). Some practices such as tourism, agriculture, and animal husbandry are significant threats to the area. In addition, it has been reported that some parts of the area are dried due to the existing airport and the agricultural fields (Şahin 2019b), and invasive fish species (*Carassius gibelio, Gambusia* sp.) were introduced into the lake several times (Şahin, 2019b).

Koca Lake wetland is one of Türkiye's richest and most sensitive in terms of ecosystem and species diversity due to its geographical location. Because it is an essential habitat for two endemic freshwater fish, *Capoeta aydinensis*, *Ladigesocypris irideus*, a butterfly species called Ottoman fire (*Lycaena ottomana*), Mediterranean monk seal (*Monachus monachus*), otter (*Lutra lutra*), the sea turtle (*Caretta caretta*), and many aquatic avian species, which are endangered on a world scale (Kıraç and Suseven, 2021), despite its national and global importance as a significant wetland supporting a wide diversity of animal taxa, but little information has been gathered about fish fauna. Yılmaz et al., (2006) conducted a taxonomic study on fish species in some inland waters of Muğla, including Lake Koca. There is no published article on the fish fauna of Lake Koca and its physicochemical properties. Research is urgently needed to understand sustaining biodiversity and ecosystem services and the value of floodplain lakes. The overall goal of this study is to describe spatial and seasonal variation in fish community structure (species richness, diversity, abundance, and evenness) in a wetland lake (Lake Koca) to investigate relationships between local environmental conditions and fish community structure. It is very important to reveal the current status of fish community in such a wetland system which is under anthropogenic pressure. This article presents the first detailed study of fish community structure in the Koca Lake.

MATERIALS AND METHODS

Study site

This study was carried out in the northwestern part of Lake Koca, a wetland lake in Türkiye. The lake is located within the borders of Kapukargın Village in southwest Türkiye and has hydrological connections to the Tersakan Stream and the Mediterranean Sea (Figure 1). While Tersakan Stream had a direct connection with the sea in the past, but today this connection is provided by narrow drainage channels. The lake's depth varies between 1 to 20m (Ayaz et al., 2013). The northwestern area of the lake is flat, and its depth is less than the southeastern part of the lake. There are Kargın and Sulfur lakes around Lake Koca. The characteristic Mediterranean climate prevails, summers are hot and dry, and winters are cool and rainy. The average annual temperature in the region is 18.3 °C. The hottest month is July with an average of 27.8°C and the coldest month is January with 10.1°C (Çınar and Ardahanlıoğlu, 2015). The annual average total precipitation is relatively high with 853 mm, of which 55% in winter, 19% in spring, 25% in autumn, and 1% in summer.

The marsh vegetation is also well represented in the wetlands and marshy areas around the littoral zone of Lake Koca, which has extensive stands of aquatic emergent (*Schoenetum nigricantis, Phragmites australis, Juncus maritimus, Typha domingensis),* floating *(Lemna* sp., *Azolla* sp., etc.) and submerged aquatic macrophytes (*Ceratophyllum* sp.*, Myriophyllum* sp., *Potamogeton spp.,* etc.). The coverage percentage of littoral aquatic vegetation is 100% (Çınar and Ardahanlıoğlu, 2015). We determined two different regions in the lake: the first region was located in the littoral zone of the lake which had extensive and dense patches of submerged, floating, and emergent macrophytes; and the second was located in the limnetic zone of the lake which had characterized by mostly a mud bottom with little submerged vegetation, but no floating and emergent macrophytes (Figure 1).

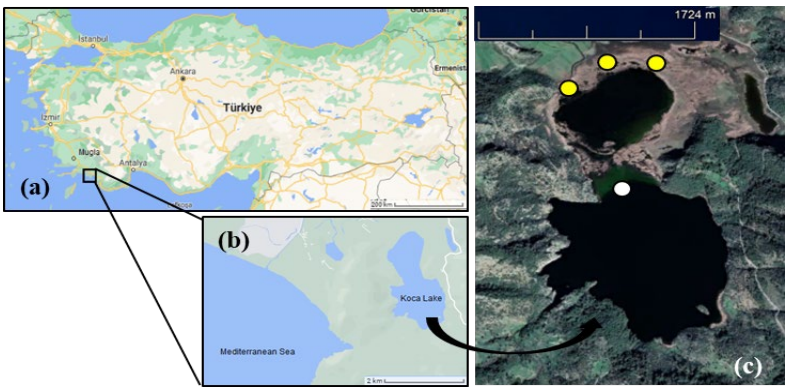

Figure 1. Location of Lake Koca in the western Mediterranean basin in Türkiye (a) and Lake Koca (b) and four study sites (c) within the lake. The yellow circles indicate the littoral sampling zone, and the white one indicates the limnetic zone

Sampling protocol

Two regions (littoral and limnetic sites) of Lake Koca were sampled for fish in winter, spring, summer, and autumn between February 2019 and December 2020. Three different locations were selected for fish sampling in the littoral site. The littoral site was sampled using overnight fyke-net sets with 17 mm mesh (mean soak time = 12 h/fyke net). Four traps for each littoral station were set as a pair with leads (15 m) facing each other and nets oriented parallel to the shoreline. Fish sampling was made from one station at the limnetic site (Figure 1). Fishes were caught using trammel nets with inner nets consisting of four 100 m long panels of 10, 25, 50, 80, and 100 -mm mesh. Trammel nets were set into the water at around 9:00 – 10:00 pm and retrieved at around 9:00 - 10:00 am the following day. After sampling, all fish individuals were treated in accordance with the guidelines of the local ethics committee (Reference number: 93773921). Captured fishes were anesthetized in MS-222 and then fixed in 4% formalin in the field. In the laboratory, samples were sorted, and identified into species (based on studies: Balık et al., 1992; Muus and Nielsen, 1999; Kottelat and Freyhof, 2007; Özuluğ and Freyhof, 2011; Güçlü et al., 2013; Turan et al., 2017; Güçlü et al., 2020), and counted.

Before trammel and fyke netting, temperature (T), dissolved oxygen concentration (DO), conductivity (Cond), and pH were measured at each survey site with a multiparameter probe (YSI Instruments). Depths (m) were measured with a meter stick at the mouth of each fyke and trammel net, and the percent macrophyte cover (MacCov) was assessed for the lead length between the wings for each net (Bhagat and Ruetz, 2011). Braun-Blanquet coverage categories were used to estimate densities from calculated percent macrophyte values (Barbour et al., 1987). For analysis of water quality parameters such as phosphate (PO_4^{3-}), nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+), a one liter sample of surface water was collected within each study station and season. Water samples were immediately placed on ice. In the laboratory, each water sample was filtered through precombusted Whatman GF/F filters (Merck, Darmstadt, Germany). The filtrate was then used to measure soluble PO_4^{3-}, NO_3^-, NO_2^- and NH_4^+ using calorimetric kits and the WTW model Photo Flex Turb (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany). To measure chlorophyll (Chl-a), 1 L of water was collected at each site and placed in polyethylene bottles. After the water samples had been filtered through a Whatman GF/F filter, the filters were placed in 90% acetone for 24 h and measured absorbance using a spectrophotometer (at 665, 664, and 750 nm) (Wetzel and Likens, 1991).

Data analysis

Due to the different fishnets used in the lake, fish densities could not be calculated and compared in terms of catches per unit effort (CPUE). Instead, they were presented as the total number of fish obtained to indicate fish abundances roughly. Spatial (site with or without aquatic vegetation) and seasonal changes in fish community metrics were analyzed. Different community metrics were determined for each site and season, including species richness (total number of species (S)), species diversity (Shannon diversity index (H')), numerical abundance (N), species evenness index (E), and species richness index (D). Higher values represent greater diversity (Cheng et al., 2012). These standard indices for each survey were calculated using the following formula:

Shannon diversity indices (H') (Krebs, 1998):

$$H^{\prime} = -\sum_{i=1}^{s} Pi \; log_2 \; Pi$$

where *Pi* = the proportion of individuals belonging to the *i*th fish species in the data set. Species richness (S) is the absolute number of species captured in a given area and time. Evenness was calculated to estimate the equitability of species abundances within each community. Species evenness index (J) (Pielou, 1966):

$$J^{\prime} = H^{\prime}/In\ (S)$$

Where *H'* = Shannon-Wiener diversity index and *S* = total number of species. Species richness index (D) (Menhinick, 1964):

$$D = S\ /\ \sqrt{N}$$

Where *S* = number of different species in the sample, *N* = total number of individuals.

A one-way analysis of variance (ANOVA), and a student's t-test were used to compare fish community metrics and environmental parameters among seasons, and sites (littoral vs. limnetic), respectively. Before the analysis of variance, all variables were tested for normality (Shapiro Wilk test), and the homogeneity of variances (Cochran tests). After macrophyte coverage was arcsine–square-root transformed and all environmental data were transformed by $\log_{10}$ (x+1), data showed normal distribution. Principal components analysis (PCA) was used to examine spatial and seasonal relationships among environmental parameters. The Pearson correlation coefficient was used to investigate relationships between environmental parameters and fish community metrics. In addition, the degree of association between fish species and environmental parameters was calculated by applying Spearmen's correlation since the abundance data of each fish species did not show normal distribution. All analyses were performed with SPSS version 23.

RESULTS

Spatial and temporal variation in environmental conditions

While seasonal variations in nitrite, nitrate, phosphate, temperature, and pH were statistically significant, depth, chlorophyll-*a,* and macrophyte cover were spatially different. Temperature was highest in summer ($F_{3,7}$=162.421; p=0.000), whereas pH and nitrite were in winter ($F_{3,7}$=17.66; p=0.009;

$F_{3,7}$=124.169, p=0.000, respectively) (Table 1). The nitrate was higher in summer but lower in winter and autumn ($F_{3,7}$=94.93, p=0.000). The phosphate concentration was the highest in autumn ($F_{3,7}$=21.86, p=0.006). The water depth was higher in the limnetic than littoral site (t_6 = -9.727, p=0.000), while chl-a concentration and macrophyte cover were greater in the littoral site (t_6 = 2.936, p=0.026; t_6 = 3.347, p=0.015, respectively) (Table 1, Figure 2).

Table 1. Seasonal and spatial variations in some environmental parameters of Lake Koca

	Winter		Spring		Summer		Autumn	
	Littoral	Limnetic	Littoral	Limnetic	Littoral	Limnetic	Littoral	Limnetic
NH_4^+(mg/l)	0.01	0.01	0.02	0.01	0.02	0.02	0.03	0.02
NO_2^-(mg/l)	1.53	0.97	-	-	0.03	0.02	0.11	0.06
NO_3^-(mg/l)	0.01	0.02	1.23	0.81	2.68	1.04	0.01	0.02
PO_4^{3-}(mg/l)	0.12	0.1	-	-	0.06	0.06	0.44	0.57
T (°C)	13.6	12.5	24.6	23.4	27.6	27	19.4	19
DO (mg/l)	9.01	9.96	10.14	11.16	9.89	10.32	6.18	8.21
pH	8.36	8.3	7.96	7.88	7.99	7.84	8	8.03
Cond (µS/cm)	418	425.63	401	419.87	448	487.6	410	400.65
Chl-a (µg/l)	3.04	1.01	5.81	2.31	6.74	3.01	7.23	3.21
MacCov	3	1	4	1	5	2	5	3
Depth (m)	3.25	11.54	1.8	9.22	1.05	6.45	2.6	10.15

The two PC axis explained 91.6% of the total variation in the season and site data (Table 2). The first PC axis described a gradient of decreasing macrophyte coverage and chlorophyll-a associated with increasing depth. The second PC axis was associated negatively with nitrite and positively with nitrate and temperature (Table 2; Figure 2).

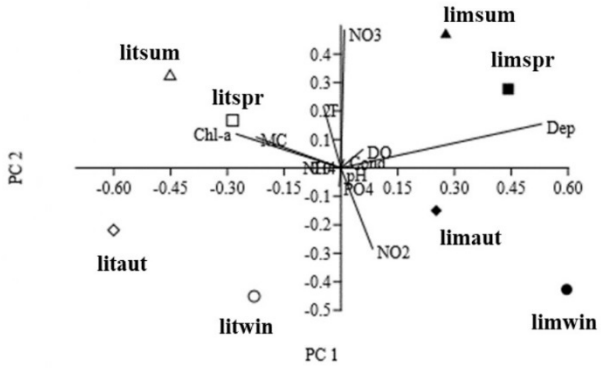

Figure 2. PCA ordination of environmental parameters (Lim: Limnetic site, Lit: Littoral site, win: winter, spr: spring, sum: summer, aut: autumn)

Table 2. PCA eigenvalues (in parentheses) and major axis loadings of environmental parameters

Environmental Parameters	PC 1 (0.198)	PC 2 (0.128)
Ammonium	0.00	0.00
Nitrite	0.13	**-0.44***
Nitrate	0.01	**0.75***
Phosphate	-0.01	-0.10
Temperature	-0.07	**0.33**
DO	0.09	0.10
pH	0.00	-0.02
Conductivity	0.01	0.05
Depth	**0.82***	0.24
Chl-a	**-0.43***	0.18
MacCov	**-0.34***	0.17

*High correlation coefficients

Fish Community Structure

A total of 12 fish taxa belonging to six families and 1.530 fish specimens were collected across four seasonal sampling events during the study. In general, the most dominant families, according to their numerical abundance, are Mugilidae (36.21%), Cichlidae (29.74%), Atherinidae (14.18%), and Cyprinidae (11.11%). Two fish species, *Capoeta aydinensis,* and *Ladigesocypris irideus* were endemic, and comprised 2.94%. In contrast, two non-native fish species (*Coptodon zillii* and *Carassius gibelio*) contributed 37.12% of the numerical abundance of the total catch. *Cyprinus carpio* comprised 3.27% of the total catch. The other fish species were the sole representatives of the Anguillidae: *Anguilla anguilla,* Siluridae: *Silurus glanis,* which comprised 6.08%, and 0.20% of the total catch, respectively.

There was no seasonal variation in the total abundance of fishes when data were pooled across all habitats ($F_{3,7}$=0.064, P= 0.976). It was determined that *C. zillii*, which was the most abundant fish species, had a very high abundance in the summer (49.61%) and autumn (38.10%) (Figure 3). When data were pooled across all seasons, the total abundance was higher in the littoral site compared to the limnetic site (t_6 = 7.132, p=0.000). Especially, *C. zillii* (39.1%) was the most abundant in the littoral site, followed by Mugilidae species (*Mugil cephalus*, *Chelon ramada* and *Chelon labrosus*) (28.4%). In the limnetic site, the most dominant species were Mugilidae species (48.98%), followed by *A. boyeri* (19.84%) and *C. zillii* (16.90%) (Figure 3).

During the study, species richness (S) and species richness index (D) varied from 6 (limnetic site during summer) to 11 (littoral site during autumn) and from 0.50 (limnetic site during summer) to 0.76 (littoral site during autumn), respectively (Figure 4). While there were no significant differences among seasons for species richness ($F_{3,7}$= 0.194, p=0.896) and D ($F_{3,7}$=0.535, p=0.683), species richness showed significant differences between sites with the highest in littoral site (t_6 = 4.127, p=0.006).

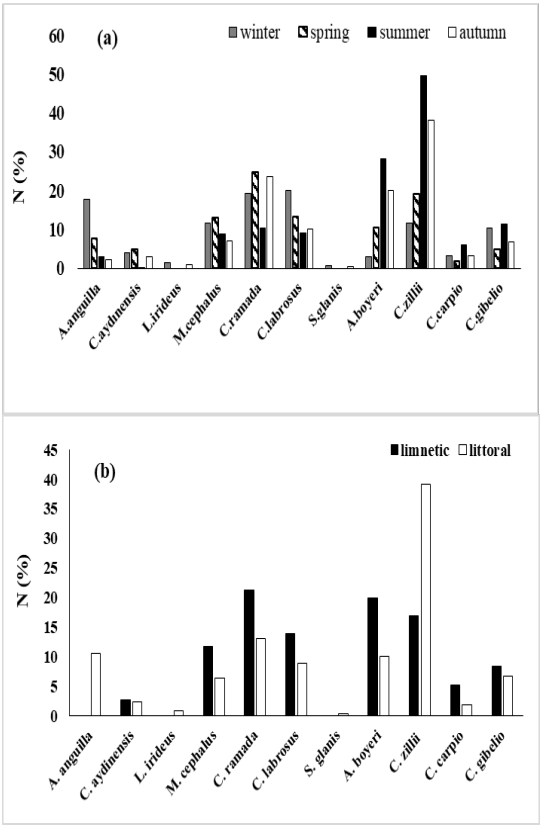

Figure 3. The temporal (a) and spatial (b) variations in the total fish abundance

Shannon-Wiener diversity index (H') and Evenness (J), which is a measure of whether the abundance of the species is evenly distributed, ranged from 1.34 (littoral, summer) to 1.98 (littoral, spring) and 0.58 (littoral, summer) to 0.96 (littoral, spring and limnetic, winter), respectively. Both H' and J did not vary seasonally ($F_{3,7}$=4.035, p=0.106; $F_{3,7}$=0.488, p=0.709, respectively) and spatially (t_6=0.48, p=0.964; t_6=1.263, p=0.253, respectively) (Figure 4).

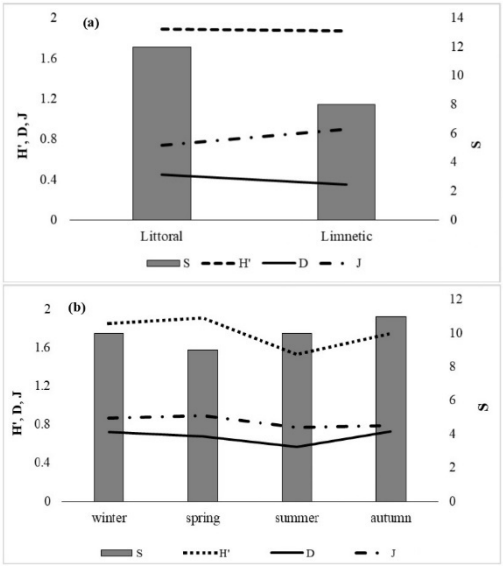

Figure 4. The spatial (a) and temporal (b) variations in mean community metrics of fishes

Water depth was negative (-0.713), but macrophyte coverage was positively (0.731) associated with species richness, whereas the opposite was true for the evenness index (0.718, -0.714, respectively). Total abundance was negatively associated with depth (-0.794) and positively correlated with chlorophyll-a concentration (0.738) and macrophyte coverage (0.765).

Spearman Correlation analysis between abundance and environmental parameters showed that *A. anguilla* had a significant negative correlation with depth. Both *S. glanis* and *L. irideus* were negatively associated with nitrate. *M. cephalus* abundance was correlated with nitrite, phosphate, and dissolved oxygen, but *C. ramada* with conductivity, *C. labrosus* with ammonium. The non-native fish species, *C. zillii,* was negatively associated with water depth while positively with chl-*a* and macrophyte coverage (Table 3).

Table 3. Spearman correlation coefficient between fish species and environmental parameters (*Aa: A. anguilla, Sg: S. glanis, Li: L. irideus, Ab: A. boyeri, Ca: C. aydinensis, Cc: C. carpio, Cg: C. gibelio, Cl: C. labrosus, Mc: M. cephalus, Cr: C. ramada, Cz: C. zillii*)

Parameters	Aa	Sg	Li	Ab	Ca	Cc	Cg	Cl	Mc	Cr	Cz
Ammonium	0.063	0.031	0.031	0.611	-0.156	-0.036	-0.548	**-0.833***	-0.419	0.419	0.405
Nitrite	0.254	0.655	0.655	-0.419	-0.228	-0.06	0.524	0.333	**-0.743***	-0.443	0.19
Nitrate	0.025	**-0.733***	**-0.733***	0.359	-0.108	0.299	-0.571	-0.19	0.551	-0.144	0.095
Phosphate	-0.076	0.483	0.483	0.012	-0.359	0.144	0.238	-0.19	**-0.766***	-0.108	0.095
T	0.101	-0.405	-0.405	**0.731***	-0.275	0.252	-0.524	-0.524	0.192	0.012	0.429
DO	-0.304	-0.592	-0.592	0.024	0.192	0.168	0	0.333	**0.886****	0.012	-0.429
pH	0.304	0.546	0.546	-0.611	0.024	-0.216	0.286	0.238	-0.575	-0.275	0.095
Cond	-0.228	-0.234	-0.234	0.311	-0.491	0.575	0.262	0.238	0.12	**-0.766***	0.024
Depth	**-0.761***	-0.234	-0.234	-0.24	0.012	0.335	0.429	0.429	0.323	0.036	**-0.881****
Chl-a	0.545	0.312	0.312	0.347	-0.06	-0.311	-0.5	-0.69	-0.599	0.216	**0.762***
MacCov	0.639	0.333	0.333	0.293	-0.11	-0.293	-0.473	-0.618	-0.64	0.073	**0.837****

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

DISCUSSION

The fish community structure of the Koca Lake wetland can be characterized by relatively low species richness but an overabundance of some species. The fish community in Lake Koca was dominated by *A. anguilla, A. boyeri, M. cephalus, Chelon ramada*, and *C. labrosus* (42.29%), characteristic of other coastal lakes of the western Mediterranean basin (Akın et al., 2005). Non-native fish species (*C. zillii,* and *C. gibelio*) were dominant in the Koca Lake wetland and represented 37.12% of the fish community. Although *C. carpio* is a native species for some regions of Türkiye (Atalay et al., 2017), it was not recorded in a previous study from Lake Koca (Yılmaz et al., 2006). Tarkan et al. (2015) has been reported as an introduced fish because this fish has been transported to almost all parts of Türkiye due to aquaculture production and stocking programs. Therefore *C. carpio* is an introduced fish species for this lake and contributed more to the fish community than endemic species. On the other hand, freshwater-dependent endemic species (*C. aydinensis* and *L. irideus*) were at a very low density (2.94%). The abundance of non-native species and the decline of native fishes could reflect the high degree of human influence on the wetland, leading to the current semi-replacement of native species. There is no study on the fish community composition or biodiversity of this wetland and only a study provides information on the fish fauna (Yılmaz et al., 2006), and reported that no non-native species, but only catadromous species were recorded between 1999 and 2003.

Our results showed that the non-native cichlid *Coptodon zillii* was the most abundant species of the Lake Koca fish community. This species is evaluated as the high invasiveness in the USA (U.S. Fish and Wildlife Service, 2014) and is generally considered to be a greedy herbivore that depends on aquatic plants for feeding, protection, or spawning (Nico et al., 2014). High fertility (developed gonads even in 0-year-old) and ecological plasticity may have contributed to successful populations of *C. zillii* (Gu et al., 2016; Çoban, 2018). We found a high abundance of *C. zillii* in the littoral site during summer and autumn. For example, the littoral site was characterized by higher chl-a and macrophyte coverage, and lower depth. However, abundance of other non-native (*C. gibelio*) and introduced fish species (*C. carpio*) did not correlate with any of the abiotic environmental parameters we identified. In fact, this result can be explained by the fact that both fish species are opportunistic generalists (Balık et al., 2003; Gül et al., 2010). Seasonal or daily habitat use preferences may vary in both fish. For example, *C. carpio* shows a significant preference for shallow, vegetated habitats during all seasons, but they migrate to deep water during the day (Zhang et al., 2020). *C. gibelio* prefers shallow waters with dense aquatic plants for feeding and spawning in spring and summer, and deep waters in autumn and winter (Giosa et al., 2014).

Species of the Mugilidae family, which are among the native catadromous species, are marine-estuarine dependent and are commercially important fishes. These species are found in greater density in the limnetic site of Lake Koca in spring and summer. Similar results have been reported in other lagoon and wetland systems in the Mediterranean region (Akın et al., 2005; Franco et al., 2008; Scapin et al., 2018). The abundance and distribution of Mugilidae species were correlated with local abiotic factors. For example, *M. cephalus* and *C. labrosus* were negatively correlated with water nutrient parameters such as nitrite, phosphate, and ammonium, respectively. The abundance of *C. ramada* was negatively correlated with conductivity. It has been reported that *C. ramada* is more tolerant to organic pollution and eutrophication than *M. cephalus* (Boglione et al., 2006). Besides, the three sympatric mullet species show interspecies differences in breeding period, habitat, and resource use to reduce competition, which may lead to differences in abundance and distribution (Cardona, 2006; Akın et al., 2005).

One of the endemic fish species, *L. irideus* has the lowest abundance (only 7 individuals) and was mainly caught in the marginal vegetation in the littoral site during winter and autumn. In addition, the abundance of *L. irideus* was found to be negatively associated with nitrate. *L. irideus* was assessed as “Near Threatened" according to the IUCN Red List 2014 (Freyhof, 2014). Although no precise data on population trends are available in the literature, the populations of this species are slowly declining due to the negative effects of anthropological activities (Yılmaz et al., 2015). Another endemic species, *C. aydinensis*, is a species that has a low tolerance to environmental changes and prefers specific habitats (Akbaş et al., 2019). *C. aydinensis* had a very low abundance and constituted 2.48% of the fish community of Lake Koca. It was determined that the abundance and distribution of this species were not correlated with the environmental variables of this wetland. Akbaş et al. (2019) also showed that this endemic fish from the Tersakan Stream which flows into the Lake Koca, was a slow-growing species, therefore the age of reaching sexual maturity is higher than other fish. The destruction of habitats by anthropogenic activities and the presence of non-native fish are critical to the survival of populations of such sensitive and slow-growing species.

Unlike species diversity, species abundance, richness, and evenness showed spatial patterns in Lake Koca. Fish sampling method and used fishing nets can explain spatial patterns (limnetic vs. littoral) in fish community structure. Besides, the fish community of Lake Koca is strongly associated with environmental parameters revealing spatial patterns. Evenness, richness, and abundance were highly correlated with macrophyte coverage and chlorophyll-a concentration. As a central component of the freshwater ecosystem, macrophytes are essential in providing resources and shelter to aquatic organisms (Silva et al., 2013). Aquatic macrophytes appear to play a powerful role in structuring the fish community in Lake Koca. Fish species richness and abundance were high in the littoral habitat, where macrophyte

coverage and chlorophyll-a concentration were high. Similar trends have been identified in many wetland and lake ecosystems (Pelicice et al., 2008; Cvetkovic et al., 2010; Thomaz and Cunha, 2010; Bhagat and Ruetz, 2011). Macrophytes increase habitat structural complexity and productivity for aquatic macroinvertebrates which are important food resources for fish. Additionally, the increase in habitat complexity provides an increase in fish species richness and associated fauna, due to the diversification of possibilities in exploring different habitats (Dias et al., 2017).

In this study, none of the fish community structure parameters showed seasonal variation. However, seasonal patterns are quite common in fish communities from estuarine and lagoon systems (Nsor and Obodai, 2016; Jin et al., 2019; Grubh and Winemiller, 2018). The seasonal variation of communities within these systems is often due to the reproductive biology of fish (reproductive migrations between marine and freshwater and seasonal dynamics) (Akın et al., 2003). Fish that spend their trophic life in fresh water and then migrate to the sea to spawn, such as eels (*A. anguilla*), and marine-estuarine opportunist fish such as Mugilidae (were commonly found in Lake Koca. Estuarine migrant Mugilidae members regularly enter the estuary, especially when juveniles, and use them as nurseries to take advantage of higher nutrient input and structured habitats to develop rapidly in their first years of life. After that, they migrate to coastal seas, where reproduction usually takes place (Rangely et al., 2023). These migratory species, albeit small in size (N. Kaymak personal observation), may have been sampled during four seasons and may have affected the species richness. In addition, some species (*C. zilli, M. cephalus, C. ramada*, etc.) that were abundant in all seasons may have affected the species diversity patterns.

CONCLUSION

The contribution of non-native fish to the fish abundance of Lake Koca was quite high and the most dominant of non-native fish was *C. zillii*. Species diversity and abundance are higher in the littoral than in the limnetic habitat, and in the summer season. All biodiversity parameters showed correlations with environmental parameters such as chlorophyll-a and macrophyte density which are related to primary productivity in the lake. Water quality parameters such as nitrate, nitrite, and phosphate were negatively correlated with native fish distribution. The results of the analysis in this study showed that environmental parameters and non-native fish species in this wetland system affect both biodiversity and fish community structure.

ACKNOWLEDGMENTS AND FUNDING

This study was supported by the Akdeniz University Research Fund (Project Number: FBA-2018-4171). We would like to thank Cihan Toslak for his actual contribution (fish sampling in the field) during the field study of this project. We also thank F. Banu Yalım for her help during the field study of this project.

AUTHOR CONTRIBUTION

Nehir Kaymak wrote the main manuscript text, Yılmaz Emre designed the project and study, Şenol Akın reviewed the draft, Nehir Kaymak and Nesrin Emre analyzed the data.

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

REFERENCES

Akbaş, F., Tarkan, A.S., Top, N., & Karakuş, U. (2019). Some biological characteristics, habitat requirements and implications for conservation of endemic freshwater fish *Capoeta aydinensis* (Turan, Küçük, Kaya, Güçlü Bektaş, 2017) in Tersakan stream (Muğla). *Turkish Journal of Bioscience and Collections*, *3*(2), 43-52. https://doi.org/10.26650/tjbc.2019000 9

Akın, S., Winemiller, K.O., & Gelwick, F.P. (2003). Seasonal and spatial variations in fish and macrocrustacean assemblage structure in Mad Island Marsh estuary, Texas. *Estuarine, Coastal and Shelf Science*, *57* (1-2), 269-282. https://doi.org/10.1016/S0272-7714(02)00354-2

Akın, S., Buhan, E., Winemiller, K.O., & Yilmaz, H. (2005). Fish assemblage structure of Koycegiz Lagoon–Estuary, Türkiye: Spatial and temporal distribution patterns in relation to environmental variation. *Estuarine, Coastal and Shelf Science, 64*(4), 671-684. https://doi.org/10.1016/j.ecss.2005.03.019

Atalay, M.A., Kirankaya, Ş. G., & Ekmekçi, F.G. (2017). The current status of gibel carp and sand smelt in Turkey's inland fisheries. *Aquaculture Studies, 17*, 41-57. https://doi.org/10.17693/yunusae.v17i26557.281581

Ayaz, S., Erdoğan, N., Beşiktaş, M., Aytış, E.A., Dereli, E.M., Aynur, S., Dilaver, M., Haksevenler, B.H.G., Uyusut, B., Dogan, Ö., Aydöner, C., Sarıkaya, O., & Akkaya, Ö. (2013). Preparation of Basin Protection Action Plans Project Western Mediterranean Basin. https://www.tarimorman.gov.tr/SYGM/Belgeler/havza%20koruma%20eylem%20planlar%C4%B1/Bati_Akdeniz_web.pdf (in Turkish)

Balık, S., Mater, S., & Ustaoğlu, M.R. (1992). Mullet Fish and Breeding Techniques. *Ministry of Agriculture, Forestry and Rural Affairs, Bodrum Fisheries Research Institute Directorate Series A* Publication No. 6. (in Turkish)

Balık, I., Karaşahin, B., Özkök, R., Çubuk, H., & Uysal, R. (2003). Diet of silver crucian carp *Carasssius gibelio* in Lake Eğirdir. *Turkish Journal of Fisheries and Aquatic Sciences*. *3*(2), 87-91.

Barbour, M.G., Rejmánek, D.M., Johnson, A.F., Pavlik, B.M. (1987). Beach vegetation and plant distribution patterns along the northern Gulf of Mexico. *Phytocoenologia*, *15*: 201–233.

Bhagat, Y., & Ruetz, III C. R. (2011). Temporal and fine-scale spatial variation in fish assemblage structure in a drowned river mouth system of Lake Michigan. *Transactions of the American Fisheries Society, 140*(6), 1429-1440. https://doi.org/10.1080/00028487.2011.630278

Boglione, C., Costa, C., Giganti, M., Cecchetti, M., Di Dato, P., Scardi, M., & Cataudella, S. (2006). Biological monitoring of wild thicklip grey mullet (*Chelon labrosus*), golden grey mullet (*Liza aurata*), thinlip mullet (*Liza ramada*) and flathead mullet (*Mugil cephalus*) (Pisces: Mugilidae) from different Adriatic sites: meristic counts and skeletal anomalies. *Ecological indicators*, *6*(4), 712-732. https://doi.org/10.1016/j.ecolind.2005.08.032

Cardona, L. (2006). Habitat selection by grey mullets (Osteichthyes, Mugilidae) in Mediterranean Estuaries: The role of salinity. *Scientia Marina, 70*(3) ,443-455. https://doi.org/10.3989/scimar.2006.70n3443

Carey, M.P., Maloney, K.O., Chipps, S.R., & Wahl, D.H. (2010). Effects of littoral habitat complexity and sunfish composition on fish production. *Ecology of Freshwater Fish*, *19*(3) 466-476. https://doi.org/10.1111/j.1600-0633.2010.00433.x

Cheng, L., Lek, S., Lek-Ang, S., & Li, Z. (2012). Predicting fish assemblages and diversity in shallow lakes in the Yangtze River basin. *Limnologica*, *42*(2), 127-136. https://doi.org/10.1016/j.limno.2011.09.007

Cvetkovic, M., Wei, A.H., & Chow-Fraser, P. (2010). Relative importance of macrophyte community versus water quality variables for predicting fish assemblages in coastal wetlands of the Laurentian Great Lakes. *Journal of Great Lakes Research*, *36*, 64-73. https://doi.org/10.1016/j.jglr.2009.10.003

Çınar, I., & Ardahanlıoğlu, Z.R. (2015). Nature Conservation and Fethiye-Göcek Special Environmental Protection Area. Sonçağ Yayıncılık Matbaacılık, Ankara.

Çoban, G. (2018). The investigation of the life-history traits of invasive fish species, *Coptodon zillii* (Gervais, 1848), from Köyceğîz Lake. MSc Thesis, Düzce University, Düzce, Türkiye.

Dias, R.M., da Silva, J.C.B., Gomes, L.C., & Agostinho, A.A. (2017). Effects of macrophyte complexity and hydrometric level on fish assemblages in a Neotropical floodplain. *Environmental Biology of Fishes*, *100*, 703-716. https://doi.org/10.1007/s10641-017-0597-y

Dustin, D.L., & Vondracek, B. (2017). Nearshore Habitat and fish assemblages along a gradient of shoreline development. *North American Journal of Fisheries Management*, *37*, 432-444. https://doi.org/10.1080/02755947.2017.1280567

Fitzgerald, D.B., Winemiller, K.O., Sabaj Perez, M.H., & Sousa, L.M. (2017). Seasonal changes in the assembly mechanisms structuring tropical fish communities. *Ecology*, *98*, 21–31. https://doi.org/10.1002/ecy.1616

Franco, A., Franzoi, P., & Torricelli, P. (2008). Structure and functioning of Mediterranean lagoon fish assemblages: a key for the identification of water body types. *Estuarine, coastal and shelf science*, *79*(3): 549-558. https://doi.org/10.1016/j.ecss.2008.05.011

Freyhof, J. (2014). *Ladigesocypris irideus.* The IUCN Red List of Threatened Species 2014: e.T61266A19009700. https://www.iucnredlist.org/

Giosa, M.D., Czerniejewski, P., & Rybczyk, A. (2014). Seasonal changes in condition factor and weight-length relationship of invasive *Carassius gibelio* (Bloch, 1782) from Leszczynskie Lakeland, Poland. *Advances in Zoology*, *7*, 678763. https://doi.org/10.1155/2014/678763

Güçlü, S.S., Küçük, F., Ertan, Ö.O., & Güçlü, Z. (2013). The fish fauna of the Büyük Menderes River (Turkey): Taxonomic and zoogeographic features. *Turkish Journal of Fisheries and Aquatic Sciences*, *13*(4), 685-698. https://doi.org/10.4194/1303-2712-v13_4_14

Güçlü, S.S., Küçük, F., Çetinkaya, O., & Yıldırım, U.G. (2020). The fish fauna of Dalaman River (Turkey): Taxonomic and zoogeographic features. In M. Doğan, E. Atay (Eds.), International Eurasian Conference on Biological and Chemical Sciences, 19-20 Mart 2020, Ankara-Türkiye Proceeding Book, 201 s.

Gül, A., Yilmaz, M., Kuşçu, A., & Benzer, S. (2010). Feeding properties of common carp (*Cyprinus carpio* L, 1758) living in Hirfanli Dam Lake. *Kastamonu Education Journal*, *18*, 545-556.

Grubh, A.R., & Winemiller, K.O. (2018). Spatiotemporal variation in wetland fish assemblages in the Western Ghats region of India. *Knowledge and Management of Aquatic Ecosystems*, *419*, 35. https://doi.org/10.1051/kmae/2018023

Gu, D.E., Mu, X.D., Xu, M., Luo, D.Q., Wei, H., Li, Y.Y., Zhu, Y., Luo, J.R., & Hu, Y. (2016). Identification of wild tilapia species in the main rivers of South China using mitochondrial control region sequence and morphology. *Biochemical Systematics and Ecology, 65*, 100e107. https://doi.org/10.1016/j.bse.2016.02.007

Jin, B.S., Winemiller, K.O., Shao, B., Si, J.K., Jin, J.F., & Ge, G. (2019). Fish assemblage structure in relation to seasonal environmental variation in sub-lakes of the Poyang Lake Floodplain, China. *Fisheries Management and Ecology, 26*, 131-140. https://doi.org/10.1111/fme.12333

Kıraç, C.O., & Suseven, B. (2021). Dalaman Plain. https://www.dogadernegi.org/wp-content/uploads/2018/11/akd003-dalaman-ovasi-onemli-doga-alanlari-kitabi.pdf (in Turkish)

Kottelat, M., & Freyhof, J. (2007). Handbook of European Freshwater Fishes. Kottelat Publication, xiv+646 p., Switzerland.

Krebs, C.J. (1998). Ecological Methodology. 2nd edition. Benjamin/Cummings, Menlo Park, California.

Lewin, W.C., Okun, N., & Mehner, T. (2004). Determinants of the distribution of juvenile fish in the littoral area of a shallow lake. *Freshwater Biology, 49*, 410–424. https://doi.org/10.1111/j.1365-2427.2004.01193.x

Marin Avendaño, C.M., & Aguirre Ramírez, N.J. (2017). Spatial and temporal variation of fish assemblage associated with aquatic macrophyte patches in the littoral zone of the Ayapel Swamp Complex, Colombia. *Acta Limnologica Brasiliensia*, *29*, e3. https://doi.org/10.1590/S2179-975X6016

Matern, S., Klefoth, T., Wolter, C., & Arlinghaus, R. (2021). Environmental determinants of fish abundance in the littoral zone of gravel pit lakes. *Hydrobiologia, 848*, 1-23. https://doi.org/10.1007/s10750-021-04563-4

Menhinick, E.F. (1964). A comparison of some species individual diversity indices applied to samples of field insects. *Ecology*, *45*, 839-861. https://doi.org/10.2307/1934933

Muus, B.J., & Nielsen, J.G. (1999). Sea fish. Scandinavian Fishing Year Book, Hedehusene, Denmark. 340 p.

Nico, L., Neilson, M., & Loftus, B. (2014). *Tilapia zillii*. USGS Nonindigenous Aquatic Species Database, Gainesville, Florida. http://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=485

Nsor, C.A., & Obodai, E.A. (2016). Environmental determinants influencing fish community structure and diversity in two distinct seasons among wetlands of northern region (Ghana). *International Journal of Ecology*, *10*, 1598701. https://doi.org/110.1155/2016/1598701

Özuluğ, M., & Freyhof, J. (2011). Revision of the Genus *Squalius* in Western and Central Anatolia, with description of four new species (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters, 22*(2), 107-148.

Pelicice, F.M., Thomaz, S.M., & Agostinho, A.A. (2008). Simple relationships to predict attributes of fish assemblages in patches of submerged macrophytes. *Neotropical Ichthyology*, *6*(4), 543-550. https://doi.org/10.1590/S1679-62252008000400001

Pielou, E.C. (1966). Species diversity and pattern diversity in the study of ecological succession. *Journal of Theoretical Biology*, *10*, 370–383. https://doi.org/10.1016/0022-5193(66)90133-0

Rangely, J., de Barros, M.S., Albuquerque-Tenório, M.D., Medeiros, R., Ladle, R.J., & Fabré, N.N. (2023). Assessing interspecific variation in life-history traits of three sympatric tropical mullets using age, growth and otolith allometry. *Fisheries Research*, *260*, 106577. https://doi.org/10.1016/j.fishres.2022.106577

Scapin, L., Zucchetta, M., Sfriso, A., & Franzoi, P. (2018). Local habitat and seascape structure influence seagrass fish assemblages in the Venice Lagoon: The Importance of conservation at multiple spatial scales. *Estuaries and Coasts*, *41*, 2410-2425. https://doi.org/10.1007/s12237-018-0434-3

Silva, T. S., Melack, J. M., & Novo, E. M. (2013). Responses of aquatic macrophyte cover and productivity to flooding variability on the Amazon floodplain. *Global change biology*. *19*(11), 3379-3389. https://doi.org/10.1111/gcb.12308

Şahin, S. (2019a). What can be done for fisheries and tourism? https://guneyege.net/balikcilik-ve-turizm-icin-neler-yapilabilir/ (in Turkish)

Şahin, S. (2019b). Muğla's largest lakes, 4 lagoons are dying. https://guneyege.net/muglanin-en-buyuk-golleri-olan-4-adet-lagunleri-oluyor/ (in Turkish)

Tarkan, A.S., Marr, S.M., & Ekmekçi, F.G. (2015). Non-native and translocated freshwater fish species in Turkey. *FiSHMED*. 003, 28 p. https://doi.org/10.29094/FiSHMED.2015.003

Thomaz, S.M., & Cunha, E.R. (2010). The role of macrophytes in habitat structuring in aquatic ecosystems: methods of measurement, causes and consequences on animal assemblages' composition and biodiversity. *Acta Limnologica Brasiliensia*, *22*(2), 218-236. https://doi.org/10.4322/actalb.02202011

Turan, D., Küçük, F., Kaya, C., Güçlü, S.S., & Bektaş, Y. (2017). *Capoeta aydinensis*, a new species of scraper from southwestern Anatolia, Türkiye (Teleostei: Cyprinidae). *Turkish Journal of Zoology*, *41*(3), 436-442. https://doi.org/10.3906/zoo-1510-43

U.S. Fish and Wildlife Service (2014). https://cupdf.com/document/redbelly-tilapia-tilapia-zillii.html?page=1 Wildlife Service

Westrelin, S., Roy, R., Tissot-Rey, L., Berge`s, L., & Argillier, C. (2018). Habitat use and preference of adult perch (*Perca fluviatilis* L.) in a deep reservoir: variations with seasons, water levels and individuals. *Hydrobiologia*, *809*, 121–139. https://doi.org/10.1007/s10750-017-3454-2

Wetzel, R.G., & Likens, G.E. (1991) Composition and Biomass of Phytoplankton. In R.G. Wetzel, G.E. Likens (Eds.), *Limnological Analyses*. Springer, New York, NY. https://doi.org/10.1007/978-1-4757-4098-1_10

Winemiller, K.O., Tarim, S., Shormann, D., & Cotner, J.B. (2000). Fish assemblage structure in relation to environmental variation among Brazos River oxbow lakes. *Transactions of the American Fisheries Society, 129*, 451-468. https://doi.org/10.1577/1548-8659(2000)129%3C0451:FASIRT%3E2.0.CO;2

Yılmaz, F., Barlas, M., Yorulmaz, B., & Özdemir, N. (2006). A Taxonomical study on the inland water fishes of Muğla. *Ege Journal of Fisheries and Aquatic Sciences,* (1-2), 27–30.

Yılmaz, F., Yorulmaz, B., & Giannetto, D. (2015). Threatened fishes of the world: *Ladigesocypris irideus* (Ladiges, 1960) (Cyprinidae). *Croatian Journal of Fisheries,* 177-180. https://doi.org/10.14798/73.3.830

Zhang, Y., Li, Y., Zhang, L., Wu, Z., Zhu, S., Li, J., & Li, X. (2020). Site fidelity, habitat use, and movement patterns of the common carp during its breeding season in the Pearl River as determined by acoustic telemetry. *Water, 12*(8), 2233. https://doi.org/10.3390/w12082233

Ziegler, J. P., Solomon, C. T., Finney, B. P., & Gregory-Eaves, I. (2015). Macrophyte biomass predicts food chain length in shallow lakes. *Ecosphere*, *6*(1), 5. https://doi.org/10.1890/ES14-00158.1

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 244-250 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.02

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Investigations on *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961) (Monogenea: Diplozoidae) found in *Capoeta aydinensis*, an endemic fish in Türkiye, based on ecological, molecular and host related factor approaches

Türkiye'nin endemik balıklarından *Capoeta aydinensis*' ten *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961) (Monogenea: Diplozoidae)'nin ekolojik, moleküler ve konak ilişkili faktör yaklaşımıyla araştırmalar

Nesrin Emre[1] ● Nurten Aydogdu[2*] ● Ali Aydogdu[3] ● Özgür Emiroglu[4]

[1]Department of Mathematics and Science Education, Faculty of Education, Akdeniz University, 07058, Antalya, Türkiye https://orcid.org/0000-0001-9047-1823
[2]Graduate School of Natural and Applied Sciences, Department of Hydrobiology, Balıkesir University, 10145, Balıkesir, Türkiye https://orcid.org/0000-0002-9926-166X
[3]Faculty of Veterinary Medicine, Department of Aquatic Animal Diseases, Bursa Uludag University, 16059, Bursa, Türkiye https://orcid.org/0000-0003-2778-0273
[4]Science and Art Faculty, Department of Biology, Eskişehir Osmangazi University, 26040, Eskişehir, Türkiye https://orcid.org/0000-0002-4433-4286

*Corresponding author: aydogdunurten98@gmail.com
Received date: 11.08.2023 Accepted date: 18.10.2023

How to cite this paper:
Emre, N., Aydogdu, N., Aydogdu, A., & Emiroglu, Ö. (2023). Investigations on *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961) (Monogenea: Diplozoidae) found in *Capoeta aydinensis*, an endemic fish in Türkiye, based on ecological, molecular and host related factor approaches. *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 244-250. https://doi.org/10.12714/egejfas.40.4.02

Abstract: The present study investigated diplozoid parasites in an endemic species, *Capoeta aydinensis* Turan, Küçük, Kaya, Güçlü & Bektaş, 2017 in Köyceğiz Lake, near Muğla province (Türkiye). The aim of this research is to improve a record of diplozoid species occurrence in *C. aydinensis*, an endemic fish species by collecting data from a previously unexplored locality situated in a different geographical region of Türkiye. A total of 187 individuals of *C. aydinensis* were collected by using fishing nets from October 2019 to July 2020 and examined for the presence of diplozoid species. Only one species of diplozoid has been recorded, known as *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961), which has been identified morphologically and confirmed through molecular analysis. The nucleotide sequences of the parasite's nuclear internal transcribed spacer (ITS2) gene marker were determined as well as phylogenetic analyses by using Bayesian inference (BI) analyses. On the basis of the molecular findings, the morphological identification of the diplozoid parasite species was confirmed. Of 187 fish sampled, 27 were infected with 117 *P. bliccae*, representing an abundance of 0.6, a mean intensity of 4.3 and a prevalence of 14.4%. The prevalence and mean intensity of infection were based on the season and sex of the host. The highest values of infection for prevalence, mean intensity and abundance were found in summer. Meanwhile, mean intensity and abundance of *P. bliccae* were higher in males, the prevalence was higher in females. To our knowledge, the present study is the first ichthyoparasitological study of *C. aydinensis* in Köyceğiz lake, near the province of Muğla in Türkiye. Furthermore, sequence data of *P. bliccae* from fish hosts in this locality were reported to GenBank for the first time as part of this study. Therefore, this study widens the host range of this parasite species in Türkiye.

Keywords: Köyceğiz lake- *Capoeta aydinensis*, *Paradiplozoon bliccae*, molecular approach, seasonal effects, host sex

Öz: Bu çalışma, Muğla (Türkiye) ili yakınlarındaki Köyceğiz Gölü' nde bulunan endemik *Capoeta aydinensis* Turan, Küçük, Kaya, Güçlü & Bektaş, 2017'in diplozoid parazitleri üzerine yapılmıştır. Araştırmanın amacı, Türkiye' nin farklı bir coğrafi bölgesinde daha önce keşfedilmemiş bir lokasyondaki endemik balık türü *C. aydinensis'* in diplozoid türlerinin varlığına ilişkin kayıtların geliştirilmesidir. Ekim 2019 – Temmuz 2020 tarihleri arasında araştırma alanından balık ağları kullanılarak toplam 187 *C. aydinensis* bireyi toplanmış ve diplozoid türlerinin varlığı açısından incelenmiştir. Ayrıntılı morfolojik tanımlamanın ardından yalnızca bir diplozoid türü, *Paradiplozoon bliccae* (Reichenbach-Klinke, 1961) tanımlanmıştır. Bu morfolojik tür tanımlamasını moleküler yöntemlerle doğrulamak için, konak balıktan alınan parazitin internal transcribed spacer 2 (ITS2)gen bölgelerinin nükleotid dizileri belirlenmiş ve Bayesian Inference (BI) algoritmaları kullanılarak filogenetik analizleri yapılmıştır. Elde edilen moleküler bulgulara göre diplozoid parazit türünün morfolojik olarak tanımlanması doğrulanmıştır. Diplozoid parazit dizileri ayrıca LT560257 erişim numarasıyla GenBank' ta saklanan *Paradiplozoon bliccae* dizileriyle de yüksek homoloji (%99.32) göstermiştir. İncelenen 187 balıktan 27'sinin toplam 117 *P. bliccae* ile 14.4%; 4.3; 0.6 sırasıyla enfeksiyon oranı, enfekte balık başına ortalama parazit sayısı ve incelenen balık başına ortalama parazit sayısı enfekte olduğu belirlenmiştir. İlave olarak, mevsime ve konakçı cinsiyetine göre enfeksiyon oranı ve enfekte balık başına ortalama parazit sayısı hesaplanmıştır. Enfeksiyon oranı, enfekte balık başına ortalama parazit sayısı ve incelenen balık başına ortalama parazit sayısı için en yüksek değerler yaz aylarında bulunmuştur. *P. bliccae*' nin enfekte balık başına ortalama parazit sayısı ve incelenen balık başına ortalama parazit sayısı erkeklerde daha yüksekken, enfeksiyon oranı dişilerde daha yüksek bulunmuştur. Bildiğimiz kadarıyla bu çalışma, Muğla ili Köyceğiz Gölü' nde *C. aydinensis*' in ilk ihtiyoparazitolojik araştırmasıdır. Ayrıca bu çalışma ile ilk kez bu lokalitedeki konak balıklardan elde edilen *P. bliccae*' nin sekans dizi verileri de GenBank'a raporlanmıştır. Böylece bu çalışma, bu parazit türünün Türkiye'deki konak yelpazesini üçe, yer sayısını ise ikiye çıkarmıştır.

Anahtar kelimeler: Köyceğiz Gölü, *Capoeta aydinensis*, *Paradiplozoon bliccae*, moleküler yaklaşım, mevsimsel etki, konak cinsiyeti

INTRODUCTION

According to Froese and Pauly (2022), 409 species of freshwater fish have been reported from Türkiye's inland waters so far. Endemic fish species represent 194 of these species (Çiçek et al., 2018). One of these endemic species,

Capoeta aydınensis Turan, Küçük, Kaya, Güçlü & Bektaş, 2017 is distributed in clear and moderately flowing waters with a substrate of stones and pebbles (Froese and Pauly, 2022). It occurs in the Büyük Menderes River as well as Dalaman, Namnam and Tersakan in the freshwater ecosystems (Froese and Pauly, 2022). Despite the increase in the number of studies on helminth parasites of endemic fish species in Türkiye in recent years, it is evident from current literature that comprehensive studies on the helminth parasites of endemic fish species are needed due to the determination of the helminth fauna of most endemic fish species distributed in Türkiye. In confirmation of the above information, there is only one ichthyo-helminthological record for *C. aydınensis* so far in Türkiye (Nejat et al., 2023). In addition to these, Diplozoid parasite species also appear to be very poorly represented in Türkiye fauna. Besides these studies, Özer (2021) found that the genus *Paradiplozoon* Akhmerov, 1974 is represented in Türkiye by six previously known species of parasites and one unspecies identified parasite plus that no previous study has found *Paradiplozoon* spp in this host fish in the locality. Moreover, *Paradiplozoon bliccae* has been recorded in only three studies in Türkiye so far (İnnal et al., 2020; Unal et.al., 2017; Nejat et.al., 2023). Diplozoids are a unique family of Monogenea that are common and widespread gill parasites of cyprinid fishes (Pecínková et. al., 2005).The frequency diplozoid parasite detections in host fish is not constant for all species, as is the case for other helminth parasites (Yunchis, 1988), and varies depending on season, host biology, host age, size, and salinity and temperature of the water of the location in the study area.

