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Authors Guidelines

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

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

GENERAL INFORMATION

Aim & Scope

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

The journal does not charge any submission and publication fees.

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

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

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

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

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

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

Language

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

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

Editorial Policy and Referee Process

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

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

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

Article Types

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

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

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

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

Reports

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

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

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

MANUSCRIPT SUBMISSION

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

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

While starting

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

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

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

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

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

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

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

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

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

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

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

First Page

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

Author Names and Affiliation

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

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

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

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

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

Abstract

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

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

Keywords

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

Following pages

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

Introduction

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

Material and Methods

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

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

Results

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

Discussion

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

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: .....)."

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

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

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

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

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

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

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

Full name/s: Conceptualization, Methodology, Software

Full name: Data curation, Writing- Original draft preparation

Full name/s: Visualization, Investigation

Full name/s: Supervision

Full name/s: Software, Validation

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

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

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

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

Examples:

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

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

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

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

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

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

Retrospective Ethics Approval

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

Data Availability

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

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

Examples:

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

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

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

Data availability: All relevant data is in the article.

Scientific Style

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

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

Equations and units

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

Abbreviations

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

Footnotes

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

References

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

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

In-Text Citation

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

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

For example: Kocataş, 1978, p. 3

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

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

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

One author:

Consider the following examples:

-…….(Kocataş, 1978)

- Kocataş (1978) states…….

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

Two authors:

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

Consider the following examples:

-…….(Geldiay and Ergen, 1972)

- Geldiay and Ergen (1972) states……

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

More than two authors:

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

See below examples:

-Geldiay et al. (1971) state…….

-……(Geldiay et al., 1971).

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

Two or more works by different author:

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

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

Two or more works by the same author:

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

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

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

For Example:

-Geldiay and Ergen, 1972a

-Geldiay and Ergen, 1972a, b

No authors:

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

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

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

No publication date:

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

Example: (Geldiay, n.d.).

Citation to secondary sources:

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

For Example:

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

Personal communication and unpublished results:

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

Example:

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

General use of websites and software:

It should be showed as below.

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

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

In References

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

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

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

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

Hanging indent paragraph style should be used.

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

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

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

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

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

For example:

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

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

For example:

Kocataş, A. (1978)

Kocataş, A., & Ergen, Z. (1972).

Kocataş, A., & Geldiay, R. (1972)

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

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

Journal Articles

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

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

Books

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

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

Chapter in books

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

E-books and chapter in e-books

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

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

Proceedings

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

Websites

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

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

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

Thesis

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

Tables and Figures

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

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

The comparison of population density of *Holothuria tubulosa* (Gmelin, 1790) and *Holothuria polii* (Delle Chiaje, 1823) between exploited and non-exploited areas in the Aegean Sea coast of Türkiye

Türkiye'nin Ege Denizi kıyılarında *Holothuria tubulosa* (Gmelin, 1790) ve *Holothuria polii* (Delle Chiaje, 1823) popülasyon yoğunluğunun av yapılan ve yapılmayan alanlar arasındaki karşılaştırılması

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Abstract: Increasing demand from the Asian market first led to the collapse of economically valuable sea cucumber stocks in the Indo-Pacific, and then demand shifted to lower value Mediterranean species. The effect of this change in sea cucumber stocks in Türkiye started to be seen after the 2010s. To address it, we carried out a study to compare the population density of the most caught *Holothuria tubulosa* and *Holothuria polii* species between exploited and non-exploited areas in the Aegean Sea coast of Türkiye. The study was carried out between September 2018 and March 2020 at 4 stations, two of which are in Çandarlı Bay, where sea cucumber fishing is free, and two in İzmir Bay, where it is prohibited. Samplings between 0-20 m depths were carried out with underwater transect line technique, and at depths deeper than 20 m, with beam trawling operations. A total of 6 sea cucumber species were identified, including *Holothuria mammata, Holothuria sanctori*, *Parastichopus regalis* and *Holothuria forskali* species, apart from the target species *H. tubulosa* and *H. polii*. Only one *P. regalis* individual was recorded in the samplings in waters deeper than 20 m. The mean densities of *H. tubulosa* and *H. polii* were found to be higher in İzmir Bay stations than in Çandarlı Bay ($p<0.05$). The results of this study clearly reveal that the population density of sea cucumber in sampling areas has decreased tens of times compared to the last 5 years.

Keywords: *Holothuria* sp., biometry, overexploitation, fisheries management, Aegean Sea

Öz: Asya pazarının artan talebi, ilk olarak İndo-Pasifik'teki ekonomik açıdan değerli deniz patlıcanı stoklarının çökmesine sebep olmuştur ve daha sonra talep daha düşük değere sahip Akdeniz türlerine yönelmiştir. Türkiye'deki deniz patlıcanı stoklarında bu değişimin etkisi 2010'lu yıllardan sonra görünmeye başlamıştır. Bunu ele almak için, Türkiye'nin Ege Denizi kıyılarında en çok avlanan *Holothuria tubulosa* ve *Holothuria polii* türlerinin popülasyon yoğunluğunu, avcılığın serbest ve yasak olduğu alanlarda karşılaştırmak için bir çalışma yaptık. Çalışma, ikisi deniz patlıcanı avcılığının serbest olduğu Çandarlı Körfezi'nde, ikisi ise yasak olduğu İzmir Körfezi'nde olmak üzere 4 istasyonda gerçekleştirilmiştir. 0-20 m derinlikler arasındaki örneklemeler sualtı çizgisel hat tekniği ile 20 m'den derindekiler ise algarna operasyonları ile gerçekleştirilmiştir. Hedef tür *H. tubulosa* ve *H. polii* dışında *Holothuria mammata, Holothuria sanctori, Parastichopus regalis* ve *Holothuria forskali* türleri olmak üzere toplam 6 deniz patlıcanı türü tespit edilmiştir. 20 m'den daha derin sularda yapılan örneklemelerde sadece bir *P. regalis* bireyi kaydedilmiştir. *H. tubulosa* ve *H. polii*'nin ortalama yoğunlukları İzmir Körfezi istasyonlarında Çandarlı Körfezi'ne göre daha yüksek bulundu ($p<0,05$). Bu çalışmanın sonuçları, deniz patlıcanı popülasyon yoğunluğunun son 5 yılda onlarca kat azaldığını açıkça ortaya koymaktadır.

Anahtar kelimeler: *Holothuria* sp., biyometri, aşırı avcılık, balıkçılık yönetimi, Ege Denizi

INTRODUCTION

The proportion of fish stocks that are within biologically sustainable levels decreased from 90 percent in 1974 to 65.8 percent in 2017, with 59.6 percent classified as being maximally sustainably fished stocks and 6.2 percent underfished stocks (FAO, 2020). As a result of this overfishing, landings from global fisheries have shifted in the last 45 years

from large piscivorous fishes toward smaller invertebrates and planktivorous fishes, especially in the Northern Hemisphere (Pauly et al., 1998). Sea cucumber species are among the invertebrates adversely affected by this change.

Sea cucumbers are deposit feeders critical to maintaining the balance of benthic microenvironments, the foundation of a highly productive and healthy marine ecosystem, including coral reefs (Uthicke, 2001; Lök et al., 2005). Sea cucumber over fishing has serious impacts on sediment health, water quality, nutrient recycling, seawater chemistry and energy transfers across the food chains in numerous marine systems globally (Conand, 2018).

Throughout the world, 66 species of sea cucumbers are commonly exploited (Purcell et al., 2010) and of particular importance in the Indo-Pacific (Choo, 2008). The global analysis of sea cucumber fisheries by Purcell et al. (2013) revealed that overexploitation and depletion of sea cucumber stocks, particularly in the Indo-Pacific, is alarmingly high. A number of species are threatened and there is evidence of local extinctions in some tropical regions (Uthicke and Conand, 2005).

Fishing of sea cucumber species has been an important source of income for small-scale fisheries in Türkiye since 1996 (González-Wangüemert et al., 2014; Lök et al., 2017; Dereli and Aydın, 2021). Sea cucumber products are exported to Asian countries, especially China (Aydın, 2008). While sea cucumber export was 247 tons in 2014, it increased to 1169 tons in 2019 (TÜİK, 2020). However, basic scientific studies such as population density, reproductive biology and habitat preferences of sea cucumber species on the Turkish coast are very limited.

The aim of this study is to compare of population densities of *H. tubulosa* and *H. polii*, which have economic value and are the most collected species between exploited (Çandarlı Bay) and non-exploited (İzmir Bay) areas.

MATERIAL AND METHODS

Study area: The study was carried out between September 2018 and March 2020 in the Çandarlı and İzmir Bays in the Aegean Sea. Heavy industry and heavy maritime traffic affect the marine ecosystem negatively in both bays where the population is dense. There are important areas where all kinds of fishing activities (large and small-scale fishing, recreational fishing and aquaculture) are carried out in both Çandarlı and İzmir Bay (Tokaç, 2017). As a result, Çandarlı and İzmir Bays are exposed to similar anthropogenic effects (DGEIAPI, 2017).

According to the regulation published by the Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture, sea cucumber fishing is free in Çandarlı Bay (Anonymous, 2020). In the same regulation, sea cucumber fishing is prohibited in the south of Izmir Bay.

Sampling: Sampling was carried out seasonally between September 2018 and March 2020. Sea cucumber samples were collected from a total of 4 stations, two from Çandarlı Bay, where fishing is free, and two from İzmir Bay, where fishing is prohibited (Figure 1).

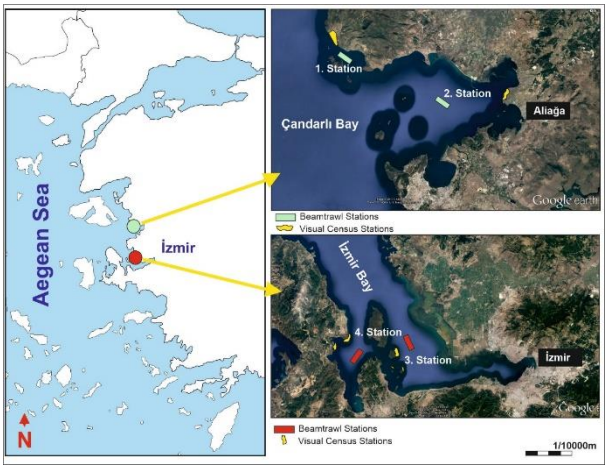

Figure 1. Study area and sampling stations

Sea cucumbers show a wide distribution depth. Thus, samplings were carried out at different bathymetries and with different methods. In this framework, depths of 0-20 meters were sampled with the diving method, and places deeper than 20 meters were sampled with beam trawl.

Samplings made with the line transect were carried out between 0-10 m and 10-20 m depth contours for each station. The transect line is marked with two buoys and a 100 m submersible rope (Figure 2).

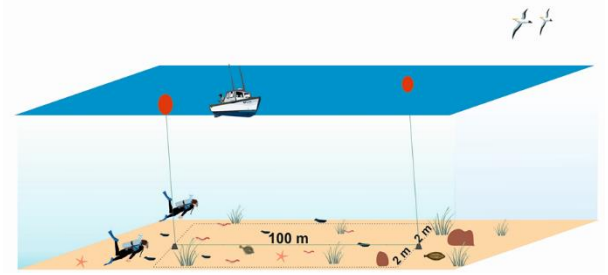

Figure 2. Transect line and dimensions used in underwater

In all four stations, the samplings in the 0-10 m transect were carried out with 6 replications and the samplings at the 10-20 m transect were carried out with 3 replications. In the preliminary studies carried out in the selected sampling areas, it was determined that sea cucumber individuals were found in the first 10 m, while they were very rarely distributed at deeper depths. When diving depth/time limits are added to this situation, the number of replications deeper than 10 meters was determined as 3. Each transect has a length of 100 m, a width of 4 m and an area of 400 m². Thus, at the end of the sampling at each station, 6 x 400 m² = 2400 m² for 0-10 m depth contour and 3 x 400 m² = 1200m² for 10-20 m depth contour, and a total of 3600 m² area was scanned.

Sea cucumber individuals sampled by the diver were placed in the net and transferred to the drums on the research vessel. The samples placed in the drums were taken out of the sea water and taken into a plastic container of known tare, and their weights (with a precision of 0.1 g) were taken. It was then transferred to plastic cuvette containing sea water. The individuals, who were kept in the cuvette for 10-15 minutes, were measured with a 1mm precision tape measure after the contractions stopped and they calmed down (maximum relaxation length value). After measuring the lenght and weight of the individuals, they were released back to the sea

Beam trawl operations were carried out to determine the population density of target species in areas deeper than 20 m. The inside width of the beam trawl frame used in the sampling is 2 m, its height is 55 cm and the cod-end length is 8 m. Polyethylene multi-monofilament (0.20 mm x 14) net with 44 mm mesh size was used in the cod-end (Figure 3).

