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Authors Guidelines

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

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

GENERAL INFORMATION

Aim & Scope

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

The journal does not charge any submission and publication fees.

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

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

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

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

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

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

Language

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

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

Editorial Policy and Referee Process

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

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

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

Article Types

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

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

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

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

Reports

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

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

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

MANUSCRIPT SUBMISSION

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

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

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

While starting

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

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

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

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

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

PREPARATION OF MANUSCRIPTS

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

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

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

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

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

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

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

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

First Page

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

Author Names and Affiliation

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

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

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

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

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

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

Abstract

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

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

Keywords

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

Following pages

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

Introduction

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

Material and Methods

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

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

Results

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

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

Conclusions

This should briefly state the major findings of the study.

Acknowledgements and Funding

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

Examples:

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

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

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

Financial support acknowledgwment should be written like the example given:

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

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

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

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

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

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

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

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

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Full name/s: Supervision

Full name/s: Software, Validation

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

Full name/s: Writing- Reviewing and Editing

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

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

Changes to Authorship

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

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

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

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

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

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

Examples:

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

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

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

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

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

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

Retrospective Ethics Approval

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

Data Availability

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

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

Examples:

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

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

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Data availability: All relevant data is in the article.

Scientific Style

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

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

Equations and units

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

Abbreviations

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

Footnotes

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

References

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

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

In-Text Citation

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

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

For example: Kocataş, 1978, p. 3

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

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

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

One author:

Consider the following examples:

-…….(Kocataş, 1978)

- Kocataş (1978) states…….

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

Two authors:

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

Consider the following examples:

-…….(Geldiay and Ergen, 1972)

- Geldiay and Ergen (1972) states……

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

More than two authors:

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

See below examples:

-Geldiay et al. (1971) state…….

-……(Geldiay et al., 1971).

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

Two or more works by different author:

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

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

Two or more works by the same author:

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

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

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

For Example:

-Geldiay and Ergen, 1972a

-Geldiay and Ergen, 1972a, b

No authors:

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

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

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

No publication date:

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

Example: (Geldiay, n.d.).

Citation to secondary sources:

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

For Example:

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

Personal communication and unpublished results:

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

Example:

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

General use of websites and software:

It should be showed as below.

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

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

In References

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

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

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

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

Hanging indent paragraph style should be used.

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

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

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

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

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

For example:

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

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

For example:

Kocataş, A. (1978)

Kocataş, A., & Ergen, Z. (1972).

Kocataş, A., & Geldiay, R. (1972)

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

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

Journal Articles

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

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

Books

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

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

Chapter in books

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

E-books and chapter in e-books

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

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

Proceedings

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

Websites

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

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

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

Thesis

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

Tables and Figures

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Effect of different hydraulic loading rates on growth of basil (*Ocimum basilicum* L. 'Genovese') in nutrient film technique aquaponics

Farklı hidrolik yükleme oranlarının nütrient film tekniği akuaponiklerde fesleğen bitkisi (*Ocimum basilicum* L. 'Genovese') üretimine etkisi

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Abstract: Aquaponics are promising and sustainable technologies consisting of fish-plant-bacteria consortia in the same system, thereby providing an environmentally friendly system by recycling water and nutrients. This study was planned to investigate the influence of varying hydraulic loading rates (HLR) on the growth of basil plant (*Ocimum basilicum* L. 'Genovese') in a low-cost of electricity nutrient film technique aquaponics (NFT) integrated with African catfish (*Clarias gariepinus* (Burchell)) under the Eastern Mediterranean climate conditions, Antalya, Türkiye. The hydraulic loading rates tested in plant-growing troughs 2, 4, 8, and 12 m^3/m^2/day. African catfish showed an excellent feed conversion ratio (0.695) over the experiment. There was no statistically significant difference in plant height, number of leaves, and stem diameter for basil plants, but a statistically significant difference was found in plant weight and leaf area. The best plant weight gain was observed in the 4 m^3/m^2/day group with 23.0±2.5 g mean weight. The optimum HLR for basil production was estimated as 4.41 m^3/m^2/day based on yield (kg/m^2) and energy consumption (KWh/kg basil) in a basil-African catfish integrated NFT aquaponics. The optimum HLR can maximize production without further increase of energy expenditure. Higher HLRs of 4.41 increase energy cost per unit of basil production.

Keywords: Aquaponics, hydraulic loading rate, *Clarias gariepinus, Ocimum basilicum*

INTRODUCTION

The tremendous increase in the human population and migration from rural to urban areas causes depletion of food resources and increase of food prices because of limited phosphorus and nitrogen resources in the earth, a trend particularly the case in developing countries (Wunderlich and Martinez, 2018). According to the United Nations' estimation, the world population will reach 9.6 billion by around 2050 and 75% of this population is going to live in cities (Gerland et al., 2014). The population growth and overcrowding in cities will make sustainable food production inevitable.

Aquaponics is considered a good alternative for environmentally-friendly (Loomis et al., 2014), self-sufficient (El-Essawy et al., 2019), healthy (Sommerville et al., 2014), and sustainable (Tyson et al., 2011) food production. This system has versatile advantages such as easy integration into urban (Kledal, 2012), unoccupied, and unfavorable (Pantanella, 2018) areas. However, it is still a field that requires the expertise of several academic disciplines. It should be well-designed and well-organized in order to be economically feasible (Li et al., 2018).

Being the most common element in the atmosphere of earth (Camargo and Alonso, 2006), nitrogen is an essential nutrient to build the structure of nucleic acids, proteins, adenosine phosphates, pyridine nucleotides, and pigments in all living organisms (Hagopian and Riley, 1998). On the other hand, supplemental nitrogen fertilization in agricultural production poses one of the major environmental problems of the world (Spiertz, 2009). The main nitrogen-dependent pollutants in nature are ammonium, nitrite, and nitrate, generally. Ammonium (NH_4^+) is the most common inorganic form and pollutant of nitrogen in aquaculture systems (Clarkson et al., 1986). Nitrite (NO_2^-) is the intermediate oxidation product of ammonium, and nitrate (NO_3^-) is the last product of the nitrification process (Endut et al., 2014). These nitrogenous pollutants, if discharged to nature uncontrollably, can cause eutrophication in freshwater resources, especially during seasonal changes (Harada and Hiramatsu, 2010). On the other hand, these macromolecules are highly available sources of nutrients for plant species. In aerobic conditions, ammonia-oxidizing bacteria (AOB) convert the ammonium to nitrite whereas nitrite-oxidizing bacteria (NOB) convert nitrite to nitrate (Hu et al., 2015), which are non-toxic fertilizers for plants.

African catfish (*Clarias gariepinus* (Burchell)) is an indigenous fish for Türkiye (Geldiay and Balık, 2007) and is considered a good candidate for aquaponics in subtropical and tropical regions (Van der Waal, 1998; Baßmann et al., 2017) due to its high tolerance for suboptimal water conditions. Basil (*Ocimum basilicum*), which has glossy and oval-shaped leaves, is an annual and commercially important plant which have medicinal (Ahmed et al., 2014), ornamental (Kaurinovic et al., 2011) and cosmetic properties (Nguyen et al., 2010). Interestingly, basil appears to be an advantageous herb to grow in aquaponics due to its better growth performance in aquaponics and hydroponic systems compared with conventional production systems (Rakocy et al., 2003; Roosta, 2014). Basil requires a high amount of water for optimal growth (Meyers, 2003), which may be a reason why it is one of the most preferred plants for growing in aquaponic systems (Love et al., 2015).

Fish feed represents one of the major operating costs (Quagrainie et al., 2018) and the feed is the ultimate source of nutrients required by fish and plants in aquaponic systems (Roosta, 2014). The feeding level of fish is important not only for water quality and fish growth but also for plant growth (Liang and Chien, 2013). The water flow rate, which is important for nutrient distribution in aquaponics, is directly related to the hydraulic loading rate (HLR) in the system. HLR has a significant impact on the effectiveness of oxygen uptake by the plant roots by supporting nitrogenase activity that is produced by nitrogen-fixing bacteria in the root environment (Wittenberg et al., 1974). Low HLR can promote denitrification in an aquaponics environment, which is an unwanted situation for ammonium-oxidizing bacteria (Shete et al., 2016). Optimum HLR in plant beds, on the other hand, promotes nitrate production, which can improve the performance and yield of plants in aquaponics (Yang, 2019; Yep and Zheng, 2019) whereas excessive loading rates can decrease the nutrient uptake by the plants due to less contact time of the root with the nutrient (Shete et al., 2016).

To prevent chlorosis that caused loss of the normal green coloration of leaves, ferrous chelating agents are used in aquaponics (Kotzen and Appelbaum, 2010). FeDTPA, Fe-EDDHA, and Fe-HBED are the most effective and commonly used ferrous chelating agents commonly used in aquaponics (Kasozi et al., 2019; Tetreault et al., 2023).

Research on the determination of optimum HLR for fish and plant growth in aquaponics has attracted significant attention. Although various flow rates showed similar growth of fish (*Cyprinus carpio*) and in an aquaponic system, an HLR of 1.8 m^3/m^2/day was reported as the most effective in terms of growth of water spinach (*Ipomea aquatica*) (Endut et al., 2009). Higher HLRs carry much more macro and micronutrients to the roots in comparison to lower water flow rates (Caron et al., 2002). An HLR of 3.3 m^3/m2/day significantly reduced the negative effects of ammonia for fish and had a positive effect on nutrient flux for plant production in aquaponics (Yang and Kim, 2020a). On the other hand, it is reported that increasing HLR could have caused lower lettuce production and worse FCR values in fish (Dediu et al., 2012). Increasing flow rate from 4 L/min to 6 L/min resulted in higher nutrient uptake of nitrogen, phosphorus, potassium, and magnesium and better plant shoot, root, biomass, and fruit performance in tomatoes maintained in a hydroponic system fed with aquaculture effluent (Khater et al., 2015).

It appears from the literature that there is no one optimum HLR for all plant species maintained in the aquaponic systems. There is a lack of information about the impacts of HLR on basil growth in aquaponic systems. Therefore, the present study was planned to investigate the impacts of different HLRs, without aeration, in NFT aquaponics integrating African catfish with basil on plant growth (g) and yield (kg/m^2).

MATERIALS AND METHODS

System setup

The NFT aquaponics with a total volume of 3.40 m^3 was established at Mediterranean Fisheries Research, Production, and Training Institute (MEDFRI), Antalya - Türkiye. The details of the system setup were given by Yeşiltaş et al. (2021). Briefly, the system consisted of a fish rearing tank (2.5 m^3), two pieces of radial flow separators (80 L apiece), two pieces of bio-filter tanks (150 L apiece), twelve troughs (21.6 L each) and a sump tank (150 L). All tanks in aquaponics including fish tank were made of polyvinyl plastic materials. This study was conducted outdoor under the climate conditions of the Eastern Mediterranean for 42 days during summer between June and July. In this study, glass wool material was preferred as a substrate in troughs, unlike the previous study. Air temperature, humidity and wind speed during the study period were recorded, daily. The water used in the aquaponic system was taken from the main water supply of the MEDFRI aquaculture facility. The water that is lost through evaporation from the system and about 10 L daily loss through the removal of settled solids concentrated in the bottom of the fish rearing tank is reinforced by using an automatic float valve installed at the sump tank supported by the aquarium unit. Water flow rates into the treatment troughs (Figure 1) were controlled by manual valves at each inlet. HLR values were tested in this experiment and HLR of the troughs were calculated using the formula of water flow rate of trough (m^3/day) / surface area of trough m^2. 2, 4, 8, and 12 m^3/m^2/day HLRs hydroponic rafts were set up for comparison with each other.

The previous study's biofilter tanks that had already included nitrification bacteria on the surface of bio-balls were reused in this experiment to utilize for the nitrification process (Yeşiltaş et al., 2021). However, the nitrification process did not work well as desired due to the lack of aeration in the system. High but not toxic to African catfish and plants ammonium concentrations were obtained in different aquaponics units. The wastewater from the fish-rearing tank flowed by gravity into the sequential radial flow separators and then to the bio-filter tanks filled with about 100 L of volume. Nitrified water in the bio-filter tanks was flowed by gravity into the hydroponics unit and then the sump/pump tank. The water was then pumped back to the fish-rearing tank with the aid of a 0.25 kWh water pump all day and night long (Pedrollo, Model Top 1, Italy) (Figure 1).

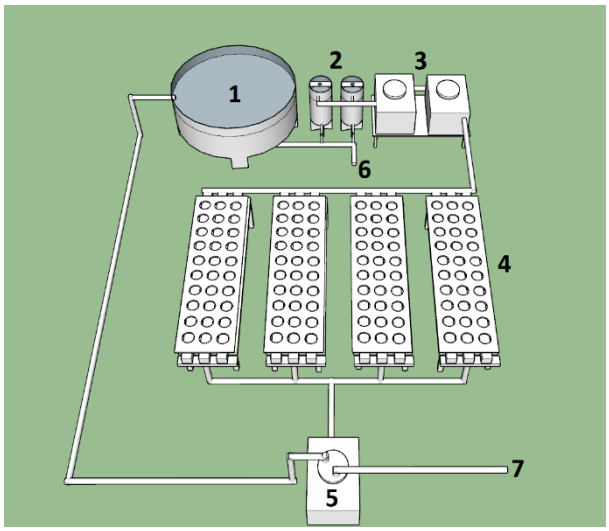

Figure 1. Schematic representation of the NFT aquaponics system used in study 1: Fish tank, 2: Separation tank, 3: Bio-filter tank, 4: Hydroponic troughs, 5: Sump/Pump tank, 6: Solid waste discharge pipe, 7: Freshwater inlet

Fish growth and rearing

African catfish used in the study were obtained from the hatchery of MEDFRI. A total of 128 fish with an average weight of 195.43 g ± 62.91 g were stocked into the fish-rearing tank at the beginning of the experiment. The initial stocking density was set as 10 kg/m^3. Fish were fed with a commercial juvenile common carp diet at predetermined levels. High protein juvenile commercial carp diet (50% crude protein, 8% crude oil) was used during the experiment (Özpekler Su Ürünleri, Denizli, Türkiye). Feed conversion ratio (FCR), specific growth rate (SGR), survival rate (SR), growth rate (GR), and fish biomass increase were calculated at the end of the experiment. FCR was determined using of "total weight of dry feed given/total wet weight gain" formula. SGR was calculated using of "(log final weight - log initial weight) x 100 / culture period (days)" formula. SR was determined by use of "(final fish number/initial fish number) x 100" formula. GR was measured using of "(final total weight – initial total weight) x 100/initial total weight" formula.

Plant growth

Each HLR was tested in triplicated troughs for 42 days of the experiment. 120 basil (*O. basilicum* L. 'Genovese') seeds were sown in seedling trays and germinated within 12-15 days. Basil seedlings were irrigated daily by hand in a greenhouse and transferred from the trays to aquaponics troughs after 22 days. Basils with approximately a mean weight of 0.1 g were planted in twelve troughs with four groups. 60 x 240 cm length styrofoam boards and 13 cm height, 7.5 cm top diameter, and 4.5 cm bottom diameter foam cups were used as plant carriers for the plantation to the hydroponic units which means 20.83 plants per square meter. The water height was settled as nearly 7 cm in the troughs at different HLRs. Thus, an air gap of 6 centimeters was created on the shelves of the hydroponic unit of NFT aquaponics. Synthetic fiber was used to fix the plant roots. At the end of the cultivation period, all plants were removed and measured for length and weight. From each trough, 5 individuals were separated for the determination of leaf areas. The samples were photographed from a vertical view to make measurements of leave areas using the Image J software program (US National Institutes of Health) (Modarelli et al., 2023). Atmospheric parameters that are directly related to plant growth are obtained from the closest weather station.

Physicochemical parameters in water

Changes in physicochemical parameters of water (temperature, salinity, dissolved oxygen, oxygen saturation, and conductivity (EC)) over the study were measured twice a day, at 09:00 and 17:00, in situ by YSI Pro DSS model handheld multi-parameter device from fish and all plant units. The total suspended solid concentration was determined using the gravimetric method of APHA (1985). Ammonium, nitrite, nitrate, phosphate, sulfate, sodium, potassium, calcium, magnesium, and chloride analyses of water from outlets of all units were performed once a week using ion chromatography (Dionex 3000, Sunnyvale, CA). Chelated iron for plants was maintained at about 2 ppm in all tanks by adding Fe-EDDHA (Doctor Tarsa, Antalya, Türkiye) when needed (Yeşiltaş et al. 2021; Wallace-Springer et al., 2022). pH values were followed by a benchtop instrument in the laboratory (Orion 4 Star, Thermo Scientific, USA).

Statistical analysis

The normality of data was tested using the Shapiro-Wilk test. One-way ANOVA followed by a post-hoc Tukey HSD test was performed for data collected from the troughs for the plant measurements except for final length and leaf number, ion compositions (with some exceptions that are tested with nonparametric analysis), and physicochemical parameters. The final plant length and leaf number of treatments were compared with ANCOVA using their initial values as covariates, followed by the Tukey HSD test for detection of significant treatments. However, the Kruskal-Wallis test was performed for ammonium, nitrite, nitrate, phosphate, and sulfate ions (followed by Dunn's multiple comparison test if required) using JMP 13 Software (SAS Institute Inc., Cary, N. C.). A significance level of $P<0.05$ was used and the values were given as mean ± standard deviation. Optimum HLR was estimated based on total yield (kg/m^2) and electricity consumption values (KWh/kg basil) using a three order polynomial regression and a nonlinear broken regression analysis in GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA).

RESULTS

Air quality parameters

Air quality parameters were monitored throughout the study. The average air temperature, average relative humidity, and average wind speed were measured as 27.42±2.82 °C, 46.95±12.35%, and 12.48±3.11 km/h, respectively. Figure 2 shows the general trends of these parameters throughout the experiment. These atmospheric parameters were used to monitor the basil plant needs.

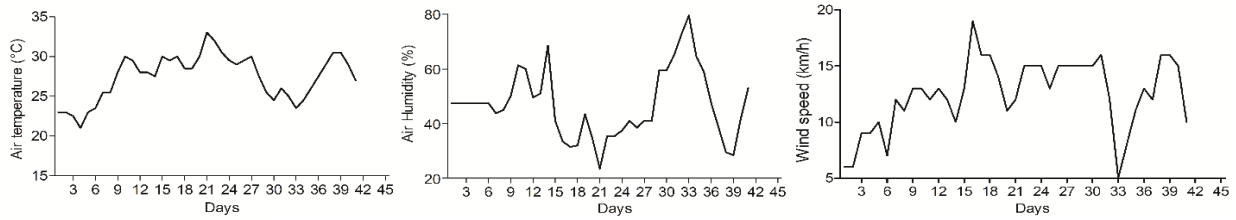

Figure 2. Air temperature, air humidity and wind speed parameters

Water quality parameters

Water temperatures were averaged as 27.19±1.74 (±SD) °C in the fish tank, 27.24±1.79 °C in the separation tank, 27.38±1.70 °C in the bio-filter tank, and 26.84±2.54 °C in the sump/pump tank. pH changed between 7.61±0.17 in the fish tank and 7.82±0.13 in the sump/pump tank. The EC and salinity varied between 1.15±0.24 mS/cm and 0.55±0.10 ppt in the sump/pump tank and 1.26±0.24 mS/cm and 0.60±0.11 ppt in the bio-filter tank. Mean dissolved oxygen concentrations were 1.67±0.74 mg/L, 0.81±0.77 mg/L, 0.27±0.24 mg/L, and 6.14±1.10 mg/L in the fish tank, separation tank, bio-filter tank, and sump/pump tank, respectively. The water physicochemical parameters including temperature, EC, salinity, pH, dissolved oxygen, oxygen saturation, and total suspended solids in fish rearing tank, radial flow separator, bio-filter tank, and sump/pump tank showed no significant differences ($p>0.05$).

The water parameters can be considered favorable for African catfish in the fish tank. However, the dissolved oxygen concentration of the fish tank showed a decreasing trend during the first 9 days, and on the other days of the experiment dissolved oxygen concentration showed a wavy trend to the end of the experiment (Figure 3). Therefore, large quantities of NH_4-N, EC, and salinity concentration changes occurred. Then 30 percent of the fish tank water changed with freshwater three times to sustain the remineralization process of the biofilter over the study. The addition of freshwater to the aquaponics, the reason why oxygen concentrations were decreased, caused limited satisfaction for the biofilter. At the first 3 weeks, extremely high levels of ammonia concentration in aquaponics because the system was not functioning optimally. In the 4th week of the experiment, the biofilter started to convert large amounts of ammonia to nitrite and nitrate. At this stage of the study nitrate levels of different units of aquaponics were at the highest concentrations (Figure 4). Total suspended solids were determined as 0.19±0.08 mg/L in the fish tank, 0.10±0.06 mg/L in the separation tank, 0.16±0.20 mg/L in the bio-filter, and 0.06±0.06 mg/L in sump/pump tanks (Figure 3). Total suspended solids showed a meaningful relationship with the dissolved oxygen concentrations of the aquaponic system. Average physicochemical parameters in different groups of HLRs have been shown in Table 1.

Table 1. Average physicochemical parameters in hydroponics in different HLRs

HLR	Temperature (°C)	EC (mS/cm)	Salinity (ppt)	Dissolved oxygen (%)	Oxygen concentration (mg/L)
2 (m^3/m^2/day)	27.49±1.70^a	1.27±0.24^a	0.60±0.11^a	3.42±2.89^a	0.29±0.25^a
4 (m^3/m^2/day)	27.45±1.69^a	1.27±0.24^a	0.60±0.11^a	3.42±2.89^a	0.30±0.25^a
8 m^3/m^2/day)	27.42±1.71^a	1.27±0.24^a	0.60±0.11^a	3.42±2.89^a	0.31±0.25^a
12 (m^3/m^2/day)	27.39±1.71^a	1.24±0.23^a	0.60±0.11^a	3.42±2.89^a	0.31±0.25^a

An initial fish of 10 kg/m^3 was almost quadrupled up to 37.3 kg/m^3 at the end of the study. The high stock density appears to have played a major role in low oxygen concentrations in the present study. However, even under these low oxygen concentrations fish performance was very well in terms of SGR and FCR.

There were no statistically significant differences found in mean ammonium concentrations, nitrite-nitrogen concentrations, nitrate-nitrogen concentrations, and phosphate-phosphorus concentrations between different units ($p>0.05$), but some significant differences were detected among the weeks ($p<0.05$) (Figure 4).

By the 4th week of the study, relatively low pH affected the ammonia-oxidized bacteria however, after the 4th week of the experiment when pH reached from 7.5 to 8 ammonia oxidation process accelerated (Figure 3, Figure 4). In the 6th week of the experiment, increasing ammonium nitrogen levels in the system pointed out the poor performance of the biofilter tank again, and the experiment ended. Average nitrite concentrations were determined as 1.03±0.33 mg/L in the fish tank, 1.41±0.66 mg/L in the separation tank, 0.95±0.33 mg/L in the bio-filter tank, and 1.36±0.55 mg/L in the sump/pump tank. Average nitrate concentrations were determined as 13.04±8.19 mg/L in the fish tank, 17.65±12.79 mg/L in the separation tank, 7.37±3.25 mg/L in the bio-filter tank, and 10.65±5.68 mg/L in the sump/pump tank. In the 4th week of the experiment, the lowest ammonium concentrations we had in all units, and the highest nitrate concentrations were detected in the system, the typical indication of completion nitrification process with accompanying decrease in nitrite concentrations.

During the last week of the experiment, 500 L/day water exchange was applied to compensate for low oxygen level in aquaponics tanks which reflected in average ammonium and phosphate concentrations as an increase.

The lack of an aerator affected the biofilter efficiency in a negative way. However, nitrite and nitrate levels in aquaponics units decreased (Figure 4). A disappearance of a significant amount of nitrification bacteria in the system appeared to be the case as a result of the compulsory water changes on the days of the 18th, 27th, and 35th of the experiment. This may also be a reason for the increase in ammonia levels in different units over the last week of the study (Figure 4). The increase in water exchange also resulted in an elevation in calcium, potassium, and phosphate concentrations.

Fish growth parameters

The final mean weight of the fish was determined as 784.26±207.13 g. Over the study period, only 8 fish died resulting in only 6.25% mortality. The SGR of fish was 3.31 %/day and relative weight gain was 3.08% although some water quality problems were experienced during the study. The growth rate of fish was calculated as 273%.

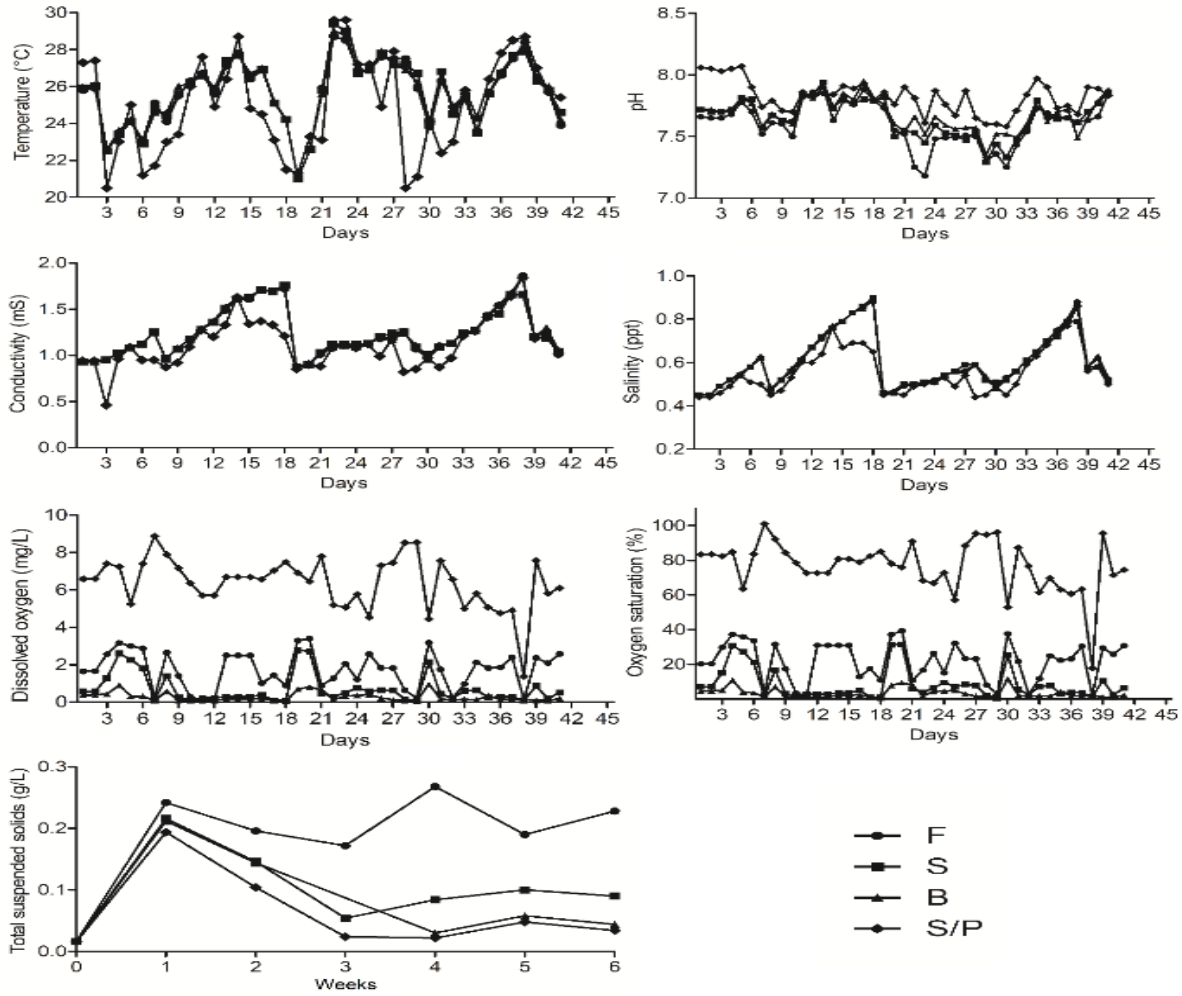

Figure 3. Physicochemical parameters in Aquaponics (F: Fish tank, S: Separation tank, B: Bio-filter tank, S/P: Sump/Pump tank)

Plant growth parameters

The tallest basils were in the group of 12 m^3/m^2/day HLR with an average value of 196.0±35.7 mm whereas the shortest plants were in the HLR group of 2 m^3/m^2/day with an average value of 173.0±48.0 mm. The growth difference between these treatments was statistically insignificant ($p>0.05$). Similarly, the best and worst groups in terms of plant weight were 4 and 2 m^3/m^2/day respectively, which were statistically significant with the values of 23.0±2.5 g and 15.5±1.7 g, respectively ($p<0.05$). There were no statistically significant differences among all groups ($p>0.05$) in terms of average leaf number with the highest value of 44.5±24.0 in the HLR of 4 m^3/m^2/day (Table 2).

Average stem diameters of basils insignificantly changed among the treatments ($p>0.05$) with the maximum and minimum values in 4 and 2 m^3/m^2/day HLR treatments, respectively. The highest average leaf area was detected as 1314±145 mm^2 in the HLR of 4 m^3/m^2/day whereas the lowest in the HLR of 4 m^3/m^2/day with 870±71 mm^2 leaf area ($p<0.05$). The annual plant yield changed between 4.66±0.33 and 7.06±1.66 kg without significant differences in the 8 and 4 m^3/m^2/day HLR groups, respectively ($p>0.05$). The best performing HLR group in terms of plant growth in the present study was 4 m^3/m^2/day, which could be due to better uptake of the nutrients by the plants.

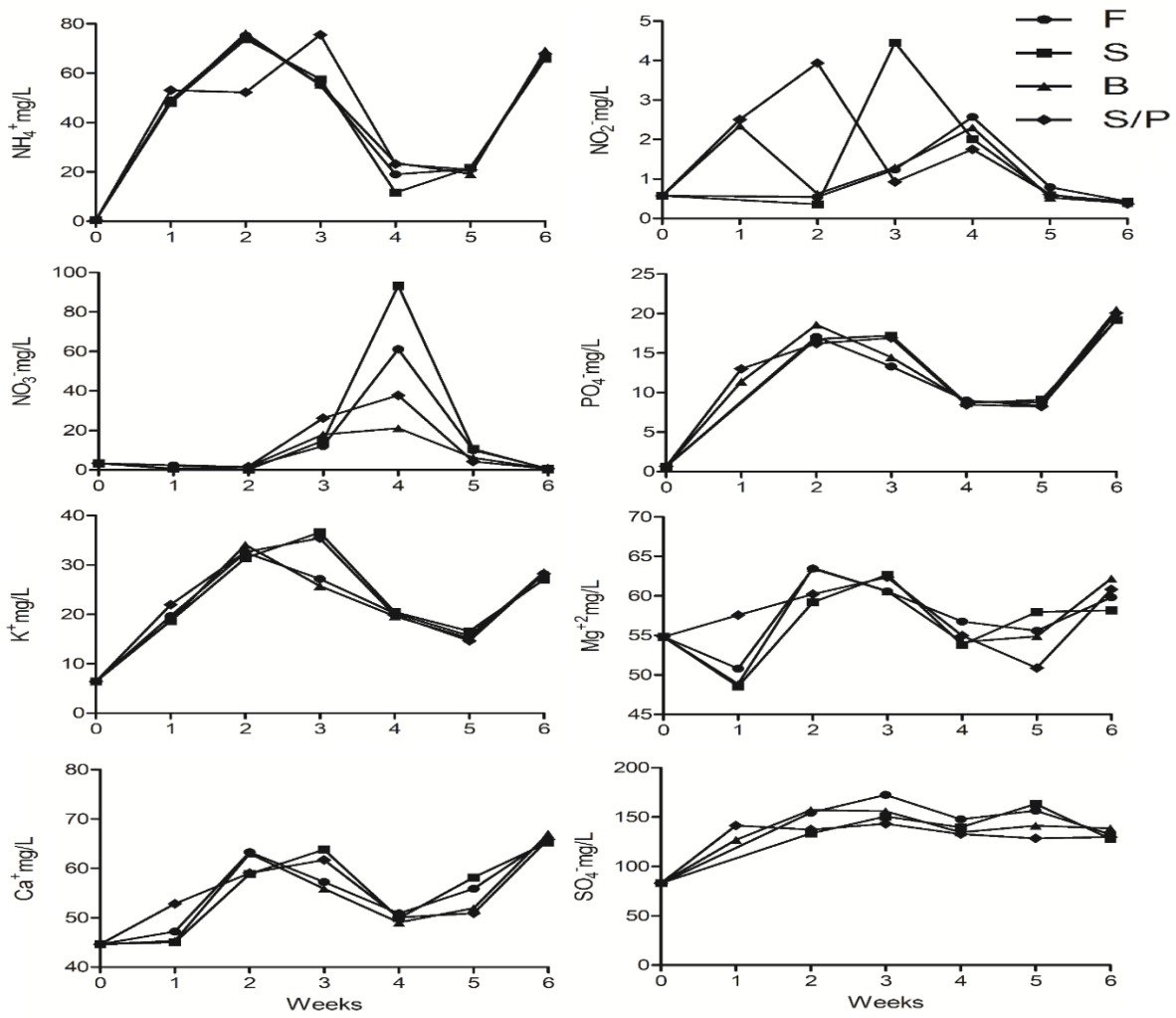

Figure 4. Ion concentrations in Aquaponics (F: Fish tank, S: Separation tank, B: Bio-filter, S/P: Sump/Pump)

Table 2. Average plant growth parameters with standard deviations

HLR	IPL (mm)	FPL (mm)	IPW (g)	FPW (g)	ILN	FLN	ISD (mm)	FSD (mm)	ILA (mm^2)	FLA (mm^2)
2 (m^3/m^2/day)	57.3±6.4^a	173.0±48.0^a	0.1±0.0^a	15.5±1.7^a	3.5±0.9^a	38.0±18.4^a	1.3±0.0^a	5.6±0.3^a	63.7±3.3^a	870±71^a
4 (m^3/m^2/day)	59.2±7.1^a	195.9±60.9^a	0.1±0.0^a	23.0±2.5^b	3.6±0.8^a	44.5±24.0^a	1.2±0.0^a	5.7±0.2^a	59.4±3.7^a	1314±145^b
8 (m^3/m^2/day)	63.2±5.0^a	175.6±32.5^a	0.1±0.0^a	15.7±1.5^{ab}	3.6±0.8^a	36.4±19.6^a	1.2±0.0^a	5.3±0.2^a	59.4±3.0^a	1119±95^{ab}
12 (m^3/m^2/day)	61.6±6.5^a	196.0±35.7^a	0.1±0.0^a	19.9±2.1^{ab}	3.9±0.1^a	40.9±17.8^a	1.2±0.0^a	5.5±0.2^a	62.4±3.7^a	1167±146^{ab}

IPL: Initial plant length, FPL: Final plant length, IPW: Initial plant weight, FPW: Final plant weight, ILN: Initial leaf number, FLN: Final leaf number, ISD: Initial stem diameter, FSD: Final stem diameter, ILA: Initial leaf area, FLA: Final leaf area

Yields of basil ranged as 0.32±0.04 kg/m^2, 0.47±0.14 kg/m^2, 0.33±0.03 kg/m^2, and 0.41±0.13 kg/m^2 in the 2, 4, 8, and 12 m^3/m^2/day HLRs, respectively without significant differences ($p>0.05$). HLR treatments of 2, 4, 8, and 12 m^3/m^2/day consumed average electricity of 20.54±2.02, 30.72±14.0, 79.50±9.48, and 102.9±34.8 kW/kg basil, respectively, with significant differences among the treatments. The third-degree polynomial regression between HLR and total yield (kg/m^2), although not strong ($R^2=0.28$; $p=0.42$), suggested the best hydraulic loading rate as 4.41 m^3/m^2/day (Figure 5). A broken line regression model for energy consumption per kg of basil production generated a breakpoint at 4 m^3/m^2/day (Figure 5), which suggests increasing HLR above 4 m^3/m^2/day will result in excessive energy expenditure for basil production.

Energy consumption of HLRs of 8 and 12 m^3/m^2/day was significantly higher than that of 2 m^3/m^2/day (Figure 6). 4.41 m^3/m^2/day HLR was determined as the best for energy consumption and productivity.

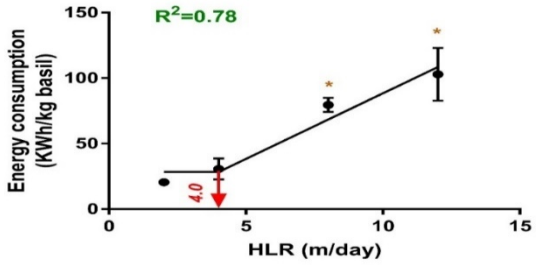

Figure 5. Relationship between HLR and energy consumption per kg of basil production in an aquaponics system (Values with * are significantly different from the treatment of 2 m^3/m^2/day based on Dunnet's test)

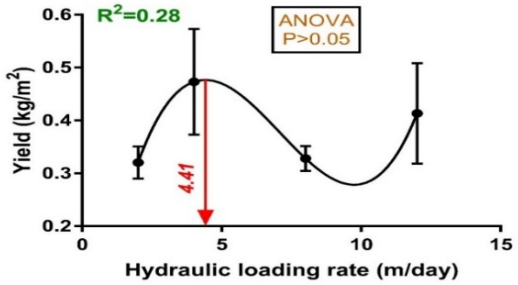

Figure 6. Relationship between HLR and basil yield in an aquaponics system

DISCUSSION

Water quality parameters

It was found that the physical and chemical parameters of the water were relatively adequate for the growth of *O. basilicum* and *C. gariepinus* (Knaus et al., 2020a; Pasch et al., 2021). The hydraulic loading rate is another important factor in the growth of basil plants. But only limited data is available on basil and HLRs in aquaponics production. But dissolved oxygen concentration in aquaponic system caused major difficulties on nitrifying bacteria effectiveness. The water temperature and the lack of aeration in the system caused the low wavy trend of oxygen concentrations in aquaponics. Low oxygen concentrations and pH of water in aquaponics restricted the nitrifying bacteria metabolism to convert ammonia to nitrate, effectively (Tyson et al., 2004). pH in aquaponics was measured as between 7 to 8 which are suitable for nitrification bacteria (Timmons et al., 2002) and African catfish (Endut et. al., 2010). The water quality parameters are consistent except for oxygen concentration and saturation with those reported by Knaus et al. (2020b) for aquaponic systems. Low oxygen is a frequent reason for fish death, but African catfish can practice aerial as well as aquatic respiration (Bovendeur et al., 1987). African catfish was reported to be highly tolerant to low dissolved oxygen as low as 0.5 mg/L (Boyd and Tucker, 1998) and able to grow well even in low oxygen levels (Akinwole and Faturoti, 2007; Ibrahim and Naggar, 2010). Average oxygen saturation levels were found as 21.16±8.39% in the fish tank, 10.03 ± 9.05% in the separation tank, 3.40±2.90% in the bio-filter tank, and 76.26±11.09% in the sump/pump tank.

In water with high levels of ammonia, nitrite, nitrate and urea, African catfish is known to be highly tolerant (Figure 4) (Bakar et al., 2015; Ip et al., 2004). Short-time NH_4-N increase in aquaponics can be acceptable (Zou et al., 2016), however in prolong duration of excess NH_4-N concentration might be toxic for fish (Yang, 2019). 80 mg/L of NH_4-N and 10 mg/L of NO_2-N were determined as upper lethal limits for African catfish in Recirculating Aquaculture Systems (Palm et al., 2018). Aquaponic systems integrated with African catfish including a wide variety of ammonium concentrations such as 0.9-1.8 mg/L by Su et al. (2020), 0.91±0.26 mg/L by Baßmann et al. (2017), and 20.46±6.53 mg/L by Knaus et al. (2020b) were compared to this study. When the concentrations in the units are considered, average ammonium concentrations were measured as 41.04±10.51 mg/L in the fish tank, 39.88±10.78 mg/L in the separation tank, 41.73±10.58 mg/L in the bio-filter tank, and 41.91±10.40 mg/L in the sump/pump tank, being higher than the findings of previous studies. In this study, these nitrogenous compounds' upper limits were not exceeded even without aeration in the system.

Nitrite and nitrate concentrations in the system were compatible with those reported by Oladimeji et al., (2020), and Yang and Kim (2020a). Mean phosphate concentrations were found as 11.21±2.33 mg/L in the fish tank, 11.73±2.45 mg/L in the separation tank, 11.88±2.54 mg/L in the bio-filter tank, and 11.94±2.50 mg/L in the sump/pump tank (Figure 4), being comparable to the results of other studies (Villarroel et al., 2011; Strauch et al., 2019; Yang and Kim, 2020a).

Not only temperature, pH, and hydrogen ions in aquaponics but also biofilter activation affected the ammonium concentrations in the system (Yavuzcan Yildiz et al., 2017). Ammonia levels of aquaponics in all units decreased at the 4th week of the experiment thanks to the activation of ammonia-oxidized bacteria (Su et al., 2020). The poor oxygen concentration related to no aeration in the system affected the used previous aquaponics' biofilter tank in a negative way. Low level of dissolved oxygen in aquaponics caused low pH as well. And pH directly affects the nitrification process in the aquaponics (Tyson et al., 2004). Average magnesium, potassium, calcium, and sulfate concentrations that directly affect plant production were found compatible with the other aquaponics studies integrating basil in aquaponics (Baßmann et al., 2018; Knaus et al., 2020a) (Figure 4).

The main aim of the biofilter tank is to enhance the oxidation of NH_4-N and NO_2-N in the recirculating water of the aquaponics (Kasozi et al., 2021). However, adequate biofiltration could not be achieved well because of the hot-summer Mediterranean climate, the increase in the amount of ammonium in parallel with the increase in fish biomass, and not including aeration in the system. In this study, the main aim was to produce fish and plants together with limited energy consumption in hot climate conditions by trying to keep the costs to a minimum.

Air parameters

The environmental factors, air temperature, relative humidity, and wind speed affected the water temperature of aquaponics directly (Figure 2, Figure 3). These parameters have been found compatible with the recordings of studies by Ghamarnia et al. (2014) and Ferrarezi and Bailey (2019), who maintained the basil in an aquaponics system and soil agriculture in a semi-arid climate. For the basil plant, the air temperature met the optimum temperature requirement throughout the study (Chang et al., 2005; Barickman et al., 2021). Air humidity changed between 20% to 80% throughout the study which is suitable for required for the optimum growth of basil (Solis-Toapanta et al., 2020; Lin et al., 2021). Wind speed (air velocity) parameters recorded between 0-20 km/h that is founded as very low compared to other studies (Cohen and Ben-Naim, 2016).