Therefore, the current study aims to: (i) Determine the *Paradiplozoon* species that infect *C. aydinensis* by collecting data from a previously unexplored location in a geographic region in Türkiye, which is different from the previous records. (ii) Increase the number of members within the Diplozoidae family documented in Türkiye. (iii) Expand our understanding of the geographical distribution and host range of Diplozoid parasite specimens in Türkiye's waters. (iv) Determine whether the influence of season and host sex on the occurrence of these parasites in *C. aydinensis*.

MATERIALS AND METHODS

Sampling and parasitological analysis

A total of 187 individuals of *Capoeta aydinensis* were caught by commercial fishermen in Köyceğiz lake, located in Muğla province. The collection was seasonal, from October 2019 to July 2020. The fish samples were placed in 12-liter cold chain plastic containers filled with ice molds on a fishing boat and were immediately transferred to the research laboratory. Then the fish were wrapped in aluminum foil to keep them separate and were placed in the freezer. On examination day, the fish were defrosted (in tap water) for approximately 20 minutes at room temperature. The remaining fish were defrosted one by one, following the completion of the previous examination. The sex of each fish was determined during dissection, and then the gills were examined for *Paradiplozoon* specimens from newly defrosted fish individuals by using an Olympus stereomicroscope. The number of parasites was counted and preserved with a mixture of glycerin–ammonium picrate (Malmberg, 1957; Khotenovsky, 1974). The identification of *Paradiplozoon* specimens were performed according to morphological keys of Gussev (1985); Bychowskaya–Pavlovskaya (1962); Khotenovsky (1985) and other available references with an optical microscope. Finally, the collected diplozoid species were fixed and stored in absolute ethanol at +4 C^0 until DNA isolation and delivered to the Laboratory of Parasitology in Department of Public Health and Infection Diseases of "MS LAB (Eskişehir, Türkiye)", for molecular identification. The photomicrographs of all parasite specimens were taken by using a photographic camera mounted Leica DMR microscope with phase contrast and an Olympus BX-50 research microscope.

Molecular Analyses

Before DNA isolation, the samples fixed in alcohol were first centrifuged at 3000 RPM in 1.5 ml microcentrifuge tubes, noting that ethanol in the upper part was removed. The Genomic DNA of the parasites were extracted using the A.B.T.™ DNA Purification Kit (Qiagen, Hilden, Türkiye), by following the standard manufacturer-recommended protocol. The purity levels of the isolated DNA samples were measured first with the Maestrogen Nano (Maestrogen, Taiwan) spectrophotometer and then with the Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) systems. After isolation, DNA samples were amplified using Promega goTaq polymerase and buffers. A fragment of ITS2 rDNA was amplified by using primers:

D (5'GGCTYRYGGNGTCGATGAAGAACGCAG-3') and

B1 (5'GCCGGATCCGAATCCTGGTTAGTTTCTTTTCCT-3').

Polymerase chain reaction (PCR) was carried out according to the reaction conditions of Matejusová et al. (2001). PCR amplicons were sent to MS LAB (Eskisehir, Türkiye) for Sanger sequencing (Applied Biosystems, Forster City, CA). Post-PCR samples were verified using the Qiagen QIAxcel Advanced capillary electrophoresis system (Qiagen, Hilden, Germany) to 293 base pairs. The resulting sequencing was analyzed to distinguish species using NCBI blast program (Altschul et al.,1990). Sequence data was published to GenBank (GenBank accession number: OP723498, http://www.ncbi.nlm.nih.gov). The acquired nucleotide sequences were compared to *Paradiplozoon* spp. sequences supplied and found to be more than 95% identical in GenBank. A phylogram was generated using the MrBayes 3.1.2 (Huelsenbeck et al., 2001) program.

For phylogenetic analyses, the sequence obtained in this study was aligned with the data in the literature using the ClustalW algorithm in the MEGA-X (Kumar et al., 2018) program. As a result of the alignment, non-informative parts of the sequences were cut at the start and at the end. Then, the Modeltest v. 2.1.5 (Darriba et al., 2012) program was used to determine the mutation model that best matched the data

obtained. Among a total of 56 mutation models, it was determined that the most appropriate mutation model for the data set was the GTR model (general time reversible model) according to the Akaike information criterion (AIC). MrBayes 3.1.2 (Huelsenbeck et al., 2001) program was used to construct the phylogenetic tree. As an outgroup, *Sindiplozoon* spp. was used. Markov Chain Monte Carlo analysis was carried out over 4 chains. In addition, analyses were carried out for 10 million generations until the split frequency fell below 0.01. When the analysis reached saturation (ESS values), it was monitored using the Tracer v.1.7 (Rambaut et al., 2018) program. 25% of the trees obtained by sampling every 1000 generations were removed by burn-in. The consensus tree was obtained as a result of the analysis that was viewed and edited in the Figtree v1.4.2 (Rambaut 2014) program (Figure 2).

Statistical analysis

Data on *Paradiplozoon* species was categorized according to the seasons and the sex of the host fish. The levels of prevalence, mean intensity and abundance of infection as defined by Bush et al. (1997) were calculated.

Standard statistical computations (standard deviation) were carried out using Microsoft Excel (Office 2000). Kruskal-Wallis (more than two groups) tests were applied to find significant differences in the mean intensity of the parasite species for the size and seasons of host fish. The Mann-Whitney U test (two groups) was used to determine the correlation between the intensity of each helminth species infection and the host sex. The significance level of $\alpha \leq 0.05$ was used. All statistics analyses were performed using SPSS v. 28 for Windows.

RESULTS

On the gills of 187 *Capoeta aydınensis* individuals, one species of diplozoid was identified. Based on our detailed studies on haptor and attachment apparatures morphology of the species, *Paradiplozoon bliccae* was identified (Figure 1A-B), and confirmed by molecular analysis. A total of 117 specimens of this parasite were found infecting 27 of the 187 fish examined, with prevalence (14.4%), a mean intensity (4.3 parasite/fish) and abundance (0.6). The seasonal variation of the infection of *P. bliccae* was also investigated in our study.

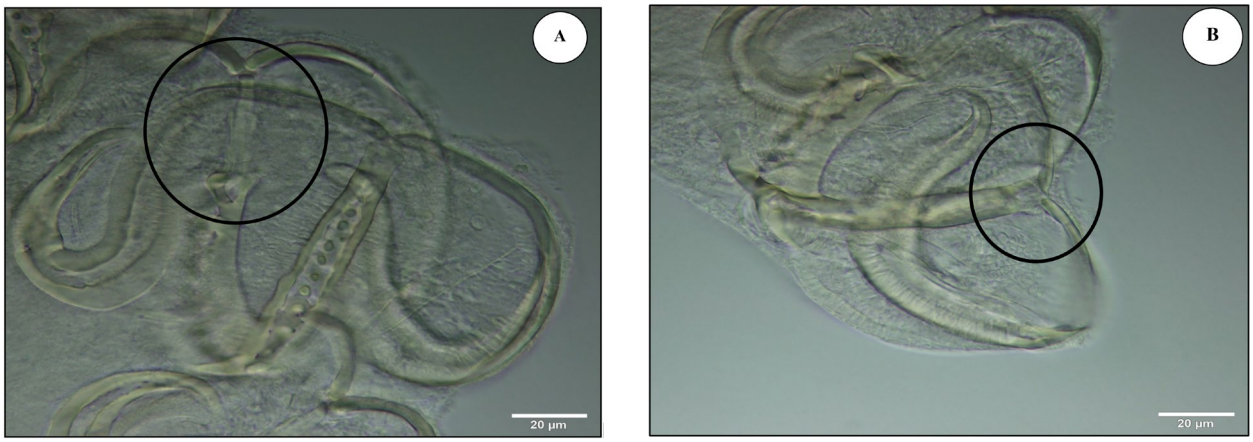

Figure 1. A clamp of *Paradiplozoon bliccae* isolated from *Capoeta aydinensis*; **A**-posterior view. - posterior end of median sclerite (circle indicates); **B**-anterior view. - anterior end of median sclerite. Detail of the trapeze spur and anterior joining sclerites forming a specific T-shape of the clamps (circle indicates)

Changes in infection parameters in relation with seasons are shown in Table 1. Seasonal prevalence, mean intensity and abundance of this parasite species differed between seasons. During spring, a total of 20 specimens of *P. bliccae* were found in 11 of 57 fish examined, yielding a prevalence of 19.2%, a mean intensity of 1.8 parasites/fish, and an abundance of 0.3. For summer, 58 fish were caught and a total of 97 parasites were found in 16 of the 58 fish examined (prevalence 27.5%, mean intensity 6 parasite/fish and abundance 1.6.

Table 1. Prevalence and intensity values of *Paradiplozoon bliccae* in *Capoeta aydinensis* from Köyceğiz Lake according to seasons and host sex

		Infection parameters			
		Prevalence (%)	**Mean intensity ± SD**	**Abundance**	**Total parasite no**
Seasons	Autumn (n=16)	-	-	-	-
	Winter (n=56)	-	-	-	-
	Spring (n=57)	19.2	1.8±1.07	0.3	20
	Summer (n=58)	27.5	6±3.15	1.6	97
Fish sex	Female (n=123)	16.2	3.3±2.36	0.5	66
	Male (n=60)	11.6	7.2±3.86	0.8	51

This species was not detected in the autumn and winter samples. Seasonal prevalence of infection was higher in summer, 27.5%, the highest mean intensity and abundance values were also found in summer (Table 1). According to Kruskal Wallis's-H test, there were statistically significant differences in number of parasites collected per season ($p<0.001$).

A total of 187 *C. aydinensis* individuals (123 females, 60 males, and 4 not identified) were examined for diplozoid parasites. *P. bliccae* was found in 7 of 60 male fish examined, with prevalence, mean intensity and abundance of infection of 11.6%, 7.2 parasite/fish and 0.8, respectively. A total of 66 specimens of this parasite species infected 20 of the 123 female examined fish (Table 1).

The prevalence of *P. bliccae* was higher in females (16.2%) when compared to males (11.6%) while the mean intensity and abundance of this species was higher in males (Table 1). There was a statistically significant sex–related difference in the number of this species based on Mann–Whitney U test ($p=0.02$). The partial sequence of 293 nucleotides of the present diplozoid species was obtained in the present study and is represented in Figure 2. The BLAST analysis of these sequences revealed high homology (99.32%) and (99.32%) with *Paradiplozoon bliccae* sequences deposited by Unal et al. (2017) (GenBank LT560257), Matějusová et al. (2001) (GenBank AJ300712), respectively and high similarity (98.98%) with *P. bliccae* collected from *Squalius cii* published by Sicard et al., (2001) (GenBank AF369761) (Figure 2). The data sequence of this present diplozoid species was deposited in GenBank (accession number: OP723498). Multiple alignments were performed on *Paradiplozoon* specimens using sequencing data from NCBI GenBank and a phylogenetic tree was generated (Figure 2).

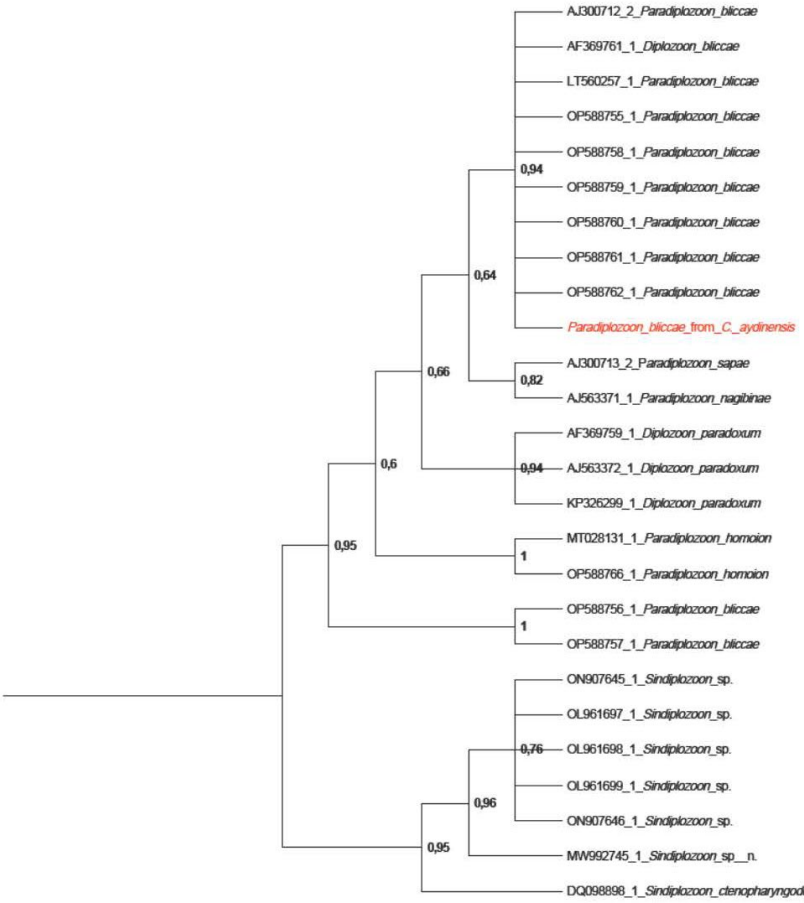

Figure 2. Phylogenetic tree for the relationships of *Paradiplozoon bliccae Paradiplozoon* spp., sequences supplied and found to be more than 95% identical in GenBank. Scale bar (=2) represents the expected number of substitutions per nucleotide

DISCUSSION

Capoeta aydinensis is one of the endemic fish species that spread in the Büyük Menderes river drainages and Dalaman, Namnam and Tersakan in South-Western Türkiye (Turan et al., 2017). To our knowledge, there is only a single ichthyohelmintological data reported for *C. aydınensis* in Türkiye so far (Nejat et al., 2023).

In this study, the diplozoid parasitesfauna was investigated in *C. aydınensis* from Köyceğiz Lake from Southwestern Anatolia, Türkiye. Only one diplozoid species was identified, namely *Paradiplozoon bliccae*. It was described based on the haptor and the attachments apparatus morphology. The identifying feature of *P. bliccae* is the typical shape of anterior

joining sclerites forming a specific T-shape and its trapeze spurs shapes, anterior joining of sclerites of the clamps and the size of the central hook sickle were distinguished it from other closely related species belonging to the genus *Paradiplozoon*. (Figure 1A-B, circle indicates). In addition to the morphological characters used to identify species belonging to the genus *Paradiplozon* above, to our best knowledge, in identifying the morphological types of monogenic parasites belonging to the Diplozoidae family, the following characteristics are used; measurements and shapes of the clamps and central hook, anterior/posterior part ratios of the body, shape of the intestinal cecum ending at the back, etc. Structures selected according to taxonomic importance are used. Situations such as the continuous growth of these structures, which are important in species identification, and the change in metric measurements even within the species, make species identification of diplozoid parasites very difficult. Therefore, it is clear that it should be confirmed using molecular analysis as well as morphological characters. For these reasons, molecular analyses were carried out. And in our study, 293 bp of ITS2 gene region was replicated and compared with the data in GenBank. The molecular identification and DNA sequences using the ITS2 gene showed that the specimens from *C. aydinensis* in Köyceğiz Lake were identified as *Paradiplozoon bliccae* (Figure 2). Our samples were clustered into *Paradiplozoon* and into *Paradiplozoon bliccae* species in the BI phylogenetic tree (98- 99). For example, the sequences of *P. homoion* are 100% similar to accession number MT417728 sequences presented by Benovics et. al., (2020). Several previous reports have indicated that in support of morphological data (Unal et. al., 2017; İnnal et al., 2020; Nejat et al., 2023), they have used the ITS2 gene 5.8S rRNA gene to confirm the morphological identification of these diplozoid parasites.

In this aspect, the present study raises the number of reports on the molecular characterization of *Paradiplozoon* specimens collected from Türkiye. In addition, sequence data of *P. bliccae* from host fish in this locality were reported to GenBank for the first time with this study. In addition, this is the first survey on the ichthyo-helmintological data for this host fish in Köyceğiz Lake in Türkiye. Therefore, this study expands localities for *P. bliccae* in Türkiye.

In the present study, a total of 117 specimens of *P. bliccae* were found in 27 of 187 *C. aydınensis* examined with prevalence and mean intensity of 14.4% and 4.3 parasite/fish respectively. *P. bliccae* has been previously reported from only two fish species living in different habitats in Türkiye: *Pseudophoxinus burduricus* Küçük, Gülle, Güçlü, Çiftçi and Erdoğan, 2013 *(*Teleostei: Cyprinidae) and *Squalius fellowesii* Gunther, 1868 (Teleostei: Cypriniformes) both collected from Doğanbaba Creek with prevalence and mean intensity varying from 23.3% and 6.1 parasite/fish to 30.9% and 3 parasite/fish respectively (Unal et al., 2017; İnnal et al., 2020). These findings are inconsistent with ours. Considering the above records, this present study adds new data to the infection parameters of this diplozoid species. Furthermore, *Blicca bjoerkna* Linnaeus, 1758 (Cyprinidae) has been designated as a main host for this parasite, despite the fact that this diplozoid species has also been recorded in different studies from various freshwater fish species in Europe and Asia (Matějusová et al., 2001; Al-Nasiri, 2009; Sobecka et al., 2014). As for the infection results of this species in their study: infection prevalence value was 13.7%, 18.2% in *Cyprinion macrostomum*, *Cyprinus carpio* respectively from Tigris River (Al-Nasiri, 2009) 1.8% *Leuciscus idus* from Dabie Lake. Moreover, as far as we know up to now, different diplozoid specimens especially, *P. homoion* have been also recorded in previous studies in Türkiye (see, for example, Koyun, 2001; Öztürk, 2005; Soylu and Emre, 2007; Aydoğdu et al., 2020a,b). In the studies conducted on different fish species distributed in different localities in Türkiye, the prevalence levels of infection of parasite specimens belonging to the genus *Paradiplozoon* varied between 1.3% and 73.6%. However, as the authors, we suggest that the formation and distribution of diplozoid parasite specimens recorded above may be due to a combination of differences in biotic and abiotic ecological characteristics variables of the geographical location. While similarities in the prevalence level of diplozoid specimens in fish living in the same habitat can be explained by similarities in biotic and abiotic factors observed in the same locality. The differences can be attributed to the diversity of biotic and abiotic factors that vary from one aquatic ecosystem to the next.

The seasonal variation of the infection of *P. bliccae* was also investigated in our study. The prevalence and mean intensity levels of this species were both higher in summer (27.5%; 6 parasite/fish respectively) (Table 1). Seasonal variation of *P. bliccae* infection rates has also been studied in different fish species in Türkiye so far (Unal et al., 2017; İnnal et al., 2020). Seasonal variation in infection rates of Aegean chub (*Squalius fellowesii*) by Unal et al., 2017) and Burdur spring minnow (*Pseudophoxinus burduricus*) by İnnal et al., 2020) from Doğanbaba Creek, Burdur have studied. They recorded the highest infection prevalence value of *P. bliccae* in the summer (59% in *S. fellowesii*, 45.4% in *P. burduricus*). However, in both studies, the researchers recorded the highest intensity values of this species in different seasons. Unal et al. (2017) found the highest mean intensity values of this species in autumn samples (3.7 parasite/fish) while İnnal et al. (2020) recorded the highest intensity value of this species in winter samples (11.5 parasite/fish). The season with the highest infection prevalence values for this species recorded in this study was consistent with the above studies, but the season with the highest mean intensity values recorded is inconsistent.

The authors (Koyun, 2001; Öztürk, 2005; Soylu and Emre, 2007; Unal et al., 2017; Aydogdu et al., 2020a,b; İnnal et al., 2020) studied the seasonal variability of *Paradiplozoon* spp. infection in different fish species in Türkiye and suggested that this might be due to a combination of differences in the parasite species, different rates of parasite development in different waters, the host and environmental conditions. We also support their suggestion.

In the present study, the mean intensity and abundance of *P. bliccae* infection were higher in male than female hosts (Table 1). However, the prevalence of this species was higher in females (16.2%) compared to males (11.6%). İnnal et al. (2020) recorded the highest prevalence levels of infection in female individuals of *P. burduricus*. The results of the present study also confirm the findings of İnnal et al., (2020). In contrast to our study, Unal et al., (2017) found the highest prevalence levels of this species in male fish in *S. fellowesii.* According to Rohde (1978) and Kennedy (1972), the parasites may infect both sexes differently. They suggested that this might be resulted to differences in colour, hormonal features, mucus, stress, the different feeding habits and habitat used between sexes. In our study, the host sex had a significant influence on *P. bliccae* infection parameters. The differences in these findings may be the result of the above-mentioned factors varying between sexes. This information may indicate that the female individuals of *P. burduricus* and *C. aydinensis* have similar behaviour or feeding habits.

CONCLUSION

In this study, a total of 187 individuals of endemic fish species were examined for diplozoid parasites from October 2019 to July 2020 in Köyceğiz Lake from Southwestern Anatolia, Türkiye. By using morphological and anatomic assessment, only one diplozoid species, *Paradiplozoon bliccae*, was identified, it was confirmed by molecular characterization that these specimens recorded in the host fish are indeed *P. bliccae*. To the best of our knowledge, this is the second ichthyoparasitological study for *C. aydinensis* in Türkiye as well as the first record of *P. bliccae* from this previously unexplored host and region. It is, therefore, a new locality for the distribution of *P. bliccae*. Furthermore, this study adds new valuable information to the molecular characterization of this species collected from Türkiye. In addition, sequence data of *P. bliccae* from host fish was reported to GenBank for the first time from Köyceğiz Lake with this study. Additionally, this study provides further insight into how the infection parameters of this species vary with seasons and host fish sex.

ACKNOWLEDGEMENTS AND FUNDING

This study was supported by Akdeniz University of Scientific Research Project Unit, FBA 2018-4175. We also thank Dr. Hasan Emre Yılmaz from Akdeniz University, for helping with Bayesian Inference-based molecular analysis and phylogenetic tree. We also thank Fatma Messaoudene from the University of Djelfa, for helping with reading the manuscript.

AUTHOR CONTRIBUTION

Nurten Aydogdu, Nesrin Emre and Ali Aydogdu contributed to the conception, coordination and design of the study. Nesrin Emre collected the samples. Nurten Aydogdu, Nesrin Emre and Ali Aydogdu performed the laboratory activities and organized the database. Nurten Aydoğdu, Nesrin Emre and Ali Aydoğdu conducted on morphological analysis studies for the identification of diplozoid parasite species. Nurten Aydogdu, Ali Aydogdu and Özgür Emiroglu conceived of the study of diplozoid species by molecular analysis, and participated in its design and coordination and helped to confirm the morphological identity of diplozoid species by molecular analysis. Nurten Aydogdu and Ali Aydogdu participated in its design and coordination and helped to draft the manuscript. Nurten Aydogdu and Ali Aydogdu critically oversaw the substantial revisions of the manuscript.

CONFLICT OF INTEREST STATEMENT

All authors have read and agreed to the published version of the manuscript. The authors declare that they have no conflicts of interest. The authors have also nothing to disclose

ETHICAL APPROVAL

No ethical approval was required, as this study did not involve clinical trials or experimental procedures. During the study, no treatment/experiment was implemented on the live animal. All sampling and laboratory work on fish have complied with the Republic of Türkiye Ministry of Agriculture and Forestry Animal Welfare Laws.

DATA AVAILABILITY

Data supporting the results are available in the manuscript.

REFERENCES

Al-Nasiri, F.S. (2009). Diplozoid species (Monogenea) parasitizing gills of some Cyprinid fishes from Tigris River passing through Tikreet city, Salah Al-Deen province. *Iraqi Journal of Agricultural Science*, *14*(5), 182- 186.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., & Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*, 403-410. htttps://doi:10.1016/S0022-2836(05)80360-2

Aydoğdu, N., Avenant-Oldewage, A., Dos Santos, Q.M., & Aydoğdu, A. (2020a). Prevalence and intensity of *Paradiplozoon homoion* (Monogenea: Diplozoidae) from Manyas spirlin, *Alburnoides manyasensis*, an endemic fish of Turkey: new host and geographical record. *Iranian Journal of Fisheries* Sciences, *19*(6), 3301-3309.

Aydogdu, N., Alemdar, A., Torcu –Koc, H., & Erdogan, Z. (2020b). A study of helminth parasites of Bitterling Fish, *Rhodeus amarus* (Pallas, 1782) (Teleostei: Cyprinidae) in Susurluk Stream (Balikesir): First record of *Paradiplozoon homoion* in *Rhodeus amarus* from Türkiye and new record for helminth parasites from Susurluk Stream new record for helminth parasites from Susurluk Stream. *Türk Tarım ve Doğa Bilimleri Dergisi*, *7*(4), 1049-1056. (in Turkish, with English abstract) https://doi.org/10.30910/turkjans.685761

Benovics, M., Vukić, J., Šanda, R., Rahmouni, I., & Šimková, A. (2020). Disentangling the evolutionary history of peri-Mediterranean cyprinids using host-specific gill monogeneans. *International Journal for Parasitology*, *50*(12), 969-984. https://doi.org/10.1016/j.ijpara.2020.05.007

Bush, A.O., Lafferty, K.D., Lotz, J.M., & Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et. al. revisited. *The Journal of Parasitology, 83*, 575-583. https://doi.org/10.2307/3284227

Bychovskaya-Pavlovskaya, Gussev, A.V, Dubinina, M.N., Izyumova, N.A., Simirnova, T.S., Sokolovskaya, I., Shtein, G.A., Shulman, S.S., & Epshtein, V.M. (1962). Key to parasites of freshwater fishes of the U.S.S.R. Moskova–Leningrad: Izdatel'stvo Academy Nauk SSR. (In Russian with English Translation – Israel Program for Scientific Translation), Jerusalem.

Çiçek, E., Fricke, R., Sungur, S., & Eagder, S. (2018.) Endemic freshwater fishes of Turkey. *FishTaxa*, *3*(4), 1-39.

Darriba, D., Taboada, G.L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, *9*, 8, 772. https://doi.org/10.1038/nmeth.2109

Froese, R., & Pauly, D. (2022). Fish Base. World Wide Web Electronic Publication. http://www.fishbase.org

Gussev, A.V. (1985). Monogenea. In O.N. Bauer (Ed.), *Key to Parasites of the Freshwater Fishes of the USSR*. Vol. 2. Nauka, Leningrad.

Huelsenbeck, J.P., Ronquist, F., Nielsen, R., & Bollback, J.P. (2001). Bayesian inference of phylogeny and its impact on evolutionary biology. *Science*, *294*, 5550, 2310–2314. https://doi.org/10.1126/science.1065889

İnnal, D., Ünal, M., Kaya, D.C.Ç., Civanova, K., & Özmen, Ö. (2020). Some parasitological features of *Paradiplozoon bliccae* (Monogenea: Diplozoidae) infestation in *Pseudophoxinus burduricus*-an endemic fish species from Doğanbaba Creek (Lake Salda, Turkey). *Acta Aquatica Turcica*, *16*(4), 498-505. https://doi.org/10.22392/actaquatr.729206

Kennedy, C.R. (1972). Parasite communities in freshwater ecosystems. *Essays in Hydrobiology. University of Exeter Press, Exeter*, 53-68.

Khotenovsky, I.A. (1974). A metod for making preparations of diplozoons. *Zoologichesky Zhurnal*, *53*, 1079- 1080.

Khotenovsky, I.A. (1985). Fauna of the USSR. Monogenea.Suborder Octomacrinea Khotenovsky. New Series No. 132. Nauka, Leningrad, 262 pp. (in Russian).

Koyun, M. (2001). *Helminth fauna of some fish species living in Enne Dame Lake*. Ph.D. Thesis. Uludağ University, Bursa. (in Turkish with English abstract)

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 6, 1547-1549. https://doi.org/10.1093/molbev/msy096

Malmberg, G. (1957). Om förekomsten av *Gyrodactylus* på svenska fiskar. *Skrifter utgivna av Södra Sveriges Fiskeriförening Årsskrift*, 19-76.

Matějusová, I., Koubková, B., D'Amélio, S., & Cunningham, C.O. (2001). Genetic characterization of six species of diplozoids (Monogenea; Diplozoidae). *Parasitology, 123*(5), 465-474. https://doi.org/10.1017/S0031182001008617

Nejat, F., Benovics, M., Řehulková, E., Vukić, J., Šanda, R., Kaya, C., & Šimková, A. (2023). Diversity phylogeny and intraspecific variability of *Paradiplozoon* species (Monogenea: Diplozoidae) parasitizing endemic cyprinoids in the Middle East. *Parasitology*, *1*, 18. https://doi.org/10.1017/S0031182023000446

Özer, A. (2021). Checklist of Marine, Freshwater, and Aquarium Fish Parasites in Turkey. *Turkish Marine Research Foundation (TUDAV)* Publication No: 62, Istanbul, Türkiye, 311 p.

Öztürk, M.O. (2005). Helminth fauna of two Cyprinid fish species (*Chalcalburnus chalcoides* Güldenstad 1972, *Rutilus rutilus* L.) from Lake Uluabat, Türkiye. *Hacettepe Journal of Biology and Chemistry*, *34*, 77-91.

Pecínková, M., Matejusova, I., Koubková, B., & Gelnar, M. (2005). Classification and occurrence of abnormally developed *Paradiplozoon homoion* (Monogenea, Diplozoinae) parasitising gudgeon *Gobio gobio*. Diseases of aquatic organisms, *64*(1), 63-68. https://doi.org/10.3354/dao064063

Rambaut, A. (2014). FigTree. v1. 4.2: Tree Drawing Tool in Computer Program and Documentation Distributed by the Author.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901-904. https://doi.org/10.1093/sysbio/syy032

Rohde, K.(1978). Latitudinal gradients in species diversity and their causes.II. Marine parasitological evidence for a time hypothesis. *Biologisches Zentralblatt*, *97*, 405-418.

Sicard, M., Desmarais, E., & Lambert, A. (2001). Molecular characterisation of Diplozoidae populations on five Cyprinidae species: consequences for host specificity. *Comptes Rendus de l'Académie des Sciences - Series III-Sciences de la Vie*, *324*, 709–717 (in French with English abstract). https://doi.org/10.1016/S0764-4469(01)01352-X

Sobecka, E., Jurkiewicz, E., & Piasecki, W. (2014). Parasite fauna of Ide, *Leuciscus idus* (L.) in Lake Dnbie, Poland. *Acta Ichthyologica Et Piscatoria*, *34*(1), 33-42. https://doi.org/10.3750/AIP2004.34.1.04

Soylu, E., & Emre, Y. (2007). Monogenean and cestode parasites of *Pseudophoxinus antalyae* Bogutskaya, 1992 and *Cyprinus carpio*, Linnaeus 1758 from Kepez Antalya, Türkiye. *Bulletin of European Association of Fish Pathologists*, *27*(1), 23-28.

Turan, D., Küçük, F., Kaya, C., Güçlü, S.S., & Bektaş, Y. (2017). *Capoeta aydinensis*, a new species of scraper from southwestern Anatolia, Türkiye (Teleostei: Cyprinidae). *Turkish Journal of Zoology*, *41*(3), 436-442. https://doi.org/10.3906/zoo-1510-43

Unal, M.C., Innal, D., Civáňová, K., Stavrescu-Bedivan, M.M., Koubková, B., Ozmen, O., & Gelnar, M. (2017). Identification of two gill parasites *Lamproglena compacta* (Copepoda: Lernaeidae) and *Paradiplozoon bliccae* (Monogenea: Diplozoidae) from endemic Aegean chub (*Squalius fellowesii*). *Bulletin of the European Association of Fish Pathologists*, *37*, 135-47.

Yunchis, O.N. (1988). Some ecological factors affecting the monogenean infection of Roach. In O.A. Skarlato (Ed.), *Investigation of Monogeans in the USSR*. Leningrad: Vsesoyuznogo Simpoziuma po Monogeneyam. 1988: 62-65.

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 251-258 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.03

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of different cooking methods on the proximate and fatty acid composition of Atlantic salmon (*Salmo salar*)

Ömer Alper Erdem[1*] ● Mehmet Tolga Dinçer[2]

[1]Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye
[2]Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye

https://orcid.org/0000-0002-1745-1751
https://orcid.org/0000-0003-1867-4305

*Corresponding author: omer.alper.erdem@ege.edu.tr

Received date: 28.07.2023 *Accepted date: 25.10.2023*

How to cite this paper:
Erdem, Ö.A., & Dinçer, M.T. (2023). Effects of different cooking methods on the proximate and fatty acid composition of Atlantic salmon (*Salmo salar*). *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 251-258. https://doi.org/10.12714/egejfas.40.4.03

Abstract: It is essential for human health to maintain a diet rich in unsaturated fatty acids, particularly polyunsaturated (PUFA), composed of omega-3. Atlantic salmon (*Salmo salar*), an important source of omega 3 long chain PUFAs, has a sizeable amount in international seafood trade because it is an abundant source of omega-3 long-chain PUFAs. Despite the fact that cooking fish to high temperatures alters its fat, protein, vitamin, and mineral content, cooked fish is preferred by consumers. The purpose of this research was to compare nutritional, physical and sensory properties of Atlantic salmon cooked in the oven, steamed and also a combination of the two cooking methods. Farmed Atlantic salmon was used to make steaks that were defrosted in the fridge the night before. Cooking methods applied were baking in the oven, steaming, and a combination of oven-baking and steaming. The results showed that the crude protein level of oven-baked salmon meat was greatest among all cooking methods. Heat treatment affected the fatty acid composition of Atlantic salmon flesh, as shown that the total saturated fatty acids of cooked fish groups were higher than those of raw salmon. It was determined that oven baking was the most effective heat treatment for maintaining all lipid characteristics of the meat, including the PUFA concentration and n-3/n-6 ratio. When EPA and DHA values of all cooking groups were compared, combination cooking group has lowest value than other cooking groups.

Keywords: Heat treatment, Atlantic salmon, omega-3, oven-baking, steaming

INTRODUCTION

Individual diet has critical situation at prophylaxis and treatment processes, especially due to preventative medicine. Unsaturated fatty acids (UFA), which the body cannot synthesize on its own, are among the required dietary components for the human body but must be received through diet. The fatty acids, especially omega 3 and omega 6 polyunsaturated fatty acids (PUFA), were mentioned many studies on effects of them on health, and relationships between them and risk factors of the health. American Heart Association (AHA) has recommended enhancing consumption of omega-3 fatty acids for risk reduction of patients with cardiovascular diseases (CVD) (Smith Jr et al., 2011). Also, different studies have given countenance to consumption of PUFAs for the risk reduction in CVD (Wang, 2018; Jackson et al., 2019). Polyunsaturated fatty acids, especially omega 3, can protect against not only CVD also inflammatory diseases, cancer, obesity, diabetes (Gu et al., 2015; Nabavi et al., 2015; Saini and Keum, 2018), Alzheimer's disease (Song et al., 2016), kidney function (Xu et al., 2016) and major depressive disorder (Bigornia et al., 2016; Husted and Bouzinova, 2016). Alpha-Linolenic acid (ALA) (C18:3n3), Eicosapentaenoic acid (EPA) (C20:5) and Docosahexaenoic acid (DHA) (C22:6) are acceptable important omega 3 fatty acids.

One of the major sources of PUFAs is oily fish and/or fish oil. Naturally, fish and seafood consumption tend to increase a few decades. Global fisheries production has totally reached 177.8 million tonnes in 2019. Atlantic salmon (*Salmo salar*), that is a rich omega 3 long chain PUFAs resource, has a special place in the international seafood trade because of its 2.615 million tonnes production from aquaculture and 17.1 billion USD nominal value (FAO, 2021).

Fish meat is obtained by removing head, viscera, frame, fin, gill, and bone from fish. Consumers generally prefer cooked fish meat except non-heat treatment processed products for example pickled, dried and/or salted fish or some ethno-traditional products like sashimi. However, a high temperature has the potential to reduce the number of PUFAs in the flesh, particularly EPA and DHA, by destroying carbon double bonds. Additionally, the application of heat treatment results in a shift in the proportions of total fat, total protein, vitamins, and minerals found in fish meat. Fish cooking uses different techniques such as oven-baking, microwaving, frying (pan- or deep-), poaching, boiling, and grilling etc. These techniques are usually used as domestic cooking. During Covid19 pandemics, most of governments all on the world introduced restrictions for preventing spread of the disease such as closing up restaurants, diners, snack bars etc. Due to the influence of social media, many people have experimented with different culinary methods at home.

Oven-baking, which is a dry-heat cooking technique (Moradi et al., 2011; Sampels, 2015), is one of most used of them. Recent studies have reported that oven baking was best method for nutritional quality and PUFAs of cooked fish (Şengör et al., 2013; Hosseini et al., 2014; Vikøren et al., 2017). In addition, Erdem and Dinçer (2019) have informed that oven baking was optimal cooking technique for nutritional properties of flesh in their study.

Already, mild- and moist-heat treatments like steaming (Moradi et al., 2011; Sampels, 2015) are also popular in household. There are several studies reported that nutritional values of steamed fish meat have been closest those of raw fish meat (Choo et al., 2018; Cano-Estrada et al., 2018; Dong et al., 2018). Oven-baking and steaming are two cooking techniques with different characteristics. The study's objective is to examine the nutritional, sensory, and occasionally physical characteristics of oven-baked, steam-cooked and also combination these methods on Atlantic salmon (*Salmo salar*). In the experimental plan, two different cooking methods were used in combination in order to identify changes in the fatty acid composition and lipid index values of fish meat.

MATERIALS AND METHODS

Fish samples

Salmon steaks from farmed Atlantic salmon (*Salmo salar*) that were shipped from Norway as frozen were acquired from a global supplier and totalled 61 pieces each of 113.89±3.31 g. 10 pieces were used as raw material for proximate composition, fatty acids composition, lipid quality indices and colour measurement. Before cooking, each sample was defrosted overnight in a refrigerator at 4°C.

Cooking methods

The cooking time of 18 minutes was determined based on the salmon meat's ability to maintain a consistent internal temperature of 63°C during the steaming process (USDA, 2023). Three different techniques were used. First batch used 17 Atlantic salmon pieces was cooked by using oven, second batch used 17 Atlantic salmon pieces was cooked by using water vapour and the last process used 17 Atlantic salmon pieces was the combination of both two cooking techniques. For oven baking, 18 minutes heating at 180°C were used within a conventional oven as preheated (Öztiryakiler Industrial Conventional Oven GN 1/1 Model, Turkey). The final internal temperature of oven-baked salmon meat was recorded at 70.8°C. For steaming, water (250 ml) was boiled in cooking pot (diameter: 20 cm; height: 12 cm) and a stainless-steel mesh strainer was placed in same pot. During steaming, salmon meat did not touch water just the vapour was used. Steaming occurred for 18 minutes at 100°C. For the combination method, steaming was firstly applied for 9 minutes, and then oven baking was performed for 9 minutes.

Proximate composition

Atlantic salmon were homogenised using an electric chopper (Arzum AR1021, Turkey). Moisture and ash analyses were gravimetrically performed salmon pieces by placing into a glass petri dish at 105°C and a porcelain crucible at 550°C, respectively (Ludorf and Meyer, 1973). Sample and reagent for crude oil analysis (chloroform / methanol; 2/1) were mixed with a homogeniser, then solvents in permeate was evaporated (Bligh and Dyer, 1959). Crude oil percentage was calculated gravimetrically. Kjeldahl method was utilised for crude protein analysis (N×6.25) via Gerhardt Vapodest 40 according to A.O.A.C. (1984) official method.

Fatty acids analysis

Atlantic salmon steak oil that had been extracted from raw and cooked salmon steak was analysed using gas chromatography (GC) (Shimadzu Corp., Kyoto, Japan) to ascertain its fatty acids composition (FAC). Methyl esters were prepared by dissolving 10 mg of extracted oil in 2 mL of n-hexane and then adding 4 mL of (2 mol/L) KOH to methanol, as described by Ichihara et al. (1996). After centrifugation at 1792g, the N-hexane layer was separated out (Özogul and Özogul, 2007). Capillary column GC (HP-88; 100 m 0.25 mm i.d., 0.20 m film thickness; Agilent Technologies International Japan, Tokyo, Japan) equipped with a flame ionisation detector was used to identify fatty acid methyl esters. First, the column was heated from 120 to 170 °C at a rate of 10 °C/min, then it was heated to 250 °C at a rate of 4 °C/min, and finally it was maintained at 250 °C for 5 minutes. The flame ionisation detector was heated to a comfortable 260°C. A 1L sample was divided and injected at a 1:50 ratio. The concentration was determined by comparing the peak area of the samples to that of the mix standard. The average percentage of the overall FAME area was used to express the results of the three replicate GC analyses.

Lipid quality

The atherogenic and thrombogenic indices (AI and TI, respectively) were derived using Ulbricht and Southgate equations (1991) to assess the likelihood that cooked samples would cause coronary heart disease. However, it is important to prioritise health while choosing a cooking method. Hypocholesterolemic/Hypercholesterolemic ratio (h/H) was calculated according to Santos-Silva et al. (2002). Flesh lipid quality index is calculated by relating the levels of the principal polyunsaturated fatty acids (PUFAs) n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the flesh to the overall lipid content of the flesh as a percentage (Abrami et al., 1992; Senso et al., 2007).

Cooking loss

Using an equation inspired by Gluchowski et al. (2020), cooking loss was determined as a percentage from the difference in weights of the Atlantic salmon flesh before and after cooking.

Colour measurement

The colour measurement method reported by Schubring (2003) was used. L^*(lightness), a^* (redness) and b^* (yellowness) values of CIElab colour space, were determined with measuring 10 times from the raw and cooked Atlantic salmon placed into a glass petri dish, after steaks mixed homogeneously by a chopper.

Sensory evaluation

Seven assessors with experience in the sensory evaluation of fish products used a methodology described by Carbonell et

al. (2002). Odour, appearance, flavour, texture, and general acceptance were all rated as sensory criteria. The evaluation of attributes ranged from 1 (weak) to 5 (intense).

Statistical analysis

The data was displayed as mean standard deviation. One-way ANOVA analysis was used to describe the variations in means. To examine for significant differences between the mean values of the various outcomes, the SPSS 25 program's Tukey test was utilised. P<0.05 was used as the significance level for all groups.

RESULTS AND DISCUSSION

Results of proximate analyses

Proximate compositional effects of different cooking techniques on salmon meat can be seen at Table 1.

Table 1. Proximate composition results (g/100 g)

Proximate composition	Raw salmon	Oven baking	Steaming	Combination cooking
Crude protein	19.33±0.44^{a}	19.90±0.76^{ab}	22.53±0.20^{b}	23.14±1.97^{b}
Crude fat	14.52±0.13^{a}	26.38±1.87^{b}	20.52±0.72^{c}	19.38±0.38^{c}
Moisture	63.00±0.42^{a}	51.26±0.54^{b}	53.74±0.60^{c}	54.53±0.59^{c}
Ash	1.24±0.17^{a}	1.14±0.01^{a}	1.24±0.08^{a}	1.08±0.02^{a}

At the level of P<0.05 significances, the same letter of meaning on the same line do not differ in any appreciable ways

In the study, the untreated group is defined as the raw salmon group (control group) to be used in comparisons to determine the effects of cooking techniques. As shown in Table 1 raw salmon steaks have the lowest crude fat percentage than those of cooked salmon groups (Table 1). The cooked groups demonstrated a significant increase in crude fat percentages as a result of water extraction in salmon meat, as anticipated due to the use of heat treatment. This situation depends on cooking time and temperature as mentioned before (Bastias et al., 2017). The findings indicate that the oven-baked salmon group exhibits the highest crude fat percentage, which aligns with the findings of previous studies (Şengör et al., 2013; Hosseini et al., 2014; Nieva-Echevarría et al., 2018). Oven-baking as a dry-heat cooking method may accelerates evaporation from salmon meat.

The moisture percentage of raw salmon is 1.23, 1.17 and 1.15 times more than oven-baking, steaming and combination cooking, respectively. Crude fat contents of oven-baking, steaming and combination cooking groups, are respectively approximately 1.82, 1.41 and 1.33 times more than uncooked salmon's. Although all cooking groups were used the same time (18 minutes), temperature used in oven-baking affected moisture and crude fat percentage. Therefore, crude fat and moisture contents of oven baking salmon meat have showed statistically significance (P<0.05). This result can be explained as the low amount of moisture in the oven environment and the high amount of evaporated water. There are studies informed that oven baked salmon meat has higher lipid percentage than steamed salmon meat vice versa moisture percentage (Larsen et al., 2010; Şengör et al., 2013; Fomena Temgoua et al., 2022). There was no statistically significant difference observed in the ash contents among all groups (P<0.05).

Cooked salmon steaks by a combination method of steaming and oven baking has shown highest crude protein content. Although, crude protein values of oven-baked salmon is higher than those of steamed salmon in recent studies (Bastias et al., 2017; Gluchowski et al., 2020; Fomena Temgoua et al., 2022), steamed salmon's protein percentage is higher than that of oven-baked salmon in the present study. There was no statistically significant difference among the groups of cooked salmon in terms of crude protein content (P>0.05). Oven-baked salmon meat has shown the lowest protein value in all cooked groups. Protein denaturalization may be discussed for oven-baked salmon meat; as a consequence, it was exposed to higher temperatures for a longer time than the others.

Fatty acids composition

Table 2 shows how different cooking techniques affect the fatty acid composition of Atlantic salmon meat. As a consequence of heat treatment, total saturated fatty acids (∑SFA) of cooked salmon groups increased according to initial value of raw salmon in Table 2. While comparing the three prepared groups, it was observed that the steamed group had the lowest increase in ∑SFA. However, it was noted that the level of palmitic acid (C16:0) in the steamed salmon meat increased, but it reduced in both the oven-baked group and the combination cooking group. There was no statistically significant difference (P<0.05) observed in the value of C16:0 between steaming salmon meat and raw salmon. Although stearic acid (C18:0) was no in raw salmon meat, was seen in both oven-baked and combination cooked salmon meat groups.

The application of the oven temperature of 180°C has the potential to induce lipid oxidation and lipid hydrolysis in crude fats of salmon meat. The oven-baking group has the lowest values of both total monounsaturated fatty acids (∑MUFA) at 47.12 and oleic acid (C18:1n9c) at 38.44%. Linoleic acid (C18:2n6c) has shown reductions for oven-baked and combination cooked salmon in comparison with the initial value because of probably high temperature and dry heating from the oven. The statistical analysis of eicosapentaenoic acid (EPA) (C20:5) and docosahexaenoic acid (DHA) (C22:6) levels in salmon meat indicates that there is a significant difference (P<0.05) between the steaming and combination cooking methods (oven baking and steaming) compared to the raw and oven baking methods. The combination cooking of salmon meat has been found to yield a statistically significant difference (P<0.05) in the α-linolenic acid (ALA) (C18:3n3) content, with the lowest value seen compared to the other groups. The EPA and ALA values of all cooked salmon groups exhibited a decrease as a result of heat treatment. Nevertheless, the cooked groups, excluding oven baking, exhibited a decrease in DHA value.

Table 2. Fatty acid composition results of groups (g/100 g of total fatty acids)

Fatty acids	Raw salmon	Oven baking	Steaming	Combination cooking
C4:0	0.04±0.00^{a}	0.04±0.00^{a}	0.04±0.01^{a}	0.05±0.00^{a}
C8:0	0.01±0.00^{a}	0.0±0.0^{a}	0.02±0.00^{b}	0.02±0.00^{b}
C10:0	0.00±0.00^{a}	0.00±0.00^{b}	0.01±0.00^{c}	0.01±0.00^{ac}
C12:0	0.04±0.01^{a}	0.03±0.00^{a}	0.04±0.00^{a}	0.03±0.00^{a}
C13:0	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}
C14:0	1.89±0.02^{a}	1.85±0.02^{a}	1.99±0.02^{b}	1.88±0.01^{a}
C15:0	0.16±0.00^{a}	0.16±0.00^{a}	0.18±0.00^{b}	0.16±0.00^{a}
C16:0	10.25±0.04^{a}	9.67±0.07^{b}	10.36±0.05^{a}	9.91±0.02^{c}
C17:0	0.17±0.00^{a}	0.16±0.00^{b}	0.18±0.00^{c}	0.17±0.00^{a}
C18:0	0.0±0.0^{a}	3.09±0.02^{b}	0.0±0.0^{a}	3.20±0.01^{c}
C20:0	0.55±0.01^{a}	0.55±0.05^{a}	0.59±0.03^{a}	0.55±0.00^{a}
C21:0	0.02±0.00^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.02±0.00^{c}
C22:0	0.24±0.00^{a}	0.24±0.03^{a}	0.25±0.03^{a}	0.24±0.00^{a}
C23:0	0.28±0.03^{a}	0.30±0.02^{a}	0.27±0.03^{a}	0.27±0.01^{a}
C24:0	0.14±0.01^{a}	0.11±0.01^{b}	0.0±0.0^{a}	0.11±0.00^{c}
∑SFA	13.80	16.21	13.94	16.63
C14:1	0.01±0.00^{a}	0.02±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}
C16:1	2.27±0.02^{a}	2.09±0.02^{b}	2.14±0.01^{c}	2.12±0.01^{bc}
C18:1n9t	0.05±0.01^{a}	0.0±0.0^{b}	0.05±0.00^{a}	0.05±0.00^{a}
C18:1n9c	39.60±0.03^{ac}	38.44±0.41^{b}	40.13±0.14^{a}	39.08±0.04^{c}
C20:1	5.43±0.04^{a}	5.39±0.06^{a}	5.78±0.02^{b}	5.56±0.01^{c}
C22:1n9	0.85±0.03^{a}	0.82±0.04^{a}	0.87±0.01^{a}	0.84±0.01^{a}
C24:1	0.47±0.02^{a}	0.40±0.0^{b}	0.47±0.02^{a}	0.42±0.00^{b}
∑MUFA	48.68	47.12	49.45	48.08
C18:2n6c	16.70±0.03^{a}	16.14±0.14^{b}	16.84±0.07^{a}	16.14±0.01^{b}
C18:3n6	0.10±0.01^{ac}	0.13±0.01^{b}	0.10±0.00^{a}	0.11±0.00^{c}
C18:3n3	10.40±0.02^{a}	10.07±0.06^{b}	10.07±0.03^{b}	9.70±0.01^{c}
C20:2	1.72±0.01^{a}	1.63±0.10^{a}	1.72±0.03^{a}	1.64±0.01^{a}
C20:3n6	0.21±0.00^{a}	0.29±0.01^{b}	0.22±0.02^{a}	0.25±0.01^{c}
C20:3n3	1.01±0.00^{a}	1.03±0.02^{a}	1.06±0.10^{a}	1.00±0.01^{a}
C22:2	0.18±0.04^{a}	0.14±0.01^{a}	0.15±0.04^{a}	0.12±0.03^{a}
C20:5	2.82±0.04^{a}	2.68±0.03^{b}	2.47±0.05^{c}	2.42±0.01^{c}
C22:6	4.37±0.03^{a}	4.53±0.06^{b}	3.97±0.03^{c}	3.89±0.01^{c}
∑PUFA	37.51	36.64	36.60	35.27
PUFA/SFA	2.71	2.26	2.62	2.12
Σn6	17.02±0.03^{a}	16.55±0.15^{b}	17.17±0.07^{a}	16.50±0.01^{b}
Σn3	18.61±0.05^{a}	18.30±0.16^{a}	17.57±0.15^{b}	17.01±0.04^{b}
Σn3/Σn6	1.09	1.10	1.02	1.03
DHA/EPA	1.55	1.69	1.60	1.61

At the level of P<0.05 significances, the same letter of meaning on the same line do not differ in any appreciable ways

The samples were analysed using a combination cooking method to find the lowest values for several parameters, including total polyunsaturated fatty acids (∑PUFA), the ratio of polyunsaturated fatty acids to saturated fatty acids (PUFA/SFA), total omega 6 fatty acids (∑n6), and total omega 3 fatty acids (∑n3). The group that undergoes oven baking exhibits the highest value for the ratios of ∑n3/∑n6 and DHA/EPA when compared to all other groups. Even though ∑n6 values for the steamed group were found to be higher than that of the uncooked samples, ∑n6 values fell after cooking the salmon meat groups using oven-baking and combination cooking methods, as seen in Table 2. However, these differences have no statistically meaning (P<0.05). DHA/EPA was seen to be lower in raw salmon compared to the three other groups, although PUFA/SFA ratio of raw salmon was the highest value.