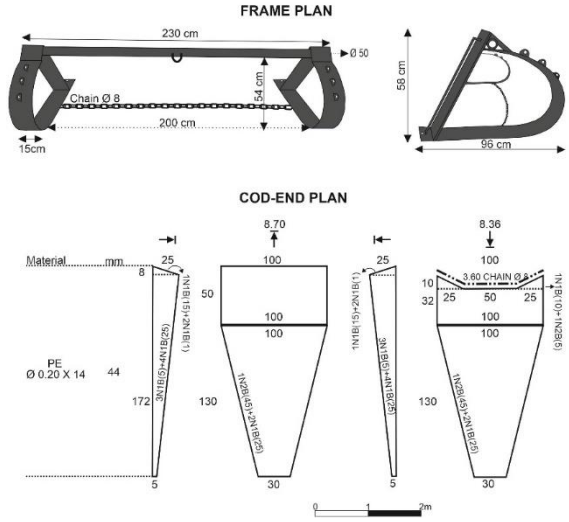

Figure 3. Technical plan of beam trawl cod-end

Beam trawl samplings were carried out at two different bathymetries. Beam trawl operations in Çandarlı Bay were carried out on the shores of Şakran for the 20-40 m depth and on the Denizköy coasts for the 40-60 m. Beam trawl operations in Izmir Bay were carried out on the shores of Gülbahçe for the 20-40 m and on the eastern shores of Hekim Island for the 40-60 m. Beam trawl towing time in one sampling was determined as 30 minutes and was carried out in 3 replicates at each station.

Data analysis: Length and weight frequency distributions were calculated to show the ranges of sizes each species and to identify the most abundant classes.

SPSS 15.0 statistical package program was used to analyse the data. Prior to the analysis, the conformity of the data to the normal distribution was examined using the Shapiro-Wilk test of normality and the homogeneity of variance was tested by Levene test. In statistical tests, the significance level was preferred as $p<0.05$ (Zar, 1999). It has been tested whether the sea cucumber population density differs between stations where fisheries are prohibited and free. In addition, the variation of sea cucumber density according to the seasons was also tested. Kruskal-Wallis one-way analysis of variance and Mann Whitney-U test from non-parametric tests were used in statistical evaluations.

RESULTS

In the study, a total of 216 successful dives were made in underwater visual census samplings carried out at four stations. 144 of these dives were made at 0-10 m depth contour, and 72 were made at 10-20 m depth contour. Diving time for a hundred-meter line varied between 20 minutes and 70 minutes, depending on the habitat type and the frequency of encounters with sea cucumber species. The scanned area in each dive sampling is 400 m². As a result, a total of 86 400m² of area, 57 600 m² at the 0-10 m depth contour and 28 800 m² at the 10-20 m depth contour, was scanned and sampled.

The length-frequency and weight-frequency distributions of all *H. tubulosa* and *H. polii* species collected in visual census samples from Çandarlı Bay and İzmir Bay stations are presented in Figures 4 and 5.

In the sampling studies carried out in Çandarlı Bay, a total of 79 *H. tubulosa* and 81 *H. polii* individuals were collected and measured. *H. tubulosa* showed the most frequent size-class in the range of 22-25 cm in length and 264-324 g in weight. For *H. polii*, these values were in the range of 8.7-10.7 cm in length and 51-81gr in weight. The average length was calculated as 19.8 cm and the average weight as 224.5 g for *H. tubulosa*. These values were found to be 10.4 cm and 82.5 g for *H. polii*. The median values of *H. tubulosa* and *H. polii* in length distribution were found to be 20.6cm and 14.5cm, respectively. Both species showed multimodal size frequency distribution in Çandarlı Bay.

In the sampling studies carried out in İzmir Bay, a total of 442 *H. tubulosa* and 1144 *H. polii* individuals were collected and measured. The most frequent size classes of *H. tubulosa* was between 21.6-23.6 cm in length and 163-213 g in weight. For *H. polii*, it was between 13.4-15.4 cm in length and 61-111 g in weight. The average length was calculated as 19.4 cm, and the average weight was 199 g for *H. tubulosa*. These values were found to be 13.2 cm and 102.3 g for *H. polii*. The median values of *H. tubulosa* and *H. polii* in length distribution were found to be 22.3 cm and 14.7 cm, respectively. *H. tubulosa* and *H. polii* showed unimodal length frequency distribution in Izmir Bay.

When the median values of the length and weigth classes of *H. tubulosa* and *H. polii* species sampled from İzmir and Çandarlı Bays were compared, the difference was not found significant ($p>0.05$).

At the end of seasonal sampling, the data of each station was analysed and population densities were calculated. Population densities of *H. tubulosa* in Table 1 and of *H. polii* in Table 2 are presented together with standard deviations.

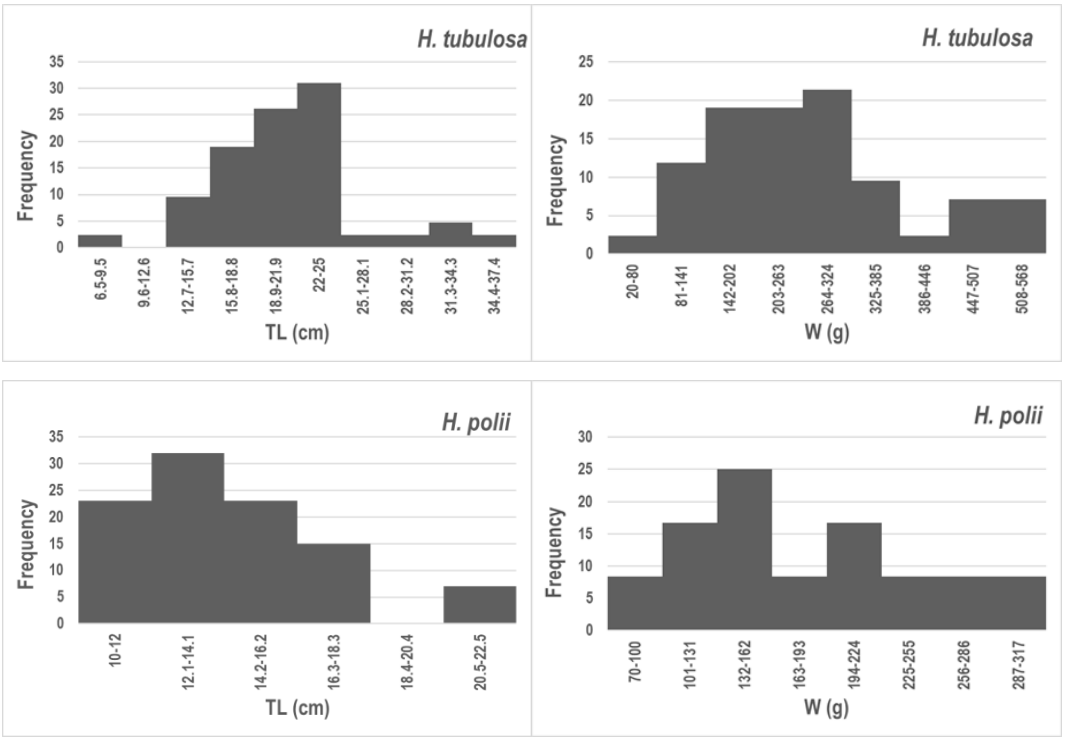

Figure 4. Length-frequency and weight-frequency distributions of *H. tubulosa* and *H. polii* species in Çandarlı Bay

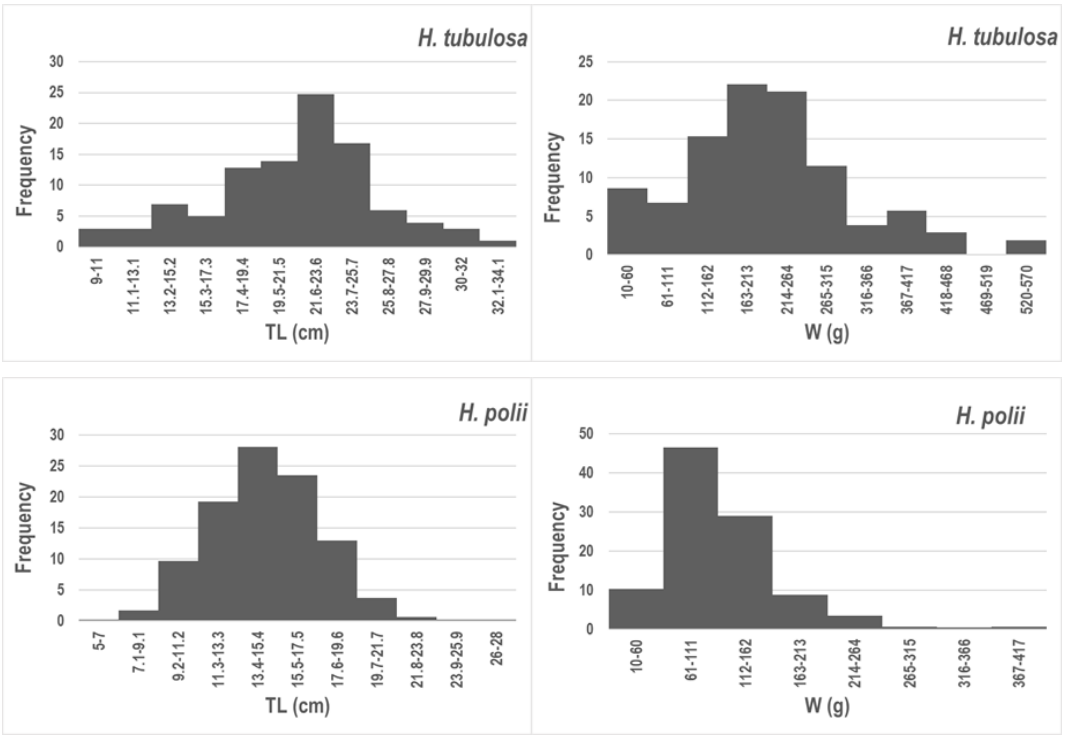

Figure 5. Length-frequency and weight-frequency distributions of *H. tubulosa* and *H. polii* species in Izmir Bay

Table 1. Mean population densities(individuals/m²±sd) of *H. tubulosa* calculated from the data obtained by the underwater transect line method

		Exploited areas		Non-exploited areas	
Season	**Depth counter**	**Denizköy**	**Şakran**	**Urla**	**Gülbahçe**
Fall 2018	0-10 m	0.0025±0.004	0.0008±0.002	0.0183±0.029	0.0058±0.008
	10-20 m	-	-	0.0117±0.013	0.0100±0.015
Winter 2019	0-10 m	0.0008±0.002	0.0008±0.002	0.0400±0.024	0.0146±0.008
	10-20 m	-	0.0008±0.001	0.0025±0.004	-
Spring 2019	0-10 m	0.0063±0.005	0.0033±0.004	0.0129±0.010	0.0008±0.0013
	10-20 m	-	0.0025±0.002	0.0058±0.005	0.0017±0.0029
Summer 2019	0-10 m	0.0063±0.009	0.0054±0.006	0.0125±0.011	0.0067±0.007
	10-20 m	-	-	-	0.0042±0.005
Fall 2019	0-10 m	0.0050±0.005	0.0019±0.002	0.0137±0.009	0.0119±0.011
	10-20 m	-	-	-	-
Winter 2020	0-10 m	0.0062±0.003	0.0012±0.002	0.0281±0.011	0.0144±0.0083
	10-20 m	-	0.0012±0.002	-	-

H. tubulosa was recorded at the 0-10 m contour at all stations and in all samples. It was not observed in any sampling on the 10-20 m contour at Denizköy station.

Considering population density values that can be calculated, the lowest value was found in Denizköy and Şakran with 0.0008 individuals/m², and the highest value was found in Urla station with 0.04 individuals/m². In general, population densities of *H. tubulosa* in Çandarlı Bay stations were found to be quite low compared to the population densities in İzmir Bay. The difference between population density values in Çandarlı Bay and İzmir Bay was found significant ($p<0.05$).

When the population densities of *H. tubulosa* were examined according to the depth contours (0-10 m and 10-20m), it was determined that the densities were higher in the 0-10 m contour ($p<0.05$). Considering the seasonal population density differences, this difference was not significant for *H. tubulosa* ($p>0.05$).

When the population density between stations was compared, the difference between Urla and Gülbahçe in İzmir Bay was significant ($p<0.05$). Urla station has a higher population density. The population density difference between Şakran and Denizköy located in Çandarlı Bay was not significant ($p>0.05$).