Fish growth

African catfish is a very sturdy fish with the ability to survive in poor water quality conditions and therefore it has a good survival rate in recirculating aquaculture systems (Akinwole and Faturoti, 2007). 25–30 °C of water temperature is specified as the optimum temperature range for the catfish culture (Putri et al., 2021). Water temperature in the system was within the optimum range for the growth of African catfish (Hogendoorn et al., 1983). Despite various water conditions, the African catfish is highly tolerant. The growth of *C. gariepinus* was satisfactory throughout the study. The feed conversion ratio (FCR) was recorded as 0.695, being consistent with our previous study by Yeşiltaş et al. (2021) and better than those reported for the same species by Endut et al. (2010) with 1.23-1.39, Palm et al. (2014) with 1.00, Baßmann et al. (2017) with 1.02, and Knaus et al. (2020a) with 0.74-0.91. Therefore, the overall growth and nutrient utilization performance of fish in this study are quite acceptable when compared with literate findings (Baßmann et al., 2017; Endut et al. 2010; Palm et al., 2014; Enyidi et al., 2017; Knaus et al., 2020a).

Plant growth

Basil adapted very well to the aquaponic system run under the summer climate conditions of the Eastern Mediterranean. During the experiment, basil seedlings in all rafts grew well and seemed healthy. Lower or higher HLRs than 4 m^3/m2/day seemed to have resulted in lower growth performance in basil in an NFT aquaponics system. Nitrogen is assumed to be the major nutrient in the aquaponics systems that influence plant growth. At low HLRs, the plant growth can be weakened due to the formation of anoxic zones with the low water current and the development of denitrification which leads to N losses from the system (Endut et al., 2010). Conversely, higher HLRs than the optimum can reduce the contact time of nutrients with the plant roots (Shete et al., 2016; Yang and Kim, 2020a), and increase the energy expenditure for each unit of plant production (Yang and Kim, 2020a).

Nutrient concentration and salinity stress in aquaponics affected the yield of the basil. Increasing trend of ammonia nitrogen, nitrite nitrogen, and phosphate phosphorus in the first three weeks and then increasing nitrate concentration in the 4th week of the experiment contributed to the growth of basil plant biomass. Electrical conductivity was not the main factor that is affecting the basil plant biomass, however, anion and cation combinations were playing important roles in obtaining a better yield (Yang and Kim, 2020b). Increasing ammonium concentrations in aquaponics in the first three weeks did not contribute as expected to basil biomass production when this data was compared to other studies (Rakocy et al., 2003). At the end of the study, the total average yield of the basil plant in the aquaponics was calculated as 311.7±54.7 g/m^2, 463.9±154.1 g/m^2, 306.1±30.47 g/m^2, and 413.6±134 g/m^2 in different HLR ratios, 2, 4, 8, and 12 m^3/m^2/day, respectively. These results were much lower than the studies conducted in the deep water technique in the literature (Rakocy et al., 2004; Rodgers et al., 2022).

Ammonium nitrogen concentration was in increasing trend in the first three weeks due to the lack of nitrifying bacteria in the biofilter. One of the other reasons for the high concentration of ammonium in water can be related to the low ammonium intake of basil plants (Nurzyńska-Wierdak et al., 2011). Nitrate concentration in aquaponics reached nearly 100 mg/L and it was higher than Roosta (2014) with a value of 34.6±3.1 mg/L. The maximum nitrate concentrations were in aquaponics, in the sump/pump tank with a range of nearly 20 to 100 mg/L.

Generally, the oxygen concentration in the root zone of plants is considered essential. Thanks to its less sensitivity to oxygen availability in water basil showed a good growth performance at low HLRs (Puccinelli et al., 2021). By using the advantage of NFT aquaponics, plant roots had enough space to respiratory from the air in the rafts. Plant length, weight, stem diameter, leaf area, and leaf number are important growth factors for basil. 27 ± 1 °C average water temperature in all tanks of the study have affected in good way to the leaf areas (Chang et al., 2005). In this study, upper and lower basil production temperatures were not exceeded.

It is difficult to compare these results with the literature findings due to different expressions used for the period per unit of yield. For instance, basil yield was founded as 42 kg/m^2 year by Savidov et al., (2005), 14.91 kg/m^2 year by Ferrerazi and Bailey (2019) and 15.2 kg/ m^2 year by Yang and Kim (2020c). Conversion of yield to a year production in the present study is impossible since the system used was outdoor that was open to seasonal environmental changes. A comparison of the treatments in the present study with the literature from an energy consumption perspective could be more reasonable.

Higher electricity consumption was calculated as 162.10 kWh/kg basil/year by Xie and Rosentrater (2015), who conducted a study on life cycle assessment and techno-economic analysis of tilapia-basil aquaponics.

CONCLUSION

The effectiveness of four different HLRs in an NFT aquaponics system was tested under the summer climate

conditions of the Eastern Mediterranean. African catfish and basil were successfully produced without aeration in very low oxygen concentrations in aquaponics. Energy savings were achieved by not using aeration. The optimum HLR for basil in the aquaponic system was between 4 and 4.41 m^3/m^2/day when the assessment was based on basil yield and energy consumption for basil production. The finding is important in terms of maximization of basil production with acceptable energy expenditure in the aquaponics. African catfish in the system showed excellent growth and feed utilization performance even in low oxygen concentrations. African catfish and basil seem a very good couple for NFT aquaponic systems thanks to their thermo-tolerant characteristics under the Eastern Mediterranean climate conditions. This type of system and HLR may be recommended to those locations that are struggling with drought and limited energy.

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CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Murat Yeşiltaş, Hüseyin Sevgili, Mehmet Ali Turan Koçer, and Edis Koru contributed to project. Murat Yeşiltaş, Mehmet Ali Turan Koçer, and Hüseyin Sevgili contributed to perform experiment. Murat Yeşiltaş and Hüseyin Sevgili contributed to data analyses, interpretation and manuscript writing.

DECLARATION OF INTEREST

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

ETHICS APPROVAL

Ethics approval for this research was obtained through Mediterranean Fisheries Research Production and Training Institute Local Ethics Committee for Animal Experiments (212809), and consent forms were signed by all participants.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Ege Journal of Fisheries and Aquatic Sciences.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Comparison of reproductive performance of Black Sea salmon broodstock (*Salmo labrax* PALLAS, 1814) reaching first sexual maturity at different ages

Farklı yaşlarda ilk cinsel olgunluğa ulaşan Karadeniz somonu (*Salmo labrax* PALLAS, 1814) anaçlarının üreme performansının karşılaştırılması

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Abstract: The aim of this study was to determine the individual first maturation age diversity in hatchery-originated F6 generation of Black Sea salmon (*Salmo labrax*) broodstock created by applying for a selective breeding program. Research was carried out in freshwater ponds and marine net cage systems between 2018-2021. In the study, 136, 87 and 3 individuals from the broodstocks that reached the first sexual maturity at the age of 22, 34 and 46 months were used, respectively. Total egg production, relative egg production, egg diameter and fertilization rates were determined at the first stripping of broodstock that reached sexual maturity at different ages. The first gonadal development controls and stripping studies were carried out in the period of 2018-2019 for 22 months old broodstock, 2019-2020 for 34 months old and 2020-2021 for 46 months old. Total fecundity of 22, 34 and 46 months old broodstocks that were stripped, were calculated at 1108.98±40.73, 3869.02±138.43 and 5899.52±1143.78 egg/kg broodstock, relative fecundity was 3024.87±87.52, 2291.90±89.52 and 1816.00±284.51 egg/kg broodstock, egg diameters were 4.40±0.01, 5.07±0.02 and 5.35±0.09 mm and fertilization rates were determined as 92.21±0.87%, 95.69±1.65% and 89.83±2.77%, respectively. The condition factor values of the rootstocks were determined as 1.05±0.02 (22 months old), 1.09±0.01 (34 months old) and 1.08±0.03 (46 months old). In the broodstock, individuals with a first maturation age of 22 months have predominantly red spots with a white halo around the perimeter, silvery coloration with black spots at 34 months of age, and silvery coloration with black spots at 46 months of age were detected. While enterprises that reared Black Sea salmon in freshwater and have restaurants prefer the red-spotted river ecotype for production, enterprises that produce in marine net cages prefer the black-spotted-silver-colored marine ecotype, whose body coloration is similar to Atlantic salmon.

Keywords: Black Sea salmon, first maturation size, reproductive traits, maturation age

Öz: Bu çalışmada, seçici ıslah programı uygulanarak oluşturulan kuluçkahane kökenli F6 nesil Karadeniz somonu (*Salmo labrax*) anaçlarında bireysel ilk üreme yaşı çeşitliliğinin belirlenmesi amaçlanmıştır. Araştırma, 2018-2021 tarihleri arasında tatlısu havuzlarında ve deniz ağ kafes sistemlerinde yürütülmüştür. Araştırmada, 22, 34 ve 46 aylık yaşta iken ilk cinsel olgunluğa ulaşan anaçlardan136, 87 ve 3 adet birey sırası ile kullanılmıştır. Farklı yaşlarda cinsel olgunluğa ulaşan anaçların ilk sağımlarındaki toplam yumurta verimi, nispi yumurta verimi, yumurta çapı ve döllenme oranları belirlenmiştir. İlk gonad gelişim kontrolleri ve sağım çalışmaları 22 aylık anaçlarda 2018-2019, 34 aylıklarda 2019-2020, 46 aylıklarda ise 2020-2021 döneminde yapılmıştır. Sağımı gerçekleştirilen 22, 34 ve 46 aylık anaçların sırasıyla toplam yumurta verimleri 1108,98±40,73, 3869,02±138,43 ve 5899,52±1143,78 adet/anaç, nispi yumurta verimleri 3024,87±87,52, 2291,90±89,52 ve 1816,00±284,51 adet/kg anaç, yumurta çapları 4,40±0,01, 5,07±0,02 ve 5,35±0,09 mm ve döllenme oranları ise %92,21±0,87, %95,69±1,65 ve %89,83±2,77 olarak hesaplanmıştır. Anaçların kondisyon faktörü değerleri 1,05±0,02 (22 aylık), 1,09±0,01 (34 aylık) ve 1,08±0,03 (46 aylık) olarak saptanmıştır. 22 aylık iken ilk üremeye ulaşan anaçlarda vücut çevresi beyaz haleli kırmızı benekler, 34 aylıklarda siyah benekli gümüşi renklenme, 46 aylıklarda ise tamamen siyah benekli gümüşi renklenme tespit edilmiştir. Tatlı suda Karadeniz somonu yetiştiren ve restoranı olan işletmeler üretim için kırmızı benekli dere ekotipini tercih ederken, denizde ağ kafeslerde üretim yapan işletmeler ise vücut renklenmesi Atlantik somonuna benzeyen siyah benekli-gümüşi renkli deniz ekotipini tercih etmektedir. Karadeniz somonu yetiştiren işletmelerde daha geç yaşta cinsel olgunluğa ulaşan bireylerden damızlık stok oluşturması, büyük boy balık üretiminde hasada kadar birçok avantaj sağlayacaktır.

Anahtar kelimeler: Karadeniz somonu, ilk üreme boyu, üreme özellikleri, cinsel olgunluk yaşı

INTRODUCTION

The aquaculture sector has been in constant search for the introduction of species with high consumer preference into aquaculture production. Salmonids, which were focused on farmed production for conservation and recreational fishing in the early days, are among the species with high commercial potential and consumer appreciation. One of these species is the Black Sea salmon. Today, anadromous lines that reach late sexual maturity are preferred by the private sector, especially

for large size trout production made in marine net cage systems. Although the Black Sea salmon, which is one of the endemic species of the Black Sea, was named differently by some researchers, *Salmo labrax* is still valid today. Slastenenko (1956) and later scientists (Svetovidov, 1984; Lelek, 1980; Solomon, 2000) named the individuals sampled from the rivers in the Caucasia and the Eastern Black Sea, as Black Sea trout and Black Sea salmon to indicate their origin and transition features to the sea. It is known that the species continues to exist in the Black Sea in three ecotypes: sea, river and lake. Svetovidov (1984), Geldiay and Balık (1996) reported that marine ecotype individuals spend most of their lives in the sea, especially the time that includes the feeding period, and they release their eggs by entering the rivers flowing into the Black Sea during the reproduction period. Fish belonging to the salmon genus can easily adapt to aquatic areas with suitable environmental conditions, more than one interspecies form can coexist in the same aquatic area. Some of these can be defined as separate species (Günther, 1866). Several studies have confirmed the coexistence of Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), as well as endemic species (*S. marmoratus, S. letnica, S. ischchan, S. carpio,* and *S. platycephalis*) (Frost et al., 1967; Behnke, 1968; Dorofeeva, 2002).

Çakmak et al. (2019) found that anadromous Black Sea salmon differs from other ecotypes with its large size, egg diameter and yield. In the study on the reproductive yield of anadromous Black Sea salmon broodstock with an average weight of 2439.21±139.28 g raised under culture conditions, the mean egg diameter was measured as 5.28±0.05 mm, and the relative fecundity was calculated as 2159±115 units/kg. Serezli et al. (2010) reported that rainbow trout, Black Sea trout and brook trout had mean hatching weights of 1357.27±406, 532±673.7 and 310.40±85.0 g, mean egg diameters of 4.95±0.2, 4.51±0.67 and 4.49±, respectively. They measured 0.21 and fecundity was calculated as 2180±676, 3558±1307 and 2571±1530 units/kg. Nikandrov and Shindavina (2007) reported that Black Sea salmon is one of the species with the largest size among migratory salmonids. In the literature, several size values have been reported for the species. Kocabaş (2009) reported that Black Sea salmon spend most of their life in the sea and can grow up to 100 cm in length and 26 kg in weight. Barach (1962) reported that a female Black Sea salmon sampled in the Kodori River was 16.7 kg and 116 cm. Similarly, Solomon (2000) reported that the largest Black Sea salmon individual obtained by fishing operations was 16 kg weight, in Batumi fish market. Kottelat and Freyhof (2007) found size of the largest Black Sea salmon as 80 cm. Çakmak et al. (2022) reported that the largest Black Sea salmon caught to date in Türkiye was 98 cm long, weighed 16.5 kg, and had an obvious marine ecotype (anadromic) character.

Adaptation of the Black Sea salmon to the culture conditions in Türkiye was achieved with the studies started in 1998. Since 2007, aquaculture operations of the species started to become more common practice in the country, especially in the Eastern Black Sea Region (Çakmak et al. 2011). In this study, F6 broodstock which has mainly anadromous individuals with high adaptation capability, was formed with a selective breeding program. This study aimed to determine the reproductive yield of 6th generation farmed broodstock of Black Sea salmon that reach their first sexual maturity at the age of 22, 34 and 46 months.

MATERIALS AND METHODS

Broodstock

In the study, hatchery originated F6 generation Black Sea salmon broodstock was used. The total number of broodstock in the F6 generation stock is 850, which is equally distributed among male and female individuals. Amongst them, 226 female and 83 male individuals were used for this study.

In this stock, 136 (32.53±0.28 cm, 364.67±8.34 g), 87 (50.45±0.42 cm, 1425.68±37.80 g) and 3 (62.6±1.10 cm, 2650.35±18.45 g) individuals reached their first sexual maturity at 22, 34 and 46 months respectively, and they were used to get reproductive data that evaluated in this study (Table 1).

Commercial trout feed with 10% moisture, 45% protein, 20% lipid, 10% ash, 3% crude fiber and 4801 Kcal/kg energy content was used in feeding. Feeding was carried out twice a day from June until one month before stripping, and once a day for the last month up to apparent satiation. All broodstock were marked with electronic markers (12 mm, 134 KHz) for individual monitoring of reproductive efficiency (Figure 1). In the study, 83 males from F6 generation Black Sea salmon broodstock of hatchery origin with an average length of 49.67±18.20 cm and a weight of 1478.58±947.32 g were used.

Broodstock rearing

The rearing of the broodstock was carried out at the Gürpınar aquaculture facility in Trabzon Province and marine cage research unit which has 17‰ salinity of the Central Fisheries Research Institute (SUMAE) in Yomra shore (Figure 2). Water temperatures were measured daily in both marine cages and freshwater units. The broodstock was transferred to the freshwater unit in June when the Black Sea water started to warm (18°C), and they were transferred again to the marine cages in February after stripping. At the freshwater units, the water change was adjusted to be 18-20 times/day. Stocking density in both freshwater ponds and marine cages was 15 kg/m³. The fish after stripping was brought to the marine cage unit with a continuous oxygen-assisted transport tank containing half fresh water and half salt water on the second day after stripping. It ensured that the water was gradually replaced with salt water in the same tank for two hours. Afterward, the broodstock was transferred to the marine cages. In the transfer from saltwater to freshwater, this procedure was reversed, the broodstock was not fed for two days before the transfer time.

Figure 1. Individual markers applied to broodstock (a: Tag reader, b: tag injector and tags, c: application of the tag to the muscle tissue, d: the view of the tag in the muscle tissue)

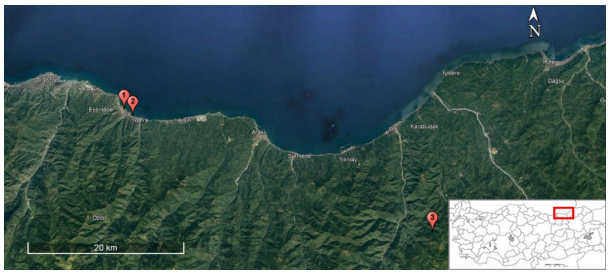

Figure 2. The sites where the broodstock was kept (**1**: SUMAE, **2**: Marine cage research unit (40°57'35,05"N, 40°19'17,44"E, Altitude:0m), **3**: Freshwater unit (40°49'20,59"N, 40°19'17,44"E, Altitude:644 m)

Reproductive controls and stripping

Controls for gonadal development of the broodstock were made 15 days in advance, taking into account the time of the previous stripping start. In all breeding season, stripping starts mostly after the second week of October. Maturity controls were performed every two weeks and continued until all broodstock were stripped. Male and female individuals, during stripping controls, were placed into tanks kept in the hatchery unit separately according to their gender. In stripping, benzocaine (Oswald, 1978) solution was used with an application dose of 50 ppm. Dry stripping method was applied. Firstly, at least 3 male individuals were stripped and sperm stock was created. The sperm stock was used to fertilize the eggs of 5 female fish. 5 minutes after fertilization, water was added until 3 cm above the eggs and until the stripping container was full after 15 minutes. In order to prevent the eggs from being without oxygen, water was added and the eggs were mixed. This treatment continued until the eggs hardened. Approximately 25 minutes later, hardened eggs were washed with hatchery water to remove residues and prepared for incubation. Seperate incubation pans were used for each broodstock's eggs and these pans were labeled with the tag number of broodstock. The incubation period of Black Sea salmon is 60 days on average (600 days/degrees) until the first feed intake, and the first feed intake date is accepted as zero in the age calculation.

Measurements and calculations

A length scale with ±1 cm precision was used for the total length measurements of the broodstock, and ±0.01 g precision scales were used for weight measurements. For the average egg diameter, 20 eggs from each broodstock were measured in a Von Bayer (1910) vessel and the mean value was calculated by dividing the number of eggs. The same eggs were weighed with a balance with a precision of 0.001 g, and the total weight of 20 eggs was found, and the weight of one egg was calculated by dividing the number by the number of eggs.

Fecundity was determined by gravimetric method (MacGregor, 1957) as total fecundity (number of eggs per broodstock) and relative fecundity (number of eggs per kg body weight). Eggs were placed in cabinet incubators with vertical flow filled with spring water using separate pans for each broodstock. One day after fertilization, the white and opaque eggs were considered unfertilized or dead and they were discarded after counting. Fertilization rate was determined by calculating the ratio of the number of unfertilized eggs to the total number of eggs. Besides, the relationship between total and relative fecundity weight was examined.

Statistical analysis

The results were analyzed with one-way ANOVA test via SPSS 14 statistical analysis program. Duncan's multiple comparison test was applied for the difference between groups. Differences were evaluated at the 5% significance level ($P<0.05$). Relationship between total and relative fecundity-weight was performed using Statistica 10 software.

RESULTS

The average water temperature of the marine net cage unit was measured at 11.27±3.06°C (min: 8.6°C, max: 20.7°C), while average water temperature was 11.91±4.28°C (min: 4.0°C, max: 18.5°C) (Figure 3).

According to the results, mean length and weights of 22, 34 and 46 months old broodstock were measured as 32.53±0.28 cm, 364.67±8.34g, 50.45±0.42 cm in length, and, 1425.68±37.80 g and 62.6±1.10 cm, 2650.35±18.45 g in weight, respectively. The proportional increase in the average length and weight of broodstock depending on the increase in first maturation age was found to be statistically significant (Table 1) ($P<0.05$).

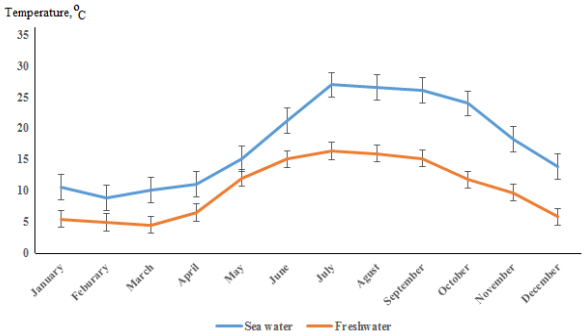

Figure 3. Water temperatures of marine net cage research unit and freshwater unit

Table 1. Reproductive data of broodstocks reaching first sexual maturity at 22, 34 and 46 months

Parameters	22 Months Old (n:136)	34 Months Old (n:87)	46 Months Old (n:3)
Length (cm)	32.53±0.28^{c}	50.45±0.42^{b}	62.6±1.10^{a}
Weight after stripping (g)	364.67±8.34^{c}	1425.68±37.80^{b}	2650.35±18.45^{a}
Condition factor	1.05±0.02	1.09±0.01	1.08±0.03
Total egg weight (g)	58.13±1.96^{c}	321.13±12.38^{b}	571.35±127.39^{a}
Egg weight (g)	0.05±0.01^{c}	0.08±0.02^{b}	0.09±0.09^{a}
Egg diameter (mm)	4.40±0.01^{c}	5.07±0.02^{b}	5.35±0.09^{a}
Egg number (egg/broodstock)	1108.98±40.73^{c}	3869.02±138.43^{b}	5899.52±1143.78^{a}
Fecundity (egg/kg)	3024.87±87.52^{a}	2291.90±89.52^{ab}	1816.00±284.51^{b}
Fertilization rate (%)	92.21±0.87	95.69±1.65	89.83±2.77

The stripping data was recorded between 2018-2021, and the reproduction was started in the second week of November and continued until the end of December. 31.62% of the broodstock reaching the first sexual maturity at the age of 22 months were stripped in November and 68.38% of them were stripped in December. Likewise, 63.22% of the broodstock reaching the first sexual maturity at the age of 34 months were stripped in November and 36.78% of those were stripped in December. However, all of the broodstock that reached the first sexual maturity at the age of 46 months were stripped in December. While the stripping of 22 and 34 months groups were continued for two months, the stripping period of the 46 months group was conducted within one month in December (Figure 4).

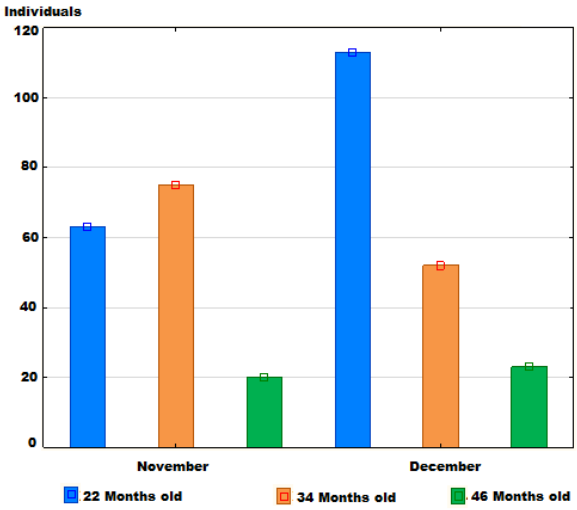

Figure 4. Number of broodstock reaching sexual maturity at different ages during the reproductive period

The total fecundity of broodstock that reached the first sexual maturity at 22, 34 and 46 months, were found as 1108.98±40.73, 3869.02±138.43 and 5899.52±1143.78 eggs/broodstock, respectively. Moreover, their relative fecundities were 3024. 87±87.52, 2291.90±89.52 and 1816.00±284.51 eggs/kg. It was observed that the increase in total fecundity in direct proportion to the first reproduction age of the fish was statistically significant. In the evaluation of the relative fecundity between stocks, it was found that relative fecundity decreased when the sexual maturation was late from 22 to 46 months (Table 1).

In addition, it was observed that the relative fecundity decreased inversely with fish weight in 34 and 46 months stocks, but this situation was statistically insignificant in 22 months old stock. This is thought to be due to the fact that this stock reaches sexual maturity at an early age and therefore in small size compared to other stocks (Table 1, Figure 5, 6, 7) ($P<0.05$).

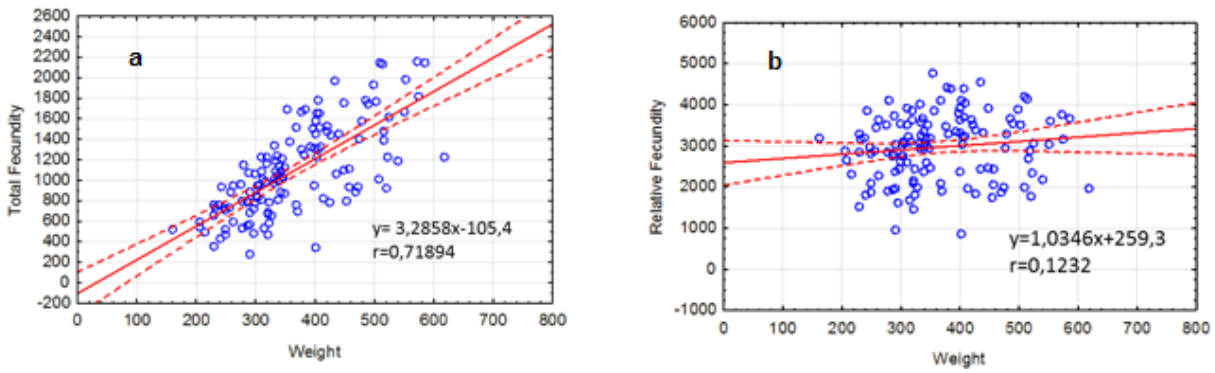

Figure 5. Relationship between total (a) and relative fecundity-weight (b) of stock reaching first sexual maturity at 22 months of age

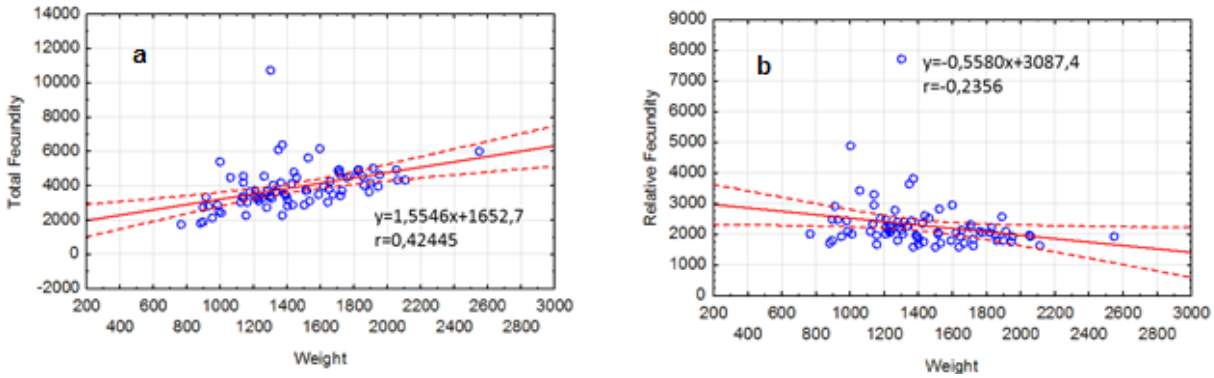

Figure 6. Relationship between total (a) and relative fecundity-weight (b) of stock reaching first sexual maturity at 34 months of age

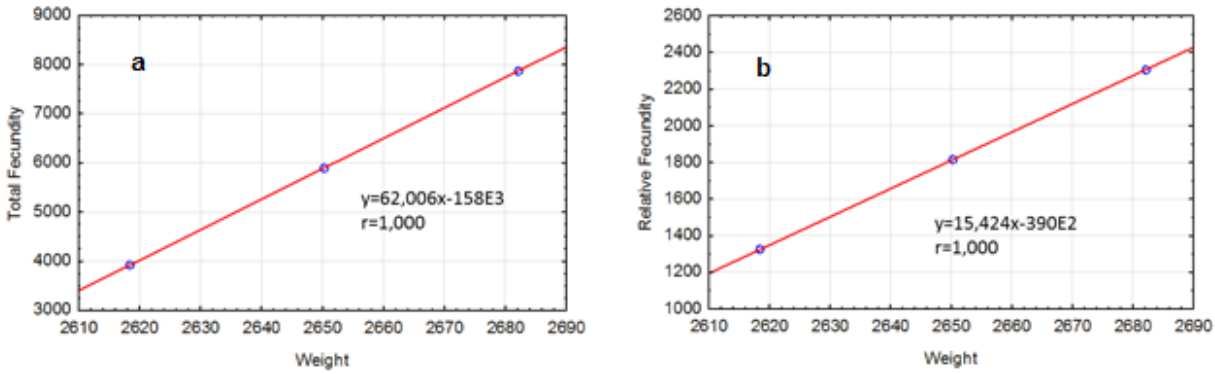

Figure 7. Relationship between total (a) and relative fecundity-weight (b) of stock reaching first sexual maturity at 46 months of age

Avarage egg diameters and weights of broodstock reaching the first sexual maturity at 22, 34 and 46 months were measured as 4.40±0.01cm-0.05±0.01g, 5.07±0.02cm-0.08±0.02g and 5.35±0.09cm-0.09±0.09g respectively. Egg diameters and weights increased in accordance with the increasing in the fish size. The difference in egg diameter and weight gain depending on fish size between stocks were statistically significant. The mean fertilization rates of broodstock that reached the first sexual maturity at 22, 34 and 46 months were calculated as 92.21±0.87%, 95.69±1.65% and 89.83±2.77, respectively (Table 1).

It has been observed that individuals reaching the first sexual maturity at the age of 22 months have red spots covered with a white halo, individuals reaching the first sexual maturity at the age of 34 months have red spots and silvery body color, while individuals reaching the first sexual maturity at the age of 46 months have black spots and silvery body color.

DISCUSSION

Several studies have been conducted regards with spawning season, natural behavior and culture characteristics of brown trout. Also, several key studies (Needham, 1945; Horton, 1961; Thomas, 1964; Moyle, 1976) have reported that brown trout give offspring within 3 months period between October and December in the northern hemisphere, similar to our findings. In contrast, in the southern hemisphere, reproduction occurs between the end of May and July (Hopkins, 1970; MacDowall, 1978).

Tabak et al. (2001) found that Black Sea salmon broodstock reproduce mainly in November and rarely until mid-December in their natural environment on the Turkish coasts. Similarly, Çakmak et al. (2022) found that the cultured Black Sea salmon started to spawn in November under culture conditions, peaking in December and finishing until late February. Salihoğlu et al. (2013) reported that this species gave offspring between the last quarter of December and the last quarter of February. In the southern hemisphere, Estay et al., (2004) determined that brown trout start to give offspring in Chile in June, reaching the highest point in July, and continues until September. Makhrov et al. (2011) revealed that the maturation and spawning times of Black Sea salmon reared in aquaculture units and natural waterways in the Northwest Caucasia show significant differences depending on environmental conditions just as temperature, mostly. It is clear fact that environmental conditions influence the reproduction period.

In this study, the broodstock that reached the first sexual maturity at 22 and 34 months of age were stripped in November and December, and the broodstock that reached the first sexual maturity at the age of 46 months was stripped in December. The stripping season were observed in this study is similar to brown trout species of northern hemisphere and

rainbow trout cultured in Eastern Black Sea region of Türkiye. Aquaculture of Black Sea salmon and rainbow trout together can be more advantageous in hatchery management considering their different reproduction periods such as early for Black Sea salmon and late for rainbow trout.

Salmonids are amongst the most demanding fish species with high nutritional value and consumer appreciation on a global scale. Thus, several studies have been carried out regarding their natural behavior, culture characteristics, and breeding. Species that reach late sexual maturity and having rapid growth rate in seawater are generally preferred in fillet production. Zama and Cardenas (1983) reported that the age of sexual maturity of brown trout (*Salmo trutta*) is 2-5, and maturation occurs mainly at 3 and 4 years old. Gjerde (1984) recommended in the creation of breeding stock of Atlantic salmon, female broodstock should be selected from fish reached sexual maturity in 4 and 5 years, in Norway. Tabak et al. (2001) found that natural individuals of Black Sea salmon reach their first sexual maturity between the ages of 2-4. In this study showed that, egg diameter of marine ecotype broodstock is 5.8±0.03 mm, relative egg production is 1747±70 units/kg, egg diameter of brook ecotype broodstock is 4.85±0,19 mm, relative fecundity is 2865±354 units/kg. Estay et al. (2004) evaluated first sexual maturity of 3 years old fish individuals in their study conducted in Chile with Brown trout (*Salmo trutta* L.), which is originated from Germany as a culture form. They found total egg production of these broodstocks varied between 1182±344 - 2744±605 units/broodstocks, the relative egg production ranged between 3577±471 - 2181±360 units/kg and the egg diameters varied between 4.64±0.11 - 5.24±0.12 mm. Heinimaa and Heinimaa (2003) reported that egg diameter of Atlantic salmon is 5.3±0.2 mm (*Salmo salar* L.) in females with an average weight of 9.0±3.0 kg having 1845±392 pieces/kg fecundity. Rainbow trout, Black Sea trout and brook trout weighted as 1.357.27±406, 532±673.7, 310.40±85.0 g, had the egg diameters of 4.95±0.2, 4.51±0.67 and 4.49±, respectively (Serezli et al., 2010). They measured it as 0.21, and the fecundity was calculated as 2.180±676, 3.558±1307, 2.571±1530 pieces/kg, respectively. Çakmak et al. (2019) found that the mean egg diameter was 5.28±0.05 mm and the relative fecundity was 2159±115 pieces/kg in Black Sea salmon broodstocks with an average weight of 2439.21±139.28 g produced under culture conditions. In general, egg production and size in fish are affected by various factors such as broodstock size, age, genotypic structure and feeding conditions (Haeley and Heard 1984; Bromage et al., 1990, 1992). In our study, body weight, egg production and egg diameters of Black Sea salmon reaching the first sexual maturity at the age of 22 months were found to be similar to those of other researchers with non-anadromic Brown trout. However, the broodstocks reaching the first sexual maturity at the age of 34 and 46 months was consistent with the studies conducted with anadromous brown trout. Besides, the difference in total egg production, relative egg production, egg diameter and egg weight of the broodstock that reach the first sexual maturity at different ages used in our study can be caused by the first reproductive age (size) and genotypic structure (anadromic characteristic) of the broodstock. In 6th generation, broodstock individuals who reached the first sexual maturity at an average age of 22 (364.67±8.34 g), 34 (1425.68±37.80 g) and 46 (2650.35±18.45 g) months are observed. This broodstock has some beneficial characteristics such as opportunity of rearing to different harvest sizes and spotting until the first breeding age. It is possible to create a breeding stock from individuals of different ages and different appearances, taking into account consumer preference and increasing culture trend of the species day by day. Tourism establishments with restaurants, can create a breeding stock from individuals with white spots and red spots (reaching the first sexual maturity at the age of 22 months) considering customer demand. The net cage enterprises that produce large sizes can create a breeding stock from individuals who reach late sexual maturity (first sexual maturity at 46 months), and operating profitability in favor of feed expenses can be achieved.

Estay et al. (2004) calculated that the fertilization rate ranged from 92.0±13.7% to 98.5±4.01% in the study they carried out with brown trout, a cultured form in Chile. Çakmak et al. (2022) calculated the fertilization rate as 93.46±5.35% in the wild Black Sea salmon production studies adapted to the culture conditions in Türkiye and reported that the fertilization rate of eggs stripped from F1, F2, F3 and F4 generation broodstock varied between 95.28±6.29 - 98.25±1.81%. It is seen that the fertilization rate findings obtained in this study are similar to the results of the study conducted with cultured brown trout.

CONCLUSION

Black Sea salmon, which is among the species with high socio-economic value for human consumption and sportive fishing, is similar to brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*) in terms of reaching to first maturation age and some characteristics. Individuals with the first reproductive age of 22 months have the typical characteristics of brown trout with early sexual maturity, red-colored white halo mottling, slow growth and being settled, while individuals with first reproductive age of 34 and 46 months have the characteristics of Atlantic salmon like late sexual maturity, black mottling, silvery coloration, rapid growth in especially marine water and being anadromous. Different ecotypes of the species, which are still in the domestication stage, are preferred by commercial enterprises with different marketing networks. The general characteristics of ecotypes should be taken into account, considering producer and consumer preferences in future studies to be carried out on genetically supported breeding of Black Sea salmon. Different ecotypes of the species, which are still in the domestication phase and whose breeding studies are still in progress, are preferred by commercial enterprises with different marketing networks. In studies to be carried out on genetically assisted breeding of Black Sea salmon, breeding efficiency, migration behavior and

morphological characteristics of ecotypes should be taken into account for stock management.

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General Directorate of Agricultural Research and Policies of Türkiye (TAGEM/HAYSUD/2017/A11/P-01/3).

AUTHORSHIP CONTRIBUTIONS

Osman Tolga Özel: Conceptualization, methodology, and design of the experiments, data analysis, validation, broodstock management, stripping, nursery. Eyüp Çakmak: Conceptualization, methodology, and design of the experiments, manuscript writing, data analysis, validation, reviewing, broodstock management, stripping, nursery. Ekrem Cem Çankırılıgil: stripping, nursery. Zehra Duygu Düzgüneş: stripping, data analysis, validation. Recayi Çimagil: stripping, nursery. Esin Batır: broodstock management, stripping, nursery, reviewing.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

ETHICAL STATEMENT

The experimental protocols were conducted in accordance with the approval of the experimental animals ethics committee of the Trabzon Central Fisheries Research Institute (protocol No.: ETIK-2017/1).

DATA AVAILABILITY STATEMENT

The data presented here is not available online.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Seasonal changes in antioxidant enzyme activities of *Garra rufa* (Heckel, 1843) in Göynük Stream (Bingöl, Türkiye)

Göynük Çayı'nda (Bingöl, Türkiye) *Garra rufa*'nın (Heckel, 1843) antioksidan enzim aktivitelerindeki mevsimsel değişimleri

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Abstract: In this study, seasonal variations of antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glucose 6-phosphate dehydrogenase (G6PD)) and malondialdehyde (MDA) levels in gill, kidney, muscle and liver tissues of *Garra rufa* (Heckel, 1843) caught from Göynük Stream, one of the most important branch of Murat River, were investigated. Fish samples were caught from two stations (Garip and Ilıcalar), which are determined regularly every month, and brought to the laboratory. The levels of biomarkers in tissues were determined by spectrophotometric methods. It was determined that the difference between the parameters in the studied tissues was statistically significant ($P < 0.05$) between the two stations, but the difference between the stations in the liver tissue in all seasons in GR enzyme was not statistically significant. However, it was determined that the differences between the seasons were significant in both stations. GR and G6PD enzyme activities were found to be lower than other enzyme activities among the enzyme groups studied, but CAT and SOD enzyme activities were found to be higher than the other enzymes., enzyme activities in muscle tissue are lower than activities in the other tissues among tissues.

Keywords: Doctor fish, *Garra rufa*, Murat River, oxidative stress, seasonal changes

Öz: Bu çalışmada, Murat Nehri'nin en önemli kollarından biri olan Göynük Çayı'ndan yakalanan *Garra rufa* (Heckel, 1843) balıklarının solungaç, böbrek, kas ve karaciğer dokularında mevsimlere bağlı olarak antioksidan enzim aktivitelerindeki seviyelerindeki değişimler incelendi. Bu amaç doğrultusunda balıklar, her ay düzenli olarak belirlenen iki istasyondan (Garip ve Ilıcalar) yakalanarak laboratuvara getirildi. Dokularda enzim aktiviteleri ve MDA seviyesi spektrofotometrik yöntemlerle belirlendi. Çalışma sonucunda, genel olarak iki istasyon arasında tüm dokularda parametreler arasındaki fark istatistiki olarak önemli bulunmuştur ($p < 0.05$). Ancak karaciğer dokusunda GR enziminde tüm mevsimlerde istasyonlar arasındaki fark istatistiki olarak önemli olmadığı belirlendi. Bununla beraber, her iki istasyonda da genel olarak mevsimler arasındaki farklılıkların önemli olduğu gözlemlendi. Çalışmada, GR ve G6PD enzim aktiviteleri diğer enzim aktiviteleri ile kıyaslandığında düşükken, CAT ve SOD aktiviteleri ise diğerlerinden yüksektir. Dokular arasında ise kas dokusundaki enzim aktiviteleri, diğer dokulardaki aktivitelerden daha düşüktür.

Anahtar Kelime: Doktor balık, *Garra rufa*, Murat Nehri, oksidatif stres, mevsimsel değişiklikler

INTRODUCTION

Garra rufa (Heckel, 1843) is a member of the Cyprinidae family that lives at the bottom of streams and rivers, clinging to underwater rocks and stones with its sticky organ under its mouth. Its mouth structure is sticky and crescent-shaped, because it feeds on zooplankton and phytoplankton. The adhesive organ consists of four parts: the anterior fold with the disc fringing, the posterior fold, the numb part, and the posterior free part of the disc (Grassberger and Hoch, 2006; Teimori et al., 2011). *G. rufa* has no teeth and they eat by breaking the dead skin with their mouth movements and offer micro-massage with their movements. *G. rufa* is a fish species that lives in Sivas thermal springs at high temperatures and is used in the treatment of many diseases. Especially, a successful result is achieved when used on psoriasis and various skin diseases (Karahan, 2007). In the literature, it has been determined that Garra species are good for many skin diseases such as eczema, pus-wound-acne and psoriasis (Duman, 2010; Karaaslan, 2010).