Oven baking, out of all the thermo-processes examined, was shown to be the most efficient heat treatment for preserving all lipid attributes of the meat, such as the PUFA content and n-3/n-6 ratio (Table 2). The effects shown in previous studies exhibit variation contingent upon the cooking manner employed and the impact of heat (Garcia-Arias et al., 2003; Gladyshev et al., 2006; Sioen et al., 2006; Schneedorferova et al., 2015). The consumption of foods that are abundant in omega-3 polyunsaturated fatty acids (PUFAs) is associated with numerous advantages, primarily attributed to their good impact on human well-being. However, the thermal treatments had a significant impact on the most widely recognised EPA and ∑PUFA, observed across all groups, with the combination cooking group exhibiting the lowest levels of these substances. A same movement was likewise detected in ALA. The three main types of n-3 polyunsaturated fatty acids, namely ALA, DHA and EPA, are predominantly obtained from seafood and are effectively utilised by the human body (Ng, 2006). According to Grosso et al. (2014), the primary omega-3 FA, ALA, can be converted into long-chain PUFA as well as DHA and EPA. The aforementioned results provide further support for the argument that ALA remains a viable precursor for the synthesis of EPA and DHA, primarily due to its limited influence during the thermal processing. The effects of various cooking techniques on the fatty acid content of different fish species have been the subject of numerous research.

According to a study conducted by Koubaa et al. (2012), the effects of steaming and oven-baking on the fatty acid profiles of cooked fish species were found to have minimal affect. Similarly, Moradi et al. (2009), indicated that the oven-baking technique resulted in little alterations in both the fat content and fatty acid composition of the fillets of *Parastromateus niager*. Furthermore, the utilisation of the oven baking technique resulted in an enhancement in the quantity of EPA and DHA in grams within the meat of New Zealand king salmon (Larsen et al., 2010). The ratio of n3/n6 fatty acids in the oven-baked salmon was found to be higher in comparison to the steamed salmon (Şengör et al., 2013). On the other hand, a previous study conducted by Fomena Temgoua et al. (2022), has reported that the concentration of n-3/n-6 and EPA+DHA in steamed salmon is significantly higher ($p < 0.05$) compared to the oven-baking treatment. The preservation of EPA and DHA content in rainbow trout is maximised through the process of steaming (Cano-Estrada et al., 2018). The findings in the present study indicate that oven-baking had a higher positive impact on the content of n3/n6, EPA, DHA, and ∑PUFA in salmon meat compared to steaming. The observed

variations in outcomes among same cooking processes, as previously discussed, may be attributed to factors such as the application method, cooking duration, or temperature.

Values of lipid quality indexes

Lipid quality indices' results can be seen in Table 3. Lower values of both AI and TI indicate superior nutritional quality of fatty acids. Consequently, diets characterised by low AI and TI values have the potential to mitigate the risk of developing coronary heart disease (CHD). Higher values of the h/H ratio are beneficial when considering the specific effects of fatty acids on cholesterol metabolism. h/H ratio possesses the capacity to offer a more accurate evaluation of the influence of fatty acid composition on cardiovascular disease (Karimian-Khosroshahi et al., 2016; Chen and Liu, 2020). FLQ index can be regarded as an adjunct to EPA + DHA, given that the absolute quantity of EPA and DHA holds greater significance (Senso et al., 2007; Chen and Liu, 2020). Oven-baked salmon flesh has lowest value of AI and highest value of FLQ and h/H. However, steamed salmon flesh has highest value of AI and, lowest value of FLQ, TI and h/H. For AI, determined raw salmon value has no statistically difference (P>0.05) when compared with oven baking and combination cooking groups, separately. Highest value of TI is 0.17 was determined in combination cooking groups. h/H ratios of raw and combination cooking salmon showed no statistically significance (P>0.05) but statistical differences were determined in oven baking and steaming groups.

Table 3. Results of lipid quality indexes (%)

Lipid Quality Indexes	Raw salmon	Oven baking	Steaming	Combination cooking
AI (%)	0.21±0.0^{ac}	0.20±0.0^{a}	0.22±0.0^{b}	0.21±0.0^{c}
TI (%)	0.14±0.0^{a}	0.15±0.02^{ab}	0.14±0.0^{a}	0.17±0.0^{b}
FLQ (%)	7.19±0.07^{a}	7.21±0.09^{a}	6.32±0.02^{b}	6.43±0.06^{b}
h/H (%)	5.75±0.02^{a}	5.88±0.04^{b}	5.65±0.03^{c}	5.74±0.02^{a}

At the level of P<0.05 significances, the same letter of meaning on the same line do not differ in any appreciable ways. AI: Atherogenic Index. TI: Thrombogenic Index. FLQ: Flesh Lipid Quality Index. h/H: Hypocholesterolemic/ Hypercholesterolemic ratio

Cooking loss results

Heat treatment effects on cooking loss values on salmon flesh can be seen at Table 4.

Table 4. Initial and cooked weights (g) and cooking loss percentages of salmon steaks

	Oven baking	Steaming	Combination cooking
Initial weight (g)	114.21±2.30^{a}	115.22±4.27^{a}	113.29±4.15^{a}
Cooked weight (g)	100.30±1.86^{a}	102.37±3.83^{a}	101.11±3.71^{a}
Cooking loss (%)	12.17±0.15^{a}	12.24±0.07^{a}	9,63±0.12^{b}

At the level of P<0.05 significances, the same letter of meaning on the same line does not differ in any appreciable ways

Water and volatiles within salmon meat retires with increase of heat and temperature. This leads to a loss at total weight. Cooking loss percentages for three cooking methods were given at Table 4. Steamed salmon steaks showed highest percentage of cooking loss. Salmon steaks by cooking combination methods had the lowest cooking loss value. Between the oven baking and steaming techniques no statistical difference was determined. However, significant differences were seen when compared with these two techniques with the combination cooking techniques. The combination method's water holding capacity was greater than that of the other procedures, as shown in Table 4. This may be the reason of heat treatment techniques and time. Combination method first treatment was steaming (9 min) this process may keep the moisture of the salmon meat later oven baking (9 min) treatment decrease the moisture of the salmon meat (evaporation). But the process time of the heat treatments were 50% lower than each techniques, this was the reason of determining higher water holding capacity (lower Cooking loss). Similar results were also determined in previous studies in Szlinder-Richert and Malesa-Ciecwierz, 2018. Although cooking loss value depends on cooking method and fish species (Szlinder-Richert and Malesa-Ciecwierz, 2018), the results of oven baked and steamed salmon steaks have nearly given the same values. However, 11.6% value of cooking loss of salmon fillet by steamed similarly above-mentioned method (Gluchowski et al., 2020) was not statistically differed the present steamed salmon steaks' value. Steaming, a relatively mild heating process, is expected reducing cooking losses (Wang et al., 2020).

Colour measurements

Following the cooking process, the lightness parameters (*L**) of the salmon steaks exhibited an increase (Table 5). Statistically significance (P<0.05) between *L** results of cooked salmon meat groups and raw salmon meat was determined. Salmon meat cooked by combination method of oven baking and steaming has highest *L** value, but lowest *a** (redness) and *b** (yellowness) value. *a** value of oven baked salmon meat increased while the others decreased after cooking and differed statistically significance (P<0.05) from other cooked salmon meat groups.

Table 5. Colour measurements

	Raw salmon	Oven baking	Steaming	Combination cooking
L*	63.42±3.71^{a}	72.40±6.30^{b}	76.44±7.90^{b}	76.55±4.17^{b}
a*	13.63±0.85^{a}	13.97±0.53^{a}	11.01±0.97^{b}	10.86±0.75^{b}
b*	21.87±1.73^{a}	20.82±2.39^{a}	20.12±1.66^{ab}	18.35±1.59^{b}

At the level of P<0.05 significances, the same letter of meaning on the same line do not differ in any appreciable ways

The observed increases in *L** values among the cooked salmon meat groups may be attributed to the denaturation of proteins and subsequent whitening of the meat that occurs during the heating process (Wan et al., 2019). Wang et al. (2020) reported the natural pink colour of the whitefish (*Coregonus peled*) muscle rapidly transitions to a pale whitish colour during the process of steam cooking. This change is accompanied by an increase in the *L** value, reaching a high

reading at a specific time point. The observed rise in *L** value during the initial phase of steaming can be attributed to the progressive denaturation of myosin and the oxidation of haemoglobin and myoglobin. As the myosin underwent incremental modifications, the pigmentation of the fish progressively transitioned to a white colour (Wan et al., 2019; Wang et al., 2020). In the context of combination cooking, the L* value exhibited a modest increase compared to that of steamed salmon meat. This might perhaps be attributed to the occurrence of browning events on involving proteins, or amines, as well as protein-lipid reactions. The changes in the *a** values of steamed and combination cooked samples were impacted by the oxidation of haemoglobin that takes place during the cooking process, which may lead to a reduction in the *a** values (Thanonkaew et al., 2006; Wan et al., 2019).

Sensory evaluation

Sensorial panel results of the groups can be seen at Figure 1. Oven-baked salmon showed highest scores in appearance, odour and flavour. Steamed salmon has given lowest results for all criteria except odour. Odour scores of oven baked, steamed and combination-cooked salmon was respectively presented 4.14±0.69, 3.42±0.78 and 3.28±0.75. No statistical differences were found in all criteria (P<0.05).

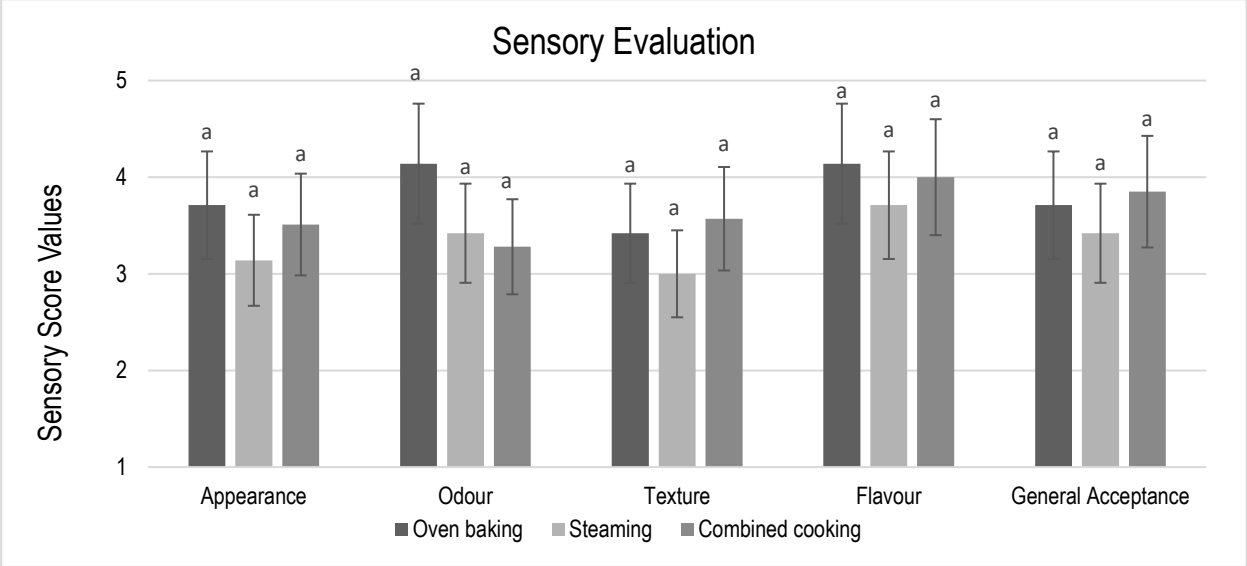

Figure 1. Sensory evaluation results of cooked salmons

Due to the results preferences in parameter of general acceptance are given as follows; Combination cooking> Oven baking>steaming. A comprehensive assessment of sensory criteria has been conducted by Larsen et al. (2011), revealing that the oven-baking procedure resulted in an enhanced preference for salmon meat. In the sensory evaluation of king salmon subjected to various heat treatments, it was observed that oven-baking was preferred above alternative methods such as steaming. In the sensory evaluation, oven-baked king salmon received the highest ratings in terms of colour, texture, and scent criteria, indicating its superior quality. Conversely, steamed king salmon was found to be less attractive in comparison (Larsen et al., 2011). The firm and chewy texture of oven-baked salmon meat is a result of the dehydration process that takes place during preparation, which reduces its moisture content. The high temperatures can also enhance the formation of corresponding smells, tastes, and flavours. In a study conducted by Alexi et al. (2019), it was shown that both steam-cooking and oven-cooking methods yielded comparable sensory profiles for meagre and gilthead seabream. The varying impact of culinary techniques on the lipid and sensory attributes of meagre and gilthead seabream suggests that a universal guideline cannot be used. Instead, the selection of a specific preparation method should be contingent upon the fish species and their corresponding fat composition.

CONCLUSION

The primary objective of this study was to investigate the effects of combining oven baking and steaming techniques on the nutritional characteristics, fatty acid content, and lipid indices of Atlantic salmon. While heat treatments of salmon meat decreased Σn3 values, oven baking salmon meat was affected at least. The Atlantic Salmon exhibited an elevation in Σn6 fatty acid content during the steaming procedure. The study findings indicate that the combination cooking method yields the most favourable value in terms of PUFA/SFA ratio. Based on the analysis of Σn3/Σn6 and DHA/EPA values, it can be concluded that oven-baking is marginally more efficient compared to other heat treatments.

ACKNOWLEDGEMENTS AND FUNDING

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.

AUTHOR CONTRIBUTIONS

Ömer Alper Erdem: Performed heat treatment processes, proximate analyses, cook loss, colour measurements, sensory evaluation, statistical analyses, writing- original draft. Mehmet Tolga Dinçer: Performed supplies frozen Atlantic salmon

steaks, heat treatment processes, cook loss, sensory evaluation, fatty acids composition, statistical analyses, writing-original draft.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

DATA AVAILABILITY

For any questions, the corresponding author should be contacted.

REFERENCES

A.O.A.C., (1984). Association of Official Analytical Chemists. 14th Ed., Method 935.47, Washington, DC, USA.

Abrami, G., Natiello, F., Bronzi, P., McKenzie, D., Bolis, L., & Agradi, E. (1992). A comparison of highly unsaturated fatty acid levels in wild and farmed eels (*Anguilla anguilla*). *Comparative Biochemistry and Physiology Part B*, *101*,79-81. https://doi.org/10.1016/0305-0491(92)90161-J

Alexi, N., Kogiannou, D., Oikonomopoulou, I., Kalogeropoulos, N., Byrne, D. V., & Grigorakis, K. (2019). Culinary preparation effects on lipid and sensory quality of farmed gilthead seabream (*Sparus aurata*) and meagre (*Argyrosomus regius*): An inter-species comparison. *Food Chemistry*, *301*, 125263. https://doi.org/10.1016/j.foodchem.2019.125263

Bastias, J.M., Balladares, P., Acuna, S., Quevedo, R., & Munoz, O. (2017). Determining the effect of different cooking methods on the nutritional composition of salmon (*Salmo salar*) and Chilean jack mackerel (*Trachurus murphyi*) fillets. *PLOS ONE*, *12*(7), Article ID e0180993. https://doi.org/10.1371/journal.pone.0180993

Bigornia, S.J., Harris, W.S., Falcon, L.M., Ordovas, J.M., Lai, C.Q., & Tucker, K.L. (2016). The omega-3 index is inversely associated with depressive symptoms among individuals with elevated oxidative stress biomarkers. *The Journal of Nutrition*, *146*(4), 758-766. https://doi.org/10.3945/jn.115.222562

Bligh, E.G., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911-917. https://doi.org/10.1139/o59-099

Cano-Estrada, A., Castañeda-Ovando, A., Ramírez-Godinez, J., & E. Contreras-López, E. (2018). Proximate and fatty acid composition in raw and cooked muscle tissue of farmed rainbow trout (*Oncorhynchus mykiss*) fed with commercial fishmeal. *Journal of Food Processing and Preservation*, *42*(8), Article ID e13674. https://doi.org/10.1111/jfpp.13674

Carbonell, I., Izquierdo, L., & Costell, E. (2002). Sensory profiling of cooked gilthead sea bream (*Sparus aurata*): sensory evaluation procedures and panel training. *Food Science and Technology International*, *8*(3), 169-177. https://doi.org/10.1177/1082013202008003641

Chen, J., & Liu, H. (2020). Nutritional indices for assessing fatty acids: A mini-review. *International Journal of Molecular Sciences*, *21*(16), 5695. https://doi.org/10.3390/ijms21165695

Choo, P.Y., Azlana, A., & Khooa, H.E. (2018). Cooking methods affect total fatty acid composition and retention of DHA and EPA in selected fish fillets. *Science Asia*, *44*, 92-101. https://doi.org/10.2306/scienceasia1513-1874.2018.44.092

Dong, X.P., Li, D.Y., Huang, Y., Wu, Q., Liu, W.T., Qin, L., Zhou, D.Y., Prakash, S., & Yu, C.X. (2018). Nutritional value and flavor of turbot (*Scophthalmus maximus*) muscle as affected by cooking methods. *International Journal of Food Properties*, *21*, 1972-1985. https://doi.org/10.1080/10942912.2018.1494196

Erdem, O.A., & Dinçer, M.T. (2019). Evaluation of nutritional, physical and sensory properties of flathead grey mullet's (*Mugil cephalus*) flesh after cooking. Journal of Food Safety And Food Quality-Archiv Fur Lebensmittelhygiene, *70*(5), 149-155.

FAO. (2021). Yearbook of Fisheries and Aquaculture Statistics 2019. Rome.

Fomena Temgoua, N.S., Sun, Z., Okoye, C.O., & Pan, H. (2022). Fatty acid profile, physicochemical composition, and sensory properties of Atlantic salmon fish (*Salmo salar*) during different culinary treatments. *Journal of Food Quality*, Article ID 7425142. https://doi.org/10.1155/2022/7425142

Garcia-Arias, M.T., Alvarez-Pontes, E., Garcia-Linares, M.C., Garcia-Fernandez, M.C., & Sanchez-Muniz, F.J. (2003). Cooking-freezing-reheating (CFR) of sardine (*Sardina pilchardus*) fillets. Effect of different cooking and reheating procedures on the proximate and fatty acid compositions. *Food Chemistry*, *83*(3), 349-356. https://doi.org/10.1016/S0308-8146(03)00095-5

Gladyshev, M.I., Sushchik, N.N., Gubanenko, G.A., Demirchieva, S.M., & Kalachova, G.S. (2006). Effect of way of cooking on content of essential polyunsaturated fatty acids in muscle tissue of humpback salmon (*Oncorhynchus gorbuscha*). *Food Chemistry*, *96*(3), 446-451. https://doi.org/10.1016/j.foodchem.2005.02.034

Głuchowski, A., Czarniecka-Skubina, E., & Rutkowska, J. (2020). Salmon (*Salmo salar*) cooking: achieving optimal quality on select nutritional and microbiological safety characteristics for ready-to-eat and stored products. *Molecules*, *25*, 5661. https://doi.org/10.3390/molecules25235661

Grosso, G., Galvano, F., Marventano, S., Malaguarnera, M., Bucolo, C., Drago, F., & Caraci, F. (2014). Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. *Oxidative Medicine and Cellular Longevity*, Article ID 313570. https://doi.org/10.1155/2014/313570

Gu, Z., Shan, K., Chen, H., & Chen, Y.Q. (2015). Polyunsaturated fatty acids and their role in cancer chemoprevention. *Current Pharmacology Report*, *1*, 283-294, 2015. https://doi.org/10.1007/s40495-015-0043-9

Hosseini, H., Mahmoudzadeh, M., Rezaei, M., Mahmoudzadeh, L., Khaksar, R., Khosroshahi, N.K. & A. Badakhani, A. (2014). Effect of different cooking methods on minerals, vitamins and nutritional quality indices of kutum roach (*Rutilus frisii kutum*). *Food Chemistry*, *148*, 86-91. https://doi.org/10.1016/j.foodchem.2013.10.012

Karimian-Khosroshahi, N., Hosseini, H., Rezaei, M., Khaksar, R., & Mahmoudzadeh, M. (2016). Effect of different cooking methods on minerals, vitamins, and nutritional quality indices of rainbow trout (*Oncorhynchus mykiss*). *International Journal of Food Properties*, *19*(11), 2471-2480. https://doi.org/10.1080/10942912.2015.1039028

Husted, K.S., & Bouzinova, E.V. (2016). The importance of n-6/n-3 fatty acids ratio in the major depressive disorder. *Medicina* (*Kaunas*), *52*, 139-147. https://doi.org/10.1016/j.medici.2016.05.003

Ichihara, K., Shibahara, A., Yamamoto, K., & Nakayama, T. (1996) An improved method for rapid analysis of the fatty acids of glycerolipids. *Lipid*s, *31*(5), 535-539. https://doi.org/10.1007/BF02522648

Jackson, K.H., Polreis, J.M., Tintle, N.L., Kris-Etherton, P.M., & Harris, W.S. (2019). Association of reported fish intake and supplementation status with the omega-3 index. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *142*, 4-10. https://doi.org/10.1016/j.plefa.2019.01.002

Koubaa, A., Mihoubi, N.B., Abdelmouleh, A., & Bouain, A. (2012). Comparison of the effects of four cooking methods on fatty acid profiles and nutritional composition of red mullet (*Mullus barbatus*) muscle. *Food Science and Biotechnology*, *21*, 1243-1250. https://doi.org/10.1007/s10068-012-0163-5

Larsen, D., Quek, S.Y., & Eyres, L. (2010). Effect of cooking method on the fatty acid profile of New Zealand King Salmon (*Oncorhynchus tshawytscha*). Food Chemistry, *119*(2), 785-790. https://doi.org/10.1016/j.foodchem.2009.07.037

Larsen, D., Quek, S.Y., & Eyres, L. (2011). Evaluating instrumental colour and texture of thermally treated New Zealand King Salmon (*Oncorhynchus tshawytscha*) and their relation to sensory properties. *LWT-Food Science and Technology*, *44*(8), 1814-1820. https://doi.org/10.1016/j.lwt.2011.03.018

Ludorff, W., & Meyer, V. (1973) Fische und Fischerzeugnisse. 2nd ed. Berlin und Hamburg: Verlag Paul Parey.

Moradi, Y., Bakar, J., Motalebi, A.A., Syed Muhamad, S.H., & Che Man, Y. (2011). A review on fish lipid: composition and changes during cooking methods. *Journal of Aquatic Food Product Technology*, *20*, 379-390.

Moradi, Y., Bakar, J., Muhamad, S.S., & Man, Y.C. (2009). Effects of different final cooking methods on physico-chemical properties of breaded fish fillets. *American Journal of Food Technology*, *4*(4), 136-145. https://doi.org/10.1080/10498850.2011.576449

Nabavi, S.F., Bilotto, S., Russo, G.L., Orhan, I.E., Habtemariam, S., Daglia, M., Devi, K.P., Loizzo, M.R., Tundis, R., & Nabavi, S.M. (2015). Omega-3 polyunsaturated fatty acids and cancer: Lessons learned from clinical trials. *Cancer and Metastasis Review*, *34*, 359-380. https://doi.org/10.1007/s10555-015-9572-2

Ng, T.K.W. (2006). Omega-3 fatty acids: Potential sources in the Malaysian diet with the goal towards achieving recommended nutrient intakes. *Malaysian Journal of Nutrition*, *12*(2), 181-188.

Nieva-Echevarría, B., Goicoechea, E., Manzanos, M.J., & Guillén, M.D. (2018). Effects of different cooking methods on the lipids and volatile components of farmed and wild European sea bass (*Dicentrarchus labrax*). *Food Research International*, *103*, 48-58. https://doi.org/10.1016/j.foodres.2017.10.029

Özogul, Y., & Özogul, F. (2007). Fatty acid profiles of commercially important fish species from the Mediterranean, Aegean and Black Seas. *Food Chemistry*, *100*, 1634-1638. https://doi.org/10.1016/j.foodchem.2005.11.047

Saini, R.K., & Keum, Y. (2018). Omega-3 and omega-6 polyunsaturated fatty acids: dietary sources, metabolism, and significance- a review. *Life Sciences*, *203*, 255-267. https://doi.org/10.1016/j.lfs.2018.04.049

Sampels, S. (2015). The effects of processing technologies and preparation on the final quality of fish products. *Trends in Food Science & Technology*, *44*, 131-146. https://doi.org/10.1016/j.tifs.2015.04.003

Santos-Silva, J., Bessa, R.J., & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. *Livestock Production Science*, *77*,187-194. https://doi.org/10.1016/S0301-6226(02)00059-3

Schneedorferova, I., Tomcala, A., & Valterova, I. (2015). Effect of heat treatment on the n3n-6 ratio and content of polyunsaturated fatty acids in fish tissues. *Food Chemistry*, *176*, 205-211. https://doi.org/10.1016/j.foodchem.2014.12.058

Schubring, R. (2003) Colour measurement for the determination of the freshness of fish. In J.B. Luten, J. Oehlenschlager, G. Olafsdottir (Eds.), *Quality of Fish from Catch to Consumer: Labelling, Monitoring and Traceability* (pp. 251-263). Netherlands: Wageningen Academic Publishers.

Şengör, G.F.U., Alakavuk, D.U., & Tosun, Y. (2013). Effect of cooking methods on proximate composition, fatty acid composition, and cholesterol content of Atlantic salmon (*Salmo salar*). *Journal of Aquatic Food Product Technology*, *22*, 160-167. https://doi.org/10.1080/10498850.2011.635839

Senso, L., Suárez, M.D., Ruiz-Cara, T., & Garcia-Gallego, M. (2007). On the possible effects of harvesting season and chilled storage on the fatty acid profile of the fillet of farmed gilthead sea bream (*Sparus aurata*). *Food Chemistry*, *101*, 298-307. https://doi.org/10.1016/j.foodchem.2006.01.036

Sioen, I., Haak, L., Raes, K., Hermans, C., De Henauw, S., De Smet, S., & Van Camp, J. (2006). Effects of pan-frying in margarine and olive oil on the fatty acid composition of cod and salmon. *Food Chemistry*, *98*(4), 609-617. https://doi.org/10.1016/j.foodchem.2005.06.026

Smith Jr, S.C., Benjamin, E.J., Bonow, R.O., Braun, L.T., Creager, M.A., Franklin, B.A., Gibbons, R.J., Grundy, S.M., Hiratzka, L.F., Jones, D.W., Lloyd-Jones, D.M., Minissian, M., Mosca, L., Peterson, E.D., Sacco, R.L., Spertus, J., Stein, J.H., & Taubert, K.A. (2011). AHA/ACCF secondary prevention and risk reduction therapy for patients with coronary and other atherosclerotic vascular disease: 2011 update. *Circulation*, *142*(22), 2458-2473. https://doi.org/10.1161/CIR.0b013e318235eb4d

Song, C., Shieh, C.H., Wu, Y.S., Kalueff, A., Gaikwad, S., & Su, K. (2016). The role of omega-3 polyunsaturated fatty acids eicosapentaenoic and docosahexaenoic acids in the treatment of major depression and Alzheimer's disease: acting separately or synergistically?. *Progress in Lipid Research*, *62*, 41-54. https://doi.org/10.1016/j.plipres.2015.12.003

Szlinder-Richert, J., & Malesa-Ciećwierz, M. (2018). Effect of household cooking methods on nutritional value of cod and salmon-twin fillet approach. *Carpathian Journal of Food Science & Technology*, *10*(5), 142-157.

Thanonkaew, A., Benjakul, S., Visessanguan, W., & Decker, E. A. (2006). The effect of metal ions on lipid oxidation, colour and physicochemical properties of cuttlefish (*Sepia pharaonis*) subjected to multiple freeze-thaw cycles. *Food Chemistry*, *95*(4), 591-599. https://doi.org/10.1016/j.foodchem.2005.01.040

Ulbricht, T.L.V., & Southgate, D.A.T. (1991). Coronary heart disease: seven dietary factors. *Lancet*, *338*, 985-992. https://doi.org/10.1016/0140-6736(91)91846-M

U.S.D.A. Food Safety and Inspection Service (2023, September 28). Safe Minimum Internal Temperature Chart. https://www.fsis.usda.gov/food-safety/safe-food-handling-and-preparation/food-safety-basics/safe-temperature-chart

Vikøren, LA., Drotningsvik, A., Bergseth, M.T., Mjos, S.A., Mola, N., Leh, S., Mellgren, G., & Gudbrandsen, O.A. (2017). Effects of baked and raw salmon fillet on lipids and n-3 PUFAs in serum and tissues in Zucker fa/fa rats. *Food & Nutrition Research*, *61*, 1333395. https://doi.org/10.1080/16546628.2017.1333395

Wan, J., Cao, A., & Cai, L. (2019). Effects of vacuum or sous-vide cooking methods on the quality of largemouth bass (*Micropterus salmoides*). *International Journal of Gastronomy and Food Science*, *18*, 100181.

Wang, D.D. (2018). Dietary n-6 polyunsaturated fatty acids and cardiovascular disease: epidemiologic evidence. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *135*, 5-9. https://doi.org/10.1016/j.plefa.2018.05.003

Wang, K., Lin, X., Zhao, W., Fan, X., Yu, W., Ma, Z., Yu, C., & Dong, X. (2020). Low-temperature steaming improves eating quality of whitefish. *Journal of Texture Studies*, *51*(5), 830-840. https://doi.org/10.1111/jtxs.12540

Xu, H., Arnlov, J., Sandhagen, B., Riserus, U., Lindholm, B., Lind, L., & Carrero, J.J. (2016). Lipophilic index, kidney function, and kidney function decline. *Nutrition, Metabolism and Cardiovascular Diseases*, *26*(12), 1096-1103. https://doi.org/10.1016/j.numecd.2016.09.006

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 259-265 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.04

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effects of feeding different densities of *Artemia* nauplii on the growth and survival of larvae of the hairy river prawn, *Macrobrachium rude* (Heller, 1862)

Sheban Mdzomba Hinzano[1]* ● Morine Mukami Ngarari[2] ● Mary Opiyo[3] ● Francis Okalo[4]

Betty Mindraa Nyonje[5] ● David Midumbi[6] ● Derrick Gitari[7]

[1]Kenya Marine and Fisheries Research Institute, Mombasa Centre, P.O. Box 81651-80100, Mombasa, Kenya https://orcid.org/0000-0003-3816-6930
[2]Kenya Marine and Fisheries Research Institute, Mombasa Centre, P.O. Box 81651-80100, Mombasa, Kenya https://orcid.org/0000-0002-5611-1784
[3]Kenya Marine and Fisheries Research Institute, Sagana Centre, P.O Box 451-10230, Sagana, Kenya https://orcid.org/0000-0001-9660-5888
[4]Kenya Marine and Fisheries Research Institute, Mombasa Centre, P.O. Box 81651-80100, Mombasa, Kenya https://orcid.org/0000-0002-0089-998X
[5]Kenya Marine and Fisheries Research Institute, Mombasa Centre, P.O. Box 81651-80100, Mombasa, Kenya https://orcid.org/0000-0003-3425-8117
[6]Kenya Marine and Fisheries Research Institute, Mombasa Centre, P.O. Box 81651-80100, Mombasa, Kenya https://orcid.org/0000-0001-8584-8067
[7]Kenya Marine and Fisheries Research Institute, Mombasa Centre, P.O. Box 81651-80100, Mombasa, Kenya https://orcid.org/0000-0002-2569-9538

*Corresponding author: mdzombahi@gmail.com

Received date: 28.07.2023 *Accepted date: 25.10.2023*

How to cite this paper:
Hinzano, S.M., Ngarari, M.M., Opiyo, M., Okalo, F., Mindraa, B.N., Midumbi, D., & Gitari, M. (2023). Effects of feeding different densities of *Artemia* nauplii on the growth and survival of larvae of the hairy river prawn, *Macrobrachium rude* (Heller, 1862). *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 259-265. https://doi.org/10.12714/egejfas.40.4.04

Abstract: The effects of feeding at different densities of *Artemia* nauplii on the growth and survival of *Macrobrachium rude* larvae were explored in this study. Two experiments were carried out. In the first trial, larvae were fed three different feeding densities: 1, 3, and 5 nauplii/mL. In the second experiment, feeding densities of 5, 10, and 15 nauplii/mL were used to determine the maximal feeding density of *Artemia* nauplii for *M. rude* larvae. There were no significant differences statistically in growth rate or survival between larvae fed 1, 3, or 5 nauplii/mL ($P>0.05$). Increased feeding density from 5 to 10 nauplii/mL resulted in growth but drastically decreased survival. Feeding above 10 nauplii/mL decreased both growth rate and survival. According to the results of the study, the optimal feeding density of *M. rude* with *Artemia* nauplii should be between 5 and 10 nauplii/mL. The study suggests further research into determining ideal feeding density at various phases of larval development in order to better understand the individual feeding requirements at each stage.

Keywords: Survival rate, total length, gain in length, water quality, broodstock

INTRODUCTION

Freshwater prawn of the genus *Macrobrachium* are decapods, palemonids and are found on all continents except Europe. *Macrobrachium* is a diverse group of about 240 species, showing great similarity (Makombu et al., 2015). *Macrobrachium* prawn has a very diverse habitat, distributed in rivers, lakes, ponds, irrigation ditches, and aqueducts in subtropical and tropical climates (Valencia and Campos, 2007). Although they spend most of their time in freshwater, the majority of prawn species migrate to brackish water, mainly during the reproductive and larval stages of development, to complete their life cycle (Lima et al., 1997; Barbieri et al., 2016). In general, *Macrobrachium* prawn reach an adult size large enough to be eaten by humans. Prawns are mainly the target of artisanal fishermen and an important source of income in many developing countries, especially in Africa (Nwosu and Wolfi, 2006). About 10 species of *Macrobrachium* prawns have been described from Africa (Makombu et al., 2015). Among the species of interest is the hairy prawn (*M. rude*), which is associated with the economy of small-scale fishermen in Thailand, India, Kenya and Tanzania (Schoonbee et al., 1989). *M. rude* is the most abundant freshwater prawn species in Kenya, accounting for about 11% of all prawns caught by small-scale fishermen (Kimani et al., 2018). It is distributed in two major freshwater rivers in Kenya, namely the Tana and Athi rivers where prawns are harvested by small-scale fishermen from these rivers and sold to local people as an important source of food, employment and livelihood for people in Tana River and Kilifi counties, Kenya (Kimani et al., 2018; Kochey, 2018). *M. rude* presence in both rivers has been reported in most months of the year except March, June, October and November, with a peak in January, April, September and May (Kochey, 2018). Due to overfishing and seasonality of river species, catch rates have plummeted, affecting the incomes of small-scale fishermen and fish-dependent traders (Kimani et al., 2018). It is also reported that female prawns with eggs are more preferred by consumers and are sold by the majority of fishermen (Kochey, 2018). Due to the high local demand, the sustainability of the prawn fishery is threatened and it justifies a sustainable production technique to ensure sufficient supply for the local people. Prawn farming can be a sustainable alternative to prawn fishing in Kenya. Several commercially important species of *Macrobrachium* have been bred in captivity with great success, and in some cases, others are being grown commercially. These include but are not limited to

M. rosenbergii, De Man, 1879 and *M. nipponesis*, De Haan 1849 (Weimin and Xianping, 2002), *M. australe*, Guérin-Méneville, 1838, *M. lar*, Holthuis 1980 (Williams, 2018), *M. vollenhovenii*, Herklots 1857 (Ndao et al., 2019), *M. olfersii*, Wiegmann 1836 (Barbieri et al., 2016), *M. amazonicum*, Heller 1861 (Soeiro et al., 2016) and *M. malcolmsonii*, Milne-Edwards 1844 (Soeiro et al., 2016 and Nair and Salin, 2012). For *M. rude*, some grow out trials in ponds at different stocking densities have been undertaken in Bangladesh (Awal et al., 2021). However, no breeding initiative for *M. rude* has been done even though it has contributed to the economy of some fishing communities, particularly in Kenya, where it accounts for a large share of the prawn population hence the need for this study. A major challenge in freshwater prawn breeding is feeding the larvae for the first time. The feeding of *Macrobrachium* prawn larvae is generally fraught with serious problems, especially poor survival and growth during the first months of independent life (González et al., 2009, 2011). *Artemia* nauplii remain the most essential food for marine crustacean larvae and fish due to their nutritional quality, digestibility, ability to combine the benefits of live and dry diets, and convenience of dry cysts that can hatch within 24 h (Bardócz et al., 1999; Nkambo et al., 2019; Van Stappen et al., 2020). Although *Artemia* nauplii have been widely used in other freshwater prawn larval culture (Kovalenko et al., 2002; Barros and Valenti, 2003; De Aviz et al., 2018), their use in larval rearing of *M. rude* larvae has not been documented. In addition, optimal feeding density of *Artemia* nauplii is essential for the survival of *M. rude* and avoid overfeeding, which can lead to poor water quality, high costs of tank cleaning and maintenance, and stress on larvae (De Aviz et al., 2018). This study was conducted to determine the optimal feeding density of *Artemia* nauplii for *M. rude* larvae.

MATERIALS AND METHODS

Study Area

The study was conducted at the Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa Center (Figure 1). The station, which is located between 4° 03' 19.29" and 39° 40' 54.53", is served by a well and seawater.

Figure 1. A map of Kenya showing the location of KMFRI's Mombasa station where the study was conducted

Broodstock collection

Marobrachium rude broodstock was obtained from local fisherman fishing with prawn seine nets and traps in the Sabaki River near Sabaki Bridge (3° 8' 55" S, 40° 7' 31" E), Kilifi County. Captured broodstock were kept in the river using pre-established hapa nets and were selected based on egg development stage, health status and appearance. Only healthy broodstock with all appendages intact and bearing eggs were chosen for females. As for the males, only those with blue claws were selected. Selected broodstock was delivered to the KMFRI Mombasa Center for spawning in polyethylene fish packaging bags filled with pure oxygen. The broodstock were acclimated before being placed in aerated circular tanks filled with freshwater. The broodstock were fed commercial feed (Skretting ME-0.5 GR starter 315-630 µm) that contained 40% crude protein. Natural photoperiod (12 h light/12 h dark) and a temperature range of 26-28°C were maintained (Valenti et al., 2010). To induce hatching, gravid females bearing grayish-black eggs were put into individual plastic hatching basins with 5 ppt salty water and kept under continuous aeration until the eggs hatched. When the eggs hatched, the female was removed, and the larvae were gathered with a 100 µm net and put into 12 ppt saline water.

Experimental design

One day after hatching, batches of 100 larvae were collected and put in a 10 L glass aquarium with 5 L of 12 ppt saline water at a stocking density of 20 larvae/L. Two experiments were carried out. The larvae were treated to three feeding densities of *Artemia* nauplii/mL in triplicate for 42 days in the first experiment. *Artemia* nauplii were obtained by decapsulating and hatching *Artemia franciscana* cysts grown in Kenyan artisanal salt ponds at Kadzuhoni (20 58'54" S, 400 08' 37" E) under ideal hatching circumstances according to Lavens and Sorgeloos (1996). After 18 hours of incubation, instar 1 *Artemia* nauplii were gathered using a 100 m net and washed, then concentrated in a 1 L glass beaker with autoclaved saltwater. After thorough mixing, six samples of 250 L were collected from the beaker and counted under a dissecting microscope (Carl Zeiss Microscopy GmbH, Germany) to determine concentration. The beaker containing *Artemia* was kept in a refrigerator at 4 °C. For 42 days, the larvae were fed once a day. In addition, 10 days after larvae stocking, 1 g of commercial feed was added to each tank once a day as a supplement. Every morning, dead larvae and other wastes were drained from the bottom of the aquaria, and 50% of the water was replaced. Based on the results of experiment I, a second experiment was conducted using the same procedure as in experiment I. The feeding densities, however, were reviewed to 5, 10, and 15 nauplii/mL. The second experiment's culture time was 14 days because 100% larval mortality occurred after day 14.

Data collection

A YSI professional plus multi-parameter water probe (Model No. 6050000, YSI Industries, Yellow Springs, OH, USA)

was used to measure water temperature, dissolved oxygen (DO), and pH on a regular basis. Every day, the salinity was measured with a portable refractometer (Extech Instruments RF20, USA). Fortnightly growth sampling was performed by selecting five larvae from each tank, fixing them with 1% Lugol's solution, and measuring the total length (TL) of each larva using a STEMI 305 dissecting microscope equipped with an Axiocam (RS5S) camera (Carl Zeiss Microscopy GmbH, Germany). Active swimming larvae were physically counted for survival using a Petri plate and a micropipette. The following formulas were used to compute the growth parameters below:

i. Specific growth rate (SGR, %) = 100 × [(Ln final length (g) – Ln initial length (g)) / days of the experiment]

ii. Percentage Survival (%) = 100 × (final number of prawns)/ (initial number of prawns)

iii. Length gain (LG) = final length - initial length of larvae at stocking.

Data analysis

Shapiro-Wilk test was used to check the normality of the collected data. Descriptive statistics such as mean growth, survival rate and standard error were calculated on Microsoft Excel spreadsheet (Version 2016) using formulas (i), (ii) and (iii) below. Leven's test and one-way analysis of variance (ANOVA) were used to evaluate the equality of variance and the respective means significant difference between treatments. The Tukey HSD test was used to compare each pair of treatments. All statistical tests were performed using R statistical software (version 4.1.0 for Windows) and considered significant at the 95% confidence interval. The results were presented using tables.

RESULTS

Experiment 1

Water quality

Water quality parameters were monitored during the feeding trial (Table 1). The average temperature ranged from 24.32 to 24.34°C while the salinity ranged from 13.78 to 14.00 g/L. The mean dissolved oxygen (DO) ranged from 4.87 to 5.23 mg/L and the pH ranged from 7.82 to 7.90, respectively. The combined results showed that the water quality parameters amongst the feeding densities had small differences but were not statistically significant at the 95% confidence level (*P>0.05*).

Growth parameters

Table 2 shows the growth parameters during the feeding trial. For all feed administrations, the mean total length rose gradually over the culture period. The final total length was greatest in larvae fed 5 nauplii/mL, followed by 3 nauplii/mL, and lowest in larvae fed 1 nauplii/mL. Overall mean length increase, SGR, and SR were all higher in the 5 nauplii/mL treatment than in the 3 nauplii/mL treatment, with the lowest in the 1 nauplii/mL treatment. A one-way analysis of variance in all parameters examined, however, revealed no significant difference amongst the feed treatments (*P>0.05*).

Table 1. Water quality parameters during larval rearing of *M. rude* fed different feeding densities of *Artemia* nauplii

Parameter	Feeding density			
	1 np/mL	3 np/mL	5 np/mL	*P* value
Temperature (°C)	24.34±0.23[a]	24.32±0.22[a]	24.33±0.22[a]	*P>0.05*
Salinity (ppt)	13.78±0.15[a]	14.00±0.00[a]	14.00±0.00[a]	*P>0.05*
pH	7.82±0.27[a]	7.86±0.25[a]	7.90±0.26[a]	*P>0.05*
Dissolved oxygen (mg/l)	5.23±0.12a	5.05±0.10[a]	4.87±0.11a	*P>0.05*

Values are presented as mean± standard error. All statistical tests were considered significant at *P<0.05*. Superscript letters compare mean values between groups. Different letters in a row represent significant differences between groups.

Table 2. Growth parameters of *M. rude* larvae fed different densities of *Artemia* nauplii

Parameter	Feeding density			
	1 np/mL	3 np/mL	5 np/mL	*P* value
Initial TL (mm)	1.92±0.28[a]	1.92±0.28[a]	1.92±0.28[a]	*P>0.05*
Final TL (mm)	3.95±0.06[a]	4.05±0.14[a]	4.24±0.21[a]	*P>0.05*
Mean TLG (mm)	1.71±0.06[a]	1.81±0.14[a]	2.00±0.21[a]	*P>0.05*
SGR (TL) (%)	1.35±0.04[a]	1.41±0.08[a]	1.51±0.12[a]	*P>0.05*
Survival rate (%)	11.67±0.09[a]	11.67±0.09[a]	18.00±3.51[b]	*P>0.05*

TL=Total length, TLG=Total length gain, SGR=Specific growth rate, SR=Survival rate and np/mL=nauplii of *Artemia* per millilitre. Values are presented as means ± standard error. Superscript letters compare mean values between groups. Different letters in a row show significant differences between groups.

Experiment 2

Water quality

The water quality parameters for experiment 2 are presented in Table 3. The results showed that the water quality parameters did not differ between the feeding densities.

The average temperature ranged from 24.53 to 24.59°C, the salinity ranged from 13.97 to 14.29 g/l. The average dissolved oxygen (DO) content ranged from 4.80 to 4.88 mg/l and the corresponding pH ranged from 7.94 to 8.01.

Growth parameters

Total final length also varied significantly between dietary treatments (*P<0.05*). The highest total final length was observed in the 10 nauplii/mL treatment, followed by the 5 nauplii/mL treatment and the lowest in the 15 nauplii/mL treatment. A similar observation of total final length was also observed for SGR and total length gain (Table 4). Survival varied significantly between different dietary treatments (*P<0.05*). The highest mean survival was observed in the 5 nauplii/mL treatment, while the lowest survival was observed in the 10 and 15 nauplii/mL treatments.

Table 3. Water quality parameters during larval rearing of *M. rude* fed different feeding densities of *Artemia* nauplii

Parameter	Feeding density			
	5 np/mL	10 np/mL	15 np/mL	*P* value
Temperature (°C)	24.59±0.08^{a}	24.58±0.07^{a}	24.53±0.08^{a}	*P>0.05*
Salinity (ppt)	13.97±0.10^{a}	14.29±0.07^{a}	14.18±0.32^{a}	*P>0.05*
pH	8.01±0.20^{a}	7.94±0.18^{a}	7.99±0.19^{a}	*P>0.05*
Dissolved oxygen (mg/l)	4.88±0.18^{a}	4.84±0.12^{a}	4.80±0.07^{a}	*P>0.05*

Values are presented as mean± standard error. All statistical tests were considered significant at *P<0.05*. Superscript letter compares mean values between groups. Different letters in a row show significant differences between groups.

Table 4. Growth parameters of *M. rude* larvae fed different densities of *Artemia* nauplii

Parameter	Feeding density			
	5 np/mL	10 np/mL	15 np/mL	*P* value
Initial TL (mm)	1.03±0.01^{a}	1.03±0.01^{a}	1.03±0.01^{a}	*P>0.05*
Final TL (mm)	1.93±0.03^{a}	2.36±0.18^{b}	1.82±0.05^{a}	*P<0.05*
TLG (mm)	0.90±0.03^{a}	1.33±0.18^{b}	0.80±0.05^{a}	*P<0.05*
SGR (% day^{-1})	4.48±0.09^{a}	5.86±0.56^{b}	4.08±0.21^{a}	*P<0.05*
SR (%)	20.00±1.40^{a}	5.71±0.80^{b}	5.71±0.80^{b}	*P<0.05*

Values are presented as means ± standard error. TL=Total length, TLG=Total length gain, SGR=Specific growth rate, SR=Survival rate and np/mL=nauplii of *Artemia* per millilitre. Superscript letters compare mean values between groups. Different letters in a row show significant differences between groups.

DISCUSSION

Freshwater prawns like many crustaceans undergo a complex life cycle characterized by a planktonic larval phase and a benthic adult phase. The transition from one life style to another is accompanied by morphological changes in internal organs, tissue systems, and most importantly, the gastrointestinal tract (Anger, 2006; Korzelecka-Orkisz et al., 2012). Due to the rapid changes, the larval stage differs from the adult stage in several respects, including behaviour, nutrition, and physiology. Newly hatched larvae are lecithotrophic and are completely dependent on yolk mass to meet nutritional requirements lasting a few days. When external feeding is initiated, the larvae must accumulate enough food reserves to survive through the different metamorphosis stages into juvenile and adult stages (Le Vay and Gamboa-Delgado, 2011).

Therefore, determining the optimal feeding density of *Artemia* nauplii for *M. rude* larvae are essential to maximize growth, development and survival rates. In experiment 1 of the study, three feeding densities of *Artemia* nauplii were compared, namely 1, 3 and 5 nauplii/mL. The results showed that the growth parameters of *M. rude* larvae were not significantly different between the 3 feed densities. The observation depicted a similar intake pattern of *M. rude* larvae treated with feed densities of 1, 3 and 5 nauplii/mL (Table 2). According to Kurmaly (1990), a small percentage of decapod larvae search for food, while the majority depend on chance encounters to capture food. Therefore, increasing *Artemia* densities from 1 to 5 nauplii/mL did not provide a better opportunity to encounter and capture the food by *M. rude* larvae. A similar result has also been reported in other studies. For example, Barros and Valenti (2003) observed similar growth parameters in *M. rosenbergii* larvae fed 2 and 4 *Artemia* nauplii/mL. In experiment 2, *M. rude* larvae survived better at the feeding density of 5 nauplii/mL compared with the feed densities of 10 and 15 nauplii/mL. Overall, a downward trend in survival was observed from 5 nauplii/mL to the highest

feeding density, 15 nauplii/mL. The poor survival at the highest feeding density could be associated with evolution of ammonia from the excess *Artemia* nauplii supplied that remained uneaten in the culture tanks. According to De Aviz et al. (2018), increasing *Artemia* feeding deensities is wastefull and results in increased waste in the culturing tanks in this case nitrogenous waste since Artemia is highly proteinous. Another pattern was observed for growth, where the highest mean total length was observed at a feeding density of 10 nauplii/mL, followed by 5 nauplii/mL with lowest observed in larvae fed 15 nauplii/mL. Increasing the feeding density from 5 to 10 nauplii/mL increased feed intake, as demonstrated by mean total length, total length gain and SGR, but significantly decreased survival. Growth was affected at 15 nauplii/mL, which could be a result of excess feed intake. According to El-Sayed (2002), increased feed density in water may increase feed intake, but high intake of particles may cause feed to flow rapidly through the intestine, resulting in poor digestion and poor assimilation of nutrients hence the poor growth perfomance observed in the prawn larvae fed at high *Artemia* nauplii densities. These results are consistent with the study of De Aviz et al. (2018), who noted that the survival rate of *M. rosenbergii* decreased when *Artemia* nauplii density increased from 5 to 10 and 20 nauplii/mL. In another study, Maciel et al. (2012) observed an increase in feed intake as feed density increased, but this did not lead to an increase in yield.