Table 2. Mean population densities (individuals/m²±sd) of *H. polii* calculated from the data obtained by the underwater transect line method

		Exploited areas		Non-exploited areas	
Season	**Depth**	**Denizköy**	**Şakran**	**Urla**	**Gülbahçe**
Fall 2018	0-10 m	0.0004±0.001	0.0075±0.012	0.0133±0.018	0.0242±0.025
	10-20 m	-	-	0.0033±0.004	0.0175±0.024
Winter 2019	0-10 m	0.0121±0.013	-	0.0308±0.014	0.0004±0.001
	10-20 m	-	-	0.0033±0.006	-
Spring 2019	0-10 m	-	0.0008±0.001	0.0871±0.012	0.0133±0.014
	10-20 m	-	0	0.0150±0.011	0.0100±0.017
Summer 2019	0-10 m	0.0025±0.003	0.0025±0.005	0.0388±0.018	0.0196±0.009
	10-20 m	-	-	-	0.0250±0.019
Fall 2019	0-10 m	0.0019±0.002	-	0.0521±0.030	0.0450±0.014
	10-20 m	-	-	0.0025±0.002	0.0137±0.012
Winter 2020	0-10 m	0.0106±0.001	0.0012±0.001	0.0550±0.018	0.0950±0.041
	10-20 m	-	-	-	-

H. polii was not detected in all samples at the 10-20 m contour at Denizköy and Şakran stations. This species was not observed in Urla and Gülbahçe stations only in two different seasons. Among the calculated density values, the lowest density was found in Denizköy with 0.0004 individuals/m² and the highest density in Gülbahçe with 0.095 individual/m². The population densities of *H. polii* were found to be higher in İzmir Bay stations than in Çandarlı Bay. Statistically, the difference between the mean density was significant ($p<0.05$). When the population densities of *H. polii* were examined according to the depth contours (0-10 m and 10-20 m), the difference was found to be higher in favor of the 0-10 m contour ($p<0.05$). The difference between the seasonal population densities of *H. polii* was not found significant ($p>0.05$).

When the population density of *H. polii* between stations were compared, the difference between Urla and Gülbahçe in İzmir Bay was higher in favor of Gülbahçe ($p<0.05$), while the

difference between Şakran and Denizköy located in Çandarlı Bay was not significant (p>0.05).

The population density of sea cucumber species in waters deeper than 20 m was tried to be determined by beam trawl towings. At the end of the study, a total of 72 valid towings were taken. As a result of all beam trawl operations, one *P. regalis* species was caught at a depth of 40-60 m in Çandarlı Bay for the spring season of 2019 g.

DISCUSSION

This study is more comprehensive than other similar studies conducted in the Mediterranean in terms of the sampling area and sampling time. The results demonstrated that there is a big difference in population densities between studies. The comparison of population density results obtained in this study with other studies conducted in similar species and geography is shown in Table 3.

Table.3 The comparison of the results of this study with other studies

Study	Area/Station	Species	Density (individual/m²)
Coulon and Jangoux (1993)	Naples Bay, Italy	*H. tubulosa*	3.77
Kazanidis et al. (2010)	Pagasitikos Bay, Greece	*H. tubulosa*	0.01
Aydın (2019)	Çandarlı Bay, Şakran, Türkiye	*H. tubulosa*	1.09
		H. polii	0.08
	Izmir Bay, Hekim Island, Türkiye	*H. tubulosa*	0.20
		H. polii	1.52
Aydın et al. (2009)	Aegean Sea, Türkiye	*H. tubulosa*	1.00
		H. polii	1.00
Dereli et al. (2016)	Çanakkale Strait, Türkiye	*H. tubulosa*	0.21
This study (2020)*	Çandarlı Bay, Şakran, Türkiye	*H. tubulosa*	0.002
		H. polii	0.002
	İzmir Bay, Urla, Türkiye	*H. tubulosa*	0.02
		H. polii	0.05

*Mean population density values were used

In a study conducted by Coulon and Jangoux (1993) on the Ischia Island of Italy, the population density of *H. tubulosa* at 9m depth was found to be 3.77 individuals/m². In addition, it has been reported that as the depth increases and the meadows become sparse, the population density decrease to 0.34 individuals/m² at 30 m depth. In this study, higher population densities were found for both species at the 0-10m contour. Furthermore, *H. tubulosa* and *H. polii* individuals were not found in algarna operations carried out from 20 m to 60 m depths. Considering that sea cucumber fisheries started in the late 1990s in the Mediterranean, it would not be surprising to find a high population density in this study conducted in 1993.

In the study carried out by Kazanidis et al. (2010) in Pagasitikos Bay on the shores of the western Aegean Sea, the population density of *H. tubulosa* was determined as 0.01 individuals/m² between 0-10 m depths. This value is close to the values found in Urla station (0.02 individuals/m²) in this study. However, the results at the other three stations are well below this value. In addition, the median value (29.8 cm) of the length distribution found in this study is considerably higher than the value found in our study (22.3 cm in İzmir Bay and 20.6 cm in Çandarlı Bay).

The study conducted by Aydın (2019) on the Aegean Sea coast between 2014 and 2015 is based on one-time sampling at 11 stations. Two of these 11 stations are in similar locations with the Urla and Şakran stations of our study. When we compare the Urla station population density results, it is seen that there is a 10-fold decrease in *H. tubulosa* and a 30-fold decrease in *H. polii*. Likewise, a 545-fold decrease in *H. tubulosa* and a 40-fold decrease in *H. polii* is observed at Şakran station.

In a study by Dereli et al. (2016) on the reproduction and population structure of *H. tubulosa* in the Dardanelles Strait, the density of the species was found to be 0.21 individuals/m². This value is considerably higher than the population density values we found. This situation may be due to the fact that the Dardanelles Strait is a prohibited area for secucumber fisheries, and that the protection activities are carried out more effectively due to the strategic importance of the Strait.

González-Wangüemert et al. (2015) studied the effects of sea cucumber fishing ban on the biometric and genetic characteristics of *H. tubulosa* and *H. polii* in the Aegean Sea. They were compared biological parameters (size distribution and weight classes) in fishery area (Ayvalık) and non-fishery area (Kuşadası). As a result, the mean weights of the samples taken from Ayvalık were found to be lower than the samples taken from Kuşadası. While similar results were found for *H. polii* in our study, there was no difference between stations for

H. tubulosa. This situation in *H. tubulosa* may be due to the higher value of the species.

González-Wangüemert et al. (2016) conducted biometric studies on four sea cucumber species, including *H. tubulosa* and *H. polii*, at 6 different stations extending from the Western Mediterranean to the Eastern Mediterranean. The range and values of length and weight classes found in this study are wider and higher than our study. This confirms the assessment of González-Wangüemert et al. (2016) that the lenght and weight values obtained at stations in the western Mediterranean are greater than those in the eastern Mediterranean. Researchers explained the reason for this by the fact that the amount of nutrients in the western basin is higher in the Mediterranean scale than in the eastern basin. In addition, it may be necessary to take into account that fishing pressure is also a factor in the reduction in length and weight of sea cucumbers.

Habitat and depth are one of the factors affecting the distribution of many marine organisms. Sea cucumbers are the most common and largest deposit-feeder species distributed in Posidonia meadows (Bulteel et al., 1992). In all stations where this study was conducted, the 0-10m depth contour is covered with Posidonia meadows, while after 12-15 m sandy-muddy or muddy ground is dominant. The fact that both sea cucumber species were found to be more abundant in areas with Posidonia meadows (2-4 m for Çandarlı Bay, 4-6 m for İzmir Bay) at all stations supports this finding. In addition, in the study conducted by Dereli et al. (2016) in the Dardanelles Strait, although the samplings were carried out at depths of 0-30 m, the finding of *H. tubulosa* individuals between 0-10 m is consistent with our study.

The results of the Bulteel et al. (1992) demonstrate that *H. tubulosa* are distributed according to a depth gradient, with the smallest individuals in the shallowest part of the meadow and the largest ones in its deepest part. In our study, length measurement was not performed during underwater visual counting sampling. However, all research divers observed small individuals of both species at shallower depths than 5 m, and large individuals at deeper depths.

CONCLUSION

The results of this study clearly reveal that the population density of sea cucumber has decreased tens of times compared to the last 5 years. Considering the density of 3.77 individuals/m^2 found at the end of the study conducted by Coulon and Jangoux (1993) in the years when sea cucumber fishing was not done in the Mediterranean, this determination can be clearly understood.

Within the framework of fisheries management in the Turkish coasts, many administrative tools are used for the regulation of sea cucumber fishing in terms of the closed area, closed season, rotational harvesting regulation, licensing, fishing gear restriction and fishing capacity. In addition to these administrative tools, a Circular was published and implemented by the General Directorate of Fisheries and Aquaculture on the quota application in sea cucumber fishing between January 01, 2020 and May 31, 2020. But despite all these legal regulations, illegal fishing is the biggest problem in sea cucumber fishery. This study showed that there is no difference between fishing and non-fishing areas in terms of mean length and weight of sea cucumber species. Another important problem is that the registration of sea cucumbers caught is not done according to the species. This can cause significant mistakes in fisheries management. Although some of the problems in sea cucumber fisheries can be solved regionally, international cooperation should be sought for comprehensive solutions.

While the importance of sea cucumber species in the coastal ecosystem is obvious, ecological problems that exist especially on the shores of the Aegean Sea also threaten these species. *Posidonia oceanica* meadows, which are crucial for sea cucumber species in our coasts where human activities are very intense (such as İzmir Bay, Çandarlı Bay, Edremit Bay) are withdrawing and their area is decreasing (Duman et al., 2019). Added to this negative picture was the mass death of *Pinna nobilis*, the largest bivalve and water filtration of the coastal ecosystem, as a result of a disease that spread throughout the Mediterranean in 2017. This shows us that we cannot solve problems if we focus on a small piece of the picture (sea cucumber) without considering the big picture (ecosystem).

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AUTHORSHIP CONTRIBUTIONS

Altan Lök: Conceptualization, project administration, funding acquisition, methodology, resources, investigation, writing-reviewing and editing. Aytaç Özgül: Conceptualization, investigation, formal analysis, visualization. Tuğçe Şensurat Genç: Investigation. Evrim Kurtay: Resources, investigation. Aynur Lök: Conceptualization, resources, investigation.

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

Detection and public health risk assessment of microplastics in disposable (PET) bottled water produced and sold locally in the Aegean Region

Ege Bölgesinde üretilen ve yerel olarak satılan tek kullanımlık (PET) şişelerdeki sularda mikroplastik tespiti ve halk sağlığı risk değerlendirmesi

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Abstract: Intensive use of plastic has led to the accumulation of plastics in all ecosystems and inevitable environmental pollution. Plastic wastes have undergone structural degradation with the effect of environmental factors and have been disintegrated into nano and microparticles; thus, might accumulate in living organisms and reach unpredictable levels in the food chain. In recent years, the impacts of these particles called "microplastics" (MP's) have become one of the most important issues in the scientific world. The aim of this study is to evaluate the possible presence of MP's in drinking water, that represents the most important nutrition element for human beings. For this purpose, samples of 6 different brands of disposable (PET) bottles produced and sold locally were examined. A total of 36 samples in bottles with 2 different volumes were analyzed in accordance with international standards and the results were evaluated. As a result of the study, the presence of MP's was detected in all samples analyzed. A total of 207 MP's were found in 36 samples. As a result of the analysis, a mean of 7.35 ± 9.66 MP L^{-1} particles was detected. It was determined that the most dominant type in terms of shape was fiber (91%), the most dominant type in terms of color was blue (57%) and the most dominant type in terms of size was 0.1-1 mm (71%). When Estimated Daily Intake (EDI) rates were calculated for public health risk assessment, it was determined that the most affected group is the 3-6 age group ($EDI_{(avg)}$ = 0.42). The importance of making recycling more widespread, raising awareness of consumers and making the necessary legal regulations on the issue was emphasized in order to reduce the problem at its source.

Keywords: Plastic, microplastic, environmental pollution, public health

Öz: Plastiğin yoğun kullanımı nedeniyle tüm ekosistemlerde plastik atıkların birikmesi kaçınılmaz bir çevre kirliliğine yol açmıştır. Plastik atıklar çevresel faktörlerin etkisiyle yapısal bozulmaya uğrayarak nano ve mikro partiküllere ayrışmakta; böylece canlı organizmalarda birikebilmekte ve besin zincirinde öngörülemeyen seviyelere ulaşabilmektedir. Son yıllarda "mikroplastik" olarak adlandırılan bu parçacıkların etkileri bilim dünyasının en önemli konularından biri haline gelmiştir. Bu çalışmanın amacı, insanlar için en önemli besin öğesi olan içme suyundaki olası mikroplastik varlığının tespit edilmesidir. Bu amaç doğrultusunda, yerel olarak üretilen ve satılan 6 farklı markanın tek kullanımlık (PET) şişelerindeki numuneler incelenmiştir. İki farklı hacme sahip şişelerde toplam 36 adet numune uluslararası standartlara uygun olarak analiz edilmiş ve sonuçlar değerlendirilmiştir. Çalışma sonucunda analiz edilen tüm numunelerde mikroplastik varlığı tespit edilmiştir. Analizler sonucunda, 36 örnekte toplam 207 mikroplastik bulunmuştur. Ortalama olarak ise 7,5 ± 9,66 MP L^{-1} tespit edilmiştir. Şekil açısından en baskın türün fibril (%91), renk açısından en baskın türün mavi (%57) ve boyut açısından en baskın türün 0,1-1 mm (%71) olduğu belirlenmiştir. Halk sağlığı risk değerlendirmesi için hesaplanan günlük alım (EDI) oranları göre en fazla etkilenen grubun 3-6 yaş arası grup olduğu ($EDI_{(avg)}$ = 0,42) tespit edilmiştir. Çalışma sonucunda, sorunu kaynağında azaltmak için geri dönüşümün yaygınlaştırılması, tüketicilerin bilinçlendirilmesi ve konuyla ilgili gerekli yasal düzenlemelerin yapılmasının önemi vurgulanmıştır.