Many different kinds of biomarkers have been used all around the world to assess the impact of pollutants on aquatic life. They track biological reactions such as the induction of biotransformations (Wong et al., 2000), the induction of protein levels (Linde et al., 2001), the suppression of enzyme activity (Peebua et al., 2007), the integrity of DNA (Ergene et al., 2007), etc. Therefore, these biomarkers offer a technique for definitive early warning signs of water pollution for fish species (Strmac and Braunbeck, 2000). Fish are useful bioindicators because of their abundance, richness, and relatively simple

identification. They are ideal for assessing regional and global environmental changes because they are less vulnerable to natural microenvironmental changes caused by lesser organisms (Gadzala-Kopciuch et al., 2004). In fact, changes in environmental conditions due to seasonal variations are likely to have an impact on enzyme activity. Therefore, it can be difficult to interpret biomarker data. Also, different levels of an enzyme biomarker may represent natural fluctuations in a species' annual physiological cycle, rather than exposure to chemical pollution (Robillard et al., 2003; Kırıcı et al., 2022a). Seasonallly, environmental conditions and metabolic activities have an effect on the responses of enzymatic activity. Therefore, knowing how biomarkers naturally evolve can help interpret field findings and distinguish between the beginning of a biological disturbance and natural variability (Figure 1) (Bocchetti and Regoli, 2006; Barda et al., 2014).

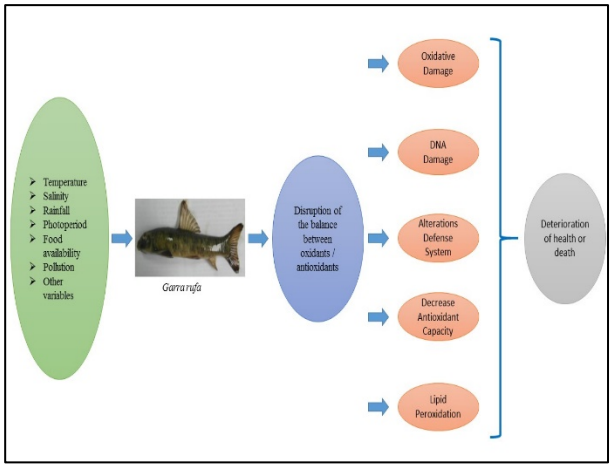

Figure 1. A schematic representation of the damages caused by different factors in *Garra rufa* in aquatic resources

MATERIALS and METHODS

Study area and stations

This study was carried out at Garip and Ilıcalar stations on Göynük Stream. Göynük Stream is one of the branches of the Murat River, which flows through the Genç District of Bingöl Province (Figure 2). These locations were chosen considering that there would be no problem in the supply of fish throughout the year with the observations made in the region. Additionally, Ilıcalar Station is a location with hot springs, thus the temperature values there are higher than seasonal averages. Garip Station, located within the borders of Genç district, has generally cold water. It is known that *Garra rufa* frequently travels to warm waters. Hence, Ilıcalar and Garip stations were chosen according to the migration requirements of the species. Gill nets and cast nets with different mesh sizes were used in the fish samplings.

Taking tissue samples from fish

In the study, 221 *G. rufa* (average weight: 11.26 ± 4.82 g; length: 9.7 ± 3.16 cm) fish were caught in one year. The caught fish were brought to the laboratory in tanks with air stones in the water taken from their natural habitat. The fish were transported as carefully as possible and kept away from stress factors. The fish were anesthetized in the laboratory with anesthetic matter (50 ppm, benzocaine) and tissues were removed rapidly. The abdominal region was opened by cutting the fish with the non-pointed part of the scissors from the anus region. The kidney, liver, gill, and gonad tissues used in the study were carefully removed. The kidney, liver, gill, and gonad tissues of each fish were placed in separate tubes. Until the time of the study, the tissues were stored at -80 °C (Kırıcı et al., 2017).

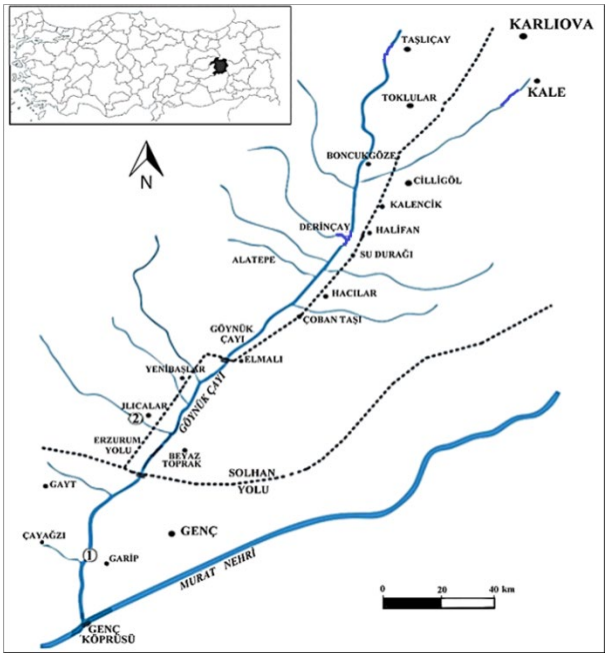

Figure 2. Stations (Garip (1) (38°47'13.2"N 40°32'56.9"E); Ilıcalar (2) (38°58'57.7"N 40°41'04.4"E) (Modified from Koyun et al., 2018; Kırıcı et al., 2022a)

Preparation of homogenate

Frozen tissues taken from -80 °C were expected to thaw at +4 °C. After the melting process, 0.9% NaCl was used to remove the blood from the tissues in the tube they were in. The kidney, gonad, liver and gill tissues of each fish were weighed separately and homogenized. Each tissue sample was cut into small pieces with the help of scissors. This fragmented tissue sample was placed in a porcelain mortar that had been pre-cooled to -80 °C. Liquid nitrogen was then added and the mixture was crushed to a paste-like consistency. The crushed tissue sample was taken into a tube and 3 times its weight of KH_2PO_4 buffer solution was added on it. Then the tissue sample was centrifuged at +4 °C at 13000 rpm for two hours. Supernatant existing on the upper section was carefully taken with a dropper. Following this, the precipitated part was removed. The supernatant was used to determine enzyme activity (Beutler, 1971).

Determination of lipid peroxidation

Malondialdehyde is a lipid peroxidation product and is a good stress marker. The MDA level was determined by reading

on a spectrophotometer (Shimadzu UV/VIS-1201) at a maximum of 532 nm. For this, 200 µl of sample from each extracted tissue was taken into the tube and 800 µl of phosphate buffer was added. It was suspended with 25 µl of BHT to stop oxidation. Then µl of 30% TCA was added. Samples were voxed, mixed in vortex and kept at -20 ºC for 2 hours. Then centrifugation was carried out at 2000 rpm for 15 minutes. 1 ml sample was taken from the upper phase and 75 µl of EDTA Na_2H_2O and 250 µl of TBA were transferred on it, respectively. After vortexing, it was kept in a water bath with a temperature of 90 ºC for about 15 minutes. Readings were taken when the samples were at room temperature (Slater, 1984).

Measurement of levels of antioxidant enzymes

G6PD activities were determined by measuring the change in absorption at 340 nm at 37 ºC, as described by Beutler. For the determination of the activity of the enzyme, NADPH formed at the end of the reaction is taken into account. Because the activity of the G6PD enzyme, which catalyzes the production of 6 phosphoglucanolac from glucose-6-phosphate, is directly proportional to the decreasing amount of NADP in this process (Beutler, 1975).

In the measurement of the activity of the GR enzyme, it is measured by the maximum absorbance of the reacting NADPH at 340 nm. In the reaction catalyzed by the GR enzyme, it causes a decrease in NADPH. Enzyme activity was determined by monitoring this decrease spectrophotometrically at 340 nm (Carlberg and Mannervik, 1985).

SOD activity, Sun et al. (1988) using the method suggested. The nitroblue tetrazolium (NBT) is reduced via the xanthine-xanthineoxidase system, which generates superoxide, as the foundation of this technique. SOD activity was measured in units per gram of tissue protein (U/g).

GPx activity was investigated using Beutler (1975) method. In the presence of hydrogen peroxide, GPx catalyzes the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG). When hydrogen peroxide is in the environment, GR and NADPH work together to convert the GSSG produced by GPx into GSH. By measuring the decrease in absorbance at 340 nm caused by the oxidation of NADPH to $NADP^+$.

CAT determination was made according to the Aebi (1983) method. In summary; When the sample is added to the H_2O_2 solution, which is prepared in phosphate buffer (pH 7.4) and gives approximately 0.500 absorbance at 240 nm. In the meantime, the decrease in absorbance was followed on the monitor and the activity was calculated from the slope (Aebi, 1983).

Determination of protein in fish tissue

Lowry et al. (1951) performed spectrophotometrically reported tissue protein determination. This approach relies on complexes of protein peptide bonds with copper ions forming in an alkaline media. The blue-violet hue created by the copper-peptide complexes and the foline reagent reaction is read at 750 nm using a blind versus spectrophotometer.

Statistics

Using the SPSS 23.0 computer program, it was evaluated whether there was a significant difference between the stations and seasons in the changes of enzyme activities in fish tissues, according to One-Way ANOVA and Duncan test. Data were given as mean ± standard error. P<0.05 value was considered statistically significant.

RESULTS

MDA level, a marker of oxidative stress, in kidney tissue was found to be higher in Garip Station tissue values in autumn and spring seasons compared to Ilıcalar Station, and the difference was statistically significant. However, kidney tissue MDA level did not show a statistically significant difference between stations in the summer and winter seasons. Significant differences were detected between stations in all seasons in the enzyme levels of SOD, CAT and GR, which are markers of oxidative stress. In G6PD kidney tissue level activity, the difference between stations in autumn was statistically significant, but the difference between summer, winter and spring seasons was not statistically significant. In GPx activity in autumn, winter and spring seasons a statistically significant difference was found (P<0.05) (Table 1).

Table 1. Levels of gill tissue oxidative stress markers in *Garra rufa*

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	3.81 ± 0.71^{a,*}	0.11 ± 0.05^{a}
	Autumn	1.26 ± 0.22^{b,*}	0.24 ± 0.07^{a}
	Winter	0.95 ± 0.18^{b}	0.84 ± 0.78^{b}
	Spring	1.97 ± 0.29^{b,*}	0.27 ± 0.11^{a}
SOD	Summer	34.46 ± 3.30^{a,*}	0.33 ± 0.01
	Autumn	15.71 ± 1.19^{b,*}	0.17 ± 0.03
	Winter	19.91 ± 1.24^{b,*}	0.21 ± 0.03
	Spring	11.60 ± 2.00^{b,*}	0.14 ± 0.02
CAT	Summer	470.1 ± 23.0^{a,*}	23.70 ± 1.30^{a}
	Autumn	308.4 ± 19.4^{b,*}	36.47 ± 2.40^{b}
	Winter	289.0 ± 16.9^{b,*}	48.39 ± 2.90^{c}
	Spring	395.1 ± 17.4^{c,*}	27.20 ± 1.10^{a}
GR	Summer	1.08 ± 0.16^{a,*}	4.47 ± 0.97^{a}
	Autumn	5.01 ± 1.73^{b,*}	2.81 ± 0.39^{b}
	Winter	2.18 ± 0.76^{a}	2.34 ± 0.35^{b}
	Spring	2.75 ± 0.32^{a}	3.24 ± 1.76^{b}
G6PD	Summer	1.10 ± 0.29	1.82 ± 0.40^{a}
	Autumn	0.84 ± 0.18*	5.67 ± 0.82^{b}
	Winter	1.04 ± 0.22	2.45 ± 0.54^{a}
	Spring	0.97 ± 0.13	1.64 ± 0.32^{a}
GPx	Summer	17.00 ± 3.11^{a}	19.18 ± 1.93^{a}
	Autumn	12.47 ± 2.18^{b,*}	38.36 ± 3.02^{b}
	Winter	8.33 ± 1.47^{c,*}	24.17 ± 2.35^{a}
	Spring	9.03 ± 1.80^{c,*}	23.13 ± 2.80^{a}

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

Statistically significant differences were found between stations in MDA level in spring, summer and autumn seasons in gill tissue. SOD and CAT enzyme activities were found to be

significantly different between Garip and Ilıcalar stations in all seasons. Significant differences were found in GR activity in summer and autumn seasons. A statistically significant difference was found between stations in GPx activity in all seasons except summer. In the gill tissue, there was no statistical difference between SOD activities at Garip Station and G6PD activities at Ilıcalar Station in all the seasons (P<0.05) (Table 2).

A statistically significant difference was found between stations in MDA levels of liver tissue in all the seasons except for spring. There are statistically significant differences between the stations in SOD, CAT and G6PD activities in all seasons. On the other hand, no significant difference was detected between Garip and Ilıcalar stations in GR activity of liver tissue. No significant difference was detected in autumn and winter months, while a statistically significant difference was detected between stations in GPx activity in summer and spring seasons. There was no significant difference between the GR activities at Garip Station and G6PD activities at Ilıcalar Station between seasons (P<0.05) (Table 3).

A statistically significant difference was found between the stations in all seasons in MDA level, SOD, CAT, G6PD and GPx activities of gonad tissue. Only, there was a statistically significant difference between stations in GR activity in the summer season. No significant difference could be detected in G6PD activities between the seasons, in Ilıcalar Station. Significant statistical differences were detected in MDA levels and enzyme activities at other stations (P<0.05) (Table 4).

Table 2. Levels of gill tissue oxidative stress markers in *Garra rufa*

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	3.81 ± 0.71^{a,*}	0.11 ± 0.05^{a}
	Autumn	1.26 ± 0.22^{b,*}	0.24 ± 0.07^{a}
	Winter	0.95 ± 0.18^{b}	0.84 ± 0.78^{b}
	Spring	1.97 ± 0.29^{b,*}	0.27 ± 0.11^{a}
SOD	Summer	34.46 ± 3.30^{a,*}	0.33 ± 0.01
	Autumn	15.71 ± 1.19^{b,*}	0.17 ± 0.03
	Winter	19.91 ± 1.24^{b,*}	0.21 ± 0.03
	Spring	11.60 ± 2.00^{b,*}	0.14 ± 0.02
CAT	Summer	470.1 ± 23.0^{a,*}	23.70 ± 1.30^{a}
	Autumn	308.4 ± 19.4^{b,*}	36.47 ± 2.40^{b}
	Winter	289.0 ± 16.9^{b,*}	48.39 ± 2.90^{c}
	Spring	395.1 ± 17.4^{c,*}	27.20 ± 1.10^{a}
GR	Summer	1.08 ± 0.16^{a,*}	4.47 ± 0.97^{a}
	Autumn	5.01 ± 1.73^{b,*}	2.81 ± 0.39^{b}
	Winter	2.18 ± 0.76^{a}	2.34 ± 0.35^{b}
	Spring	2.75 ± 0.32^{a}	3.24 ± 1.76^{b}
G6PD	Summer	1.10 ± 0.29	1.82 ± 0.40^{a}
	Autumn	0.84 ± 0.18*	5.67 ± 0.82^{b}
	Winter	1.04 ± 0.22	2.45 ± 0.54^{a}
	Spring	0.97 ± 0.13	1.64 ± 0.32^{a}
GPx	Summer	17.00 ± 3.11^{a}	19.18 ± 1.93^{a}
	Autumn	12.47 ± 2.18^{b,*}	38.36 ± 3.02^{b}
	Winter	8.33 ± 1.47^{c,*}	24.17 ± 2.35^{a}
	Spring	9.03 ± 1.80^{c,*}	23.13 ± 2.80^{a}

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

Table 3. Levels of liver tissue oxidative stress markers in *Garra rufa*

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	8.95 ± 2.58^{a,*}	4.65 ± 0.73^{a}
	Autumn	10.11 ± 2.98^{a,*}	15.98 ± 1.53^{b}
	Winter	4.38 ± 1.16^{b,*}	10.88 ± 1.99^{c}
	Spring	5.55 ± 1.37^{b}	7.36 ± 1.09^{d}
SOD	Summer	48.36 ± 2.26^{a,*}	7,11 ± 1.45^{a}
	Autumn	82.42 ± 2.38^{b,*}	8.30 ± 2.47^{a}
	Winter	40.79 ± 2.17^{a,*}	6.90 ± 1.08^{a}
	Spring	84.07 ± 2.26^{b,*}	4.07 ± 1.27b
CAT	Summer	54.46 ± 3.30^{a,*}	224.35 ± 34.82^{a}
	Autumn	31.55 ± 2.99^{b,*}	241.84 ± 27.75^{a}
	Winter	28.18 ± 1.43^{b,*}	325.73 ± 48.20^{b}
	Spring	40.92 ± 3.09^{c,*}	198.03 ± 17.39^{a}
GR	Summer	3.70 ± 1.67^{a}	2.47 ± 0.27
	Autumn	3.80 ± 1.86^{a}	2.91 ± 0.39
	Winter	1.56 ± 0.66^{b}	3.04 ± 0.35
	Spring	1.95 ± 0.75^{b}	3.45 ± 0.34
G6PD	Summer	0.79 ± 0.03*	16.70 ± 5.21^{a}
	Autumn	0.60 ± 0.01*	21.67 ± 6.01^{b}
	Winter	0.71 ± 0.02*	16.35 ± 4.07^{a}
	Spring	1.02 ± 0.02*	15.19 ± 2.18^{a}
GPx	Summer	4.93 ± 1.08^{a,*}	8.39 ± 2.72^{a}
	Autumn	8.64 ± 2.13^{b}	9.06 ± 2.81^{a}
	Winter	5.61 ± 1.98^{a}	7.28 ± 2.02^{b}
	Spring	2.76 ± 0.83^{c,*}	9.64 ± 1.94^{a}

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

Table 4. Levels of gonad tissue oxidative stress markers in *Garra rufa*

Parameters	Seasons	Ilıcalar	Garip
MDA	Summer	4.88 ± 0.49^{a,*}	0.26 ± 0.03^{a}
	Autumn	7.13 ± 0.51^{b,*}	0.08 ± 0.02^{b}
	Winter	4.42 ± 0.47^{a,*}	0.04 ± 0.02^{b}
	Spring	3.99 ± 0.25^{a,*}	0.05 ± 0.01^{b}
SOD	Summer	6.21 ± 0.78^{a,*}	13.36 ± 1.87^{a}
	Autumn	9.37 ± 1.96^{b,*}	14.21 ± 2.00^{a}
	Winter	5.93 ± 1.24^{a,*}	21.08 ± 2.96^{b}
	Spring	6.23 ± 0.81^{a,*}	14.89 ± 2.01^{a}
CAT	Summer	29.42 ± 4.58^{a,*}	8.18 ± 1.32^{a}
	Autumn	11.16 ± 2.73^{b,*}	19.11 ± 4.47^{b}
	Winter	19.48 ± 3.47^{b,*}	7.88 ± 1.55^{a}
	Spring	19.92 ± 3.18^{b,*}	8.34 ± 2.35^{a}
GR	Summer	0.30 ± 0.09^{a,*}	4.2 ± 0.47^{a}
	Autumn	0.69 ± 0.12^{b}	0.9 ± 0.04^{b}
	Winter	0.41 ± 0.09^{a}	1.3 ± 0.09^{b}
	Spring	0.19 ± 0.06^{a}	0.8 ± 0.03^{b}
G6PD	Summer	0.19 ± 0.11*	5.4 ± 0.88^{a}
	Autumn	0.23 ± 0.09*	8.6 ± 2.19^{b}
	Winter	0.15 ± 0.01*	5.8 ± 0.93^{a}
	Spring	0.10 ± 0.01*	5.0 ± 0.91^{a}
GPx	Summer	4.28 ± 0.25^{a,*}	0.11 ± 0.02^{a}
	Autumn	8.20 ± 0.23^{b,*}	0.13 ± 0.07^{a}
	Winter	3.41 ± 0.15^{a,*}	0.94 ± 0.48^{b}
	Spring	6.28 ± 0.25^{a,b,*}	0.18 ± 0.09^{a}

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

DISCUSSION

The Murat River, the largest branch of the Euphrates River, is one of Türkiye's most important water resources, passing through the borders of Bingöl province. Murat River is a 722 km long in

Eastern Anatolia, formed by the merging of branches originating from Aladağ and Muratbaşı Mountain in the north of Van Lake, and flowing to the Keban Dam by moving westward, passing through the north of Bingöl province Genç district. Its total length in Bingol Province is 96 km and it is the most important water source of Bingöl Province. Today, water resources are heavily threatened by various ecological problems such as natural pollution, pesticides and household wastes (Kırıcı et al., 2016a).

In ecotoxicology; biomarkers are used in describe of interactions between a biological system and a chemical, physical or biological environmental pollutant. Inhibition or induction of biomarkers is a useful approach to determine the effects and potential effects of xenobiotics on living organisms *in vivo* (RendÓn-von Osten et al., 2005; Taysı et al., 2021a). Fish can be used as biomonitors to test for pollution and determine the ecological health of the aquatic environment because they are highly sensitive to it. Resistance of aquatic organisms to contamination; it is affected by many factors, including its phylogenetic location, ecological and biological characteristics of the living thing, physiological conditions and the presence of effective detox mechanisms (Hotard and Zou, 2008). In this study, it was aimed to correlate the effects of different seasonal changes in the aquatic ecosystem on fish with changes in biomarker response and to identify reliable biomarkers for monitoring. *G. rufa* fish, a species belonging to the Cyprinidae family, naturally distributed in Türkiye, Syria, Iran, Iraq and Jordan, can be used as a biomarker for the detection of seasonal biomarker changes and the pollution of rivers. In this study, *G. rufa* was chosen as a marker organism due to the ease of sampling, high adaptability to the region, and high ecological and economic importance of *G. rufa* from Göynük Stream on the Murat River. Most of the enzymatic activities in poikilothermic species vary with the environmental temperature. In fact, the level of many enzyme activities is not directly dependent on ambient temperature, but on physiological activity, which is tightly correlated with water temperature. Variations in biotic parameters such as sex, size, age, gonadal maturity or hunger are known to affect biomarkers and complicate the interpretation of the environmental significance of the markers (Forget et al., 2003).

Oxidative stress is a result of an imbalance between the generation and elimination of reactive oxygen species (ROS) in aquatic species, which can be brought on by a variety of anthropogenic and natural stimuli (xenobiotics) (Halliwell and Gutteridge, 2007; Yonar et al., 2016). An excellent cleaning potential against oxidative stress is provided by antioxidant defenses. Enzymatic and non-enzymatic antioxidant defense components are frequently used as biomarkers in environmental monitoring investigations (Sheehan and Power, 1999). Indicators of xenobiotic exposure and/or consequences, biomarkers are cellular, biochemical, molecular, or physiological alterations assessed in an organism's cells, body fluids, tissues, or organs (Lam and Gray, 2003; Kırıcı et al., 2015; Kırıcı et al., 2016b; Taysı et al., 2021b). A promising approach for biomonitoring aquatic systems is the evaluation of the enzyme biomarkers superoxide dismutase (SOD) and catalase (CAT), glutathione (GSH) content, and malondialdehyde (MDA) production activities (Livingstone, 2001; Yonar et al., 2011; Ispir et al., 2017). In a number of marine and freshwater animals, antioxidants including CAT, SOD, and GSH have been proposed as indicators of pollutant or seasonally related oxidative stress, and their activation represents a response to pollution (Borkovic et al., 2005; Yonar et al., 2012; Topal et al., 2014). However, environmental variables such as temperature, dissolved oxygen and food availability are known to influence oxidative stress responses through their effects on metabolism and reproduction (Sheehan and Power, 1999; Kırıcı et al., 2022b). The results obtained in this study show that seasonal factors have a significant effect on the antioxidant defense system. External factors such as temperature (Verlecar et al., 2007), salinity (Prevodnik et al., 2007), pH (Lima et al., 2007), nutrient source (Khessiba et al., 2005), and reproductive cycle (Filho et al., 2001), seasonal changes can be effective from complex interactions between endogenous factors.

Danabas et al. (2015) investigated some oxidative stress parameters (SOD, CAT and GPx activities and MDA levels) determined in the gill tissues of *Capoeta umbla* caught in different seasons from 10 sampling areas in Uzuncayir Dam Lake. They indicated that changes in SOD and CAT activities for the four seasons were found to be statistically significant ($p < 0.05$). The highest SOD enzyme activity was found in September 2011 in region 6, while the lowest in region 2 was found in March 2012. The highest CAT enzyme activity was found in the 3rd region in March 2012, while the lowest value was determined in the 4th region in September 2011. GPx activities were statistically insignificant in regions 2, 6 and 9 according to the four seasons ($p > 0.05$). The lowest GPx activity was found in September 2011 at region 8. Changes in MDA levels between seasons were statistically significant ($p < 0.05$). The highest MDA level was detected in zone 1 in September 2011, while the lowest was detected in zone 5 in March 2011. In this study, while the highest activity in kidney tissue was measured in CAT activity in Garip station in autumn season, the lowest activity was measured in GR activity in Garip station in winter. In the gill tissue, CAT activity was measured as the highest activity in the summer at Ilıcalar station, while the MDA level was measured as the lowest in the Garip station in the summer. In liver tissue, the highest CAT activity was measured at Garip station in winter, while the lowest was measured in G6PD activity in autumn at Ilıcalar station. In the gonad tissue, the highest CAT activity was measured at Ilıcalar station in summer, while the lowest MDA level was measured at Garip station in winter.

In a study evaluating the liver antioxidant enzyme activities of *Salmo trutta caspius, Salmo trutta labrax* and *Salmo trutta macrostigma* species, it was reported that these enzymes were

not affected by seasonal temperature changes, but there were changes in antioxidant enzyme activities in autumn. It has been stated that this oxidative enzyme change, which is an indicator of oxidative stress, may be related to pre-reproduction. It was emphasized that the rapid decrease in temperature, heavy rainfall and increase in daylight could trigger stress in fish. In addition, the same research team generally determined the liver SOD, GPx, CAT, G6PD, GR and GST enzyme activities in 3 different species (*Salmo trutta caspius, Salmo trutta labrax* and *Salmo trutta macrostigma*) found higher (Aras et al., 2009). They determined that GPx activities in all 3 fish, liver and gill tissues were higher than other enzyme activities studied in all seasons. In this study, CAT activity was generally found to be higher than other enzyme activities in all tissues. Especially, CAT activity was highest in the gill tissue at Ilıcalar Station in summer. In a study conducted in Munzur Alası of Munzur Stream, it was reported that the activities of glutathione peroxidase, catalase, superoxide dismutase and superoxide dismutase antioxidant enzymes in the muscle, liver and gill tissues of *Salmo* sp. increase in summer months. In addition, it was reported that liver tissue superoxide dismutase activity was statistically significant between seasons, and muscle, gill and liver tissues glutathione peroxidase activity was statistically significant in summer (Can et al., 2017).

CONCLUSIONS

As a result of this study, it was observed that antioxidant enzyme activities and MDA levels changed according to the seasons. Although the levels of oxidative stress parameters vary according to the seasons, these parameters can also be modified by various factors such as sex, sexual maturity, reproductive parameters and pollutants. The number of studies similar to this study should be done more by increasing the parameters.

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AUTHORSHIP CONTRIBUTIONS

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

CONFLICT OF INTEREST

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

ETHICAL STATEMENT

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

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Determination of lipid quality and mercury levels of sardine and rainbow trout cooked with different methods

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Abstract: This study aimed to investigate the effects of baking and pan-frying methods on the lipid quality and mercury (Hg) levels of two important fish species in Türkiye, namely, fileted sardine (*Sardina pilchardus*) and rainbow trout (*Oncorhynchus mykiss*). The results revealed that sardines significantly decreased n-3 fatty acids depending on the cooking process, while the best n-6/n-3 ratio was observed in baked sardines, with higher rates found in pan-fried fish. Notably, pan-fried rainbow trout cooked with butter showed the highest atherogenic index (AI) of 0.71±0.32 and thrombogenic index (TI) of 0.61±1.43, as well as a hypocholesterolemic/hypercholesterolemic index (HH) of 0.79 ± 0.17. Conversely, fried sardines exhibited lower atherogenic and thrombogenic scores, with fried sardines cooked in sunflower oil having a hypocholesterolemic/hypercholesterolemic index of 4.85 ± 0.3. There were no significant variations in Hg content between raw and cooked fish. However, when compared to the raw control, the rise in Hg content for baked fish was substantial ($p < 0.05$) (baked rainbow trout 0.18 mg/kg and sardine 0.29 mg/kg). The decrease in FAs (Fatty Acids) due to cooking methods can be ordered as follows: Raw sardine > baked > fried. Conversely, the increase in FAs due to the cooking methods can be ordered as follows: fried > baked > raw sardine. Baked rainbow trout had much lower levels of palmitic acid, stearic acid, and myristic acid than the raw material. However, these levels were significantly increased in rainbow trout cooked in a frying pan. Additionally, oleic acid levels in fried rainbow trout were detected to be lower than in other preparations, whereas they were equivalent in baked rainbow trout.

Keywords: Fatty acids composition, pan-frying; baking, lipid quality, mercury, sardine, rainbow trout

INTRODUCTION

Because of its high polyunsaturated fatty acids (PUFAs) content with a significant amount of omega 3, which is not naturally present in the human body, and its low content of saturated fatty acids (SFA), fish is preferred for consumption. (Erdem and Dinçer, 2019). Additionally, long-chain n-3 PUFA is abundant in the lipids of fish meat, and seafood (Erdem et al., 2020). They are key cell membrane constituents that contribute to a variety of membrane activities. (EFSA, 2012a). It is well known that the amount of nutrients and toxins accumulated in the body depends on the species and the amount consumed. Heavy metals, such as lead, cadmium, and mercury, can accumulate in the tissues of fish, especially in their flesh. These metals can come from a variety of sources, including pollution, industrial waste, and contaminated waterways. Mercury is one of the most concerning heavy metals found in fish, as it can have toxic effects on the nervous system, particularly in young children and pregnant women. Certain types of fish, such as large predatory fish like swordfish and sharks, tend to have higher levels of mercury due to their position in the food chain. The abundance of heavy metals and mercury in fish flesh is a significant public health concern, and guidelines exist to help consumers make informed decisions about which types of fish are safe to eat and how often to consume them. Cooking fish and seafood before eating is a common practice. This heat treatment is a necessary precaution to ensure that the food offers the desired texture, flavor, and color during the cooking process or food preparation, in addition to ensuring food cleanliness and safety (Erdem and Dinçer, 2019).

The effects of several cooking methods on nutritional quality, particularly fatty acid (FA) profiles and mercury concentration have recently been examined (Karimian-Khosroshahi et al., 2016; Zhang et al., 2019; Islam et al., 2020). Methods of cooking, including frying, boiling, baking, microwaving, and steaming, affect the composition of various fatty acids, with PUFAs being altered by these cooking methods and fish species (Farag, 2013; Flaskerud et al., 2017; Alexi et al., 2019). In Türkiye, sunflower oil, which contains approximately 71% polyunsaturated fatty acids (PUFA), is popular culinary oil (Demirtas Erol et al., 2022). Sardines (*Sardina pilchardus*) and rainbow trout (*Oncorhynchus mykiss*) are popular types of fish consumed in many parts of the world, including Türkiye. Sardines are a staple food in Mediterranean countries, and they are also commonly consumed in Asia, South America, and Africa. On the other hand, rainbow trout is widely consumed in Europe and North America.

In Türkiye, sardines are a popular seafood item, especially in the coastal regions. They are typically grilled or fried and served with salad or bread. Rainbow trout are also consumed in Türkiye, particularly in the Black Sea region, where it is abundant in the local rivers, and it is mostly served pan-fried with butter. These two species were selected to investigate possible changes induced by different cooking methods.

MATERIALS AND METHODS

Fish

Sardines (*Sardina pilchardus*) with an approximate weight of 94.6 g ± 10.2 and length of 15.6 cm ± 1.4 were freshly bought from the fish market in Buca, İzmir, Türkiye. Farm-raised rainbow trout (*Oncorhynchus mykiss*) with an approximate weight of 250 g ± 35 and length of 20.1 cm ± 1.4 were obtained from an aquaculture facility in Manisa, Türkiye. The fish were transferred to the laboratory under a cold chain. Afterward, the fish, with their skins on, were filleted and cooked depending on cooking methods.

Fish heat treatment

Cooking techniques were based on previously published methods (Flaskerud et al., 2017; Farag, 2013). For this study, pan-frying and baking were selected as the cooking methods. In Türkiye, rainbow trout is a widely consumed freshwater fish species, and it is often prepared by frying. However, in Turkish households, the use of saturated fats for pan-frying is being increasingly replaced by healthier unsaturated vegetable oils, especially olive oil. Some families still prefer using butter for pan-frying (Bilgin et al., 2010). Therefore, sardine is a widely caught and consumed marine fish species. The temperature and time used in this study were chosen based on references used for cooking methods. However, there have been numerous studies on the effect of different cooking methods on sardines and rainbow trout. The goal of this study, different from other research, is to reveal the effect of different cooking techniques on both mercury and fatty acids.

Sardine fillets were pan-fried in sunflower oil for 10 minutes on both sides at 180°C. The rainbow trout fillets were pan-fried in butter for 12 minutes on each side at 180°C and then gently drained for approximately 2 minutes. For the baking process, sardines were placed in the oven (Öztiryakiler, OKFE 101, İzmir, Türkiye) and baked at 180°C for 22 minutes, while rainbow trout fillets were baked for 30 minutes at the same temperature. The cooking procedure was considered complete when a quartz electronic thermometer indicated that the fillet's interior temperature ranged between 60 and 70°C. Once the necessary temperature was obtained for all cooking methods, the samples were cooled and tested.

Fatty acid analysis

Fatty acid analyses were carried out using the IUPAC II.D.19 method (IUPAC, 1979). Fatty acids of the anchovy and anchovy oil were analyzed using a Perkin Elmer Auto system XL Gas Chromatograph equipped with SP-2330 and a flame ionization detector (FID). Separation of fatty acid methyl esters was achieved on a fused silica capillary column (30 m x 0.25 mm x 0.20 µm film thicknesses the oven temperature was 120°C for 2 min, and programmed to 220°C at a heating rate of 5°C/min, then held for 15 min. The injector and detector temperatures were maintained at 240°C and 250°C, respectively. The carrier gas was helium 10psi with a split ratio of 1/50. The air and hydrogen pressure were 338 ml/min and 45 ml/min respectively. Fatty acids were identified by comparing the retention times of fatty acid methyl esters (FAME) with a standard 37-component FAME mixture (Supelco- Catalog No:18919-1Amp.) Results were expressed as the percentage of each fatty acid concerning the total fatty acids. The GC analyses were performed in triplicate, and the results were expressed as % of total FAME area as the mean value of a percentage.

Lipid quality indices

The thrombogenic index (TI) and atherogenic index (AI) were calculated due to FA composition by using the method of Ulbricht and Southgate (1991). The hypocholesterolemic/ hypercholesterolaemic ratio (HH) was calculated according to fatty acid composition by using the method Santos-Silva et al. (2002) using the following equations:

$$AI = \frac{12:0+(4x14:0)+16:0}{\sum UFA}$$

$$TI = \frac{14:0+16:0+18:0}{(0.5MUFA)+(0.5n\text{-}6PUFA)+(3n\text{-}3PUFA)+((n.3PUFA)/(n\text{-}6PUFA))}$$

$$HH = \frac{18:1n\text{-}9+18:2n\text{-}6+20:4n\text{-}6+18:3n\text{-}3+20:5n\text{-}3+22:5n\text{-}3+22:6n\text{-}3}{12:0+14:0+16:0}$$

[1]*MUFA: monounsaturated fatty acid*

Determination of total mercury (Hg) content

For the quantitative analyses of total mercury (Hg), fish samples were digested. Wet samples and HNO_3 were taken in the tube and digested according to the program of eicosapentaenoic acid (EPA) Methods (1994). After digestion, each sample was transferred to a 50 ml volumetric flask and filled up to the mark with deionized water. The sample was filtered and further diluted by four times to be analyzed by ICP-MS (Agilent 7500CE, USA). The standard solutions were prepared by diluting the required amount of the solution from the stock solution, manufactured by Agilent, Germany.

Statistical analysis

Statistical analysis was performed using IBM SPSS (statistical package for the social sciences) Statistics 22.0 and expressed as mean ±SD of the three replicated cooking processes. To define the significance of differences in proximate value, fatty acid content, and nutritional quality before and after cooking, analysis of variance ANOVA) using one way followed by Tukey's significant difference test (p <0.05). All data are expressed as mean ± standard deviation. Principal component analysis (PCA) explored differences in the three groups' compositions.

RESULTS

FA composition of pan-fried and baked sardine

Fatty acids were classified as SFA, MUFA, and PUFA, and a total of 33 fatty acids were examined. Different cooking techniques resulted in various alterations in the fatty acid composition. The fatty acids of raw, fried, and baked sardines are shown in Table 1. All cooking methods reduced the total SFA, HUFA, and n-3 PUFA, while they increased total MUFA, PUFA, and n-6 PUFA in baked and fried sardines when compared to raw sardines. Palmitic acid (C16:0) was the major constituent of SFA. Myristic, palmitic, palmitoleic, stearic, linoleic, and eicosapentaenoic acids decreased in baked and fried sardines compared to raw sardines. The decrease in FAs due to cooking methods can be ordered as follows: Raw sardine > baked > fried. Among the MUFAs, oleic acid was the most abundant in sardines. Additionally, oleic, linoleic, and docosahexaenoic acids increased in baked and fried sardines compared to raw sardines. The increase in FAs due to the cooking methods can be ordered as follows: fried > baked > raw sardine. PUFA in cooked fish significantly differed from those in raw fish ($p<0.001$).

Table 1. Fatty acids (%) profile of cooked by different methods and raw sardine

Fatty acids composition	Raw	Baked	Fried
Caproic acid (C6:0)	0.02 ± 0.001	nd	nd
Caprylic acid (C8:0)	0.01 ± 0.005	nd	nd
Capric acid (C10:0)	0.03 ± 0.02	nd	nd
Undecanoic acid (C11:0)	0.01 ± 0.002	nd	nd
Lauric acid (C12:0)	0.13 ± 0.02^{a}	0.08 ± 0.05^{ab}	0.04 ± 0.02^{b}
Tridecanoic acid (C13:0)	0.05 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.02^{a}
Myristic acid (C14:0)	5.12 ± 0.1^{a}	3.36 ± 0.04^{b}	1.99 ± 0.22^{c}
Myristoleic acid (C14:1)	0.02 ± 0.01	nd	nd
Pentadecanoic acid (C15:0)	0.86 ± 0.01^{a}	0.57 ± 0.03^{b}	0.31 ± 0.02^{c}
Palmitic acid (C16:0)	22.95 ± 0.05^{a}	17.02 ± 0.08^{b}	12.38 ± 0.12^{c}
Palmitoleic acid (C16:1)	5.58 ± 0.42^{a}	3.77 ± 0.73^{b}	2.28 ± 0.67c
Heptadecanoic acid (C17:0)	0.62 ± 0.04^{a}	0.44 ± 0.06^{b}	0.23 ± 0.09^{c}
Stearic acid (C18:0)	4.86 ± 0.04^{a}	4.14 ± 0.06^{b}	3.57 ± 0.44c
Elaidic acid (C18:1n9t)	0.15 ± 0.02^{a}	0.06 ± 0.01^{b}	0.04 ± 0.01^{b}
Oleic acid (C18:1n9c)	14.87 ± 0.13^{a}	20.5 ± 0.5^{b}	26.01 ± 2.99^{c}
Linoleic acid (C18:2n6c)	2.39 ± 0.01^{a}	19.71 ± 0.28^{b}	33.67 ± 1.33^{c}
Arachidic acid (C20:0)	0.77 ± 0.02^{a}	0.59 ± 0.03^{b}	0.43 ± 0.13c
ɣ-Linolenic acid (C18:3n3)	0.13 ± 0.03^{a}	0.09 ± 0.04^{ab}	0.05 ± 0.01^{b}
11-Eicosenoic acid (C20:1)	1.63 ± 0.03^{a}	1.04 ± 0.04^{b}	0.7 ± 0.17a
α-Linolenic acid (C18:3n3)	1.9 ± 0.1^{a}	1.31 ± 0.07^{b}	0.8 ± 0.4c
Heneicosanoic acid (C21:0)	0.03 ± 0.01^{a}	0.02 ± 0.02^{a}	0.01 ± 0.05^{a}
Eicosadienoic acid (C20:2)	3.75 ± 0.05^{a}	2.58 ± 0.12^{b}	1.6 ± 0.7c
Behenic acid (C22:0)	0.25 ± 0.02^{a}	0.41 ± 0.09^{b}	0.5 ± 0.05^{b}
8,11,14-Eicosatrienoic acid (C20:3n6)	0.09 ± 0.01^{a}	0.06 ± 0.04^{a}	0.04 ± 0.01^{a}
Erucic acid (C22:1n9)	0.26 ± 0.01^{a}	0.17 ± 0.06^{b}	0.11 ± 0.01^{b}
11,14,17-Eicosatrienoic acid (C20:3n3)	0.17 ± 0.02^{a}	0.12 ± 0.04^{ab}	0.07 ± 0.01^{b}
Arachidonic acid (C20:4n6)	0.4 ± 0.08^{a}	0.34 ± 0.04^{a}	0.18 ± 0.03^{b}
13,16-Docosadienoic acid (C22:2)	0.86 ± 0.04^{a}	0.62 ± 0.04^{b}	0.38 ± 0.04^{c}
Lignoceric acid (C:24:0)	0.09 ± 0.01^{a}	0.15 ± 0.05^{ab}	0.18 ± 0.03^{b}
5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	8.06 ± 0.04^{a}	5.9 ± 0.4^{b}	3.72 ± 0.24^{c}
Nervonic acid (C24:1)	0.53 ± 0.03^{a}	0.4 ± 0.07^{b}	0.23 ± 0.09c
7,10,13,16,19-Docosapentaenoic acid (C22:5n3)	0.9 ± 0.2^{a}	0.64 ± 0.11^{a}	0.4 ± 0.05^{b}
4,7,10,13,16,19-Docosahexaenoic acid (c22:6n3)	11.19 ± 0.31^{a}	8.14 ± 0.36^{b}	5.13 ± 0.18^{c}
∑ SFA	35.8 ± 0.1^{a}	26.81 ± 0.02^{b}	18.53 ± 0.02^{c}
∑ MUFA	23.04 ± 0.04^{a}	25.94 ± 0.06^{b}	35.83 ± 0^{c}
∑ HUFA	19.25 ± 0.05^{a}	14.04 ± 0.02^{b}	7.39 ± 0.02c
∑ PUFA	29.84 ± 0.16^{a}	39.51 ± 0.37^{b}	39.85 ± 0.01b
∑ PUFA (n-3)	22.35 ± 0.01^{a}	16.2 ± 0.15^{b}	10.6 ± 0.06^{c}
∑ PUFA (n-6)	2.88 ± 0.02^{a}	20.11 ± 0.11^{b}	27.87 ± 0.01^{c}

Means in the same line with the same letter do not differ significantly at the level of 0.05 significance. nd: not detected; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid. n:3 (arithmetic mean±SD)

Fatty acids composition of pan-fried and baked rainbow trout

The fatty acid composition of rainbow trout was given in Table 2. Palmitic acid, stearic acid, and myristic acid were dramatically reduced in baked rainbow trout compared to raw material. However, they increased significantly in pan-fried rainbow trout. Additionally, the oleic acid content in baked rainbow trout remained similar to the raw one, while it was lower in fried rainbow trout compared to the other cooking methods.