The results of the present study are in contrast to those reported by Daniel et al. (2019) on the increase in larval growth parameters of Amazon aquarium fish when the *Artemia nauplii* feeding density was increased from 50 to 150 nauplii/mL. The survival rate of *M. rude* larvae in the present study ranged from 6 to 20% in both experiments. This is consistent with the study of Makombu et al. (2014), who observed survival rates of 3 to 9% when rearing *Macrobrachium vollenhovenii* larvae. The survival rates observed in the present study were much lower than those associated with other *Macrobrachium* species. For example, research by Gomes et al. (2014) reported survival rates of 56 to 78% with *Macrobrachium equidens*. In another study, Habib et al. (2014) reported a 64% survival rate with *Macrobrachium rosenbergii* larvae. The survival observed in the present study was also lower when compared to other crustaceans. The study of Rodríguez-Serna et al. (2010) on Mexican crayfish (*Procambarus llamasi*) fed different farm animal feed reported 100% survival whereas Kaldre et al. (2015) observed 89% and 78% survival with marbled crayfish (*P. virginalis*) fed with carp and discus feeds. Amanyazov and Karadal (2023), on the other hand stated 58-75% survival with red swamp crayfish (*P. clarkii*) fed with three different commercial aquarium feeds. The survival of prawn larvae is related to several factors. Brown (2005) associated poor survival with sub-optimal nutritional factors that render larvae incapable of transitioningfrom one developmental stage to another.

In contrast, Armstrong et al. (1976) and Aquacop (1983) linked prawn larval survival to the maintenance of good water quality, the quality and quantity of feed as well as the ability of the larvae to obtain food from the water. In the present study, the water quality parameters monitored (Table 1 and 3) were within the optimal range for prawn larval development. In addition, feed density treatments were also within the recommended range, consistent with previous studies. Therefore, the overall lower survival rates observed in the present study as compared to other studies could be due to handling and other zootechnical challenges (Brown, 2005).

CONCLUSION

In summary, the results of this study have important implications in determining the density of *Artemia* nauplii fed to *M. rude* larvae to optimize larval growth and survival. From the study, it was clear that feeding *Artemia* nauplii at lower densities (1-5 nauplii/mL) gave similar results in terms of growth rate and survival.

The study also demonstrated that increasing the density of *Artemia* nauplii fed to *M. rude* larvae would increase growth parameters, but there was an optimal range of 5 to 10 nauplii/mL. According to the study, increasing the feeding densities beyond 10 nauplii/mL not only leads to wasted feed but also reduces the growth and survival rates of the prawn larvae. The study recommends repeating the same work but with *Artemia* nauplii enriched with highly unsaturated fatty acids (HUFA) to determine if survival could be improved.

ACKNOWLEDGEMENTS AND FUNDING

The authors would like to thank the Western Indian Ocean Marine Sciences Association (WIOMSA) for funding this study under the MASMA project "Optimizing Artemia Production Technology for Sustainable Aquaculture Development (APTSAD), Food Security, and Economic Growth for East African Coastal Communities" grant number MASMA/OP/2020/02. The Kenya Marine and Fisheries Research Institute is appreciated for providing the study team and equipment.

AUTHORSHIP CONTRIBUTIONS

Sheban Mdzomba Hinzano: Conceptualization, data curation, formal analysis, investigation, methodology, software, supervision, validation, visualization, writing original draft, writing review and editing. Morine Mukami Ngarari: Conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, visualization, writing original draft, writing review and editing. Mary Opiyo: Conceptualization, funding acquisition, methodology, project administration, resources, supervision, review and editing. Francis Okalo: Conceptualization, funding acquisition, resources, writing review and editing. Betty Nyonje Mindraa: Conceptualization, project administration, supervision, writing review and editing. David Midumbi: Data curation, investigation, methodology, writing review and editing. Derrick Gitari: Data curation, investigation, methodology, writing review and editing.

CONFLICTS OF INTEREST

The authors state that there is no conflict of interest to declare.

ETHICS APPROVAL

The experiment was carried out in accordance with the Kenya Marine and Fisheries Research Institute (KMFRI) guidelines for animal handling, as registered with the National Commission for Science, Technology, and Innovation (NACOSTI) registration number NACOSTI/2016/05/001, and in accordance with the Prevention of Cruelty to Animals Act 1962, CAP 360 (Revised 2012) of Kenyan laws, and the EU regulation (EC Directive 86/609/EEC).

DATA AVAILABILITY

All relevant data is in the article.

REFERENCES

Amanyazov, V., & Karadal, O. (2023). Growth performance, molting frequency and carapace coloration of two different size classes of red swamp crayfish (*Procambarus clarkii*) fed with different diets. *Journal of Limnology and Freshwater Fisheries Research*, *9*(1), 1-10. https://doi.org/10.17216/limnofish.1120574

Anger, K. (2006). Contributions of larval biology to crustacean research: A review. *Invertebrate Reproduction and Development*, *49*(3), 175-205. https://doi.org/10.1080/07924259.2006.9652207

Aquacop (1983). Intensive larval rearing in the clear water of *Macrobrachium rosenbergii* (De Man, 1879 Anuenue stock) at the Centre Oceanologique du Pacifique, Tahiti. In J.P. Mcvey, J.R. Moore (Eds.), *CRC Handbook of Mariculture, Crustacean Aquaculture*, *1*, 179 –187, CRC, Florida.

Armstrong, D.A., Stephenson, M.J., & Knight, A.W. (1976). Acute toxicity of nitrite to larvae of the giant Malaysian prawn, *Macrobrachium rosenbergii*. *Aquaculture*, *9*, 39-46. https://doi.org/10.1016/0044-8486(76)90046-6

Awal, M.D.R., Yesmine, S., Khanom, M., Chowdhury, P., Kohinoor A.H.M., & Mahmud, Y. (2021). Culture potentialities of gura chingri (*Macrobrachium rude*) in pond at different stocking densities. *Bangladesh Journal of Fisheries Research*, *20*(1–2), 123–130.

Barbieri, E., Moreira, P., Luchini, L.A., Ruiz Hidalgo, K., & Munoz, A. (2016). Assessment of acute toxicity of carbofuran in *Macrobrachium olfersii* (Wiegmann, 1836) at different temperature levels. *Toxicology and Industrial Health*, *32*(1), 7-14. https://doi.org/10.1177/0748233713484655

Bardócz, T., Kovács, É., Radics, F., & Sándor, Z. (1999). Experiments for the improved use of decapsulated *Artemia* cysts in intensive culture of African catfish larvae. *Journal of Fish Biology*, *55*, 227-232. https://doi.org/10.1111/j.1095-8649.1999.tb01058.x

Barros, H.P., & Valenti, W.C. (2003). Food intake of*Macrobrachium rosenbergii* during larval development. *Aquaculture*, *216*(1-4), 165-176. https://doi.org/10.1016/S0044-8486(02)00505-7

Brown, J.H. (2005). Joint study on market led opportunities in sustainable shrimp farming in West Africa: "Potential for shrimp culture in Africa." Report prepared for Sahel and West Africa Club (SWAC)/Organisation for Economic Cooperation and Development (OECD) Africa–West Africa Business Cooperation, Paris. http://www.oecd.org/swac/publications/38524477.pdf

Daniel, A.V., Matheus, H.C.M., Odinilson, P.M., Lorena, B.D.M., Bruno, J.C.F.E., Marcos, F.B., & Galileu, C.V. (2019). Effects of feeding rates and feeding frequencies on growth performance, uniformity of the batch, and survival rate of Amazon ornamental fish larvae. *International Journal of Fisheries and Aquaculture*, *11*(2), 23-28. https://doi.org/10.5897/IJFA2018.0705

De Aviz, M.A.B., Abrunhosa, F.A., Maciel, M., & Maciel, C.R. (2018). On feeding of the freshwater prawn larvae *Macrobrachium rosenbergii*. *Boletim do Instituto de Pesca*, *44*(4). https://doi.org/10.20950/1678-2305.2018.44.4.373

El-Sayed, A.F.M. (2002). Effects of stocking density and feeding levels on growth and feed efficiency of Nile tilapia (*Oreochromis niloticus*) fry. *Aquaculture Research*, *33*(8), 621–626. https://doi.org/10.1046/j.1365-2109.2002.00700.x

Gomes, J.N., Abrunhosa, F.A., Costa, A.K., & Maciel, C.R. (2014). Feeding and larval growth of an exotic freshwater prawn *Macrobrachium equidens* (Decapoda: Palaemonidae), from Northeastern Pará, Amazon Region. *Anais of the Brazilian Academy of Sciences*, *86*, 1525-1536. https://doi.org/10.1590/0001-3765201420130079

González R., Celada J.D., Carral J.M., González Á., Sáez-Royuela M., & García V. (2009). Decapsulated *Artemia* cysts as dietary supplement for juvenile crayfish (*Pacifastacus leniusculus*, Astacidae) at different food supply frequencies from the onset of exogenous feeding under controlled conditions. *Aquaculture*, *295*, 200−204. https://doi.org/10.1016/j.aquaculture.2009.07.009

González, Á., Celada, J.D., Carral, J.M., Sáez-Royuela, M., García, V., & González, R. (2011). Additional supply of decapsulated *Artemia* cysts for various periods in intensive rearing of juvenile crayfish (*Pacifastacus leniusculus*, Astacidae). *Knowledge and Management of Aquatic Ecosystems*, *401*, 15. https://doi.org/10.1051/kmae/2011030

Habib, A., Das, N.G., & Hossain, M.B. (2014). Growth performance and survival rate of *Macrobrachium rosenbergii* (De Man, 1979) larvae using different doses of probiotics. *Pakistan Journal of Biological Sciences*: PJBS, *17*(7), 920-924. https://doi.org/10.3923/pjbs.2014.920.924

Kaldre, K., Haugjärv, K., Liiva, M., & Gross, R. (2015). The effect of two different feeds on growth, carapace colour, maturation and mortality in marbled crayfish (*Procambarus fallax* f. *virginalis*). *Aquaculture International*, *23*, 185-194. https://doi.org/10.1007/s10499-014-9807-1

Kimani E.N., Aura M.C., & Okemwa G.M. (2018). *The status of Kenya fisheries: towards the sustainable exploitation of fisheries resources for food security and economic development.* Kenya Marine and Fisheries Research Institute (KMFRI), Mombasa, pp 18-33

Kochey, K.J. (2018). *Evolutionary ecology, utilization patterns and aquaculture of the fresh/brackish water* Macrobrachium *spp. along rivers Tana and Athi in Kenya*. Doctoral dissertation, Johann Wolfgang Goethe-Universität in Frankfurt.

Korzelecka-Orkisz, A., Szalast, Z., Pawlos, D., Smaruj, I., Tański, A., Szulc, J., & Formicki, K. (2012). Early ontogenesis of the angelfish, *Pterophyllum scalare* Schultze, 1823 (Cichlidae). *Neotropical Ichthyology*, *10*, 567-576. https://doi.org/10.1590/S1679-62252012005000017

Kovalenko, E.E., D'Abramo, L.R., Ohs, C.L., & Buddington, R.K. (2002). A successful microbound diet for the larval culture of freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture*, *210*(1–4), 385–395.

Kurmaly, K. (1990). *Studies on the acceptability and digestibility of artificial diets by Crustacea.* Doctoral dissertation, University of Wales, Bangor.

Lavens, P., & Sorgeloos, P. (1996). *Manual on the production and use of live food for aquaculture*. FAO, Rome, 79-250.

Le Vay, L., & Gamboa-Delgado, J. (2011). Naturally-occurring stable isotopes as direct measures of larval feeding efficiency, nutrient incorporation and turnover. *Aquaculture*, *315*(1-2), 95-103. https://doi.org/10.1016/j.aquaculture.2010.03.033

Lima, A.G., McNamara, J.C., & Terra, W.R. (1997). Regulation of hemolymph osmolytes and gill Na+/K+-ATPase activities during acclimation to saline media in the freshwater prawn *Macrobrachium olfersii* (Wiegmann, 2b 1836) (Decapoda, Palaemonidae). *Journal of Experimental Marine Biology and Ecology*, *215*(1), 81-91. https://doi.org/10.1016/S0022-0981(97)00016-6

Maciel, C.R., New, M.B., & Valenti, W.C. (2012). The Predation of *Artemia* nauplii by the larvae of the Amazon River prawn, *Macrobrachium amazonicum* (Heller, 1862), is affected by prey density, time of day, and

ontogenetic development. *Journal of the World Aquaculture Society*, *43*(5), 659-669. https://doi.org/10.1111/j.1749-7345.2012.00599.x

Makombu, J.G., Oben, P.M., Oben, B.O., Gaudin, G.L., Motto, I.S., Makoge, N., Syapze, J.K., Brown, J.H., Ngueguim, J.R., & Mialhe, E. (2014). Complete larval development of the freshwater prawn *Macrobrachium vollenhovenii* in Cameroon. *Journal of Applied Aquaculture*, *26*(4), 310-328. https://doi.org/10.1080/10454438.2014.934170

Makombu, J.G., Oben, B.O., Oben, P.M., Makoge, N., Nguekam, E.W., Gaudin, G.L., & Brummett, R.E. (2015). Biodiversity of species of the genus *Macrobrachium* (Decapoda, Palaemonidae) in Lokoundje, Kienke and Lobe Rivers, South Region, Cameroon. *Journal of Biodiversity and Environmental Science*, *7*, 68-80.

Nair, C.M., & Salin, K.R. (2012). Current status and prospects of farming the giant river prawn *Macrobrachium rosenbergii* (De Man) and the monsoon river prawn *Macrobrachium malcolmsonii* (HM Edwards) in India. *Aquaculture Research*, *43*(7), 999-1014. https://doi.org/10.1111/j.1365-2109.2011.03074.x

Ndao, P.D., Kantoussan, J., Seck, M., Jouanard, N., Savaya, A.A., Ndione, R.A., & Thiaw, O.T. (2019). Breeding and larval rearing in hatchery of *Macrobrachium vollenhovenii* (Herklots, 1857) in a perspective of biological control against human schistosomiasis in tropical area. *Journal of Marine Biology and Aquaculture*, *5*(1), 1-9. https://doi.org/10.15436/2381-0750.19.2019

Nkambo, M., Mwanja, M., Balirwa, J.S., & Bugenyi, F.W. (2019). Use, production and existence of local *Artemia* resources in Uganda and Africa: A review. *Journal of Natural Sciences Research*, *9*(18), 30-36. https://doi.org/10.7176/JNSR/9-18-04

Nwosu, F.M., & Wolfi, M. (2006). Population dynamics of the giant African river prawn *Macrobrachium vollenhovenii* Herklots 1857 (Crustacea, Palaemonidae) in the Cross River estuary, Nigeria. *West African Journal of Applied Ecology*, *9*(1) 1-14. https://doi.org/10.4314/wajae.v9i1.45681

Rodríguez-Serna, M., Carmona-Osalde, C., & Arredondo Figueroa J.L. (2010). Growth of juvenile crayfish *Procambarus llamasi* (Villalobos 1955) fed different farm and aquaculture commercial foods. *Journal of Applied Aquaculture*, *22*(2):140-148. https://doi.org/10.1080/10454431003736417

Schoonbee, H.J., Cort, M., Ferreira, J.T., & Visser, J. (1989). Species records of the river prawn *Macrobrachium* (Decapoda: Natantia: Palaemonidae) from Lake Cubhu, Zululand, Natal. *Water South Africa*, *15*(1), 49-51. https://doi.org/10.10520/AJA03784738_1554

Soeiro, R.K.S., Rocha, C.P., Maciel, M., Abrunhosa, F.A., & Maciel, C.R. (2016). Relationship between the coastal origin of the freshwater prawn *Macrobrachium amazonicum* and salinity levels in the hatchery. *Boletim do Instituto de Pesca*, *42*(3), 691-703. https://doi.org/10.20950/1678-2305.2016v42n3p691

Valencia, D.M., & Campos, M.R. (2007). Freshwater prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea: Decapoda: Palaemonidae) of Colombia. *Zootaxa*, *1456(*1), 1-44. https://doi.org/10.5281/zenodo.176325

Valenti, W.C., Daniels, W.H., New, M.B., & Correia, E. S. (2010). Hatchery systems and management. In M. Bernard, W.C. Valenti, J.H. Tidwell, L.R. D'Abramo, M.N. Kutty (Eds.), *Freshwater Prawns: Biology and Farming*, pp. 55–85. https://doi.org/10.1002/9781444314649.ch5

Van Stappen, G., Sui, L., Hoa, V.N., Tamtin, M., Nyonje, B., de Medeiros Rocha, R., Sorgeloos, P., & Gajardo, G. (2020). Review on integrated production of the brine. *Reviews in Aquaculture*, *12*, 1054-1071. https://doi.org/10.1111/raq.12371

Weimin, M., & Xianping, G. (2002). Freshwater prawn culture in China: An overview. *Aquaculture Asia*, *7*(1), 9-12.

Williams, L.V. (2018). *Assessment of the capture and culture potential of two native freshwater prawns (*Macrobrachium lar *(Fabricius, 1798) and* Macrobrachium australe *(Guérin-Méneville, 1838) in the Fiji Islands.* Master's thesis, University of South Pacific.

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 266-275 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.05

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Bioinformatics studies and comparison of mRNA transcription of glutathione S-transferase gene in some tissues of common carp (*Cyprinus carpio*) and brown trout (*Salmo trutta*)

Badrul Islam Elsevar[1] ● Mehtap Bayır[2*]

[1]Department of Agricultural Biotechnology, Faculty of Agriculture, Atatürk University, 25240, Erzurum, Türkiye https://orcid.org/0000-0003-3447-0628
[2]Department of Agricultural Biotechnology, Faculty of Agriculture, Atatürk University, 25240, Erzurum, Türkiye https://orcid.org/0000-0002-7794-1058

*Corresponding author: mehtap.bayir@atauni.edu.tr

Received date: 14.08.2023 Accepted date: 09.11.2023

How to cite this paper:
Elsevar, B.I., & Bayır, M. (2023) Bioinformatics studies and comparison of mRNA transcription of glutathione S-transferase gene in some tissues of common carp (*Cyprinus carpio*) and brown trout (*Salmo trutta*). *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 266-275. https://doi.org/10.12714/egejfas.40.4.05

Abstract: Bioinformatics has revolutionized the way we study gene expression and regulation, enabling researchers to analyze large-scale genomic data with unprecedented speed and precision. In this study, we use bioinformatics tools and methods to compare mRNA transcription of glutathione S-transferase (*gstr*) gene in two different fish species: common carp and brown trout. In this study, liver, intestine, muscle, brain, heart, eye, spleen, gill, kidney, stomach, ovary and testis samples were taken from male and female brown trout and common carp, and total RNA was isolated from each tissue to synthesize cDNA from these tissues. Then, the transcript amounts of the *gstr* gene were determined by qPCR from all tissue samples. Gene structures, conserved gene synteny design, phyogenetic tree analyzes and similarity-identity ratios with other vertebrates were determined. When the transcriptional differences between male and female tissues for the brown trout *gstr* gene were examined, it was seen that the intestine, gill, kidney, stomach, muscle and gonads were significantly higher in male fish ($p<0.05$), but the differences between other tissues were not statistically significant. It has been determined that the highest gene expression was liver ($p<0.05$) and brain, eye, spleen, kidney, heart and spleen tissues have significantly lower *gstr* gene expression than other tissues in both male and female in common carp. In addition, the in-silico analysis determined that the brown trout *gstr* gene shared the highest similarity and identity ratio with rainbow trout, and the common carp *gstr* gene shared the highest similarity and identity ratio with goldfish.

Keywords: Brown trout, common carp, in silico analysis, *gstr*, gene expression

INTRODUCTION

Aquaculture is an important industry that helps meet the growing demand for seafood while reducing pressure on wild fish populations (Chen et al., 2021). Brown trout and common carp are two popular species that are extensively farmed for their economic and nutritional benefits (Adamek et al., 2023; Franěk et al., 2021). Common carp farming has been shown to improve glucose metabolism disorder in fish. Carp farming, however, can also contribute to increases in turbidity and internal nutrient load by resuspending sediments, which may eventually reduce the water quality (Arlinghaus and Mehner, 2003). Brown trout is another important aquaculture species that faces challenges due to global warming and a changing climate (Keiz et al., 2023). Inland fisheries, including aquaculture, contribute significantly to food security and economic security by providing primary sources of animal protein, essential for human health and well-being (Lynch et al., 2016).

Glutathione S-transferase (GST) is an enzyme that plays a crucial role in the detoxification of xenobiotics and endogenous compounds by catalyzing the conjugation of glutathione to electrophilic substrates. GSTs are encoded by a large gene family, and their expression is induced by various environmental stressors, including microcystin-LR, cadmium, and weathered polyethylene microplastics. GSTs have been studied in various fish species, including common carp (Chen et al., 2017), Nile tilapia (Liang et al., 2007), and zebrafish (Glisic et al., 2015; Tierbach et al., 2018). The expression of GST genes varies among different tissues and fish species. For example, alpha-class GST gene expression was higher than that of rho-class GST gene in both exposed and control fish of silver carp and grass carp, whereas rho-class GST gene expression was higher than that of alpha-class GST gene in both exposed and control fish of Nile tilapia (Liang et al., 2007). The induction of GST enzyme activity corresponds to *gstr* gene expression at the latter stages of exposure to weathered polyethylene microplastics (Pandi et al., 2022). In cadmium-exposed river pufferfish, seven genes of the GST family were cloned and expressed, and GST1.18 was found to play a critical role in detoxification pathways (Kim et al., 2010). GSTs also play an important role in phase II detoxification of lipid peroxides and demonstrate the functions such as glutathione peroxidase activity towards (Rudneva et al., 2010). Overall, GSTs have an important role in the detoxification of xenobiotics and endogenous compounds in fish (Glisic et al., 2015).

Bioinformatics studies of mRNA transcription in fish have become increasingly popular as they provide insights into the biological mechanisms involved in various physiological processes (Qian et al., 2014). In genetics research, bioinformatics plays a crucial role in studying genetic information, such as DNA and RNA sequences, and their interactions with various biological processes (Bayat, 2002).

In this study, bioinformatics tools and techniques were used to compare the mRNA transcription of the *gstr* gene in the tissues of common carp and brown trout. By employing bioinformatics tools and techniques, we aim to gain a deeper understanding of the expression patterns of the *gstr* gene in different tissues of these two species, and explore potential differences in expression levels between them. Our findings may have significant implications for understanding the role of *gstr* gene expression in aquatic organisms and its potential effects on their health and survival in varying environmental conditions.

MATERIALS AND METHODS

In silico analysis

In-silico analysis for the identification of the *gstr* gene in brown trout and common carp were performed using bioinformatics tools such as Ensembl, NCBI, and UniProt databases. The cDNA sequences of brown trout and common carp *gstr* were obtained from the Ensembl database and their accuracy was confirmed by performing a BLAST search on the NCBI database. It was observed that both brown trout and common carp have one isoform of the *gstr* gene, which was identified through Ensembl database searches. In the study, to determine the mRNA expression of the *gstr* gene in both brown trout and common carp, as well as the reference genes for common carp, actin beta 1 (*actb*) and glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), primers were designed according to the exon-exon junction model (Table 1). The primer sequences used for beta-actin and elongation factor 1a (*eef1a*) genes, which were reference genes for brown trout, were obtained from Özdemir and Bayır (2023) (Table 2). Additionally, genomic primers were designed (Table 3) to amplify the desired regions and obtain the sequence of PCR products from the beginning and end parts of the open reading frame by designing the primers from the closest regions possible to the start and end of the open reading frame. The PCR products obtained from the designed primers for gDNA were placed in three nuclease-free Eppendorf tubes containing 30 µL of PCR product each and sent to a specialized sequencing company for Sanger sequencing.

The design of the conserved gene synteny manually using the Ensembl database (Figure 1). The chromosomes and regions where the *gstr* gene are found in brown trout and common carp are recorded. The other genes found outside the *gstr* gene in the identified chromosomes and regions, as well as the chromosomal regions of these genes in another organism, rainbow trout, are also identified. A conserved gene synteny is created based on the common genes and their chromosomal locations in the genomes of these three organisms. The CLUSTALW BioEdit program (http://www.mbio.ncsu.edu/bioedit/page2.html) was used to determine the phylogenetic relationship of brown trout and common carp using the *gstr* gene, and to construct a phylogenetic tree (Figure 2). The nucleotide sequences of the *gstr* genes in brown trout and common carp were determined using the Ensembl database. Separate nucleotide sequences were designed for both species, indicating the exons, introns, amino acids synthesized by the exons, 5' and 3' ends, TATA box, poly-A signal, and stop codon of the *gstr* gene (Figure 3, 4). The similarity-identity ratios between the *gstr* genes of common carp and goldfish, zebrafish, rainbow trout, Atlantic salmon, brown trout, and gilthead seabream were calculated using the BioEdit program based on the protein sequences synthesized by these genes (Figure 5, 6).

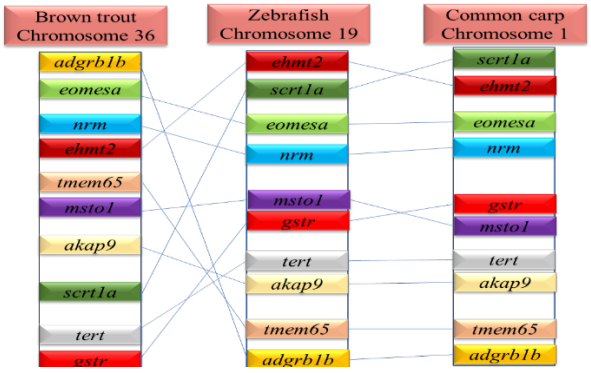

Figure 1. Conserved gene synteny among brown trout, common carp, and zebrafish *gstr* gene

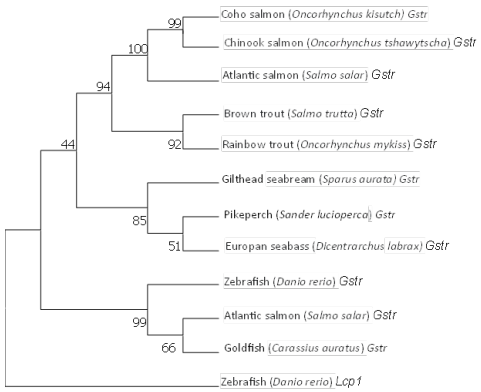

Figure 2. The phylogenetic relationship of brown trout and common carp *gstr* genes with those of other fish species

The protein sequence accession numbers used in the phylogenetic tree, created using the maximum likelihood method (Felsenstein, 1989), are as follows: Atlantic salmon (*Salmo salar*) ENSSSAG00000056150, common carp (*Cyprinus carpio*) ENSCCRG00000013532, brown trout (*Salmo trutta*) ENSSTUG00000037965, rainbow trout (*Oncorhynchus mykiss*) ENSOMYG00000034408, Chinook salmon (*Oncorhynchus tshawytscha*) ENSOTSG00005006610, coho salmon (*Oncorhynchus kisutch*) ENSOKIG00005022094, goldfish (*Carassius auratus*) ENSCARG00000006155, zebrafish (*Danio rerio*) ENSDARG00000042620, gilthead seabream (*Sparus aurata*) ENSSAUG00010003870, European seabass (*Dicentrarchus labrax*) ENSSLUG00000008281, Nile tilapia (*Oreochromis niloticus*) ENSONIG00000034559, European seabass (*Dicentrarchus labrax*) ENSDLAG00005030493.

Table 1. Primer sequences *gstr*, *actb1,* and *gapdh* genes of common carp

Common carp	Forward primer (5´→3´)	Reverse primer (5´→3´)	Tm (°C)
gstr	CCAGAGCTCAGGTTCCAACT	GGTCTCAAACATTCGCTGGT	62
actb1	CCCAGGCATCAGGGAGTGA	TCCATATCATCCCAGTTGGTCA	62.5
gapdh	CAACATGGGGATTTGGCCGT	AGACGGTGATAGCGTGACCA	60

Table 2. Primer sequences for *gstr, actb*, and *ef1α* genes of brown trout

Brown trout	Forward primer (5´→3´)	Reverse primer (5´→3´)	Tm (°C)
gstr	GGACAGCTCCCTGCTTTCAA	CGGGGACACGGTAGTTGTAG	62
b-actin	ATGGAAGGTGAAATCGCC	TGCCAGATCTTCTCCATG	52.1
ef1α	GTCMMTGGAACGCACTCG	CTACTGATTGGCTGCTTCGC	59.45

Table 3. Genomic primers for brown trout and common carp *gstr* genes.

gstr	**Forward primer (5´→3´)**	**Reverse primer (5´→3´)**	**Tm (°C)**
Brown trout	CCAGAGCTCAGGTTCCAACT	GGTCTCAAACATTCGCTGGT	61
Common carp	TAACACAAGCGCACCACTG	AGACTGTTAATGTGCGCTGC	59

```
5’tatacaggtaactagctgagattaggagcacactcttaaagggagtgctcctaatctc
agctcgttacctgtataaaagacacctgggagccagaaatctttctgattgagaggggt
caaatacttatttccctcattaaaatgcaaatcaatttataacatttttgacatgcgttt
ttctggatttttagttgttattctgtctctcagtgttcaaattaacctaccattaaaat
TATAgactgatcatttctttgtcagtgggcaaacgtacaaaatcagcaggggatcaaata
+1
CTTTTTCCCCTCACTGTATATTGGTTCTTAACTTCCCTGAAAGTTGCATATTGCCGGGGC
TATTCGATTCTAATGCGTACTATTTCCATTTTTCTATTTTTCCTGTTTCTTACTTTTTAA
CTGTGCATTGTTTGGAAAGAGCTCGTACTGTAACTAAGCGTTTCACGGTAAAGTCTACAC
CTGTTGTATTCGGCGCAGGTGACAAACACAATTTGATATGACTTCCTTTATGCTGTAGCC
AACATGACTACGCGGAATTCATGTGTTGATAGAAGACCAGTAGAACTGGACTGTCATGAC
   -M--T--T--R--N--S--C--V--D--R--R--P--V--E--L--D--C--H--D-
TCGTACATTAAGgtgac’N361’agcagATTTCGACCATCATGGCCAAGGACATGACACT
-S--Y--I--K-                  -I--S--T--I--M--A--K--D--M--T--L
GCTGTGGGGCTCCGGCTCTCCTCCGTGCTGGCGTGTCATGATCGCTCTGGAGGAGAAGAA
--L--W--G--S--G--S--P--P--C--W--R--V--M--I--A--L--E--E--K--K
ACTGCAGGGATACAATCACAAACTTCTCTCCTTCGAGAAAGCAGAGCACAAGTCTAAAGA
--L--Q--G--Y--N--H--K--L--L--S--F--E--K--A--E--H--K--S--K--E
AGTCCTGGATATCAATCCCAGAGGACAGgtagt’N448’cccagCTCCCTGCTTTCAAAC
--V--L--D--I--N--P--R--G--Q-                  -L--P--A--F--K--
ACGGAGACAACATACTCAACGAGTCATATGCAGCATGCATGTACCTGGAGgtaag’N2045’
H--G--D--N--I--L--N--E--S--Y--A--A--C--M--Y--L--E-
tacagAGCCGGTTCAGGTCCCAGGGACCCCAGTTGATTCCTGAGGGCCAACTAGAGCAGG
     -S--R--F--R--S--Q--G--P--Q--L--I--P--E--G--Q--L--E--Q--
CCCTGATGTACCAGCGCATGTTTGAGATCCTCAACCTCAGTGACAAACTCAgtaag’N415’
A--L--M--Y--Q--R--M--F--E--I--L--N--L--S--D--K--L--
cccagGTAACGTCATCTACTACAACTACCGTGTCCCCGAGGGAGAGAGACATGACTCTGC
     S--N--V--I--Y--Y--N--Y--R--V--P--E--G--E--R--H--D--S--A
TATCAAGAGGAACAAGGAGAACCTGGCCACGGAAATCAAACTGTGGGAGGGATACTTTCA
--I--K--R--N--K--E--N--L--A--T--E--I--K--L--W--E--G--Y--F--Q
GAAGgtgca’N756’tccagATGGAGGTGGGTTCTTACCTGGCAGGAAAAGCCTTCTCAT
--K-                  -M--E--V--G--S--Y--L--A--G--K--A--F--S--
TGGCTGACGTTATTGTCTTCCCTGTGATTGCCTACGCCTTCCGCTTTGGgtaag’N67’t
L--A--D--V--I--V--F--P--V--I--A--Y--A--F--R--F--G
ccagGCTGTCTACGGAGCGTTACCCCAAACTGGGAGCATACTACGATATGATGAAGGAAA
    --L--S--T--E--R--Y--P--K--L--G--A--Y--Y--D--M--M--K--E--
GACCCAGCGTTAAAGCTACCTGGCCCCCACACTGGCTGGAGAACCCTCAGGGAGGGGACG
R--P--S--V--K--A--T--W--P--P--H--W--L--E--N--P--Q--G--G--D--
CTCTCAAGGAGTTCTGAgacacacaggaacaacacagcacattatcttaaggatgttaat
A--L--K--E--F--*-
cgtcacttcctgtatatcactgttgtaaccacgggaaacgcaagttgctttaaatgtacg
tttcctcagatgagtatcagtcaagtagttttccactaagtgaacacaatttttttgcat
tgcctttctgggggtttttgtaacaaatgctttttttttttacttctatatatacacttt
aactgaaacattaaacacaaagtgtgtttttacgaacatgactttataataacagtacat
cctccatataatttctgtgttttgtgtacagaccacatacactggtgtggAATTAAtaaa
aaaaatcataccaag 3’
```

Figure 3. Exon-intron organization of the brown trout glutathione S-transferase (*gstr*) gene

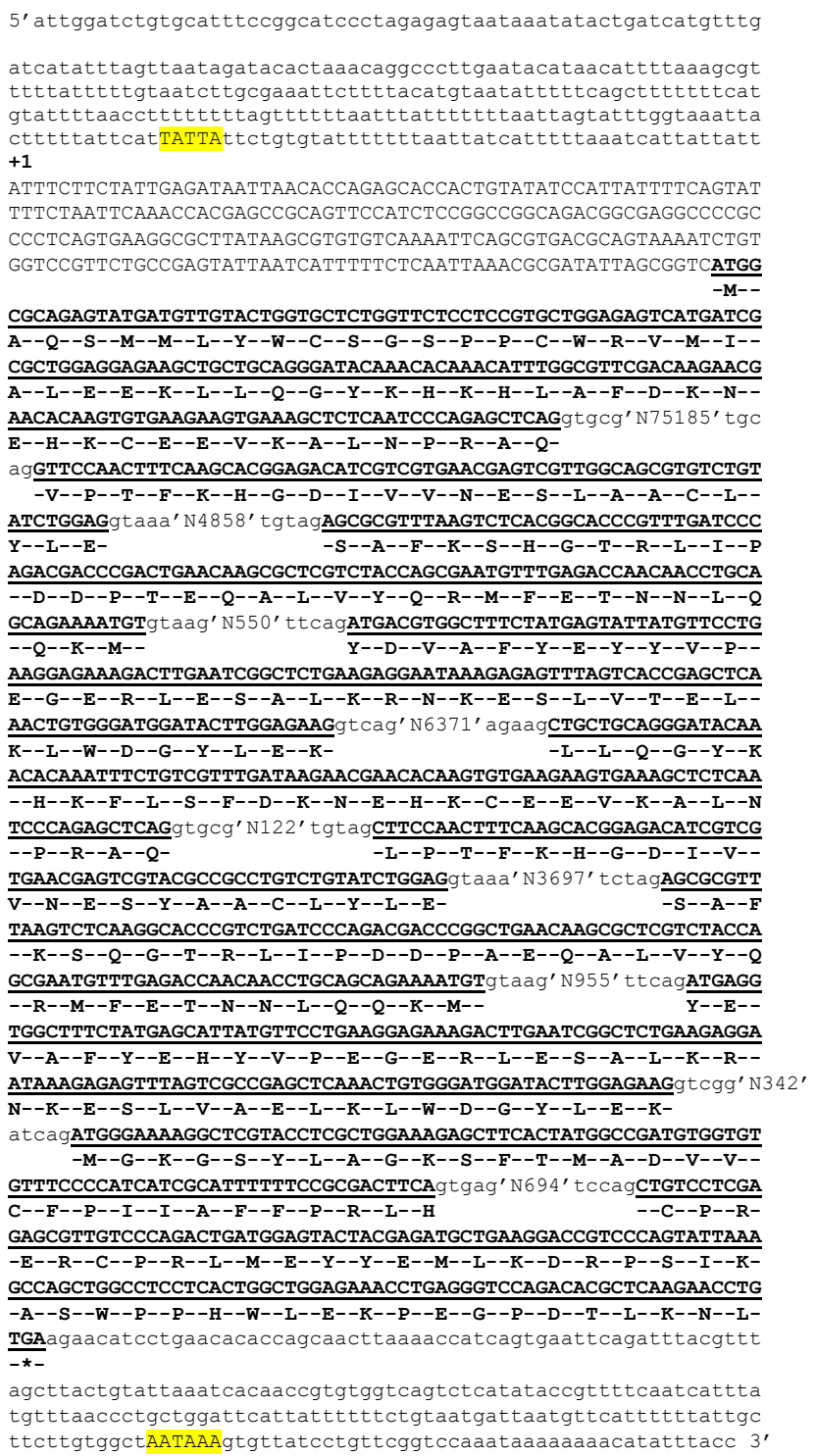

Figure 4. Exon-intron organization of the common carp glutathione S-transferase (*gstr*) gene

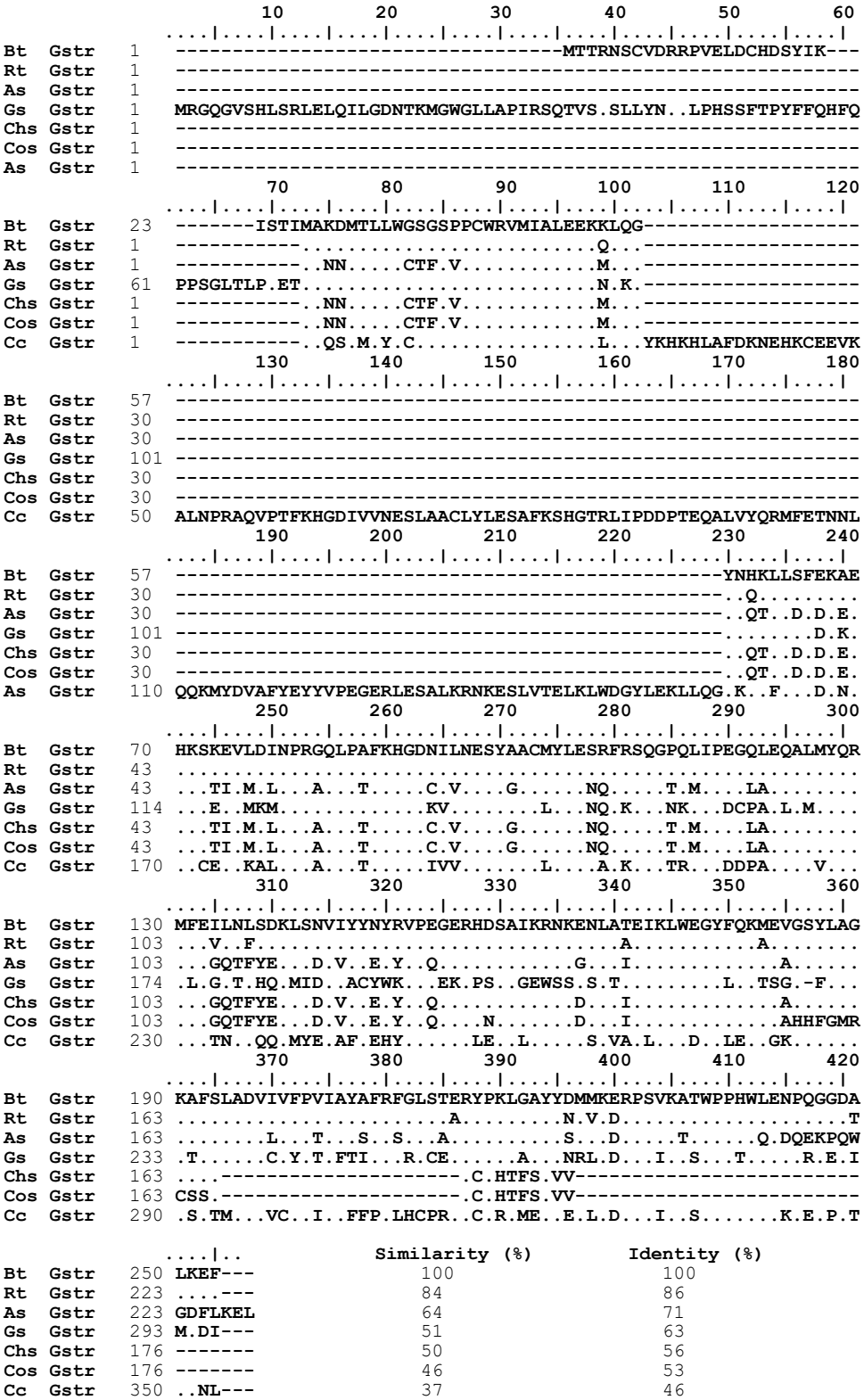

Figure 5. Similarity-Identity rates among *gstr* genes of brown trout (Bt) and rainbow trout (Rt), Atlantic salmon (Ats), gilthead seabream (Gs), chinook salmon (Chs), coho salmon (Cos), and common carp (As)

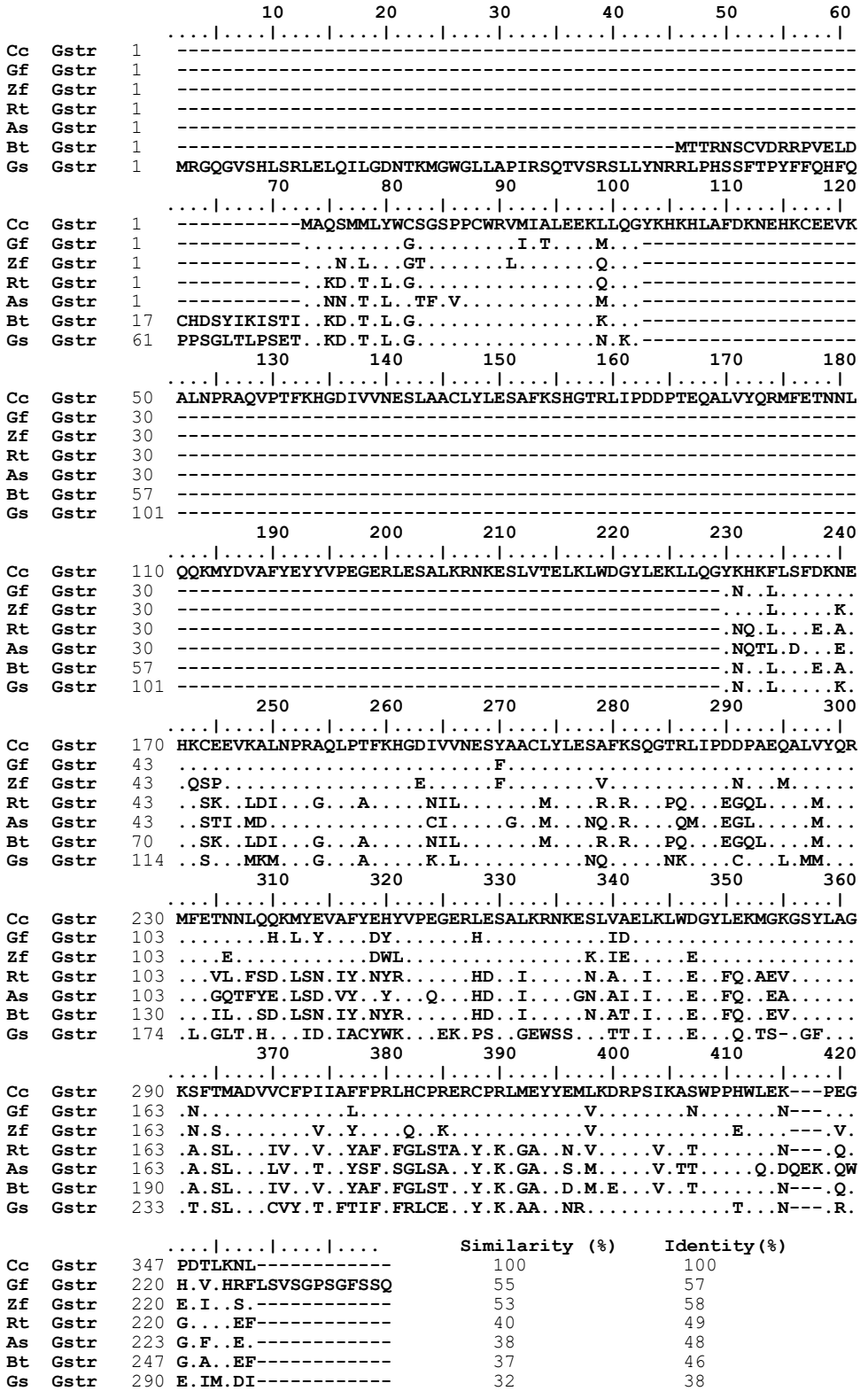

Figure 6. Similarity-identity rates between the brown trout (Bt) and the rainbow trout (Rt), Atlantic salmon (As), Gilthead seabream (Gs), zebrafish (Zf), and common carp (Cc), goldfish (Gf) *gstr* genes.

Husbandry and dissection of fish

The study obtained three adult female and three adult male brown trout, in addition to three female and three male common carp from the Faculty of Fisheries at Atatürk University.These fish were housed in a 100-liter aquarium at temperatures maintained at 29 ± 1°C for common carp and 9 ± 1°C for Brown trout. They were fed a commercial diet twice daily until they were fully satiated. The stocking density was set at 100 fish per cubic meter, and the pH level was maintained at 7.5 for common carp and 7 for Brown trout. A diurnal light: dark cycle of 12:12 hours was provided by fluorescent lighting. Molecular analyses were conducted at the Agricultural Biotechnology Laboratory. In the study, liver, intestinal muscle, brain, heart, eye, spleen, gill, kidney, stomach, and gonad samples were taken from all fish. The samples were placed in 2 ml Eppendorf tubes containing 1 ml of RNA later and stored at +4°C for 24 hours and then at -80°C until the day of analysis. Prior to sample collection, the fish were anesthetized using clove oil. Before the dissection process, the dissecting instruments and the work area were sterilized and cleaned using RNase ZAP (Invitrogen™). The entire study was conducted in accordance with the rules of the Local Ethics Committee for Animal Experiments at Atatürk University (29.04.2021/E-75366018-000-2100117626).

RNA isolation and reverse transcriptase (RT) and real-time PCR (qPCR) analysis

To extract total RNA, liver and gill tissue samples were taken out of RNAlater and homogenized using trizol reagent (Life Technologies). The concentration of RNA was measured using a Nanodrop 8000 spectrophotometer, and the quality of the total RNA was assessed through agarose gel-electrophoresis. For cDNA synthesis, 2 µg of RNA from each tissue was utilized. The RNA underwent DNase treatment (DNase I, Amplification Grade, Life Technologies) and was then converted into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Life Technologies). After RNA isolation, the isolated RNA samples were quantitatively analyzed using nanodrop measurements. RNA samples with quantities ranging from 800 to 1000 ng/µl and OD260/OD280 ratio between 1.8-2 were used. In cases where the RNA concentrations were high, dilutions were performed.

The quantity of brown trout and common carp *gstr* transcript (copy number/µL) was determined using the SYBR Green PCR Kit method on a qPCR instrument. Each qPCR tube contained 10 µL SYBR Green, 5 µL DNAse/RNAse-free water, 2 µL forward primer, 2 µL reverse primer, and 1 µL cDNA. For each sample, two replicates were performed, and a negative control was included in each analysis. The qPCR procedure consisted of an initial denaturation at 95°C for 15 minutes, followed by 40 cycles of denaturation at 95°C for 20 seconds, annealing at the optimum temperature determined for each gene for 30 seconds, and elongation at 72°C for 30 seconds.

Statistical analysis

The statistical analyses were conducted using GraphPad Prism 9 software in the United States. The data underwent one-way ANOVA, and significance was determined using Duncan's multiple range post hoc test. These statistical tests were used to compare the levels of *gstr* gene expression in different tissues of both brown trout and common carp. All data are presented as mean ± SEM. Values were considered statistically significant when $p < 0.05$.

RESULTS

Bioinformatics studies of *gstr* gene in brown trout and common carp

The *gstr* gene and other genes such as *adgrb1b*, *eomesa, nrm, tmem65, msto1, akap9, scrt1a,* and *tert* which are conserved among these organisms, were found on chromosome 36 in brown trout, chromosome 1 in common carp, and chromosome 19 in zebrafish.

The in-silico analysis of the *gstr* gene in brown trout and common carp aimed to provide basic data for the development of modern strategies to protect against the harmful effects of oxidative stress in both cultured fish and other vertebrates. The analysis revealed that the brown trout *gstr* gene has 7 exons and 6 introns, while common carp *gstr* gene has 6 exons and 5 introns, both with a highly conserved exon-intron organization. Alignment analysis of the brown trout and common carp Gstr/GSTR sequences using CLUSTAL W revealed that the polypeptide identity and similarity rates between brown trout and other species, such as rainbow trout, Atlantic salmon, sea bream, Chinook salmon, Coho salmon, and common carp, were quite high. Similarly, the polypeptide identity and similarity rates between common carp and goldfish, zebrafish, rainbow trout, Atlantic salmon, brown trout, and sea bream were also quite high. The analysis also revealed that the brown trout *gstr* gene shared the highest similarity and identity rates with rainbow trout, while common carp *gstr* gene had the highest similarity and identity rates with goldfish.

Tissue-specific transcription of gstr gene in brown trout and common carp

In this study, the tissue-specific distribution of the *gstr* gene was determined in female and male brown trout and common carp using qPCR (Figure 7). For female brown trout, the tissue-specific distribution of the *gstr* gene was determined as follows: liver 25.66 ± 1.49, intestine 13.68 ± 0.61, muscle 0.42 ± 0.02, brain 1.73 ± 0.39, heart 2.89 ± 0.43, eye 2.35 ± 0.18, spleen 0.96 ± 0.21, gill 14.27 ± 0.82, kidney 0.98 ± 0.17, stomach 1.25 ± 0.15, and ovary 7.39 ± 0.32. For male brown trout, the tissue-specific distribution was determined as follows: liver 32.60 ± 1.70, intestine 22.08 ± 0.59, muscle 4.01 ± 0.25, brain 1.38 ± 0.15, heart 4.79 ± 0.25, eye 1.84 ± 0.13, spleen 1.33 ± 0.08, gill 24.70 ± 1.14, kidney 1.83 ± 0.08, stomach 2.74 ± 0.13, and testis 15.86 ± 0.83. The results showed that the liver had higher gene expression than all other tissues, and the intestine and gill

had significantly higher gene expression than the liver in both female and male brown trout.

The ovary and testis tissues had the third-highest *gstr* gene expression. When the transcriptional differences between male and female tissues were examined, it was observed that the intestine, gill, kidney, stomach, muscle, and gonads had significantly higher expression in male brown trout, while other tissues did not show significant differences between male and female brown trout.

The tissue-specific distribution of the *gstr* gene in common carp (Figure 8) was also determined, and the *gstr* gene in female common carp showed the following expression levels: liver 39.06 ± 3.63, intestine 29.48 ± 2.98, muscle 19.32 ± 1.32, brain 4.07 ± 0.50, heart 5.96 ± 0.39, eye 2.11 ± 0.08, spleen 1.09 ± 0.093, gill 8.43 ± 0.33, kidney 1.79 ± 0.21, stomach 3.00 ± 0.43, and ovary 12.11 ± 0.62.