Anahtar kelimeler: Plastik, mikroplastik, çevre kirliliği, halk sağlığı

INTRODUCTION

In 1972, the world became aware of the presence of micro-sized plastic particles in the aquatic environment for the first time, when it was reported that large numbers of small floating plastic particles were found in the Sargasso sea surface water (Carpenter and Smith, 1972). In 2004, these small particles were defined as microplastics (Thompson et al., 2004). Then, plastics smaller than 5 mm were accepted as microplastics by the Steering Committee of the National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program. According to this definition, plastics were specified as Macroplastic ≥ 25 mm, Mesoplastic 25 – 5 mm, Microplastic ≤ 5 mm –1 µm and Nanoplastic < 1 µm.

As microplastics are highly absorbent, they attract pollutants such as heavy metals, endocrine disrupting chemicals and durable organic pollutants (Yurtsever, 2015; GESAMP, 2015). These harmful substances cause harmful effects such as birth defects, cognitive development disorders, reproductive problems and cancer. Although studies on microplastic pollution have increased today, there are not enough scientific research on the determination of

microplastic pollution in food and its effects on human health (Li et al., 2018). Although microplastics do not have a direct negative effect on human health, it is necessary to increase the number of scientific studies that may adversely affect human health due to the dangerous substances in the structure of microplastics or adhere to the surface. It has been suggested that nanoplastics rather than microplastic particles adversely affect human health (Rist and Hartmann, 2018). In a study on microplastics ingested by rodents, it was determined that microplastics were very difficult to absorb in the blood. In the same study, it was observed that a very small part of the microplastics could reach the blood circulation via lymph, but it was also determined that this amount could not penetrate the organs and was probably eliminated via the spleen (Bouwmeester et al., 2015). In another study, it was determined that most of the ingested microplastics and nanoplastics were removed from the human body (Smith et al., 2018). Ragusa et al. (2021) even reported its presence in the human placenta. Conti et al. (2020) demonstrated the presence of microplastics in vegetables and fruits for the first time in the world. Zuccarello et al. (2019) detected microplastics in drinking water bottled in Italy. Microplastics cannot pass through cell membranes due to their size. However, the effects of microplastics such as inflammation in the intestines are possible and thus can affect the immune system. In addition, microplastics found in stool samples taken from people living in different countries showed that microplastics negatively affected the digestive system (Schwabl et al., 2019).

The main purpose of this study was to evaluate the possible amounts of microplastics (MP) in drinking water brands, which are produced and distributed locally. Also, it was investigated whether the different volumes of the drinking water bottles could have an effect on the amount of microplastic. In this way, it is aimed to increase the awareness of both consumers, producers and local authorities.

MATERIAL and METHODS

Collection of Samples

In this study, 0.5 and 1.5 L samples were taken from 6 different brands of drinking water produced locally in Izmir, Aydın, Muğla and Denizli provinces and sold in markets in Muğla Province, and the possible presence of microplastics was analyzed. Three samples were taken from different volume sub-samples of each brand of water. The samples were immediately placed in a portable freezer and brought to Muğla University Faculty of Fisheries Water Quality Laboratory at a constant temperature of 4 °C and stored in the dark, under appropriate conditions until the analysis.

Analysis of samples

Various chemical and/or physical pretreatments were applied to detect the presence of microplastics in samples (sediment, water and/or living tissue) taken from different compartments of the natural ecosystem. No chemical treatment was required to detect the presence of MP in water samples. For this reason, the standard method used to detect the presence of MP in waters all over the world was also used to detect the possible presence of MP in the sampled drinking waters in this study. The samples were filtered using GF/F Whatman® brand glass fiber filter papers with a diameter of 47 mm and a mesh size of 0.7 µm, using a vacuum pump (approximately 100 mbar under a maximum pressure of 2 bar).

Each of the filter papers on which the samples were filtered were taken into glass petri dishes separately, placed in a closed fume hood and left to dry at room temperature (23 °C). Then, each dried filter paper was examined and photographed twice by different researchers under two identical (twin) BOECO 55 MST 606 stereo microscopes, and the microplastics on it were counted and grouped according to their size, color and shape. The sizes of MP's were divided into two subgroups as 0.1–1.0 mm and 1.0-5.0 mm. The detection limit of stereomicroscopes of the particles was ~100 µm. As the stereomicroscopes may not accurately separate natural and synthetic particles, it has been suggested to use both microscopy and other advanced techniques (eg. FTIR, RAMAN etc.) to identify the possible MP's particles. Also, the results can be influenced by the quality of the microscope, the researcher, and the particle sizes which may lead to an underestimation or overestimation of amount of MP's (Song et al., 2015; Loder and Gerdts, 2015). For smaller size MP's (<500 µm), visual identification may not reliable at all times. As the sizes of microplastics decrease, the amount increases, and the accuracy of identification is reduced, which may cause considerable challenges in microplastic identification. To visually determine whether a particle is of plastic, the criteria of Hidalgo-Ruz et al. was met (Hidalgo-Ruz et al., 2012; Baltic, 2017), even though, a subset of samples was confirmed by Raman-spectroscopy. Microplastics were classified as red, blue, green, yellow, white and other in terms of color and as fragments, fibers and film particles in terms of shape, and counting was carried out in line with these groupings (Nuelle et al., 2014; Lots et al., 2017; Yabanlı et al., 2019).

Prevention of possible microplastic interferences during laboratory studies

All equipment (washing bottle, volumetric flask, petri dish, beaker etc.) used during the analyzes carried out in the laboratory were cleaned with pre-filtered (GF/F glass fiber filter paper used in this study /0.7 µm- 47 mm diameter) distilled water in order to remove possible particles in it and were preserved in a fume hood, carefully covered with blotting paper. Nitrile gloves were used during the analyzes and all the doors and windows of the laboratory were always closed during the procedures in order to cut off the air flow (Torre et al., 2016; Crawford and Quinn, 2016). In addition, in order to detect any contamination that may occur by air, some filter

papers (GF/F glass fiber) (Field blanks) were left for control at 4 different points of the laboratory throughout the entire study. These results were deduced from all the results obtained by counting the possible plastics on these filter papers and it was tried to minimize the errors caused by the interferences that might arise from the environment. During the laboratory study, it was calculated that the time taken for each sample to be ready for analysis after it was brought from the natural environment was approximately 30 minutes (± 3 minutes). It was calculated how much interference the microplastics detected on the filters placed in different parts of the laboratory for control purposes could do, according to the 30-minute time frame and based on this number, the environment-based interventions found were subtracted from all the results obtained, and the most accurate findings were tried to be reached (Lusher et al., 2014; Catarino et al., 2017; La Daana et al., 2017). As a result of the study, 0.056 < 1 piece in a 30-minute period for all MP's in the form of white fibers were detected in the field blanks.

Identification of polymers by micro-Raman

In order to capture the MP's image accumulated on the filters in the clearest way, the surface of the entire filter was scanned in 20-fold magnification. For the scanning area, the closest dimensions to the surface area of the filter, 4.4 mm × 4.4 mm (≅ 69 mm^2), were chosen (The diameter of the filter is 47 mm and its area is ≅ 74 mm^2 including the hollow partition at the edges). Thanks to the special computer software of the device, MP's quantity, size and morphology of the particles (image analysis) were determined. For scanning in the device, 532 nm excitation laser, 5 s integration time, laser intensity of 12%, laser spot size about 0.7 mm, spectral resolution of 5 cm^{-1} and spectral range between 500-4000 cm^{-1} were set. In order to define the results, they were compared with the sample data used for the identification of plastic-derived substances in the reference library within the device itself. Library matches with a rank ≥550 to ≤700 was analyzed and interpreted separately (Woodall et al., 2014). The particles suspected to be MP's were identified by scanning all surface areas of the filters. The particles suspected to be plastic with a similarity rate of more than 51% as a result of the scan were evaluated. The mean of similarity was 57.8% for all samples. Only those with a plastic-based structure were evaluated in the results of the study.

Estimation of microplastic intake by humans

The estimated daily intake (EDI) of MPs in bottled water was calculated using the following formula:

$$EDI = \frac{C \times IR}{bw}$$

EDI; Estimated Daily İntake (MP kg^{-1} d^{-1}),

IR; Ingestion Rate (L d^{-1}),

C; Concentration of Mps (particles L^{-1}),

bw; body weight (kg).

Estimated Daily intake (EDI) of MP's is calculated based on the Daily average consumption of drinking water (IR), concentration of MP's in drinking water (C) and body weight (*bw*). The data of water consumption and body weight were estimated based on the 4 different target groups (infants, children, teenager and adults). The average water consumption rates (IR) in infants (0–2 years old), children (3–6 years old), teenagers (7–16 years old) and adults (≥ 17 years old) were 0.08, 0.85, 2 and 2.5 L day^{-1}, respectively. Body weight of target groups were considered 10, 15, 50 and 78 kg, respectively (Ghoochani et al., 2017; Yousefi et al., 2018). In addition to this IR, for the year 2022, an average of 63 L of water in plastic and glass bottles per adult person in Türkiye per year (31.5 L on average for a pet bottle $year^{-1}$ $person^{-1}$) was consumed (SUDER, 2022).

Statistical Analyses

STATISTICA STAT 10.0 program was used in the statistical evaluation of the obtained data. A comparison was made between sample groups with 2 different volumes (0.5 L and 1.5 L) using Analysis of Variance (ANOVA), and it was revealed whether there was any statistically significant difference. In cases where there was a difference between groups, Tukey post-hoc test was used after ANOVA to determine which group the difference originated from. Obtained results were evaluated at $p < 0.05$ confidence level.

RESULTS

Presence of MP's in the samples

In the study, a total of 36 samples of drinking water in two different volumes (0.5 and 1.5 L) belonging to 6 different brands, which were bottled in the Aegean region and sold locally within the borders of Muğla Province, in disposable bottles were used. As a result of the examination of all samples, a total of 207 microplastic particles were detected (Figure 1).

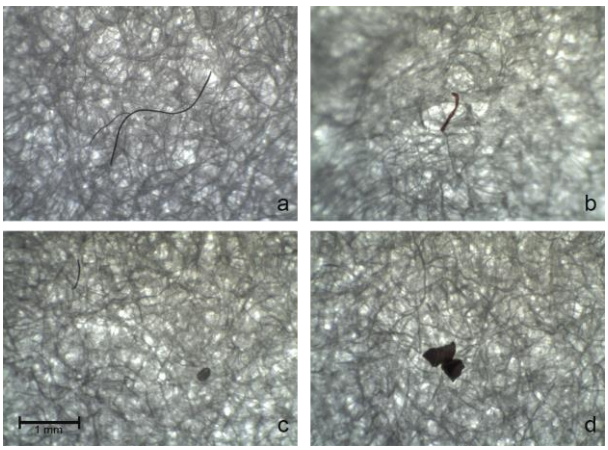

Figure 1. Microplastic samples detected on filters during the study. (a) blue fiber longer than 1 mm, (b) red fiber smaller than 1 mm, (c) black fragment and white fiber smaller than 1 mm, (d) black film smaller than 1 mm

The microplastics accumulated on the filter papers placed at 4 different points of the laboratory throughout the study in order to prevent microplastic contamination that might come from the air during laboratory studies, were counted under the microscope. As a result of the countings, it was determined that the amount of microplastics that could have interfered with the air was a very low value for each type or color per 30 minutes, which was the average processing time, and it was ignored (0.056 < 1 piece in a 30-minute period for all).

In the light of the findings, a total of 95 microplastics were detected in bottles with a volume of 0.5 L (mean 5.27 ± 5.35 particle bottle^{-1}) and a total of 112 microplastics in bottles with a volume of 1.5 L (mean 6.22 ± 11.21 particle bottle^{-1}). As a result of the study, a minimum of 1 particle bottle-1 and a maximum 22 particle bottle^{-1} in bottles with a volume of 0.5 L, and the presence of a minimum of 1 particle bottle^{-1} and a maximum of 50 particles in a bottle^{-1} in bottles with a volume of 1.5 L were found.