Total SFA was the highest in fried rainbow trout. Total MUFA was the highest in raw rainbow trout but close to that of baked rainbow trout. Total HUFA, PUFA, n-3 PUFA, and n-6 PUFA were the highest in baked rainbow trout. Total n-3 PUFA was higher in baked rainbow trout than in raw and fried ones. The order for the n-3 PUFA content of rainbow trout is as follows: baked > raw rainbow trout > fried. Linoleic, eicosapentaenoic, and docosahexaenoic acids were higher in baked rainbow trout than in the other cooking methods.

Table 2. Fatty acids (%) profile of cooked by different methods and raw rainbow trout

Fatty acids composition	Raw	Baked	Fried
Caproic acid (C6:0)	0.13 ± 0.13^{a}	nd	0.72 ± 0.07^{b}
Caprylic acid (C8:0)	0.08 ± 0.08^{a}	nd	0.65 ± 0.07^{b}
Capric acid (C10:0)	nd	nd	0.68 ± 0.04
Undecanoic acid (C11:0)	0.04 ± 0.04^{a}	nd	0.13 ± 0.1^{b}
Lauric acid (C12:0)	0.07 ± 0.07^{a}	0.04 ± 0.02^{a}	0.76 ± 0.24^{b}
Tridecanoic acid (C13:0)	0.02 ± 0.02^{a}	0.01 ± 0.01^{a}	nd
Myristic acid (C14:0)	4.42 ± 0.81^{a}	1.94 ± 0.17^{b}	5.98 ± 0.53^{c}
Myristoleic acid (C14:1)	nd	0.02 ± 0.02^{a}	0.08 ± 0.02^{b}
Pentadecanoic acid (C15:0)	0.32 ± 0.32^{a}	0.15 ± 0.08^{a}	0.51 ± 0.06^{a}
Palmitic acid (C16:0)	24.45 ±1.87^{a}	11.43 ± 0.89^{b}	29.15 ± 0.3^{c}
Palmitoleic acid (C16:1)	3.69 ± 0.27^{a}	2.91 ± 0.14^{b}	1.66 ± 0.16^{c}
Heptadecanoic acid (C17:0)	0.33 ± 0.33^{a}	0.14 ± 0.05^{a}	0.41 ± 0.02^{a}
Stearic acid (C18:0)	7.14 ± 1.27^{a}	3.38 ± 0.46^{b}	9.21 ± 0.23^{c}
Elaidic acid (C18:1n9t)	0.1 ± 0.1^{a}	0.12 ± 0.02^{a}	0.04 ± 0.01^{b}
Oleic acid (C18:1n9c)	32.61 ± 2.6^{a}	33.12 ± 2.09^{a}	21.26 ± 2.79^{b}
Linoleic acid (C18:2n6c)	6.57 ± 0.99^{a}	24.47 ± 0.5^{b}	5.05 ± 0.9^{a}
Arachidic acid (C20:0)	0.37 ± 0.37^{a}	0.21 ± 0.11^{a}	0.44 ± 0.11^{a}
ɣ-Linolenic acid (C18:3n3)	0.08 ± 0.08^{a}	0.29 ± 0.05^{b}	0.02 ± 0.02^{a}
11-Eicosenoic acid (C20:1)	2.28 ± 1.28^{a}	2.15 ± 0.05^{a}	0.89 ± 0.09^{a}
α-Linolenic acid (C18:3n3)	0.89 ± 0.11^{a}	3.32 ± 0.18^{b}	0.44 ± 0.22^{b}
Heneicosanoic acid (C21:0)	0.02 ± 0.01^{a}	0.01 ± 0.006^{a}	nd
Eicosadienoic acid (C20:2)	0.61 ± 0.24^{a}	1.56 ± 0.09^{b}	0.25 ± 0.27^{a}
Behenic acid (C22:0)	0.18 ± 0.05^{a}	0.2 ± 0.15^{a}	0.52 ± 0.13^{b}
8,11,14-Eicosatrienoic acid (C20:3n6)	0.16 ± 0.07^{a}	0.45 ± 0.2^{b}	nd
Erucic acid (C22:1n9)	0.28 ± 0.28^{ab}	0.28 ± 0.08^{a}	0.11 ± 0.05^{b}
11,14,17-Eicosatrienoic acid (C20:3n3)	0.09 ± 0.09^{a}	0.32 ± 0.14^{a}	nd
Arachidonic acid (C20:4n6)	0.09 ± 0.06^{a}	0.4 ± 0.04^{b}	0.04 ± 0.005^{a}
13,16-Docosadienoic acid (C22:2)	0.16 ± 0.16^{a}	0.6 ± 0.2^{b}	0.03 ± 0.01^{c}
Lignoceric acid (C:24:0)	0.07 ± 0.1^{a}	0.08 ± 0.02^{a}	nd
5,8,11,14,17-Eicosapentaenoic acid (C20:5n3)	0.45 ± 0.09^{a}	1.75 ± 0.12^{b}	0.25 ± 0.11^{a}
Nervonic acid (C24:1)	0.27 ± 0.25^{a}	0.21 ± 0.12^{a}	0.09 ± 0.01^{a}
7,10,13,16,19-Docosapentaenoic acid (C22:5n3)	0.32 ± 0.14^{a}	0.63 ± 0.3^{a}	0.37 ± 0.12^{a}
4,7,10,13,16,19-Docosahexaenoic acid (c22:6n3)	1.25 ± 0.14^{a}	3.97 ± 0.05^{b}	0.83 ± 0.11^{c}
∑ SFA	37.64 ± 2.66^{a}	17.59 ± 1.36^{b}	49.16 ±3.47^{c}
∑ MUFA	39.23 ± 2.01^{a}	38.81 ± 1.42^{a}	24.13 ±2.23^{b}
∑ HUFA	1.7 ± 0.07^{a}	5.72 ± 1.53^{b}	1.08 ± 1.08^{a}
∑ PUFA	10.67 ± 0.91^{a}	37.76 ± 1.49^{b}	7.28 ± 0.57^{c}
∑ PUFA (n-3)	3.08 ± 0.67^{a}	10.28 ± 2.52^{b}	1.91 ± 0.3^{c}
∑ PUFA (n-6)	6.82 ± 1.54^{a}	25.32 ± 1.8^{b}	5.09 ± 0.19^{a}

Means in the same line with the same letter do not differ significantly at the level of 0.05 significance. nd: not detected; SFA: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; HUFA, highly unsaturated fatty acid. n:3 (arithmetic mean±SD)

In fried sardines cooked with sunflower oil, a significant decrease ($p < 0.05$) was observed in myristic, palmitic, palmitoleic, stearic, linolenic, arachidic, and docosahexaenoic acid (DHA). However, there was a significant increase

($p < 0.05$) in oleic and linoleic acid. The DHA content was decreased in all fried groups.

Indices of lipid quality

These indices take into account the many consequences that fatty acids may have on human health, especially the likelihood that atherosclerosis and/or thrombus formation may become more common. The atherogenic index (AI) and thrombogenicity index (TI) were respectively found 0.71±0.32 and 0.61±1.43 to be highest in butter-fried rainbow trout.

Therefore, the hypocholesterolemic / hypercholesterolemic (HH) index of rainbow trout fried in butter (0.79±0.17) was found to be the best in terms of nutritional quality. The atherogenic and thrombogenic index were determined lower in all groups of sardines. The hypocholesterolemic / hypercholesterolaemic index of fried sardine (4.85±0.3) in sunflower oil was found to be the best in terms of nutritional quality (Table 3).

Table 3. Lipid quality indices in sardine and rainbow trout after cooked by different methods

	Rainbow trout AI	Rainbow trout TI	Rainbow trout HH	Sardine AI	Sardine TI	Sardine HH
Raw	0.29 ± 0.19^{a}	0.23 ± 0.02^{a}	1.46 ± 0.54^{a}	0.06 ±0.01^{a}	0.02±0.01^{a}	1.4±0.23^{a}
Baked	0.01 ± 0.16^{a}	0.01 ± 0.02^{b}	5.05 ± 0.71^{b}	0.02 ±0.02^{b}	0.01± 0.01^{a}	2.76±0.14^{b}
Fried	0.71 ± 0.32^{b}	0.61 ± 0.43^{c}	0.79 ± 0.17^{a}	0.01±0.02^{b}	0.01 ± 0.00^{a}	4.85±0.3^{c}

Means in the same column with the same letter do not differ significantly at the level of 0.05 significance. n:3 (arithmetic mean±SD)

Mercury (Hg) content

The content of Hg is given in Table 4. The Hg content of raw rainbow trout was found 0.08 mg/kg. This value is 0.11 mg/kg in raw sardine. In both fish baking increased the Hg levels than frying. Fried rainbow trout was significantly different from raw and baked ones ($p < 0.05$).

Therefore, baked sardine was significantly different from raw and fried one. Fish had higher Hg contents after cooking, according to several studies (Girard et al., 2018; Burger et al., 2003).

Table 4. Mercury (Hg) content (mg/kg) of raw and cooked rainbow trout and sardine

Rainbow trout			Sardine		
Raw	**Baked**	**Fried**	**Raw**	**Baked**	**Fried**
0.08 ± 0.03^{a}	0.18 ± 0.07^{a}	0.08 ± 0.02^{b}	0.11 ± 0.1^{a}	0.29 ± 0.04^{b}	0.15 ± 0.03^{a}

Means in the same line in the same group with the same letter do not differ significantly at the level of 0.05 significance. n: 3 (arithmetic mean±SD)

DISCUSSION

Due to the association between these fatty acids and health benefits, the quantity of n-3 PUFAs in fish, particularly EPA and DHA, can be used to determine nutritional quality.

Our findings are consistent with those of Karimian-Khosroshahi et al. (2016). The nutritional value of rainbow trout was estimated by studying the effects of baking and pan-frying. The study examined the chemical composition, lipid quality indexes, fatty acid profile, and mercury levels of rainbow trout. Ideal n-6/n-3 human nutrition values are considered to be 1-1.5 or less (Larrieu and Layé, 2018). Baked sardines represent the optimum n-6/n-3 ratio, whereas these rates are quite high in pan-fried fish.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to have numerous health benefits, including reducing inflammation, lowering blood triglyceride levels, and reducing the risk of heart disease. According to the AHA, adults should aim to consume at least two servings of fatty fish per week, which can provide about 500 milligrams of EPA and DHA for daily intake. The European Food Safety Authority (EFSA) suggests a range of 250–500 mg/day based on cardiovascular risk concerns for European adults (Kris-Etherton et al., 2002).

The increased level of SFA in fried rainbow trout fillets is assumed to be caused by the butter used. Dairy products, in particular butter, have been considered to increase the risk for cardiovascular diseases in humans because, in comparison to other lipid sources, they contain a higher proportion of lauric, myristic, and palmitic acids and a lower proportion of unsaturated fatty acids (Sacks and Katan, 2002) proposed an atherogenic index (AI) for lipids as a dietary risk indicator for cardiovascular disease. Sunflower oil contains approximately 15% saturated, and 85% unsaturated fatty acid and consists of 14–43% oleic and 44–75% linoleic acids in its unsaturated fatty acid content (Akkaya, 2018).

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Effects of the cooking methods on lipid quality were related to the containing of meat dehydration, fat migration to the frying oil, and oil penetration to meat. Frying had the greatest impact on lipid quality, but its impact varied depending on the species.

Due to their ability to prevent the development of plaque and lower levels of cholesterol, phospholipids, and esterified fatty acids, unsaturated fatty acids are thought to be anti-atherogenic. As a result, consuming meals or goods with a lower AI can lower LDL-C and total cholesterol in blood plasma values. HH values for shellfish range from 1.73 to 4.75, except for *Loxechinus albus*. For fish, the values varied from 1.54 to 4.83, except for *Opisthonema oglinum*, which has an HH value of 0.87. For dairy products and meat, the ranges are 1.27–2.786, and 0.32–1.29, respectively (Chen and Liu, 2020). Fish consumption is the primary pathway through which people are exposed to mercury. Seafood is widely used in traditional cuisines around the world even though it quickly bioaccumulates mercury (Hg). Only a small number of previous studies on Hg in cooked seafood took into account both MeHg and Hg(II); the majority concentrated on total mercury (Liao et al., 2019). Although Hg levels (Burger et al., 2003; Khansari et al., 2005; Kalogeropoulos et al., 2012; Jadán-Piedra et al., 2017: Liao et al., 2019; Dahl et al., 2020) are typically highest in well-distributed fish organs like the liver, spleen, and kidney (Sandheinrich and Wiener, 2011; Matos et al., 2015) the greatest pool of Hg in fish is found in the muscle. In the fish muscle,> 95% of the Hg(II) is present as MeHg (Bloom, 1992).

It has been hypothesized that this has something to do with weight loss brought on by moisture and fat loss during cooking (Morgan et al., 1997). Multiple studies on mercury in fish have found that cooking leads to an increase in the wet weight content of mercury in fish, most likely as a result of moisture loss during preparation (Girard et al., 2018; Perugini et al., 2016). Since we also saw a slight drop in moisture after baking, our findings corroborate these mercury-related ones.

CONCLUSION

In conclusion, the atherogenicity (AI) and thrombogenicity (TI) indexes are two important predictors of future cardiovascular problems. They are calculated based on the concentrations of various FAs in the diet, and a higher value indicates a higher risk of developing cardiovascular disease.

On the other hand, the hypocholesterolaemic / hypercholesterolaemic (HH) index in fish fatty acids measures the effect of fish consumption on cholesterol levels in the body. A higher HH index indicates a more hypocholesterolemic effect, which may have potential health benefits. Overall, these indices provide essential information about the health effects of different types of dietary fatty acids and can be useful for developing personalized dietary recommendations.

The n3/n6 ratio, HH, AI, and TI are the best nutritional quality indices in fish. The atherogenicity (AI) and thrombogenicity (TI) indexes were found to be lower in fried sardines. The hypocholesterolaemic/hypercholesterolaemic (HH) index of fried sardine in sunflower oil were found to be the best in terms of nutritional quality

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AUTHORSHIP CONTRIBUTION STATEMENT

Şükran Çaklı: Conceptualization, methodolgy; Nida Demirtaş Erol: Formal analysis, resources; Evren Burcu Şen Yılmaz: Resources, formal analysis; Pınar Baldemir: Formal analysis; Atilla Çaklı: Formal analysis.

DECLARATION OF COMPETING INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study

DATA AVAILABILITY

All relevant data is in the article

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

A checklist and some new records on the teuthofauna of Türkiye in the Northeastern Mediterranean Sea

Kuzeydoğu Akdeniz Türkiye teuthofaunası kontrol listesi ve bazı yeni kayıtlar

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Abstract: The cephalopod species observed in this study were caught during two concurrent projects on the demersal fisheries resources conducted along the coasts of Türkiye in the Northeastern Mediterranean Sea. The samples were collected by a bottom trawl at depths ranging from 50 to 800 meters, following the guidelines set by MEDITS (International Bottom Trawl Survey in the Mediterranean). Through the study, a total of 22 cephalopod species were determined. Among them, *Heteroteuthis dispar* (Rüppell, 1844), *Chiroteuthis veranii* (A. Férussac, 1834), *Onychoteuthis banksii* (Leach, 1817), *Octopus salutii* Vérany, 1839 are being reported first time in the Mediterranean coasts of Türkiye.

Keywords: Cephalopoda, distribution, Eastern Mediterranean, Turkish coasts

Öz: Bu çalışmada incelenen sefalopod türleri Akdeniz'in kuzeydoğusunda Türkiye'nin kıyılarındaki demersal balıkçılık kaynaklarına yönelik yapılan iki çalışmada yakalanmıştır. Örnekler, MEDITS (Akdeniz'de Uluslararası Dip Trolü Araştırmaları) tarafından belirlenen yönergeler izlenerek 50 ila 800 metre arasında değişen derinliklerde dip trolü ile toplanmıştır. Bu çalışmada 22 sefalopod türü tespit edilmiş olup bunlardan *Heteroteuthis dispar* (Rüppell, 1844), *Chiroteuthis veranii* (A. Férussac, 1834), *Onychoteuthis banksii* (Leach, 1817), *Octopus salutii* Vérany, 1839 Türkiye'nin Akdeniz kıyıları için ilk kez rapor edilmektedir.

Anahtar kelimeler: Cephalopoda, dağılım, Doğu Akdeniz, Türkiye kıyıları

INTRODUCTION

Cephalopods are one of the most preferred seafood by humans due to either their high-level nutritive characteristics or their tastes. Increasing populations of the countries are led to the import of seafood from abroad, and they are also placed in Turkish fish markets, both fresh and frozen. As per the data from FAO (2022), in 2014, humans captured nearly 5 million tonnes of cephalopods, which plays a role in the food web of the ocean ecosystem. Moreover, the importance of cephalopods in the marine food web is overmuch than being human food, and Clarke (1996) stated that the expected annual consumption of cephalopods by only cetaceans, except for the other known predators, such as fishes, seabirds, and seals, was between 120 and 320 million tonnes.

Cephalopod fauna of the Mediterranean Sea occupied 10% of the known species in the world, and most of the species were reported from the surrounding seas of Türkiye, excluding the Black Sea, in different studies (Katagan et al., 1993; Salman et al., 2002; Salman, 2015; 2016). Although taxonomic studies on the group are comparatively diverse in Turkish seas, studies on their stocks and fisheries productivity are limited. (Salman et al., 1997; Salman and Katagan, 2004; Dereli et al., 2021). Until now, faunistic observations on cephalopods were generally taken into fisheries studies using demersal trawl, a preferred sampling gear for many benthic and demersal cephalopod species (D'Onghia et al., 1992; Belcari and Sartor, 1993; Salman et al., 1997; Salman and Katagan, 2004; Dereli et al., 2021). To determine the pelagic cephalopod species and their paralarvae, however, Isaacs-Kidd Midwater Trawl (IKMT) (Roper, 1974), which has a mouth opening of 2-3 m or Hamburg Plankton Net (HPN) was mainly used (Salman et al., 2003).

Because some cephalopod species are difficult to catch through fishing operations or other sampling methods mentioned above, their ecological roles in the ecosystem are inferred from the feeding habits of their natural predators. Understanding the distribution of cephalopod species is crucial,

and one way to gain insight is by analyzing the beaks found in the stomachs of predator fishes or cetaceans. However, this data may not always be entirely accurate due to the prolonged stability of the beaks in the stomach from a few weeks to several months and the predator's ability speed to migrate (Xavier et al., 2011).

The various physicochemical conditions and ecology of the seas surrounding Türkiye led to notable differences in the distribution of cephalopod species. Furthermore, the scarcity of studies on cephalopod fauna and stock structures is one of the main reasons for the present uncertainty regarding their taxonomy and biology in the Turkish seas. This study aims to provide a clear understanding of the current state of the cephalopod fauna living on the Mediterranean coast of Türkiye.

MATERIALS AND METHODS

The cephalopod specimens were collected using a bottom trawl at 41 stations chosen between the locations of 36°25'85"N - 30°31'03"E and 36°40'20"N - 36°10'99"E on the Turkish Mediterranean coast (Figure 1). Approximately 450 trawl operations were succeeded at these stations with depths ranging from 50 to 800 m by *R/V Akdeniz Araştırma 1* between 2015 and 2022. All trawl haulings were performed according to the MEDITS (International Bottom Trawl Survey in the Mediterranean) (Anonymous, 2016; 2017)

Trawl operations were carried out only during the daytime, starting 30 minutes after sunrise and ending 30 minutes before sunset. The hauling duration was limited to 30 min at depths shallower than 200 m and extended to one hour at depths deeper than 200 m. Before each sampling, whether the bottom structure was suitable for the trawling operation was checked with Simrad EK 60 scientific echo-sounder. Simrad PX II sensors were used to monitor crucial information such as trawling depth and trawl mouth opening throughout every trawling process. Cephalopod specimens were fixed in a 10% formalin solution, according to Roper and Sweeney (1983). Identification of the cephalopod species belonging to Sepiolida, Teuthida and Octopoda orders in the study was made according to Jereb and Roper (2005), Jereb and Roper (2010) and Jereb et al. (2016) respectively. After identification, their mantle lengths were measured by a fish measuring board to the nearest 0.1 mm and relatively rare species were recorded at the ESFM (Ege University Faculty of Fisheries Museum) in the international coding system.

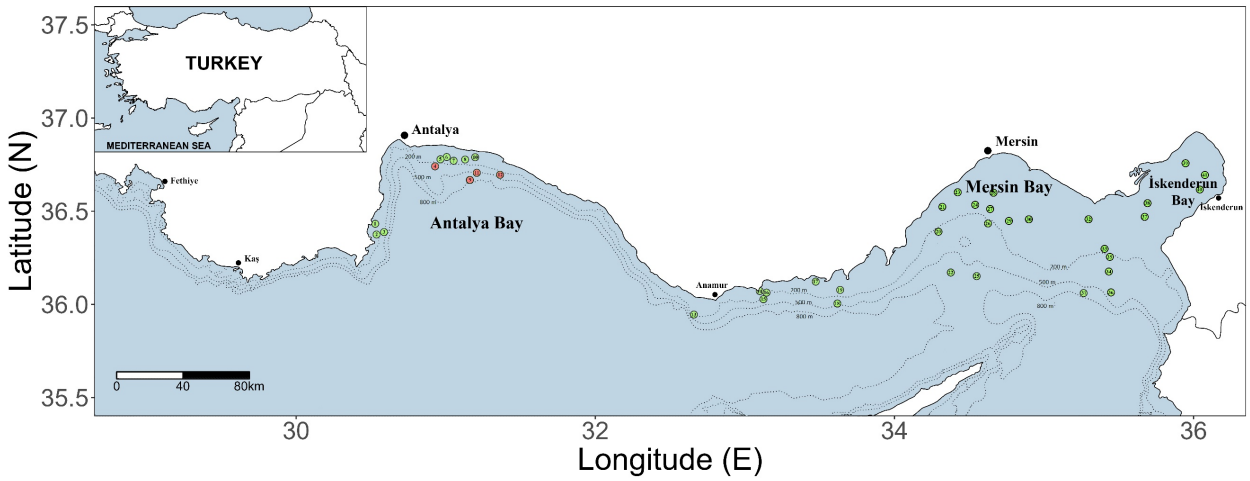

Figure 1. Trawling stations along the Mediterranean coast of Türkiye in the study- (The red colour stations in Antalya Bay indicated the locations for *Heteroteuthis dispar* (Sta. 4 and 11), *Chiroteuthis veranii* (Sta. 9), *Onychoteuthis banksii* (Sta. 11) and *Octopus salutii* (Sta. 12)).

RESULTS

A checklist of the cephalopods in the present study is given in Table 1. The columns about fish and cetaceans' stomachs could be considered a prediction of the species in the area due to the abovementioned reasons. During the present study, 22 cephalopod species were identified from the trawl compositions. From them, *Heteroteuthis dispar*, *Chiroteuthis veranii*, *Onychoteuthis banksii* and *Octopus salutii* were reported for the first time here with body morphological traits of the whole specimens, and all were caught in Antalya Bay (Figure 1).

The following species were recorded at the ESFM museum because of their rarity and obtaining difficulties;

Order: SEPIOLIDA

Family: SEPIOLIDAE

Heteroteuthis dispar (Rüppell, 1844)

Two specimens were recorded in Antalya Bay in 2017. One of them was sampled at 300 m depth (36°44'N-30°53'E) has a mantle length of 11 mm (ESFM-CEP-2017-006), and the other specimen from 560 m depth (36°39'N-31°13'E) has 19 mm mantle length (Figure 1; Sta. 4 and 11).

Table 1. Checklist of cephalopods in the Northeastern Mediterranean off Turkish coasts (T: Trawl; P: Plankton; ST-F: Stomach content of fishes; ST-M: Stomach content of mammals).

	Species	T	P	ST-F	ST-M
SEPIIDA					
Sepiidae	*Sepia officinalis* Linnaeus, 1758	2,5,6,8,**17**			
	Sepia elegans Blainville, 1827	5,6,8,**17**			
	Sepia orbignyana Férussac, 1826	6,**17**			
SEPIOLIDA					
Sepiolidae	*Heteroteuthis dispar* (Rüppell, 1844)	**17**		9,10	7,12
	Sepiola steenstrupiana Lévy, 1912	6			
	Rondeletiola minor (Naef, 1912)	6			
	Sepietta oweniana (d'Orbigny, 1841)	6,**17**			
	Sepietta neglecta Naef, 1916	6			
	Rossia macrosoma (Delle Chiaje, 1830)	6,**17**			
TEUTHIDA					
Loliginidae	*Loligo vulgaris* Lamarck, 1798	2,5,6,8,**17**	11		
	Loligo forbesii Steenstrup, 1856	6,**17**			
	Alloteuthis media (Linnaeus, 1758)	6,**17**			
	Alloteuthis subulata (Lamack, 1798)	6			
	Sepioteuthis lessoniana d'Orbigny, 1826	4			
Ancistrocheiridae	*Ancistrocheirus lesueurii* (d'Orbigny, 1842)			9	7,15,15
Brachioteuthidae	*Brachioteuthis riisei* (Steenstrup, 1882)				7,12,15
Chiroteuthidae	*Chiroteuthis veranii* (A. Férussac, 1834)	**17**		10	7,12,15
Chtenopterygidae	*Chtenopteryx sicula* (Vérany, 1851)			9	7,12
Enoploteuthidae	*Abralia veranyi* (Rüppell, 1844)	6, **17**			7,12
Histioteuthidae	*Histioteuthis bonnellii* (A. Férussac, 1835)				7,12,15
	Histioteuthis reversa (A. E. Verrill, 1880)	14,16,**17**		10	7,15
Octopoteuthidae	*Octopoteuthis sicula* Rüppell, 1844	13,**17**		10	7,12,15
Ommastrephidae	*Illex coindetii* (Vérany, 1839)	6,8,**17**		9	
	Todaropsis eblanae (Ball, 1841)	6,**17**		9	
	Todarodes sagittatus (Lamarck, 1798)	6,**17**		9,10	7
	Ommastrephes bartramii (Lesueur, 1821)	1		10	7
Onychoteuthidae	*Onychoteuthis banksii* (Leach, 1817)	**17**		9,10	7,12
	Ancistroteuthis lichtensteinii (A. Férussac [in A. Férussac & d'Orbigny], 1835)			10	12,15
Pyroteuthidae	*Pyroteuthis margaritifera* (Rüppell, 1844)	6, **17**		10	7,12,15
	Pterygioteuthis giardi H. Fischer, 1896			10	12
OCTOPODA					
Octopodidae	*Octopus vulgaris* Cuvier, 1797	2,5,6,8,**17**			
	Octopus salutii Vérany, 1839	**17**			
	Amphioctopus cf. aegina/kagoshimensis (Gray, 1849)	3,5,6			
	Callistoctopus macropus (Risso, 1826)	6,8			
	Macrotritopus defilippi (Vérany, 1851)	5,6			
	Pteroctopus tetracirrhus (Delle Chiaje, 1830)	6			
	Scaeurgus unicirrhus (Delle Chiaje [in Férussac & d'Orbigny], 1841)	6,**17**		10	
	Eledone cirrhosa (Lamarck, 1798)	8		10	
	Eledone moschata (Lamarck, 1798)	2,5,6,8,**17**			
Argonautidae	*Argonauta argo* Linnaeus, 1758			9,10	
Tremoctopodidae	*Tremoctopus violaceus* delle Chiaje, 1830			9,10	7
Total number of species by different sampling methods		**33**	**1**	**18**	**16**

1= Katagan et al. (1993); 2= Gücü and Bingel (1994); 3= Salman et al. (1999); 4= Salman (2002); 5= Duysak et al. (2004); 6= Salman and Katağan (2004); 7= Öztürk et al., (2007); 8= Duysak et al. (2008); 9= Karakulak et al. (2009); 10= Salman and Karakulak (2009); 11= Salman (2012); 12= Dede et al. (2016); 13= Jereb et al. (2016); 14= Gökoğlu et al. (2021); 15= Tonay et al. (2021); 16= Üstüner and Gökoğlu (2022); 17= Present study

Order: TEUTHIDA

Family: CHIROTEUTHIDAE

Chiroteuthis veranii (A. Férussac, 1834)

Two specimens were found in Antalya Bay (36°41'N-31°14'E) at 654 m depth in the Northeastern Mediterranean Sea during 2022 surveys. Only one has a whole body (ML= 64 mm) (ESFM-CEP-2022-001). Unfortunately, only the head of the second specimen was observed (Figure 1; Sta. 9)

Family: ENOPLOTEUTHIDAE

Abralia veranyi (Rüppell, 1844)

Five specimens were recorded in Antalya Bay (36°41'N-31°14'E) 2016 at 560 m depth. The mantle lengths of the specimens were between 45-52 mm (ESFM-CEP-2016-001). In addition, five juvenile specimens were observed in the same survey at 435 m depth (36°'42'N – 31°15'E) with mantle lengths between 11-25 mm. Also, in 2022, four specimens from Antalya Bay (36°41'N-31°14'E) were caught at 654 m depth mantle lengths ranging from 36 to 44 mm (ESFM-CEP-2022-002).

Family: HISTIOTEUTHIDAE

Histioteuthis reversa (A. E. Verrill, 1880)

Three specimens were recorded in 2015 and 2017. The first specimen Antalya Bay (36°41'N-31°07'E), at 600 m depth ML=89 mm (ESFM-CEP-2015-002), second specimen in Antalya Bay (36°39'N-31°05'E) at 560 m depth ML=27 mm (ESFM-CEP-2017-003) and last one from off Silifke coasts, in Mersin Bay (36°09'N-34°25'E) 620 m depth ML=26 mm (ESFM-CEP-2017-002)

Family: OCTOPOTEUTHIDAE

Octopoteuthis sicula Rüppell, 1844

One specimen was caught off Silifke coasts in Mersin Bay (36°09'N-34°23'E) at 650 m depth in the Northeastern Mediterranean Sea in 2017 (ML= 39 mm) (ESFM-CEP-2017-001).

Family: OMMASTREPHIDAE

Todaropsis eblanae (Ball, 1841)

One specimen was found in Antalya Bay (36°02'N-33°09'E) at 255 m depth in the northeastern Mediterranean Sea. (ML= 93 mm). No record number was given because the species already have specimens in EFSM.

Todarodes sagittatus (Lamarck, 1798)

Four specimens were found in Antalya Bay. Two of them (36°44'N-30°53'E) were caught at 440 m, has ML= 308-245 mm and others (36°39'N-31°05'E) were caught at 300 m has ML=227-229 mm. No record number was given because the species already have specimens in EFSM.

Family: ONYCHOTEUTHIDAE

Onychoteuthis banksii (Leach, 1817)

Two juvenile specimens were found in Antalya Bay (36°39'N-31°13'E), the Northeastern Mediterranean Sea at 560 m. (ML= 21 and 33 mm) (ESFM-CEP-2017-005) (Figure 1; Sta. 11).

Family: PYROTEUTHIDAE

Pyroteuthis margaritifera (Rüppell, 1844)

One specimen was found (36°39'N-31°05'E) at 560 m depth in Antalya Bay in 2016 (ML= 23 mm) (ESFM-CEP-2016-003).

Order: OCTOPODA

Family: OCTOPODIDAE

Octopus salutii Vérany, 1839

One specimen was found in Antalya Bay (36°41'N-31°21'E) at 300 m in the Northeastern Mediterranean Sea. (ML= 71 mm). No record number was given because the species already have specimens in EFSM (Figure 1; Sta.12).

Scaeurgus unicirrhus (Delle Chiaje [in Férussac & d'Orbigny], 1841)

One specimen was caught in Antalya Bay (36°43'N-31°09'E) at 440 m in 2017 (ML= 22 mm) (ESFM-CEP-2017-007).

DISCUSSION

Although faunistic cephalopod studies were mainly based on trawling operations in Turkish seas (Gücü and Bingel, 1994; Salman and Katağan, 2004; Duysak et al., 2004, 2008), some additional records were identified from paralarva and juvenile specimens in plankton samples (Salman, 2012). Besides that, cephalopod beaks could be seen in the stomachs of predatory fishes (Karakulak et al., 2009; Salman and Karakulak, 2009) and cetaceans (Öztürk et al., 2007; Dede et al., 2016; Tonay et al., 2021). However, these beaks could remain undigested in the stomachs of predators for nearly a few months, leading to inaccurately identifying the species' origin (Xavier et al., 2011). For this reason, more robust results of the faunistic studies on cephalopods are only being enabled by identifying the species using body morphological traits from specimens in the whole condition.

If we combine 22 cephalopod species observed in the study and the remaining 11 species from the other studies on the Mediterranean coasts of Türkiye, we could say that 33 species are distributed in the area, and that is roughly half of the known 67 species (Salman, 2015) in the Mediterranean basin. That also indicates that the faunistic studies in the area have been insufficient until now. If we consider the cephalopod beaks found in stomachs to represent the exact location of the species, the number could potentially increase to 41 (Table 1). In this context, however, four species namely, *Heteroteuthis dispar*, *Chiroteuthis veranii*, *Onychoteuthis banksii* and *Octopus salutii* were reported from their remaining beaks in the

stomach contents of fishes or cetaceans incidentally or in planned studies in the Northeastern Mediterranean before the present study, they have reported by the first time here with body morphological traits of the whole specimens.

On the other hand, the occurrence of planktonic cephalopod paralarva randomly once from zooplankton sampling in the past (Table 1) clearly shows that the knowledge on the ontogeny of these species almost none, and there were no planned studies conducted on the subject up to now. Also, the origin of the rare cephalopod species, such as reported by Gökoğlu et al. (2021) and Üstüner and Gökoğlu (2022), obtained from incidental catches of commercial trawling operations is another sign of that. Moreover, faunistic studies on the pelagic cephalopods and paralarvae were started by Degner (1925) and were followed by Roper (1974) and Salman (2012).

Consequently, cephalopods are primarily consumed by top predators in marine ecosystems, but they also provide a significant food source for humans. Despite their ecological and commercial importance, research on cephalopods has been insufficient, and systematic research on cephalopod ontogeny, both in the pelagic and deep sea, is needed to fill these knowledge gaps. Moreover, it is also be accounted fishery management and sustainability studies on commercially important species should be improved.

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AUTHORSHIP CONTRIBUTIONS

The first author was responsible for identifying the species, designing the manuscript, and writing. The second and third authors contributed to collecting the specimens in the field studies and manuscript editing. The last author was involved in the field studies and manuscript editing.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL

No specific ethical approval was required for this study.

DATA AVAILABILITY

All relevant data is inside the article.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Investigation of otolith mass asymmetry in three stocks of European sardine, *Sardina pilchardus* (Walbaum, 1792) from Türkiye

Türkiye'den üç sardalya, *Sardina pilchardus* (Walbaum, 1792) stoğunun otolit kütle asimetrisinin incelenmesi

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Abstract: It was aimed to investigate sagittal otolith mass asymmetry *Sardina pilchardus* sampled from Aegean, Marmara and Mediterranean seas of Türkiye in present study. In this study, differences between right and left otoliths were statistically significant for Marmara and Mediterranean seas (P<0.05) not significant for Aegean Sea, (P>0.05). The mean values of otolith mass asymmetry (X) were found between 0.0393 and 0.0144 according to Aegean Sea, Marmara Sea and Mediterranean Sea, respectively. In addition, absolute mass asymmetry $|X|$ were calculated as 0.03226±0.00514, 0.02057±0.00439 and, 0.05141±0.00755 for *S. pilchardus* samples according to Aegean Sea, Marmara Sea and Mediterranean Sea, respectively. The present study showed that the otolith mass asymmetry in *S.pilchardus* does not depend on fish length and otolith growth. Also, when there were no significant differences between localities for otolith mass asymmetry (P>0.05), there were significant differences for absolute otolith mass (P<0.05). The value of the otolith mass asymmetry can reveal information about pollutants such heavy metals, pesticides, stressors, and changes in the physico-chemical characteristics of water in relation to the environment of fishes. This is the first study about otolith mass in three stocks of *S. pilchardus* (Walbaum, 1792) from Türkiye.

Keywords: *Sardina pilchardus*, otolith, mass asymmetry, stock, Türkiye

Öz: Bu çalışmada, Türkiye'nin Ege, Marmara ve Akdeniz kıyılarından örneklenen *Sardina pilchardus*'un sagittal otolit kütle asimetrisi çalışılmıştır. Bu çalışmada, sağ ve sol otolitler arasındaki fark Marmara ve Akdeniz için istatistiksel olarak önemli (P<0,05), Ege Denizi için önemsiz (P>0,05) olarak bulunmuştur. Ortalama X değerleri sırasıyla Ege Denizi, Marmara Denizi ve Akdeniz'e göre 0,0393 ile 0,0144 arasında bulunmuştur. Ayrıca, *S. pilchardus* örneklerinin $|X|$ değerleri Ege Denizi, Marmara Denizi ve Akdeniz'e göre sırasıyla 0,03226 ±0,00514, 0,02057±0,00439 ve 0,05141± 0,00755 olarak hesaplanmıştır. Bu çalışmanın sonuçlarına göre, *S. pilchardus*'ta otolit kütle asimetrisinin (X) balık boyu ve otolit büyüme hızına bağlı olmadığı görülmüştür. Ayrıca, otolit kütle asimetrisi lokaliteler arasında anlamlı farklılık göstermezken (P>0,05), mutlak otolit kütle asimetrisi lokaliteler arasında farklılık göstermiştir (P<0,05) Otolit kütle asimetri değeri; ağır metaller, pestisitler, stres faktörleri, suyun fiziko-kimyasal özellikler gibi balıkların yaşadığı ortamla ilgili değişiklikler ve kirlilik faktörleri hakkında bilgi verebilir. Bu çalışma, Türkiye'den üç *S. pilchardus* (Walbaum, 1792) stokunda otolit kütlesi ile ilgili ilk çalışmadır.

Anahtar kelimeler: *Sardina pilchardus*, otolit, kütle asimetrisi, stok, Türkiye

INTRODUCTION

The fish otoliths, often known as "ear bones," are calcareous structures found in the inner ear of vertebrates that serve as sound receptors and balance organs (Schulz-Mirbach and Ladich, 2016; Tuset et al., 2021). In most species the sagitta is large, but in some species, the asteriscus is larger like Cypriniformes, Siluriformes, Characiformes and Gymnotiformes (Berra and Aday, 2004). These calcareous structures, in which the life histories of fishes are hidden, have been the most basic element of many different studies until today (Yu et al., 2014; Yilmaz et al., 2015; Bostanci and Yedier, 2018; Chesalin, 2021; Ozpicak et al., 2021; Pavlov, 2022; Jawad et al., 2023). The morphology of otoliths is extremely unique and varies greatly throughout fish families, however, it can also be quite species-specific (Maisey, 1987). Many studies published in recent years have revealed that otolith mass asymmetry (OMA) is a very important study area because of playing a major role in acoustic functionalities (Lychakov et al., 2006; Jawad et al., 2011; Jawad et al., 2017; Yedier et al., 2018; Bouriga et al., 2021). Because the otoliths of teleost fishes are easily quantified, they make an excellent biological model for analyzing the physiological significance of OMA (Lychakov et al., 2006).

In fishes, otoliths should be bilaterally symmetrical, but in some circumstances, weight discrepancy between the left and right otolith masses is seen, which is known as otolith mass asymmetry (Yedier et al., 2018). When fishes are subjected to weightlessness during parabolic or space flight, OMA may be at least partly responsible for anomalous behavior in fish (Lychakov et al., 2006). In order to analyze otolith displacements with respect to OMA and otolith mass under sound stimulation or gravity, mathematical models were used (Lychakov and Rebane, 2005). According to various studies on OMA, X values range between -0.2 and +0.2 (Jawad, 2013; Bouriga et al., 2021). In this concept, if X has an absolute value greater than 0.2, a fish's ability to produce sound may change (Lychakov et al., 2006). Therefore, OMA is a very important field of study both for the environment in which the fish live and

for the adaptation of the fish to their environment.

Pilchard or sardine, *Sardina pilchardus* (Walbaum, 1792) is one of the most popular sea fish worldwide. Sardine is an economically important and short-lived pelagic fish (Silva et al., 2019), widely distributed throughout the Mediterranean Sea (Jemaa et al., 2015), Northeast Atlantic, from the Celtic and North Seas to Mauritania and Senegal (ICES, 2018), with smaller populations in the Archipelagos of Azores, Madeira, and the Canary Islands and, is also found in the Mediterranean, Marmara, and Black seas (Parrish et al., 1989). Commercially valuable marine pelagic fish populations are frequently being driven to extinction due to excessive overfishing (Pauly et al., 2003; Atarhouch et al., 2006). European sardine catches have decreased in several areas during the past ten years, and nearly all of its geographic range has been identified as having totally or extensively exploited (FAO, 2018, 2019; ICES, 2018). There are many different studies on the genetics (Atarhouch et al., 2006;), ecology (Chouvelon et al., 2014; Castalago et al., 2015), fishing (Marçalo et al., 2006; Molina-Fernandez et al., 2015), feeding (Garrido et al., 2008; Costalago and Palomera, 2014), age and growth (Dahel et al., 2016; Baldè et al., 2022), and morphology (Silva, 2003; Baibai et al., 2012) of this fish species, which is very important in economically. Although the increase in OMA research throughout the world, there is no study about OMA of *S. pilchardus* in Türkiye.