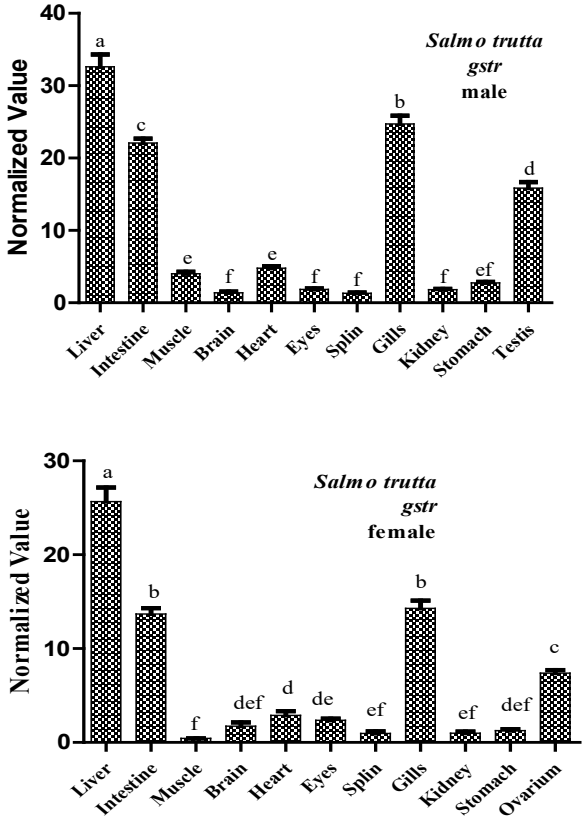

Figure 7. The tissue-specific distribution of brown trout *gstr* gene

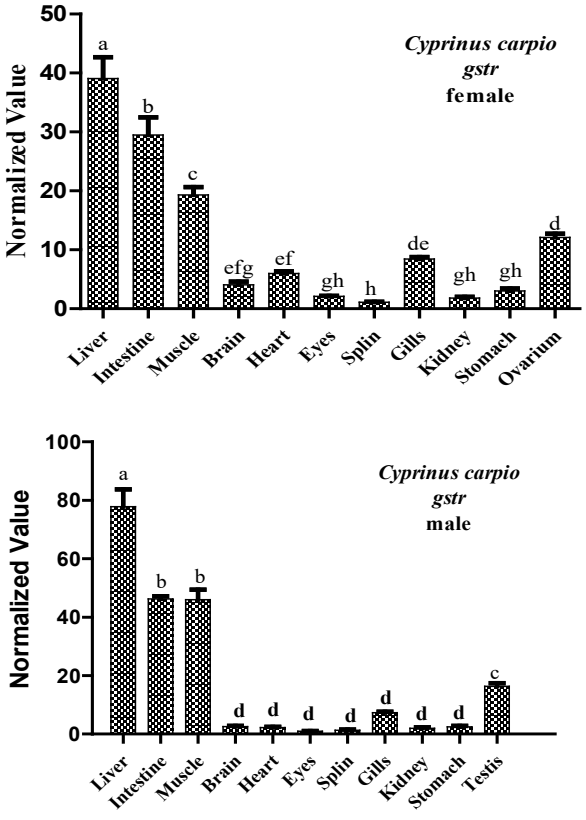

Figure 8. The tissue-specific distribution of common carp *gstr* gene

In male common carp, the tissue-specific distribution showed the following expression levels: liver 77.81 ± 5.95, intestine 46.25 ± 0.91, muscle 45.95 ± 3.42, brain 2.56 ± 0.23, heart 2.20 ± 0.25, eye 0.92 ± 0.081, spleen 1.32 ± 0.18, gill 7.27 ± 0.37, kidney 1.99 ± 0.27, stomach 2.46 ± 0.33, and testis 16.29 ± 1.16. The highest gene expression was observed in the liver for both female and male common carp, while the second-highest gene expression in females was in the intestine, and in males, it was in both the intestine and muscle. The brain, eye, spleen, kidney, heart, and gill tissues showed significantly lower *gstr* gene expression in both female and male common carp. In brown trout, the *gstr* gene exhibits the highest gene expression in the liver tissue in both females and males ($p<0.05$), while the intestine and gills are identified as tissues with the second-highest gene expression.

The differences between these two tissues are statistically insignificant in both female and male fish. The results indicate that the liver has the highest gene expression among all tissues, the intestine and gills have significantly lower *gstr* gene expression compared to the liver, and ovaries and testes have the third-highest *gstr* gene expression. When examining transcriptional differences between genders, the intestine, gills, kidney, stomach, muscle, and gonads show significantly higher gene expression in males ($p<0.05$), while the differences among other tissues are statistically not significant.

In common carp, the *gstr* gene shows the highest gene expression in the liver tissue in both females and males ($p<0.05$). In females, the intestine has the second-highest gene expression, while in males, both the intestine and muscle tissues exhibit the highest gene expression.

DISCUSSION

Gene expression analysis to determine the effects of various sources of stress on cells compared to healthy cells is commonly used in the diagnosis and treatment of disease

(Aubrecht and Caba, 2005). This approach can also be used to develop compounds that bind to expressed proteins and to identify transcriptional regulators that cause changes in expression levels. The common carp (*Cyprinus carpio*) and brown trout (*Salmo trutta*) will be used in this study to identify and characterize *gstr* gene which is antioxidant enzyme (AE) gene and to determine the biological significance of a signaling pathway. The tissue-specific distribution of the glutathione s transferase (*gstr*) gene in common carp and brown trout will be studied, and the results will be used as essential and fundamental precursor data for other studies. Antioxidant enzymes play a vital role in the antioxidant defense system in biological systems. Therefore, this study will be important for developing gene therapy for stress-induced diseases in the future.

Bioinformatics Studies of of *gstr* gene in brown trout and common carp

The designed conserved gene synteny indicates that the *gstr* gene in brown trout and common carp resulted from teleost whole-genome duplication (TTGD). Based on the synteny, it can be said that the conservation rate of the *gstr* gene is quite high (Figure 1). After the teleost-specific genome duplication in teleost fish, many genes have duplicate copies (Braasch and Postlethwait, 2012). However, it was determined that both brown trout and common carp have only one copy of the *gstr* gene. Therefore, it is suggested that this gene underwent duplication first and then one of the copies was lost.

In brown trout and common carp, in-silico analyses were conducted to characterize and identify the *gstr* gene. Especially, valuable data for developing molecular strategies to protect against the effects of reactive oxygen species in cultured fish were obtained and presented to the scientific community. In this study, the *gstr* gene in the brown trout and common carp genomes was found to have 7-6 and 6-5 exon-intron counts, respectively, based on Ensembl database searches. Alignment analyses of the *gstr* gene of brown trout and common carp with their orthologs in rainbow trout, Atlantic salmon, sea bream, sea bass, Coho salmon, and common carp, and Japanese medaka, zebrafish, rainbow trout, Atlantic salmon, brown trout, and sea bream, respectively, using CLUSTAL W (Thompson et al., 1994) revealed that brown trout has high identity and similarity rates with rainbow trout (Figure 5). On the other hand, common carp was found to have the highest identity and similarity rates with Japanese medaka (Figure 6).

Tissue-specific transcription of *gstr* gene in brown trout and common carp

Genetic expression changes are primary responses in fish, making genomic analyses a valuable advantage for research, and measurements of gene expression could facilitate the early detection and assessment of adverse effects on fish caused by various stressors (Larsen et al., 2010; Rojas-Hernandez et al., 2019). Approaches to gene expression have the potential to identify sensitive, mechanism-based biomarkers that can also reveal long-term harmful effects (Voelker et al., 2007). When examining the applications of genomic analysis in aquaculture, it has been observed that responses to stress factors might involve not only small changes in gene expression but also a series of gene interactions (Guo et al., 2023). Core genes generally regulate metabolic pathways, and alterations in these core genes can lead to various outcomes observable through genomic responses (Papin et al., 2003).

CONCLUSION

In conclusion, genomic analysis and measurements of gene expression are valuable tools for assessing the effects of stressors on fish and identifying sensitive, mechanism-based biomarkers that can reveal long-term harmful effects. The *gstr* gene exhibits the highest gene expression in the liver tissue of both brown trout and common carp, with statistically significant differences observed between tissues. Additionally, transcriptional differences between genders were observed in several tissues. The importance of examining gene interactions and alterations in core genes that regulate metabolic pathways when examining responses to stress factors in fish. Overall, the use of genomic analysis and gene expression measurements can provide valuable insights into the health of aquatic ecosystems and the effects of environmental contaminants on fish populations.

ACKNOWLEDGMENTS AND FUNDING

The present research was financially supported by Atatürk University Scientific Research Project (FYL-2021-9372).

AUTHOR CONTRIBUTIONS

The manuscript, produced from Badrul Islam Elsevar's master thesis, involves collaborative contributions from the authors. Badrul Islam Elsevar has taken on responsibilities such as literature review, drafting, writing, laboratory experiments, and data analysis and management. Meanwhile, the role of another author, referred to as Mehtap Bayır, includes conceptualization, drafting, writing, review, editing, and supervision. It is important to note that all authors have collectively reviewed and endorsed the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ETHICAL APPROVAL

The research adhered to all relevant international, national, and institutional guidelines for the ethical care and use of animals. Approval was granted by the Local Ethics Committee for Animal Experiments of Atatürk University (27.05.2021/No:127)

DATA AVAILABILITY STATEMENTS

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

REFERENCES

Adamek, M., Matras, M., Surachetpong, W., Rakus, K., Stachnik, M., Bauer, J., Falco, A., Jung-Schroers, V., Piewbang, C., Techangamsuwan, S., Abd El Rahman, S., Paley, R., Reichert, M., & Steinhagen, D. (2023). How susceptible are rainbow trout and brown trout to infection with tilapia lake virus at increased water temperature – Is there any potential for climate change driven host jump? *Aquaculture*, 571, 739469. https://doi.org/10.1016/j.aquaculture.2022.739469

Arlinghaus, R., & Mehner, T. (2003). Socio-economic characterisation of specialised common carp (*Cyprinus carpio* L.) anglers in Germany, and implications for inland fisheries management and eutrophication control. *Fisheries Research*, *61*(1-3), 373-389. https://doi.org/10.1016/S0165-7836(02)00243-6

Aubrecht, J., & Caba, E. (2005). Gene expression profile analysis: An emerging approach to investigate mechanisms of genotoxicity. *Pharmacogenomics*, *6*(4), 419. https://doi.org/10.1517/14622416.6.4.419

Bayat, A. (2002). Science, medicine, and the future: Bioinformatics. *British Medical Journal*, ;*324*(7344), 1018-22. https://doi.org/10.1136/bmj.324.7344.1018

Braasch, I., & Postlethwait, J.H. (2012). Polyploidy in fish and the teleost genome duplication. In P.S. Soltis, D.E. Soltis (Eds.), *Polyploidy and Genome Evolution*, 341-383. https://doi.org/10.1007/978-3-642-31442-1_17

Chen, B., Peng, W., Xu, J., Feng, J., Dong, C., & Xu, P. (2017). Genomic analysis of glutathione S-transferases (GST) family in common carp: Identification, phylogeny and expression. *Pakistan Journal of Zoology*, *49*(4), 1437-1448. https://doi.org/10.17582/journal.pjz/2017.49.4.1437.1448

Chen, L., Xu, J., Sun, X., & Xu, P. (2021). Research advances and future perspectives of genomics and genetic improvement in allotetraploid common carp. *Reviews in Aquaculture*, *13*(1), 16 26. https://doi.org/10.1111/RAQ.12636

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, *17*(6), 368-376. https://doi.org/10.1007/BF01734359

Franěk, R., Kašpar, V., Shah, M.A., Gela, D., & Pšenička, M. (2021). Production of common carp donor-derived offspring from goldfish surrogate broodstock. *Aquaculture*, *534*, 736252. https://doi.org/10.1016/j.aquaculture.2020.736252

Glisic, B., Mihaljevic, I., Popovic, M., Zaja, R., Loncar, J., Fent, K., Kovacevic, R., & Smital, T. 2015. Characterization of glutathione-S-transferases in zebrafish (*Danio rerio*). *Aquatic Toxicology*, *158*, 50-62. https://doi.org/10.1016/j.aquatox.2014.10.013

Guo, C., Duan, Y., Ye, W., Zhang, W., Cheng, Y., Shi, M., & Xia, X.Q. (2023). FishGET: A fish gene expression and transcriptome database with improved accuracy and visualization. *iScience*, *26*(4), 106539. https://doi.org/10.1016/j.isci.2023.106539

Keiz, K., Ulrich, S., Wenderlein, J., Keferloher, P., Wiesinger, A., Neuhaus, K., Lagkouvardos, I., Wedekind, H., & Straubinger, R.K. (2023). The development of the bacterial community of Brown Trout (*Salmo trutta*) during ontogeny. *Microorganisms*, *11*(1), 211. https://doi.org/10.3390/microorganisms11010211

Kim, J.H., Dahms, H.U., Rhee J.S., Lee, Y.M., Lee J., Han, K.N., Lee,J.S. 2010. Expression profiles of seven glutathione S-transferase (GST) genes in cadmium-exposed river pufferfish (*Takifugu obscurus*). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 151*(1), 99-106. https://doi.org/10.1016/j.cbpc.2009.09.001

Larsen, P.F., Schulte, P.M., & Nielsen, E.E. (2010). Gene expression analysis for the identification of selection and local adaptation in fishes. *Journal of Fish Biology*, *77*(9), 1745-1776. https://doi.org/10.1111/j.1095-8649.2010.02834.x

Liang, X.F., Li, G.G., He, S., & Huang Y., 2007. Transcriptional responses of alpha- and rho-class glutathione S-transferase genes in the liver of three freshwater fishes intraperitoneally injected with microcystin-LR: Relationship of inducible expression and tolerance. *Journal of Biochemical and Molecular Toxicology*, *21*(5), 289-98. https://doi.org/10.1002/jbt.20188

Lynch, A.J., Cooke, S.J., Deines, A.M., Bower, S.D., Bunnell, D.B., Cowx, I.G., Nguyen, V.M., Nohner, J., Phouthavong, K., Riley, B., Rogers, M.W., Taylor, W.W., & Woelmer, W. (2016). The social, economic, and environmental importance of inland fish and fisheries. *Environmental Reviews*, *24*(1), 75-91. https://doi.org/10.1139/er-2015-0064

Özdemir, E., & Bayır, M. (2023). Molecular cloning and characterization of Cu-Zn superoxide dismutase (sod1) gene in brown trout and its expression in response to acute aquaculture stressors. *Animal Biotechnology*, *34*(6), 1968-1978. https://doi.org/10.1080/10495398.2022.2061505

Pandi, P., Madhuvandhi, J., Priya, K.K., Thiagarajan, R., Gopalakrishnan, S., Elumalai, S., & Thilagam, H. (2022). Weathered polyethylene microplastics exposure leads to modulations in glutathione-S-transferase activity in fish. *Frontiers in Marine Science*, *9*, 1521. https://doi.org/10.3389/fmars.2022.990351

Papin, J. A., Price, N. D., Wiback, S. J., Fell, D. A., & Palsson, B. O. (2003). Metabolic pathways in the post-genome era. *Trends in Biochemical Sciences*, *28*(5), 250-258. https://doi.org/10.1016/S0968-0004(03)00064-1

Qian, X., Ba, Y., Zhuang, Q., & Zhong, G. (2014). RNA-Seq technology and its application in fish transcriptomics. *OMICS: A Journal of Integrative Biology*, *18*(2), 98–110. https://doi.org/10.1089/omi.2013.0110

Rojas-Hernandez, N., Véliz, D., & Vega-Retter, C. (2019). Selection of suitable reference genes for gene expression analysis in gills and liver of fish under field pollution conditions. *Scientific Reports*, *9*, 3459. https://doi.org/10.1038/s41598-019-40196-3

Rudneva, I.I., Kuzminova, N.S., & Skuratovskaya E. N., 2010. Glutathione-S-Transferase Activity in Tissues of Black Sea Fish Species. *Asian Journal of Experimental Biological Sciences*, *1*(1),141-150.

Thompson, J. D., Higgins, D. G., Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*(22), 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Tierbach, A., Groh, K.J., Schönenberger, R., Schirmer, K., & Suter, M.J. (2018). Glutathione S-transferase protein expression in different life stages of zebrafish (*Danio rerio*). *Toxicological Sciences*, *162*(2), 702–712. https://doi.org/10.1093/toxsci/kfx293

Voelker, D., Vess, C., Tillmann, M., Nagel, R., Otto, G. W., Geisler, R., Schirmer, K., & Scholz, S. (2007). Differential gene expression as a toxicant-sensitive endpoint in zebrafish embryos and larvae. *Aquatic Toxicology*, 81(4), 355-364. https://doi.org/10.1016/j.aquatox.2006.12.014

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 276-285 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.06

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The gonadal health status of Cyprinidae fish species collected from the river impacted by anthropogenic activities

Antropojenik aktivitelerden etkilenen nehirden toplanan Cyprinidae familyasına ait balık türlerinin gonad sağlığı durumları

Hatice Dane[1] ● Turgay Şişman[2*]

[1]Department of Biology, Science Faculty, Atatürk University, 25240, Erzurum, Türkiye https://orcid.org/0000-0001-7623-7037
[2]Department of Molecular Biology and Genetics, Science Faculty, Atatürk University, 25240, Erzurum, Türkiye https://orcid.org/0000-0002-1098-1741

*Corresponding author: tsisman@atauni.edu.tr
Received date: 09.08.2023 Accepted date: 13.11.2023

How to cite this paper:
Dane, H., & Şişman, T. (2023). The gonadal health status of Cyprinidae fish species collected from the river impacted by anthropogenic activities. *Ege Journal of Fisheries and Aquatic Sciences,* 40(4), 276-285. https://doi.org/10.12714/egejfas.40.4.06

Abstract: Many freshwater ecosystems are contaminated with heavy metals released by municipal wastewater, cultivation and factory wastewater. The aim of the study was to evaluate the negative impact of metal pollution on the fish reproductive system. It was performed using the gonadal histopathology, hepatosomatic index (HSI), and gonadosomatic index (GSI) of three fish species, *Capoeta damascina*, *Squalius semae* and *Alburnus sellal* inhabiting in Karasu River (Erzurum, Turkey) contaminated by various anthropogenic activities. The highest GSI values were determined for each sex according to the seasons, and lower GSI values were observed in polluted stations in all three species. It was observed that HSI values in fish varied significantly from station to station. In addition, an increase in ovarian and testicular lesions (degeneration in oocyte and spermatocytes, atresia, congestion, infiltration, edema, vascular hypertrophy, fibrosis) was detected in the polluted areas. The results clearly showed that the metal pollution in the river adversely affected the reproductive system of the fish species living in the river.

Keywords: Freshwater fish, Karasu River, gonad histology, GSI, HSI, water pollution, histopathology

Öz: Birçok tatlı su ekosistemi, belediye atık suları, tarım ve fabrika atık sularında bulunan ağır metallerle kirlenmektedir. Bu çalışmanın amacı, metal kirliliğinin balıkların üreme sistemi üzerindeki olumsuz etkilerini değerlendirmektir. Bunun için çeşitli antropojenik faaliyetlerle kirlenen Karasu Nehri'nde (Erzurum) yaşayan üç balık türünde (*Capoeta damascina*, *Squalius semae* ve *Alburnus sellal*) gonadal histopatoloji, hepatosomatic indeks (HSI) ve gonadosomatik indeks (GSI) analizleri yapıldı. Mevsimlere göre en yüksek GSI değerleri her cinsiyet için belirlenmiş ve her üç türde de kirli istasyonlarda düşük GSI değerleri gözlenmiştir. HSI değerleri ise istasyonlar arası önemli oranda değişkenlik göstermiştir. Ayrıca özellikle kirli bölge balıklarının yumurtalık ve testislerinde tespit edilen patolojik lezyonlarda (oosit ve spermatositlerde dejenerasyon, atrezi, konjesyon, infiltrasyon, ödem, vasküler hipertrofi, fibrosis gibi) belirgin artışların olduğu saptanmıştır. Sonuçlar nehirdeki metal kirliliğinin nehirde yaşayan balık türlerinin üreme sistemini olumsuz etkilediğini açıkça göstermiştir.

Anahtar kelimeler: Tatlısu balığı, Karasu Nehri, gonad histolojisi, GSI, HSI, su kirliliği, histopatoloji

INTRODUCTION

Pollutants merged in both terrestrial and aquatic environments due to anthropogenic activities have a negative impact on both human and other organisms. It is known that anthropogenic chemical production has increased in recent years. This production reaches up to 400 million tons annually (Gavrilescu et al., 2015). This increase causes a rise in the amounts of substances merging in aquatic system. There is not enough information on the potential environmental risks of these substances as of now (Naidu et al., 2016). Mixing of unwanted substances in aquatic systems results in an unbalance regarding physical, chemical, and biological properties of water ecologically (Yadav et al., 2018). Among these substances, industrial wastes cause water pollution, thereby posing a threat to both aquatic plants and animals (Gupta et al., 2015). This pollution leads to a constant decrease in water flora and fauna, especially in fish population. All aquatic species, including fish, either absorb pollutants directly from water or take them through food chain (Łuczyńska et al., 2018). Fish, being the most important species used in the estimation of pollutant levels in water, provide some advantages in terms of identifying natural properties of aquatic systems and evaluating habitat changes (Martinez-Haro et al., 2015). In addition, due to being in most of the aquatic systems exposed to pollutants, their effects on the structure of food chain, and their ecological suitability, fish are considered as a main bioindicator in evaluating the quality of aquatic ecosystems (Corredor-Santamaría et al., 2019).

Exposure to pollutants may cause an exposure to acute or chronic toxicity in fish organs, especially gonads. This leads to a deficiency in reproduction. The health of the fish reproductive process is an important indicator that the organism is self-sustainable (Zulfahmi et al., 2018). Pesticides, heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, alkylphenolic compounds, phthalates, and endocrine disrupting chemicals may damage the reproductive physiology of fish populations (Agbohessi et al., 2015). In some studies, it

was reported that gonadal abnormalities, decreased gonadosomatic index values, varied hormone levels, delay in plasma vitellogenin levels and gametogenesis occurred in fish which were found in areas with wastewater containing agricultural and industrial chemicals (Gilroy et al., 2012; Kaptaner, 2015). However, there is not enough information on the ecological risks caused by reproductive disruption following exposure to toxic substances in freshwater fish species in Turkey and the subject needs further research.

Capoeta damascina (Güldenstädt, 1773), *Squalius semae* (Linnaeus, 1758) and *Alburnus sellal* (Heckel, 1843) species used in this study belong to Cyprinidae family. These species are widespread in Euphrates and Tigris River systems in the Eastern and Southeastern Anatolia regions in Turkey (Geldiay and Balık, 2009). Wastewater in Erzurum province, agricultural activities in Karasu Basin and industrial wastes pollute the surface water of the Karasu River (Sönmez et al., 2012; Anonymous, 2016; Aydoğan et al., 2017; Dane and Şişman, 2017). In fact, previous reports have shown that the surface water and sediment of the river are particularly contaminated with metals (Dane and Şişman 2020a, 2020b). In this study, it was aimed to evaluate histopathologically how the fish species found in the natural fauna of selected locations are affected in terms of gonadal health due to metal pollution.

MATERIALS AND METHODS

Karasu River is unfortunately polluted by discharge of domestic and industrial (cement, paint, and plaster factories) wastes. Wastewater caused by industrial facilities located in the organized industrial zone in Erzurum is connected to municipality's sewage, and the sewage waste is discharged into Karasu River (Anonymous, 2016). In the study, four different stations were selected on the river. The stations were chosen by paying attention to the pollution load of the river and the regions where the fish species were observed. The first station was Dumlu (1st station 40° 05' 36.1" N 41° 22' 49.0" E). Other stations were as follows: Ilıca (2nd station 39° 57' 10.6" N 41° 04' 15.2" E), Aşkale 1 (3rd station 39° 54' 52.7" N 40° 40' 29.4" E) and Aşkale 2 (4th station 39° 56' 15.1" N 40° 37' 25.9" E) (Figure 1).

Dumlu station was taken as the reference region. Livestock and pasture farming are carried out around it and there is a settlement with a population of 1413. There are two factories around the Ilıca (2nd) and agricultural activities are carried out. In addition, the sewage water of the city is given to the river from here. Aşkale stations (3rd and 4th) are the last areas where the river leaves the Erzurum plain and where all the wastes of the city mix, and there are paint and cement factories nearby. Average heavy metal levels and physico-chemical parameters of the surface waters of the sampling stations are shown in Table 1 (Dane and Şişman, 2020a).

Species belonging to the natural fauna of the river, *Capoeta damascina*, *Squalius semae* and *Alburnus sellal* were caught from the stations using nets in May, June, July, August, and September (2015-2016). A total of 158 mature fish were caught from the river, including 54 *C. damascina* (28 ♀♀ 26 ♂♂), 52 *A. sellal* (29 ♀♀ 23 ♂♂) and 52 *S. semae* (24 ♀♀ 28 ♂♂). Permissions required for the study were taken from the relevant authorities before the study was initiated. The fish were brought to the laboratory alive and taken to intensely air-conditioned aquariums. No morphological abnormalities were observed in the fish. The mean total lengths and weights of the fish were 20.79 ± 3.42 cm and 89.08 ± 21.32 g (*C. damascina*), 17.83 ± 3.96 cm and 44.22 ± 5.37 g (*A. sellal*), 16.07 ± 2.03 cm and 48.09 ± 9.26 g (*S. semae*).

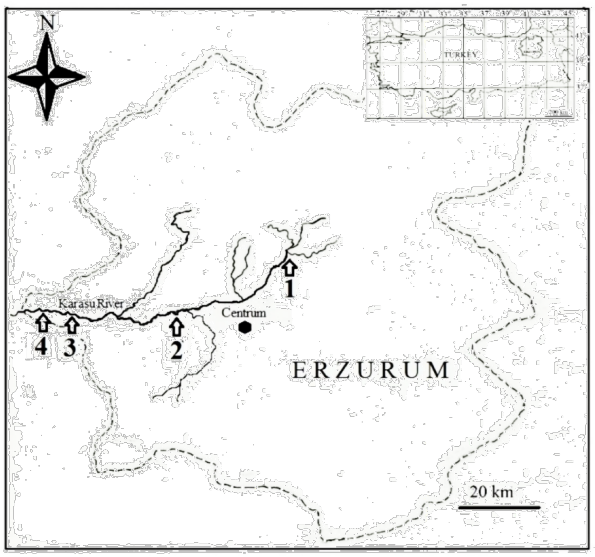

Figure 1. General view of the sampling stations

The hepatosomatic index (HSI) is expressed as the ratio of liver weight to body weight, and provides information about the health status of the fish and the quality of water. The fish were decapitated and the liver were removed. Hence the total organ weight of liver was taken for HSI. HSI was calculated according to the following formula: HSI=(Liver weight (g) / Fish weight (g) × 100 (Sadekarpawar and Parikh, 2013).

The gonadosomatic index (GSI) represents the ratio of fish gonad weight to body weight and used in identifying gonadal development in fish. For GSI calculation, fish were euthanized using MS 222 (75 mg/L). After measuring fish weight, the abdomen was cut from the front of the anus to the bottom of the gill, and organs were taken out the body. After measuring gonadal weight, the following formula was used in GSI calculation. GSI = (Gonadal weight) / (Total body weight) x 100 (Sadekarpawar and Parikh, 2013).

Fifteen fish from each station were used in histological analysis. Gonad samples were fixed in 10% neutral buffered formalin for up to 48 hours and subjected to a standard histological procedure. Parrafin embedded samples were cut with microtome with 5µm thickness. Slides were stained with hematoxylin and eosin (H&E) (Gautier, 2011) and microscopically analyzed with Leica DM750 light microscope and LASEZ software programme. The 10 sections of the gonads of each fish were analyzed. Twenty fields per section of tissue were observed.

Table 1. Current data of the mean metal levels and physico-chemical parameters of surface water samples of the stations (mg/L), and the water quality classes of the river water according to Turkish Water Pollution Control Regulation, and maximum acceptable limits of metal for river waters according to United Nations Economic Commission for Europe (UNECE, 1994), (Dane and Şişman, 2020a)

Metals	1. Station	2. Station	3. Station	4. Station	TS IV	UNECE 1994
Cr	8.55 ± 1.5^{a,b}	6.7 ± 1.2^{a,b}	12.1 ± 2.1^{a,b,*}	13.1 ± 1.8^{a,b,*}	>0.2	0.016
Mn	5.5 ± 1.1^{a}	5.7 ± 1.3^{a}	9.3 ± 1.5^{a,*}	7.7 ± 1.4^{a,*}	>3.0	
Fe	4.1 ± 0.9	5.5 ± 1.1^{a,*}	8.4 ± 1.3^{a,*}	7.8 ± 1.6^{a,*}	>5.0	
Co	0.7 ± 0.1^{a}	0.8 ± 0.1	1.26 ± 0.2^{a,*}	1.2 ± 0.1^{a,*}	>0.2	
Ni	0.8 ± 0.1^{a}	0.74 ± 0.1^{a}	1.17 ± 0.1^{a}	1.01 ± 0.1^{a}	>0.2	1.400
Cu	0.51 ± 0.1^{a,b}	0.57 ± 0.1^{a,b}	0.88 ± 0.1^{a,b,*}	0.82 ± 0.1^{a,b,*}	>0.2	0.018
Zn	0.42 ± 0.1^{b}	0.46 ± 0.1^{b}	0.66 ± 0.1^{b}	0.61 ± 0.1^{b}	>2	0.120
As	1.14 ± 0.1^{a,b}	1.27 ± 0.1^{a,b}	1.55 ± 0.2^{a,b,*}	1.46 ± 0.2^{a,b,*}	>0.1	0.360
Se	0.11 ± 0.01^{a}	0.20 ± 0.01^{a,*}	0.24 ± 0.01^{a*}	0.35 ± 0.05^{a,*}	>0.02	
Pb	0.29 ± 0.01^{a,b}	0.69 ± 0.1^{a,b,*}	2.55 ± 0.5^{a,b,*}	2.96 ± 0.6^{a,b,*}	>0.05	0.082
Physico-chemical parameters						
Temperature (ºC)	21±4.1	23±4.8*	25±5.7*	25±5.5*	>30	-
pH	7.81±1.5	7.74±1.6	8.03±1.2	8.06±1.4	9.0	-
Dissolved oxygen (mg/L)	7.62±1.0	6.32±1.5	5.60±1.1*	4.31±0.8*	<3	-

Values are expressed as mean ± standard errors. Asterisk shows statistical difference compared to reference station (1st Station). TS IV; Turkish Standard IV means the surface water are polluted. The letter "a" indicates that metal concentration exceeds the TS IV value, and the letter "b" indicates that metal concentration exceeds the UNECE limit.

Histological damage detected in gonads was assessed via the Degree of Tissue Change (DTC). Damages detected in tissues are grouped as the following according to DTC parameter: 0 (no abnormality), 1 (low abnormality frequency), 2 (medium abnormality frequency), and 3 (high abnormality frequency) (Abdel-Moneim et al., 2012). Abnormalities in gonads were classified according to the damage phase in DTC calculation. These phases are as follows: 1st Phase: Tissue has a normal function. 2nd Phase: Normal function of the tissue is disrupted at medium severity. 3rd Phase: An irreversible damage has occurred in the tissue (Bernet et al., 1999). DTC values were found using the formula: DTC = (1 x ΣI) + (10 x ΣII) + (100 x ΣIII). In the formula, ΣI, ΣII and ΣIII indicate the total number of abnormalities observed in the phases respectively. After finding the DTC value for each fish, the mean index was calculated for each station. This index was analyzed according to following criteria. DTC: between 0-10: organ functions are normal; between 11-20: mild organ damage; between 21-50: medium-level organ damage; between 51-100: severe organ damage; above 100: irreversible organ damage (Poleksic and Mitrovic-Tutundzic, 1994).

The general evaluation of GSI and HSI were performed by ANOVA. Duncan test was used for multiple comparisons in variance analysis. The data were interpreted by considering $p < 0.05$ significance level.

RESULTS

Mean HSI values of the species caught according to stations are given in Table 2. The highest HSI values for *C. damascina* and *S. semae* were recorded in the 3rd station, The highest value for *A. sellal* was obtained in the 4th station. When a comparison was made between the stations in terms of HSI values, it was determined that the HSI values were significantly higher in the other three stations compared to the 1st station ($p < 0.05$).

Table 2. HSI values of the species according to stations

Stations	*Capoeta damascina*	*Alburnus sellal*	*Squalius semae*
1. Station	0.34 ± 0.06^{c}	0.40 ± 0.07^{c}	0.46 ± 0.06^{d}
2. Station	0.63 ± 0.07^{b}	0.95 ± 0.08^{b}	0.90 ± 0.07^{c}
3. Station	1.34 ± 0.03^{a}	1.29 ± 0.04^{a}	1.70 ± 0.10^{a}
4. Station	1.11 ± 0.06^{a}	1.43 ± 0.08^{a}	1.38 ± 0.08^{b}

Data are presented as mean ± SD. Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$)

The spawning time of the fish caught in the Karasu River was determined by analyzing the gonadosomatic index (GSI) of the male and female individuals. In Table 3, the mean GSI values of female and male individuals are presented for three species. The highest GSI values were recorded in June, whereas the lowest values were recorded in August for *C. damascina*. On the other hand, the highest GSI value was found in *A. sellal* females in June, males in May, and the lowest in both sexes in August. The mean highest value for both female and male individuals of *S. semae* was recorded in May, whereas the lowest value was recorded in July. When the GSI values of fish were compared, the highest average index among the species was observed in *A. sellal*. When the values of the female and male individuals were compared, it was found that the GSI values of the female individuals were higher than the males in all three species. According to the data, the reproductive period for the species was found to be May and June.

The change in mean GSI values in these species by station is given in Table 4. When GSI values of species were analyzed by station, the lowest value for *C. damascina* was recorded in the 3rd station, and the highest value was recorded in the 1st station. The highest mean GSI value for *A. sellal* was obtained in the 1st station, and the lowest GSI value was recorded in the 4th station. The lowest mean value for *S. semae* was recorded in the 3rd station, and the highest mean value was recorded in the 1st station.

Table 3. GSI values of the species by months

Period	*Capoeta damascina*		*Alburnus sellal*		*Squalius semae*	
	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂
May	3.86 ± 1.08^{b}	2.52 ± 0.33^{b}	5.04 ± 0.25^{b}	4.75 ± 0.58^{a}	4.72 ± 0.65^{a}	3.24 ± 0.16^{a}
June	5.87 ± 1.01^{a}	3.85 ± 1.05^{a}	7.65 ± 1.99^{a}	3.81 ± 0.35^{b}	3.65 ± 0.11^{b}	2.59 ± 0.12^{b}
July	1.24 ± 0.03^{d}	0.98 ± 0.03^{d}	2.56 ± 0.35^{c}	2.02 ± 0.22^{c}	0.82 ± 0.05^{e}	0.25 ± 0.08^{e}
August	1.04 ± 0.05^{e}	0.39 ± 0.02^{e}	0.92 ± 0.07^{e}	0.87 ± 0.01^{e}	1.06 ± 0.03^{d}	0.96 ± 0.07^{c}
September	1.36 ± 0.02^{c}	1.14 ± 0.02^{c}	1.26 ± 0.03^{d}	1.12 ± 0.02^{d}	1.20 ± 0.02^{c}	0.81 ± 0.04^{d}

Data are presented as mean ± SD. Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$)

Table 4. GSI values of the species according to stations

Stations	*Capoeta damascina*	*Alburnus sellal*	*Squalius semae*
1. Station	5.90 ± 0.20^{a}	6.13 ± 0.94^{a}	5.58 ± 1.04^{a}
2. Station	5.56 ± 0.10^{a}	4.28 ± 0.51^{b}	4.03 ± 0.23^{b}
3. Station	3.34 ± 0.15^{b}	3.41 ± 0.18^{c}	3.01 ± 0.05^{c}
4. Station	4.12 ± 0.13^{b}	3.06 ± 0.12^{d}	3.27 ± 0.12^{c}

Data are presented as mean ± SD. Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$)

According to these data, it was concluded that mean GSI values were remarkably low, especially in the 3rd and 4th stations, for all three species ($p < 0.05$). When the current state of the surface waters of the stations where the fish samples were taken, the physico-chemical parameters were within the allowable limit values, while the metal levels (Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br and Pb) were higher at the third and fourth stations compared to the other stations (Table 1). On the histological slides of fish caught from Station 1, follicles containing multiple oocysts at different developmental stages (chromatin nucleolar, perinucleolar, cortical alveolar, vitellogenical phase, and maturation stage) were observed (Figure 2).

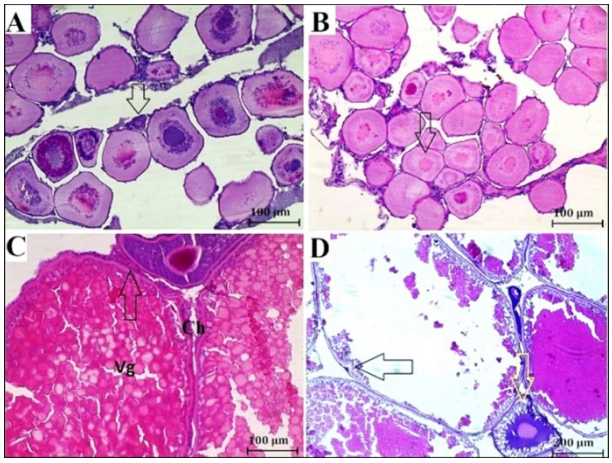

Figure 2. Normal ovarian histology of fish from the Karasu River. A) Chromatin nucleolar oocyte (arrow), (*C. damascina*). B) Perinucleolar oocyte (arrow). C) Vitellogenic oocyte (arrow), Vg: vitellus granule, Ch: chorion (*C. damascina*). D) Cortical alveolar oocyte (yellow arrow), and mature oocyte (black arrow: animal invagination) (*A. sellal*). H&E

When the ovaries were evaluated according to the stations, histopathological damage was observed at varying rates in all three species, especially from the last two stations. In the section in which oocystes were placed belonging the chromatin nucleolar phase in the fish analyzed, congestion in blood vessels, melanomacrophage, mononuclear cell infiltration, and edema were observed (Figure 3A). In addition to the frequently observed enlargement of the follicular epithelium of the cortical alveolar oocyte (Cao) (Figure 3B), mononuclear cell infiltration damage (Figures 3C and 4A), and a severe case of fibrosis (Figure 3C) were detected. Cao shows the cell membrane of the oocyte. The abnormality showed that the vacuoles borders in the cell and the cytoplasm were mixed together in some places. Disorder of cytoplasm and vacuole in the Cao (Figure 3D), proteinaceous fluid and edema in the interstitial space (Figure 4A), as well as atresia (Figure 4B) were identified. Other pathologies were degeneration and malformation in oocytes (Figure 4C) and degeneration in the cytoplasm of the transformed cortical alveolar oocyte (Figure 4D).

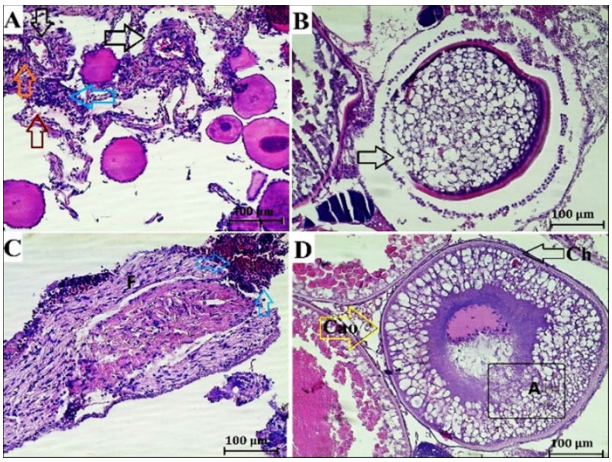

Figure 3. The ovarian pathologies detected in fish species of Karasu River. A) Congestion (black arrows), melanomacrophage (orange arrow), infiltration (blue arrow), edema (red arrow), (*C. damascina*). B) Enlargement of the follicular epithelium (arrow), (*A. sellal*). C) Infiltration (blue arrows) and fibrosis (F), (*C. damascina*). D) Cytoplasm and vacuoles disorder (square), Cao; cortical alveolar oocyte, Ch; chorion, A; alveol (*A. sellal*). H & E

In Table 5, the histological damages detected in the ovary tissues of fish species according to the stations were given with

DTC frequencies. Accordingly, it was concluded that there was no difference between the species in terms of the type of histopathological abnormality, and the frequency of histopathological damage from the 1st station to the others increased even more for all three species.

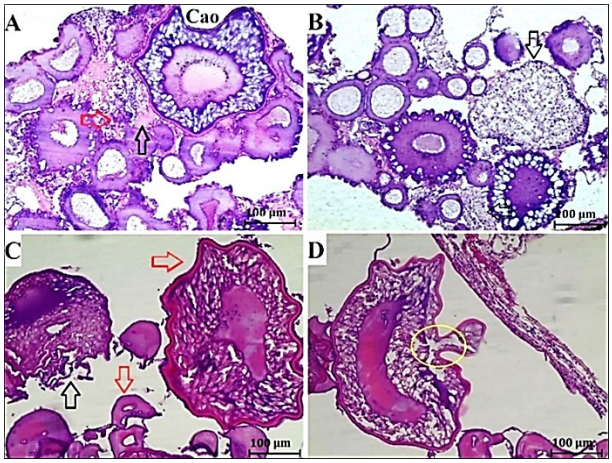

Figure 4. The ovarian pathologies detected in fish species of Karasu River. A) Edema and interstitial proteinaceous fluid (black arrow), infiltration (red arrow), Cao; cortical alveolar oocyte, (*A. sellal*). B) Atresia (arrow), (*A. sellal*). C) Degeneration in the oocyte (black arrow) and oocyte deformity (red arrows), (*S. semae*). D) Degeneration in the oocyte cytoplasm of the deformed cortical alveolar oocyte (yellow circle), (*S. semae*). H & E

When the testicular tissue of the fish caught from Station 1 was examined, a normal structure consisting of numerous seminiferous tubules and interstitial spaces between the tubules, curved, oval or round shaped and of different sizes, containing germ cells at different developmental stages was observed. Secondary spermatocytes that were dark stained in mature testis were observed as clusters in an area close to the tubule lumen. Being the smallest cells, spermatids that were coloured darker was on the lumen. In many sections, it was found that sperms filled the inside of tubules completely (Figure 5).

On the other hand, histological damages were detected at varying frequencies in the testicles of fish caught from other stations. In fish testes analyzed, especially vascular hypertrophy (Figure 6A) and severe congestion (Figures 6A and 6B) were remarkable. In addition, degenerations in the efferent duct and seminiferous tubules (Figure 7A), proteinaceous fluid and inflammatory infiltrate (Figure 7A), edema (Figure 7A and 7B), hypertrophy and degeneration in spermatocytes (Figure 8A), separation in the interstitial area (Figure 6B, 7A, and 8B), and fibrous formations (Figure 6B, 7A, and 8B) were observed. While the lümen of the efferent duct should be smooth, a part of the lumen surface was fragmented in polluted area fish testis (Figure 7A).

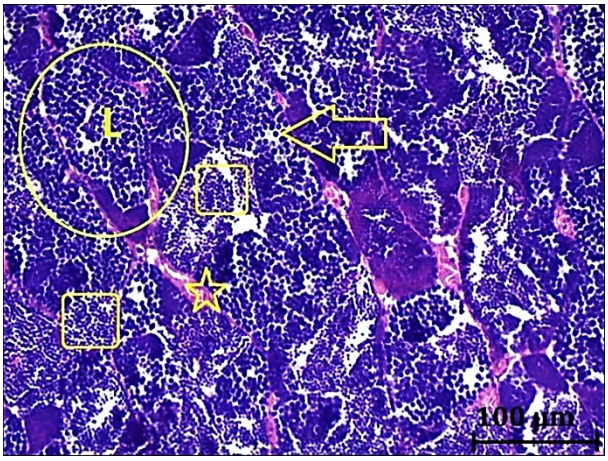

Figure 5. Normal testis histology of fish from the Karasu River (*C. damascina*). Blood vessel (star), spermatids (squares), secondary spermatocyte (arrow), lumen (L), and seminiferous tubule (circle). H & E

In many testes sections, the borders of epithelium surrounding tubules could not be seen clearly, and tissue integrity was disrupted (Figure 8A). Major degeneration was also noted in the seminiferous tubule epithel (Figure 8B). Congestion in tissues with abnormalities has been observed as a common pathology (Figures 8A and 8B).

Table 5. Histological abnormalities and DTC frequencies detected in the ovaries of the species according to the stations

Stations		**1. St**			**2. St**			**3. St**			**4. St**		
Fish species		**C**	**A**	**S**	**C**	**A**	**S**	**C**	**A**	**S**	**C**	**A**	**S**
Ovarium pathologies	**DTC level**												
Atresia	I	1	1	1	1	1	1	1	2	2	1	2	1
Infiltration	I	0	0	0	1	0	1	2	2	2	1	2	2
Proteinaceous fluid	I	0	0	0	0	0	1	1	2	2	1	2	2
Congestion	I	0	0	0	0	0	0	2	1	2	2	2	2
Melanomacrophage center	I	1	1	1	1	1	1	2	1	3	1	3	3
Oocyte deformity	I	0	0	0	0	0	0	1	0	1	0	1	1
Edema	I	0	0	0	0	0	1	1	1	2	1	1	1
Enlargement of the follicular epithelium	II	0	0	0	0	0	0	1	2	2	0	1	1
Degeneration in the oocyte cytoplasm	II	0	0	0	0	1	0	0	1	2	1	1	1
Degeneration of oocytes	II	0	0	0	0	0	1	1	1	2	1	1	1
Fibrosis	III	0	0	0	0	0	0	1	0	1	0	1	0

0: no alteration, 1: mild and focal, 2: moderate and multi-focal, 3: severe and diffuse alterations. St; Station, C; *Capoeta damascina*, A; *Alburnus sellal*, S; *Squalius semae.*

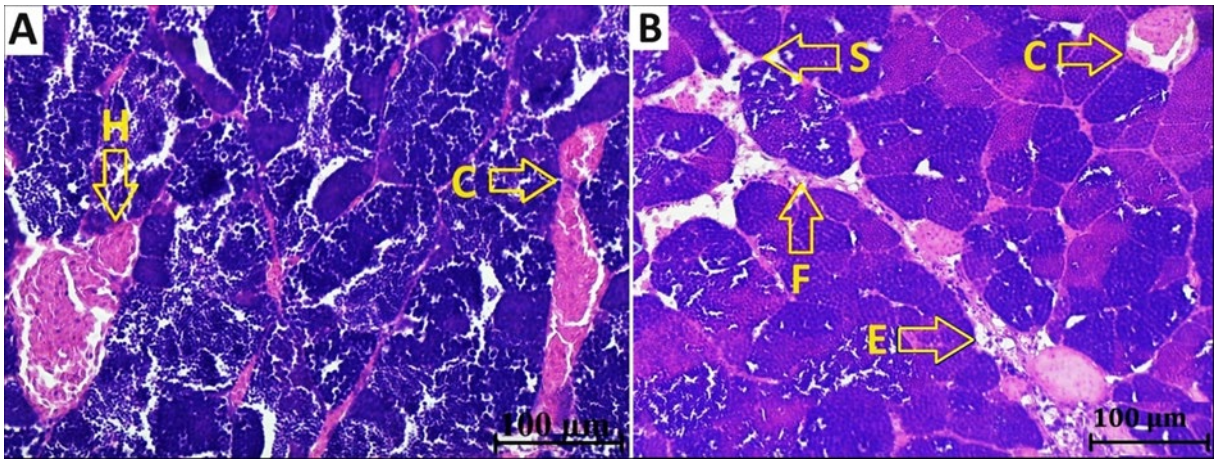

Figure 6. The testis pathologies detected in fish species of Karasu River. A) Vascular hypertrophy (H) and congestion (C), (*C. damascina*).B) Congestion (C), fibrosis (F), separation in the interstitial area (S) and edema (E). H & E

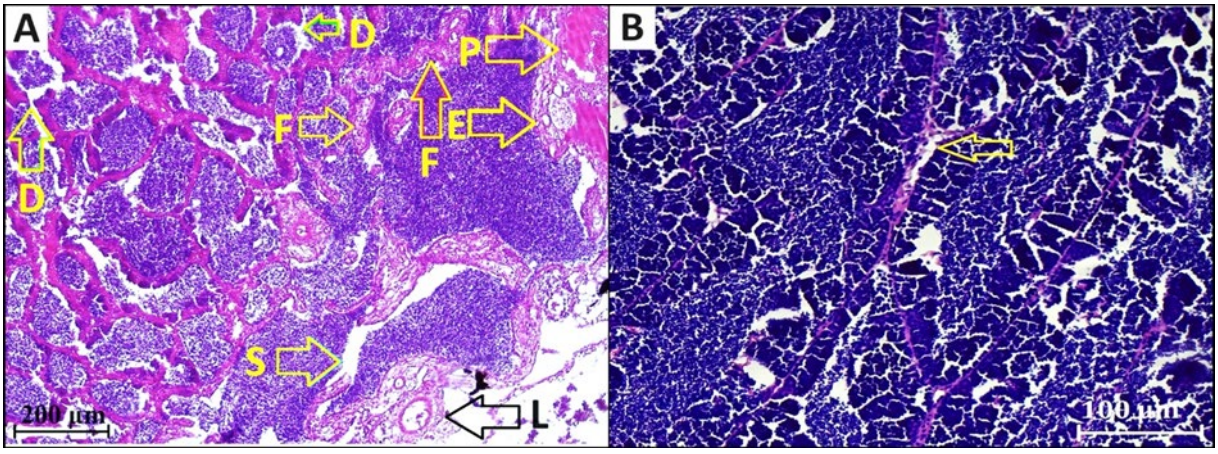

Figure 7. The testis pathologies detected in fish species of Karasu River. A) Proteinaceous fluid and inflammatory infiltrate (P), degeneration of seminiferous tubule (D), fibrosis (F), edema (E), separation in the interstitial area (S), lumen degeneration in the efferent duct (L), (*A. sellal*). B) Edema (yellow arrow) (*A. sellal*). H & E

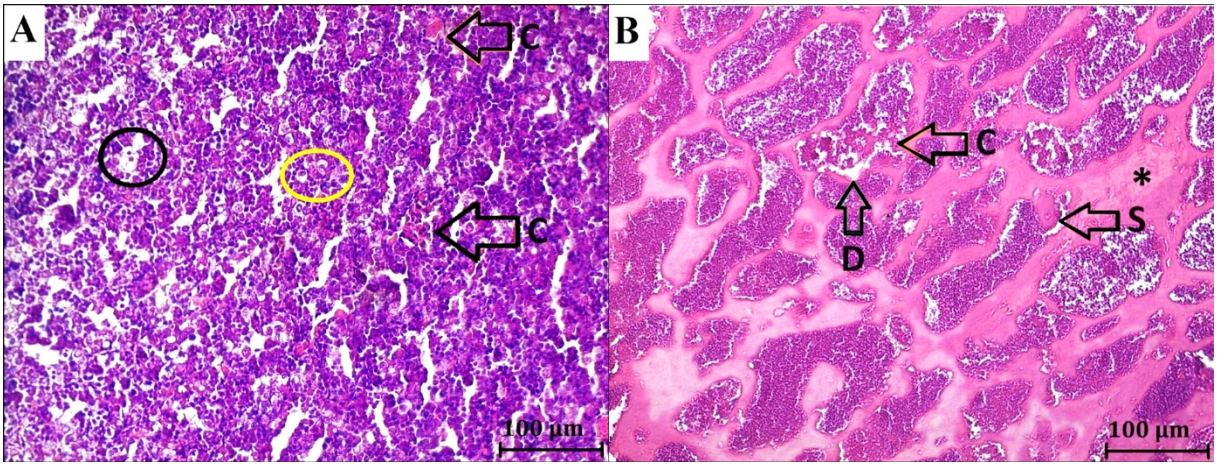

Figure 8. The testis pathologies detected in *S. semae*. A) Degeneration in primary spermatocyte (black circle), hypertrophy in primary spermatocyte (yellow circle), congestion (C), and undetermined seminiferous tubule boundaries (general view). B) Degeneration of the seminiferous tubule epithelium (D), congestion (C), separation in the interstitial area (S), and severe fibrosis (asterisk). H & E

The histological damages detected in the testes of fish species according to stations were given with DTC frequencies in Table 6. Accordingly, it was concluded that there was no difference among fish species in terms of the type of histopathological damage.