Concentration of MP's per liter

All results were calculated according to 1 L volume in order to compare the MP accumulation amounts in drinking water samples in bottles of different volumes. According to the results of these calculations, a total of 265 microplastics (average 7.35 ± 9.66 MP L^{-1}) were detected in all samples (n=36). A total of 190 particles (mean 10.56 ± 10.71 MP L^{-1}) were detected in 0.5 L volume bottles and 74.67 particles (mean 4.15 ± 7.47 MP L^{-1}) in 1.5 L volume bottles. As a result of the analysis, when all samples (n=36) were taken into account, a mean of 7.35 ± 9.66 MP L^{-1} particles were detected in the drinking water used in the study.

According to the ANOVA results applied to the sample group, when comparing the amount of MP in water bottles of different volumes, it was determined that there was a statistically significant difference between the mean amount of MP in 0.5 L water bottles and the mean MP amount determined from 1.5 L water bottles in 1 L volume (F= 4.33; p < 0.045).

Types of MP's in the samples

The MP found in the filters examined during the study was classified into 3 subgroups (Fiber, fragment and film) in terms of type. In line with the findings, a total of 188 fibers, 17 fragments and 2 film-shaped microplastics were detected in all filters. In all samples, fiber-shaped microplastics with 47 pieces, fragment-shaped microplastics with 2 pieces and film-shaped microplastics with 2 pieces were counted at the highest value in a single filter.

As a result of the countings made by grouping the samples according to the types of microplastics, it was determined that fiber-shaped MP was more dominant (90.8%) in both 1.5 L and 0.5 L water bottles (Figure 2).

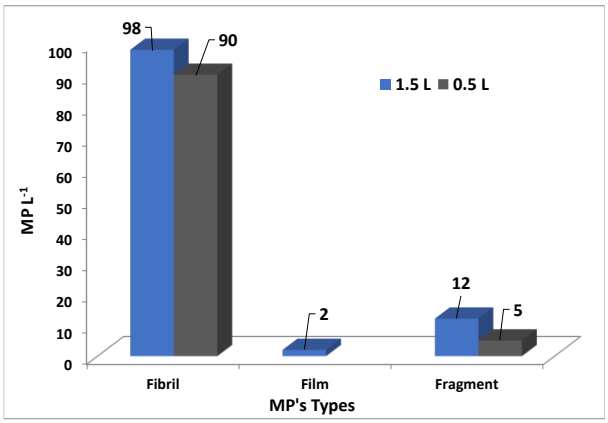

Figure 2. Dominant microplastic accumulations in 0.5 and 1.5 L water bottles in terms of shape

According to the results of the ANOVA test, which was carried out in line with the findings obtained according to the countings made by grouping in terms of shape, a statistically significant difference was determined between the shape of MP's in the drinking water samples sold in disposable bottles (F= 5.98; p<0.004). Fiber-shaped MP's were statistically more abundant than other shaped microplastics (Figure 3).

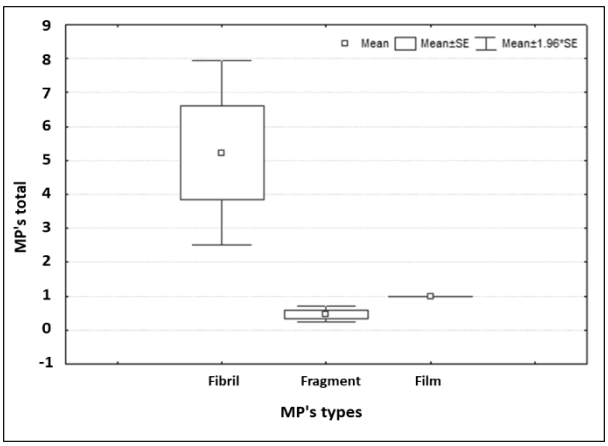

Figure 3. ANOVA results show that there is a difference between microplastic accumulation in terms of shape

In terms of MP colors in the filters examined during the study, they were classified into 6 subgroups as white, red, blue, yellow, green and other. In accordance with the visual countings, it was determined that the blue colored MP was the most dominant microplastics in terms of colors with 118 particles and other colored MPs with 62 units. In all samples, blue colored microplastics were counted with 42 pieces and other colored microplastics were counted as the highest value in a single filter with 5 pieces.

By grouping the MP colors in the samples and the volumes of the bottles (0.5 and 1.5 L), it was determined that the blue colored MP was dominant in both water bottle types (51.58% at 0.5 L volume and 61.61% at 1.5 L volume), followed by the other colored MP (32.63% at 0.5 L volume and 27.68% at 1.5 L volume). (Figure 4).

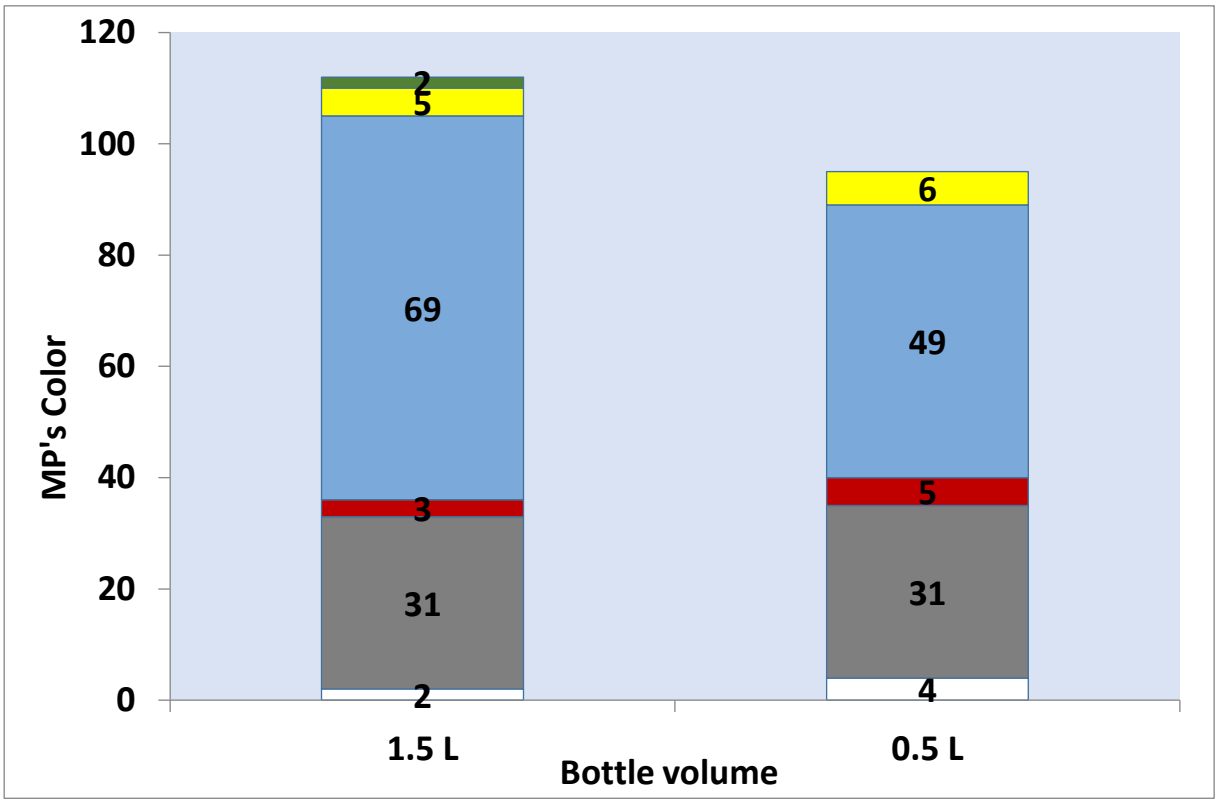

Figure 4. Microplastic accumulation dominant in terms of color groups in 0.5 and 1.5 L water bottles

In terms of color in all samples, it was determined that the MP in the blue and other colored group was more dominant (57% and 30% respectively), but according to the ANOVA results, it was determined that the amount of MP in different colors in the disposable water bottles did not have any statistical significance between the color groups. (F= 2.330; p= 0.051) (Figure 5).

The lengths of microplastics detected during visual counting of the filters were determined in two subgroups (0.1-1.0 mm and 1 -5 mm) using scaled oculars. The analysis showed that 146 of the total microplastics detected were in the size range of 0.1-1 mm and 61 of them were in the size range of 1-5 mm (Figure 6). As a result of the statistical analysis, no significant difference was found between the groups.

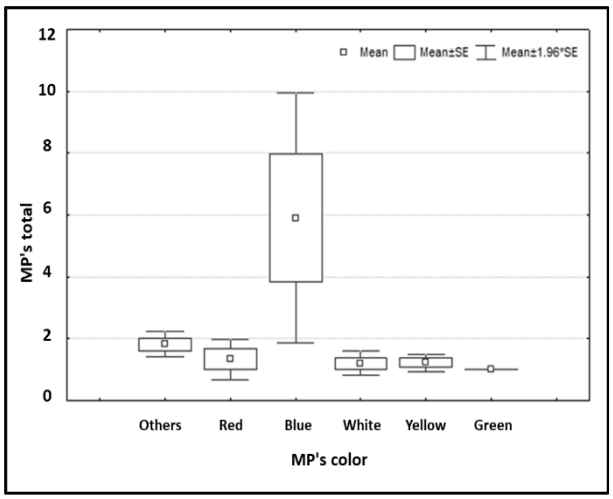

Figure 5. ANOVA results showing that there was no difference between microplastic accumulation in terms of color groups

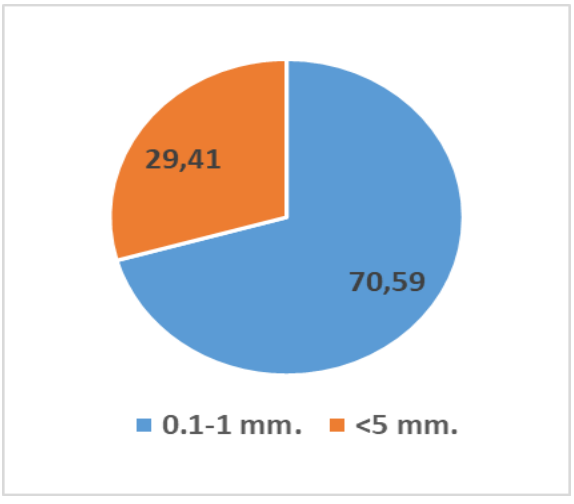

Figure 6. Distribution of MP's in terms of size groups (mm)

Qualitative assessment of MP's in the samples

According to the results of Raman spectroscopy, it was determined that MPs in the samples examined had Polyethylene (PET), polypropylene (PP) and Polyamid-Nylon (PA) type polymer structures. The most dominant of these plastics is PET with 78%, while the rarest is PA (Nylon) with 6%. The average identification rate of Raman spectroscopy was 57.8% (Figure 7).

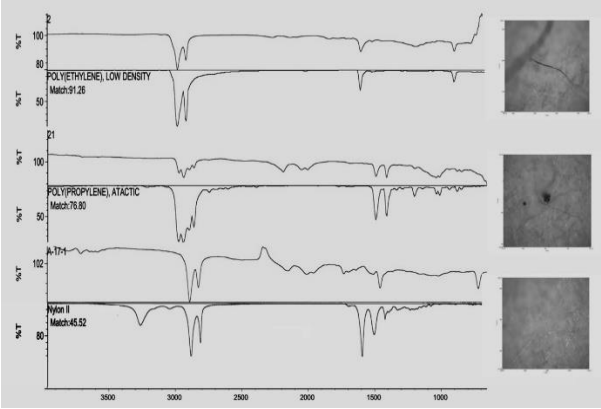

Figure 7. According to the Raman spectroscopy results, the spectrums determined with the highest detection ratio

Public health risk assessment

As a result of EDI calculations, it was determined that MPs in drinking water in 0.5 L and 1.5 L plastic bottles exposed the 3-6 age group the most (Table 1). In the light of the findings, the results of EDI $_{(0.5\ L)}$, EDI $_{(1.5\ L)}$ and EDI $_{(avg)}$ were close to each other in each age group. Calculated by taking the mean of all results with 0.5 and 1.5 L bottles and using the annual average water consumption value in adults, the values of Annual Ingestion Rate (AIR) were found as 2.25, 2.52 and 2.32 kg person^{-1} year^{-1} respectively.

Table 1. EDI (kg d^{-1}) values were calculated in terms of water consumption according to each age group in relation to the results obtained from 0.5 and 1.5 L bottles

Ages	EDI $_{(0.5\ L)}$	EDI $_{(1.5\ L)}$	EDI $_{(avg)}$	IR (L day^{-1})	*Bw* (kg)
0-2	0.04	0.05	0.05	0.08	10
3-6	0.32	0.35	0.33	0.85	15
7-16	0.22	0.25	0.23	2	50
>17	0.18	0.20	0.18	2.5	78

According to the EDI calculation based on MP's accumulation in 0.5 and 1.5 L bottles compared to 1 L, it was determined that the most exposed group was again the 3-6 age group (Table 2). However, in this calculation, the EDI 1 L (0.5 L) value was found to be significantly higher than the other groups. Considering the annual average water consumption value in adults, the AIR 1 L (0.5 L), AIR 1 L (1.5 L) and AIR 1 L (avg) values for each 1 L of water consumption were calculated as 4.26, 1.68 and 2.97 kg person^{-1} year^{-1}, respectively.