According to literature, the harvested sardines from northern coasts such as Marmara and Aegean seas, are smaller than samples from Mediterranean Sea (Sarmasık et al., 2008). The temperature effect on development rate (hot Mediterranean waters versus cooler Aegean and Marmara seas) may be the only explanation for this, although size disparities may also be influenced by the genetic background of different stocks. Therefore, these differences can also affect the otolith characteristics. In addition, this study is aimed to investigate otolith mass asymmetry and compare total length-OMA and total length-absolute otolith mass asymmetry relationships in three stocks of *S. pilchardus* from Aegean, Marmara and Mediterranean seas.

MATERIALS AND METHODS

Study material and sampling

Samples of sardine were obtained from commercial fishermen from Aegean Sea (AS) (n=50), Marmara Sea (MS) (n=49), and Mediterranean Sea (MEDS) (n=50) in Türkiye. Specimens were defrosted for laboratory analysis about one month later to ensure that all fish were analyzed after a similar period of being frozen. The total length (L_T) of each sample was measured to ± 0.1 cm.

Otolith extraction and mass asymmetry

Sagittal otolith pairs were removed by making right and left distinctions. Otoliths were dried and kept in eppendorf tubes after cleaned with distilled water. Otolith pairs were weighted (± 0.0001g). Otolith mass asymmetry (X) was determined by using the following formula:

$$X = (M_R - M_L) / M,$$

M_R and M_L are the otolith masses of cleaned right and left otoliths and M is the average mass of M_R and M_L. Theoretically, otolith mass asymmetry (X) could vary from -2 to +2. 'Zero' ($M_R = M_L$) value denotes the absence of the mass asymmetry, whereas '-2' or '+2' values imply greatest asymmetry. When the value of X is positive, the right otolith mass is greater than the left otolith mass, and when it is negative, the opposite is true.

Additionally, the formula X=a×TL+b was used to compute the relationship between (X) and total length, as well as absolute otolith mass asymmetry ($|X|$) and total length. The paired t-test was used to examine the left and right otoliths and examine any variations between variables across all samples. Also, otolith mass asymmetry was compared with ANOVA between localities. Statistical analyses were performed in SPSS 21.0, Minitab 17.0 software, and the Microsoft Excel packages.

RESULTS

Sardina pilchardus samples were collected from Aegean Sea (12.93±0.13 cm TL), Marmara Sea (13.02±0.10 cm TL) and Mediterranean Sea (22.84±0.17 cm TL). Descriptive statistics of otolith weight in *S. pilchardus* individuals were in Table1.

Table 1. Descriptive statistics of *S. pilchardus* otolith weights according to localities

Locality	N	Side	Mean	±SE	±SD	Minimum	Maximum	*P*
Aegean Sea	50	R	0.0013	0.00004	0.0003	0.0006	0.0019	0.077
		L	0.0013	0.00004	0.0003	0.0006	0.0020	
Mediterranean Sea	49	R	0.0032	0.00012	0.0008	0.0022	0.0051	0.000*
		L	0.0033	0.00012	0.0009	0.0020	0.0051	
Marmara Sea	50	R	0.0012	0.00004	0.0003	0.0007	0.0021	0.007*
		L	0.0013	0.00004	0.0003	0.0007	0.0021	

*There are statistically differences between the right and left otolith pairs; *n*, number of samples R, Right; L, Left; SE, Standard Error; SD, Standard Deviation; Mean, Average otolith weight; *P*, Significance

Otolith mass asymmetry was calculated as -0.10081≤X≤ +0.10081, -0.10081≤X≤ +0.10081, and -0.20161≤X≤ +0.10081 for Aegean Sea, Marmara Sea and Mediterranean Sea, respectively (Table 2). According to results, right otoliths are heavier than left otoliths (70% for Aegean Sea, 65% for Marmara Sea and 51% for Mediterranean Sea). In addition, the mean values of $|X|$ were calculated as 0.03226 ±0.00514, 0.02057±0.00439 and, 0.05141± 0.00755 for *S. pilchardus* samples according to Aegean Sea, Marmara Sea and Mediterranean Sea, respectively.

Table 2. Descriptive statistics of otolith mass asymmetry of *S. pilchardus* according to localities

Locality	Mean	SE	SD	Minimum	Maximum
Aegean Sea	-0.0121	0.0067	0.0473	-0.1008	0.10081
Mediterranean Sea	-0.0393	0.0089	0.0630	-0.20161	0.10081
Marmara Sea	0.0144	0.0049	0.0341	-0.10080	0.10081

Mean, Average otolith mass asymmetry; SE, Standard Error; SD, Standard Deviation; *P*,Significance

Moreover, when there were no significant differences between localities for otolith mass asymmetry (Kruskal-Wallis test, $P>0.05$), also, there were significant differences for absolute otolith mass ($P<0.05$). In addition, the correlation coefficients r^2 and regression equations were calculated for all of the localities (Figure 1, Figure 2). Correlation coefficients and regression equations were y = -0.0094x + 0.1091; r^2 = 0.0306, y = -0.0005x-0.0079; r^2= 0.0001 and, y = -0.0005x-0.0079; r^2= 0.0917 for Aegean Sea, Marmara Sea and Mediterranean Sea, respectively (Figure 1a, b and c).

Based on the results of regression analysis of total length-absolute otolith mass asymmetry, the correlation coefficients and regression equations were calculated as y = 0.0076x - 0.0663; r^2= 0.0342, y = -0.0022x + 0.0492; r^2 = 0.0026, and y = 0.013x -0.2447; r^2 = 0.0809 for Aegean Sea, Marmara Sea and Mediterranean Sea, respectively (Figure 2a, b and c). The results do not support the hypothesis since no significant correlation between *X*-total length ($0.0001 \leq r^2 \leq 0.0917$) and **|***X***|**-total length was found ($0.0026 \leq r^2 \leq 0.0803$).

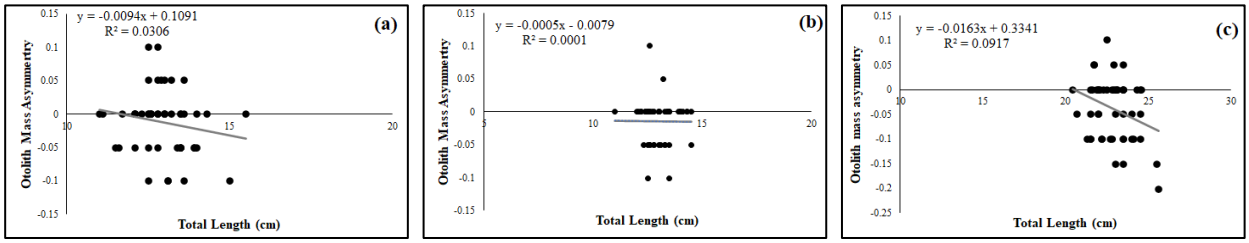

Figure 1. Sagittal OMA (X) and total length relationship in *S.pilchardus* (a) Aegean Sea, (b) Marmara Sea, (c) Mediterranean Sea

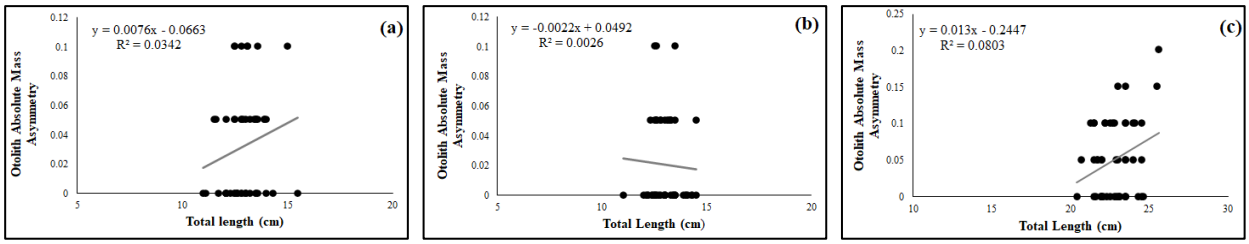

Figure 2. I*X*I and total length relationship in *S.pilchardus* (a) Aegean Sea, (b) Marmara Sea, (c) Mediterranean Sea

DISCUSSION

In fisheries science there are many investigations on OMA, and the otolith weight asymmetry values were found to be in the range of $-0.2<X<+0.2$ for marine and freshwater species (Lychakov, 1992; Lychakov and Rebane, 2005; Yedier et al., 2018; Kontas et al., 2019; Jawad and Quasim, 2020; Bouriga et al., 2021; Jawad et al., 2021; Jawad and Adams, 2022). Our results showed to fall within that range ($-0.20161 \leq X \leq +0.10081$). In addition, the otolith weight asymmetry was less than 0.06, which was lower than the value found for many marine species (Lychakov et al., 2006) and was unaffected by the otolith growth stage. Reduced acoustic and vestibular functionality in fish ears is thought to be a result of OMA (Lychakov and Rebane, 2005). Bouriga et al. (2021) were calculated *X* between $-0.3636 \leq X \leq 0.1538$ for *S. pilchardus* from Gulf of Tunis. Differences in OMA may be affected by the ecological conditions, physiological state of species and its habitat (Grønkjær, 2016). Both genetic and environmental conditions have an impact on the morphological variability of sagitta (Lombarte et al., 2010; Annabi et al., 2013). Additionally, changes in otolith mass asymmetry can have a deleterious impact on other aspects of fish life, particularly their ability to hear and balance. In accordance with other fish species' conditions, the otolith weight difference grows with fish length. (Lychakov and Rebane, 2004).

Additionally, Lychakov and Rebane (2005) demonstrated that only fish with big otoliths and $|X|$ >0.2 may, in principle, experience difficulty with sound handling as a result of improper and inconsistent movement of the fish's two otoliths on either side of its head. As a result, because their otolith weight non-symmetry is below acute levels, the majority of fish species can flee with effective incapacity. According to Lychakov and Rebane (2005), only fish with the biggest otoliths and $|X|$ >0.2 might theoretically experience issues with sound processing because of the disparity and peculiar movement of the fish's otoliths on either side of its head. In the present study, otolith absolute mass asymmetry is very low, $|X|$ was found between 0.02057±0.0043 and 0.05141±0.00755 according to

localities. There are similar results in the literature, too (Jawad et al., 2017; Bouriga et al., 2021; Jawad et al., 2021).

In several investigations, the relationship between the total length and the otolith mass asymmetry has been examined. Although, sagittal otolith mass disparities rise with fish length, this is a phenomena in bottom fish more than pelagic fish (Lychakov et al., 2006). However, results of present study do not support the hypothesis since no significant relation between mass asymmetry-total length ($0.0001 \leq r^2 \leq 0.0917$) and absolute otolith mass asymmetry-total length was found ($0.0026 \leq r^2 \leq 0.0803$) (Jawad, 2013; Yedier et al., 2018; Bouriga et al., 2021). Similiar to the literature, there is no correlation between fish size and otolith mass asymmetry in *S.pilchardus* which is a pelagic fish species. Fish length and otolith mass asymmetry are thought to be related in a complicated trend.

Numerous research conducted throughout the world examined the otolith mass and shape asymmetries. In Türkiye, otolith mass asymmetry studies are limited (Yedier et al., 2018; Kontaş et al., 2019). However, in Türkiye, which is surrounded by seas on three sides, it plays a very important role in fisheries studies to examine the lives of fishes and to obtain maximum efficiency in them. Exposure to domestic, industrial, and agricultural wastes causes ongoing degradation of the aquatic environment, and the harm that pollution is doing to the ecosystem is getting worse (Turgut and Özgül, 2009). Stress is brought on in aquatic animals by pollution in their surroundings. In fish, this stress may lead to developmental instability. The fact is that, according to earlier research in otolith mass asymmetry, there is a connection between environmental stress and pollution-related asymmetry (Jawad et al., 2012). Currently, otolith mass asymmetry may be a result of environmental stress.

Somatic development and otolith accumulation are indirectly impacted by environmental variables. Additionally, the otolith mass asymmetry is a low-cost method to assess the environmental health state, and as a suggestion, populations of other fish species should be checked for this kind of bilateral asymmetry. It should be evident that there is a special physicochemical mechanism for paired otolith formation that keeps the otolith mass a disparity as low as possible. This mechanism is still not fully understood. However, contrary to other data, the otolith weight does not appear to play a role in the feedback regulation of its growth (Lychakov, 2002).

Future studies on otolith mass asymmetry in marine fish are expected to use the results of the current study as a baseline, enabling researchers to compare the otolith mass asymmetry of *Sardina pilchardus* populations from Türkiye and overseas.

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AUTHORSHIP CONTRIBUTIONS

Melek Ozpicak: Conceptualization; Investigation, methodology, resources, software, validation, writing – original draft, writing – review and editing. Semra Saygin: Investigation, methodology, resources, software, validation, writing – original draft, writing – review and editing.

CONFLICT OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Aflatoxin, bacterial and heavy metal load in *Scomber scombrus* and *Clupea harengus* from two selected coldroom facilities in Kwara State, Nigeria

Nijerya Kwara eyaletinde seçilmiş iki soğuk oda işletmesinde *Scomber scombrus* ve *Clupea harengus*'taki aflatoksin, bakteri ve ağır metal birikimi

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Abstract: Impurities found in fish have been a major cause of disease and illness to consumers. This study's objective was to evaluate the total aflatoxin, heavy metal and microbial load in two frozen fish: *Scomber scombrus* and *Clupea harengus* from two (2) cold room facilities in Omu-Aran and Ilorin. Heavy metals, aflatoxin and microbial load were investigated using standard methods. Health risk was also determined using health risk index (HRI), daily intake of metals (DIM), and health quotient (HQ), and total toxicity of metals (TTM). Cd, Cu, Ni, Pb, Mn and Cr did not differ significantly ($p>0.05$) in both species from both locations. Nickel was lower than the recommended limit by World Health Organization (WHO). Level of Mn and Cr were higher in both species. Mn load was higher in the muscles of the sampled fish than in the gills from September through to November with highest value of 1.26±0.08 and 1.30±0.12 obtained for *S. scombrus* and *C. harengus*. Highest concentrations of all metals was observed in the gills except manganese and copper [Cd = 0.03 (*S. scombrus*), Cr = 1.22±0.13 (*S. scombrus*), Ni = 0.025±0.04 (*S. scombrus*), Pb = 0.06±0.02 (*S. scombrus*)]. HRI was > 1 in the different age groups for the different metals. TTM was > 1 in both species. Total aflatoxin level was higher in the gills (4.25 – 5) ppb than in the muscle (1.5 - 3) ppb for both locations respectively. *Vibrio* spp. and non-coliform bacteria were high in both species from both locations. The study concludes that heavy metal loads (Mn, Cd, Cu, Cr, and Ni) were more than the permitted limitations imposed by FAO, WHO and EU legislation for fish and fish products placing consumers at health risk.

Keywords: Frozen fish, muscle, gill, heavy metal, bacterial load, aflatoxin

INTRODUCTION

In Nigeria, fish is recognized and cherished as a healthy, safe and a cheap source of protein (Amuneke et al., 2020) adding an average of 17% to the protein intake (Boyd et al., 2022), and per capita consumption of 20.2 kg per annum in 2020, and a total consumption of 157 million Mt (FAO, 2022). Fish importation as reported by the Minister of Agriculture and Rural Development accounts for about 75% of the total consumption. White croaker (*Umbrina canosai*), African mackerel (*Scomber scombrus* Linnaeus, (1758)), and herring (*Clupea harengus* Linnaeus, (1758)) are three popular frozen fish in the Nigerian markets, and they make up a sizable portion of the imported fish consumed in Nigeria. However, water body pollution due to the presence of heavy metal attributable to industrialization, and agricultural activities has grown to be a significant public health and environmental concern.

Nigeria has many public frozen fish sales outlets and retail marketplaces where both retailers and consumers regularly purchase frozen seafood items. Though, several investigations has been conducted on heavy metal loads of frozen foods in Nigeria market (Kareem et al., 2016; Ogundiran, et al., 2014), there are only a few of these studies that considered their sources (Abubakar et al., 2015). Frozen fish enters into the Nigeria market from different countries of which Russia, Netherlands, Chile are popular. Other countries are Mauritania, Faroe Island, Ireland, Japan, Norway, Peru, and Morocco. Besides the differences in source, the safety regulations in these countries varies, and the state of the water body also differ.

Although, fish is appreciated as one of the healthiest and cheapest source of protein, if contaminated fish are consumed, it can result in associated health risk hazard. According to Bintsis (2017), perhaps the most pervasive health issue of our time is foodborne disease, which also contributes significantly to lower economic output. Numerous elements, including Sodium (Na), Potassium (K), Iron (Fe), Calcium (Ca), Boron (B), Magnesium (Mg), Selenium (Se), Copper (Cu), and Zinc (Zn) are present in both the fish and its surroundings. The World Health Organization (WHO) as well as the Food and

Agriculture Organization of the United Nations (FAO) listed eight (8) elements that are present in fish that requires constant monitoring. They include; Mercury (Hg), Cadmium (Cd), Lead (Pb), Arsenic (As), Copper (Cu), Zinc (Zn), Iron (Fe), and Selenium (Sn), while screening of others—while not required—might be advantageous (Simpson and Uche, 2019).

This research was carried out in order to assess the amount of heavy metals present in two commonly consumed imported frozen fish bought from cold room facilities in Ilorin and Omu-Aran, Kwara State, and obtained from different countries/sources. Also, to examine the microbial load and total aflatoxin level of the species, and to assess the detrimental effects on human health from eating such fish.

MATERIALS AND METHODS

Purchase of fish

Fish samples were purchased from two cold rooms in Ilorin and Omu-Aran, Kwara State, Nigeria. 5 kg fish samples each was bought per carton of 20 kg for both species (*S. scombrus* and *C. harengus*) per month. A total of three (3) cartons per species were examined (*S. scombrus* (n=70) and *C. harengus* (n=144). Sources of the fish samples used for the study were from Holland and Japan. Fish samples were kept in an ice chest and transported to the wet laboratory of the Department of Animal Science, Landmark University, Omu-Aran, Kwara State, Nigeria. The choice for using these fish species for the study, stemmed from consumers preference for these species in the country. Purchase was done once every third week of the month, for three months (September, October and November). This is because the cold rooms' operators informed that new batch of importation arrive at such interval most of the times.

Laboratory procedure

Using an electronic weigh balance, Camry (Model EK3250.5 kg), the weight of the fish samples was immediately determined and recorded. Five (5) whole fish were taken from *S. scombrus* and 10 from *C. harengus* monthly from each location for heavy metal determination.

Gills and muscles of *S. scombrus*(n=8) and *C. harengus* (n=14) were aseptically separated monthly for heavy metal analysis. Samples were preserved individually in well-marked plastic bags for digestion and microbiology study. Microbiology was investigated for the first and third months. For total aflatoxin determination, gill and muscle was collected from *S. scombrus* monthly. They were kept in the freezer until further use. Samples for heavy metal determination were then blended separately using an electronic blender (Binatone BLG-595 MK2). For the heavy metal determination, comparison was between the species: whole fish, gills, and muscles for the three months.

Digestion of fish samples

This study used a wet acid digestion method (Olalekan et al., 2019). From the milled samples, 0.5 g of each sample was weighed into a beaker. To the sample in the beaker, 4 ml of nitric acid was added. The beaker holding the mixture was placed on a hot plate for 15 minutes until the solution became clear, and was made up to 50 ml using distilled water and poured into sterile bottles, and left at room temperature until use.

Fish digest was exposed to Atomic Absorption Spectrophotometry (AAS) (Model 211 VGP Buck Scientific) for heavy metal analysis using the calibration plot technique (Adedire et al., 2021) at Afe Babalola University, Ado-Ekiti, Nigeria. The samples were analyzed with the concentration of the metals present being displayed in parts per million (ppm) after extrapolation from the standard curve.

Isolation, and identification of bacteria from experimental fish

Methods used in the process of bacteria isolation and identification are as described in the Cowan and Steel's manual for identification of medical bacteria (Barrow and Feltham, 1993). Composite samples of the whole fish samples from both species were grinded using sterile mortal and pestle. One gram of the composite whole fish sample was prepared for incubation in a suitable medium for culture. The cultures media used include nutrient agar (NA), McConkey agar (MA), nutrient broth (NB), blood agar (BA), phosphate buffer saline (PBS), Xylose lysine De-carboxylase Agar (XLD) agar and Thiosulfate–citrate–bile salts–sucrose agar (TCBS). All the fish samples were thoroughly homogenized in sterile water, cultured, and incubated for 24 hours at 37°C. Isolated bacteria were identified using cultural characteristics, cellular morphology, and biochemical test.

Cultural characteristics

Colonial cultural morphology of each isolates was examined according to their size, shape, colour, edges, elevation and haemolysis.

Gram staining

A drop of water was dispensed on the glass slide. Using the sterilized inoculating loop, a colony of organism was picked from the petri dish, and smeared on the glass slide and allowed to air-dry. This process was repeated for all the slides needed. The air-dried slide was then heat fixed and stained using crystal violet dye followed by grams iodine, acetone and safranine. Then the dried slide was examined under the microscope(100X) using immersion oil.

Biochemical identification

This was done to further characterize the bacteria isolates

according to World Health Organization (WHO) manual for laboratory investigation of bacteria organisms as described by Dawodu and Akanbi (2021).

a. Oxidase test

Electron transfer is demonstrated with this test, which is also used to distinguish enterobacteriacea from other bacterial species. In a petri dish, filter paper was covered with reagent using a inoculating loop. The suspected colony was applied to the moist filter paper using a plastic loop, and any color changes were checked. Within 10 seconds, purple coloration appeared over the streak, indicating electron transport. A change of colour is positive, if the colour remains the same, it is negative. Positive sample means they contain the enzyme "oxidase" that breaks down oxidase.

b. Catalase test

A drop of 3% H_2O_2 was dispensed on a glass slide. Using a heated and cooled wire loop, bacteria colony was picked from the petri-dish and smeared on the glass slide with H_2O_2 and observed for reaction. An effervescence reaction indicate positive for catalase.

c. Indole test

Gram negative bacilli are distinguished using this indole production test. Overnight, the organism grew on peptone water. The water culture was given a few drops of Kovac's reagent before being left for 24 hourst. Positive indole result was attested by the presence of a red ring above the peptone water.

d. Simmons citrate agar

This test is used to determine whether an organism can use citrate as a source of energy.

Citrate agar was prepared, heated and autoclaved, the test tubes were slanted after autoclaving. Inoculating loop was used to smear a colony of organism on the medium, which was then incubated for 24 hours at 37°C. A colony of organism was smeared on the agar and incubated for 24 hours. A positive test result was determined by a blue coloration, while the negative still retains the green colour of the agar .

e. Methyl red test

This is a method of enterobacteria differentiation. It determines when there is a enough amount of acid produced during the fermentation of glucose. A young culture of the organism was lightly injected into the medium, which was then incubated for 48 hours at 35°C. To the culture, five drops of methyl red indicator were added. A positive reaction was denoted by the color red.

Serial dilution of sample

Serial dilution was done for preparation of microbial load count of the intestine. A sample of the intestine sub sample (0.5g in 5ml of potassium buffer saline) was serially diluted using syringe in a sterile environment. The serial 10-fold dilution was prepared in 5ml dilution tubes for each of the samples, of which the first bottle containing the prepared sample and 9 sterile tubes containing 4.5ml of phosphate buffer solution each were placed on the rack. 0.1ml of the processed sample was mixed with 4.5ml of the first bottle of buffer solution, which produced the 10^{-1} dilution. Then 0.1ml from the first bottle was mixed with the second bottle solution and 0.1ml from the second to the third and serially in the same order until the last bottle, which is the 10^{-9} diluted sample.

Bacterial count of sample

Bacterial count was done as described by (Ogur, 2022). The samples obtained from serial dilution were inoculated on nutrient agar medium in petri dishes for microbial count. Firstly, 0.1ml was taken from the 10^{-1} dilution sample and seeded at a triangular distance on a nutrient agar media. This process is repeated until the 10^{-9} dilution sample, giving 9 inoculated plate with each plate from a dilution. After incubation at 37°C for 24hours three round shaped colonies from the nutrient agar medium were observed on the dish which were counted meticulously to determine the microbial load count.

Total aflatoxin in *Scomber scombrus* from different location

Total aflatoxin test protocol was carried out as described in the Romer labs test kits with slight modification (Avrameas, 1969). Five (5) g was weighed from the composite fish gill and muscle separately and was placed in beaker containing 25 mls of 70% methanol and left for 10 minutes for extraction of aflatoxin. Then the mixture was filtered using a No. 1 Whatman filter paper. A one-tenth dilution was then made by adding 100 µl of the filtrate to 900 µl of 70% methanol. 50 µl of the diluent and 100 µl of conjugate: fumonisin was then dispensed into the green-bordered well. 100 µl from the filtrate-conjugate mixture was taken and dispensed into the antibody coated wells and incubated at room temp for 15 minutes. The content of the well was then discarded and washed with distilled water 3 times after which 100 µl of urea peroxidase (substrate) was added to the well and incubation was done for 5 minutes. It was then observed for colour change (different shades of blue to colourless) after which a 100 µl of stop solution (sulphuric acid (1 mol/dm^3)) was added and the plates were read with ELISA plate reader at 450 nm wavelength. Optical density of the samples was recorded and multiplied by 10.

On Microsoft Excel, a graph was plotted of the standard concentrations versus optical densities. From this graph, extrapolations were made to determine levels of total aflatoxin in fish samples.

Risk assessment

Using hazard quotient (HQ) (Khan et al., 2015), health risk

index (HRI) (Abubakar et al., 2015), total toxicity of mixtures (TTM) index (Anzecc and Armcanz, 2000), and daily intake of metal (DIM) (Okunola et al., 2011), risk evaluation was conducted to measure the danger presented by human consumption of tainted fish samples.

Hazard quotient (HQ) fomulae

HQ was determined using the equation;

$$\mathrm{HQ} = \frac{Wfish * Mfish}{RfD * Bo})$$

Where,

Wfish = daily dry weight of fish that is eaten (gd-1). For nutritional needs, adults with body weight 79.96 kilograms and more, consume 20.9 grams of fish daily, children weighing 49.7 kilograms and below, consume 10.1 grams daily, and 6.2 grams per person (0 years – 9 years) weighing 17.3 kilograms was advised.

Mfish ($mgkg^{-1}$) = metal concentration in fish,

RfD ($mgkg^{-1}d^{-1}$) = metal reference dose used; Iron (0.7), Manganese (0.014), Zinc (0.3), Copper (0.04), Nickel (0.02), Cadmium (0.001).

Bo (kg) = average body weight

Daily intake of metals (DIM)

The DIM formula was developed to estimate the daily loading of metals into the human system from fish intake.

$$\mathrm{DIM} = (Cmetal\frac{Dfish}{Bo})$$

Where,

Cmetal = concentration of heavy metals in the fish ($mgkg^{-1}$),

Dfish = daily nutritional intake of fish (gday-1),

Bo = average body weight (Kg)

Health risk index (HRI)

The Health Risk Index (HRI) was calculated using the formula below.

$$\mathrm{HRI} = \frac{DIM}{RfD}$$

A Health Risk Index (HRI) value of less than one (1) denotes a safe exposure to such a heavy metal and is regarded as acceptable; otherwise, the fish may be at danger for exposure to heavy metals.

Total toxicity of mixtures (TTM)

Total Toxicity of Mixtures (TTM) for heavy metals was calculated using TTM index.

$$\mathrm{TTM} = \Sigma(1\frac{Ci}{GVi})$$

Where,

Ci = Concentration of the 'ith' component of mixture

GVi = Value to use as a guide for the 'ith' component. Values that should be used as triggers for low-risk livestock water consumption. Iron not enough hazardous, Lead 0.1 mg/L, Manganese not sufficiently toxic, Nickel 1 mg/L, Zinc 20 mg/L, Cadmium 0.01 mg/L, Chromium 1 mg/L, Copper 0.4 - 5 mg/L.

TTM >1= The mixture was shown to be above the Guideline value

Statistical analysis

Weight of *S. scombrus* and *C. harengus* and heavy metal data in the whole fish, gills, and muscles of sampled fish were analysed using simple descriptive statistics on the Statistical Package for Social Sciences version (SPSS) version 20. Health Risk index were calculated from the mean of the heavy metals concentration in the whole fish.

RESULTS

Weight of sampled *Scomber scombrus* and *Clupea harengus*

Average weight of *S. scombrus* and *C. harengus* used for the study are represented in Table 1. *S. scombrus* purchased from Ilorin (385.22 ± 88.04) were heavier than those gotten from Omu-Aran. *C. harengus* gotten from Omu-Aran, recorded highest weight (214.33 ± 31.09).

Table 1. Weights of sampled *Scomber scombrus* and *Clupea harengus*

Species	Location	N	Mean weight (g)	Min. (g)	Max. (g)
S. scombrus	Omu-Aran	39	375.00 ± 62.75	205.01	442.78
S. scombrus	Ilorin	39	385.22 ± 88.04	225.0	657.88
C. harengus	Omu-Aran	72	214.33 ± 31.09	155.32	281.80
C. harengus	Ilorin	72	197.37 ± 40.98	115.50	277.41

Heavy metals in sampled fish

Mean concentrations of specific metals in the gills, muscle and whole *S. scombrus* and *C.* harengus for the three (3) consecutive months is shown in Tables 2, 3, 4. Mn and Cr were higher in both species than other metals. Mn load was higher in the muscles of the sampled fish than in the gills from September through to November with highest value of 1.26 ± 0.08 and 1.30 ± 0.12 obtained for *S. scombrus* and *C. harengus*. Highest concentrations of all metals was observed in the gills except manganese and copper [Cd = 0.03 (*S. scombrus*), Cr = 1.22 ± 0.13 (*S. scombrus*), Ni = 0.025 ± 0.04 (*S. scombrus)*, Pb = 0.06 ± 0.02 (*S. scombrus*)].

Table 2. Mean concentration of heavy metals in sampled fish in the first month (September)

Location	Species	Fish Part	Cadmium (ppm)	Copper (ppm)	Chromium (ppm)	Manganese (ppm)	Nickel (ppm)	Lead (ppm)
	Permissible limit →		0.000005	0.000003	0.03	0.000025	0.05	0.000002
Omu-Aran (Holland)	*S. scombrus* (n = 13)	Gills	0.018 ± 0.003	0.214 ± 0.01	1.07 ± 0.15	0.41 ± 0.05	0.009 ± 0.01	0.06 ± 0.02
		Muscles	0.001 ± 0.001	0.19 ± 0.03	0.50 ± 0.01	1.05 ± 0.07	0.001 ± 0.00	0.001 ± 0.001
		Whole	0.012 ± 0.01	0.21 ± 0.01	0.81 ± 0.43	0.66 ± 0.25	0.005 ± 0.01	0.04 ± 0.03
	C. harengus (n = 24)	Gills	0.008 ± 0.01	0.32 ± 0.01	0.75 ± 0.12	0.61 ± 0.01	0.000 ± 0.001	0.02 ± 0.08
		Muscles	0.007 ± 0.001	0.21 ±0.003	0.39 ± 0.02	1.04 ± 0.07	0.001 ± 0.00	0.015 ± 0.002
		Whole	0.008 ± 0.001	0.26 ± 0.05	0.58 ± 0.18	0.81 ± 0.21	0.001 ± 0.001	0.02 ± 0.01
Ilorin (Japan)	*S. scombrus* (n = 13)	Gills	0.003 ± 0.00	0.28 ± 0.01	0.89 ± 0.02	0.48 ± 0.01	0.005 ± 0.001	0.03 ± 0.001
		Muscles	0.001 ± 0.001	0.15 ± 0.01	0.35 ± 0.01	1.205 ± 0.02	0.004 ± 0.003	0.006 ± 0.001
		Whole	0.001 ± 0.001	0.148 ± 0.005	0.35 ± 0.007	1.205 ±0.02	0.004 ± 0.003	0.006 ± 0.001
	C. harengus (n = 24)	Gills	0.01 ± 0.00	0.29 ± 0.02	0.524 ± 0.01	0.56 ± 0.01	0.01 ± 0.001	0.04 ± 0.002
		Muscles	0.002 ± 0.001	0.12 ± 0.01	0.53 ± 0.01	0.87 ± 0.14	0.003 ± 0.001	0.02 ± 0.002
		Whole	0.006 ± 0.005	0.199 ± 0.09	0.52 ± 0.01	0.73 ± 0.17	0.006 ± 0.004	0.03 ± 0.02

Means are presented as mean ± SD.

Table 3. Mean concentration of heavy metals in sampled fish in the second month (October)

Location	Species	Fish Part	Cadmium (ppm)	Copper (ppm)	Chromium (ppm)	Manganese (ppm)	Nickel (ppm)	Lead (ppm)
	Permissible limit →		0.000005	0.000003	0.03	0.000025	0.05	0.000002
Omu-Aran (Holland)	*S. scombrus* (n = 13)	Gills	0.02 ± 0.01	0.29 ± 0.05	0.93 ± 0.28	0.41 ± 0.07	0.009 ± 0.01	0.06 ± 0.02
		Muscles	0.002 ± 0.003	0.22 ± 0.03	0.38 ± 0.05	1.26 ± 0.08	0.004 ± 0.002	0.02 ± 0.02
		Whole	0.017 ± 0.01	0.26 ± 0.05	0.66 ± 0.32	0.74 ± 0.44	0.009 ± 0.01	0.04 ± 0.03
	C. harengus (n = 24)	Gills	0.008 ± 0.01	0.34 ± 0.07	0.81 ± 0.26	0.51 ± 0.08	0.000 ± 0.001	0.03 ± 0.008
		Muscles	0.002 ± 0.01	0.21 ±0.05	0.35 ± 0.09	1.23 ± 0.29	0.001 ± 0.001	0.006 ± 0.002
		Whole	0.014 ± 0.001	0.28 ± 0.1	0.57 ± 0.29	0.88 ± 0.42	0.001 ± 0.001	0.02 ± 0.01
Ilorin (Japan)	*S. scombrus* (n = 13)	Gills	0.01 ± 0.00	0.32 ± 0.04	0.96 ± 0.28	0.57 ± 0.11	0.006 ± 0.01	0.04 ± 0.007
		Muscles	0.002 ± 0.003	0.204 ± 0.08	0.28 ± 0.09	1.06 ± 0.03	0.003 ± 0.003	0.005 ± 0.01
		Whole	0.006 ± 0.004	0.22 ± 0.1	0.52 ± 0.43	0.85 ± 0.25	0.005 ± 0.004	0.02 ± 0.02
	C. harengus (n = 24)	Gills	0.01 ± 0.005	0.29 ± 0.05	0.67 ± 0.05	0.57 ± 0.11	0.011 ± 0.01	0.06 ± 0.01
		Muscles	0.00 ± 0.00	0.14 ± 0.03	0.42 ± 0.03	1.30 ± 0.12	0.004 ± 0.004	0.006 ± 0.003
		Whole	0.007 ± 0.007	0.21 ± 0.09	0.54 ± 0.14	0.92 ± 0.38	0.009 ± 0.007	0.03 ± 0.03

Means are presented as mean ± SD.

Table 4. Mean concentration of heavy metals in sampled fish in the third month (November)

Location	Species	Fish Part	Cadmium (ppm)	Copper (ppm)	Chromium (ppm)	Manganese (ppm)	Nickel (ppm)	Lead (ppm)
	Permissible limit →		0.000005	0.000003	0.03	0.000025	0.05	0.000002
Omu-Aran (Holland)	*S. scombrus* (n = 13)	Gills	0.03 ± 0.01	0.38 ± 0.18	1.22 ± 0.13	0.65 ± 0.17	0.025 ± 0.04	0.05 ± 0.01
		Muscles	0.005 ± 0.003	0.34 ± 0.06	0.3 ± 0.1	0.69 ± 0.06	0.001 ± 0.000	0.01 ± 0.01
		Whole	0.02 ± 0.02	0.33 ± 0.1	0.84 ± 0.54	0.66 ± 0.13	0.022 ± 0.05	0.03 ± 0.02
	C. harengus (n = 24)	Gills	0.22 ± 0.11	0.34 ± 0.06	0.91 ± 0.29	0.58 ± 0.12	0.006 ± 0.01	0.05 ± 0.1
		Muscles	0.007 ± 0.003	0.31 ±0.008	0.31 ± 0.008	1.15 ± 0.07	0.005 ± 0.001	0.001 ± 0.001
		Whole	0.14 ± 0.15	0.32 ± 0.1	0.63 ± 0.36	0.87 ± 0.27	0.005 ± 0.001	0.04 ± 0.12
Ilorin (Japan)	*S. scombrus* (n = 13)	Gills	0.02 ± 0.006	0.27 ± 0.02	0.61 ± 0.07	0.37 ± 0.07	0.005 ± 0.002	0.03 ± 0.002
		Muscles	0.006 ± 0.003	0.48 ± 0.05	0.23 ± 0.02	0.83 ± 0.04	0.005 ± 0.002	0.008 ± 0.002
		Whole	0.014 ± 0.01	0.39 ± 0.12	0.36 ± 0.18	0.64 ± 0.25	0.005 ± 0.002	0.02 ± 0.02
	C. harengus (n = 24)	Gills	0.02 ± 0.008	0.36 ± 0.06	0.98 ± 0.24	0.59 ± 0.15	0.013 ± 0.002	0.03 ± 0.004
		Muscles	0.02 ± 0.01	0.42 ± 0.03	0.304 ± 0.02	0.77 ± 0.13	0.009 ± 0.005	0.01 ± 0.007
		Whole	0.016 ± 0.006	0.38 ± 0.06	0.65 ± 0.38	0.65 ± 0.18	0.01 ± 0.002	0.02 ± 0.005

Means are presented as mean ± SD.

Risk assessment index of metals

Tables 5, 6, 7 showed calculated health quotient (HQ), daily intake of metal (DIM), and health risk index (HRI) for different age groups. HRI was >1 in all the age categories for all metals excluding nickel for all purchases in the two species of fish sampled.

Total toxicity of metals (TTM) was >1 for *S. scombrus* and *C. harengus* (Table 8).