Similarly, in all three species, a significant increase was observed in the frequency of the damages at stations 3 and 4. Mean DTC values of fish gonads are given in Table 7. The DTC values demonstrated that gonads had a normal function in the 1st station in all three species, whereas gonads had mild or medium-level damage in other stations. DTC values for *C. damascina* revealed that gonads had mild damage in the 4th station, medium-level damage in the 3rd station, and had normal structure in the 2nd station. DTC values for *A. mossulensis* revealed that the organ had medium-level damage in the 3rd and 4th stations, and mild damage in the 2nd station. DTC values for *S. semae* revealed that gonads had medium-level damage in the 3rd and 4th stations, and mild damage in the 2nd station.

When DTC values for these species were compared, it was seen that the highest values belonged to *S. semae*. While the highest DTC values for *C. damascina* and *S. semae* were recorded in the 3rd station, the highest value for *A. sellal* was recorded in the 4th station. It was found that the differences in DTC values were statistically significant ($p < 0.05$).

Table 6. Histological abnormalities and DTC frequencies detected in the testes of the species according to the stations

Station		1. St			2. St			3. St			4. St		
Fish species		**C**	**A**	**S**	**C**	**A**	**S**	**C**	**A**	**S**	**C**	**A**	**S**
Testis pathologies	**DTC level**												
Proteinaceous fluid and inflammatory infiltrate	I	0	0	0	0	1	1	2	1	3	1	2	2
Separation in the interstitial area	I	0	0	0	0	0	1	2	1	2	1	1	1
Congestion	I	1	1	1	1	1	1	2	2	3	2	2	2
Edema	I	0	0	0	0	0	0	1	1	2	1	2	1
Hypertrophy in spermatocyte	I	0	0	0	0	0	0	1	1	2	1	1	1
Deformity of the seminiferous tubule	I	0	0	0	0	0	1	1	2	2	1	1	1
Undetermined seminiferous tubule boundaries	I	0	0	0	1	1	1	2	2	3	2	2	3
Vascular hypertrophy	I	0	0	0	1	1	1	1	2	2	1	1	2
Lumen degeneration of efferent duct	II	0	0	0	0	0	0	1	1	1	1	1	1
Degeneration in spermatocyte	II	0	0	0	0	0	0	1	1	1	1	1	1
Degeneration of seminiferous tubule	II	1	1	1	1	1	1	3	2	3	2	3	3
Fibrosis	III	0	0	0	0	0	0	1	0	1	0	1	1

0: no alteration, 1: mild and focal, 2: moderate and multi-focal, 3: severe and diffuse alterations. St; Station, C; *Capoeta damascina*, A; *Alburnus sellal*, S; *Squalius semae.*

Table 7. Average DTC values for three fish species from the stations

Stations	*Capoeta damascina*	*Alburnus sellal*	*Squalius semae*
1. Station	5.85 ± 0.22^{d}	8.03 ± 0.31^{d}	8.07 ± 0.35^{d}
2. Station	8.61 ± 0.32^{c}	16.02 ± 0.25^{c}	18.20 ± 0.14^{c}
3. Station	28.60 ± 0.34^{a}	23.16 ± 0.27^{b}	47.63 ± 0.25^{a}
4. Station	18.54 ± 0.19^{b}	40.10 ± 0.24^{a}	39.50 ± 0.36^{b}

Data are presented as mean ± standard deviation. Differences between the averages indicated by the different letters in the same column are statistically significant ($p < 0.05$).

DISCUSSION

Heavy metal pollution is considered to be a serious problem especially in aquatic systems (Joshi, 2011). The reason for the pollution is the increasing industrial, agricultural and mining activities. These activities are increasing day by day (Ashraf et al., 2012). The Karasu River takes all the pollutants of the Karasu Basin and the pollution in the river unfortunately continues. In previous studies, it was reported that the metals analyzed in the water and sediment of the river were above the reported standard values (Sönmez et al., 2012; Aydoğan et al., 2017; Dane and Şişman, 2017), and that the surface waters were extremely polluted water of 4th class quality. In addition, in 2015-2016, concentrations of 6 elements each (V, Mn, Fe, Co, Ni, Sr) in 3rd station and (Ti, Cr, Cu, Zn, As, Pb) in 4th station in water samples were found to be higher compared to the others, and the abiotic parameters (temperature, pH, and dissoved oxygen) did not exceed the standard (Dane and Şişman, 2020a, 2020b). Therefore, it is highly probable that the low GSI values and high gonad histopathologies detected in the fish caught from the 3rd and 4th stations in the current study are the effects of heavy metals in the water.

The GSI value was analyzed on a monthly basis in order to identify gonad development in the fish. In Karasu River, the highest mean GSI value was recorded in June and the lowest value in August for *C. damascina* female and male population. Similarly, Sen et al. (2008) reported that the spawning period for *C. damascina* was between the first week of June and second week of July, while the highest GSI value was recorded in late May for females, and in early June for males, and the lowest values were recorded in July. For *A. sellal*, the highest GSI value was recorded in June for females, and in May for males, while the lowest value was recorded in August. In many studies, it was reported that the highest GSI values of the species were in June, as it was the case in our study (Uçkun and Gökçe, 2015). In Karasu River, the highest mean GSI

value was recorded in May and the lowest value in July for *S. semae*. The spawning period for *S. semae* was also reported to be in May and July in Karakaya Dam Lake and Muş Karasu Stream (Kalkan et al., 2005; Sen and Saygin, 2008). GSI is also an indicator of reproductive success being sensitive to various chemical pollutants. Monitoring pollutants which have negative effects on germ cells development and spawning of fish is necessary for preserving their genetic distribution in their region and ecological processes (Corsi et al., 2003). The comparison of GSI values between the stations in the study revealed that the mean GSI values were significantly lower in the 3rd and 4th stations compared to the 1st station for all three species. At the same time, this finding showed that these stations are the most polluted locations of the river. Similarly, low GSI values were reported at stations with the highest pollution density in different fish species (Ribeiro et al., 2013; Kaptaner, 2015). Sadekarpawar and Parikh (2013) reported that pollution in freshwater resources negatively affects the gonads of *Oreochromis mossambicus* based on GSI values and histopathological analysis results.

Aquatic pollution causes an increase in HSI value. HSI is accepted as a good indicator for chemical pollution and provides information on the fish health (Pyle et al., 2005). In the current study, significant differences between HSI values and stations were determined. There are some studies that support this situation. Authman (2011) reported that HSI value in fish exposed to high-dose Al was higher than control group. Monsefrad et al. (2012) reported that some heavy metals were determined in the liver and muscle of fish caughted the Caspian Sea, and the metals increased the fish liver weight and there was a positive correlation between Cd levels and HSI values.

Histopathological abnormalities detected in gonads of the species analyzed in the study has revealed that metal pollution causes serious damages for all three species. In the study, the increase in the frequency of ovarian histopathology in polluted stations was remarkable. The atresia process observed in the study may occur as a normal physiological mechanism in the fish ovary. On the other hand, it is known that atresia is an important response to water pollution. Atresia observed especially in previtellogenical oocyte is an important indicator of a pathological case (Kaptaner, 2015). Hyperplasia and hypertrophy in granulosa cells taking part in the atresia process is a typical sign of atresia (İşisağ Üçüncü and Çakıcı, 2009). The increase in melanomacrophage centers storing xenobiotic substances and containing pigment in ovary in terms of number and size reflects the stress conditions surrounding fish such as chemical pollution. Blazer (2002) reported that severe fibrosis detected in ovaries could be a chronic tissue response to damages caused by pollutants. Studies investigating the effects of heavy metal pollution on the ovaries have reported deformation in oocytes and a decrease in the number of oocytes (Ambani, 2015; Khillare et al., 2017). On histopathological changes in oocysts, which is one of the damages detected in this study, Berois et al. (2011) noted that oocyte sheaths, and particularly zona radiata, are useful bioindicators to identify changes in the aquatic environment. Later, membrane damage detected in primary oocysts at later stages was accepted as an epithelial response to pollution.

Testes, an important part of male reproductive system, is one of the most affected tissues by inner and outer environment changes. Hormones, defects in growth factors or chemical substances may pose serious problems to testis. It was reported that mercury, a xenobiotic metal, caused proliferation in the interstitial tissue of mature individuals' testes in *Rasbora dandia* as well as a decrease in GSI and spermatozoa, thereby decreasing reproductive success (Rajan and Kuzhivelil, 2015). Bashir et al. (2022) reported that there were histopathological abnormalities such as testes degeneration, generalized tissue degeneration produced by fragmentation and detachment of basement membrane, necrosis, and fibrosis in the testis of the fish collected from a metal-polluted river. Another study also demonstrated that environmental pollutants affect estradiol and testosterone levels in fish (Hayati et al. 2022). Panti et al. (2017) demonstrated that pollutants also affect the cell and tissue structure of fish testes resulting in changes in the size of the seminiferous tubules. Many other studies have shown that there are serious changes (various inflammations, degenerative lesions, increase in melanomacrophage centers, disintegration in the seminiferous borders, accumulation of proteinaceous water in the interstitial area of the ovaries, follicular atresia) in fish gonads exposed to various chemicals in their habitats (Feist et al., 2015; Torres-Martinez et al., 2017). In light of all these studies on gonads and our findings, it could be easily said that irreversible histopathological damages in fish may alter the reproductive success and population balances of the species in the future.

CONCLUSION

The findings of the present study clearly showed that the reproductive system of the natural fish species of Karasu River was affected by the pollution. Although species are histologically affected, they can still survive despite the pollution on the river. However, if pollution continues in this way, it is obvious that reproductive health of the fish species will be disrupted. Since the reproductive success will decrease due to pollution, there is a possibility that a distinct decrease will be seen in these populations in the near future. Therefore, further studies both on the field and in the laboratory are needed in order to identify pollutants and mixtures in the river and to analyze how specific changes in fish are related to reproductive health and population effects.

ACKNOWLEDGEMENTS AND FUNDING

This study was prepared from Hatice Dane's Phd thesis. The authors thank to Dr. Şeymanur Adil for helping with the histological analysis. The authors received no financial support for the research, authorship, and/or publication of this article.

AUTHORSHIP CONTRIBUTIONS

Hatice Dane performed experimental parts. Turgay Şişman purposed the research idea, analyzed histological slides, designed the study protocol, wrote the manuscript draft and prepared the final version of the paper, and supervised the whole research. All authors approved the submission and publication of this manuscript.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

The legal permissions was obtained from Atatürk University Animal Experiments Local Ethics Committee (ATA36643897/25.09.2013).

DATA AVAILABILITY

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

REFERENCES

Abdel-Moneim, A.M., Al-Kahtani, M.A., & Elmenshawy, O.M. (2012). Histopathological biomarkers in gills and liver of *Oreochromis niloticus* from polluted wetland environments, Saudi Arabia. *Chemosphere*, *88*, 1028-1035. https://doi.org/10.1016/j.chemosphere.2012.04.001

Agbohessi, P.T., Toko, I.I., Ouedraogo, A., Jauniaux, T., Mantiki, S.N.M., & Kastemont, P. (2015). Assessment of the health status of wild fish inhabiting a cotton basin heavily impacted by pesticides in Benin (West Africa). *Science of the Total Environment*, *506-507*, 567-584. https://doi.org/10.1016/j.scitotenv.2014.11.047

Ambani, M.M. (2015). Effects of reproductive biology on heavy metal pollution on the histopathological structure of gonads in India. *Advances in Aquaculture and Fisheries Management*, *3*, 223-227.

Anonymous (2016). Erzurum governorship provincial directorate of environment and urbanization. Erzurum province 2016 environmental status report. Available at: https://webdosya.csb.gov.tr/db/ced/editordosya/Erzurum_icdr2016.pdf (Accessed 3 June 2016) (in Turkish).

Ashraf, M.A., Maah, M.J., & Yusoff, I. (2012). Bioaccumulation of heavy metals in fish species collected from former tin mining catchment. *International Journal of Environmental Research, 6*, 209-218. https://doi.org/10.22059/IJER.2011.487

Authman, M.M.N. (2011). Environmental and experimental studies of aluminium toxicity on the liver of *Oreochromis niloticus* (Linnaeus, 1758) fish. *Life Science Journal*, *8*(4): 764-776.

Aydoğan, Z., İncekara, Ü., Şişman, T., & Gürol, A. (2017). Monitoring chemical contamination levels in Karasu River based on the use of *Enochrus* spp. (Hydrophilidae) and *Chondrostoma regium* (Cyprinidae). *The Turkish Journal of Occupational/Environmental Medicine and Safety, 1*, 486-492.

Bashir, W., Sultana, S., Sultana, T., Al-Misned, F., & Riaz, M.N. (2022). Histopathology unveiling the structural damage in gonads of *Catla catla* due to freshwater contamination. *Journal of King Saud University-Science*, *34*(8), 102369. https://doi.org/10.1016/j.jksus.2022.102369

Bernet, D., Schmidt, H., Meier, W., Burkhardt-Holm, P., & Wahli, T. (1999). Histopathology in fish: proposal for a protocol to assess aquatic pollution. *Journal of Fish Diseases, 22*, 25-34. https://doi.org/10.1046/j.1365-2761.1999.00134.x

Berois, N., Arezo, M.J., & Papa, N.G. (2011). Gamete interactions in teleost fish: the egg envelope. Basic studies and perspectives as environmental biomonitor. *Biological Research, 44*, 119-124. https://doi.org/10.4067/S0716-97602011000200002

Blazer, V.S. (2002). Histopathological assessment of gonadal fish tissue in wild fishes. *Fish Physiology and Biochemistry, 26*, 85-101. https://doi.org/10.1023/A:1023332216713

Corredor-Santamaría, W., Torres-Tabares. A., & Velasco-Santamaría, Y.M. (2019). Biochemical and histological alterations in *Aequidens metae* (Pisces, Cichlidae) and *Astyanax gr. bimaculatus* (Pisces, Characidae) as indicators of river pollution. *Science of the Total Environment*, *692*, 1234-1241. https://doi.org/10.1016/j.scitotenv.2019.07.187

Corsi, I., Mariottini, M., Sensini, C., Lancini, L., & Focardi, S. (2003). Cytochrome P450, acetylcholinesterase and gonadal histology for evaluating contaminant exposure levels in fishes from a highly eutrophic brackish ecosystem: the Orbetello Lagoon, Italy. *Marine Pollution Bulletin*, *46*, 203-212. https://doi.org/10.1016/s0025-326x(02)00359-4

Dane, H., & Şişman, T. (2017). A histopathological study on the freshwater fish species chub (*Squalius cephalus*) in the Karasu River, Turkey. *Turkish Journal of Zoology*, *41*, 1-11. https://doi.org/10.3906/zoo-1509-21

Dane, H., & Şişman, T. (2020a). Effects of heavy metal pollution on hepatosomatic ındex and vital organ histology in *Alburnus mossulensis* from Karasu River. *Turkish Journal of Veterinary and Animal Sciences, 44*, 607-617. https://doi.org/10.3906/vet-1904-50

Dane, H., & Şişman, T. (2020b). A morpho-histopathological study in the digestive tract of three fish species influenced with heavy metal pollution. *Chemosphere*, *242*, 125212. https://doi.org/10.1016/j.chemosphere.2019.125212

Feist, S.W., Stentiford, G.D., Kent, M.L., Santos, A.R., & Lorance, P. (2015). Histopathological assessment of liver and gonad pathology in continental slope fish from the northeast Atlantic Ocean. *Marine Environmental Research*, *106*, 42-50. https://doi.org/10.1016/j.marenvres.2015.02.004

Gautier, J.C. (2011). *Drug Safety Evaluation: Methods and Protocols, Methods in Molecular Biology*. Springer Science Business Media: Berlin/Heidelberg, Germany. https://doi.org/10.1007/978-1-60761-849-2

Gavrilescu, M., Demnerová, K., Aamand, J., Agathos, S., & Fava, F. (2015). Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *New Biotechnology*, *32*, 147-156. https://doi.org/10.1016/j.nbt.2014.01.001

Geldiay, R., & Balık, S. (2009). Freshwater Fish Species. Ege Üniversitesi Su Ürünleri Fakültesi Yayınları No: 46 VI. Baskı Bornova, İzmir. (in Turkish)

Gilroy, È.A., McMaster, M.E., Parrott, J.L., Hewitt, L.M., Park, B.J., Brown, S.B., & Sherry, J.P. (2012). Assessment of the health status of wild fish from the Wheatley Harbour Area of Concern, Ontario, Canada. *Environmental Toxicology and Chemistry*, *31*, 2798-2811. https://doi.org/10.1002/etc.2021

Gupta, N., Yadav, K.K., & Kumar, V. (2015). A review on current status of municipal solid waste management in India. *Journal of Environmental Sciences*, *37*, 206-217. https://doi.org/10.1016/j.jes.2015.01.034

Hayati, A., Pramudya, M., Soepriandono, H., Astri, A. R., Kusuma, M. R., Maulidah, S., Adriansyah, W., & Dewi, F. R. P. (2022). Assessing the recovery of steroid levels and gonadal histopathology of tilapia exposed to polystyrene particle pollution by supplementary feed. *Veterinary World*, *15*(2), 517–523. https://doi.org/10.14202/vetworld.2022.517-523

İşisağ Üçüncü, S., & Çakıcı, Ö. (2009). Atresia and apoptosis in preovulatory follicles in the ovary of *Danio rerio* (zebrafish). *Turkish Journal of Fisheries and Aquatic Sciences*, *9*(2), 215-221. https://doi.org/10.4194/trjfas.2009.0214

Joshi, P.S. (2011). Impact of zinc sulphate on behavioural responses in the freshwater fish *Clarias batrachus* (Linn.). *Online International Interdisciplinary Research Journal*, *1*, 76-82.

Kalkan, E., Yilmaz, M., & Erdemli, A.Ü. (2005). Some biological properties of the *Leuciscus cephalus* (L., 1758) population living in Karakaya Dam Lake in Malatya (Turkey). *Turkish Journal of Veterinary and Animal Sciences*, *29*, 49-58.

Kaptaner, B. (2015). Relation between increased oxidative stress and histological abnormalities in the ovaries of *Alburnus tarichi* in Lake Van, Turkey. *Environmental Monitoring and Assessment*, *187*, 1-11. https://doi.org/10.1007/s10661-015-4936-1

Khillare, K., Khillare, Y.K., & Wagh, U. (2017). Histological changes in gonads of fresh water fishes due to heavy metal pollution. *World Journal of Pharmaceutical Sciences, 6*, 601-609. https://doi.org/10.20959/wjpps201777-8743

Łuczyńska, J., Paszczyk, B., & Łuczyński, M.J. (2018). Fish as a bioindicator of heavy metals pollution in aquatic ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health. *Ecotoxicology and Environmental Safety*, *153*, 60-67. https://doi.org/10.1016/j.ecoenv.2018.01.057

Martinez-Haro, M., Beiras, R., Bellas, J., Capela, R., Coelho, J.P., Lopes, I., & Marques, J.C. (2015). A review on the ecological quality status assessment in aquatic systems using community based indicators and ecotoxicological tools: what might be the added value of their combination? *Ecological Indicators*, *48*, 8-16. https://doi.org/10.1016/j.ecolind.2014.07.024

Monsefrad, F., Imanpour, N.J., & Heidary, S. (2012). Concentration of heavy and toxic metals Cu, Zn, Cd, Pb and Hg in liver and muscles of *Rutilus frisii kutum* during spawning season with respect to growth parameters. *Iranian Journal of Fisheries Sciences*, *11*(4), 825-839.

Naidu, R., Espana, V.A.A., Liu, Y., & Jit, J. (2016). Emerging contaminants in the environment: risk-based analysis for better management. *Chemosphere*, *154*, 350-357. https://doi.org/10.1016/j.chemosphere.2016.03.068

Panti, C., Rysky, E., Pedà, C., & Maricchiolo, G. (2017). Gene expression in liver of European Sea Bass *Dicentrarchus labrax* experimentally exposed to PVC microplastics. Conference: SETAC Europe 2017. 27th Annual Meeting. https://doi.org/10.13140/RG.2.2.12883.68640

Poleksic, V., & Mitrovic-Tutundzic, V. (1994). Fish gills as a monitor of sublethal and chronic effects of pollution. In R. Müller, R. Lloyd (Eds.), *Sublethal and Chronic Effects of Pollutants on Freshwater Fish*, Cambridge Univ. Press, Cambridge, 339-352.

Pyle, G.G., Rajotte, J.W., & Couture, P. (2005). Effects of industrial metals on wild fish populations along a metal contamination gradient. *Ecotoxicology and Environmental Safety, 61*, 287-312. https://doi.org/10.1016/j.ecoenv.2004.09.003

Rajan, P., & Kuzhivelil, B.T. (2015). Mercury induced histopathological changes in the testis of the freshwater fish, *Rasbora dandia. International Journal of Science and Research, 4*, 252-254.

Ribeiro, C.A.D.O., Katsumiti, A., França, P., Maschio, J., Zandoná, E., Cestari, M.M., & Filipak Neto, F. (2013). Biomarkers responses in fish (*Atherinella brasiliensis*) of paranaguá bay, southern Brazil, for assessment of pollutant effects. *Brazilian Journal of Oceanography*, *61*, 1-11. https://doi.org/10.1590/S1679-87592013000100001

Sadekarpawar, S., & Parikh, P. (2013). Gonadosomatic and hepatosomatic indices of freshwater fish *Oreochromis mossambicus* in response to a plant nutrient. *World Journal of Zoology*, *8*, 110-118. https://doi.org/10.5829/idosi.wjz.2013.8.1.7268

Sen, F., Elp, M., & Kankaya, E. (2008). Growth and reproduction properties of *Capoeta capoeta* (Guldenstaedt, 1772) in Zernek Dam Lake, Van, Turkey. *Journal of Animal and Veterinary Advances*, *7*, 1267-1272.

Sen, F., & Saygin, F. (2008). Biological properties of chub (*Leuciscus cephalus* L., 1758) in Karasu Stream (Mus/Turkey). *Journal of Animal and Veterinary Advances*, *7*(8), 1034-1037.

Sönmez A, Hisar, O., & Yanık, T. (2012). Determination of heavy metal pollution in Karasu River and classification of water quality. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 43*, 69-77. (in Turkish with English abstract)

Torres-Martínez, A., Sánchez, A.J., Alvárez-Pliego, N., Hernández-Franyutti, A.A., López-Hernández, J.C., & Bautista-Regil, J. (2017). Gonadal histopathology of fish from La Polvora Urban Lagoon in the Grijalva Basin, Mexico. *Revista internacional de contaminación ambiental*, *33*, 713-717. https://doi.org/10.20937/RICA.2017.33.04.14

Uçkun, A.A., & Gökçe, D. (2015). Assessing age, growth, and reproduction of *Alburnus mossulensis* and *Acanthobrama marmid* (Cyprinidae) populations in Karakaya Dam Lake (Turkey). *Turkish Journal of Zoology*, *39*, 1-14. https://doi.org/10.3906/zoo-1211-13

UNECE (1994). Standard statistical classification of surface freshwater quality for the maintenance of aquatic life. In *Readings in International Environment Statistics*, United Nations Economic Commission for Europe, United Nations, New York and Geneva, 53-64.

Yadav, K.K., Gupta, N., Kumar, V., Choudhary, P., & Khan, S.A. (2018). GIS-based evaluation of groundwater geochemistry and statistical determination of the fate of contaminants in shallow aquifers from different functional areas of Agra city, India: Levels and spatial distributions. *RSC Advances*, *8,* 15876-15889. https://doi.org/10.1039/C8RA00577J

Zulfahmi, I., Muliari, M., Akmal, Y., & Batubara, A.S. (2018). Reproductive performance and gonad histopathology of female Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) exposed to palm oil mill effluent. *The Egyptian Journal of Aquatic Research*, *44*, 327-332. https://doi.org/10.1016/j.ejar.2018.09.003

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 286-291 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.07

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Investigation of toxic effects of BPA and BPA analogues (BPS and BPAF) on *Spirulina* sp., *Desmodesmus subspicatus* and *Chlorella vulgaris*

Duygu Turan[1*] ● Özlem Çakal Arslan[2]

[1]Centre for Environmental Studies, Ege University, 35100, Bornova, İzmir, Türkiye
[2]Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye

https://orcid.org/0000-0003-2909-7240
https://orcid.org/0000-0001-7777-3886

*Corresponding author: duyguturan35@gmail.com

Received date: 08.02.2023 *Accepted date: 20.11.2023*

How to cite this paper:
Turan, D., Çakal Arslan, Ö. (2023). Investigation of toxic effects of BPA and BPA analogues (BPS and BPAF) on *Spirulina* sp., *Desmodesmus subspicatus* and *Chlorella vulgaris*. *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 286-291. https://doi.org/10.12714/egejfas.40.4.07

Abstract: Bisphenols (BPs) are produced for many applications for used in industry. BPs have been found all part of aquatic environments such as sediment and surface water that is poses a risk to the aquatic ecosystem. Restricting the use of BPA, environmental concentrations of bisphenol S, and bisphenol AF begin to increase. The present study aims to indicate that toxicity BPA and BPA analogues (BPS and BPAF) by algal growth inhibition test for the green algae *Chlorella vulgaris*, *Spirulina* sp., *Desmodesmus subspicatus*. In this way, result of this study present the nominal effective concentrations of BPA analogues and the suitability of the species for use as a biomarker in ecotoxicology tests. IC_{50} values (growth rate inhibition by 50%, respectively) for three toxicants were determined separately. Results of this study showed the effects of these chemicals on photosynthesis (primer production). The result of algal growth inhibition test showed that BPAF (72h EC_{50} 3.80 mg/L) was found to be more toxic than BPS (3d EC_{50} 6.31 m L^{-1}) for *Spirulina* sp. BPS (3d EC_{50} 2.43 mg/L) showed the most toxic effect on the growth of *C. vulgaris*, followed by BPAF with 3d EC_{50} 3.32 mg/L. BPS (3d EC_{50} 0.88 mg/L) and BPAF (3d EC_{50} 6.48 mg/L) were found to be toxic for *D. subspicatus*, respectively, from highest to lowest toxicity. These results indicate that bisphenol analogues are hazardous to primer production. Therefore, it is necessary to study their combined effects as well as to study how they act individually.

Keywords: Bisphenols, toxicity, freshwater algae, aquatic environment, aquatic ecology

INTRODUCTION

Plastic pollution threat to marine ecosystems due to its widespread use in all areas. So that, it has several impacts on aquatic organisms, many of which have not been investigated (Uibel, 2016). The use of a wide variety of plastic products has increased considerably in recent years due to their social benefits such as ease of use, practicality, etc. Being durable and light, plastic has become the preferred base material for many applications, especially industrial applications. On the other hand, the multifaceted use of plastic has led to an increase in environmental pollution and a threat to natural life. Certain additives/chemical compounds are used in order to have the desired properties (durability, etc.) and to facilitate the production of plastics during the production phase. The most widely used of these compounds, bisphenol A (BPA), is used in the production of polycarbonate and epoxy resins (Huang et al., 2012). BPA is one of the important chemicals with the highest production volume in industrial areas worldwide (Abraham and Chakraborty, 2019).

BPA is commonly used as a stabilizer, an antioxidant in polycarbonate plastic (Grignard et al., 2012). BPA has a wide range of uses, such as food packaging, bottles, straws, thermal receipt paper, toys, CDs and medical devices (European Commission, 2018). The burning and photo degradation of plastics cause a BPA contamination in aquatic environment (Kang et al., 2007). Because of the decomposition of BPA occurs rapidly in UV light, heat, acidic or basic environments, it causes pollution in the environment and human exposure to natural life (Frenzilli et al., 2021). The toxic effects of bisphenol A, has received great attention that it acts as a xenoestrogen and causes endocrine disruption. Today, due to the ban on the use of BPA in many countries (Liu et al., 2021). There are many studies concerning that BPA has toxicity to fish and invertebrates (LC_{50} 1.1 to 10 mg/L) (Colborn et al., 1996).

Because of the lack of data especially its toxicities at low dose exposure Today, believed that the BPA alternatives are "safer". BPS and BPAF are the second and third most abundant analogues in the environment, detected at even higher levels than BPA in surface waters (Liu et al., 2021). A few studies have documented that BPS may be equally or more harmful than BPA (Rochester and Bolden, 2015). So, new researchers advised that necessary to investigate the current alternatives used instead of BPA. Chen et al. (2016) have identified the potentially toxic effects of BPA alternatives on non-target organisms. Furthermore, these BPA analogues have also been determined as endocrine-disrupting chemicals (Moreman et al., 2017). A large number of studies showed that BPS, BPF, and BPAF are found lower concentrations in water, sediment (Liao et al., 2012; Chunyang and Kurunthachalam, 2013; Chen et al., 2016) and bio accumulate in the body of several animal species (Wang et al., 2021). Restricting the use of BPA leads to greater use of BPA alternatives and increases their production. Therefore, concentrations of BPA alternatives are expected to increase in all areas of the environment. The predicted no-effect concentration (PNEC) reported as 1500 ng/L by European Union (Morales et al., 2020).

Effects of pollutants on natural ecosystems can defined by Ecotoxicology. The toxicity of chemicals was ranged according

to species (Hammer et al., 2006). Algae and aquatic plants are the most important primary producer's waters and provide oxygen and shelter for many aquatic organisms. Because of this, they are the most important parts of the aquatic food chain. Algae have been reported as more sensitive than animals (Ferreira and Graça, 2002) and have been widely used in toxicity tests.

Recent studies have shown that BP analogues are detected in different environmental media, such as water, air, soil, biomass and sediment (Song et al., 2012; Liu et al., 2016). The use of BPS and BPAF in the production of BPA-free products leads to their detection in the aquatic environment at concentrations ranging from ng/L to µg/L (Chen et al., 2016; Zhao et al., 2019).

Their presence in the aquatic environment, BPS and BPAF have caused some risks for primary producers (Barboza et al., 2020; Czarny et al., 2021). As primary producers, microalgae at the base of the aquatic food chain are of essential for important in aquatic ecology (Fromme et al., 2002). Their sensitivity to toxic substances is the main reason for microalgae to be preferred as good testing organisms. Their readily available, small individual size, and rapid reproduction allow to for rapid assessment of chemical concentrations and generational effects of multiple populations (Abdel-Hamid, 1996). According to previous study report, BPA inhibited the growth and accumulation of chlorophyll in the test organisms. *C. mexicana* had a higher effective concentration value than *C. vulgaris.* Biodegradation and bioaccumulation of BPA were observed in both microalgae. *C. vulgaris was* exposed to concentrations of 1, 10, and 100 mg L-1 BPS, and the inhibition rate of *C. vulgaris* was 41.6%, 103.7%, and 238.4%, respectively (Ding et al., 2020).

New studies have indicated that bisphenols affect ecosystem health (Ji et al., 2013), but studies on the comparative toxicity of bisphenol analogues are limited. Little data reported the toxic effects of BPS and BPAF on growth of phytoplankton. Especially no data available about the effects of BPS and BPAF on *Spirulina* sp., *Desmodesmus subspicatus* and *Chlorella vulgaris*.

The aim of this study is to obtain more data on the acute effects of BPA and BPA analogues on freshwater algae, and the acute toxic effects of BPA and its analogues (BPS and BPAF) on *Spirulina* sp., *Desmodesmus subspicatus* and *Chlorella vulgaris* were examined. This study is a part of the doctoral thesis.

MATERIALS AND METHODS

Chemicals

The chemicals used for the phytotoxicity tests were bisfenol A (BPA) CAS No. 80-05-7, bisphenol S (BPS) CAS No. 80-09-1, bisphenol AF (BPAF) CAS No. 1478-61-1 from Sigma-Aldrich (St. Louis, MO, USA). BPA, BPS and BPAF were prepared according to the conditions recommended by the manufacturer. Dilutions of 1/10 of the stock solution prepared from each chemical were used to prepare intermediate stock solutions.

Test species and culture conditions

Test organisms *Spirulina* sp., *D. subspicatus* and *C. vulgaris* were obtained from Ege University Fisheries Faculty Aquaculture Department and the cultures were grown in the Algae Culture unit of Ege University Ecotoxicology Laboratory.

To reproduce the pure cultures of the phytoplankton to be used in the study, the necessary medium and appropriate environmental conditions were provided for each of them. *Spirulina* sp. the standard Zarrouk broth medium was prepared according to the method Madkour et al. (2012) for the propagation of the pure culture of phytoplankton. The enrichment and environmental conditions suitable for *D. subspicatus* were prepared according to the protocol (OECD, 2011). This algae culture was grown at 21±2 ^{0}C in 4000 lux lighting and 24 h of light. BBM (bold basal medium) was prepared for the enrichment of *Chlorella vulgaris*. The pH value of BBM was adjusted to 6.8. *Chlorella vulgaris* culture was grown at 23±2 ^{0}C in 4000 lux lighting and 24 h of light. A shaker was used to prevent the samples from sticking to the surfaces of the erlenmeyer. For the growth of phytoplankton, firstly, 10 ml of the main algae stock was taken and added to the erlenmeyer containing 20 ml of enrichment. The cultures, which were left to grow under suitable conditions, were transferred to erlenmeyer with volumes of 150 and 200 ml, respectively, as their volumes increased. This process was repeated for all three phytoplankton.

Toxicity test

Algal growth inhibition tests were performed as described in (OECD, 2011). Experiments were started when the cell numbers for *C. vulgaris* and *D. subspicatus* phytoplankton reached 10^5 - 10^6 per ml. *Spirulina* sp.'s long filamentous structure is not suitable for visual microscope counting. For this reason, the cell density of *Spirulina* sp. was measured by fluorimetry (µg/L chlorophyll-a) (Turner Designs the Aquafluor Handheld Fluorometer 54555). Experiments were set up in 20 ml volume. The total duration of the experiments is three days. The experiments were performed in triplicate and cell counts were made at 0.h and 72. h. The determined growth curves were compared with the control group (under the same conditions without adding bisphenol analogues) and the percent inhibition was calculated. 7 different chemical concentrations (0.5, 1, 1.5, 3, 5, 10, 15 mg/L) for *Spirulina* sp., 10 different chemical concentrations (0.5, 0.8, 1, 1.5, 2, 3, 5, 7, 9, 15 mg/L) for *D. subspicatus* and *C. vulgaris* were tested. Algae growth rate and percent inhibition were calculated for each organism.

Cultures exposed to BPA, BPS and BPAF were grown at 23±2 ^{0}C in 4000 lux lighting and 24 h of light. The measurements of the experiments were calculated with the help of algae growth rate and inhibition (%) exponential function.

Algae growth rate (μ), $\mu = (\ln x_j - \ln x_0) / (t_j - t_0)$ (day^{-1})

X_0: number of cells counted at time t_0 (cells/ml) (for *D. subspicatus* and *C. vulgaris)*

Xj: number of cells counted at time tj (cells/ml) (for *D. subspicatus* and *C. vulgaris)*

tj: days until the last measurement of the experiment

% Inhibition= [(μc -μr) / μc] x 100

μc= control group growth rate

μr= concentration group growth rate

The toxicity of the chemicals used in the study on the test phytoplankton was classified according to the (The European Commission, 2013). According to this report, classifies substances according to their effective concentrations values as follows:

Effective concentration 50 (EC_{50}) values in different classes:

a) 1–10 mg L^{-1} (toxic)

b) < 1 mg/L (very toxic)

c) 10–100 mg/L (harmful) for aquatic organisms

Substances with an EC_{50} above 100 mg/L are not classified.

Statistical calculations

IC_{50} values were calculated from the inhibition - concentration curve as 50% growth inhibition of test population compared to control treatment, based on growth rate. Data analysis. The 72 h IC_{50} values were calculated according to the "area under the curve" method prescribed by the OECD. IC_{50}-value was determined by nonlinear regression analysis. All results are presented as mean ± SD. Differences were considered significant at $P < 0.05$. The SPSS Statistics 25 computer programmer was used in the data analysis (Hocking, 1996). The data of growth rates were compared with controls by Dunnet test.

RESULTS

The aim of this study is to identify the acute toxic effects of BPA, BPS and BPAF on *Spirulina* sp., *C. vulgaris* and *D. subspicatus*. According to the results of the studies, *Spirulina* sp. gave different answers. As the toxic effects of three chemicals on the growth of the test organism were compared, BPA accelerated growth, while BPS and BPAF showed a limiting effect on growth. The toxic effect of BPAF has the most toxic effect on growth compared to the other two chemicals. The EC_{50} value for bisphenol A exposure could not be calculated for *Spirulina* sp. because BPA exposure caused the organism to grow.

BPS has a more toxic effect than BPA and BPAF for *D. subspicatus*. It has a more toxic effect on *D. subspicatus* than bisphenol A and bisphenol AF. EC_{50} value could not be calculated for BPA due to overgrowth.

BPS is more toxic to *C. vulgaris* than BPAF. The acute toxicity of BPS is greater than BPA and BPAF for *C. vulgaris*. According to the research results, bisphenol A increased the growth of *Spirulina* sp. instead of stopping it. *Spirulina* sp. and *C. vulgaris* were recorded as the most resistant species to BPA.

As a result, it was observed that BPS and BPAF showed more toxic effects for all three species compared to BPA. Compared to the other two species, *D. subspicatus* is more sensitive to BPS and BPAF.

The EC_{50} values found for the three selected species are given in Table 1.

Table 1. EC_{50} values for phytoplankton species

	EC_{50} (mg/L)		
Test Species	**BPA**	**BPS**	**BPAF**
C.vulgaris	26.5	2.43	3.32
Spirulina sp.	-	6.31	3.80
D.subspicatus	-	0.88	6.48

EC_{50}: effective concentration; the dosage at which the desired response is present for 50 percent of the population

In the first experiments, an increase in the growth of *Spirulina* sp. was observed after three days of BPA exposure. BPA stimulated the growth of the organism. While the growth rate is less at low concentrations (0.5, 1, 1.5, 3 mg L^{-1}), the growth rate is quite high at high concentrations (5, 10, 15 mg/L). The increase in negative inhibition is greater at higher concentrations (5, 10 and 15 mg/L). A decrease in the growth rate and the inhibition % of *Spirulina* sp. were increased after three days of exposure to BPS. In addition, after 3 days of exposure, while the growth rate was 0.58 in the control, parallel to increasing concentrations the growth rates were increased. The growth rate decreased at increasing BPS concentrations. Determination that the inhibition as a function of growth rate. While the growth rate for BPAF was 0.78 in the control group, in highest chemical concentrations (3, 5, 10 and 15 mg/L) it was observed as; -0.17, 0,21, -0.22, -0,19. These results showed that inhibition increasing with parallel to increasing BPS concentrations. BPAF exposure of *Spirulina* sp. resulted in increased inhibition percentage and decreased exposure to growth rate. The growth rate and inhibition graphs of *Spirulina* sp. as a result of BPA, BPS and BPAF exposures are as shown in (Figure 1). According to the results obtained, it is more toxic BPAF (3d EC_{50}= 3.80 mg/L) than BPS (72 h EC_{50}= 6.31 mg/L).

The effects of the chemicals were examined in all three phytoplankton species separately. BPA stimulated the growth of *D. subspicatus*. As compared to the growth rate of the control group (0.60), more growth was observed than the control at all concentrations of BPA exposure. BPS limited growth at all concentrations. It caused inhibition of BPAF in *D. subspicatus*. The highest concentration (15 mg/L) of BPAF showed a high inhibitory effect for *D. subspicatus* (Figure 2).

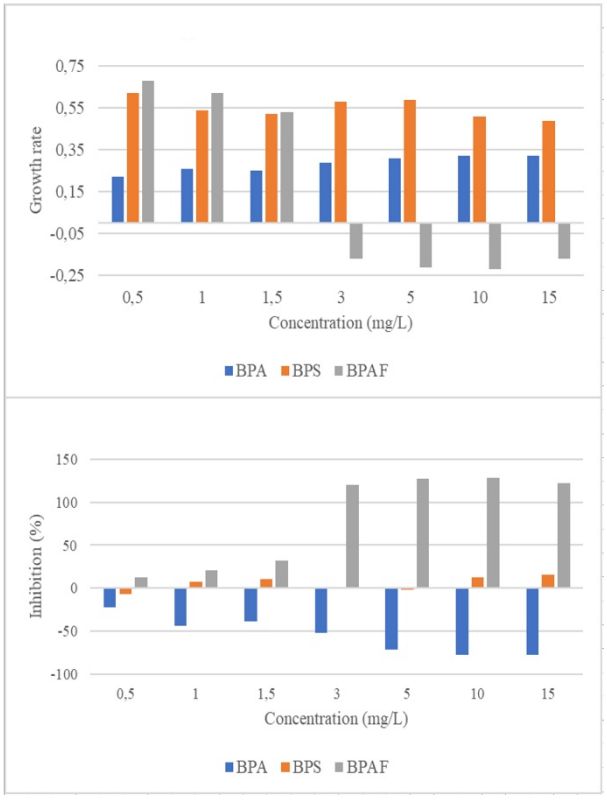

Figure 1. Effects of BPA, BPS and BPAF on growth rate and inhibition of *Spirulina* sp.

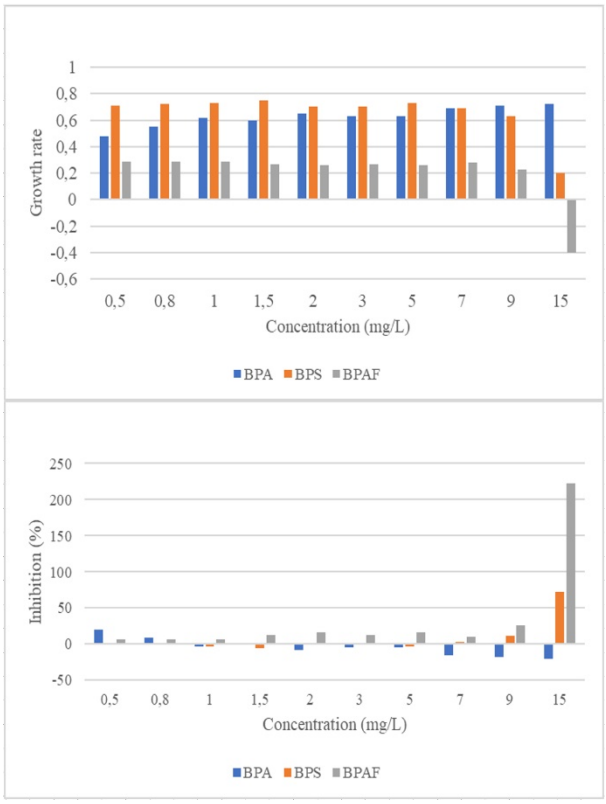

Figure 2. Effects of BPA and BPS, BPAF on growth rate and inhibition for *D. subspicatus*

The EC_{50} value for BPS was calculated as 0.88 mg L^{-1}, the EC_{50} value for BPAF was 6.48 mg/L. EC_{50} value could not be calculated for BPA due to overgrowth. It has been noted that BPS and BPAF inhibit D. *subspicatus*. According to the results obtained, BPS showed a more toxic effect than BPA and BPAF.

According to the results of the experiments conducted with *C.vulgaris*, BPS showed more toxic effects than BPAF and BPA. The EC_{50} value for BPA was calculated as 26,5 mg L^{-1}, the EC_{50} value for BPS was 2,43 mg/L and the EC_{50} value for BPAF 3,32 mg/L. According to these calculations, the acute toxicity of BPS is greater than BPA and BPAF. As a result of exposure to the chemical bisphenol A, the organism continued to grow at low concentrations (0.5, 1, 1.5, 2, 3 mg/L). At higher concentrations (5, 7, 9, 15 mg/L), growth was reduced compared to the control (0.92). A growth arresting effect was observed at all chemical concentrations applied for bisphenol S. BPAF had a growth-limiting effect on *C. vulgaris* from the lowest concentration at which exposure began (Figure 3). Results of this study, BPA and BPAF was found to be less toxic than BPS for *C. vulgaris*.

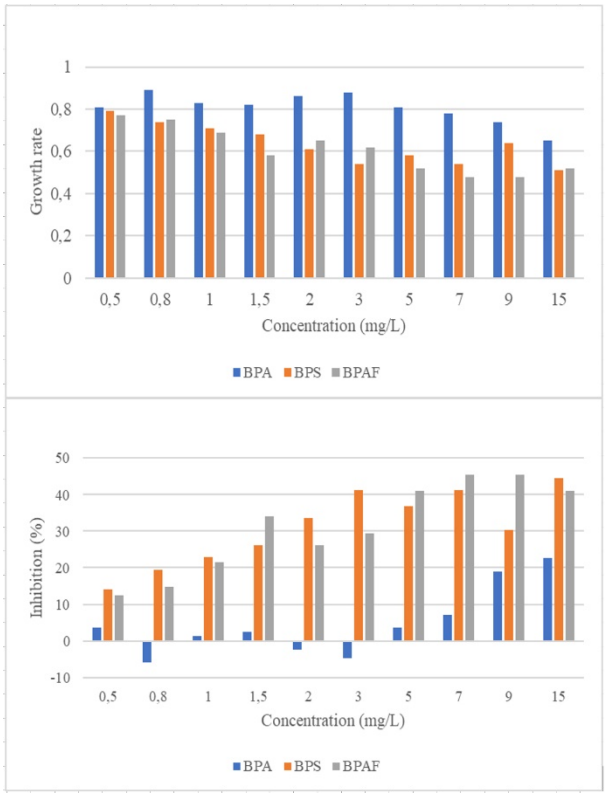

Figure 3. Effects of BPS and BPAF on growth rate and inhibition for *C. vulgaris*

DISCUSSION

Bisphenols are widely available as alternatives to BPA in various environmental and biological samples. There are only few available data about toxic effects of analogues of BPA to microalgae. For example; Libralato et al. (2011) reported that

the ecotoxicological characterization of Lignin and tannin on testing species the marine alga *Phaeodactylum tricornutum* (Bohlin). This research showed that the Lignin and tannin effected the algae an EC_{50} of 113.84 (100.90–128.45) mg/L and 26.04 (20.10–33.95) mg/L, respectively. They are also reported the NOEC and LOEC values as <0.1 mg/L and 0.1 mg/L for lignin and tannin. Seoane et al. (2021) noted that the toxicity of the emerging pollutant bisphenol A with three marine microalgae (*Tetraselmis suecica*, *Phaeodactylum tricornutum* and *Nannochloropsis gaditana*). Results of their studies showed that *P. tricornutum* was the most affected species. Researcher reported that After 96 h of exposure to three BPA concentrations, treated cultures of *P. tricornutum* and significant reduction ($p < 0.05$) was observed. These results indicate that *P. tricornutum* growth was the most affected by BPA and also 96 h-EC_{50} values of BPA were reported as 0.6 mg L^{-1}.

The investigation of Czarny-Krzymińska et al. (2022), showed that because of the water solubility of Bisphenol analogues (log_{Kow} values of BPs were 3.64–6.56= $log_{Kow} > 3$) its easily cross the cell wall of microalgae and bioaccumulate. Furthermore researcher reported the toxicity of bisphenol A, its six analogues, on the the green algae *Chlorella vulgaris* (bisphenol AF for *C. vulgaris* 14 days, EC_{50}: 22.39 mg L^{-1}) and *Desmodesmus armatus* (EC_{50}: 42.29 mg L^{-1} for Bisphenol A, and bisphenol AF EC_{50}: 27.16 mg L^{-1}) (Czarny-Krzymińska et al., 2022). Tisler et al. (2016) reported that IC_{50} values (3 days) were 3.00 mg-BPAF/L for *Desmodesmus subspicatus* and also showed that the BPAF was more harmful to *Desmodesmus subspicatus* than BPA. Ding et al. (2020) found that bisphenol S showed high toxicity to *C. vulgaris* than bisphenol A, and the obtained EC_{50} values (2 d) were 3.16 and 41.43 mg L^{-1}, respectively. Ding et al. (2020) emphasized that the acute toxicity of BPS in the aquatic ecosystem should be more attention than BPA. In our study, BPS was found toxic for *C. vulgaris*. Czarny-Krzymińska et al. (2022) carried out the first study explaining the effects of toxicity of bisphenol A and its derivatives on microalgae. According to this study with *D. armatus* and *C. vulgaris*, BPAF was found to be more toxic than BPA. The toxicity of BPF, BPA and BPAF on *D. magna*, *D. rerio* and *D. subspicatus* was investigated and BPAF concentrations in the surface waters were observed to pose a risk for aquatic organisms (Tišler et al., 2016).

In our study, the effect of BPA, BPS and BPAF on the growth of freshwater microalgae *Chlorella vulgaris*, *Spirulina* sp., *D. subspicatus* was investigated. The toxicities of BPA, BPS and BPAF chemicals on this three phytoplankton are different. bisphenol A increased the growth of *Spirulina* sp. *C. vulgaris* and *Spirulina* sp. were recorded as the most resistant species to BPA among the test organisms. At concentrations of 5 mg/L and above, BPAF dramatically reduced the growth rate of *Spirulina* sp. BPS at high concentrations (7 mg/L and above) caused inhibition by slowing the growth rate of both *Desmodesmus subspicatus* and *C. vulgaris*. Comparison of calculated EC_{50} values of chemicals tested for their toxicity on three phytoplankton was made according to The European Commission (2013). EC_{50} values obtained from the study are shown in Table 1. As a result of comparing the EC_{50} values obtained from the study with the report, it was determined that BPS and BPAF had toxic effects on all three species. *D. subspicatus* is more sensitive to BPS than BPAF. The most resistant phytoplankton to BPAF exposure is *D. subspicatus* and the most sensitive is *C. vulgaris.*

When the toxic effects of individual and mixed bisphenol analogues on cyanobacteria were examined, it was observed that the mixture of bisphenol analogues had a stronger toxic effect than BPA (Czarny et al., 2021).

In addition to these studies, the effects of BPS and BPAF on more phytoplankton are still unknown. These effects should be identified by acute and chronic toxicity tests and the presence of these chemicals in the aquatic system should be reduced. Investigation of the effect of BPA and analogues on phytoplankton in the first step of the aquatic system should be expanded.

These types of studies are important in predicting the toxic effects of chemicals on living organisms. Light of previous and our studies, BPS and BPAF concentrations in the environment may not be hazardous at present time. But BPA analogues such as; BPS and BPAF concentrations in aquatic environment must be monitoring for the ecosystem health. Furthermore, this study will enable us to obtain more information about BPA and its analogues by examining the toxic effects of widely used BPA derivatives, BPS and BPAF, on the primary producers, phytoplankton.

ACKNOWLEDGMENTS AND FUNDING

This study was supported by the Turkish Scientific and Technological Research Institution (Grant number: 119Y246).

AUTHOR CONTRIBUTIONS

Material preparation and research were carried out by Duygu Turan. The article was written and edited by Duygu Turan and all authors have read and approved the article.

CONFLICT OF INTEREST STATEMENT

The author declares no conflict of interest.

ETHICAL APPROVAL

Ethical approval is not required for this study.

DATA AVAILABILITY

All relevant data is inside the article.

REFERENCES

Abdel-Hamid, M. I. (1996). Development and application of a simple procedure for toxicity testing using immobilised algae. *Water Science and Technology, 33*(6), 129-138. https://doi.org/10.1016/0273 1223(96)00289-2

Abraham, A., & Chakraborty , P. (2019). A review on sources and health impacts of bisphenol A. *Reviews on Environmental Health, 35*(2), 201-210. https://doi.org/10.1515/reveh-2019-0034

Barboza, L.G.A., Cunha, S.C., Monteiro, C., Fernandes, J.O., & Guilhermino, L. (2020). Bisphenol A and its analogues in the muscle and liver of fish from the North East Atlantic Ocean in relation to microplastic contamination. Exposure and risk to human consumers. *Journal of Hazardous Material*, 122-419. https://doi.org/10.1016/j.jhazmat.2020.122419

Chen, D., Kannan, K., Tan, H.L., Zheng, Z.G., Feng, Y.L., Wu, Y., & Widelka, M. (2016). Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity – A review. *Environmental Science & Technology*, *50*, 5438-5453. https://doi.org/10.1021/acs.est.5b05387

Chunyang, L., & Kurunthachalam, K. (2013). Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. *Journal of Agricultural and Food Chemistry*, *61*, 4655– 4662. https://doi.org/10.1021/jf400445n

Colborn, T., Dumanoski, D., & Myers, J.P. (1997). *Our stolen future: Are we threatening our fertility, intelligence, and survival?--a scientific detective story*. Penguin.