Table 2. EDI (kg d^{-1}) values were calculated in terms of the results obtained from the comparison of 0.5 and 1.5 L bottles to 1 L according to each age group

Ages	EDI$_{1\ L\ (0.5\ L)}$	EDI$_{1\ L\ (1.5\ L)}$	EDI$_{1\ L\ (avg)}$	IR (L day^{-1})	*Bw* (kg)
0-2	0.08	0.03	0.06	0.08	10
3-6	0.60	0.24	0.42	0.85	15
7-16	0.42	0.17	0.29	2	50
>17	0.34	0.13	0.24	2.5	78

DISCUSSION

As a result of this study, MP presence was detected in all 36 samples obtained from drinking water of 6 different brands sold in disposable bottles with two different volumes (0.5 and 1.5 L). One of the most important needs for the continuity of life is drinking water and these waters are suitable for consumption in terms of human health. Research on the presence and amount of MP in food products and the impacts of these pollutants on foods such as directly consumed drinking water is a recently emerged gray area. International standards regarding the risk, hazard and extent of exposure associated with MP entry into the human metabolism are not yet fully clarified.

There are some studies in the world whose subject is similar to this study. In a study conducted in 2018, the presence of MP in disposable (PET) bottles of 11 different brands was investigated and a mean of 14 ± 14 MP L^{-1} particles (varying in the range of 2-44 MP L^{-1}) were detected (Schymanski et al., 2018). In 2018, Mason et al. investigated the presence of MP in 279 drinking water samples belonging to 11 different brands that were stored in 500-600 mL bottles and sold to 5 different continents, and the presence of MP was detected in 93% of all samples and an average of 10.4 MP L^{-1} (> 100 µm) was found. In the study of Cox et al., in which they investigated the presence of MP in drinking water samples taken from the tap and bottled water in Canada in 2019, an average of 94.37 MP L^{-1} particles were detected in bottled water and an average of 4.23 MP L^{-1} particles in tap water. In a study carried out to detect the presence of MP in the drinking water of 10 different brands sold in single-use bottles in the markets in Thailand, the presence of MP was detected in all of the samples and the average was found to be 12 ± 1 MP L^{-1} (> 50 µm) (Kankanige and Babel, 2020). In another study investigating the presence of MP in drinking water, which focused on distribution networks of drinking water and as a result, the possible pollution risk in 5 different water supplies was revealed. A mean of 174 ± 405 MP m^{-3} particles was determined in all drinking water distribution systems examined (Kirstein et al., 2021).

Although the results of this study show some similarities when compared with previous studies conducted in the world, there are also studies with very different findings. There may be some obvious reasons for these differences. The most important of these are methodological differences. Most of the studies (including this study) use the counted particles for which they could fully confirm the polymeric nature using Raman spectroscopy method. On the other hand, in some studies, sample filters are painted with dyes with different luminescence properties (eg. Nile red) and assuming that these dyes highlight the plastic particles, counting and identification processes are performed only on particles that glow. Some studies use bottled drinking water, while others have sampled tap water directly. The number of samples used in the studies also varies widely. As a result, the subject of standardization in studies related to MP remains blurry. There is an urgent need for the organizations leading such studies in the world to explain more clear and universal methodologies that may be applicable by all scientists in the world.

The results of this study are compatible with the results of studies similar to this subject in the world. (Table 3).

Table 3. Comparison of study results with similar studies conducted in the world

Container	Liquid type	Sample number (*n*)	Mean microplastic number (MP L^{-1})	Study
Single use (PET bottle)	Drinking water	295	10*	Mason et al., 2018
Single use (PET bottle)	Drinking water	32	16	Oßmann et al., 2018
Single use (PET bottle)	Drinking water	38	14*	Schymanski et al., 2018
Single use (PET bottle)	Drinking water	3	3	Kosuth et al., 2018
Single use (PET bottle)	Drinking water	18	8	Winkler et al., 2019
Single use (PET bottle)	Drinking water	65	12*	Kankanige and Babel, 2020
Single use (PET bottle)	Drinking water	69	3	Zhou et al., 2021
MEAN		**74**	**9**	
Single use (PET bottle)	Drinking water	18	7*	**Current study**

*> 50 - 100 µm

It was determined that the most dominant polymer chain detected by Raman spectroscopy in the study was PET. The predominance of PET type MPs might be due to the material from which the bottles were made. Although the rules to be followed in facilities where drinking water is stored and bottled in containers of different volumes are very strict, the hygiene rules taken to prevent factors that may endanger human health might be taken into consideration more. Considering the process in which drinking water is put into bottles, the method used is the technique of filling the bottles as soon as possible with pressure. Thus, during this process, due to pressure, fractures and ruptures may have occurred on the inner surfaces of the bottle. In addition, pressurized water may create stress fractures on the inner surface of the bottle, and these fractures may cause ruptures in later processes (transportation, storage, etc.) (Kankanige and Babel, 2020). In addition, if the bottles are kept in conditions where the thermal impact may occur, such as sunlight or hot temperatures, where water is stored, these stress fractures may cause degradation. Therefore, the MP release from the packaging material is an outcome of its natural degradation and other causes in the production process, distribution and storage, and consumer behavior.

According to the results of Raman spectroscopy, the rarest polymer was found as PA (Nylon). PA is the most common type of MP in airborne particles in a laboratory environment. Studies have shown that atmospheric microfiber wastes in urban areas, for 2–355 microfibers m^{-2} particles, 50% are cotton/ wool (which are PA nylon) with 17% referring to other plastics (Dris et al., 2016). It is thought that white colored fibril-shaped plastic cotton/wool PA nylon particles may originate from the white coats worn by the laboratory team. As a result, the small number of PA detected in the samples may be due to air circulation in the laboratory environment.

The leading organizations of the world (FAO, ATSDR, WHO, GESAMP etc.) have not yet made valid and accepted regulations about the possible negative impacts of MP's detected in foods on human health. There are legal restrictions, the validity of which is open to question, only for certain foods that have the possibility of direct contact with the contaminant and can be consumed directly with very little pre-treatment (such as mussels). Potential risks posed by MP's for drinking water have just started to be studied, and there is no legal limit accepted yet. As a result, it should be noted that many parameters have an impact on the EDI, including the consumption behavior and quality of the bottle material. Consequently, further research is necessary to determine the permissible daily intake of MPs in drinking water.

CONCLUSION

Plastic has become one of the basic materials that human beings cannot give up using due to its ease of production and availability, and its high reuse rate. However, due to its intensive and unconscious production and/or use, its

uncontrolled presence in nature in recent years has led to undeniable environmental problems. So much so that these residual wastes of plastics, which are divided into particles too small to be seen by environmental factors and called "*microplastics*", have been heavily involved in the food chain and even started to be detected in the structure of basic nutrients that are directly consumed as human food. Current study and similar studies in the world have shown that even in drinking water, which is in the most basic food group and bottled untouched, MP is present. Although microplastic has been recognized as an environmental problem in the first quarter of the 21st century, it is difficult to say that there is sufficient research on this issue. Although human exposure to microplastic is accepted as a global problem, it is still an open question what precautions should be taken related to many different parameters such as uncertainty, variability and lifetime accumulation. Currently, available information on the potential adverse effects of MP on human health is insufficient, scattered and yet far from the desired level. There are no regulations regarding microplastics as a food pollutant in the world and in our country. Although there is a general tendency to draw a legal framework regarding MP found in foods and natural ecosystems, various information gaps complicate the issue at this point. As a result of future studies, ensuring the unity in analytical methods, determining the toxic effects of MP and eliminating the deficiencies in monitoring data will also guide the legal regulations to be made in this regard.

In order to reduce the problem at its source, making recycling more widespread, raising awareness of consumers and initiating the necessary legal actions on the subject are important for both food safety, human and animal health and ecological balance.

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AUTHORSHIP CONTRIBUTIONS

All phases of the study, such as conceptualization, methodology, research, verification, software, etc. were carried out by Aykut Yozukmaz.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Integrated assessment with biomarker responses and metal concentrations on some fish species from İzmir Bay: A preliminary investigation

İzmir Körfezi'ndeki bazı balık türlerinde biyoişaretçi yanıtlarının metal konsantrasyonları ile birlikte değerlendirilmesi: Bir ön araştırma

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Abstract: Antioxidant related biomarkers (superoxide dismutase, catalase, malondialdehyde) were investigated to evaluate metal (Hg, Cd, Pb, Cr, Cu, Zn, and Mn) bioaccumulation in some organs of fish species (*Sparus aurata*, *Chelon labrosus*, *Diplodus vulgaris*) from the İzmir Bay. Samples were collected at November 2019 from Inner and Outer Bays. Metal and biomarker analyses were carried out by Atomic Absorption Spectrometer and microplate reader, respectively. For metal analyses in organs, higher metal bioaccumulations were found at liver tissues. Higher Hg and Zn concentrations were found in *S. aurata*, higher Cd, Cr, Cu and, Mn concentrations were detected in *C. labrosus* and higher Pb concentrations were determined in *D. vulgaris*. In biomarker results, superoxide dismutase (SOD) and catalase (CAT) activities were generally higher in gills, however, MDA contents were higher at liver. The highest biomarker results were detected at *C. labrosus*. Statistical analyses were demonstrated that especially MDA content were expressed strong responses for the metal bioaccumulations. Also, Mn levels were considerably correlated with all biomarkers as expected. This study revealed that combined utilization of biomarkers and metal concentrations could be a vital indicator to investigate health status of the marine ecosystems.

Keywords: Catalase, superoxide dismutase, malondialdehyde, trace metals, mullet, Sparidae sp.

Öz: İzmir Körfezi'ndeki balık türlerinin (*Sparus aurata*, *Chelon labrosus*, *Diplodus vulgaris*) organlarında metal (Hg, Cd, Pb, Cr, Cu, Zn ve Mn) biyobirikimini değerlendirmek için antioksidanla kökenli biyoişaretçiler (süperoksit dismutaz, katalaz, malondialdehit) incelenmiştir. Balıklar Kasım 2019'da İç ve Dış Körfez'den toplanmıştır. Metal ve biyoişaretçi analizleri sırasıyla Atomik Absorpsiyon Spektrometresi ve mikroplaka okuyucu ile yapılmıştır. Organlarda bulunan metal değerlerine göre karaciğer dokularında daha yüksek metal biyobirikimleri bulunmuştur. *S. aurata*'da daha yüksek Hg ve Zn konsantrasyonları, *C. labrosus*'ta daha yüksek Cd, Cr, Cu ve Mn konsantrasyonları ve *D. vulgaris*'te ise daha yüksek Pb konsantrasyonları tespit edilmiştir. Biyoişaretçi sonuçlarına göre, solungaçlarda SOD ve CAT aktiviteleri genel olarak daha yüksek, ancak MDA içerikleri karaciğerde daha yüksek bulunmuştur. En yüksek biyoişaretçi bulguları ise *C. labrosus*'ta tespit edilmiştir. İstatistiksel hesaplamalar, özellikle MDA içeriğinin, metal biyobirikimleri için kayda değer cevaplar verdiğini göstermiştir. Ayrıca Mn seviyeleri, beklendiği gibi tüm biyoişaretçiler ile anlamlı korelasyonlar göstermiştir. Bu çalışma, biyoişaretçilerin ve metal konsantrasyonlarının birlikte kullanımının deniz ekosistemlerinin sağlık durumunu araştırmak için önemli bir belirteç olabileceğini ortaya koymuştur.

Anahtar kelimeler: Katalaz, süperoksit dismutaz, malondialdehit, iz metaller, kefal, Sparidae sp.

INTRODUCTION

Due to natural components of Earth's crust, metals interfere with living resources all the time. Main routes of metals trough the aquatic environment can be defined as two separate ways: natural and anthropogenic activities. Natural phenomenon includes erosion, volcanic eruptions, evaporation, and corrosion. Besides, industrial, agricultural, urban run-offs can be defined as anthropogenic sources. Eventually, aquatic organisms are exposed to essential and toxic metals which they can be fatal when they reach to the food chain with biomagnification mechanisms. (Briffa et al., 2020).

As it is known, the concentrations of pollutants in the aquatic ecosystem increase as a result of domestic and industrial activities. For this reason, environmental monitoring studies are needed to identify, evaluate and manage risks of the negative effects of organic and inorganic pollutants, on the environment and natural resources (Cajaraville et al., 2000). These environmental monitoring programs generally focus on determining the levels of physical (such as salinity, temperature, pH) and chemical (nutrients and pollutants) parameters in the water column. Although particle/grain size analysis from sediment, determination of pollutants and

physicochemical analyses provide the information about contamination status of the environment, they cannot provide sufficient information about the effects of pollutants on the marine biological systems (Lam, 2009).