Table 5. HQ, DIM and HRI for individual responses to heavy metal accumulation in fish samples (mgkg^{-1}) in the first month

Location	Species	Metals	Mean±SD (ppm)	DIM (Age categories)			HRI (Age categories)			HQ (Age categories)		
				A	B	C	A	B	C	A	B	C
Omu-Aran (Holland)	*S. scombrus*	Cd	0.012 ± 0.01	0.003	0.002	0.004	3	2	4	3.1366	2.4386	4.3006
		Cu	0.21 ± 0.01	0.055	0.043	0.078	1.375	1.0 75	1.95	1.3723	1.0669	1.8815
		Cr	0.81 ± 0.43	0.2117	0.1646	0.2903	70.5667	54.8667	96.7667	70.5727	54.8692	96.7630
		Mn	0.66 ± 0.25	0.1725	0.1341	0.2365	12.3214	9.5786	16.8929	12.3222	9.5803	16.8951
		Ni	0.005 ± 0.01	0.0013	0.001	0.0018	0.065	0.05	0.09	0.0654	0.0513	0.0895
		Pb	0.04 ± 0.03	0.0105	0.0081	0.0143	2.625	2.025	3.575	2.6138	2.0322	3.5838
	C. harengus	Cd	0.008 ± 0.001	0.0021	0.21	0.029	2.1	2.0	2.9	2.0911	1.6257	2.8671
		Cu	0.26 ± 0.05	0.068	0.0528	0.0932	1.7	1.32	2.33	1.6990	1.3209	2.3295
		Cr	0.58 ± 0.18	0.1516	0.1179	0.2079	50.5333	39.3	69.3	50.5336	39.2891	69.2871
		Mn	0.81 ± 0.21	0.2117	0.1646	0.2903	15.1214	11.7571	20.7357	15.1227	11.7577	20.7349
		Ni	0.001 ± 0.001	0.003	0.002	0.0004	0.015	0.01	0.02	0.1307	0.1016	0.1792
		Pb	0.02 ± 0.01	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919
Ilorin (Japan)	*S. scombrus*	Cd	0.001 ± 0.001	0.0003	0.0002	0.0004	0.3	0.2	0.4	0.2614	0.2032	0.3584
		Cu	0.148 ± 0.005	0.0387	0.0301	0.0530	0.9675	0.7525	1.325	0.9671	0.7519	1.3260
		Cr	0.35 ± 0.007	0.915	0.0711	0.1254	30.5	23.7	41.8	30.4944	23.7089	41.8112
		Mn	1.205 ±0.02	0.315	0.2449	0.4319	22.5	17.49	30.85	22.4974	17.4914	30.8464
		Ni	0.004 ± 0.003	0.0011	0.0008	0.0014	0.055	0.04	0.07	0.0523	0.0406	0.0717
		Pb	0.006 ± 0.001	0.0016	0.0012	0.0022	0.4	0.3	0.55	0.3921	0.3048	0.5376
	C. harengus	Cd	0.006 ± 0.005	0.0016	0.0012	0.0022	1.6	1.2	2.2	1.5683	1.2193	2.1503
		Cu	0.199 ± 0.09	0.0520	0.0404	0.0713	1.3	1.01	1.7825	1.3004	1.0110	1.7829
		Cr	0.52 ± 0.01	0.1360	0.1057	0.1864	45.3333	35.2333	62.1	45.3090	35.2247	62.1195
		Mn	0.73 ± 0.17	0.1908	0.1483	0.2616	13.6286	10.5929	18.6857	13.6291	10.5964	18.687
		Ni	0.006 ± 0.004	0.0016	0.0012	0.0022	0.08	0.06	0.11	0.0784	0.0610	0.1075
		Pb	0.03 ± 0.02	0.0078	0.0061	0.0108	1.95	1.525	2.7	1.9604	1.5242	2.6879

A = adults age 20 years and above. B = children age 10 years – 19 years. C = children age 0 – 9 years

Table 6. HQ, DIM and HRI for individual responses to heavy metal accumulation in fish samples (mgkg^{-1}) in the second month

Location	Species	Metals	Mean±SD (ppm)	DIM (Age categories)			HRI (Age categories			HQ (Age categories)		
				A	B	C	A	B	C	A	B	C
Omu-Aran (Holland)	*S. scombrus*	Cd	0.017 ± 0.01	0.0044	0.0035	0.0061	4.4	3.5	6.1	4.4435	3.4547	6.0925
		Cu	0.26 ± 0.05	0.0680	0.0528	0.0932	1.7	1.32	2.33	1.6990	1.3209	2.3295
		Cr	0.66 ± 0.32	0.1725	0.1341	0.2365	72.4167	44.7	78.8333	57.5038	45.3469	78.8439
		Mn	0.74 ± 0.44	0.1934	0.1504	0.2652	13.8143	10.7429	18.9429	13.8158	10.7416	18.9430
		Ni	0.009 ± 0.01	0.0024	0.0018	0.0032	0.12	0.009	0.16	0.1176	0.0915	0.1613
		Pb	0.04 ± 0.03	0.0105	0.0081	0.0143	2.625	2.025	3.575	2.6138	2.0322	3.5838
	C. harengus	Cd	0.014 ± 0.001	0.0037	0.0029	0.0050	3.7	2.9	5.0	3.6593	2.8451	5.7103
		Cu	0.28 ± 0.1	0.0732	0.0569	0.1004	1.83	1.4225	2.51	1.8297	1.4225	2.5087
		Cr	0.57 ± 0.29	0.1490	0.1158	0.2043	49.6667	38.6	68.1	49.6623	38.6117	68.0925
		Mn	0.88 ± 0.42	0.2300	0.1788	0.3154	16.4286	12.7714	22.5286	16.4296	12.7327	22.5268
		Ni	0.001 ± 0.001	0.0003	0.0002	0.0004	0.015	0.01	0.02	0.0131	0.0102	0.0179
		Pb	0.02 ± 0.01	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919
Ilorin (Japan)	*S. scombrus*	Cd	0.006 ± 0.004	0.0016	0.0012	0.0022	1.6	1.2	2.2	1.5683	1.2193	2.1503
		Cu	0.22 ± 0.1	0.0575	0.0447	0.0789	1.4375	1.1175	1.9725	1.4376	1.1177	1.9711
		Cr	0.52 ± 0.43	0.1360	0.1057	0.1864	45.3333	35.2333	62.1333	54.0187	41.9987	74.0655
		Mn	0.85 ± 0.25	0.2222	0.1727	0.3046	15.8571	12.3357	21.7571	15.8695	12.3383	21.7589
		Ni	0.005 ± 0.004	0.0013	0.0010	0.0018	0.065	0.05	0.09	0.0653	0.0508	0.0896
		Pb	0.02 ± 0.02	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919
	C. harengus	Cd	0.007 ± 0.007	0.0018	0.0014	0.0025	1.8	1.4	2.5	1.8297	1.4225	2.5087
		Cu	0.21 ± 0.09	0.0550	0.0430	0.0780	1.375	1.075	1.95	1.3723	1.0669	1.8815
		Cr	0.54 ± 0.14	0.1412	0.1097	0.1935	47.0667	36.5667	64.5	49.0485	36.5795	64.5087
		Mn	0.92 ± 0.38	0.2405	0.1869	0.3297	17.1786	13.014	23.55	17.1765	13.3544	23.5508
		Ni	0.009 ± 0.007	0.0024	0.0018	0.0032	0.12	0.09	0.16	0.1176	0.0915	0.1613
		Pb	0.03 ± 0.03	0.0005	0.0004	0.0007	0.125	0.1	0.175	1.9604	1.5242	2.6879

A = adults age 20 years and above. B = children age 10 years – 19 years. C = children age 0 – 9 years

Table 7. HQ, DIM and HRI for individual responses to heavy metal accumulation in fish samples ($mgkg^{-1}$) in the third month

Location	Species	Metals	Mean±SD (ppm)	DIM (Age categories)			HRI (Age categories)			HQ (Age categories)		
				A	B	C	A	B	C	A	B	C
Omu-Aran (Holland)	*S. scombrus*	Cd	0.02 ± 0.02	0.0052	0.0041	0.0072	5.2	4.1	7.2	5.2276	4.0644	7.1676
		Cu	0.33 ± 0.1	0.0862	0.0671	0.1183	2.155	1.6775	2.9575	2.1564	1.6766	2.9567
		Cr	0.84 ± 0.54	0.2196	0.1707	0.3011	73.2	56.9	100.3667	73.1866	56.9014	100.3468
		Mn	0.66 ± 0.13	0.1725	0.1341	0.2365	12.3214	9.5786	16.8929	12.3222	9.5803	16.8951
		Ni	0.022 ± 0.05	0.0052	0.0041	0.0072	0.26	0.205	0.36	0.2614	0.2032	0.3584
		Pb	0.03 ± 0.02	0.0078	0.0061	0.0108	1.95	1.525	2.7	1.9604	1.5242	2.6879
	C. harengus	Cd	0.14 ± 0.15	0.0366	0.0285	0.0508	36.6	28.5	50.8	36.5933	28.4507	50.1734
		Cu	0.32 ± 0.1	0.0865	0.0650	0.1147	2.1625	1.625	2.8675	2.0911	1.6258	2.8671
		Cr	0.63 ± 0.36	0.1647	0.1280	0.2258	54.9	42.6667	75.2667	54.8900	42.6761	75.2601
		Mn	0.87 ± 0.27	0.2274	0.1768	0.3118	16.2429	12.6286	22.2714	16.2429	12.6286	22.2709
		Ni	0.005 ± 0.001	0.0013	0.0010	0.0018	0.065	005	0.09	0.0653	0.0508	0.0896
		Pb	0.04 ± 0.12	0.0105	0.0081	0.0143	2.625	2.025	3.575	2.6138	2.0322	3.5838
Ilorin (Japan)	*S. scombrus*	Cd	0.014 ± 0.01	0.0037	0.0029	0.0050	3.7	2.9	5.0	3.6593	2.8451	5.0173
		Cu	0.39 ± 0.12	0.1020	0.0793	0.1398	0.3	1.9825	3.495	2.5485	1.9814	3.4942
		Cr	0.36 ± 0.18	0.0941	0.0835	0.1290	31.3667	27.8553	43	31.3657	24.3863	43.0058
		Mn	0.64 ± 0.25	0.1673	0.1301	0.2294	11.95	9.2929	16.3857	11.9488	9.2900	16.3832
		Ni	0.005 ± 0.002	0.0013	0.0010	0.0018	0.065	0.05	0.09	0.0653	0.0508	0.0896
		Pb	0.02 ± 0.02	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919
	C. harengus	Cd	0.016 ± 0.006	0.0042	0.0033	0.0057	4.2	3.3	5.7	4.1821	3.2515	5.7341
		Cu	0.38 ± 0.06	0.0993	0.0772	0.1362	2.4825	1.93	3.405	2.4831	1.9306	3.4046
		Cr	0.65 ± 0.38	0.1699	0.1321	0.2330	56.6333	44.0333	77.6667	56.6325	44.0309	77.6493
		Mn	0.65 ± 0.18	0.1699	0.1321	0.2330	11.9214	9.4357	16.6429	12.1355	9.4352	16.6391
		Ni	0.01 ± 0.002	0.0026	0.0020	0.0036	0.13	0.10	0.18	0.1307	0.1016	0.1792
		Pb	0.02 ± 0.005	0.0052	0.0041	0.0072	1.3	1.025	1.8	1.3069	1.0161	1.7919

A = adults age 20 years and above. B = children age 10 years – 19 years. C = children age 0 – 9 years

Table 8. TTM for individual responses to heavy metal accumulation in pooled fish samples

Species	Metals	Mean±SD ($mgkg^{-1}$)	Guideline value (mg/L)	Ci/Gvi	TTM
S. scombrus	Cd	0.010 ± 0.01	0.01	1.00	
	Cu	0.23 ± 0.07	5.0	0.046	
	Cr	0.64 ± 0.35	1	0.64	3.536
	Mn	0.77 ± 0.35	0.5	1.54	
	Ni	0.01 ± 0.01	1	0.01	
	Pb	0.03 ± 0.03	0.1	0.3	
C. harengus	Cd	0.01 ± 0.01	0.01	1.00	
	Cu	0.24 ± 0.01	5.0	0.048	
	Cr	0.55 ± 0.19	1.0	0.55	3.622
	Mn	0.86 ± 0.34	0.5	1.72	
	Ni	0.004 ± 0.01	1.0	0.004	
	Pb	0.025 ± 0.02	0.1	0.3	

Total aflatoxin in sampled organs of *Scomber scombrus*

Aflatoxin levels in the organs of *Scomber scombrus* from different coldrooms and sources revealed that there was no significant difference. Highest levels of aflatoxin were recorded in the gills of the fish from both sources Table 9.

Table 9. Aflatoxins level in sampled organs of *Scomber scombrus*

Location	Gills (ppb)	Muscles (ppb)
Omu-Aran (Holland, n = 24)	5.00	1.50
Ilorin (Japan, n = 24)	4.25	3.00

Microbial load in sampled fishes

Table 10 shows the bacteria and fungi found in the muscles of the experimental fish. 2 cfu/100µl and 80 cfu/100µl of the coliform and non-coliform bacteria were present in *S. scombrus* bought from Omu-Aran market in the month of September Table 10. Other bacteria identified include *Enterobacter intermedius* and *Shigella sonnei*. Coliform and non-coliform bacteria load in the month of November were observed to be higher to too numerous to count in both species. Other bacteria identified are *Citrobacter diversus* and *Shigella sonnei* Table 11.

DISCUSSION

Mn concentration which was noticed in this study to have exceeded the recommended permissible limit for fish and fish products 0.000025 $mgkg^{-1}$ according to (Skovgaard, 2003) is an indication of the water pollution levels from which the fish was captured. In addition, the activities ongoing around the fish environment and the effluents deposited into the water body could be a factor. Dissimilar report was document in the study by (Benzer et al., 2013).

Table 10. Microbial load and identification in sampled fishes from both sources in September

Source	Species	Coliform cfu/100µ	Non-Coliform cfu/100µl	*E. coli*	*Salmonella* spp.	*Vibrio* spp. cfu/100µl	*Streptococcus* spp.	Other bacterial isolated
Holland	*S. scombrus*	2 x 10^4	8 x 10^5	-	-	0	-	*Enterobacter intermedius*
	C. harengus	0	0	-	-	0	-	*Shigella sonnei*
Japan	*S. scombrus*	0	0	-	-	0	-	
	C. harengus	0	0	-	-	0	-	

Key: TNTC - Too numerous to count, CFU – Colony forming unit

Table 11. Microbial load and identification in sampled fishes from both sources in November

Source	Species	Coliform cfu/100µ	Non-Coliform cfu/100µl	*E. coli*	*Salmonella* spp.	*Vibrio* spp. cfu/100µl	*Streptococcus* spp.	Other bacterial isolated
Holland	*S. scombrus*	4.9x10^5	TNTC	-	-	4.7x10^5	-	*Citrobacter diversus*
	C. harengus	5.6x10^5	TNTC	-	-	4.4 x10^5	-	*Shigella sonnei*
Japan	*S. scombrus*	2.2x10^5	4.96x10^6	-	-	2.9 x10^5	-	
	C. harengus	2.1x10^5	TNTC	-	-	1.20 x10^6	-	

Key: TNTC - Too numerous to count, CFU - Colony forming unit

Differences could be attributable to the differences in location from which the sampled species were obtained. Mn levels in the analyzed *S. scombrus* and *C. harengus* are beyond the prescribed risk allowance for consumers (HRI value of Mn across the different ages was > 1) and therefore, a source of danger to people's health.

Cr levels also reported in this study to exceed the permissible limit for fish and fish products, though, play a key function in glucose metabolism in its biological usable form, is also a notable hazardous metal. Daily, intake of chromium would be regarded as adequately taken if its 35 µg/day and 25 µg/day for young men and women, and could be less for younger persons (Trumbo et al., 2001). That means, the level reported in this study could be hazardous to the consumers of the fish when it accumulates in the body overtime. In addition, risk assessment indices calculated in this study was high for this metal across the different age classification (DIM, HQ, HRI, and TTM). Findings of this study corroborates with the report of Hothem et al. (2007).

Cu, Pb, Cd, which were also higher than the permissible levels for fish and fish products in the current study aligns with (Abubakar et al., 2015). Though, the essentiality of Cu in maintaining good health cannot be overruled, but if taken above the permissible level can result in liver and kidney damage (Ahmad et al., 2022). The mean concentrations of copper reported here as opposed to previous studies, was lower (Frías-Espericueta et al., 2014; Kareem et al., 2016). In addition, the HRI of Cu and Cd were >1 making the consumption of the fish sampled hazardous to the consumers.

Aflatoxin, produced by fungi in agricultural crops such as maize, cottonseed, etc. could have found its way into the water body due to runoff of wastewater from livestock feed mills and food producing industries into the high seas and oceans. The total aflatoxins recorded in the gills of sampled fish from Holland and Japan reported to have exceeded the permissible limits (4 ppb) could be due to its direct contact with the water and thus, the major organ of accumulation of aflatoxins. The gill is the first barrier of defense and the first organ to be exposed to suspended particles in water (Ahmed et al., 2015). Though, recorded to be lower in the muscle of the fish than the permissible limit, it is too close to the maximum aflatoxin level for human food (4ppb) according to the Commission of the European Communities (2001). Aflatoxins are immunosuppressive, mutagenic, teratogenic, and carcinogenic, if found in large quantities in foods, it can cause several health hazards (nausea, vomiting, abdominal pain, convulsions, and other signs of acute liver injury) to consumers (Dhakal et al., 2022; Azizi and Rouhi, 2013).

The discrepancy in the microbial load recorded for coliform, and non-coliform bacteria, and fungi during the study period could be due to the source of the fish, handling during processing and by buyers, contamination introduced from the fishing vessel, and during storage. It could also be due to the sample collection method. The total coliform bacteria count observed were within the standard recommended by NAFDAC for public health (5.0 × 10^5 and 1.0 × 10^6 cfu g-1) (Taiwo et al., 2021). Total coliform bacteria being indicator of sewage contamination of the fish sample could be a reflection of the source of the fish, and especially if the source of water flowing into the water body comes from human residents and livestock farms. *Vibrio* spp. observed to be present in the experimental fish species especially in the last purchase is the aetiology of diarrhea and are not to be present in fresh and frozen fish in accordance with the International Association of Microbiology Society's guidelines (Sanjee and Karim, 2016). *Vibrio cholerae* is reported to be the third-highest cause of shellfish-related diseases, next to noncholera *Vibrio* spp. (Wittman and Flick, 1995).

CONCLUSION

The heavy metal loads in this study were respectively above the permissible limits set by FAO, WHO and EU legislation for fish and fish products. Thereby posing a risk to the final consumer of the fish species as revealed by the risk indices of DIM, HQ, HRI, and TTM, and this calls for serious

public health concern. Total aflatoxin levels in the fish muscle sampled did not pose any threat to consumers as it was below the permissible limit. The study recommends that government should provide screening centres at the various entry points to ensure proper monitoring and screening of imported frozen fish before entry into the country.

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AUTHORSHIP CONTRIBUTION

Oghenebrorhie Mavis Oghenochuko, Adeyinka Olamide Agbato: Conceptualization, idea, design. Rachael Oluwatosin Kolawole, Olasunkanmi Peter Olajide, Adeyinka Olamide Agbato: Data curation. Rachael Oluwatosin Kolawole: Writing-Original draft preparation. Oghenebrorhie Mavis Oghenochuko, Adeyinka Olamide Agbato, Olasunkanmi Peter Olajide: Revised first draft. Oghenebrorhie Mavis Oghenochuko, Rachael Oluwatosin Kolawole, Olasunkanmi Peter Olajide, Adeyinka Olamide Agbato: Revised final draft. Oghenebrorhie Mavis Oghenochuko: Supervision.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

ETHICS APPROVAL

No specific ethical approval was necessary for this study as frozen fish were used.

DATA AVAILABILITY

All relevant data is in the article.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

A mutagenicity investigation of sediment from İzmir Inner Bay using Ames genotoxicity assay

İzmir İç Körfezi sedimentinin Ames genotoksisite testi kullanılarak mutajenitesinin incelenmesi

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Abstract: İzmir Bay is one of the most important ecosystems of Aegean Region. Impacts of environmental pollution in the aquatic environments, especially impacts of pollution with mutagenic and carcinogenic substances on human health is an important area of research. Thus, it is required to incorporate short-term biological research methods to the molecular chemical analysis methods. By means of Ames's assay, it is possible to determine mutagenic potential of several chemicals, environmental pollutants, sediments, and waste waters. After the treatment facility was established in İzmir Bay in 2000, no mutagenicity studies were carried out in the sediment. Ames's mutagenicity assay without S9 fraction using TA98 and TA100 strains of *Salmonella typhimurium* was done at four different concentrations (125 µg, 250 µg, 375 µg, and 500 µg) in the sediment samples from six stations on İzmir Inner Bay in order to detect presence of chemicals that may cause mutagenic effects. Based on the results of Ames's assay, it was found that especially Turan Area (Station 3, on which shipyard is located) among 6 stations on İzmir Bay was under mutagenic and toxic effect and Bostanlı Area (Station 6) was under intense toxic effect. Turan Area was under the influence of environmental pollutants that may cause frameshift mutations. According to the present study, İzmir Inner Bay was contaminated by mutagenic and toxic substances.

Keywords: *Salmonella typhimurium*, TA98-TA100, sediment, pollution, Ames test, İzmir Bay

Öz İzmir Körfezi, Ege Bölgesi'nin en önemli ekosistemlerinden biridir. Su ortamlarında çevre kirliliğinin etkileri, özellikle mutajenik ve kanserojen maddelerle oluşan kirliliğin insan sağlığı üzerindeki etkileri önemli bir araştırma konusudur. Bu nedenle kısa süreli biyolojik araştırma yöntemlerinin moleküler kimyasal analiz yöntemleriyle birleştirilmesi gerekmektedir. Ames testi ile çeşitli kimyasalların, çevresel kirleticilerin, sediment ve atık suların mutajenik potansiyelini belirlemek mümkündür. İzmir Körfezi'nde 2000 yılında arıtma tesisi kurulduktan sonra sedimentte mutajenite çalışması yapılmamıştır. Bu amaçla İç Körfez'deki altı istasyondan alınan sediment örneklerinde, mutajenik etki potansiyeline sahip kimyasal maddelerin olup olmadığını tespit etmek için dört farklı konsantrasyonda (125 µg, 250 µg, 375 µg ve 500 µg) *Salmonella typhimurium* TA98 ve TA100 suşları kullanılarak S9 fraksiyonu olmadan Ames'in mutajenite tarama testi yapıldı. Ames testinin sonuçlarına göre, İzmir Körfezi'ndeki altı istasyondan özellikle Turan Bölgesi'nin (tersanenin bulunduğu İstasyon 3) mutajenik ve toksik etki altında olduğu, Bostanlı Bölgesi'nin (İstasyon 6) ise yoğun toksik etki altında olduğu belirlendi. Turan Bölgesi, çerçeve kayması mutasyonlarına sebep olabilen çevresel kirleticilerin etkisi altındadır. Bu çalışmaya göre, İzmir İç Körfezi mutajenik ve toksik etkili maddelerle kirlenmiştir.

Anahtar kelimeler: *Salmonella typhimurium*, TA98-TA100, sediment, kirlilik, Ames testi, İzmir Körfezi

INTRODUCTION

Aquatic ecosystems have been exposed to several pollutants and pollution has increased rapidly in recent years as a consequence of rapidly increasing production and use of artificial chemicals in progressively increasing urban population, and intense industrial activities.

The sediment layer underlying the seas is an appropriate substance to understand history of the pollution in the aquatic environments and to interpret on its future situation (Atgin et al., 2000). Many hazardous toxic substances are known to be accumulated in the sediment by several mechanisms of transportation (i.e., direct solid/fluid discharge, drainage from the land, atmospheric precipitates, shipping activities). Currently, the chemicals have become indispensable part of daily life. Hazardous chemicals are those leading to acute or chronic damage to the environment. The registry of Chemical Abstracts Service (CAS) includes more than 140 million chemicals. Annually, about twenty-five thousand new chemicals are added to the list. Number of the chemicals used in the market has been estimated to be around 350.000, but it has been reported that only about one per cent of them has been evaluated in terms of safety on human health and environment.

As can be seen readily, procedures for assessing the chemical substances are not up-dated and sufficient for steadily increasing number of chemicals currently available in the market (Anonymous, 2018). This is the case chemicals contaminants, including organochlorine compounds, herbicides, domestic and municipal wastes, petroleum products and heavy metals are now recognized to have adverse ffects on ocean and sea environment, even when released at low levels (Haynes and Johnson, 2000; Pinto et al., 2003).

Determining the chemicals with impacts on human health

and on the environment is an important research object in environmental and medical science. Impacts of environmental pollution, especially the pollution from the mutagenic and carcinogenic chemicals on human health is an important research object. The most important source of mutagenic and carcinogenic substances is industrial and agricultural activities. Xenobiotics originating from these sources sooner or later come into contact with aquatic ecosystems. Environmentally relevant foreign chemicals, named xenobiotics, are members of very different categories, including polyaromatic hydrocarbons (PAHs), polychlorinated biphenlys (PCBs), dioxins, dib enzofurans, organometals, arylamines, nitroaromatics, organophosphates, phatalate esters, organochlorines, etc. (Sheehan, 2007; Livingstone, 1993).

Although considered as an important part of the basic mechanisms of evolution, mutations more often have a detrimental effect for individuals and their offspring. Furthermore, increased mutation rates due to environmental pollution may adversely impact populations. There is consensus on close proximity of DNA damage, mutations and the induction of various types of cancer. It is the dominant paradigm in genetic toxicology that the ability of a chemical to cause mutation presages its ability to cause cancer (Zeiger, 2001). Even though carcinogenesis is a complex, multi-step process that is still not fully unravelled, growing evidence shows that it involves multiple mutations eventually leading to uncontrolled cell proliferation (OSPAR, 2002).

Detecting the chemicals exactly in the aquatic environments, however, is challenging and practically impossible if not literally impossible because of complex molecular structure of the organic substances. On the other hand, using the pollutants detected so far as a base and searching them in certain environments is a practical way to predict pollution and toxicity in those environments. Thus, incorporating the short-term biological research methods to molecular chemical analysis methods makes a practical and validated method to explore toxic substances in the environmental samples (Shuetzle and Lewtas, 1986). Among them, Ames's assay is one of the most important assays continuing to be relevant. Several chemicals can be investigated using Ames's assay (Grifoll et al., 1990). Aquatic sediments act as repositories for a variety of industrial and domestic pollutants. Our primary objective was to enhance the utility of the Ames assay for screening these complex chemical mixtures for mutagenicity (Papoulias et al., 1996). In most cases, mutagenic potential of pure chemicals has been studied. Ames's assay, however, has also been used to analyze complex mixtures extracted from a variety of sources, such as effluents from pulp mills (Douglas et al.,1980; Kamra, et al., 1983), petroleum refineries (Metcalfe,1985), and wastes from the wood-preserving industry (Donnelly, 1987). Several studies have been carried out in order to detect mutagenicity of sediment samples using the *Salmonella* Mutagenicity Assay (Ames's Assay) (Hollert et al.,1999; Vargas et al., 2001; Boyacıoğlu et al., 2008; Çakmak and Demir, 2018).

The first study on mutagenic characteristics of sediment from İzmir Bay was conducted by Boyacıoğlu as a doctoral dissertation along with BAP project in 1999 followed by a few genotoxicity studies (Boyacıoğlu, 2004; Arslan et al., 2010; Boyacıoğlu et al., 2011). Pollution status of the sediment which was a complex mixture hasn't been explored mutagenically in İzmir Inner Bay since the treatment facility (Big Canal Project) was launched in 2000. Thus, a mutagenicity study was aimed to be performed in order to re-explore potential mutagenicity status of sediment from İzmir Inner Bay 20 years after the Treatment Facility was launched.

MATERIALS AND METHODS

İzmir Inner Bay being polluted rapidly from 1960s was one of the areas with heaviest pollution in Mediterranean Sea before the treatment facility run as a part of Big Canal Project. Organic matters, hydrocarbons, metals, and pathogenic organisms created big threats in terms of aesthetic and health. Fifty percent of these basic pollutants affecting the water quality of İzmir Bay came from industrial wastes, 15% from raindrop, 10% from the rivers, 10% from the agricultural sources, and 15% from other sources (İZSU, 2012; İZSU, 2016). Until the establishment of the Treatment Facility, there were approximately 10 creek and discharge points in the Inner Bay (Boyacıoğlu,1999).

Study area

İzmir Bay is located on the coast of Aegean Sea on 38º25´–38º42´ North Altitudes and 29º 25´–27º 10´ East Longitudes. Its total length is 64 km with surface area of 500 km^2. The part of İzmir Bay between Karaburun Peninsula and Gediz Delta is called "Outer Bay" whereas the part of it from the end of Outer Bay to Yenikale Lighthouse is called "Middle Bay" and rest of it from Yenikale Lighthouse is called Inner Bay.

In the present study, sediment samples collected using a grab sampler at 6 stations in the Inner Bay were used. (St.1=Bayraklı, St.2=Alsancak, St.3=Turan, St.4=Pasaport, St.5=Konak, St.6=Bostanlı) (Table 1) (Figure 1).

Table 1. Data on the stations from which the sediment samples were taken and their coordinates

Sampling Stations	Latitude	Longitude
Bayraklı (St. 1)	38˚ 27' 34.93'' N	27˚ 9' 0.05'' E
Alsancak (St. 2)	38˚ 26' 59.19'' N	27˚ 9' 35.85'' E
Turan (St. 3)	38˚ 27' 18.1'' N	27˚ 8' 24.23'' E
Pasaport (St. 4)	38˚ 26' 23.14'' N	27˚ 7' 47.88'' E
Konak (St. 5)	38˚ 25' 11.88'' N	27˚ 7' 12.07'' E
Bostanlı (St. 6)	38˚ 26' 41.56'' N	27˚ 6' 36.26'' E

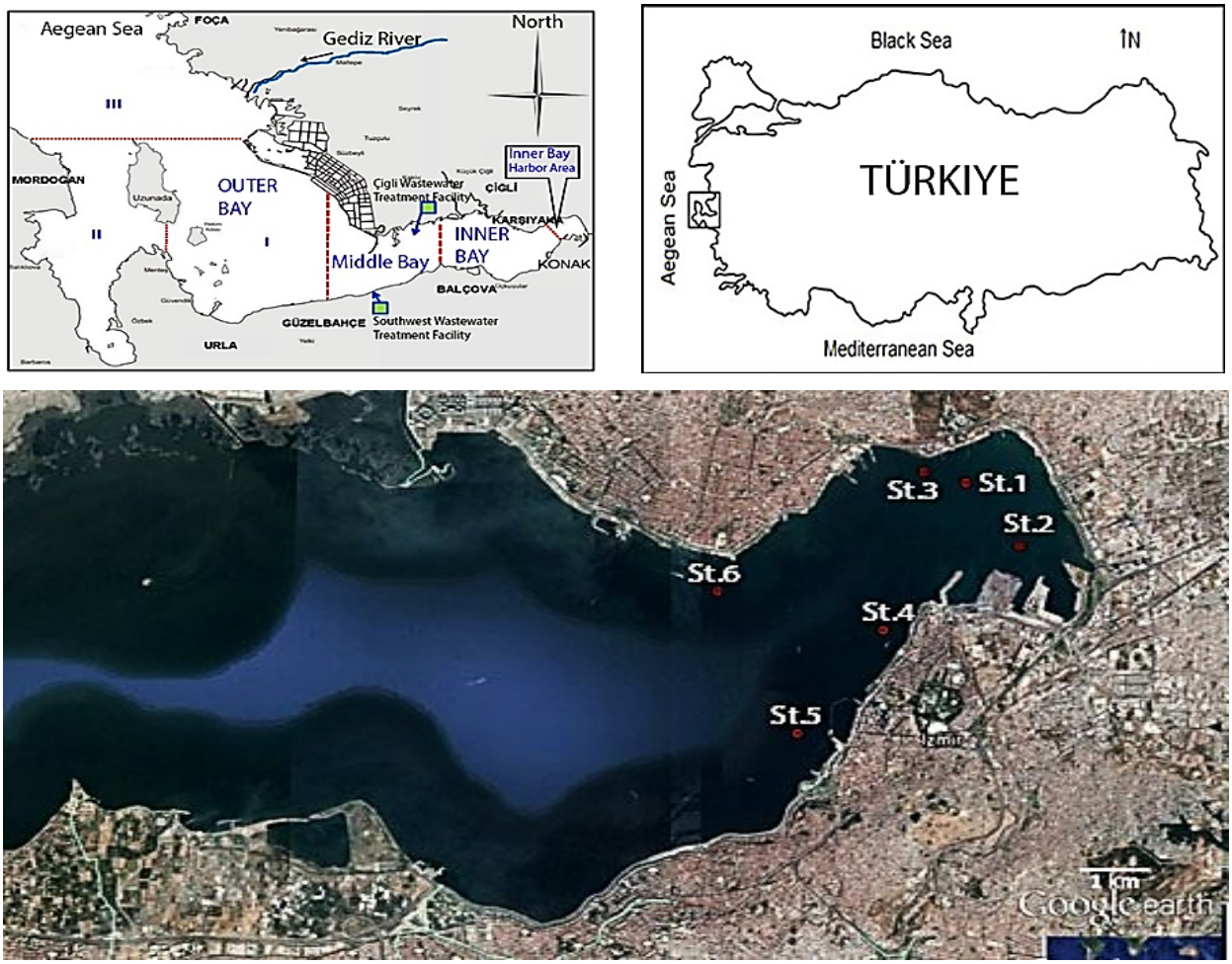

Figure 1. Six stations selected from İzmir Inner Bay

Extraction of the samples

Sediment samples were collected by a grab sampler from 6 stations in İzmir Bay (Figure 1). They were kept cool in ice-box until returning to the laboratory. Sediment samples were kept cool in ice-box at 4°C until returning to the laboratory. Air-dried samples for 24-48 hours in a fume-hood prior to extraction were crash to powder using net with pore size of 63 µm and then placed in portions of 2 g into teflon tubes and mixed with hexane/chloroform/acetone (1:1:1: v: v: v) (Maccubin, 1991). Subsequently, supernatants were taken after centrifugation for 10 Min at 4°C at 5600 g in *Sigma K3* cooled centrifuge. This procedure was repeated for 3 times and supernatant was collected. Organic solvents of the sediment samples were evaporated and dissolved with 2 mL Dimethylsulfoxide (DMSO) and added to give a final residue concentration of 2 g sediment mL (Kotelevtsev and Stepanova, 1995).

The mutagenicity assay

Salmonella mutagenicity assay was performed using the standard plate incorporation method (Maron and Ames, 1983) with the TA98 and TA100 strains of *Salmonella typhimurium,* and without S9-derived metabolic activation. Organic extracts (sediments) were diluted to 125 µg 250 µg, 375 µg, and 500 µg. of residue for each sample and tested for mutagenicity using the standard plate incorporation protocol (Maron and Ames, 1983). For testing mutagenicity, 100 µl of organic extract of sediment was mixed with 100 µl of an overnight culture of bacteria and 2 ml of melted agar containing 0.5 mM histidine and biotin. The molten top agar was then poured onto a minimal glucose agar base plate and incubated at 37°C for 2 days. Daunomicyne (0.6 µg/plate) and Mitomycin-C (0.5 µg/plate) were used as positive controls. Each dilution of extracts and controls was assayed in triplicate. Following incubation, the number of revertant colonies (His^- revertants) was counted (Maron, and Ames,1983).

Statistical methods

In the present study, the mean and standard deviation of 3 parallel results for each concentration were given in Table 2.3. The results were not subtracted from the results of negative controls. Analysis of variance (ANOVA) was used to test the significance of the difference among the variables. Calculations

with TA100 strain showed a statistically significant difference compared to negative controls at each concentration of all stations ($p < 0.005$). In mutagenicity assay with TA98 strain, statistically significant difference was found for 3 concentrations on Station 3 (Turan Area) and for concentration of 500 µg on Station 6 ($p < 0.005$).

RESULTS

According to the results of the present study, mutagenicity was observed on the Station 3 (Turan area), meaning that the number of revertant colonies of TA98 strain of *S. typhimurium* exceeded 10 folds of the control negative ($p<0.005$). Highest level of toxicity was observed on Turan and Bostanlı (Station 3, Station 6) at concentration of 500 µg (based on Ames's mutagenicity criteria) (Dugan et al., 1990) (Table 2, Figure 2).

Based on the results for TA100 strain, numbers of the revertant colonies were found to be below the negative controls at all concentrations from all 6 stations ($p< 0.005$), and the numbers fallen below half of the negative controls at concentration of 500 µg on Turan and Bostanlı Areas ($p<0.005$) (based on Ames's mutagenicity criteria) (Dugan et al., 1990), (St.6 and St. 3) (Table 3, Figure 2).

Table 2. Number of the revertant colonies in the mutagenicity analysis of sediment samples using *S. typhimurium* assay with TA98 strain in the absence of metabolic activation.

Stations	Concentration of the sediment extracts per plate					
	NC[a]	125 µg	250 µg	375 µg	500 µg	A-Cr**
Bayraklı St. 1	23.6 ± 3.21	35 ± 2	19.66±4.04	17.66 ± 4.1	21 ± 0	NM
Alsancak St. 2	23.6 ± 3.21,	33.3 ± 6.5	34.3±5.5	27 ± 5.5	20 ± 5	NM
Turan St. 3	23.6 ± 3.21	639.33 ±120*	402.6± 20.5*	391.66±9.5*	0 ± 0*	M
Pasaport St. 4	23.6 ± 3.21	26 ± 3	28.33±3.78	26 ± 3	23 ± 3	NM
Konak St. 5	23.6 ± 3.21	20 ± 3	26 ± 1	29.33 ± 3.51	24 ± 2	NM
Bostanlı St. 6	23.6 ± 3.21	22 ± 6.08	19.33 ± 3.51	18 ± 1	7.6±5.6*	TOX

[a] NC (Negative Control): DMSO (not subtracted from the values in the table)
Number of Spontaneously revertant colonies: 32-38 (not subtracted from the values in the table)
Number of the revertant colonies of positive control with Mitomycin-C (0.5µg/plate): No growth
*Analysis of mutagenic activity of sediments from Inner Bay as the number of His^+ revertant colonies in Ames test without metabolic activation system; significant data are shown in (*) ($p<0.005$)
A-Cr**: Ames Criteria, SM: strongly mutagenic; M: moderately mutagenic; WM: weakly mutagenic; NM: non-mutagenic; TOX: toxic (Dugan et al.,1990).

Table 3. Number of the revertant colonies in the mutagenicity analysis of sediment samples using *S. typhimurium* assay with TA100 strain in the absence of metabolic activation.

Stations	Concentration of the sediment extracts per plate					
	NC[a]	125 µg	250 µg	375 µg	500 µg	A-Cr**
Bayraklı St. 1	180.6±1.5	109±17.3*	117±1.5*	104±14*	100.6±6.6*	NM
Alsancak St. 2	180.6±1.5	117±10*	135±5*	112±11*	121±2.8*	NM
Turan St. 3	180.6±1.5	54±9.2*	47±3.05*	67±15.3*	79±7.2*	NM
Pasaport St. 4	180.6±1.5	137±11.26*	124±3.6*	110.33±9.2*	110.33±14.4*	NM
Konak St. 5	180.6±1.5	171±16.04	114±11.5*	109±5.1*	118±7.8*	NM
Bostanlı St. 6	180.6±1.5	107.3±11.5*	83.33±0.5*	97.33±5.8*	81.66±8.5*	NM

[a]NC (Negative Control): DMSO (not subtracted from the values in the table)
Number of the spontaneously revertant colonies: 165-218 (not subtracted from the values in the table)
Number of the revertant colonies of pozitive control with Daunomycine (6.0 µg/plate) :47-68
*Analysis of mutagenic activity of sediments from Inner Bay as the number of His^+ revertants in Ames test without metabolic activation system; significant data are shown in (*) ($p<0.005$).
**A-Cr (Ames criteria): SM: strongly mutagenic; M: moderately mutagenic; WM: weakly mutagenic; NM: non-mutagenic; TOX: toxic (Dugan et al.,1990).

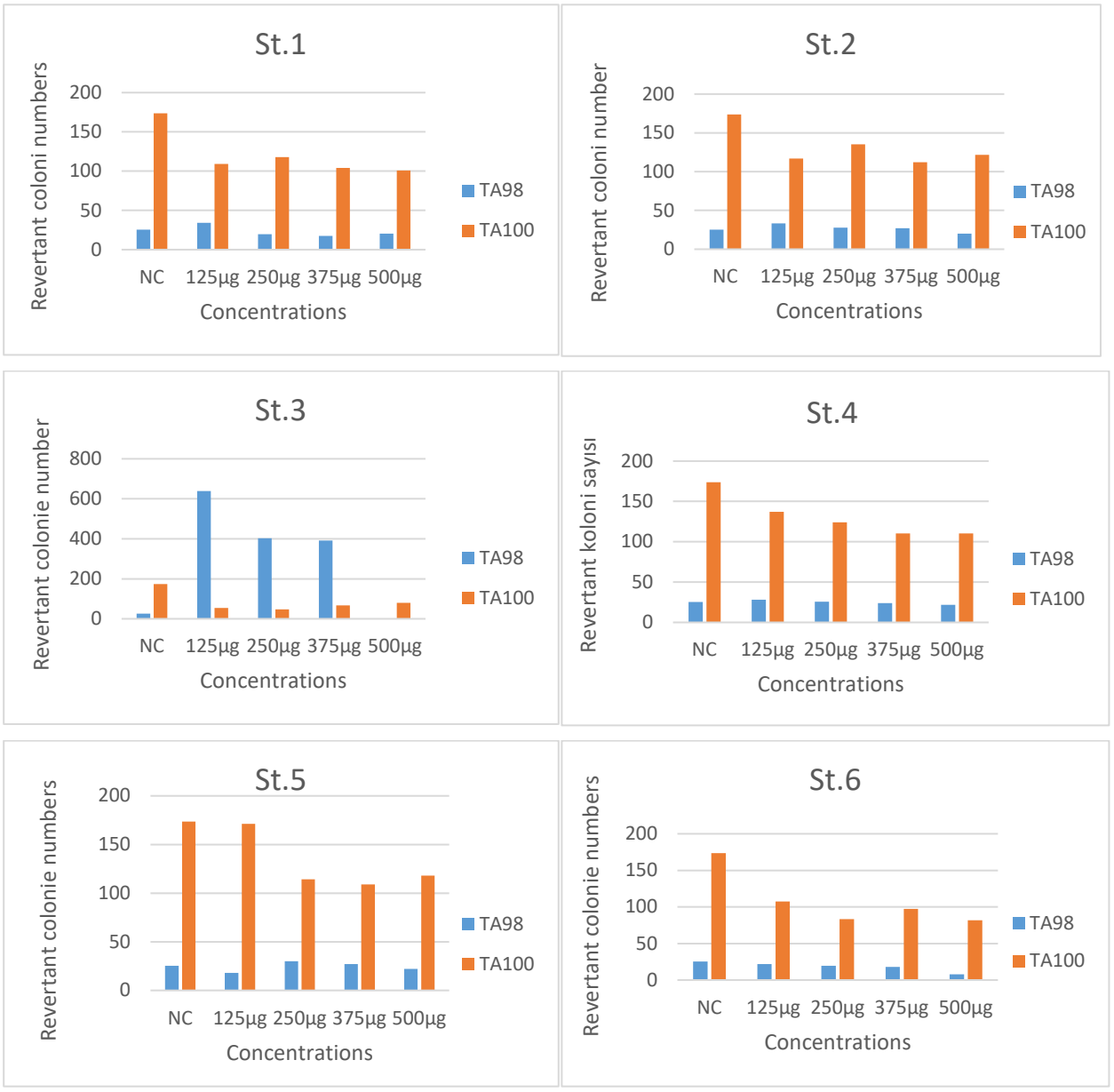

Figure 2. Number of the revertant colonies in the mutagenicity analysis of sediment samples using *S. typhimurium* assay with TA98 and TA100 strain in the absence of metabolic activation [(NC (Negative Control): DMSO (not subtracted from the values in the graphic); Concentrations: Concentration of the sediment extracts per plate (125 µg, 250 µg, 375 µg, 500 µg)]

DISCUSSION

İzmir Bay is known to be contaminated by organic materials, hydrocarbons, heavy metals, nutrients and pathogenic organisms (Balcı and Türkoğlu, 1993; Balkas and Juhasz, 1993; Kaymakci et al., 2001).

Aközcan and Görgün (2013) determined trace metals in surface sediments collected from two important areas (İzmir Bay and Didim Area) from the eastern Aegean coast and measured trace metals (Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in the sediment samples. The trace metal results showed that the İzmir Bay was exposed to trace metal pollution.

Küçüksezgin et al. (2006) reported that the heavy metal concentrations in various fish in İzmir Bay ranged between 4.5 and 520 µg/kg for Hg, 0.10 and 10 µg/kg for Cd, and 0.10 and 491 µg/kg for Pb µg/kg; in the same study, metal concentrations in the sediments were found to be as follows: Hg, 0.05-1.3; Cd, 0.005-0.82; Pb, 14-113, and Cr, 29-316 µg/g.

Cellular biomarkers and heavy metal concentration were investigated in mussels (*Mytilus galloprovincialis* L.) collected from eight different regions of İzmir Bay, and as a result, different levels of heavy metal pollution in İzmir Bay are an environmental problem (Katalay et al., 2022). Especially the

coastal areas of the gulf have been heavily affected by anthropogenic effects due to the increasing population.

Oral et al. (2012) found the highest concentrations of heavy metals as well as total PAH from the same stations on İzmir Bay (anthracene [A], fluoranthene [Fluo], benzo(ghi)perylene [BPer], benzo(b) fluoranthene [BbF], benzo(k)fluoranthene [BkF], benzo(a)pyrene [BaP] and indeno(1,2,3-cd) pyrene [IP]). Total PAH concentration was reported as 885,5+-244,5 ng/g dw.

There is a scarcity of ecotoxicological studies sediment of İzmir Bay sediment (Boyacıoğlu, 1999; Boyacıoğlu, 2004; Kutlu et al., 2008, Arslan et.al., 2010). The mutagenicity study of Ames conducted by Boyacıoğlu on İzmir Bay in 1999, is the first study conducted before the establishment of the Treatment Facility (2000) in the bay. This study was carried out approximately 18 years after the establishment of the Treatment Facility.