Czarny, K., Krawczyk, B., & Szczukocki, D. (2021). Toxic effects of bisphenol A and its analogues on cyanobacteria *Anabaena variabilis* and *Microcystis aeruginosa*. *Chemosphere*, *263*, 128-299. https://doi.org/10.1016/j.chemosphere.2020.128299

Czarny-Krzymińska, K., Krawczyk, B., & Szczukocki, D. (2022). Toxicity of bisphenol A and its structural congeners to microalgae *Chlorella vulgaris* and *Desmodesmus armatus*. *Journal of Applied Phycology, 34*(3), 1397–1410. https://doi.org/10.1007/s10811-022-02704-3

Ding, T., Li, W., Yang, M., Yang, B., & Li, J. (2020). Toxicity and biotransformation of bisphenol S in the freshwater green alga *Chlorella vulgaris*. *Science of the Total Environment, 747*, 141–144. https://doi.org/10.1016/j.scitotenv.2020.141144

European Commission. (2018). Regulation (EU) 2018/213. https://eur-lex.europa.eu/eli/reg/2018/213/oj

Ferreira, R., & Graça, M. (2002). A comparative study of the sensitivity of selected aquatic plants to mining effluents. *Limnetica*, *21*(1-2), 129-134. https://doi.org/10.23818/limn.21.12

Frenzilli, G., Martorell-Ribera, J., Bernardeschi, M., Scarcelli, V., Jönsson, E., Diano, N., Moggio, M., Guidi, P., Sturve, J., & Asker, N. (2021). Bisphenol A and bisphenol S induce endocrine and chromosomal alterations in brown trout. *Frontiers in Endocrinology, 12, 645519*. https://doi.org/10.3389/fendo.2021.645519

Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J., & Wenzel, A. (2002). The occurrence of phthalates and bisphenol A and F in the environment. *Water Research, 36*(6), 1429-1438. https://doi.org/10.1016/S0043-1354(01)00367-0

Grignard, E., Lapenna, S., & Bremer, S. (2012). Weak estrogenic transcriptional activities of Bisphenol A and Bisphenol S. *Toxicology in Vitro*, *26*(5), 727-731. https://doi.org/10.1016/j.tiv.2012.03.013

Hammer, K., Carson, C., Riley, T., & Nielsen, J. (2006). A review of the toxicity of *Melaleuca alternifolia* (tea tree) oil. *Food and chemical toxicology*, *44*(5), 616-625. https://doi.org/10.1016/j.fct.2005.09.001

Hocking, R. (1996). *Methods and Applications of Linear Models*: *Regression and the analysis of variance*. New York: Wiley.

Huang, Y.Q., Wong, C.K.C., Zheng, J.S., Bouwman, H., Barra, R., Wahlström, B., Neretin, L., & Wong, M.H. (2012). Bisphenol A (BPA) in China: A review of sources, environmental levels, and potential human health impacts. *Environment International, 42*, 91-99. https://doi.org/10.1016/j.envint.2011.04.010

Ji, K., Hong, S., Kho, Y., & Choi, K. (2013). Effects of bisphenol S exposure on endocrine functions and reproduction of zebrafish. *Environmental Science & Technology*, *47*(15), 8793-8800. https://doi.org/10.1016/j.ecoenv.2008.05.012

Kang, J.H., Aasi, D., & Katayama, Y. (2007). Bisphenol A in the aquatic environment and its endocrine-disruptive effects on aquatic organisms. *Critical Reviews in Toxicology*, *37*(7), 607-625. https://doi.org/10.1080/10408440701493103

Liao, C., Liu, F., & Kannan, K. (2012). Bisphenol S, a new bisphenol analogue, in paper products and currency bills and its association with bisphenol Aresidues. *Environmental Science & Technology*, *46*, 6515–6522. https://doi.org/10.1021/es300876n

Libralato, G., Avezzù, F., & Ghirardini, A. V. (2011). Lignin and tannin toxicity to *Phaeodactylum tricornutum* (Bohlin). *Journal of Hazardous Materials*, *194*, 435-439. https://doi.org/10.1016/j.jhazmat.2011.07.103

Liu, D., Liu, J., Guo, M., Xu, H., Zhang, S., Shi, L., & Yao, C. (2016). Occurrence, distribution, and risk assessment of alkylphenols, bisphenol A, and tetrabromobisphenol A in surface water, suspended particulate matter, and sediment in Taihu Lake and its tributaries. *Marine Pollution Bulletin*, *112*(1-2), 142-150. https://doi.org/10.1016/j.marpolbul.2016.08.026

Liu, J., Zhang, L., Lu, G., Jiang, R., Yan, Z., & Li, Y. (2021). Occurrence, toxicity and ecological risk of Bisphenol A analogues in aquatic environment–A review. *Ecotoxicology and Environmental Safety*, *208*, 111-481. https://doi.org/10.1016/j.ecoenv.2020.111481

Madkour, F. F., Kamil, A. E. W., & Nasr, H. S. (2012). Production and nutritive value of Spirulina platensis in reduced cost media. *The Egyptian Journal of Aquatic Research*, *38*(1), 51-57. https://doi.org/10.1016/j.ejar.2012.09.003

Morales, M., de la Fuente, M., & Martín-Folgar, R. (2020). BPA and its analogues (BPS and BPF) modify the expression of genes involved in the endocrine pathway and apoptosis and a multi drug resistance gene of the aquatic midge *Chironomus riparius* (Diptera). *Environmental Pollution, 265, 114806*. https://doi.org/10.1016/j.envpol.2020.114806

Moreman, J., Lee, O., Trznadel, M., David, A., Kudoh, T., & Tyler, C. R. (2017). Acute toxicity, teratogenic, and estrogenic effects of bisphenol A and its alternative replacements bisphenol S, bisphenol F, and bisphenol AF in zebrafish embryo-larvae. *Environmental Science & Technology*, *51*(21), 12796-12805. https://doi.org/10.1021/acs.est.7b03283

OECD (2011). OECD Guidelines for the testing of chemicals. Freshwater alga and cyanobacteria, growth inhibition test. *Organisation Economic Co-operation Development,* 1-25.

Rochester, J.R., & Bolden, A.L. (2015). Bisphenol S and F: A systematic review and comparison of the hormonal activity of bisphenol A substitutes. *Environmental Health Perspectives*, *123*, 643–650. https://doi.org/10.1289/ehp.1408989

Seoane, M., Cid, A., & Esperanza, M. (2021). Toxicity of bisphenol A on marine microalgae: Single-and multispecies bioassays based on equivalent initial cell biovolume. Science of the Total Environment, 767, 144363. https://doi.org/10.1016/j.scitotenv.2020.144363

Song, S., Ruan, T., Wang, T., Liu, R., & Jiang, G. (2012). Distribution and preliminary exposure assessment of Bisphenol AF (BPAF) in various environmental matrices around a manufacturing plant in China. *Environmental Science & Technology, 46*, 13136-13143. https://doi.org/10.1021/es303960k

The European Commission (2013). Commission directive 2011/8/EU of 28 January 2011 amending directive 2002/72/EC regarding the restriction of use of bisphenol A in plastic infant feeding bottles. *Official Journal of the European Union, L26*, 11-14.

Tišler, T., Krel, A., Gerželj, U., Erjavec, B., Dolenc, M., & Pintar, A. (2016). Hazard identification and risk characterization of bisphenols A, F and AF to aquatic organisms. *Environmental Pollution, 212*, 472-479. https://doi.org/10.1016/j.envpol.2016.02.045

Uibel, N.C. (2016). *Effects of bisphenol-a and styrene on fertilization and development of the purple sea urchin (Strongylocentrotus purpuratus)*. Master's Theses, The Faculty of California Polytechnic State University. https://doi.org/10.15368/theses.2016.50

Wang, R., Tan, T., Liang, H., Huang, Y., Dong, S., Wang, P., & Su, X. (2021). Occurrence and distribution of bisphenol compounds in different categories of animal feeds used in China. *Emerging Contaminants*, *7*, 179-186. https://doi.org/10.1016/j.emcon.2021.08.001

Zhao , X., Qiu , W., Zheng , Y., Xiong , J., Gao , C., & Hu , S. (2019). Occurrence, distribution, bioaccumulation, and ecological risk of bisphenol analogues, parabens and their metabolites in the Pearl River Estuary, South China. *Ecotoxicology and Environmental Safety*, *180*, 43-52. https://doi.org/10.1016/j.ecoenv.2019.04.083

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 292-297 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.08

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The effect of feeds containing different protein levels on growth and survival rates of European sea bass (*Dicentrarchus labrax* L., 1758) juveniles grown in freshwater

Farklı protein seviyesi içeren yemlerin tatlısuda yetiştirilen Avrupa levreği (*Dicentrarchus labrax* L., 1758) yavrularının büyüme ve yaşama oranları üzerine etkisi

Kutsal Gamsız[1]* ● Ali Yıldırım Korkut[2] ● Aysun Kop[3]

[1]Aquaculture Department, Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye https://orcid.org/0000-0003-3277-9488
[2]Aquaculture Department, Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye https://orcid.org/0000-0002-1096-5725
[3]Aquaculture Department, Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye https://orcid.org/0000-0003-1724-5672

*Corresponding author: kutsal.gamsiz@ege.edu.tr
Received date: 18.08.2023 Accepted date: 20.11.2023

How to cite this paper:
Gamsız, K., Korkut, A.Y., & Kop, A. (2023). The effect of feeds containing different protein levels on growth and survival rates of European sea bass (*Dicentrarchus labrax* L., 1758) juveniles grown in freshwater. *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 292-297. https://doi.org/10.12714/egejfas.40.4.08

Abstract: In this study, the effect of the use of feeds containing different protein ratios on the growth performance of juvenile sea bass (*Dicentrarchus labrax* L., 1758) reared in freshwater was investigated. Sea bass fry with an average body weight of 1.0 ± 0.03 g were stocked in 120 liter tanks in triplicate after their adaptation to freshwater. The feeds used in the study had different protein/similar fat content. Accordingly, the experimental groups were named as 45CP (45% CP / 18% CF), 50CP (50% CP / 18% CF) and 55CP (55% CP / 18% CF). At the end of the experiment, the highest body weight gain was 5.84 ± 0.03 g in the 55CP group, while the other groups were 5.73 ± 0.09 g (45CP) and 5.8 ± 0.08 g (50CP). SGR rates were similar for all three groups and there was no statistical difference between the groups (P>0.05). SGR values of the groups were calculated as 1.94 ± 0.04 (45CP), 1.89 ± 0.01 (50CP), 1.91 ± 0.02 (55CP), respectively. There was no statistical difference between the 45CP and 55CP groups, while the 50CP group showed a statistically lower FCR rate than the other groups (P<0.05). FCR values were calculated as 1.36 ± 0.05, 1.29 ± 0.03, 1.37 ± 0.04, respectively. There was no difference between the survival rates of the groups. According to the results of the study, it was concluded that sea bass fish can be raised in freshwater, especially in the fry stage, and that it is more effective than the commercial feeds currently used.

Keywords: Sea bass, *Dicentrarchus labrax*, freshwater, protein, growth performance, survival rate

Öz: Bu çalışmada tatlı suda yetiştirilen yavru levrek (*Dicentrarchus labrax* L.,1758) balıklarının beslenmesinde farklı protein oranları içeren yemlerin kullanımının büyüme performanslarına üzerine etkisi araştırılmıştır. Ortalama canlı ağırlıkları 1,0 ± 0,03 gram olan levrek yavruları, tatlı suya adaptasyonlarından sonra 120 litre hacimdeki tanklarda üçer tekrarlı olacak şekilde stoklanmıştır. Çalışmada kullanılan yemler farklı protein / benzer yağ içeriğindedir. Buna göre deneme grupları 45CP (%45 HP / %18 HY), 50CP (%50 HP / %18 HY) ve 55CP (%55 HP / %18 HY) olarak isimlendirilmiştir. Deneme sonunda en yüksek canlı ağırlık artışı 5,84 ± 0,03 g ile 55CP grubunda iken diğer gruplarda 5,73 ± 0,09 g (45CP), 5,8 ± 0,08 g (50CP) olarak tespit edilmiştir. SGR oranları her üç grup için benzer oranlarda olup gruplar arasında istatistiki bir farklılık bulunmamaktadır (P>0,05). Grupların SGR değerleri sırası ile 1,94 ± 0,04 (45CP), 1,89 ± 0,01 (50CP), 1,91 ± 0,02 (55CP) olarak hesaplanmıştır. FCR değerlerine bakıldığında 45CP ve 55CP grupları arasında istatistiki bir farklılık tespit edilmemişken, 50CP grubu istatistiki olarak diğer gruplardan daha düşük bir FCR oranı göstermiştir (P<0,05). FCR değerleri sırası ile 1,36 ± 0,05, 1,29 ± 0,03, 1,37 ± 0,04 olarak hesaplanmıştır. Grupların yaşama oranları arasında bir farklılığa rastlanmamıştır. Çalışma sonucuna göre özellikle yavru dönemlerinde levrek balıklarının tatlı suda yetiştirilebileceği, halen kullanılmakta olan ticari yemlere göre daha düşük oranlarda protein içeren yemlerle de balıkların beslenebileceği gözlenmiştir.

Anahtar kelimeler: Levrek, *Dicentrarchus labrax*, tatlı su, protein, büyüme performansı, yaşama oranı

INTRODUCTION

Aquaculture production is increasing rapidly all over the world. World aquaculture production in 2020 was 87.5 million tons in total, including 33.1 million tons of marine production and 54.4 million tons of production in inland waters (FAO, 2022). Türkiye's aquaculture production in 2022 was 514,815 tons, of which 368,742 tons were produced at sea and 146,63 tons were produced in inland waters (Turkish Statistical Institute, 2022). While most of the production in the sea in Türkiye is European sea bass and sea bream species, almost all of the production in inland waters is trout production. The limited production areas in the seas will limit the increase in production capacity in the next years. Therefore, increasing recirculated aquaculture systems in terrestrial areas is an important issue for aquaculture. Water supply is the most important issue in recirculated or open production systems built on land. Even in closed aquaculture systems, 3-10% of the total water volume needs to be replaced with new water daily. This causes additional costs and problems for facilities that are

far from the sea or have difficult access to salty or brackish water from underground.

The adaptation period of European sea bass to freshwater has been investigated in many studies and it has been reported that direct adaptation of European sea bass to freshwater is possible, but long-term adaptation can reduce stress effects in fish (Nebel et al., 2005; Kokou et al., 2019).

The quantity and quality of protein in feed are very important for somatic growth in fish. Protein sources play a crucial role in providing adequate levels of amino acids needed to synthesize new tissue protein (Jana et al., 2021). Aquaculture with feeds with balanced essential amino acid composition and optimum protein levels will ensure better growth of fish as well as economically viable and environmentally friendly production.

In their natural habitat, the nutrient requirements of European sea bass are 48-52% crude protein and 14-16% crude fat during the fry period and 43-45% crude protein and 18-20% crude fat during the growth period (Oliva-Teles, 2000; Ghisaura et al., 2014). However, there are no studies on the optimum protein values that should be used in the diets of freshwater European sea bass fry. Understanding the interactions between growth performance and food consumption of European sea bass fry kept in freshwater is important for the success of aquaculture. For this purpose, in this study, the effects of using feeds with different protein contents on the survival and growth rates of European sea bass fry adapted to freshwater were investigated.

MATERIALS AND METHODS

Fish and experimental plan

Sea bass fry, which are widely cultivated in Türkiye, were used in the study. The study was carried out at Ege University Center for Research on Laboratory Animals, Aquaculture Research Laboratory. The fish used in the experiment had an average live weight of 1.0±0.03 grams and were obtained from a private hatchery (Akvatek, Çandarlı-Izmir) and transferred to the experimental unit with a fish transport tank. Three experimental groups were formed in the study. Accordingly, the fish were stocked in 9 cylindrical conical polyester tanks with a volume of 120 liters, each group was stocked with 35 individuals/tank with 3 replicates for a total of 315 individuals. The salinity in the experimental tanks was gradually reduced from 38 ppt to 0 ppt over 4 days.

The experimental system used in the study was a recirculated aquaculture system (RAS) and a sand filter, protein skimmer, biological filter and UV filter were used for water filtration. The current capacity of the system was maintained by adding freshwater to the system. Water flow rates were set at 2 l/minute so that 100% of the total volume was changed in one hour. Water temperature was kept between 21.4-22°C and dissolved oxygen between 7.1-7.3mg/l. Lighting was set to 12 hours day and 12 hours night. Water parameters (temperature, dissolved oxygen, pH and salinity) and daily mortality rates were measured and recorded every morning before feeding started. YSI P1100 model multi-parameter device was used for the measurements.

The study was initiated with the adaptation of the fish to freshwater. The study lasted 75 days and biometric measurements (body weight and total length) of the fish were measured at the beginning of the experiment, on the 25th, 50th and 75th days. Visceral and hepatosomatic index values were measured and calculated at the end of the experiment.

Feed and nutrition

The fish adapted to freshwater were fed with 3 different proteins (45, 50, 55%) and 18% fixed lipid ratios. Feed groups and their nutrient contents are given in Table 1.

Table 1. Ingredients and nutritional values of the feeds used in the study

	45CP (45/18%)	50CP (50/18%)	55CP (55/18%)
Ingredients			
Fish Meal (65.5% CP)	33	46.5	59
Soybean Meal (48% CP)	23	23	15.5
Wheat Meal (15% CP)	3	3	0
Corn Gluten Meal (66% CP)	10	10	12
Sun Flower Meal (35.18% CP)	15.5	3	0
Fish Oil (Anchovy)	14.5	13.5	12.5
Vitamin Mix*	0.4	0.4	0.4
Mineral Mix*	0.3	0.3	0.3
Methionin	0.1	0.1	0.1
Binder	0.2	0.2	0.2
Total	100	100	100
Chemical Composition (%DM)			
CP % (min)	45.26	49.73	54.57
CF % (min)	18.29	18.21	18.16
CS % (max)	3.2	2.72	2.04
DM % (min)	92.9	90.83	90.41
Ash % (max)	12.4	10.56	10.48
ME Kcal/kg**	4308.79	4568.19	4892.73
DE Kcal/kg***	4290.05	4370.93	4400.72
NFE %****	13.75	9.58	5.16
P/E***** (mg protein/Kcal DE)	105.49	113.56	123.82

*Commercial premixes were used for European sea bass fry feeds. Vitamin A 15000 IU/Kg, Vitamin D3 2500 IU/Kg, Vitamin E 350 mg/Kg, Vitamin K 20 IU/Kg, Vitamin C 350 mg/Kg, P 1.5%, Ca 1-3%, Se 0, 1%.
**Metabolic Energy (ME) = 0.239. [10.(CP.0,9.18.64+HY.0,9.39.57+NFE.0,88.17.17)] (Kcal/kg)
***Digestible Energy (DE) = 0.239. [10.(CP.0,9.23.66+HY.0,9.39.27+NFE.0,88.17.17)] (KCal/kg)
****Nitrogen Free Extractives= 100-(%CP+%HY+%HK+%HS+%Moisture)
*****Protein Energy Ratio=[(%CP/SE(Kcal/100g))X1000]

The raw materials used in feed production were purchased from a company producing commercial fish feed. The feeds were produced as a pellet form by using a meat grinder in the Fish Nutrition and Feed Technology Laboratory. After drying, they were ground to 0.8-2mm in diameter in accordance with the size of the experimental fish.

The experimental groups were named as 45CP group (45% CP/18% HY), 50CP group (50% CP/18% HY) and 55CP

group (55% CP/18% HY). Feeding was made 3 times a day at a rate of 4% of the total body weight at the beginning of the experiment and 3% of the total body weight from the second half of the experiment due to the increase in the body weight of the fish. Feed amounts were recalculated after body weight measurements every 25 days, taking into account survival rates. Feed amounts given to the experimental tanks were recorded daily in order to determine the feed utilization rates.

Depending on the feeds, the growth performance of the fish was evaluated according to the following formulas (Halver, 1976; Metailler, 1986; Hoşsu et al., 2005; Mritunjoy et al., 2022).

Live weight gain(g)=Final weight(g)-Initial weight(g)

Feed conversion ratio (FCR)=(Total feed consumed(g))/(Body weight gain(g))

Specific growth rate (SGR,%day^{-1})=[Ln(Final weight(g))-Ln(Initial weight(g))]/(Trial duration(days))×100

Protein efficiency ratio (PER)=Weight gained(g)/Crude protein consumed

Condition factor (CF)=(Total live weight(g))/(Total height(cm))^{3}x100

Visceral index (VSI)=(Visceral weight(g)/Body weight(g))x100

Hepatosomatic index (HSI)=(Liver weight(g)/Body weight(g))x100

Statistical analysis

Live weight gain, growth performance (CF, FCR, SGR and Survival), VSI and HSI values of fish fed with diets produced with different protein and fixed fat ratios were statistically tested by analysis of variance (ANOVA). When significant differences among the variables were detected, Tukey's honestly significant difference (HSD) test was used to determine variables were different. Significance levels were set at P<0.05. The SPSS ver. 25 statistical programs were used to evaluate data.

RESULTS

During the 4-day adaptation period, European sea bass fry showed normal behaviour, swimming and feeding activities. No mortality was observed during the freshwater adaptation period.

Since the experimental tanks operated in a closed-water system, no big changes were observed in water temperatures. Accordingly, the average water temperature was determined as 21.8±0.1˚C. During the experiment, the lowest water temperature was 21˚C and the highest water temperature was 22.4˚C. The average dissolved oxygen value was 7.2mg/l, with a minimum of 6.8 and a maximum of 7.7mg/l. The pH was 7.3 on average, and nitrite, ammonia and phosphorus values were observed at appropriate values.

There was a statistical difference between the 45CP group and the 55CP group in terms of body weight gain, while there was no difference between the 50CP group and other groups (P>0.05). The highest live weight gain was observed in the 55CP group with 5.84±0.03g, while the lowest weight gain was observed in the 45CP group with 5.73±0.09g. In the 50CP group, the weight gain was 5.8±0.08g. SGR rates were similar for all three groups and there was no statistical difference between the groups (P>0.05). While there was no statistical difference between the 45CP and 55CP groups in terms of FCR, the 50CP group showed a statistically lower FCR rate than the other groups (P<0.05). While there was no statistical difference between the 45CP and 50CP groups in PER values, the 55CP group was statistically different from the other 2 groups (P<0.05). It was observed that the PER value was inversely proportional to the protein value in the feed (Table 2).

When survival rates were evaluated, no difference was found between the groups (Table 2).

Table 2. Comparison of growth and development performance between groups

	45CP	50CP	55CP
Initial body weight (g)	0.97±0.19^a	1.01±0.19^a	1.01±0.22^a
Final body weight (g)	6.71±1.24^a	6.81±0.94^{ab}	6.86±1.30^b
Live weight gain (g)	5.73±0.09^a	5.8±0.08^{ab}	5.84±0.03^b
SGR	1.94±0.04^a	1.89±0.01^a	1.91±0.02^a
FCR	1.36±0.05^a	1.29±0.03^b	1.37±0.04^a
PER	1.63±0.06^a	1.55±0.3^a	1.32±0.08^b
Survival %	97^a	96^a	97^a
CF	1.25±0.03^a	1.23±0.07^a	1.22±0.02^a
VSI	11.37±0.9^c	12.33±0.3^b	13.26±0.5^a
HSI	1.52±0.05^a	1.91±0.04^b	1.98±0.1^b

CF values were similar for all groups (P<0.05). VSI values were statistically different between all groups; (P<0.05). HSI values of the 45CP group were statistically different from the other 2 groups, while there was no difference between the 50CP and 55CP groups (P<0.05).

DISCUSSION

Survival rates determined during adaptation to freshwater showed that European sea bass can adapt to freshwater without any problems within 4 days. Dendrinos and Thorpe (1985) reported that European sea bass fish can be adapted to a minimum of 0.5 ppt water, death occurs at 0 ppt, and feed intake and growth decreases as salinity decrease. However, in many other studies, in agreement with our study, the direct or gradual transfer of European sea bass fish to freshwater has been reported to be successful (Cataudella et al., 1991; Venturini et al., 1992; Varsamos et al., 2005; Kokou et al., 2019).

Many studies have reported different survival rates in European sea bass fry kept in freshwater, indicating that there are different physiological capacities in adaptation to freshwater within the same species (Dendrinos and Thorpe, 1985; Allegrucci et al., 1994; Jensen et al., 1998; Nebel et al., 2005; Giffard-Mena et al., 2008). Considering the results obtained in the study, the survival rate of all groups is quite high with 97%, but it is lower than the 100% value reported by

Eroldoğan and Kumlu (2002). It has been reported by various researchers that European sea bass in the Mediterranean region have genetic differences and therefore their adaptation to environmental conditions is different (Caccone et al., 1997; Allegrucci et al., 1997). The difference in survival rates may be due to the fact that the experimental fish in our study and the study of Eroldogan and Kumlu (2002) were European sea bass taken from different regions of Türkiye. Thus, more studies should be carried out on this subject.

In general, growth rate is positively correlated with protein levels in feed in many species. However, in some studies, it has been reported that fish change their feed intake due to the effect of salinity and this has an effect on growth. Fish growth is reduced or unaffected when fish are fed lower or higher feed protein than the optimum level (Talukdar et al., 2019; Kim and Lall, 2001). In such a situation, there is no protein synthesis, and there is a decrease in protein conversion efficiency, depending on the percentage of protein catabolized (Deng et al., 2014). In addition, although there is high protein in the feed, an insufficient non-protein energy source in the feed also decreases growth performance. (Winfree and Stickney, 1981). The carbon chains constituting amino acids due to the increase in the protein ratio in the feed are used in energy production. In addition, the amino parts cannot be used and must be excreted from the body. In addition, giving too much protein disrupts the amino acid balance and deamination is observed. This leads to increased nitrogen excretion and energy loss and protein efficiency decreases (Hoşsu et al., 2005). It has been reported that there is a negative correlation between protein efficiency ratio (PER) and feed protein level and feed protein/energy level ratio for almost every growth period of European sea bass. (Kousoulaki et al., 2015). In our study, it was observed that PER values decreased as the protein content of the feed increased. This may be due to the increase in nitrogen excretion in freshwater due to protein content.

Oliva-Teles (2000) reported that the optimum protein/ener gy ratio of European sea bass diets is higher than that of trout and sea bream and that feeds should contain 45 to 50% protein and a minimum of 9-12% lipids. Furthermore, the optimal DP/DE ratio for European sea bass fry was recommended to be 21-22 mg DP/kJ DE (0.525 kcal/kg) (Peres and Oliva-Teles, 1999). Saleem et al. (2022) reported that feeds containing 42% protein and 17.4% protein were suitable for the growth of European sea bass raised in seawater. The protein contents of the feeds used in the study were within the limits given in the above literatures. The fat ratios used in the feed are the ratios used in commercial feeds and are slightly higher than those given in the literatures. In future studies, different fat and energy contents should be tried to increase feed efficiency.

It has been reported that increasing the fish meal ratio in the feed causes an increase in SGR and FCR in European sea bass adapted to freshwater (Shalaby et al., 2023). In addition, it has been reported that decreasing the fish meal ratio and adding salt to the feed increases feed intake, SGR and FCR values (Cnaani et al., 2012). In the study conducted by Rimoldi et al. (2015), the addition of 3% salt to a diet containing only 10% fishmeal resulted in feed intake, SGR and FCR values similar to those of fish fed a diet containing 30% fishmeal. Therefore, in future studies, it will be important to try to reduce the utilization rate of fish meal by using salt in the diet of fish adapted to freshwater. Also, osmoregulation capacity may differ according to fish developmental stages and new studies should be conducted to include the whole production period (Bernardino and Fernandes, 2016).

The value of the viscerosomatic index increases as a result of visceral fat accumulation. Lovell (1989) reported that when fish are fed a high protein diet, excessive fat accumulation in the visceral cavity and tissue is reported due to an imbalance in the digestible energy/crude protein ratio. The highest HSI and VSI values obtained in the group fed low protein diets may be attributed to an imbalance in the P/E ratio due to the conversion of carbohydrates from raw materials into liver glycogen and lipid, thus increasing liver weight, and not as a result of freshwater rearing (Brown et al., 1992). As observed in this study, higher HSI and VSI values indicate poorer growth compared to the other groups. According to the condition factor data obtained in our study, it was observed that the VSI and HSI differences observed in the internal organs and liver did not cause a morphological difference. This shows that the feeds used were suitable in terms of nutrition and feeding rates.

CONCLUSION

According to the results obtained, at the end of 75 days, there was no significant difference between the groups fed with feed containing 45% protein and feed containing 50% protein. Accordingly, it was observed that European sea bass fish can be adapted to freshwater at the fry stage and can be fed with feeds containing lower protein ratios than the currently used commercial feeds, and this will not have a negative effect on the growth of the fish. This will help to reduce the cost of production.

In addition, the cultivation of European sea bass in freshwater will make a great contribution to the development of aquaculture. The fact that European sea bass is not affected by water temperature increases as much as trout and can live in higher water temperatures will enable the utilization of freshwater resources that cannot be used for trout production during hot periods and remain empty.

AUTHORSHIP STATEMENT

All authors contributed to the idea and design of the study. Material preparation and research was carried out by (Kutsal Gamsız), (Ali Yıldırım Korkut) and (Aysun Kop). The writing and editing of the manuscript was done by (Kutsal Gamsız), (Ali Yıldırım Korkut) and (Aysun Kop) and all authors have read and approved the manuscript.

CONFLICT OF INTEREST

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

ETHICS APPROVAL

Approval was obtained from Ege University Animal Experiments Ethics Committee (25.12.2019/2019+-116).

DATA AVAILABILITY STATEMENT

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

REFERENCES

Allegrucci, G., Fortunato, C., Cataudella, S., & Sbordoni, V. (1994). Acclimation to freshwater of the sea bass: Evidence of selective mortality of allozyme genotypes. In A.R. Beaumont, (Ed.), *Genetic and Evolution of Aquatic Organisms,* 486-502, Chapman and Hall, London.

Allegrucci, G., Fortunato, C., & Sbordoni, V. (1997). Genetic structure and sllozyme variation of sea bass (*Dicentrarchus labrax* and *D. punctatus*) in the Mediterranean Sea. *Marine Biology*, *128*, 347–358. https://doi.org/10.1007/s002270050100

Bernardino, R.J., & Fernandes, C. (2016, July). Growth performance for European sea bass fingerlings, *Dicentrarchus labrax*, reared at different salinities. In *IMMR International Meeting on Marine Research*. https://doi.org/10.3389/conf.FMARS.2016.04.00031

Brown, M., Nematipour, G.R., & Gatlin, G.M. (1992). Dietary protein requirement of juvenile sunshine bass at different salinities. *The Progressive Fish-Culturist*, *54*(3), 148-156. https://doi.org/10.1577/1548-8640(1992)054<0148:DPROJS>2.3.CO;2

Caccone, G., Allegrucci, G., Fortunato, C., & Sbordoni, V. (1997). Genetic differentiation within the European sea bass (*D. labrax*) as revealed by RAPD-PCR assays. *Journal of Heredity*, *88*(4), 316–324. https://doi.org/10.1093/oxfordjournals.jhered.a023109

Cataudella, S., Allegrucci, G., Bronzi, P., Cataldi, E., Cioni, C., Corti, M., Crosetti, D., de Merich, D., Fortunato, C., Garibaldi, L., Loy, A., Marino, G., Sola, L., & Sbordoni, V. (1991). Multidisciplinary Approach to the optimization of sea bass (*Dicentrarchus labrax*) rearing in freshwater. In Aquaculture Europe '91. International Conference, Dublin, Ireland. *EAS Special Publication*, *14*, 55-57.

Cnaani, A., Stavi, A., Smirnov, R., & Harpaz, S. (2012). Rearing white grouper (*Epinephelus aeneus*) in low salinity water: effects of dietary salt supplementation. *Israeli Journal of Aquaculture–Bamidgeh*, *64*, 1-5. https://doi.org/10.46989/001c.20633

Dendrinos, P., & Thorpe, J.P. (1985). Effects of reduced salinity on growth and body composition in the European bass *Dicentrarchus labrax* (L.). *Aquaculture*, *49*(3-4), 333-358. https://doi.org/10.1016/0044-8486(85)90090-0

Deng, J., Zhang, X., Han, X., Tao, L., Bi, B., & Kang, B. (2014). Dietary protein requirement of juvenile dianchi golden-line barbell, *Sinocyclocheilus grahami*. *Journal of World Aquaculture Society*, *45*, 421–429. https://doi.org/10.1111/jwas.12137

Eroldoğan, O.T., & Kumlu, M. (2002). Growth performance, body traits and fillet composition of the European sea bass (*Dicentrarchus labrax*) reared in various salinities and freshwater. *Turkish Journal of Veterinary & Animal Sciences*, *26*(5), 993-1001.

FAO (2022). *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation*. Rome, FAO. https://doi.org/10.4060/cc0461en

Ghisaura, S., Anedda, R., Pagnozzi, D., Biosa, G., Spada, S., Bonaglini, E., Cappuccinelli, R., Roggio, T., Uzzau, S., & Addis, M.F. (2014). Impact of three commercial feed formulations on farmed gilthead sea bream (*Sparus aurata*, L.) metabolism as inferred from liver and blood serum proteomics. *Proteome Science*, *12*, 1-18. https://doi.org/10.1186/s12953-014-0044-3

Giffard-Mena, I., Lorin-Nebel, C., Charmantier, G., Castille, R., & Boulo, V. (2008). Adaptation of the sea-bass (*Dicentrarchus labrax*) to freshwater: role of aquaporins and Na+/K+-Atpases. *Comparative Biochemistry and Physiology. Part A: Molecular & Integrative Physiology*, *150*(3), 332–338 https://doi.org/10.1016/j.cbpa.2008.04.004

Halver, J.E. (1976). The nutritional requirements of cultivated warmwater and coldwater fish species. *FAO Technical Conference on Aquaculture*, Kyoto (Japan).

Hoşsu, B., Korkut, A.Y., & Fırat ,A., (2005). Fish feeding and feed technology 1 (Fish Feeding Physiology and Biochemistry). *Ege University Fisheries Faculty Publication* No: 50. Bornova, İzmir (in Turkish)

Jana, P., Prasad Sahu, N., Sardar, P., Shamna, N., Varghese, T., Dharmendra Deo, A., Harikrishna, V., Paul, M., Panmei, H., Gupta, G., Nanda, C. & Krishna, G. (2021). Dietary protein requirement of white shrimp, *Penaeus vannamei* (Boone, 1931) juveniles, reared in inland ground water of medium salinity. *Aquaculture Research*, *52,* 2501-2517. https://doi.org/10.1111/are.15100

Jensen, M.K., Madsen, S.S., & Kristiansen, K. (1998). Osmoregulation and salinity effects on the expression and activity of Na+, K+-Atpase in the gills of European sea bass, *Dicentrarchus labrax* (L.). *Journal of Experimental Zoology, 282*, 290–300. https://doi.org/10.1002/(SICI)1097-010X(19981015)282:3<290::AID-JEZ2>3.0.CO;2-H

Kim, J., & Lall, S.P. (2001). Amino acid composition of whole body tissue of atlantic halibut (*Hippoglossus hippoglossus*), yellowtail flounder (*Pleuronectes ferruginea*) and Japanese flounder (*Paralichthy solivaceus*). *Aquaculture*, *187,* 367–373. https://doi.org/10.1016/S0044-8486(00)00322-7

Kokou, F., Con, P., Barki, A., Nitzan, T., Slosman, T., Mizrahi, I., & Cnaani, A. (2019). Short- and long-term low-salinity acclimation effects on the branchial and intestinal gene expression in the European sea bass (*Dicentrarchus labrax*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 231,* 11-18. https://doi.org/10.1016/j.cbpa.2019.01.018

Kousoulaki, K., Saether, B. S., Albrektsen, S., & Noble, C. (2015). Review on European seabass (*Dicentrarchus labrax* Linnaeus, 1758) nutrition and feed management: A practical guide for optimizing feed formulation and farming protocols. *Aquaculture Nutrition*, *21,* 129-151. https://doi.org/10.1111/anu.12233

Lovell, R.T. (1989). *Nutrition and feeding of fish*. New York, Springer. https://doi.org/10.1007/978-1-4757-1174-5

Metailler, R. (1986). Experimental in nutrition. In A. Bruno (Ed.), M*editerranean Regional Aquaculture Project*, *Nutrition in Marine Aquaculture*, (pp.1-11). Lisbon. https://www.fao.org/3/s5347e/S5347E12.htm

Mritunjoy, P., Parimal, S., Narottam, P., Ashutosh, D., Tincy, V., Nazeema, S., Prasanta, J., & Gopal, K. (2022). Effect of dietary protein level on growth and metabolism of gift juveniles reared in inland ground saline water of medium salinity. *Journal of Applied Aquaculture*, *35*(4), 948-974. https://doi.org/10.1080/10454438.2022.2054672

Nebel, C., Romestand, B., Nègre-Sadargues, G., Grousset, E., Aujoulat, F., Bacal, J., Bonhomme, F., & Charmantier, G. (2005). Differential freshwater adaptation in juvenile sea-bass *Dicentrarchus labrax*: Involvement of gills and urinary system. *The Journal of Experimental Biology*, *208*, 3859–3871. https://doi.org/10.1242/jeb.01853

Oliva-Teles, A. (2000). Recent advances in European sea bass and gilthead sea bream nutrition. *Aquaculture International*, *8*, 477–492. https://doi.org/10.1023/A:1009236517555

Peres, H., & Oliva-Teles, A. (1999). Effect of dietary lipid level on growth performance and feed utilization by European sea bass juveniles (*Dicentrarchus labrax*). *Aquaculture 179*, 325-334. https://doi.org/10.1016/S0044-8486(99)00168-4

Rimoldi, S., Bossi, E., Harpaz, S., Cattaneo, A. G., Bernardini, G., Saroglia, M., & Terova, G. (2015). Intestinal B(0)AT1 (SLC6A19) and PEPT1 (SLC15A1) mRNA levels in European sea bass (*Dicentrarchus labrax*)

reared in freshwater and fed fish and plant protein sources. *Journal of Nutritional Science, 4*(21), 1-13. https://doi.org/10.1017/jns.2015.9

Saleem, B., Orma, O., Abd El-Wahab, A., & Ibrahim, T. (2022). Growth performance parameters of European sea bass (*Dicentrarchus labrax*) cultured in marine water farm and fed commercial diets of different protein levels. *Mansoura Veterinary Medical Journal*, *23*(1), 10-17. https://doi.org/10.21608/mvmj.2022.229849

Shalaby, S.M, El-Dakar, A.Y., Osman, M.F., & Mohammed, A.S. (2023). Reduction of dietary fishmeal inclusion in practical diets of the seabass, (*Dicentrarchus labrax*), juveniles by plant protein feedstuff. *Egyptian Journal of Aquatic Biology and Fisheries,* 27(1), 589-604. https://doi.org/10.21608/ejabf.2023.289748

Talukdar, A., Kumar, S., Varghese, T., Jain, K. K., Sahu, N. P., & Sahoo, S. (2019). Feeding gelatinized carbohydrate in the diets of magur, *Clarias batrachus* (Linnaeus, 1758): Effects on growth performance, enzyme activities and expression of muscle regulatory factors. *Aquaculture Research*, *50*, 765–777. https://doi.org/10.1111/are.13933

Turkish Statistical Institute (2022). Aquaculture statistics. Ankara. https://data.tuik.gov.tr/Bulten/Index?p=Su Urunleri-2022-49678 (in Turkish)

Varsamos, S., Nebel, C., & Charmantier, G. (2005). Ontogeny of osmoregulation in postembryonic fish: A review. *Comparative Biochemistry and Physiology, Part A, 141*, 401-429. https://doi.org/10.1016/j.cbpb.2005.01.013

Venturini, G., Cataldi, E., Marino, G., Pucci, P., Garibaldi, L., Bronzi, P., & Cataudella, S. (1992). Serum ions concentration and ATPase activity in gills, kidney and esophagus of European sea bass (*Dicentrarchus labrax*, Pisces, Perciformes) during acclimation trials to freshwater. *Comparative Biochemistry and Physiology Part A: Physiology*, *103*(3), 451-454. https://doi.org/10.1016/0300-9629(92)90271-Q

Winfree, R.A., & Stickney, R.R. (1981). Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of *Tilapia aurea*. *Journal of Nutrition*, *111*(6):1001-1012. https://doi.org/10.1093/jn/111.6.1001

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 298-303 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.09

SHORT COMMUNICATION KISA MAKALE

First record of alien gastropods *Epitonium aranea* Bonfitto, 2018 and *Stosicia annulata* (Dunker, 1860) (Mollusca) from the Mediterranean Sea

İki yabancı gastropod türünün [*Epitonium aranea* Bonfitto, 2018 ve *Stosicia annulata* (Dunker, 1860) (Mollusca)] Akdeniz'den ilk defa kaydedilmesi

Bilal Öztürk[1*] ● Murat Recevik[2]

[1]Faculty of Fisheries, Ege University, 35100, Bornova, İzmir, Türkiye
[2]484 Commercial St, Provincetown, MA, 02657, USA

https://orcid.org/0000-0002-5638-2496
https://orcid.org/0009-0006-8987-3941

*Corresponding author: bilal.ozturk@ege.edu.tr; ozturkbilal1955@gmail.com

Received date: 10.07.2023 Accepted date: 25.09.2023

How to cite this paper:
Öztürk, B., & Recevik, M. (2023). First record of alien gastropods *Epitonium aranea* Bonfitto, 2018 and *Stosicia annulata* (Dunker, 1860) (Mollusca) from the Mediterranean Sea. *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 298-303. https://doi.org/10.12714/egejfas.40.4.09

Abstract: In a benthic material taken from the Levantine coast of Türkiye, *Epitonium aranea* Bonfitto, 2018, a species previously known from the Red Sea only, and *Stosicia annulata* (Dunker, 1860) distributed in Japan Sea, Persian Gulf and Central and East Indian Ocean were recorded for the first time from the Mediterranean Sea. Along with two unknown species from the region, herein have also been dealing with *Melanella* sp., *Oscilla virginiae* Peñas, Rolán and Sabelli, 2020 and *Zafra pumila* (Dunker, 1860) which are poorly known species from the area, and some remarks of the taxonomy and distribution of the studied taxa are discussed.

Keywords: Mollusca, alien species, Mediterranean Sea, new record, Turkish coast

Öz: Türkiye'nin Levant Denizi kıyılarından alınan bir bentik materyalde, daha önce sadece Kızıldeniz'den bilinen *Epitonium aranea* Bonfitto, 2018 ile Japon Denizi, Basra Körfezi ve Orta ve Doğu Hint Okyanusu'nda dağılım gösteren *Stosicia annulata* (Dunker, 1860) türleri Akdeniz'de ilk defa bulunmuştur. Bu çalışmada, bölgeden bilinmeyen bu iki türün yanı sıra, *Melanella* sp., *Oscilla virginiae* Peñas, Rolán ve Sabelli, 2020 ve bölgeden az bilinen yumuşakçalar arasında yer alan *Zafra pumila* (Dunker, 1860) türleri de bulunmuş olup, incelenen türlerin taksonomik ve dağılım özellikleri tartışılmıştır.

Anahtar kelimeler: Mollusca, yabancı tür, Akdeniz, yeni kayıt, Türkiye kıyıları

INTRODUCTION

The Mediterranean Sea, which is a semi enclosed ecosystem, is one of the prominent hotspots of marine bioinvasions on earth (Rilov and Galil, 2009) and most of the known species in this ecosystem are assessed to have successfully established, being recorded for multiple times (Zenetos et al., 2022).

The alien species being successfully established in an introduced area may restructuring established food webs, importing new diseases and competition with native organisms for food and space. Other ecological changes may occur when the invading organisms reproduce with native species, altering the gene pool (Occhipinti Ambrogi, 2001). Invaders are also capable of colonizing every ecosystem on earth, changing the ecological relations within communities, altering evolutionary processes, and causing dramatic changes in native populations, including extinctions (Mack et al., 2000). Gastropods, which are in the majority among the alien species, cause a multitude of environmental and socioeconomic impacts in many habitats, represent a major threat to native plants and animals. They also consist one of the significant problems in agriculture, resulting in economic losses by reducing the yield (Kesner and Kumschick, 2018).

Regarding the housed alien species number, there are significant differences among the Mediterranean basins. Eastern Mediterranean, being close to the Suez Canal, which is one of the main pathways for entering of alien species, includes highest number species, where the taxa with Indo-Pacific origin dominated. The lowest alien species diversity occurs in the western Mediterranean, where fouling and ballast water of ships seem to be the main vector for transportation of alien species (Zenetos et al., 2012; Galil et al., 2018).

Among the Mediterranean countries, Türkiye is one of the most impacted one. İskenderun Bay, which is found in close distance to the Suez Canal and the dense maritime traffic occurring due to the oil transportation from the Ceyhan Oil Terminal, attracts attention as a hotspot area of the region from where was reported 77 alien species (Bitlis Bakır et al., 2012). Çınar et al. (2021) reported 539 species belonging to 18 taxonomic groups, 404 of which have been estimated as established ones, including the list of alien species distributed on the Turkish coasts by the end of 2020. In terms of the number of species, molluscs rank first with 123 species. The number of alien molluscs has been increased from 105 species (Çınar et al., 2011) to 123 for a period nearly one decade. *Epitonium vaillanti* (Jousseaume, 1912) recorded from the Turkish Levantine coast (Öztürk et al., 2023a), *Phosinella digera* (Laseron, 1956) found on the Turkish Levantine coast

(in Taşucu) and *Circulus octoliratus* (Carpenter 1856) recorded from Taşucu and İskenderun Bay (Levantine coast of Türkiye) (Ovalis and Mifsud, 2019; Öztürk et al., 2023b) should be added to the last published checklist (Çınar et al., 2021). *P. digera* and *C. octoliratus* have been overlooked in the recently published checklist by Çınar et al. (2021).

In the present study is dealing with two new record gastropod species for the Mediterranean Sea (*E. aranea* and *S. annulata*), recorded in the İskenderun Bay (Levantine coast of Türkiye), along with some other poorly known alien species.

MATERIALS AND METHODS

The material was sampled from off Konacık Sütunlu Liman (Colonnade Port) (İskenderun Bay), eastern Levantine coast of Türkiye. Konacık Sütunlu Liman is located within the borders of Konacık village (Arsuz-Hatay) and is in a distance approximately 8 km from Arsuz district center (Figure 1). On the coast of Konacık village there are the ruins of an ancient port city from the Hellenistic period, and for this reason, it was called the "Colonnade Port" by the local people.

Figure 1. Map of sampled area and locality

The benthic material, in which the species dealing with herein were found, was sampled from a depth of about 48 m (36°23'46"N- 35°50'19"E) on August 23, 2022 (Figure 1). The material was muddy sand with shell fragments and sea urchin skeletons and was fixed with 4% formaldehyde. In the laboratory, the material was washed with tap water on a 0.5 mm mesh and then sorted under a stereomicroscope. Both living individuals and empty shells were identified and counted. In the present study, only the alien gastropods have been taken into consideration. The nomenclature of the studied species is given according to WoRMS (World Register of Marine Species).

The studied specimens of each species, with individual catalogue numbers, are deposited in the museum collections of the Faculty of Fisheries (ESFM), Ege University, İzmir/Türkiye.

RESULTS AND DISCUSSION

The examination of the sampled benthic material revealed 16 specimens and 5 shells belonging to nine alien mollusc taxa, from which four species are within the subclass Caenogastropoda [*Epitonium aranea* Bonfitto, 2018; *Melanella* sp., *Stosicia annulata* (Dunker, 1860) and *Zafra pumila* (Dunker, 1860)] and five species are from the subclass Heterobranchia [*Leucotina natalensis* Smith, E. A., 1910; *Cingulina isseli* (Tryon, 1886); *Oscilla virginiae* Peñas, Rolán and Sabelli, 2020; *Pyrgulina pupaeformis* (Souverbie, 1865) and *Pyrunculus fourierii* (Audouin, 1826)]. According to the recent publication by Zenetos et al. (2022), including the alien species distributed in the Mediterranean Sea, *E. aranea* and *S. annulata* were recorded for the first time from the Mediterranean Sea. On the other hand, *O. virginiae* and *Melanella* sp. are new records for the Turkish mollusc fauna as reported by this study. Among the other taxa, some are well known species such as *L. natalensis*, *C. isseli*, *P. pupaeformis* and *P. fourierii*. All the previously known species were recorded from the Turkish Levantine coast, except for *L. natalensis* and *P. fourierii* which were also reported from the Aegean coast of Türkiye.

Some taxonomic, ecologic, and distributional features of newly recorded or poorly known species are given below.

Taxonomic account

Epitoniidae Berry, S. S., 1910

Epitonium aranea Bonfitto, 2018 (Figure 2)

Epitonium aranea; Bonfitto, 2018: 119-129, fig. 4, A-K

Material examined: 1 specimen (ESFM-GAS/2022-01).

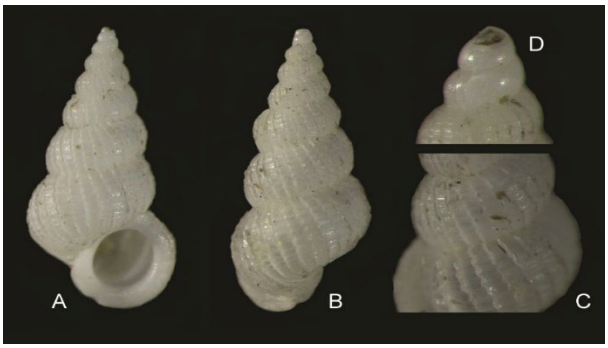

Figure 2. *Epitonium aranea*: ventral (A) and dorsal (B) views of the sampled specimen, axial ribs under magnification (C) and the protoconch of the specimen (D). (A=B=4.3 mm)

Remarks: The type locality of the species is the Red Sea (off Yemen), found on muddy sand at 76 m depth (Bonfitto, 2018: 124). The species is with a glossy protoconch of about 3.5 whorls and is characteristic having a reticulated sculpture of regular meshes caused by the crossing of axial ribs and spiral threads. Axial ribs (23 on the body whorl) are thin, stripe-

like, and sinuous (Figure 2). Aperture oval, outer lip thick and columella auriculate.

Distribution: The species was hitherto known from its type locality (Red Sea) only (Bonfitto, 2018). The present record is the first one for the Mediterranean Sea. The location, where the species was found, being in the close distance to the Suez Canal, it is suggested the species to have penetrated the Mediterranean by this pathway.

Eulimidae Philippi, R. A., 1853

Melanella sp. (Figure 3)

Melanella sp. 2; Mbazios et al., 2020: 35, fig. 7a.

Melanella conoidea; Manousis et al., 2021: 39, fig. 5e.

Material examined: 1 specimen (ESFM-GAS/2022-02).