Since the second half of the 20th century, researches on early detection of the pollution effects on aquatic life have become increasingly important. The indicators obtained as a result of these researches are called “biomarkers”. Basically, biomarkers defined as “measurable biological responses in a biological system that occur as a result of exposure to pollutants”.

Biomarker responses are measured in cells, body fluids, tissues or organs; although they are limited to chemical, molecular or physical changes and these are an indicator of the effects of pollutants (Lam and Gray, 2003).

Biomarkers could be classified in three sub-groups for the effects in organisms; a) stress-sensitive biomarkers, b) genotoxicological biomarkers, and c) exposure biomarkers (Viarengo et al., 2007). While catalase (CAT) and superoxide dismutase (SOD) were associated into the stress-sensitive biomarkers class; malondialdehyde (MDA) is one of the exposure biomarkers (Viarengo et al., 2007).

Atmospheric O_2 has a distinctive feature because it has two unpaired electrons in its ground state. For this important property, O_2 is described as "paramagnetic" and therefore has limited interaction with organic molecules unless there is an activation. Univalent reduction of molecular oxygen results in mostly unstable intermediates such as superoxide radical (O_2^{-}), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^{-}), before producing water (H_2O) (Markovitch, 2005). These structures are defined as reactive oxygen species (ROS). Since ROS products are involved in many metabolic reactions, they can cause cellular damage and also have disease-causing effects such as DNA damage (Lee and Steinert, 2003; Lesser, 2006). In addition to metabolic reactions in living cells, organic and inorganic pollutants such as metals and pesticides also cause the formation of free radicals.

There are some antioxidant enzymes that help remove the affection of ROS in the cell. Superoxide dismutase (SOD), an antioxidant enzyme, is a metalloprotein that distributes in different organelles or cytoplasm inside the cell and contains different metals (such as Mn, Fe, and Zn) as cofactors. Main function of SOD catalyzes the conversion reaction of O_2^{-} radical ion to H_2O_2 and O_2 molecules. Catalase (CAT), another oxidative stress biomarker, is an enzyme containing a heme group; It enables the conversion of H_2O_2 to H_2O and O_2. In the use of hydroxyl (-OH) scavenging agents, SOD and CAT determine the role of oxygen radicals involved in biological processes. (Lesser, 2006; Di Giulio et al., 1989).

Lipid peroxidation (LPO) is a mechanism that occurs under the control of enzymes in biological systems without radical ions. One of the final products formed in this reaction is malondialdehyde (MDA). MDA molecule is formed by the breakdown of unsaturated polylipids by ROS. MDA can have a mutagenic effect by binding to DNA as a result of the reactions it enters in the cell. The presence of MDA or other LPO products is expressed as an important “fingerprint” about the entry of xenobiotics that cause oxidative stress in the cell (Pryor and Stanley, 1975; Romero et al., 1998).

Chelon labrosus (Risso, 1827) (thicklip grey mullet) in one of the common species of Mugilidae family. Its individuals generally distribute coasts and stream mouths, especially rocky and sandy bottoms. It also prefers living with larger groups (Parrino et al., 2021). *Chelon labrosus* is an omnivorous scavenger that feeds with small organisms and particles from water column and sediment (Abumourad et al., 2014).

Sparus aurata (Linneaus, 1758) (gilthead seabream) is one of the common fish species at the Eastern Atlantic Ocean and the Mediterranean Sea. It generally lives about 0 to 30 meters depth, but it can excess to 150 m (Bauchot and Hureau, 1990; Seginer, 2016). This species is carnivore and usually feeds on generally molluscs and arthropods (Hadj Taieb et al., 2013).

Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817) (common two-banded seabream) is a demersal marine fish that lives generally inshore waterbeds and posidonia covered areas with 0 – 90 m depth (Cengiz et al., 2019). Along with *S. aurata*, it is also member of Sparidae family (Da Palma, 2002). Main diet of this species contains crustacea such as amphipoda, isopoda, prawns, fish and polychaeta (Osman and Mahmoud, 2009).

These species have spawning period between late autumn – winter session (Abumourad et al., 2014; Hadj Taieb et al., 2013; Osman and Mahmoud, 2009).

İzmir Bay, chosen as the study area, is heavily under the influence anthropogenic and industrial pollution. Various industrial facilities (such as agricultural production, food processing, tanneries, paper production, textile, metal industries and beverage manufacturing) and urban wastes discharge to the Bay and these are prime reasons for the pollution (Kucuksezgin et al., 2006).

Many streams and domestic discharge channels carry the pollution load to the bay. Gediz River, one of the most important sources of pollution, is located in the northern part of the bay. Inner Bay has intensity of industrial and anthropogenic activity. In addition, İzmir Port, one of Türkiye's most important natural ports, operates actively in the Inner Bay. Thanks to the wastewater treatment plant, which was put into operation in 2000, the water quality and number of living organisms in the bay has increased. Accordingly, the people of İzmir can hunt fish with high economic value such as sea bream, sea bass and mullets, especially in the Inner Bay. According to many studies (Uluturhan Suzer et al., 2015; Kacar et al., 2016; Taş et al., 2018; Taş and Sunlu, 2019), the pollution in the sediment continues despite the improvement in water quality in the bay. For this reason, organisms like fish and mussels caught from

Izmir Bay, a potential risk to public health in seafood via consumption continues (Çakal Arslan et al., 2010; Kucuksezgin et al., 2011).

In this context, the aim of this study is integrated evaluation of the metal concentrations and the investigation of the metabolic responses by using biomarkers sensitive to reactive oxygen species distributed in fish species in the İzmir Bay

MATERIALS AND METHODS

Sampling: Fish samples were collected coastal sites of Foça (Outer Bay) and İnciraltı (Inner Bay) in November 2019 by fishing rods (Figure 1). While *Sparus aurata*, *Diplodus vulgaris* and *Chelon labrosus* were sampled in Foça, only *C. labrosus* individuals were sampled in İnciraltı (Table 1).

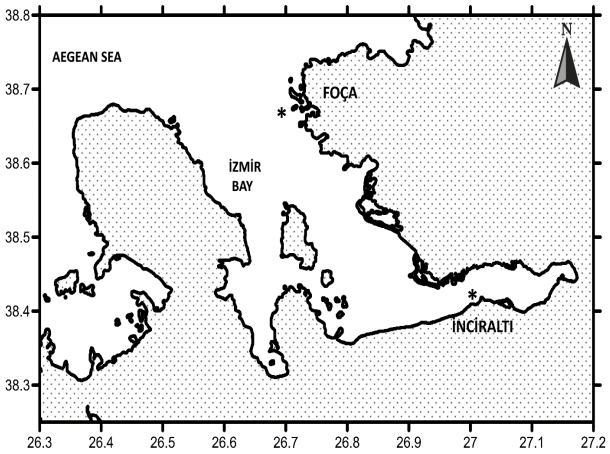

Figure.1. Sampling points (Foça and İnciraltı) at Izmir Bay (*)

The fish were sampled and quickly decapitated. Muscle, liver and gill tissues were separated from fish. Samples that used in biomarker analysis were placed in cryo-tubes and stored in a liquid nitrogen tank (-196 °C), while tissues for metal analysis were stored in deep freezers (-18 °C) in plastic bags (UNEP/FAO/IAEA/IOC 1984).

Table 1. Biometric data of sampled fish species in November 2019

Locations	Species	n	Weight (g)	Fork Length (cm)
Foça	*S. aurata*	3	254-271	24.6-25.7
Foça	*C. labrosus*	3	361-455	29.5-33.0
Foça	*D. vulgaris*	3	209-237	18.8-23.0
İnciraltı	*C. labrosus*	8	252-272	26.5-37.0

Metal analysis: Firstly, tissue samples were first be subjected to drying using a lyophilizer and then, the dried samples were weighed approximately 0.50 g in at least triplicate and digested in a Milestone microwave digestion system in an acid mixture of 5 mL HNO_3 and 1 mL $HClO_4$. The mixture with fragmented samples were completed to 25mL and stored in HDPE bottles. Within the scope of metal analysis, mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), zinc (Zn) and manganese (Mn) analyzes were carried out. Varian Atomic Absorption Spectrometer (AAS) was performed on 280FS and 280Z instruments using flame technique (Cu, Zn and Mn), graphite furnace (Pb, Cd and Cr) and cold vapor (Hg) (UNEP/FAO/IAEA/IOC 1984; UNEP 1990). Recovery rates were set with standard solutions and the percentages of ratios were set 100%±10 to verify the accuracy of analyses. All metal analyses were expressed as "mg/kg dry weight" unit.

Biomarker analyzes: SOD, CAT and MDA analyzes were performed with Biotek Synergy HTX model microplate reader. Homogenization processes were carried out with ISOLAB homogenizer.

Determination of SOD activity: This method was described by Sun et al. (1988) and adapted to the microplate reader. The supernatants from the homogenates prepared with Tris-HCl (pH 7.5) containing EDTA were measured at 560 nm wavelength against blank reading in the presence of xanthine/xanthine oxidase reaction and nitroblue tetrazolium. Depending on the inhibition rate obtained, the SOD activity was determined as mU/mL.

CAT activity determination: CAT activity method was adapted from the methods of (Aebi, 1984) for the microplate reader. The activity was determined at 230 nm as mU/mL by kinetic reading against H_2O_2.

Determination of Total Protein: Total protein analysis was performed to calculate SOD and CAT activities as specific activities. Protein determination was measured at a wavelength of 562 nm by using the "Pierce™ BCA Protein Assay Kit" from ThermoFisher Scientific. As a result of protein determination, CAT and SOD results were calculated as mU/mg protein.

Determination of MDA content: Concentration of MDA content was determined by adapting the method of Ohkawa et al. (1979) to the microplate. It was determined by spectrophotometric measurement of the tissues against 1,1,3,3-tetramethylpropane (TMP) at a wavelength of 532 nm after the reaction with a mixture of thiobarbituric acid and acetic acid and incubation at 95 °C for 1 hour. The level of MDA contents is expressed in pmol MDA/mg tissue.

Statistical analysis and evaluation of data: Statistical calculations were carried out JASP statistical software (version 0.16.3.0). Evaluation of metal and biomarker analyses; by using parametric (ANOVA) and non-parametric (Kruskal-Wallis) statistical tests, the interactions of the analysis results with each other were examined by considering the changes depending on the region, species and tissues ($p < 0.05$). Data were log-transformed where necessary to meet normal distribution and Levene's homogeneity tests. In addition, the interactions of metals and biomarkers were investigated by Pearson Product-Moment Correlation tests ($p < 0.05$).″R≥ |0.40|" values were evaluated and interpretation of absolute magnitudes on correlation coefficients were described as

Schober and Schwarte (2018). Also, Network Analysis were carried out to exhibit interferences of sub-groups (metals and biomarkers) with each other.

RESULTS AND DISCUSSION

Metal analyses

Metal concentrations at organs of fish species were given at Table 2. According to the tissue-based investigation, the highest Hg concentrations were detected at muscle tissues for *S. aurata* (0.68 mg/kg). In addition, highest levels of Cd (1.41 mg/kg), Pb (3.98 mg/kg), Cu (79.6 mg/kg), and Zn (368 mg/kg) were found at livers, while the highest levels of Cr (1.30 mg/kg) and Mn (26.7 mg/kg) were obtained at gills (Table 2).

For *C. labrosus*, maximum Hg (0.51 mg/kg), Cd (4.51 mg/kg), Cr (1.50 mg/kg), Cu (775 mg/kg), and Zn (180 mg/kg) levels found at liver tissues. Also, maximum Pb (4.28 mg/kg) and Mn (33.2 mg/kg) concentrations were determined at gills (Table 2).

For, *D. vulgaris* the highest concentrations of Hg (0.60 mg/kg) were detected at muscle tissues. The highest Cu (41.3 mg/kg), Cd (3.82 mg/kg) and Zn (166 mg/kg) levels were obtained at liver, while the highest Pb (16.5 mg/kg), Cr (0.75 mg/kg) and Mn (25.3 mg/kg) levels were found at gills.

To evaluate minimum concentrations for all species; only Hg levels of *S. aurata* and *D. vulgaris* were detected at gills, other minimum levels of metals were all found at muscle tissues (Table 2).