Many studies have been reported about mutagenic characters of sea, river and lake waters. It has also been reported that this mutagenic character was primary originated from PAHs (Kutlu et al., 2008)

Kutlu et al. (2008) performed *Salmonella* mutagenicity analysis on water samples in İzmir Çamaltı saltern without S9 fraction. They concluded that low PAH toxicity of central İzmir Bay might be considered as a reason of negative results on the mutagenicity investigation of Çamaltı Saltern.

As well known, TA100 strain of *S. typhimurium* is used to detect the mutagens causing alterations in base-pairs of the DNA chain while TA98 strain is used to detect the mutagens causing frameshift mutations (Maron and Ames, 1983). TA98 strain is rather sensitive to the PAH derivatives (i. e. benz(a)anthracene, 7-ethylbenz (a)anthracene) (Ames et al., 1972). Technically TA 98 strain of *S. typhimurium* indicate and sensitive to the occurrence of PAH in the media (Chen and White 2004). TA100 strain, on the other hand, is rather sensitive to the compounds causing alterations in the base-pairs such as acridine dyes, nitrous acid, hydroxylamine, alkylating agents, aldehydes, hydroperoxidases, aromatic amines, benzydine, toluidine, and dianisidine (Prival et al., 1979) (Prival and Mitchell,1982). The present study showed that Turan area (Station 3) was polluted by the mutagens against which TA98 strain of *S. typhimurium* were sensitive (causing frameshift mutations). This was considered to be due to presence of a military shipyard on Turan area. Repair and maintenance activities commonly carried out at shipyards include hull cleaning, repair and painting, electrical and machine work, carpentry, steel fabrication, pipe-fitting, and sand blasting of parts. Paint stripping and painting activities are significant sources of pollution from shipyards, and their waterfront locations increase the potential for pollutants to reach bodies of water. Many of the coatings used on hulls contain anti-fouling. heavy metals, such as copper and zinc. The metals are toxins added to marine coatings to prevent marine organisms from building up on ship hulls, which reduces speed and fuel efficiency (Turner, 2010).

It was found that amount of the mutagens responding to the TA98 strain of *Salmonella typhimurium* increased remarkably since 1999 and toxic effect was found on Station 6 (Bostanlı Area). It is possible to explain the reason for toxic effect on Bostanlı Area (Station 6) as follows: Cyclonic gyre exists on Inner İzmir Bay (Beşiktepe et al., 2011) and pollution burden from Turan Area (Station 3) as a result of this cyclonic gyre possibly affects this area as well.

When results of the present study were compared to those from the mutagenicity study conducted by Boyacioglu (1999) using TA98 strain of *S. typhimurium*, the mutagens responding to TA98 strain of *S. typhimurium* were observed to again mutagen in sediments from Station 3 (Table 2, Figure 2).

In the study with TA100 strain, numbers of the revertant colonies at all concentrations from six stations were found to be lower than the negative control and numbers of revertant colonies were observed to decrease almost to half values at the highest concentration of 500 µg in the samples from Turan Area (St.3) and Bostanlı Area (St.6). Although the values were non-mutagenic, toxic effect was observed according mutagenicity criteria of the Ames' Assay ($P < 0.005$) (Dugan et al., 1990) (Table 3; Figure 2).

When results of the present study were compared to those from the mutagenicity study conducted by Boyacıoğlu (1999) using TA100 strain of *S. typhimurium*, the mutagens responding to TA100 strain of *S. typhimurium* were observed to change from mutagenic to toxic characteristics (Dugan et al., 1990) in sediments from all 6 stations (Table 3, Figure 2).

CONCLUSION

In conclusion, based on the results of Ames's assay, it was found that especially Turan Area (Station 3, on which shipyard is located) and Bostanlı Area (Station 6) among 6 stations on İzmir Bay was under frameshift mutagenic and toxic effect. Although the pollution burden from the Inner Bay decreased after İzmir Treatment Facility was operated in 2000, according to the results of this study, the pollution burden from the shipyard continues to pollute the Inner Bay with mutagenic and toxic influences. The military shipyard needs to take some precautions about environmental pollution as soon as possible. Additional experiments should be performed using rat liver homogenate (S9) in order to determine the genotoxic potentials in mammals. We believe that this study will shed light on future ecotoxic studies.

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AUTHORSHIP CONTRIBUTIONS

Meltem Boyacıoğlu: Conceptualization, funding acquisition, methodology, resources, investigation, writing-

reviewing and editing. Yiğit Egüz: Conceptualization, investigation, methodology, formal analysis, writing-reviewing.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

All relevant data is inside the article.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Evaluation of health risks from heavy metals in the creeks feeding Mogan Lake, Türkiye

Mogan Gölü'nü (Türkiye) besleyen derelerde ağır metallerden kaynaklanan sağlık risklerinin değerlendirilmesi

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Abstract: The non-carcinogenic and carcinogenic health risks arising from potential exposure to heavy metals pose a negative threat to human health. In this study, heavy metals (Hg, As, Cd, Cr, Pb, Ni, Cu, Zn) analyses were conducted in the waters of four creeks in the Mogan Lake Basin (Sukesen Creek, Başpınar Creek, Yavrucak Creek, Gölcük Creek) under anthropogenic pressure. In the water samples taken from the creeks that also contribute to Mogan Lake, which has significant recreational importance in the basin: a) The non-carcinogenic health risks (HQ) of exposure to heavy metals through ingestion and dermal routes were identified for adults and children, b) The total potential non-carcinogenic health risks for adults and children were determined using the hazard index (HI). The total HI (THI) value was calculated as the sum of individual HIs ($HI_{ingestion}$ + HI_{dermal}), c) Carcinogenic health risk (CR) values were calculated for three heavy metals (Cr, Ni and As). According to the findings: a) Ingestion HQ values were found to be higher in adults and children due to As compared to dermal HQ values. According to the HI values, there was a high level of non-carcinogenic health risk in terms of heavy metals in Sukesen, Başpınar, and Gölcük Creeks, varying according to the month and age group. However, for Yavrucak Creek, there has not been appeared to be a non-carcinogenic health risk for adults and children, b) According to the calculated HQ_{dermal} and HI_{dermal} values, there was no significant adverse health risk due to dermal exposure for adults and children, c) Children hazard index values were found to be higher than adult hazard index values, highlighting that children were at higher health risk most particularly when it comes to the considered heavy metals, d) The THI values for Sukesen and Başpınar Creeks indicated a significant non-carcinogenic health risk possibility for both adults and children in all sampled months. In Gölcük Creek, a serious non-carcinogenic health risk probability was observed for adults in April and for children during the sampling period, e) The calculated CR values for chromium, nickel, and arsenic indicated that the ingestion pathway poses a higher risk compared to the dermal route, expressing the likelihood of cancer incidence in adults and children. In the context of potential health hazards, to take administrative measures regarding heavy metal contamination, particularly in Başpınar and Sukesen Creeks, is important not only for the protection of public health but also for the sustainability of Mogan Lake.

Keywords: Heavy metals, health risk assessment, pollution, creeks, Mogan Lake

Öz: Ağır metallere potansiyel maruziyetten kaynaklanan kanserojenik olmayan ve kanserojenik sağlık riskleri, insan sağlığını olumsuz yönde tehdit etmektedir. Bu çalışmada, antropojenik baskı altındaki Mogan Gölü Havzası'ndaki dört dere (Sukesen Deresi, Başpınar Deresi, Yavrucak Deresi, Gölcük Deresi) sularında ağır metal (Hg, As, Cd, Cr, Pb, Ni, Cu, Zn) analizleri yapılmıştır. Havzadaki rekreaktif öneme sahip Mogan Gölü'nü de besleyen derelerden alınan su örneklerinde; a) Ağır metallere sindirim ve deriden emilim yoluyla maruz kalmanın, insan sağlığı üzerindeki kanserojenik olmayan riskleri (HQ) yetişkinler ve çocuklar bazında saptanmış, b) Yetişkinler ve çocuklarda toplam potansiyel kanserojenik olmayan sağlık riskleri tehlike katsayısı (HI) kullanarak belirlenmiştir. Toplam HI (THI) değeri; $HI_{sindirim}$ ile HI_{dermal} değerlerinin toplamından elde edilmiştir, c) Kanserojenik sağlık risk değerleri (CR) ise üç ağır metal (Cr, Ni ve As) için hesaplanmıştır. Bulgular doğrultusunda; a) $HQ_{sindirim}$ değerleri, As nedeniyle yetişkinler-çocuklarda, HQ_{dermal} değerlerinden daha yüksek bulunmuştur. HI kanserojenik sağlık değerine göre, Sukesen, Başpınar ve Gölcük Derelerinde ay ve yaş gruplarına göre değişmek üzere, ağır metaller açısından yüksek düzeyde kanserojenik olmayan sağlık riski söz konusudur. Yavrucak Deresi içinse yetişkinler-çocuklar açısından kanserojenik olmayan sağlık riski olası gözükmemektedir, b) Hesaplanan HQ_{dermal} ve HI_{dermal} değerlerine göre, yetişkinler-çocuklar açısından dermal yolla maruziyetten dolayı belirgin bir olumsuz sağlık riski bulunmamıştır, c) Çocuklar için tehlike katsayısı değerleri yetişkinler için tehlike katsayısı değerlerinden daha yüksek bulunmuş olup, özellikle dikkate alınan ağır metallere karşı çocukların daha fazla sağlıksal riskle karşı karşıya kaldıkları belirlenmiştir, d) Sukesen ve Başpınar Deresi'ne ilişkin THI değerleri; yetişkinler ve çocuklar için örnekleme yapılan tüm aylarda, Gölcük Deresi'nde, yetişkinler için nisan ayında, çocuklar içinse örnekleme periyodu esnasında ciddi düzeyde kanserojenik-olmayan sağlıksal risk olasılığını ortaya koymuştur, e) Krom, nikel ve arsenik için hesaplanan CR değerleri, sindirim yoluyla maruziyetin dermal yola göre daha riskli olduğunu ve yetişkinlerde-çocuklarda kansere yakalanma olasılığını ifade etmektedir. Potansiyel sağlık tehlikeleri bağlamında, özellikle Başpınar ve Sukesen Dere'lerinde ağır metal kontaminasyonuna ilişkin yönetsel tedbirlerin alınması, halk sağlığının korunması yanında Mogan Gölü'nün sürdürülebilirliği açısından da önem taşımaktadır.

Anahtar kelimeler: Ağır metaller, sağlık risk değerlendirmesi, kirlilik, dereler, Mogan Gölü

INTRODUCTION

Surface waters are among the water bodies most affected by water pollution. Heavy metals, which constitute a significant aspect of water pollution, are transported to water sources through various artificial means such as industrial, domestic, and agricultural wastewater as well as through natural sources and acid rain (Pulatsü and Topçu, 2015; Bat and Arıcı, 2018).

Uptake metals in aquatic ecosystems by humans through the ingestion of water, contact with water through the skin, and the consumption of fish and/or agricultural products irrigated

with contaminated water. From a human health perspective, the estimation of risks arising from exposure to pollutants such as heavy metals in surface waters is of significant importance (Xiao et al., 2019; Joseph et al., 2022; Zhang et al., 2022). The contamination of surface and groundwater with heavy metals can result in cytotoxic, carcinogenic, and mutagenic effects of the heavy metals that enter the human body, particularly through ingestion. In more severe cases, it can lead to an increased risk of cancer development as a result of alterations in the expression patterns of various genes (Castresana et al., 2019). Carcinogenic and non-carcinogenic health risk assessments are based on methodologies that involve determining the concentration of heavy metals and evaluating the differences between groups, considering equations based on chronic exposure. For this purpose, hazard quotient (HQ), hazard index (HI), and carcinogenic risk (CR) data, which are developed as tools based on equations recommended by the United States Environmental Protection Agency (USEPA), are widely used. Methods for predicting the risk are continuously revised to characterize the accuracy and magnitude of the mentioned risk. Health experts focus on evaluating all pathways of exposure that humans experience after the release of chemicals into receiving waters, with particular emphasis on the ingestion of surface water when it is appropriate, as well as dermal exposure to this water (ATSDR, 2018). Due to the pollutants that widely reach the soil, plants, surface waters, and groundwater depending on the quality of water used today, health risk assessment studies on surface waters have gained momentum in our country, as well as worldwide, in recent years.

Mogan Lake is located within the 'Gölbaşı Special Environmental Protection Area,' which is one of the 15 designated protected areas in Türkiye. The lake is not only an important wetland and recreational area in its geography but also one of the significant natural habitats in the country in terms of its flora and fauna. In order to control the external pollution load on Mogan Lake, various rehabilitation and improvement works have been carried out on Sukesen Creek, which is the most important creek that passes through the town center and feeds the lake, as well as Tatlım, Kaldırım, and Gölcük Creeks located to the east. For the creeks that flow into Çökek Marsh within the basin area, some purification measures have been proposed as well. However, the development of the capital city, Ankara, and particularly the increasing population of Gölbaşı District located within the Gölbaşı Special Environmental Protection Area, have led to an increase in urbanization and industrial activities around the lake. Although agricultural areas are gradually shrinking due to urbanization, the use of chemical fertilizers and agricultural pesticides is still prevalent in the remaining agricultural lands. In addition to that, the increasing number of allotment gardens, especially in recent years, is among the factors that exert pressure on the lake ecosystem. Furthermore, the presence of numerous mining processing facilities around the lake, particularly andesite processing plants, is another factor contributing to the exposure of surface waters in the basin to heavy metal pollution. In this context, inevitably that any pollution that may occur in the water sources that feed the lake would have a negative impact on the entire wetland ecosystem.

The aim of this research is to assess the health risks faced by the population in the Mogan Lake Basin who are exposed to heavy metals in the creeks that flow into the lake, using various indices. For this purpose, two different groups, adults and children, were considered. The following values were determined: a) For the assessment of non-carcinogenic health risk: The Average Daily Doses (ADD) through ingestion and dermal absorption, hazard quotient (HQ), hazard index (HI), b) For the assessment of carcinogenic health risk: Cancer risks (CRs) and total cancer risks (TCR) values. It is believed that in addition to contributing to the development of rational strategies focused on heavy metal control about creeks in the context of public health, the findings will also play a role in the sustainability of the recreational Mogan Lake.

MATERIALS AND METHODS

Study area

The Mogan Lake, which is one of the important wetlands in our country nominated for Ramsar Site, has a significantly low groundwater supply, and the input of water mostly occurs during summers through irregularly flowing creeks, which often dry up. The most important of these creeks are Sukesen, Başpınar, Gölova, Yavrucak, Çolakpınar, Tatlım, Kaldırım, and Gölcük Creeks, located in the eastern and northwestern parts of the basin (Figure 1). The waters of Mogan Lake flow into Eymir Lake, which is entirely located within the Middle East Technical University (METU) campus, under the control of the regulator located in the northeast (Anonymous, 2017).

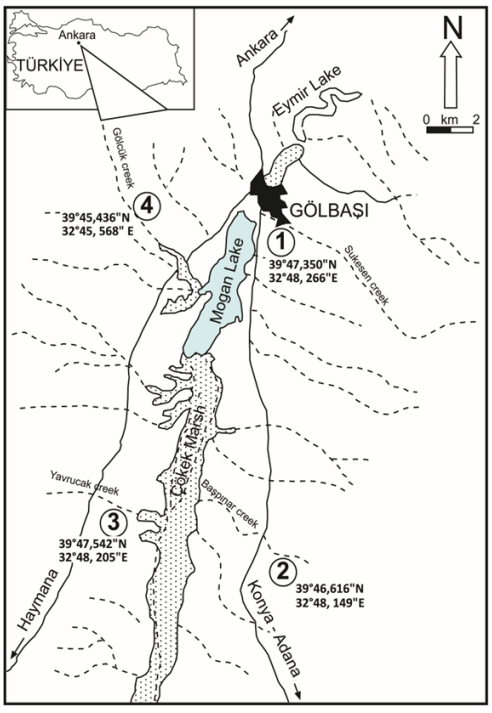

Figure 1. Study area and selected creeks

As shown in Figure 1, four creeks that contribute to the inflow of Mogan Lake and transport both point and non-point source pollutants to the lake. Wastewaters are broughtcome to the lake from stone quarries and residential areas in Sukesen Creek, from residential areas and agricultural activities in Başpınar Creek, from agricultural activities in Yavrucak Creek and Gölcük Creek.

Methods

Within the scope of the study, sampling was conducted three times from the creeks that feed Mogan Lake, in December 2022, February 2023, and April 2023. In determining the sampling times, months with a high probability of receiving heavy rainfall were taken into consideration based on previous meteorological data. The water samples were transported to the laboratory in a dark and cool environment. The heavy metal analysis of the water samples (Hg, As, Cd, Cr, Pb, Ni, Cu, Zn) was conducted in an accredited laboratory following the TS EN ISO 17294-1.24 standard. The analysis was performed with four replicates. Within the scope of the assessment of human health risk, the presence of a significant difference between adult and child health risk indices was determined using the non-parametric Wilcoxon test (Kolassa, 2020). The analysis was conducted using the SPSS 22 software package.

Assessment of human health risks

- Non-carcinogenic health risk

To calculate the potential non-cancer health risk through ingestion and dermal exposure of surface water, child - adult were considered as the target groups. Parameters and their authoritative value used for exposure assessment of heavy metals through ingestion and dermal adsorption of waters are presented in Table 1. The Average Daily Doses (µg/ kg-day) through ingestion (ADD_i) and dermal (ADD_d) absorption were calculated using the following equation (1) and equation (2) as outlined by the US Environmental Protection Agency (USEPA, 2004).

$$ADD_i = C_w \times IR \times EF \times ED / BW \times AT \quad (1)$$

$$ADD_d = C_w \times SA \times K_p \times ET \times EV \times EF \times ED / BW \times AT \quad (2)$$

HQ is the ratio between exposure through individual pathways and the reference dose (RfD). The non-carcinogenic health quotient (HQ) of heavy metals through ingestion and dermal adsorption of water for the population in the area was calculated using the following Eq. (3):

$$HQ_{i/d} = ADD_{i/d} / RfD_{i/d} \quad (3)$$

Table 1. Input assumptions in used to calculate non-carcinogenic and carcinogenic human health risks due to metal exposure through ingestion and dermal pathways

Definitions	Symbols	Units	Values		References
			Ingestion	Dermal	
Measured metal concentration	C_w	µg/ L			
Ingestion rate-adult	IR	L/day	2.2	-	USEPA (2004) Wang et al. (2017) Saleem et al. (2019)
Ingestion rate-child	IR	L/day	0.64	-	
Exposure time-adult	ET_a	h /event	1	0.58	
Exposure time-child	ET_c	h /event		1	
Exposure frequency	EF	days /year	350		USEPA (2004) Saleem et al. (2019)
Exposure duration-adult	ED_a	year	70	30	
Exposure duration-child	ED_c	year	6		
Conversion factor	CF	L/cm^3	0.001		
Body weight-adult	BW_a	kg	70		
Body weight-child	BW_c	kg	20		
Average time-adult (365xED_a)	$AT_{a\text{-}re}$	days	25550		
Average time-child (365xED_c)	AT_c	days	2190		
Skin surface area-adult	SA_a	cm^2	18000		
Skin surface area-child	SA_c	cm^2	6600		
Dermal permeability coefficient	Kp	cm/h	Cd, As, Cu: 0.001; Pb: 0.0001; Ni:0.0002; Zn: 0.0006; Cr:0.002; Hg:0.001		USEPA (2004)
Ingestion reference dose	$RfD_{ingestion}$	µg/ kg-day	Cr: 3; Ni: 20; Cu: 40; Zn: 300; As:0.3; Cd: 0.5; Hg: 0.3; Pb: 1.4		USEPA (2004) Qu et al. (2018)
Dermal reference dose	RfD_{dermal}	µg/ kg-day	Cr: 0.075; Ni: 0.8; Cu: 8; Zn: 60; Cd: 0.025; As: 0.123 Hg: 0.3; Pb: 0.42		USEPA (2004) Kumar et al. (2019)
Cancer slope factor	CSF	µg/ kg-day	Cr: 0.0005; Ni: 0.0017; As: 0.0015		Mohammadi et al. (2019) Kumar et al. (2019)

The total potential non-carcinogenic risks were assessed by hazard index (HI), which was the sum of the HQs for each element in each exposure pathway (Eq. 4). Total HI (THI) for each receptor was calculated by summing the HIs in each exposure pathway (Eq. 5).

$$HI_{ingestion/dermal} = HQ_{Cr} + HQ_{Ni} + HQ_{Cu} + HQ_{Zn} + HQ_{As} + HQ_{Cd} + HQ_{Hg} + HQ_{Pb} \quad (4)$$

$$THI = HI_{ingestion} + HI_{dermal} \quad (5)$$

If the values of HI and THI > 1, indicates that there may be a potential for adverse non-carcinogenic health effects to

occur, while HI and THI values < 1 indicate that non-carcinogenic health effects are not expected (USEPA, 2004).

- Carcinogenic health risk

The carcinogenic health risk (CR) was calculated for three metals (Cr, Ni and As) due to exposure to a potential carcinogen in this study. Potential carcinogenic risk possibilities that an individual may develop cancer over a lifetime of exposure are calculated by multiplying the $ADD_{i/d}$ and cancer slope factor (CSF) together (USEPA 1989, 2004). The slope factor (CSF) is a toxicity value that describes the association between dose and response (Table 1). The ADD of ingestion and dermal exposure of the above-mentioned carcinogens was considered in the calculation of total CR (TCR) for creeks. The CR and TCR were evaluated using the following equations Eqs. (6-8):

$$CR_{ingestion} = ADD_{ingestion} \times SF \quad (6)$$

$$CR_{dermal} = ADD_{dermal} \times SF \quad (7)$$

$$TCR = CR_{ingestion} + CR_{dermal} \quad (8)$$

A value of CR > 1.0×10^{-4} is considered unacceptable; 1.0×10^{-4} < CR < 1.0×10^{-6} is considered an acceptable range depending on the exposure conditions; CR<1.0×10^{-6} is considered not to have significant health effects (Mohammadi et al., 2019; Custodio et al., 2020).

RESULTS

The potential non-carcinogenic risks (HQ) and the total potential non-carcinogenic risks caused by different pathways (HI) for each creek, for adults and children, during the periods of December 2022, February 20023, and April 2023, were presented in Table 2,3,4,5.

In Sukesen Creek, during the three months of sampling, the values of the potential non-carcinogenic risks (HQ) related to ingestion were found to be greater than the values of dermal exposure (HQ_{dermal}) for both adults and children. Among the risk values of $HQ_{ingestion}$ and HQ_{dermal}, As has the highest contribution. In adults, values exceeding 1, indicating non-carcinogenic health risk, were observed for February and April. Similarly, in children, values above 1 were detected for all three months, indicating non-carcinogenic health risk.

Table 2. Heavy metal concentrations and non-carcinogenic (HQ and HI) risks of adults and children for Sukesen Creek

Months	Heavy metals	Concentration (µg/L) (mean±SD)	$HQ_{ingestion}$		HQ_{dermal}	
			Adult	Child	Adult	Child
December	Cr	0.36±0.02	3.62×10^{-3}	4.91×10^{-3}	5.88×10^{-4}	4.05×10^{-3}
	Ni	3.45±0.40	5.20×10^{-3}	7.06×10^{-3}	5.29×10^{-5}	3.64×10^{-4}
	Cu	-	-	-	-	-
	Zn	0.21±0.07	2.11×10^{-5}	2.86×10^{-5}	1.29×10^{-7}	8.86×10^{-7}
	As	8.16±0.63	8.20×10^{-1}	1.11×10^{0}	4.07×10^{-3}	2.80×10^{-2}
	Cd	-	-	-	-	-
	Hg	-	-	-	-	-
	Pb	-	-	-	-	-
	$HI_{ingestion}$ - HI_{dermal}		8.20×10^{-1}	1.12×10^{0}	5×10^{-3}	3.20×10^{-2}
	THI	Adult	0.83×10^{0}			
		Child	1.16×10^{0}			
February	Cr	0.64±0.10	6.44×10^{-3}	8.73×10^{-3}	1.05×10^{-3}	7.20×10^{-3}
	Ni	3.51±0.66	5.29×10^{-3}	7.18×10^{-3}	5.38×10^{-5}	3.70×10^{-4}
	Cu	0.87±0.09	6.55×10^{-4}	8.90×10^{-4}	6.67×10^{-6}	4.59×10^{-5}
	Zn	0.71±0.04	7.13×10^{-5}	9.68×10^{-5}	4.35×10^{-7}	3.00×10^{-6}
	As	11.18±1.13	1.12×10^{0}	1.52×10^{0}	5.57×10^{-3}	3.83×10^{-2}
	Cd	0.18±0.08	1.08×10^{-2}	1.47×10^{-2}	4.41×10^{-4}	3.04×10^{-3}
	Hg	0.02±0.01	2.01×10^{-3}	2.73×10^{-3}	4.09×10^{-6}	2.81×10^{-5}
	Pb	0.08±0.03	1.72×10^{-3}	2.34×10^{-3}	1.17×10^{-6}	8.04×10^{-6}
	$HI_{ingestion}$ - HI_{dermal}		5.18×10^{-1}	1.56×10^{0}	1.0×10^{-3}	5.0×10^{-2}
	THI	Adult	0.52×10^{0}			
		Child	1.61×10^{0}			
April	Cr	0.76±0.07	7.65×10^{-3}	1.04×10^{-2}	1.24×10^{-3}	4.62×10^{-3}
	Ni	2.34±0.30	3.53×10^{-3}	4.79×10^{-3}	3.59×10^{-5}	1.33×10^{-4}
	Cu	1.54±0.0	1.16×10^{-3}	1.58×10^{-3}	1.18×10^{-5}	4.39×10^{-5}
	Zn	0.55±0.19	5.53×10^{-5}	7.50×10^{-5}	3.37×10^{-7}	1.25×10^{-6}
	As	14.14±0.0	1.42×10^{0}	1.93×10^{0}	7.05×10^{-3}	2.62×10^{-2}
	Cd	0.10±0.02	6.03×10^{-3}	8.18×10^{-3}	2.45×10^{-4}	9.11×10^{-4}
	Hg	0.03±0.02	3.01×10^{-3}	4.09x10-03	6.13×10^{-6}	2.28×10^{-5}
	Pb	0.87±0.15	1.87×10^{-2}	2.54×10^{-2}	1.27×10^{-5}	4.72×10^{-5}
	$HI_{ingestion}$ - HI_{dermal}		1.46×10^{0}	1.98×10^{0}	9.0×10^{-3}	3.0×10^{-2}
	THI	Adult	1.47×10^{0}			
		Child	2.01×10^{0}			

Table 3. Heavy metal concentrations and non-carcinogenic (HQ and HI) risks of adults and children for Başpınar Creek

Months	Heavy metals	Concentration (µg/ L) (mean±SD)	$HQ_{ingestion}$		HQ_{dermal}	
			Adult	Child	Adult	Child
December	Cr	0.29±0.04	$2.92x10^{-3}$	$3.95x10^{-3}$	$4.74x10^{-4}$	$3.26x10^{-3}$
	Ni	0.37±0.28	$5.58x10^{-4}$	$7.57x10^{-4}$	$5.67x10^{-6}$	$3.90x10^{-5}$
	Cu	-	-	-	-	-
	Zn	0.53±0.24	$5.32x10^{-5}$	$7.23x10^{-5}$	$3.25x10^{-7}$	$2.24x10^{-6}$
	As	17.94±1.45	$1.80x10^{0}$	$2.45\ x10^{0}$	$8.94x10^{-3}$	$6.15x10^{-2}$
	Cd	-	-	-	-	-
	Hg	-	-	-	-	-
	Pb	-	-	-	-	-
	$HI_{ingestion}$ - HI_{dermal}		$1.81\ x10^{0}$	$2.45\ x10^{0}$	$9.03\ x10^{-3}$	$6.50\ x10^{-2}$
	THI	Adult	$1.82\ x10^{0}$			
		Child	$2.52\ x10^{0}$			
February	Cr	2.18±0.23	$2.19x10^{-2}$	$2.97x10^{-2}$	$3.57x10^{-3}$	$2.45x10^{-2}$
	Ni	1.57±0.0	$2.37x10^{-3}$	$3.21x10^{-3}$	$2.41x10^{-5}$	$1.66x10^{-4}$
	Cu	0.63±0.21	$4.75x10^{-4}$	$6.44x10^{-4}$	$4.83x10^{-6}$	$3.32x10^{-5}$
	Zn	1.64±0.42	$1.65x10^{-4}$	$2.24x10^{-4}$	$1.01x10^{-6}$	$6.92x10^{-6}$
	As	26.39±1.85	$2.65x10^{0}$	$3.60x10^{0}$	$1.32x10^{-2}$	$9.05x10^{-2}$
	Cd	0.39±0.06	$2.35x10^{-2}$	$3.19x10^{-2}$	$9.56x10^{-4}$	$6.58x10^{-3}$
	Hg	0.07±0.02	$7.03x10^{-3}$	$9.55x10^{-3}$	$1.43x10^{-5}$	$9.84x10^{-5}$
	Pb	0.08±0.03	$1.72x10^{-3}$	$2.34x10^{-3}$	$1.17x10^{-6}$	$8.04x10^{-6}$
	$HI_{ingestion}$ - HI_{dermal}		$9.93x10^{-1}$	$3.68\ x10^{0}$	$2.0\ x10^{-3}$	$1.20\ x10^{-1}$
	THI	Adult	$1.00\ x10^{0}$			
		Child	$3.80\ x10^{0}$			
April	Cr	2.41±0.18	$2.43x10^{-2}$	$3.29x10^{-2}$	$3.95x10^{-3}$	$1.46x10^{-2}$
	Ni	2.00±0.68	$3.01x10^{-3}$	$4.09x10^{-3}$	$3.06x10^{-5}$	$1.14x10^{-4}$
	Cu	0.49±0.31	$3.69x10^{-4}$	$5.01x10^{-4}$	$3.75x10^{-6}$	$1.40x10^{-5}$
	Zn	0.33±0.05	$3.32x10^{-5}$	$4.50x10^{-5}$	$2.02x10^{-7}$	$7.52x10^{-7}$
	As	27.41±2.13	$2.75\ x10^{0}$	$3.74\ x10^{0}$	$1.37x10^{-2}$	$5.08x10^{-2}$
	Cd	0.01±0.01	$6.03x10^{-4}$	$8.18x10^{-4}$	$2.45x10^{-5}$	$9.11x10^{-5}$
	Hg	0.04±0.04	$4.02x10^{-3}$	$5.46x10^{-3}$	$8.17x10^{-6}$	$3.04x10^{-5}$
	Pb	0.55±0.19	$1.18x10^{-2}$	$1.61x10^{-2}$	$8.03x10^{-6}$	$2.98x10^{-5}$
	$HI_{ingestion}$ - HI_{dermal}		$2.79\ x10^{0}$	$3.80\ x10^{0}$	$1.80\ x10^{-3}$	$7.0\ x10^{-2}$
	THI	Adult	$2.82\ x10^{0}$			
		Child	$3.86\ x10^{0}$			

Additionally, it was determined that regardless of the metal, the children hazard index values ($HI_{ingestion}$ and HI_{dermal}) were higher than the adult hazard index values during all three months. Statistically, a significant difference was found between the children hazard index (HI) and adult hazard index (HI) values ($p < 0.05$). The THI-adult value was found to be above 1 in April, while the THI-child value was above 1 throughout all three months (Table 2). In this context, considering the heavy metals involved, there appear to be potential adverse health effects in terms of non-carcinogenic risks for adults in April and for children throughout all the sampled months.

As it can be seen in Table 3, in Başpınar Creek, considering the $HQ_{ingestion}$ and HQ_{dermal} values for adults and children, the contribution of As is significant in the high non-carcinogenic risk. In all months, the $HQ_{ingestion}$ values were found to be higher than the HQ_{dermal} values. Except for February, the HI-adult values were found to be above 1, indicating non-carcinogenic health risk. Similarly, the HI-child values were also above 1 throughout the three months, indicating non-carcinogenic health risk. Indeed, this situation indicates an emerging health concern. Regardless of the specific metals, it was observed that the health risk values for children every month basis were higher than the health risk values for adults.

In Yavrucak Creek, during the sampled months, the $HQ_{ingestion}$ values were determined to be higher than the HQ_{dermal} values. For this creek, both the HI-adult and HI-child values were found to be below 1 (Table 4). Since the THI values for all months were found to be below 1 in comparison to the other three creeks, there is no significant adverse health effect on adults and children due to exposure to heavy metals through ingestion and dermal contact pathways. When considering the three-month period regardless of the specific metals, there is a significant difference ($p < 0.05$) in the health risk values between adults and children. However, no significant difference was observed ($p > 0.05$) between the measurements of HI-adult and HI-child.

Table 4. Heavy metal concentrations and non-carcinogenic (HQ and HI) risks of adult and child for Yavrucak Creek

Months	Heavy metals	Concentration (µg/ L) (mean±SD)	$HQ_{ingestion}$		HQ_{dermal}	
			Adult	Child	Adult	Child
December	Cr	0.58±0.00	-	-	-	-
	Ni	0.71±0.21	$1.07x10^{-3}$	$1.45x10^{-3}$	$1.09x10^{-5}$	$7.49x10^{-5}$
	Cu	-	-	-	-	-
	Zn	-	0	0	-	-
	As	1.28±0.31	$1.29x10^{-1}$	$1.75x10^{-1}$	$6.38x10^{-4}$	$4.39x10^{-3}$
	Cd	-	-	-	-	-
	Hg	-	-	-	-	-
	Pb	-	-	-	-	-
	$HI_{ingestion}$ - HI_{dermal}		$1.30x10^{-1}$	$1.76 x10^{-1}$	$1.0 x10^{-3}$	$4.0 x10^{-3}$
	THI	Adult	$1.30 x10^{-1}$			
		Child	$1.80 x10^{-1}$			
February	Cr	0.16±0.03	$1.61x10^{-3}$	$2.18x10^{-3}$	$2.62x10^{-4}$	$1.80x10^{-3}$
	Ni	0.45±0.16	$6.78x10^{-4}$	$9.21x10^{-4}$	$6.90x10^{-6}$	$4.75x10^{-5}$
	Cu	0.40±0.09	$3.01x10^{-4}$	$4.09x10^{-4}$	$3.06x10^{-6}$	$2.11x10^{-5}$
	Zn	0.36±0.11	$3.62x10^{-5}$	$4.91x10^{-5}$	$2.21x10^{-7}$	$1.52x10^{-6}$
	As	1.90±0.29	$1.91x10^{-1}$	$2.59x10^{-1}$	$9.47x10^{-4}$	$6.52x10^{-3}$
	Cd	0.05±0.03	$3.01x10^{-3}$	$4.09x10^{-3}$	$1.23x10^{-4}$	$8.44x10^{-4}$
	Hg	0.02±0.01	$2.01x10^{-3}$	$2.73x10^{-3}$	$4.09x10^{-6}$	$2.81x10^{-5}$
	Pb	0.02±0.02	$4.31x10^{-4}$	$5.84x10^{-4}$	$2.92x10^{-7}$	$2.01x10^{-6}$
	$HI_{ingestion}$ - HI_{dermal}		$1.0 x10^{-1}$	$2.70 x10^{-1}$	$1.0 x10^{-4}$	$1.0 x10^{-2}$
	THI	Adult	$1.0 x10^{-1}$			
		Child	$2.80 x10^{-1}$			
April	Cr	0.58±0.06	$5.83x10^{-3}$	$7.91x10^{-3}$	$9.48x10^{-4}$	$3.52x10^{-3}$
	Ni	2.50±0.11	$3.77x10^{-3}$	$5.11x10^{-3}$	$3.83x10^{-5}$	$1.42x10^{-4}$
	Cu	0.05±0.02	$3.77x10^{-5}$	$5.11x10^{-5}$	$3.83x10^{-7}$	$1.42x10^{-6}$
	Zn	0.18±0.04	$1.81x10^{-5}$	$2.45x10^{-5}$	$1.10x10^{-7}$	$4.10x10^{-7}$
	As	2.00±0.17	$2.01x10^{-1}$	$2.73x10^{-1}$	$9.97x10^{-4}$	$3.70x10^{-3}$
	Cd	0.02±0.01	$1.21x10^{-3}$	$1.64x10^{-3}$	$4.90x10^{-5}$	$1.82x10^{-4}$
	Hg	0.02±0.01	$2.01x10^{-3}$	$2.73x10^{-3}$	$4.09x10^{-6}$	$1.52x10^{-5}$
	Pb	0.95±0.12	$2.05x10^{-2}$	$2.78x10^{-2}$	$1.39x10^{-5}$	$5.15x10^{-5}$
	$HI_{ingestion}$ - HI_{dermal}		$2.34x10^{-1}$	$3.20 x10^{-1}$	$2.0 x10^{-3}$	$1.0 x10^{-2}$
	THI	Adult	$2.40 x10^{-1}$			
		Child	$3.20 x10^{-1}$			

As presented in Table 5, in Gölcük Creek, similar to the other three creeks, As holds significant importance when considering the ingestion and dermal exposure pathways. Although the $HQ_{ingestion}$ values were found to be higher than the HQ_{dermal} values, the $HQ_{ingestion}$ values for both children and adults, except for April, were below the non-carcinogenic health risk level of 1. Similar to the above three creeks, the health risk values for children were relatively higher compared to the health risk values for adults. Regardless of the specific metals, similar to the other creeks, no significant difference was found between the measurements of HI-adult and HI-child in Gölcük Creek ($p > 0.05$). According to the THI values, except for April, the heavy metals do not pose a significant non-carcinogenic health risk in terms of potential adverse effects.

In the scope of the study, it was determined that the carcinogenic risk values ranged from $1.0x10^{-4}$ to $1.38x10^{-3}$ for adults and from $1.30x10^{-4}$ to $1.88x10^{-3}$ for children. CR and TCR values ranging from 10^{-6} to 10^{-4} represent the probability of developing cancer within a lifespan of 70 years. Especially for Başpınar Creek, the data for the month of April were found to be significantly above the acceptable carcinogenic risk range. As shown in Figure 2, during the sampling months, the ∑CR child values in all four creeks were found to be higher than the ∑CR adult values. This finding indicates that children are more vulnerable to carcinogenic risks compared to adults when exposed to heavy metals in creek waters.

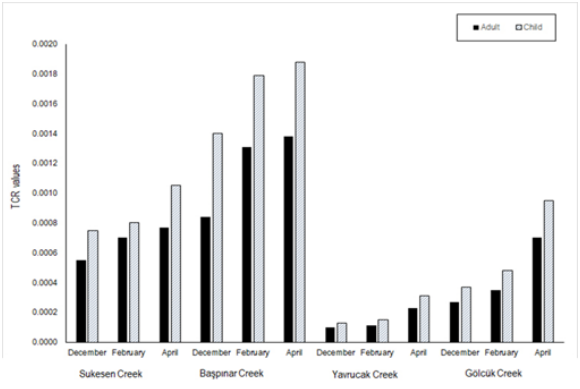

Figure 2. Total carcinogenic values for adults and children at each creek

Table 5. Heavy metal concentrations and non-carcinogenic (HQ and HI) risks of adult and child for Gölcük Creek

Months	Heavy metals	Concentration (µg/L) (mean±SD)	$HQ_{ingestion}$		HQ_{dermal}	
			Adult	Child	Adult	Child
December	Cr	-	-	-	-	-
	Ni	0.80±0.14	$1.21x10^{-3}$	$1.64x10^{-3}$	$1.23x10^{-5}$	$8.44x10^{-5}$
	Cu	-	-	-	-	-
	Zn	-	-	-	-	-
	As	5.11±0.10	$5.13x10^{-1}$	$6.97x10^{-1}$	$2.55x10^{-3}$	$1.75x10^{-2}$
	Cd	0	-	-	-	-
	Hg	0	-	-	-	-
	Pb	0	-	-	-	-
	$HI_{ingestion}$ - HI_{dermal}		$3.0x10^{-3}$	$6.90x10^{-1}$	$3.0x10^{-3}$	$2.0x10^{-2}$
	THI	Adult	$6.0 x10^{-3}$			
		Child	$0.72 x10^{0}$			
February	Cr	0.25±0.0	$2.51x10^{-3}$	$3.41x10^{-3}$	$4.09x10^{-4}$	$2.81x10^{-3}$
	Ni	3.06±0.0	$4.61x10^{-3}$	$6.26x10^{-3}$	$4.69x10^{-5}$	$3.23x10^{-4}$
	Cu	0.75±0.07	$5.65x10^{-4}$	$7.67x10^{-4}$	$5.75x10^{-6}$	$3.96x10^{-5}$
	Zn	0.50±0.07	$5.02x10^{-5}$	$6.82x10^{-5}$	$3.06x10^{-7}$	$2.11x10^{-6}$
	As	4.22±0.38	$4.24x10^{-1}$	$5.76x10^{-1}$	$2.10x10^{-3}$	$1.45x10^{-2}$
	Cd	0.21±0.07	$1.27x10^{-2}$	$1.72x10^{-2}$	$5.15x10^{-4}$	$3.54x10^{-3}$
	Hg	0.02±0.01	$2.01x10^{-3}$	$2.73x10^{-3}$	$4.09x10^{-6}$	$2.81x10^{-5}$
	Pb	0.06±0.04	$1.29x10^{-3}$	$1.75x10^{-3}$	$8.76x10^{-7}$	$6.03x10^{-6}$
	$HI_{ingestion}$ - HI_{dermal}		$2.70x10^{-1}$	$6.10 x10^{-1}$	$4.0 x10^{-3}$	$2.0 x10^{-2}$
	THI	Adult	$2.70 x10^{-1}$			
		Child	$0.63 x10^{0}$			
April	Cr	0.06±0.02	$6.03x10^{-4}$	$8.18x10^{-4}$	$9.81x10^{-5}$	$3.65x10^{-4}$
	Ni	2.98±0.15	$4.49x10^{-3}$	$6.10x10^{-3}$	$4.57x10^{-5}$	$1.70x10^{-4}$
	Cu	0.04±0.01	$3.01x10^{-5}$	$4.09x10^{-5}$	$3.06x10^{-7}$	$1.14x10^{-6}$
	Zn	0.02±0.01	$2.01x10^{-6}$	$2.73x10^{-6}$	$1.23x10^{-8}$	$4.56x10^{-8}$
	As	12.02±0.91	$1.21 x10^{0}$	$1.64 x10^{0}$	$5.99x10^{-3}$	$2.23x10^{-2}$
	Cd	0.31±0.07	$1.87x10^{-2}$	$2.54x10^{-2}$	$7.60x10^{-4}$	$2.83x10^{-3}$
	Hg	0.01±0.01	$1.00x10^{-3}$	$1.36x10^{-3}$	$2.04x10^{-6}$	$7.59x10^{-6}$
	Pb	0.61±0.19	$1.31x10^{-2}$	$1.78x10^{-2}$	$8.90x10^{-6}$	$3.31x10^{-5}$
	$HI_{ingestion}$ - HI_{dermal}		$1.24x10^{0}$	$1.69x10^{0}$	$7.0x10^{-3}$	$3.0x10^{-2}$
	THI	Adult	$1.25x10^{0}$			
		Child	$1.72x10^{0}$			

DISCUSSION

In this study, the hazard index (HI) value was obtained by summing up the total potential health risks (HQs from ingestion and dermal contact exposure pathways) caused by heavy metals. Within the scope of the study, ingestion hazard quotient ($HQ_{ingestion}$) values were found to be higher than dermal absorption hazard quotient (HQ_{dermal}) values. In different studies, it was reported that the non-carcinogenic health risk values associated with metal ingestion were higher than the risk values associated with dermal exposure (Wu et al., 2009; Wang et al., 2017; Qu et al., 2018; Castresana et al., 2019; Mohammadi et al., 2019; Varol and Tokatlı, 2023). According to our findings, non-carcinogenic risks associated with water ingestion were identified in the other creeks, particularly in April, for both children and adults, except for Yavrucak Creek. It is possible that individuals exposed to the water of these creeks may experience adverse health effects However, Kutlu and Sarıgül (2023) reported that they found all HQ and HI values to be below 1 in surface water samples taken from the Munzur River area (Ramsar Site) in Türkiye for both adults and children.