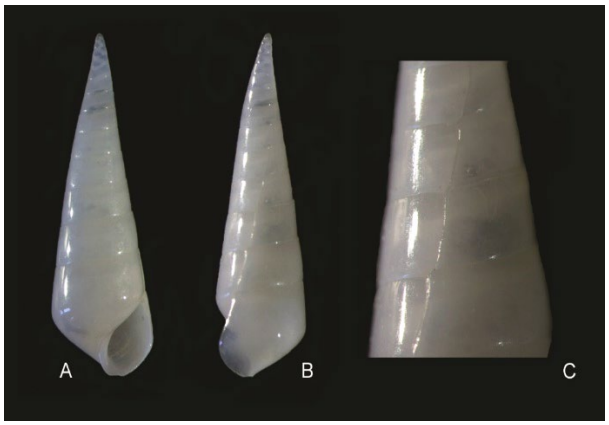

Figure 3. *Melanella* sp.: ventral (A), lateral (B) and incremental scars (C) views of the found specimen (A=B=6.3 mm)

Remarks: The shell in grey-white colour, conical, shiny and consists of 11 flat teleoconch whorls. Apical part sinuous. Incremental scars evident. The species is characteristic with subangulated body whorl and angulated outer lip of the aperture. By its shell shape and characteristics of the body whorl, the specimen looks very similar to that of *Melanella conoidea* (Kurtz and Stimpson, 1851), which is a species distributed along the Atlantic coasts from North Carolina to Uruguay at depths between 0-538 m (Rosenberg et al., 2009: 643), but more specimens need to be studied to understand whether we are dealing with a known species or a new undescribed one.

A similar specimen was reported by Mbazios et al. (2020: 35, fig. 7A) as *Melanella* sp. 2, which specimen was compared by the authors to *Parvioris ibizenca* (Nordsieck, 1968), *Melanella conoidea* (Kurtz and Stimpson, 1851) and fossil records of Chirli (2009) and, although existence of some differences, the specimen was found quite similar to *Melanella conoidea* and *M. lactea* (Grateloup, 1838). After a while, a similar specimen was identified as *Melanella conoidea* by Manousis et al. (2021: 39, fig. 5e). The authors were also in opinion that the specimen considered by Mbazios et al. (2020) as *Melanella* sp. 2 was a *Melanella conoidea* (Manousis et al., 2021: 32).

Distribution: *Melanella* sp. is distributed along the Hellenic (Mbazios et al., 2020; Manousis et al., 2021) and Turkish coasts (the present study).

Zebinidae Coan, 1964

Stosicia annulata (Dunker, 1860) (Figure 4)

Rissoina annulata; Dunker, 1860: 235 (*not figured*).

Material examined: 2 specimens (ESFM-GAS/2022-3).

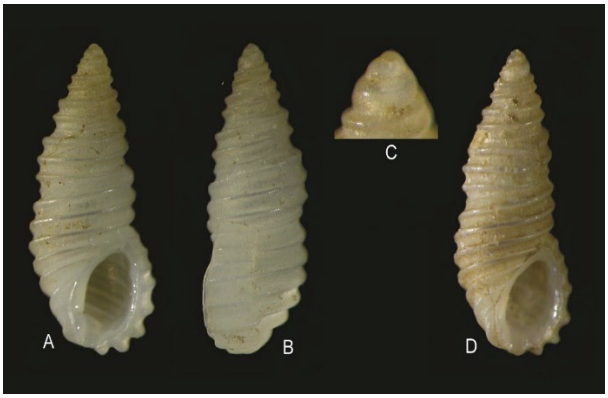

Figure 4. *Stosicia annulata*: ventral (A, D) and dorsal (B) views of the sampled specimens and the protoconch (C) of the specimen A (A=B=3.4 mm; D=3.4 mm).

Remarks: The type locality of the species is Japan coasts (WoRMS).

Shell thick, consists of about 4 teleoconch whorls and a smooth protoconch of nearly 3 whorls (Figure 4C). On the whorls strong spiral ribs with canaliculated interspaces. On the body whorl 6 ribs, 3 ribs on the penultimate whorl and two ribs on the initial whorls. Aperture ovoid, outer lip thickened. In whitish colour.

Distribution: The species is known to be distributed in Japan Sea to Kyushu (Okutani, 2000: 160), Persian Gulf and Gulf of Oman (Bosch et al., 1995: 48), western Indian coast, Indochina, Central and East Indian Ocean (Mukhopadhyay, 2015: 92-93). Its report in the present study is the first one for the Mediterranean Sea.

Pyramidellidae Gray, 1840

Oscilla virginiae Peñas, Rolán and Sabelli, 2020 (Figure 5)

Material examined: 2 shells (ESFM-GAS/2022-04).

Remarks: The type locality of *O. virginiae* is Aqaba (Jordan), 5-15 m (Peñas et al.,2020).

Shell minute, solid and consists of 4 teleoconch whorls with spiral ribs on. On the first two whorls there is two ribs, of which adapical one is wider. On the third whorl the upper rib bifurcates and on the body whorl they become three ribs, of which the upper two ribs being closer to each other. One of the shells (A) was with eroded protoconch and the other shell (B) has a protoconch with submerged nucleus. No visible axial elements. Aperture oval-shaped, an evident tooth on the columella

and thin outer lip. The differences of the species from the similar ones (*C. isseli*, *Oscilla galilae* and *Miralda* sp.) were clarified in the study by Albano et al. (2021: 46).

Distribution: Red Sea (Jordan and Egypt coasts) (Peñas et al., 2020) and eastern Mediterranean Sea (Israeli coast) (Albano et al., 2021). The present record is the second one from the Mediterranean Sea.

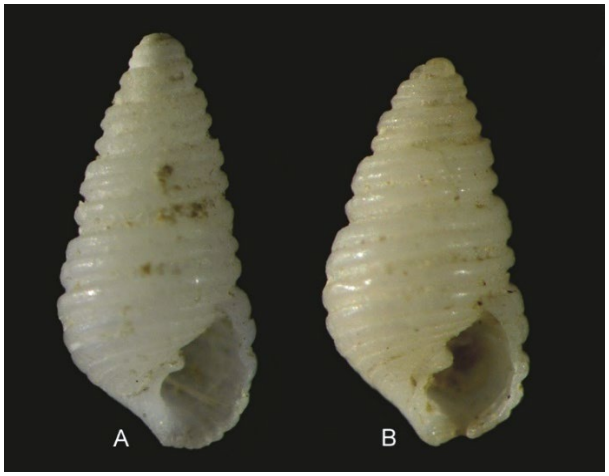

Figure 5. *Oscilla virginiae*: ventral views of the sampled shells (A=2.9 mm; B=2.3 mm).

Columbellidae Swainson, 1840

Zafra pumila (Dunker, 1860) (Figure 6)

Columbella pumila; Dunker, 1860: 224 (*not figured*).

Material examined: 2 specimens (ESFM-GAS/2022-05).

Figure 6. *Zafra pumila*: ventral (A) and dorsal (B) views of one of the sampled specimens (A=B=4.0 mm).

Remarks: The type locality of the species is Decima (Japan) (Monsecour and Köhler, 2006).

In the Mediterranean Sea, the species was first recorded from the Turkish Levantine coast in June 2010 (Öztürk et al., 2015). According to Monsecour and Köhler (2006), the specimens belonging to the species may being of two-colour morphs: one is brown overall and the second one in yellowish colour with two narrow brown spiral bands on the body whorl and one spiral band on the spire whorls, nearer to the lower suture. Along the Turkish Levantine coast both colour morphs have been recorded (Figure 6A, B and Öztürk et al., 2015 fig. 5 A, B, C). Recently, 7 specimens of *Z. pumila* was also found in Taşucu (Tisan Bay) (Levantine cost of Türkiye) in a sandy substrate taken at 8 m depth (Panayotis Ovalis, pers. comm.).

Distribution: *Zafra pumila* is widespread in the Indo-Pacific area. The species is also distributed along the shoreline from the Red Sea south to Natal (South Africa), and on the Japanese and Polynesian coasts (Monsecour and Köhler, 2006). Since June 2010 it also occurs in the Mediterranean Sea (Öztürk et al., 2015).

The other alien species detected in the present study (*L. natalensis*, *C. isseli*, *P. pupaeformis* and *P. fourierii*) are widely distributed in the Mediterranean and have been assessed as established species (Figure 7). All above-mentioned species are distributed on the Levantine coast of Türkiye (Çınar et al., 2021), except for *L. natalensis* and *P. fourierii*, which are also distributed along the Turkish Aegean coast (Öztürk et al., 2017).

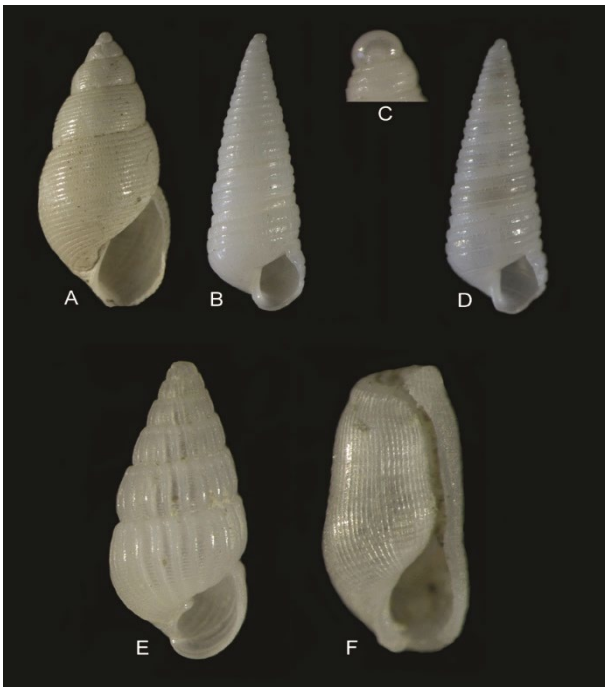

Figure 7. The alien species detected within the present study and widely distributed in the Mediterranean Sea. **A.** *Leucotina natalensis*; **B.** *Cingulina isseli*; **C.** protoconch of the specimen B; **D.** *C. isseli*; **E.** *Pyrgulina pupaeformis* and **F.** *Pyrunculus fourierii* (A=7.2 mm; B=5.9 mm; D=5.5 mm, E=3.7 mm and F=2.3 mm)

The penetration of non-indigenous species into the Mediterranean Sea is a process that has been going on for years, although it has increased in last decades depending on

many factors such as widening of the Suez Canal, intensive marine transport, and rapid climate warming.

In a recent study by Zenetos et al. (2022), comprising the alien species in the Mediterranean Sea reported to the end of 2021, 1366 alien taxa were examined, of which 751 species are assessed as established, 232 taxa as casual, and 70 species were evaluated as questionable ones. However, the number of alien species in different groups also varies depending on the years, and the establishment success or status of some species can be changed in a shorter period, due to their adaptation to the environmental conditions in the recipient ecosystem. For example, after the year 2020 only, 21 more mollusc species have been assessed to be in established status in the Mediterranean (Zenetos et al., 2022). The same fact is also valid for the Turkish coasts, where some species such as *Pseudorhaphitoma iodolabiata* (Hornung and Mermod, 1928), *Circulus novemcarinatus* (Melvill, 1906), *Zafra pumila* (Dunker, 1860) and *Varicopeza pauxilla* (Adams, A., 1855) which have been increased to the established rank for a period nearly one decade (since 2012), being recorded for multiple times from different localities on the Turkish Levantine coast (Bilal Öztürk, pers. data). On the other hand, out of 230 alien mollusc taxa known from the Mediterranean Sea (Zenetos et al., 2022), 123 species were also detected on the Turkish coasts, which number was increased by 23% since 2011 (Çınar et al., 2021). Their number is varied according to the seas and most species (113 species) are known from the Turkish Levantine coast, whereas the lowest number species (3 species) were detected along the Turkish Black Sea coast. With the species dealing with herein, is being added two more species to the Mediterranean mollusc fauna and four species to the Turkish marine mollusc inventory.

ACKNOWLEDGEMENT AND FUNDING

The authors thank to the anonymous referees for their contribution during the reviewing process of the manuscript. 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.

AUTHOR CONTRIBUTIONS

Bilal Öztürk: Designing of the study, identification of the investigated species, writing of the draft, submission, writing-review, and editing. Murat Recevik: Sampling and sorting of the materials.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for the study.

DATA AVAILABILITY

For any questions the corresponding author should be contacted.

REFERENCES

Albano, P.G., Steger, J., Bakker, P.A.J., Bogi, C., Bošnjak, M., Haim T.G., Huseyinoglu, M.F., LaFollette, P.I., Lubinevsky, H., Mulas, M., Stockinger, M., Azzarone, M., & Sabelli, B. (2021). Numerous new records of tropical non-indigenous species in the Eastern Mediterranean highlight the challenges of their recognition and identification. *ZooKeys*, *1010*, 1-95. https://doi.org/10.3897/zookeys.1010.58759

Bitlis Bakır, B., Öztürk, B., Doğan, A., & Önen, M. (2012). Mollusc fauna of İskenderun Bay with a checklist of the region. *Turkish Journal of Fisheries and Aquatic Sciences*, *12*, 171-184. https://doi.org/10.4194/1303-2712-v12_1_20

Bonfitto, A. (2018). New species of Epitoniidae (Gastropoda: Epitonioidea) from the Red Sea. *Molluscan Research*, *38*, 119-129. https://doi.org/10.1080/13235818.2017.1385168

Bosch, D.T., Dance, S.P., Moolenbeek, R.G., & Oliver, P.G. (1995). *Seashells of Eastern Arabia*. Dubai, Motivate publishing, 296 p.

Chirli, C. (2009). *Malacofauna Pliocenica Toscana. Vol. 7. Ptenoglossa.* Firenze, Tavernele Val di Pesa, 98 p.

Çınar, M.E., Bilecenoğlu, M., Öztürk, B., Katağan, T., Yokeş, M.B., Aysel, V., Dağlı, E., Açık, Ş., Özcan, T., & Erdoğan, H. (2011). An updated review of alien species on the coasts of Turkey. *Mediterranean Marine Sciences*, *12*, 257-315. https://doi.org/10.12681/mms.34

Çınar, M. E., Bilecenoğlu, M., Yokeş, B., Öztürk, B. Taşkın, E., Bakır, K., Doğan, A., & Açık, Ş. (2021). Current status (as of end of 2020) of marine alien species in Turkey. *Plos One*, *16*, Article e0251086. https://doi.org/10.1371/journal.pone.0251086

Dunker, W. (1860). Neue japanische Mollusken. *Malakozoologische Blätter*, *6*, 221-240.

Galil, B. S., Marchini, A., & Occhipinti-Ambrogi, A. (2018). East is east and West is west? Management of marine bioinvasions in the Mediterranean Sea. *Estuarine, Coastal and Shelf Science*, *201*, 7-16. https://doi.org/10.1016/j.ecss.2015.12.021

Kesner, D., & Kumschick, S. (2018). Gastropods alien to South Africa cause severe environmental harm in their global alien ranges across habitats. *Ecology and Evolution*, *8*(16), 8273-8285. https://doi.org/10.1002/ece3.4385

Kurtz, J.D., & Stimpson, W. (1851). *Several new species of shells from the Southern Coast. Proceedings of the Boston Society of Natural History,* 4. Cambridge: Printed by H.O. Houghton and Company, 415p.

Mack, R.N., Simberloff, D., Londsdalle, W.M., Evans, H., Clout, M. & Bazzaz, F.A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, *10*, 689-710. https://doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

Manousis, T., Zaminos, G., Kontadakis, C., Bazios, G., Porfyris, A., & Galinou-Mitsoudi, S. (2021). New records of Mollusca for the Mediterranean and the Hellenic Seas. *Xenophora Taxonomy*, *31*, 30-44.

Mbazios, G., Kontakidis, C., Galinou-Mitsoudi, S., & Manousis, T. (2020). New gastropod records for the Mediterranean and the Hellenic seas. *Xenophora Taxonomy*, *27*, 34-58.

Monsecour, K., & Köhler, F. (2006). Annotated list of columbellid types held in the Malacological Collection of the Museum für Naturkunde, Berlin (Mollusca, Caenogastropoda,Columbellidae). *Zoologische Reihe*, *82*, 282-306. https://doi.org/10.1002/mmnz.200600015

Mukhopadhyay, A. (2015). New records of three minute gastropods from west coast of India. *Records of the Zoological Survey of India*, *115*(1), 91-94.

Occhipinti Ambrogi, A. (2001). Transfer of marine organisms: A challenge to the conservation of coastal biocoenoses. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *11*, 243-251. https://doi.org/10.1002/aqc.450

Okutani, T. (2000). *Marine Mollusks in Japan*. *1st edition*. Tokyo, Tokai University Press.

Ovalis, P., & Mifsud, C. (2019). *Circulus octoliratus* (Carpenter, 1856) and *Phosinella digera* (Laseron, 1956): Two new non-indigenous gastropod species for the Mediterranean Sea. *Iberus*, *37*, 267-270.

Öztürk, B., Recevik, M., & Geyran, K. (2015). New alien molluscs in the Mediterranean Sea. *Cahiers de Biologie Marines*, *56*, 205-212.

Öztürk, B., Bitlis, B., Doğan, A., & Türkçü, N. (2017). Alien marine molluscs along the Turkish coast, with a new record of *Varicopeza pauxilla* (A. Adams, 1855) (Mollusca: Gastropoda) from the Mediterranean Sea. *Acta Zoologica Bulgarica*, Supplement 9, 83-92.

Öztürk, B., Türkçü, N., & Bitlis, B. (2023a). New records of gastropods (Caenogastropoda and Heterobranchia) from the Turkish coasts with observations on some poorly known species. *Turkish Journal of Zoology*, *47*, 135-146. https://doi.org/10.55730/1300-0179.3125

Öztürk, B., Bitlis, B., & Türkçü, N. (2023b). *Circulus* (Mollusca-Gastropoda) species of the Turkish coasts with a note on the presence of *Circulus octoliratus* (Carpenter, 1856). *Ege Journal of Fisheries and Aquatic Sciences*, *40*(1), 77-80. https://doi.org/10.12714/egejfas.40.1.11

Peñas, A., Rolán, E., & Sabelli, B. (2020). The family Pyramidellidae in the Red Sea. I. The tribe Chrysallidini. *Iberus*, *38*, Supplement 8, 1-93.

Rilov, G., & Galil, B. (2009). Marine Bioinvasions in the Mediterranean Sea-History, Distribution and Ecology. In G. Rilov, J.A. Crooks (Eds.) *Biological Invasions in Marine Ecosystems. Ecological Studies* (pp. 549–575). Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-79236-9_31

Rosenberg, G., Moretzsohn, F., & García, E.F. (2009). Gastropoda (Mollusca) of the Gulf of Mexico. In D.L. Felder, D.K. Camp (Eds.) *Gulf of Mexico–Origins, Waters, and Biota. Biodiversity* (pp. 579–699). Texas A & M, University Press College Station.

Zenetos, A., Gofas, S., Morri, C., Rosso, A., Violanti, D., Raso, J.G., Çınar, M. E., Almogi-Labin, A., Ateş, A.S., Azzurro, E., Ballesteros, E., Bianchi, C.N., Bilecenoğlu, M., Gambi, M. C, Giangrande, A., Gravili, C., Hyams-Kaphzan, O., Karachle, P.K., Katsanevakis, S., Lipej, L., ……., & Verlaque, M. (2012). Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways. *Mediterranean Marine Sciences*, *13*, 328-52. https://doi.org/10.12681/mms.327

Zenetos, A., Albano, P.G., Garcia, E.L., Stern, N., Tsiamisi, K., & Galanidis, M. (2022). Established non-indigenous species increased by 40% in 11 years in the Mediterranean Sea. *Mediterranean Marine Science*, *23*(1), 196-212. https://doi.org/10.12681/mms.29106

WoRMS World Register of Marine Species. https://molluscabase.org/ Accessed online 27 April 2023.

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(4), 304-309 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.4.10

SHORT COMMUNICATION KISA MAKALE

A new oligochaeta record for Türkiye, *Rhyacodrilus falciformis* Bretscher, 1901 (Annelida: Naididae: Rhyacodrilinae)

Türkiye Oligochaeta faunası için yeni kayıt, *Rhyacodrilus falciformis* Bretscher, 1901 (Annelida: Naididae: Rhyacodrilinae)

Melek Zeybek Yünlü[1]* ● Eylem Aydemir Çil[2] ● Ton van Haaren[3] ● Seray Yıldız[4]

[1]Department of Biology, Faculty of Arts and Sciences, Süleyman Demirel University, İsparta, Türkiye https://orcid.org/0000-0002-2982-8616
[2]Department of Environmental Engineering, Faculty of Engineering and Architecture, Sinop University, Sinop, Türkiye https://orcid.org/0000-0003-2405-1155
[3]Eurofins Aqua Sense H.J.E. Wenckebachweg 120, 1114 AD Amsterdam Duivendrecht, Netherlands https://orcid.org/0000-0001-5119-1714
[4]Department of Marine-Inland Waters Sciences and Technology, Faculty of Fisheries, Ege University, İzmir, Türkiye https://orcid.org/0000-0002-2848-7469

*Corresponding author: melekzeybek@sdu.edu.tr
Received date: 11.08.2023 Accepted date: 17.10.2023

How to cite this paper:
Zeybek Yünlü, M., Aydemir Çil, E., van Haaren, T. & Yıldız, S. (2023). A new oligochaete record for Türkiye, *Rhyacodrilus falciformis* Bretscher, 1901 (Annelida: Naididae: Rhyacodrilinae). *Ege Journal of Fisheries and Aquatic Sciences, 40*(4), 304-309. https://doi.org/10.12714/egejfas.40.4.10

Abstract: The aim of this paper is to contribute to the aquatic Oligochaeta fauna of Türkiye. The study area selected for this research is Karasu Stream, located in the Black Sea Region of Türkiye, in the province of Sinop. Benthic macroinvertebrate samples were collected on a monthly basis between February 2013 and January 2014 from 10 selected stations in the study area. As a result of the study, *Rhyacodrilus falciformis* Bretscher, 1901 is reported as a new record for the Oligochaeta fauna of Türkiye. Besides being recognized as a new record species, this record also extends the species' distribution area to the Asian part of Türkiye. Additionally, this study provides detailed information on the biological, ecological, and morphometric characteristics, as well as the distribution of *R. falciformis*, identified as a new record for the fauna of Türkiye.

Keywords: Oligochaeta, Rhyacodrilinae, new record, Karasu Stream, Sinop, Türkiye

Öz: Bu makalenin amacı, Türkiye'nin sucul Oligochaeta faunasına katkıda bulunmaktır. Bu araştırma için seçilen çalışma alanı, Türkiye'nin Karadeniz Bölgesi'nde, Sinop ilinde bulunan Karasu Çayı'dır. Bentik makroomurgasız örnekleri, Karasu Çayı'nda 10 istasyondan Şubat 2013 ile Ocak 2014 arasında aylık olarak toplanmıştır. Araştırma alanında tespit edilen, *Rhyacodrilus falciformis* Bretscher, 1901, Türkiye'den ilk kez bildirilmektedir. Türkiye Oligochaeta faunası için yeni kayıt olarak tanınmasının yanı sıra, bu araştırma ile türün dağılım alanı Türkiye'nin Asya kısmına kadar genişlemektedir. Ayrıca, bu çalışma, Türkiye faunası için yeni bir kayıt olarak tanımlanan *R. falciformis*'in biyolojik, ekolojik ve morfometrik özellikleri ile dağılımı hakkında detaylı bilgiler de sağlamaktadır.

Anahtar kelimeler: Oligochaeta, Rhyacodrilinae, yeni kayıt, Karasu Çayı, Sinop, Türkiye

INTRODUCTION

The Oligochaeta order in freshwater ecosystems comprises approximately 1100 known species (Martin et al., 2008). The family Naididae is one of the most significant groups of aquatic Oligochaeta. Rhyacodrilinae is a subfamily within the family Naididae, and its representatives are commonly found in rivers and marine environments. The genus Rhyacodrilus, now placed in the family Naididae from Tubificidae, is a cosmopolitan and large genus (Ohtaka, 1995). There have been 54 valid species of *Rhyacodrilus* identified worldwide (Martin et al., 2023). Globally, 20 genera are recognized within this subfamily (Martin et al., 2023).

However, from Türkiye, only five species belonging to this subfamily have been reported so far: *R. coccineus* (Vejdovsky 1876) in Gümüş River by Öntürk and Arslan (2003); in Tunca River by Çamur-Elipek et al. (2006); in Balıkdamı Wetland by Arslan et al. (2007); in Lake Uluabat by Kökmen et al. (2007); in Porsuk River and in the Trace Region by Arslan and İlhan (2010); in Trace Region-Taş et al. (2012); in Tuzla Stream by Odabaşı et al. (2018); *Branchiura sowerbyi* Beddard, 1892 in Buldan Dam Lake by Balık et al. (2004); *Monopylephorus irroratus* (Verrill, 1873) in Hazar Lake by Şahin and Baysal (1972); *Epirodrilus moubayedi* Giani and Martinez-Ansemil (1981) in Balıkdamı Wetland by Arslan et al. (2007); and *Bothrioneurum vejdovskyanum* Stolc, 1886 in Karamenderes Stream by Odabaşı et al. (2017) have been reported from different parts of Türkiye.

The objective of this study is to report *R. falciformis* Bretscher, 1901 as a new record for the Oligochaeta fauna of Türkiye. In addition, this record also contributes to the knowledge of the species distribution by extending its range to the Asian part of Türkiye.

MATERIALS AND METHODS

Between February 2013 and January 2014, samples were collected from 10 stations in Karasu Stream, located in the Black Sea Region of Türkiye, at monthly intervals (Figure 1).

The kick sampling method was used to collect zoobenthic

samples from a 1 m² area at each station, with a 5-minute collection standard (kick-net mesh size: 180 µm) (Letovsky et al., 2012). After fixing the samples with 4% formaldehyde in the study area, they were transported to the laboratory and washed again through a 180 µm sieve. Oligochaeta samples separated from the debris were preserved in 70% alcohol with CMCP 10 solution for identification. Species identification was carried out using Timm's (1999 and 2009) keys.

Digital photos of the species were taken using a digital camera (Camedia, C-7070, Olympus) connected to compound and stereo microscopes. The specimens are housed in the Museum of the Faculty of Fisheries, Ege University. All measurements are given in parentheses.

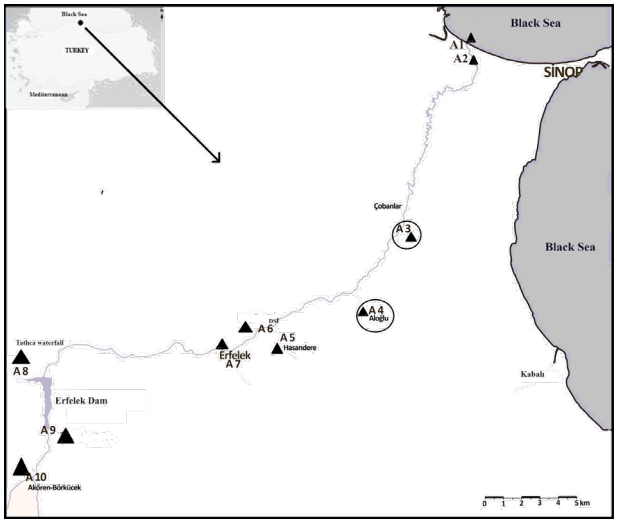

Figure 1. Sampling stations and map of the study area (Çil et al., 2021)

RESULTS

As a result of the samplings, *R. falciformis* was found at only two stations (Stations A3 and A4) (Figure 2, Figure 3).

Figure 2. Station 3 (A3)

Figure 3. Station 4 (A4)

The ecological, biological, and morphometric characteristics, as well as the distribution, of the Oligochaeta species determined as a new record for the fauna of Türkiye are explained in detail below.

Rhyacodrilus falciformis Bretscher, 1901 (Figure 4 and 5)

Material

A total of 15 specimens were obtained, and they were found only at two stations in the study area (Station 3=A3: Karasu Stream 41°57'59.24"N, 35°01'26.45"E, and Station 4=A4: Tributary of Karasu 41°54'45.10"N, 34°59'33.17"E) (Figure 1). One individual was obtained from Station 3 in February, while 11 were collected from Station 4 in February and 1 in March.

Description

Total length = 8-10 mm, Number of segments = 38-48

The anterior and posterior locomotory chaetae are similar, with upper teeth 2-6 in anterior bundles and 1-2 in posterior bundles, measuring 72-81 µm in length. Near the male pores (XI), single large sickle-shaped penial chaetae are usually hidden inside the body with a simple tip, measuring 94-148 µm in length and 12-14 µm in thickness. The presence of abundant large coelomocytes in the body cavity gives the living worm a white color, similar to enchytraeids (Timm, 1999).

Diagnosis

Total length = 5.75-9.25 mm, Number of segments = 31-45

The anterior and posterior locomotory chaetae are similar, with upper teeth 2-6 in anterior bundles and 1-2 in posterior bundles, measuring 57.5 µm-61 µm in length. Near the male pores (XI), single large sickle-shaped penial chaetae are usually hidden inside the body with a simple tip, measuring 96-117 µm in length and 18.5 µm-22.5 µm in thickness.

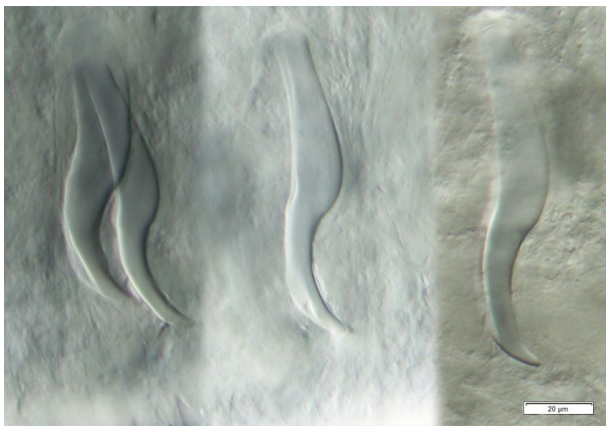

Figure 4. *Rhyacodrilus falciformis*, genital area (XI. ventral segment) showing large modified penial setae

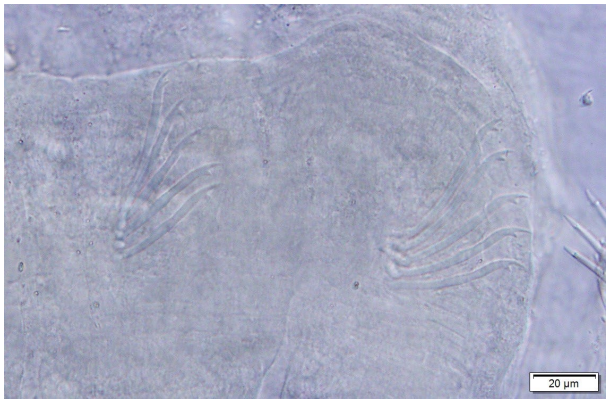

Figure 5. *Rhyacodrilus falciformis*, anterior dorsal setae III-IV with distinct longer distal tooth

Differential characteristics

R. falciformis shows similarities with *Bothrioneurum vejdovskyanum* Štolc, 1886, but *B. vejdovskyanum* bears sensory prostomial pits and only the foremost chaetae have considerably longer upper teeth, while the teeth become progressively equal in subsequent preclitellar segments.

The sickle-shaped penial setae of this species are a distinctive feature. A similar species described from France (*R. pigueti*) has larger penial chaetae of 150 µm and is more straight compared to the sickle-shaped penial chaetae of *R. falciformis* (Martinsson et al., 2013).

In this study, the penial setae of *R. falciformis* vary in length from 96-117 µm, which falls within the normal range for this species (100-140 µm: Martinsson et al., 2013).

Although penial setae are normally between 94-148 µm long (Kasprzak, 1979, 1981; Timm, 2009), Martin and Boughrous (2012) have shown penial setae of approximately 175 and 200 µm (Table 1).

Environment

This species can be found in groundwater, springs, clean lakes, and in soil (van Haaren, 2002).

Habitat

R. falciformis in habits freshwater. Karasu Stream originates in Boyabat district, passes along through Erfelek town (Sinop Province) and pours into the Black Sea (Figure 1). This approximately 80 km long stream provides drinking water to the surrounding settlement areas together with the Erfelek Dam.

Station 3 (A3): This stream, which passes through the middle of a village surrounded by forest, is closely associated with agricultural activities and animal husbandry. The water flow rate at this station shows significant changes throughout the year. The bottom structure of the stream consists of stones, gravel, and sand.

Station 4 (A4): This station has a rich riparian zone and merges with the Karasu Stream. The main source of pollution at this station is the domestic waste discharged from nearby settlement areas.

Distribution

Europe, possibly introduced in North America (Timm, 2009).

Table 1. Penial setae lengths according to literature

References	Region	Length penial chaetae (µm)
Piguet (1906) as *R. lemani*	Lake Geneva	124
Michaelsen (1908) as *Taupodrilus lemani*	Germany	116
Piguet and Bretscher (1913); Piguet (1913)	Switzerland	138
Hrabě (1935)	Bohemia and Moravia	117.6
Čhekanovskaya (1962); Brinkhurst (1963)	Europe	138
Hrabě (1981)	Czech Rep	128-140
Kasprzak (1979, 1981)	Poland	94-148
Timm et al. (1996)	Sweden	100-122
Martin and Boughrous (2012)	Maghreb	~175 (fig no 58C) and ~200um (fig no 58D)
Unpublished measurements (van Haaren, T.)	Netherlands	100-130 (n=7)
This study	Türkiye	(78-)96-117

DISCUSSION

The main distribution area of *R. falciformis* is in Central Europe (van Haaren, 2002) (Figure 6).

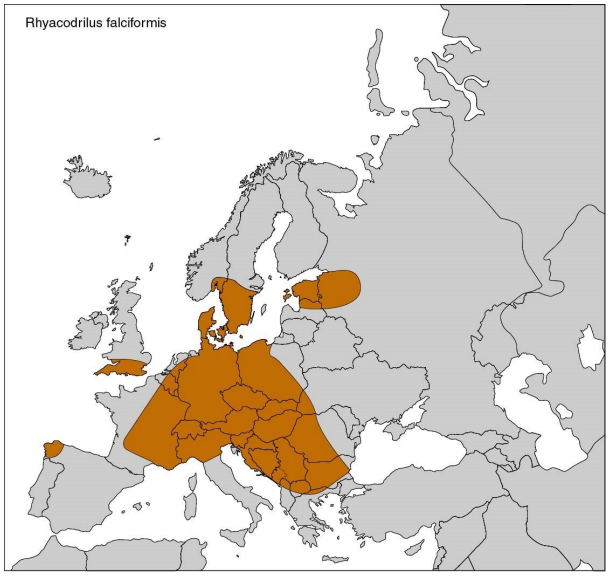

Figure 6. Distribution map of *Rhyacodrilus falciformis* Bretscher, 1901 in Europe (van Haaren, 2002).

R. falciformis Bretscher, 1901, is a rarely encountered groundwater species. This genus was first described in 1900 from materials collected from the Swiss Alps (Bretscher, 1901). Subsequently, it was rediscovered from samples collected at Geneva Lake in France (Juget, 1967) and from the Pieniny Mountains in the southern part of Poland (Kasprzak, 1979). According to van Haaren (2002), the distribution of *R. falciformis* in Europe is as follows:

Records of *R. falciformis* are from the following locations: Holland (Verdonschot et al., 1992), Susaa River in Denmark (Berg, 1948), source of the Fulda River, and Schlei in Germany (Wachs, 1967; von Bülow, 1957), Vättern Lake in Sweden (Brinkhurst and Jamieson, 1971), Geneva Lake in Switzerland (Brinkhurst, 1964; Brinkhurst and Jamieson, 1971), South Dorset in England (Ladle and Bird, 1980), Jantra and Struma rivers in Bulgaria (Uzunov and Kapustina, 1993), Tambre River and Porto do Cabo in the Iberian Peninsula (Martínez-Ansemil and Giani, 1980; Martínez-Ansemil, 1984; Martínez-Ansemil and Collado, 1996), source of the Adige basin in Italy (Di Chiara Paoletti and Sambugar, 1996), Estonia (Timm, 1999), Dyje River in Czechoslovakia (Wolgemuth and Schenkova, 1999), Poland (Dumnicka, 2001), and various locations in France including Annecy Lake, Argens River (l'Eau Salée), Geneva Lake, and the (subterranean) underflow of the upper Rhône, as well as the southern and western foothills of the Carpathian mountains in caves along the Hungarian/Slovakian border (Brinkhurst and Jamieson, 1971; Giani and Martinez-Ansemil, 1981; Juget, 1984; IUCN, 1998; Dumnicka, 2001). There are also records from Scandinavia (Milbrink, 1978) and Norway (Bremnes and Sloreid, 1994) and in Magnesian Limestone Plateau of County Durham, UK (Standen et al., 2009). Recently, Martinsson et al. (2013) described a slightly different new species from France, *R. pigueti* sp. n., and successfully distinguished it from *R. falciformis* using DNA barcoding, based on the shape of the penial chaetae. In *R. falciformis,* the penial chaetae are sickle-shaped, while in the new species, they are straight and somewhat spoon-shaped.

In North America, *R. falciformis* is extremely rare. According to Timm (2009), this species was possibly introduced to North America. It was first recorded from Airport Creek in British Columbia (Brinkhurst, 1978), and since then, it has been documented from Cascade Cave (Vancouver Island), the Hudson River in New York, Fraction Run in Illinois, and Montana (Brinkhurst, 1986; Wetzel, 1992; Kathman and Brinkhurst, 1998). The collection of *R. falciformis* from Mystery Cave expands its range southward and is the second report of this species from a cave in North America (Wetzel and Taylor, 2001).

Apart from the distribution of *R. falciformis* in these continents, Martin and Boughrous (2012) reported that *R. falciformis* has also been collected in the upper courses of the Oued Aïssi River in Algeria by Lounaci (1987).

CONCLUSION

Numerous studies have been conducted on freshwater oligochaeta species in different regions of Türkiye, yet to date; there have been no verified records of the stygophilous species *R. falciformis*. With this study, it has been confirmed that the distribution of *R. falciformis* extends to the Asian part of Türkiye. Ongoing research on the oligochaeta fauna in Türkiye is expected to yield more records regarding the distribution of this species and other oligochaeta species that were previously unrecorded in the country's biodiversity records.

ACKNOWLEDGMENTS AND FUNDING

This study has been financed by Sinop University Scientific Research Council (BAP) (Project no: SÜF-1901-12-12). Authors deeply grateful to them for this support.

AUTHOR'S CONTRIBUTION

Melek Zeybek Yünlü, Eylem Aydemir Çil and Seray Yıldız conceived the idea and developed the experimental protocol. Seray Yıldız and Ton van Haaren have organized the assignments and data related to the species. All authors contributed critically to the draft and gave the final approval for publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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.

REFERENCES

Arslan, N., Timm, T., & Erséus, C. (2007). Aquatic Oligochaeta (Annelida) of Balıkdamı wetland (Turkey), with description of two new species of Phallodrilinae. *Biologia, 62*(3), 323-334. https://doi.org/10.2478/s11756-007-0055-y

Arslan, N., & İlhan, S. (2010). Distribution and abundance of oligochaeta (Annelida) species and environmental variables of Porsuk Stream (Sakarya River, Turkey). *Review of Hydrobiology*, *3*(1), 51-63.

Balık, S., Ustaoğlu, M.R., Özbek, M., Taşdemir, A., & Yıldız, S. (2004). Benthic fauna of Buldan Reservoir (Denizli, Turkey) (in Turkish with English abstract). *Ege Journal of Fisheries and Aquatic Sciences, 21*(1-2), 139-141.

Berg, K. (1948). Biological studies on the river Susaa. *Folia Limnologica Scandinavica*, *4*, 1-54.

Bremnes, T., & Sloreid, S.E. (1994). Oligochaetes of freshwater. Distribution in south Norway. *NINA Utredning*, *56*, 1-42.

Bretscher, K. (1901). Beobachtungen über Oligochaeten den Schweiz (French). *Revue Suisse de Zoologie*, *9*, 189-223. https://doi.org/10.5962/bhl.part.82517

Brinkhurst, R.O. (1963). Taxonomical studies on the Tubificidae (Annelida, Oligochaeta). *Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie Systematische Beihefte*, *2*, 1-89.

Brinkhurst, R.O. (1964). Observations on the biology of lake dwelling Tubificidae. *Archiv für Hydrobiologie, 60*, 385-418.

Brinkhurst, R.O. (1978). Freshwater oligochaeta in Canada. *Canadian Journal of Zoology, 56*, 2166-2175. https://doi.org/10.1139/z78-292

Brinkhurst, R.O. (1986). *Guide to the fresh water aquatic microdrile oligochaetes of North America.* Ottawa, Canadian Special Publication of Fisheries and Aquatic Sciences.

Brinkhurst, R.O., & Jamieson, B.G.M. (1971). *Aquatic oligochaeta of the world*. Ontario, University of Toronto Press. https://doi.org/10.4319/lo.1972.17.1.0166a

Čekanovskaya, O.V. (1962). *Vodnye malostetinkovye cervi fauny SSSR. Opredeliteli po faune SSSR*. Mocsow, Izdatelstvo Akademii Nauk SSSR.

Çamur-Elipek, B., Arslan, N., Kirgiz, T., & Oterler, B. (2006). Benthic macrofauna in Tunca River (Turkey) and their relationships with environmental variables. *Acta Hydrochimica et Hydrobiologica, 34*, 360-366. https://doi.org/10.1002/aheh.200500631

Çil, E.A., Özbek, M., Yardım, Ö., Yıldız, S., Taşdemir, A., Rasouli, H., & Gürbüzer, P. (2021). Diversity of benthic macroinvertebrates and water quality of Karasu Stream (Black Sea). *Ege Journal of Fisheries and Aquatic Sciences*, *38*(4), 467-477. https://doi.org/10.12714/egejfas.38.4.08

Di Chiara Paoletti, A., & Sambugar, B. (1996). Aquatic oligochaeta in Italy, with special reference to Naididae. *Hydrobiologia, 334*, 37-49. https://doi.org/10.1007/BF00017352

Dumnicka, E. (2001). *Distribution of Oligochaeta in the surface and underground water on the example of karstic area in the Sudety Mts (south-western Poland)*. Slawkowska, 38. Karol Starmach Institute of Freshwater Biology Polish Academy of Sciences.

Giani, N., & Martinez-Ansemil, E. (1981). The aquatic oligochaetes of the Argens basin (Var, France). *Annales De Limnologie, 17*, 121-141. https://doi.org/10.1051/limn/1981017

Hrabě, S. (1935). Uber *Moraviodrilus pygmaeus* n. g. n. sp., *Rhyacodrilus falciformis* Br., *Ilyodrilus bavaricus* Oschm. und *Bothrioneurum vejdovskyanum* St. *Spisy vyddavane Prirodovedeckou Fakultou Masarykovy Univversity, Brno*. *209*, 1-19.

Hrabě, S. (1981). Aquatic oligochaeta of Czechoslovakia. *Biologia,* 1-168.

IUCN, International Union for Conservation of Nature and Natural Resources. (1998). *Potentia Natural World Heritage Sites in Europe.* https://portals.iucn.org/library/sites/library/files/documents/Rep-1998-075.pdf

Juget, J. (1967). Some new data on the oligochaeta of lake leman: composition and origin of the population. *Annales De Limnologie*, *3*, 217-229. https://doi.org/10.1051/limn/1967019

Juget, J. (1984). Oligochaeta of the epigean and under- ground fauna of the alluvial plain of the French upper Rhône (biotypological trial). *Hydrobiologia*, *115*, 175-182. https://doi.org/10.1007/BF00027913

Kasprzak, K. (1979). Oligochaetes (Oligochaeta) of the Pieniny Mountains. II. Naididae, Tubificidae, Haplotaxidae, Lumbriculidae, Branchiobdellidae. *Fragmenta Faunistica, 24*, 57-80. https://doi.org/10.3161/00159301FF1979.24.2.057

Kasprzak, K. (1981). Enchytraeids (Oligochaeta, Enchytraeidae) of Warsaw and Mazovia. *Memorabilia Zoologica*, *34*, 59-67.

Kathman, R.D., & Brinkhurst, R.O. (1998). *Guide to the freshwater oligochaetes of North America*. Tennessee, College Grove Aquatic Resources Center.

Kökmen, S., Arslan, N., Filik, C., & Yılmaz, V. (2007). Zoobenthos of Lake Uluabat, a Ramsar site in Turkey and their relationship with environmental variables. *Clean*, *35*(3), 266-274. https://doi.org/10.1002/clen.200700006

Ladle, M., & Bird, G.J. (1980). Aquatic Oligochaeta of Southern England. In R.O. Brinkhurst, & D.G. Cook (Eds.), *Aquatic Oligochaete Biology* (pp. 165-174). Boston: Springer. https://doi.org/10.1007/978-1-4613-3048-6_9

Letovsky, E., Myers, J.E., Canepa, A., & McCabe, D.J. (2012). Differences between kick sampling techniques and short-term Hester-Dendy sampling for stream macroinvertebrates. *Bios*, *83*(2), 47-55. https://doi.org/10.1893/0005-3155-83.2.47

Lounaci, A. (1987). *Recherches hydrobiology quessur les peuplementsd' invertébrés benthiques du bassin de l'ouedAïssi (Grande–Kabylie)* (in French). Thèse de Magister, Université des Sciences et de la Technologie Houari Boumediene, Alger.

Martin, P., Martínez-Ansemil, E., Pinder, A., Timm, T., & Wetzel, M.J. (2008). Global diversity of Oligochaetous clitellates ("Oligochaeta"; Clitellata) in freshwater. *Hydrobiologia, 595*, 117-127. https://doi.org/10.1007/s10750-007-9009-1

Martin, P., & Boughrous, A. (2012). *Guide taxonomique des oligochètes dulçaquicoles du Maghreb* (in French with English abstract). Brussels, Royal Belgian Institute of Natural Sciences.

Martin, P., Reynolds, J., & Van Haaren, T. (2023). *World List of Marine Oligochaeta. Rhyacodrilinae Hrabe, 1963*. https://marinespecies.org/aphia.php?p=taxdetails&id=137343

Martínez-Ansemil, E. (1984). Freshwater oligochaetes of Galicia: Catalogue and several ecological aspects. *Limnética*, *1*, 311-320.

Martínez-Ansemil, E., & Giani, N. (1980). First records of aquatic oligochaetes from the Iberian Peninsula. *Annales De Limnologie, 16*, 43-54. https://doi.org/10.1051/limn/1980007

Martínez-Ansemil, E., & Collado, R. (1996). Distribution patterns of aquatic oligochaetes inhabiting water-courses in the northwestern Iberian Peninsula. *Hydrobiologia, 334*, 73-83. https://doi.org/10.1007/BF00017355

Martinsson, S., Achurra, A., Svensson, M., & Erseus, C. (2013). Integrative taxonomy of the freshwater worm *Rhyacodrilus falciformis* (Clitellata: Naididae), with the description of a new species. *Zoologica Scripta*, *42*(6), 612-622. https://doi.org/10.1111/zsc.12032

Milbrink, G. (1978). Indicator communities of oligochaetes in Scandinavian lakes. *Internationale Vereinigung für theoretische und angewandte Limnologie, Verhandlungen*, *20*(4), 2406-2411.

Michaelsen, W. (1908). Die Oligochäten Westindiens (in Deutsch). *Zoologische Jahrbücher / Abteilung für Systematik, Geographie und Biologie der Tiere,* (Supplement) *11*, 13-32.

Odabaşı, S., Arslan, N., & Cirik, S. (2017). A new Rhyacodrilin (Oligochaeta) record (*Bothrioneurum vejdovskyanum* Štolc, 1886) for Turkey. *Süleyman Demirel University Journal of Eğirdir Fisheries Faculty*, *13*(2), 179-185. https://doi.org/10.22392/egirdir.293118

Odabaşı, S., Cirik, S., & Arslan, N, (2018). Aquatic oligochaeta (Annelida: Clitellata) assemblages in the streams of Biga Peninsula (Marmara-Turkey) and their seasonal variations. *Comu Journal of Marine Science and Fisheries*, *1*(2), 72-81.

Ohtaka, A. (1995). A new species of the genus *Rhyacodrilus* Bretscher 1901 (Oligochaeta, Tubificidae) from Japanese lakes. *Zoological Science*, *12*(4), 491- 498. https://doi.org/10.2108/zsj.12.491

Öntürk, T., & Arslan, N. (2003). A preliminary study for the determination on the Oligochaeta and Chironomidae fauna of Gümüş Stream (Mardin–Kızıltepe). *Book of Fisheries and Aquatic Science Symposium* 2003 (pp. 82-86). Elazığ, Turkey: Proceedings Book.

Piguet, E. (1906). Oligochetes de la Suisse francais. *Revue suisse de Zoologie, 14*, 389-398. https://doi.org/10.5962/bhl.part.82527

Piguet, E. (1913). Notes sur les Oligochetcs (in French). *Revue suisse de Zoologie*, *21*, 111-146. https://doi.org/10.5962/bhl.part.37153

Piguet, E., & Bretscher, K. (1913). *Catalogue des Invertébrés de la Suisse Oligochètes* (in French). Geneve: Muséumd'histoire naturelle de Genève.

Standen, V., Graefe, U., Schmelz, R. M., & Schlaghamerský, J. (2009). Diversity of Enchytraeidae (Annelida: Clitellata) in habitats within the Magnesian Limestone Plateau of County Durham, U.K. *Soil Organisms*, *81*(2), 137-143.

Şahin, Y., & Baysal, A. (1972). *Benthic fauna of Lake Hazar and its distribution*. İstanbul, Publications of the Hydrobiological Research Institute Faculty of Science University of İstanbul.

Taş, M., Çamur-Elipek, B., Kırgız, T., Arslan, N., & Yıldız, S. (2012). The aquatic and semi-aquatic Oligochaeta fauna of Turkey Thrace. *Journal of Fisheriessciences.com, 6*(1), 26-31. https://doi.org/10.3153/jfscom.2012004

Timm, T. (1999). *A Guide to the Estonian Annelida*. *Naturalist's Handbooks 1*. Tartu - Tallinn, Estonian Academy Publishers.

Timm, T. (2009). A guide to the freshwater Oligochaeta and Polychaeta of Northern and Central Europe. *Lauterbornia, 66*, 1-235.

Timm, T., Erséus, C., & Lundberg, S. (1996). New and unusual records of freshwater Oligochaeta from the Scandinavian peninsula. *Nordic Journal of Freshwater Research, 72*, 15-29.

Uzunov, Y., & Kapustina, L. (1993). Current review on Oligochaeta from macrozoobenthic communities of the Bulgarian rivers. *Lauterbornia*, *13*, 73-83.

van Haaren, T. (2002). Eight species of aquatic Oligochaeta new for the Netherlands (Annelida). *Nederlandse Faunistische Mededelingen, 16*, 39-55.

Verdonschot, P.F.M., Higler, L.W.G., van der Hoek, W.F., & Cuppen, J.G.M. (1992). A list of macroinvertebrates in dutch water types: a first step towards an ecological classification of surface waters based on key factors. *Hydrobiological Journal, 25*, 241-259. https://doi.org/10.1007/BF02270810

von Bülow, T. (1957). Systematisch-autökologische Studienaneulitoralen Oligochaeten der Kimbrischen Halbinsel (in Deutsch). *Kieler Meeresforschungen, 13*, 69-116.

Wachs, B. (1967). Die Oligochaeten Fauna der Fliessgewasser under besonderer Berticksichtigung der Beziehungen zwischen der Tubificiden-besiedlung und dem Substrat (in Deutsch). *Archiv für Hydrobiologie*, 63, 310-386.

Wetzel, M.J. (1992). Aquatic Annelida of Illinois: Intro- duction and checklist of species. *Transactions of the Illinois State Academy of Science, 85*(1-2), 87-101.

Wetzel, M.J., & Taylor, S.J. (2001). First records of freshwater oligochaetes (Annelida, Clitellata) from caves in Illinois and Missouri, USA. *Journal of Cave and Karst Studies, 63*(3), 99-104.

Wohlgemuth E. & Schenkova J. (1999). Annelida: Aeolosomata, Oligochaeta. In V. Opravilova, J. Vanhara, & I. Sukop (eds). *Aquatic Invertebrates of the Palava Biosphere Reserve of UNESCO. Folia Facultatis scientiarium naturalium Universitatis Masarykianae Brunensis Biologia*, *101*, 89-95.