According to our study, As has been determined as the heavy metal with the highest contribution in the HI ingestion pathway, which is in line with similar findings reported in different research studies. For example, Wu et al. (2009) reported that in surface water samples from the Yangtze River in China, all metals except As were found to have Hazard Quotient values below 1. Xiao et al. (2019) on the other hand, indicated a potential hazard by finding high $HQ_{ingestion}$ and $HI_{ingestion}$ values for As in a river water sample from the Lös Plateau in China. In their study, Kumar et al. (2019), examined the heavy metal content of surface waters worldwide and found that As was the main pollutant in terms of ingestion-dermal exposure hazard quotients for both adults and children. According to Custodio et al. (2020), the waters from rivers exposed to mining impacts in the Central Andes of Peru showed higher hazard indexes, determined by the ingestion of heavy metals, including As, exceeding the threshold value ($HI_{ingestion} > 1$). The findings of the present study align with the results reported by Li and Zhang (2010) in their study on surface water samples from the Upper Han River. They found that As posed a greater risk to human health, with a hazard quotient (HQ) exceeding the threshold value of 1, and identified it as the most significant pollutant causing non-carcinogenic and carcinogenic concerns, especially in children, with a carcinogenic risk greater than 10^{-4}. Our findings parallel the results reported by Varol (2019) for Keban Dam Lake, where

he found that As contributed to approximately 70% of the hazard index (HI) through the ingestion pathway for both residential and recreational receptors. Additionally, our findings align with the study conducted by Tokatlı and Ustaoğlu (2020) on the Meriç River Delta, where they identified As as the most hazardous toxicant.

In this study, dermal contact hazard quotient (HQ_{dermal})values were found to be <1 in all the creeks and sampling months, which is consistent with the findings of Mazel et al. (2022) for Loutété River in Southeast Congo, where they reported a HQ_{dermal} lower than 1 for all heavy metals. Varol (2019) indicated in his study conducted in Keban Dam Lake that Cr contributed approximately 79% to the HI for both residential and recreational receptors through the dermal contact pathway. In our study, Cr was identified as the second heavy metal contributing to the HQ_{dermal} value, following arsenic, including the December.

In this study, it was determined that regardless of the metal, the hazard index values ($HI_{ingestion}$ and HI_{dermal}) for children were higher than those for adults in all three sampling months. This result is consistent with the findings of a study (Varol 2019; Canpolat et al., 2020) that reported higher health risks for children compared to adults based on the HQ, HI, and THI values determined for Keban Dam Lake (Türkiye). In the study conducted by Saleem et al. (2019) in three freshwater reservoirs in Pakistan during three seasons (pre-monsoon, monsoon, and post-monsoon), it was indicated that Cr, Cd, Co, Pb, and Ni were associated with particularly high risks ($HI_{ingestion}$ > 1) for children. In a study conducted by Castresana et al. (2019) evaluating the health risks of the local population exposed to pollution in the Atoyac River (Mexico), the hazard index (HI) results were found to be higher in children due to the consumption of drinking water during the dry season, which is consistent with our research findings.

Different countries have reported carcinogenic risk (CR) values above the acceptable level of 10^{-4} for potential cancer risks associated with arsenic in surface waters (Qu et al., 2018; Shil and Singh 2019; Zhang et al., 2022; Selvam et al., 2022). In some studies conducted in our country, specifically in dam lakes and rivers, the carcinogenic risk values for As and Cr are below the target risk level of 1×10^{-4} (Canpolat et al., 2020; Varol et al., 2021; Kutlu and Sarıgül 2023). In our study, the cancer index values for Cr, Ni, and As were found to be higher than the range of $1x10^{-6}$ and $1x10^{-4}$ recommended by USEPA (1989, 2004) for both adults and children in all months and creeks. In this context, it has been determined that Cr, Ni, and As are potential heavy metals that have negative effects on health. Kumar et al. (2019) and Mohammadi et al. (2019) have also reported that heavy metals such as Cr, Ni, and Cd, in addition to As, exhibit values that can be considered as high risk for cancer formation through ingestion compared to dermal exposure. In addition, our finding that the carcinogenic risk values arising from oral exposure in children are higher than those in adults is consistent with the findings reported by Castresana et al. (2019), Custodio et al. (2020) and Joseph et al. (2022).

CONCLUSION

Water pollution caused by heavy metals as a result of intense anthropogenic pressure poses a threat to aquatic environments and consequently human health. Heavy metals can cause significant adverse effects on human health by serving as both non-carcinogenic and carcinogenic risk factors. In this study, hazard quotient (HQ) values were determined through ingestion and dermal pathways in four major streams that feed Lake Mogan, for both adults and children. The potential positive and negative health effects of heavy metals were evaluated based on exposure through these two pathways. Based on the findings, it was determined that As was the primary contaminant posing the highest risk to human health. According to the HQ-ingestion pathway values, while the heavy metals posing the highest risk after As vary among the creeks and months, it has been observed that Cr has a significant contribution in the HQ-dermal pathway after As, specifically in Sukesen, Başpınar, and Yavrucak Creeks. According to the $HI_{ingestion}$ and HI_{dermal} values, the children hazard index values were found to be higher than the adults hazard index values, indicating that children are exposed to higher health risks, particularly related to the considered heavy metals. The total hazard index (THI) values, considering the selected heavy metals in Sukesen and Başpınar Creeks for all sampled months in both adults and children, as well as in Gölcük Creek specifically for children in April, revealed that heavy metals pose a significant risk to the health of both adults and children. Based on the THI data, the ranking of the studied creeks in terms of potential adverse health effects is as follows: Sukesen Creek > Başpınar Creek > Gölcük Creek > Yavrucak Creek. The possible reason why Yavrucak Creek is ranked last may be that it is under less pressure than other creeks in terms of residential areas. The carcinogenic risk values determined for Cr, Ni, and As reflect a high risk of cancer for adults and particularly for children, indicating that the ingestion pathway is more risky compared to the dermal route.

Based on the study findings, it was determined that the heavy metals in Sukesen, Başpınar, and Gölcük Creeks in the Mogan Lake Basin pose a risk to human health and may contribute to the emergence of adverse health effects. In this context, considering the necessity of protecting public health, measures should be developed to reduce heavy metal contamination in the surface waters of the basin, especially in the mentioned creeks. Administrative measures regarding the sources of pollution in the lake basin are not only important for protecting human health but also for the sustainability of the lake ecosystem.

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AUTHORSHIP CONTRIBUTION

All authors took part in a part of the article and contributed to the design of the research, collection and writing of the article.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL STATEMENT

There are no ethical issues with the publication of this manuscript.

DATA AVAILABILITY

The authors confirm that the data that supports the findings of this study are available within the article.

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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Farklı dezenfektanların balık işleme tesisinden izole edilen *Staphylococcus aureus* ve *Pseudomonas fluorescens* üzerine etkinliklerinin incelenmesi

An investigation of the efficacy of different disinfectants on *Staphylococcus aureus* and *Pseudomonas fluorescens* isolated from fish processing plant

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Öz: Sağlıklı ve güvenli gıda üretimi, uluslararası düzeyde ihracat yapan işleme tesisleri için önemli bir konudur. Dezenfeksiyon uygulamaları kullanılarak, işleme tesisinin her aşamasında, çeşitli kaynaklardan gelerek balıkları kontamine edebilecek bakteri sayısı en düşük seviyede tutulmaya çalışılır. Bu çalışmada, balık işleme tesisinin kalıcı mikroflorasını oluşturan patojen bakteri izolatları üzerinde, farklı dezenfektanların etkili konsantrasyonlarının belirlenmesi amaçlanmıştır. Dezenfektan olarak; %0,5; %1, %2 konsantrasyonlarda klor, kuarterner amonyum, gluteraldehid ve hidrojen peroksitin, balık işleme tesisinden izole edilen *Staphylococcus aureus* ve *Pseudomonas fluorescens* üzerinde kantitatif süspansiyon testi ile antibakteriyel etkinliği değerlendirilmiştir. Çalışmamızda tüm dezenfektanların %2 konsantrasyonda izole edilen bakteriler üzerine etkili olduğu tespit edilmiştir. Klor bileşiklerinin düşük konsantrasyonlarda (%0,5 ve %1) *S. aureus* ve *P. fluorescens* izolatlarında bakterisidal etkinlik göstermediği belirlenmiştir. Kuarterner amonyum bazlı dezenfektanların tüm izolatlar üzerine, hidrojen peroksit bileşiklerinin ise *Pseudomonas fluorescens* üzerine tüm konsantrasyonlarda etkinliği saptanmıştır.

Anahtar kelimeler: Balık işleme tesisi, dezenfektan, *Staphylococcus aureus, Pseudomonas fluorescens*, antibakteriyel aktivite

Abstract: Healthy and safe food production is an important issue for processing plants that export internationally. With cleaning and disinfection, the number of bacteria that can contaminate fish by coming from various sources at every stage of the processing plant is kept to a minimum. In this study, it was aimed to determine the effective concentrations of different disinfectants on the bacterial strains forming the resident microflora of the fish processing plant. In the study, the effectiveness of 4 different disinfectants (chlorine, quaternary ammonium compounds, gluteraldehyde and hydrogen peroxide) was termined on bacteria isolated from different parts of the fish processing plant. The antimicrobial efficacy of different disinfectant concentrations was determined by the Quantitative Suspension Assay. In our study, it was determined that all disinfectants were effective on bacteria isolated at 2% concentration. It was determined that chlorine compounds did not show bactericidal activity at low concentrations (0.5%, 1%) on *Staphylococcus aureus* and *Pseudomonas fluorescens* isolates. The effectiveness of quaternary ammonium-based disinfectants on all isolates and hydrogen peroxide compounds on *P. fluorescens* at all concentrations was determined.

Keywords: Fish processing plant, disinfectant, *Staphylococcus aureus*, *Pseudomonas fluorescens*, antimicrobial activity

GİRİŞ

Balık işleme endüstrisinde mikrobiyolojik kontaminasyon riskini kontrol etmek için, temiz ve dezenfekte edilmiş balık temas yüzeyleri çok önemlidir. Kontaminasyon, farklı temizlik ve sanitasyon teknikleri kullanılarak giderilmeye çalışılmaktadır (Martowitono, 2011). Bu amaçla gıda işleme ortamlarında mikroorganizmaların kontrolü için farklı kimyasal formülasyonlara sahip dezenfektanlar kullanılmaktadır (Iñiguez-Moreno vd., 2017). Gıda endüstrisinde en sık kullanılan dezenfektanlar klor bileşikleri (örn; hipoklorit ve klordioksit), organik asitler (örn; perasetik asit, laktik, asetik, propiyonik, sitrik ve benzoik asit), kuarterner amonyum bileşikleri (örn; benzalkonyum klorür ve didecyl dimethyl ammonium chloride), iyot bileşikleridir (Xiang vd., 2019).

Kuarterner amonyum bileşikleri (QA) mikroorganizmaların yüzeyindeki negatif yüklü grupları hedef alan katyonik maddelerdir ve en çok kullanılan dezenfektanlar arasında yer almaktadır. Bu bileşikler Gram pozitif bakterilere, Gram negatif bakterilerden daha etkili olup funguslara ve zarflı virüslere karşı da iyi aktivite gösterirler (Reuter, 1998; Dvorak, 2005; Morente vd., 2013). Kuarterner amonyum bileşikleri grubundan olan didecyl dimethyl ammonium chloride (DDAC) çok sayıda üründe kullanılan bir dialkil-kuarterner amonyum bileşiğidir (Anderson vd., 2016).

Aldehit grubundan olan, gluteraldehid ve formaldehid ise geniş bir spektrumda dezenfektan etkisi gösterir ve proteinleri denatüre ederek, nükleik asitleri parçalayarak etkili olurlar. Gluteraldehid hem düşük sıcaklıktaki dezenfeksiyonda kullanılabilmesi, hem de metal, kauçuk vb. içeren yüzeylerde aşındırıcı olmamasından dolayı sıklıkla kullanılan bir dezenfektandır (McDonnell ve Russell, 1999; Dvorak, 2005).

Oksidan madde olan hidrojen peroksit ve perasetik asit de mikroorganizmaların proteinlerini ve lipidlerini denatüre ederek

etki gösteren geniş spektrumlu maddelerdir (Dvorak, 2005; Anderson vd., 2016). Perasetik asit, hidrojen peroksitten daha güçlü bir dezenfektan olarak kabul edilmektedir. Ayrıca perasetik asit, dezenfeksiyondan sonra kalıntı bırakmaması, organik madde varlığında ve düşük sıcaklıklarda etkili olması nedeniyle yaygın olarak kullanılmaktadır (Arda, 2000). Dezenfektan olarak kullanılan diğer bir grup halojen bileşiklerinden olan klor içeren sodyum veya kalsiyum hipoklorit özellikle gıda işleme tesislerinde çok yaygın olarak kullanılmaktadır. Bunlar geniş bir antimikrobiyal aktivite spektrumuna sahip olan, toksik tortular bırakmayan ve su sertliğinden etkilenmeyen bileşiklerdir (Rutala vd, 2008).

Farklı dezenfektanların mikroorganizmalar üzerine etkisinin incelendiği çeşitli çalışmalar (Can vd., 2010; Mısırlı ve Aydın, 2011; Li vd., 2014; Møretrø ve Langsrud, 2017) ve farklı dezenfaktanların su ürünleri işleme fabrikalarında kullanımı ile ilgili çok sayıda çalışma (Feliciano vd., 2010; Martowitono, 2011; Vázquez-Sánchez vd., 2014; Beeharry vd., 2016; Iñiguez-Moreno vd., 2017) yapılmasına rağmen, kalıcı bakteriyel florayı oluşturan izolatlar üzerinde dezenfektanların antibakteriyel aktivitesinin belirlendiği kapsamlı bir çalışmaya rastlanılmamıştır.

Bu amaçla yapılan çalışmada, klor, kuarterner amonyum, gluteraldahid, ve hidrojen peroksit bazlı dezenfektanların balık işleme tesisinin farklı bölümlerinden dezenfeksiyon uygulaması sonrasında izole edilen *Staphylococcus aureus* ve *Pseudomonas fluorescens* izolatları üzerinde antibakteriyel etkinliğinin belirlenmesi hedeflenmiştir.

MATERYAL VE METOT

Test edilen dezenfektanlar

Bu çalışmada, 2 farklı firmaya ait 4 farklı kimyasal formülasyon içeren dezenfektanın etkinliği incelenmiştir. Dezenfektan olarak; klor bazlı (K), kuarterner amonyum bazlı (QA), gluteraldehid bazlı (GA) ve hidrojen peroksit bazlı (HP) olanlar kullanılmıştır (Tablo 1).

Tablo 1. Farklı dezenfektan formülasyonları
Table 1. Different disinfectant formulations

Ürün	Kısaltma	Aktif bileşenler
Klor bazlı	K	%5-15 Sodyum hidroksit, <%5 Sodyum hipoklorit, <%5Aminler, C10-16 alkildimetil, N-oksitler
Kuarterner amonyum bazlı	QA	<%5 Didesildimetilamonyum klorür
Gluteraldehid bazlı	GA	%15-25 Gluteraldehid, %5-15 Dördüncül amonyum bileşikleri, benzil-12-16-alkildimetil, klorürler, <%5 Didesildimetilamonyumklorür, <%5 Tetrasodyum etilendiamintetraasetat, <%5 Fosforik asit, <%5 d-limonen
Hidrojen peroksit bazlı	HP	%99,956 hidrojen peroksit (%50'lik), %0,044 kolloid gümüş

EN 1040 "Kantitatif süspansiyon"da bildirilen zorunlu deney şartları (konsantrasyon, sıcaklık, temas süresi ve bakteriler) kullanılmıştır. Dezenfektanlar üretici firmanın tavsiye ettiği en düşük konsantrasyonda (%2) ve tavsiye edilen konsantrasyonun altındaki konsantrasyonlar (%1,%0,5) kullanılarak dilüsyonları hazırlanmıştır. Tüm dezenfektanlar testten önce taze olarak hazırlanmışlardır. Seyreltici ve dezenfektan kontrolü olarak sterilize edilmiş distile su kullanılmıştır. Her bir bakteri için işlem ikişer kez uygulanmıştır.

Test edilen bakteri türleri

Balık işleme tesis yüzeyinin 10 farklı bölümünden izole edilmiş olan 20 bakteri (*S. aureus* ve *P. fluorescens)* izolatlarının, farklı dezenfektanlara olan duyarlılıkları, farklı konsantrasyon ve sürelerde test edilmiştir. Test bakterileri, balık işleme tesis yüzeylerinden dezenfektan uygulamasından sonra izole edilmiştir. Yüzey örnekleri, sürtme (swab) yöntemi ile alınmıştır. 25 cm^2 alan steril serum fizyolojik ile nemlendirilmiş swab'lar ile taranmış ve bu swab çubukları, örnek olarak kabul edilmiştir. Bu örneklerden onluk seyreltmeler hazırlanmıştır (Collins vd., 1998; Bell vd., 2005). *Pseudomonas* spp. izolasyonunda, *Pseudomonas* Selective Agar Base (Cetrimide Agar, Merck, 105284.0500) besiyerine ekimler yapılmıştır. 37^{o}C'de 48 saat inkübasyon sonunda petriler değerlendirilmiştir. Sarı-yeşil pigmentli ve uzun dalga boylu UV lamba ile floresan ışıma veren koloniler *Pseudomonas* spp., olarak değerlendirilmiştir (Halkman, 2005). İdentifikasyon testi API 20NE test kiti ile yapılmıştır.

Staphylococcus spp., izolasyonu için, Baird – Parker Agar (Merck, 105406.0500) besiyerine ekimler yapılmıştır. Ekimden sonra petriler 37^{0}C'de 24 saat inkübasyona bırakılmıştır (ISO,2003).Tipik ya da şüpheli kolonilere Bactident Coagulase (Merck, 113306.00001) kullanılarak koagülaz testi yapılmıştır. *Staphylococcus* spp.'nin identifikasyonu, API *Staphylococcus* test kiti ve tamamlayıcı biyokimyasal yöntemlerle yapılmıştır (Halkman, 2005).

Bakterisidal aktivitenin belirlenmesi (Süspansiyon testi)

Dezenfektanların bakteriler üzerindeki etkinliği, EN 1040 "Kantitatif süspansiyon" test yöntemi kullanılarak saptanmıştır (TSE EN 1040, 2006). Nötralizasyon işleminin etkinliği de TSE EN 1040 standardına göre yapılmıştır. Nötrleştirici madde olarak Ringer solüsyonu ile hazırlanmış 30g/L Tween 80 çözeltisi kullanılmıştır. Test bakteri kullanılarak nötralizan sistemin üremeyi inhibe etmediği, dezenfektanların aktivitelerini durdurduğu kontrol edilmiştir.

Dezenfektanın etkinliğinin belirlenmesi için saf kültürler Tryptic Soy Agar (TSA) (Merck, 105458.0500) besiyerine ekilerek aktif hale getirilmiştir. Sonrasında bu ilk pasajdan aynı

yolla ikinci bir pasaj hazırlanmış ve 37^{0}C 24 saat inkübe edilmiştir. TSA petrisinden alınan bakteri kolonisi Tryptic Soy Broth (TSB) (Merck, 105458.0500) içeren tüp içine pasajlanmış ve 37^{0}C 24 saat inkübe edilmiştir. İnkübasyon süresi sonunda tüpler 2000 rpm'de 20 dk. santrifüj edilmiştir. Hücre pelletleri %0,5 Tween 80 çözeltisi ile yıkanmıştır. TSB içerisinde bulunan ve McFarland 0,5 değerine göre ayarlanmış bakteri süspansiyonundan 1 ml alınarak 9 ml dezenfektan solüsyonu içerisine eklenmiştir. Her bir dilüsyon örneği 5 ve 15 dk. temas süresinde, 20^{0}C inkübatörde (Memmert, Almanya) bekletilmiştir. Temas süresi sonunda, deney süspansiyonundan 1ml örnek alınmış ve içinde 8 ml nötürleştirici madde ile 1 ml steril distile su bulunan tüpe pipetlenmiştir. Daha sonra 20^{0}C'de 5 dk. nötralizasyon işlemi için inkübatörde bekletilmiştir. Sürenin sonunda, sayım için, seyreltici kullanılarak 10^{-1}'den 10^{-6}'a kadar seyreltme tüpleri hazırlanmıştır. Her bir dilüsyon örneğinden 100 µl alınıp TSA petrilerinin üzerine yayma plak yöntemiyle ekim yapılmıştır. Ekim yapılan petriler 37^{0}C'de 24 saat süreyle inkübe edilmiştir.

Oluşan koloniler sayılmış ve koloni oluşturan birim (kob) dilüsyon faktörüyle çarpılarak hesaplanmıştır. Mikrobiyal sayımlar $\log_{10}$ ölçeğine dönüştürülmüştür. Bakteriyel azalma (redüksiyon oranı), bir dezenfektana maruz kalmadan önceki canlı koloni sayısından, maruz kaldıktan sonraki canlı koloni sayısı çıkarılarak hesaplanmıştır ($\log_{10}$ redüksiyon= $\log_{10}$ predezenfektanlı sayım-$\log_{10}$ dezenfektanlı sayım). Antimikrobiyal etkinlik testlerinin sonuçları standartta verilen logaritmik limitlere göre değerlendirilmiştir. Bakterisidal aktivite için TSE EN 1040 gerekliliği, seçilen temas süresi içinde izolatlar üzerinde ≥5 $\log_{10}$'luk azalma veya daha büyük olması etkili antimikrobiyal aktivite olarak kabul edilmiştir.

İstatiksel Analiz

Bakteri sayımlarının istatistiksel analizleri mutlak değerler üzerinden yapılmıştır. Bakteri sayılarının redüksiyon değerleri logaritmik değerlere dönüştürülmüştür. Logaritmik değerleri analiz etmek için tek yönlü ANOVA ve Duncan'ın çoklu aralık testleri kullanılmıştır. Analizler Statistical Package for Social Sciences 26.0 for Windows (SPSS Inc., Chicago, Illinois, ABD) kullanılarak yapılmış olup, sonuçlar $p<0.05$ anlamlılık düzeyinde değerlendirilmiştir.

BULGULAR

Çalışmamızda kullanılan izolatların tamamı %2 konsantrasyonda gluteraldehid, kuarterner amonyum, hidrojen peroksit ve klora 5 dk. temas süresinde duyarlı bulunmuştur. *S. aureus* izolatları üzerinde %0,5 klor konsantrasyonu 5 ve 15 dk. temas sürelerinde etkinlik gösterememiştir. Fakat %1 ve %2 klor çözeltilerinde bakteri izolatlarının duyarlı olduğu saptanmıştır. Ayrıca %0,5 ve %1 klor bazlı dezenfektanın 20°C'da 5 ve 15 dk. temas sürelerinde *P. fluorescens* izolatlarına etkinliği gözlenmemiştir. İstatistiksel olarak farklı dezenfektan konsantrasyon düzeyleri arasında anlamlı fark belirlenmiştir. *P. fluorescens* üzerinde 5 ve 15 dk. temas sürelerinde ve %0,5 ve %1 konsantrasyonlarda istatistiksel olarak herhangi bir farklılık gözlemlenmezken, anlamlı istatiksel farklılık %2 düzeyinde mevcut olduğu gözlenmiştir (Tablo 2).

Kuarterner amonyum bazlı dezenfektanın test bakterileri üzerinde farklı azalma düzeyleri tespit edilmiştir. Fakat %0,5 ve %1 konsantrasyonlarda antibakteriyel etkinlik göstermiştir. Tüm kuarterner amonyum konsantrasyonları test edilen izolatlara karşı en etkili dezenfektan olarak belirlenmiştir. İstatistiksel olarak farklı dezenfektan konsantrasyon düzeyleri arasında anlamlı fark belirlenmiştir (Tablo 3).

Gluteraldehidin %0,5 konsantrasyonunda 5 dk. temas süresinde *S. aureus* izolatları üzerine etkili olmadığı, fakat diğer konsantrasyonlarda test edilen izolatların duyarlı olduğu saptanmıştır. *P. fluorescens* izolatları üzerinde, %0,5 gluteraldehid çözeltisi haricinde, tüm temas süresinde yeterli antibakteriyel etkinliği saptanmıştır (Tablo 4).

Test edilen *S. aureus* izolatlarına %1 hidrojen peroksitin antibakteriyel etkisi saptanmıştır (≥5 $\log_{10}$ azalma). Ancak %0,5 hidrojen peroksit çözeltilerinin 5 dk. temas süresinde *S. aureus* izolatlarına karşı aktivitesinin azaldığı gözlenmiştir. *P. fluorescens* izolatlarına karşı, hidrojen peroksitin tüm konsantrasyonlarının etkili olduğu belirlenmiştir (Tablo 5).

Tablo 2. *S. aureus* ve *P. fluorescens* suşlarının belirli temas sürelerinde klor bazlı dezenfektana karşı redüksiyon değerlerinin istatistiksel analizi sonuçları

Table 2. Statistical analysis results of reduction values of *S. aureus* and *P. fluorescens* isolates against chlorine-based disinfectant at certain contact times

Bakteri	Kontrol log10 kob/ml	Klor bazlı dezenfektan konsantrasyonları ve süreleri					
		%0,5		%1		%2	
		5 dk.	15 dk.	5 dk.	15 dk.	5 dk.	15 dk.
S. aureus	8,14±0,27^a	3,20±0,58^b	3,37±0,47^b	7,09±0,85^c	7,12±0,54^c	6,37±1,22^d	7,88±0,35^a
P. fluorescens	8,22±0,36^a	4,71±0,12^b	4,73±0,16^b	4,67±0,20^b	4,66±0,23^b	6,15±0,42^c	6,80±0,12^d

*Aynı harflerle takip edilmeyen aynı satırdaki ortalamalar, dezenfektan konsantrasyonları ve süreleri açısından istatiksel olarak anlamlı farklılık göstermektedir ($p < 0,05$). Her bir kategori için redüksiyon değerleri istatistiksel özetlenmesi 'Ortalama±Standart sapma' olarak verilmiştir. Testlerde kullanılan başlangıç hücre sayıları: 1,5 x10^8- 5x10^8

Tablo 3. *S. aureus* ve *P.fluo*rescens suşlarının belirli temas sürelerinde kuarterner amonyum bazlı dezenfektana (QA) karşı redüksiyon değerlerinin istatistiksel analiz sonuçları

Table 3 Statistical analysis results of reduction values of *S. aureus* and *P.fluorescens* isolates against quaternary ammonium-based disinfectant (QA) at certain contact times

Bakteri	Kontrol log10 kob/ml	Kuarterner amonyum bazlı dezenfektan konsantrasyonları ve süreleri					
		%0,5		%1		%2	
		5 dk.	15 dk.	5 dk.	15 dk.	5 dk.	15 dk.
S. aureus	7,69±0,47^{a}	6,39±1,15^{b}	7,12±0,15^{c}	6,99±0,21^{c}	6,93±0,20^{c}	7,15±0,06^{c}	8,06±0,18^{a}
P. fluorescens	8,47±0,38^{a}	5,93±1,04^{b}	5,60±1,18^{b}	6,13±1,10^{b}	6,29±0,97^{b}	6,39±1,15^{b}	7,32±0,55^{c}

*Aynı harflerle takip edilmeyen aynı satırdaki ortalamalar, dezenfektan konsantrasyonları ve süreleri açısından istatiksel olarak anlamlı farklılık göstermektedir ($p < 0,05$). Her bir kategori için redüksiyon değerleri istatistiksel özetlenmesi 'Ortalama±Standart sapma' olarak verilmiştir. Testlerde kullanılan başlangıç hücre sayıları: 4,6x10^{7}; 1,5 x10^{8}- 5x10^{8}

Tablo 4. *S. aureus* ve *P. fluorescens* suşlarının belirli temas sürelerinde gluteraldehid bazlı dezenfektana karşı redüksiyon değerlerinin istatistiksel analizi sonuçları

Table 4. Statistical analysis results of reduction values of *S. aureus* and *P. fluorescens* isolates against glutaraldehyde-based disinfectant (QA) at certain contact times

Bakteri	Kontrol log10 kob/ml	Gluteraldehid bazlı dezenfektan konsantrasyonları ve süreleri					
		%0,5		%1		%2	
		5 dk.	15 dk.	5 dk.	15 dk.	5 dk.	15 dk.
S. aureus	8,35±0,45^{a}	4,24±0,34^{b}	6,82±0,91^{c}	6,80±0,26^{c}	6,85±0,20^{c}	7,18±0,23^{d}	7,22±0,14^{d}
P. fluorescens	8,60±0,51^{a}	3,21±0,24^{b}	2,65±0,14^{b}	6,42±1,32^{c}	6,39±1,14^{c}	7,16±0,73^{c}	6,84±0,96^{c}

*Aynı harflerle takip edilmeyen aynı satırdaki ortalamalar, dezenfektan konsantrasyonları ve süreleri açısından istatiksel olarak anlamlı farklılık göstermektedir ($p < 0,05$). Her bir kategori için redüksiyon değerleri istatistiksel özetlenmesi 'Ortalama±Standart sapma' olarak verilmiştir. Testlerde kullanılan başlangıç hücre sayıları: 1,5 x10^{8}- 5x10^{8}

Tablo 5. *S. aureus* ve *P.fluorescens* suşlarının belirli temas sürelerinde hidrojen peroksit bazlı dezenfektana karşı redüksiyon değerlerinin istatistiksel analizi sonuçları

Table 5. Statistical analysis results of reduction values of *S. aureus* and *P. fluorescens* isolates against hydrogen peroxide-based disinfectant at certain contact times

Bakteri	Kontrol log10 kob/ml	Hidrojen peroksit bazlı dezenfektan konsantrasyonları ve süreleri					
		%0,5		%1		%2	
		5 dk.	15 dk.	5 dk.	15 dk.	5 dk.	15 dk.
S. aureus	8,23±0,36^{a}	3,55±0,45^{b}	6,89±0,09^{cd}	5,97±0,35^{e}	7,12±0,10^{d}	6,60±0,54^{c}	8,03±0,35^{a}
P. fluorescens	8,14±0,27^{a}	5,56±0,31^{b}	7,15±0,80^{cd}	5,72±0,13^{b}	7,51±0,78^{c}	6,61±1,04^{d}	7,57±0,58^{ac}

*Aynı harflerle takip edilmeyen aynı satırdaki ortalamalar, dezenfektan konsantrasyonları ve süreleri açısından istatiksel olarak anlamlı farklılık göstermektedir ($p < 0,05$). Her bir kategori için redüksiyon değerleri istatistiksel özetlenmesi 'Ortalama±Standart sapma' olarak verilmiştir. Testlerde kullanılan başlangıç hücre sayıları: 1,5 x10^{8}- 5x10^{8}

TARTIŞMA

Gıda endüstrisinde gıda ile temas eden yüzeylere, patojen bakterilerin yapışması çapraz kontaminasyon riskini artırmaktadır. Buna karşın dezenfektanların kullanımı üretim süreci boyunca gıdanın kontaminasyonunu azaltabilmektedir (Li vd., 2014). Gıdaların bozulmaması ve sağlık açısından sakıncalı hale gelmemesi için hijyen ve sanitasyon kurallarına uyulması gıda üreten ve pazarlayan işletmelerde çok önemlidir (Duong, 2005). Gıda endüstrisinde hijyene odaklanma, kimyasal dezenfeksiyonun kullanımını arttırmış ve bunun seçici bir baskıya neden olması ile dezenfektanlara dirençli mikroorganizmaların ortaya çıkmasına katkıda bulunacağı yönünde spekülasyonlar yapılmıştır (Thi vd., 2016).

Klor, düşük maliyeti ve geniş spektrumlu bir antimikrobiyal olarak hareket etme yeteneği nedeniyle gıda endüstrisinde yaygın olarak kullanılmaktadır (Sharma vd., 2022). Çalışmamızda *P. fluorescens*'in test bakterisi olarak kullanıldığı test sonuçları incelendiğinde, sodyum hipoklorit içeren dezenfektanların düşük konsantrasyonlarının (%0,5 ve %1) bakterisidal aktivite göstermediği bulunmuştur. *S. aureus*'un, klor bazlı dezenfektanlar haricinde, genellikle tüm dezenfektan konsantrasyonlarına duyarlı olduğu tespit edilmiştir. Benzer olarak, Duong (2005) yaptığı çalışmada, 50 ve 200 ppm konsantrasyonlarda hipoklorit içeren dezenfektanların, özellikle yağ varlığında, perasetik asit ve kuarterner amonyum bileşikleri (%0,25, 0,5 ve 1) içeren formülasyonlardan daha az etkili olduğunu bulmuştur. Kocot ve Olszewska (2020) yaptığı çalışmada, *P. fluorescens* ve *S. aureus* biyofilmleri üzerinde klor bazlı dezenfektanları dirençli bulmuştur. İyot ve klor gibi çeşitli kimyasalların, yüzeydeki gıda artıkları ve kir ile reaksiyona girerek daha az etkili hale geldiği ve yüzeyi gerektiği gibi dezenfekte edemediği bildirilmiştir (Sharma vd., 2022). Bu veriler üretilen hammadde türünün, uygulanan dezenfektanların zaman- konsantrasyon ilişkisini etkileyen önemli bir parametre olduğunu bildirmektedir. Buna

karşın, Thi vd. (2016), *Pangasius* filetolarının yıkama suyundaki serbest klorün, toplam psikrofil bakteri sayısını 2 ve 4 log cfu/100 ml arasında azalttığını saptamıştır. Benzer bir çalışmada, Cabeça vd., (2012) sodyum hipoklorit'in biyofilm hücrelerine karşı en etkili dezenfektan olduğunu bildirmişlerdir. DeQueiroz vd. (2007) yaptıkları çalışmada, sodyum hipokloriti alüminyum veya paslanmaz çelik yüzeyler üzerinde, *P. aeruginosa* ATCC 19142 hücrelerinin öldürülmesi ve *P. aeruginosa* biyofilmlerinin uzaklaştırılması üzerinde denemişlerdir. Hücre sayılarının, 1 dakikada 3 log'dan 4 log'a ve 5 dakika sonra 4 log'dan 6 log'a azaldığını bildirmişlerdir.

Yapılan çalışmada test edilen diğer bir dezenfektan olan kuarterner amonyum çözeltisinin farklı konsantrasyonlarının *S. aureus* ve *P. fluorescens* suşlarına karşı etkili olduğu belirlenmiştir. Benzer olarak Li vd. (2014) gıda kalıntıları (süt, sığır et suyu ve ton balığı) üzerinde, benzalkonyum klorür (BAC) ve alkil dimetilglisin hidroklorürün (AGH) bakterisit etkisini araştırmışlardır. Dezenfektan uygulamasından sonra patojen bakterileri (*Escherichia coli* O26, *P. aeruginosa*, *S. aureus*, *Bacillus cereus* ve *B. cereus* sporları) sayılarının azalmış olduğunu bulmuşlardır. Fakat düşük konsantrasyonlarda (0,5 mg/ml, 2 mg/ml), dehidrasyondan ve dezenfektanların olumsuz etkilerinden bakteri hücrelerinin korunmuş olduğunu bildirmişlerdir. Kuda vd. (2008), gıda maddesi olmadan paslanmaz çelik yüzeylere yapıştırılan *E. coli* O26, *P. aeruginosa* ve *S. aureus* bakterilerinin, benzalkonyum klorüre karşı dirençlerini incelemişlerdir. *E. coli* ve *S. aureus* bakterilerinin, benzalkonyum klorürün (BKC) (0,5 mg/ml) 10 dk. temas süresinden sonra sayılarının azaldığı rapor edilmiştir. Ayrıca P. *aeruginosa* BKC'ye direnç göstermesine rağmen, yapıştırılan diğer bakterilerin 2 mg /ml BKC ile inaktive edildiği bulunmuştur.

Gosling vd. (2017) *Salmonella*'yı ortadan kaldırmada gluteraldehid bazlı dezenfektanlardan olan gluteraldehid'in %2 konsantrasyonunun yüksek seviyeli dezenfeksiyon işlemi için etkili olduğunu, buna karşın çok fazla olumsuz etkilerinin de olduğu bildirilmiştir. Benzer şekilde dezenfektan olarak kullandığımız %2 gluteraldehid çözeltisinin *P. fluorescens*'un test izolatı olarak kullanıldığı test sonuçları incelendiğinde yeterli antimikrobiyal aktivite gösterdiği fakat dezenfektanın % 0,5 konsantrasyonun ise 20^{0}C'de tüm temas süresinde en düşük bakterisidal etki gösterdiği belirlenmiştir. Aynı zamanda % 0,5 gluteraldehid çözeltisinin *S. aureus* izolatları üzerinde 5 dk. temas süresinde etkinlik göstermemiştir. Benzer bir çalışmada Vizcaino-Alcaide vd. (2003) gluteraldehit'in %2'lik konsantrasyonun 10-20 dk. içinde, mikobakteriler ve sporlar haricinde tüm mikroflorayı öldürdüğünü bildirmişlerdir. Fakat organik madde varlığında ise, gluteraldehid'in (%2) organik maddeyi metal yüzeye sabitleyerek, 2 saat içinde korozyona neden olduğu rapor etmişlerdir. Benzer olarak Pineau vd. (2008) gluteraldehid bazlı dezenfektanların (%2) protein birikimine ve fiksasyonuna neden olduğunu bildirmişlerdir. Al-Saleh vd. (2021) diş hekimliğinde kullanılan kron kaplama malzemelerinin dezenfeksiyonunda NaOCl ve gluteraldehit dezenfektanlarını test etmiş ve *S. aureus, S. mutans* ve *E. coli* suşlarına karşı etkin olduğunu belirlemişlerdir. Test edilen dezenfektanlar arasında en iyi etki gösteren %2 gluteraldehid olarak saptanmıştır. Avcı vd. (2017) hastanelerde sık kullanılan 4 adet antiseptik ve dezenfektanın hastaneden izole edilen hastane enfeksiyonu etkeni, dirençli farklı bakteriler üzerine etkisi ve etki süresinin karşılaştırılmasının amaçlandığı çalışmasında, direkt ve ½ sulandırımda 1 dk. tüm bakterilerin üremesini inhibe eden glutaraldehid (%2) en etkili dezenfektan olarak saptanmıştır.

Dezenfektan olarak kullandığımız hidrojen peroksit tüm konsantrasyonlarda (%0,5, %1, %2) ve temas sürelerinde özellikle *P. fluorescens* izolatları üzerinde en etkili dezenfektan olarak saptanmıştır. Benzer bir çalışmada, Hassan vd. (2013) su ürünleri işleme fabrikalarındaki farklı lokasyonlarında, hidrojen peroksit ve ticari stabilize hidrojen peroksidin etkinlikleri ve etkinlik sürelerini incelemişlerdir. Ticari stabilize % 5 H_2O_2 belirgin bir şekilde daha iyi bir genel dezenfeksiyon etkisinin olduğunu tespit etmişlerdir. Başka bir çalışma da DeQueiroz ve Day (2007) hidrojen peroksit dezenfektanlarını alüminyum veya paslanmaz çelik yüzeyler üzerinde, *P. aeruginosa* ATCC 19142 hücrelerinin öldürülmesi ve *P. aeruginosa* biyofilmlerinin uzaklaştırılması üzerinde denemişlerdir. Hücre sayılarının, 5 dk. temastan sonra 7 log azaldığı ve 20 dk. temastan sonra hiçbir canlı bakteri tespit etmemişlerdir. Choi vd. (2012), paslanmaz çelik yüzeylerde *E. coli* O157;H7, *Salmonella typhimurium*, *Listeria monocytogenes*'in hayatta kalma oranlarını ve aerosol haline getirilmiş hidrojen peroksit konsantrasyonunun (%0,25; %0,5) etkinliğini araştırmışlardır. Paslanmaz çelik yüzeylerde patojenlerin hayatta kalma oranlarının bakteri suşlarına ve koşullarına bağlı olarak değiştiğini ve aerosol şeklindeki hidrojen peroksit bazlı dezenfektanın bu patojenleri yüzeyden uzaklaştırmak için etkili olduğunu göstermişlerdir.

SONUÇ

Çalışmamızda kullandığımız dezenfektanlar balık işleme tesislerinde sıklıkla kullanılmaktadır. Dezenfektanların doğru kullanılması ve seçilmesi protein ve nem açısından zengin bir ürünün işlendiği bu ortamlarda etkin bir dezenfeksiyon sağlamaktadır. Bunun için dezenfektanların istenen antibakteriyel etkinliği gösterdiğinin belirlenmesinde, işleme tesisi yüzeylerinden izole edilen kalıcı florayı temsil eden bakteriler üzerinde test edilmesi önem taşımaktadır.

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

VERİ KULLANILABİLİRLİĞİ

Mevcut çalışmaya ait veri setleri editör veya hakemlerin talebi üzerine sorumlu yazar tarafından sağlanacaktır.

KAYNAKÇA

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