Bioaccumulation order for the tissues in all fish species can be described as liver > gills > muscle. Kruskal – Wallis test was performed to the whole data to observe differences among locations, species and tissues ($p < 0.05$). All metals showed significant differences between tissues. Besides, Hg concentrations were showed significances between locations and species. On the other hand, Pb was showed statistical differences only for species ($p < 0.05$). Fish physiologies and behaviours, life cycle, and feeding habits affect variations on metal bioaccumulations such as Hg and Pb (Vetsis et al., 2021; Kontas et al., 2022). Fish individuals tend to store and magnify metals in the fatty organs such as liver (Omar et al., 2014). Previous studies also suggest that main source for the increased concentrations of heavy metals such as Hg and Cd is through the feeding (benthic organisms) for fish species (Cretì et al., 2010; Pan et al., 2022). Liver is the most efficient organ for detoxification and liver include many enzymes related to metals like metallothioneins. Therefore, liver contains high levels of metals (Cretì et al., 2010; Pan et al., 2022). In addition, metal ions were firstly deposited at gills through the passive diffusion, therefore higher concentrations of gills can be related with this natural phenomenon (da Silva et al., 2021). Increased levels of metal bioaccumulation at livers, gills, and muscle tissues of fish species are under the influenced of feeding habits, lipid contents, swimming activity, and, physiology (Vetsis et al., 2021). Similar profiles of minimum concentrations in *S. aurata* and *D. vulgaris* could be related with they are both member of Sparidae family (Da Palma, 2002).

To compare between species in Foça, the maximum concentrations of Hg and Zn were found at *S. aurata*; maximum concentrations of Cr, Cu, and Mn were detected at *C. labrossus* and the maximum Cd and Pb concentrations were determined at *D. vulgaris* (Table 2). Two-way ANOVA tests were performed to distinguish significances between tissues and species for metal levels and all variables were showed significant differences ($p < 0.05$). Previous studies claim that variations of metal bioaccumulation in different fish species may be related both exposure levels of metals, biochemical activities, reproduction and also ingestion, filtration and detoxification capabilities (Islam et al., 2015; Luoma and Rainbow, 2008; Wang and Rainbow, 2008).

Data of *C. labrosus* were evaluated from two locations. The maximum levels of Pb, Zn and Mn were found at Foça, however, maximum levels of Hg, Cd, Cr, and Cu were detected at İnciraltı (Table 2). Kruskal – Wallis tests were performed to detect significances between locations, but no statistical differences were observed ($p < 0.05$). It is well known that, inorganic (metal) pollutants that originated from natural and anthropogenic resources through the inputs such as atmospheric depositions, sewage, industrial and urban discharges (Zhang et al., 2015). İzmir Bay is heavily affected from municipal, agricultural, mining, and industrial sources where transport along with Gediz River that cause elevated concentrations of Hg, Pb and Cr (Uluturhan Suzer et al., 2015).

Biomarker analyses

Results of SOD, CAT, and MDA were given at Table 3. According to the biomarker results, *S. aurata* and *C. labrosus* generally showed similar profile. The maximum SOD and CAT activities were determined at gills, while maximum MDA contents were found at liver tissues. On the other hand, minimum levels of all biomarkers were detected at muscle tissues. For *D. vulgaris*, the highest levels of biomarker were obtained at livers, however, the lowest SOD and CAT activities were found at gills and the lowest MDA contents were found at muscle tissues (Table 3). The reason of higher concentrations on livers and gills than the muscle; liver tissues responded more for the antioxidant enzymes (SOD and CAT) and gills responded to biomarker of effects (MDA) (Gusso-Choueri et al., 2015) In addition, gills have thinnest epithelium and pollutants could be exposed through the respiration. Also, liver can concentrate metals and other pollutants to the considerable degree (Farombi et al., 2007). Kruskal – Wallis tests showed that SOD activities were reflected the significant differences for locations, besides, CAT activities and MDA contents were exhibited significant differences for tissues ($p < 0.05$). Higher activities of biomarkers were having connections with the exposure of elevated concentrations of metals and/or other pollutants (Beg et al., 2015; He et al., 2012). In our results, CAT and MDA levels were generally followed close patterns.

Table 2. Maximum and minimum metal concentrations in organs of selected fish species from inner and outer bays of Izmir Bay (mg/kg dry weight)

	Hg	Cd	Pb	Cr	Cu	Zn	Mn
S. aurata							
Foça (Outer Bay)							
Muscle	0.67-0.68	0.0008-0.0010	0.28-0.37	0.16-0.21	0.69-0.91	16.6-17.2	0.50-0.81
Liver	0.437-0.438	1.34-1.41	3.61-3.98	0.49-0.58	77.5-79.6	367-368	12.9-13.3
Gills	0.147-0.149	0.0036-0.0039	2.18-.2.21	1.18-1.30	2.83-2.99	86.4-91.8	26.1-26.7
C. labrosus							
Foça (Outer Bay)							
Muscle	0.25-0.27	0.001-0.002	0.24-0.33	0.12-0.69	0.55-0.66	12.4-14.7	0.18-0.20
Liver	0.056-0.058	3.19-3.66	2.17-2.30	1.15-1.44	567-568	162-165	3.20-3.24
Gills	0.056-0.064	0.003-0.004	3.55-4.28	0.50-0.53	2.62-2.84	56.3-61.9	31.0-33.2
İnciraltı (Inner Bay)							
Muscle	0.01-0.03	0.001-0.002	0.20-0.34	0.23-0.35	0.95-1.72	11.5-19.0	0.48-0.88
Liver	0.22-0.51	2.40-4.51	1.59-1.89	0.52-1.50	56.1-775	100-180	3.78-4.36
Gills	0.02-0.03	0.0038-0.0040	2.56-2.71	0.49-0.52	2.54-6.79	54.4-59.8	23.4-30.0
D. vulgaris							
Foça (Outer Bay)							
Muscle	0.57-0.60	0.0021-0.0023	1.00-1.16	0.11-0.12	1.34-1.63	18.4-18.7	1.09-1.25
Liver	0.20-0.22	3.30-3.82	9.78-10.3	0.43-0.58	40.9-41.3	165.5-165.9	9.05-9.36
Gills	0.11-0.12	0.0040-0.0042	16.2-16.5	0.70-0.75	2.44-3.13	84.5-91.7	24.2-25.3

This situation suggests that production of extensive amounts of H_2O_2 that cause oxidative stress. In that case, CAT activities could not able to sufficiently eliminate hydrogen peroxides, which provides lipid peroxidation products such as MDA (Vlahogianni et al., 2007).

Foça results showed that the highest biomarker levels were recorded at *C. labrosus*. The lowest activities of SOD and CAT were found at *S. aurata*, while the lowest MDA contents were detected at *D. vulgaris* (Table 3). Moreover, ANOVA tests were performed to test statistical variations between species significant differences were detected for all variables ($p < 0.05$).

Higher levels of biomarkers on *C. labrosus* could be linked their bioaccumulation of pollutants by their feeding habit. It is widely known that these sampled species can feed from bottom such as benthic organisms and sedimented detritus, also, this species has ability of feeding small organisms and suspended materials (Osman and Mahmoud, 2009; Hadj Taieb et al., 2013; Abumourad et al., 2014).The individuals of *C. labrosus* could be confronted floating or suspended pollutants such as oil spill and garbage, microplastics etc. (Bilbao et al., 2010; Chacón Aranda et al., 2021). To assess *C. labrosus* results between locations, SOD and CAT activities in Foça were higher than İnciraltı. MDA results were not able to distinguish locations. ANOVA tests also confirm these situations; SOD and CAT activities had significant differences; however, no significant differences were observed for MDA. Higher antioxidant enzyme activities could be related greater accumulations of metals. In conclusion, our results could confirm that, variations of MDA and metal levels were occurred parallel trends with each other. Marine organisms like fish species elevated enzyme activity levels may be triggered due to SOD's scavenging capacity and CAT removes H_2O_2 (Kumar et al., 2021).

Inter-relationship between metal concentrations and biomarker levels

According to the Pearson Product-Moment Correlation tests, especially MDA levels were showed significant correlations between all metals (Figure 2a). MDA contents were provided positive moderate correlations with Cd (0.509), Pb (0.476), Cr (0.589), Zn (0.627), and Mn (0.589). Also, MDA-Cu relationship were calculated as 0.394 ($p < 0.05$). Besides these, MDA levels were showed negative moderate correlations with Hg (-0.493) ($p < 0.05$). Also, Network Analysis confirmed that MDA contents have strong interaction between metals (Figure 2b).

Table 3. Maximum, minimum and (mean ± SD) levels biomarker levels in organs of selected fish species from inner and outer bays of İzmir Bay.

	CAT (mU/mg protein)	SOD (mU/mg protein)	MDA (pmol MDA/ mg tissue)
S. aurata			
Foça (Outer Bay)			
Muscle	1.89-2.88 (2.24±0.56)	36.8-37.7 (37.2±0.51)	9.11-11.0 (10.3±1.05)
Liver	1.53-2.89 (2.08±0.72)	22.3-31.3 (28.2±5.07)	26.9-31.0 (29.6±2.39)
Gills	3.80-7.60 (5.77±1.90)	69.4-90.7 (77.5±11.6)	21.7-23.2 (22.7±0.85)
C. labrosus			
Foça (Outer Bay)			
Muscle	2.47-17.3 (7.84±8.18)	23.2-27.0 (25.2±1.91)	9.97-11.3 (10.4±0.74)
Liver	4.74-7.51 (6.39±1.46)	29.5-36.9 (32.5±3.85)	31.9-33.1 (32.3±0.69)
Gills	14.9-22.4 (17.6±4.19)	111-131 (124±10.9)	28.7-30.1 (29.3±0.75)
İnciraltı (Inner Bay)			
Muscle	1.90-2.70 (2.36±0.41)	17.8-22.7 (20.5±2.47)	9.44-11.6 (10.5±1.07)
Liver	1.76-4.53 (3.01±1.41)	36.6-40.3 (38.6±1.83)	20.9-28.2 (25.0±3.76)
Gills	2.90-4.78 (3.79±0.94)	21.0-24.2 (21.3±2.73)	25.4-33.2 (30.4±4.33)
D. vulgaris			
Foça (Outer Bay)			
Muscle	2.56-3.12 (2.85±0.28)	28.1-30.6 (29.5±1.25)	8.32-8.49 (8.43±0.10)
Liver	4.70-6.27 (5.69±0.86)	48.7-51.0 49.6±1.19)	30.2-30.6 (30.4±0.22)
Gills	2.23-3.97 (3.26±0.91)	26.9-29.0 (28.1±1.08)	26.3-27.6 (26.9±0.66)

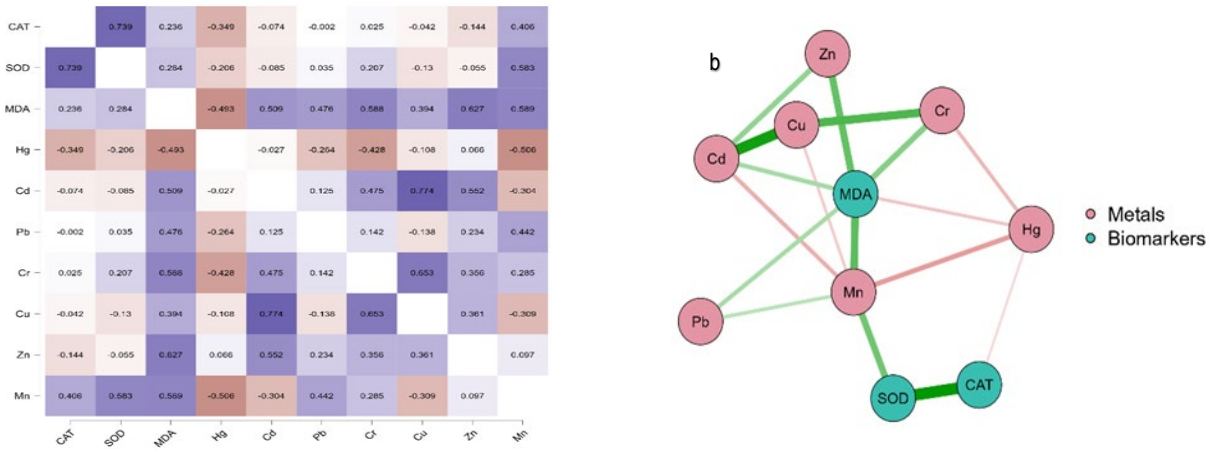

Figure 2. **a)** Heat map **b)** Network Analysis of combined correlations between biomarkers and metals

Previous studies also describe those levels of MDA contents had a strong link to essential and non-essential metals (Beg et al., 2015; Nesto et al., 2007). Lipid degradation by ROS starts a chain reaction which results MDA as products. Therefore, MDA is a vital compound to diagnose oxidative stress (Mishra, 2022). In addition, Mn concentrations were exhibited positive moderate correlations with SOD (0.583) and CAT (0.406) ($p < 0.05$). Hence one of the main components (as co-factor) of

SOD and having directly involved to ROS production mechanism, (Roche and Bogé, 1993) Mn and antioxidant biomarkers relationships were obviously revealed in this study.

CONCLUSION

Integrated approach was processed to evaluate metal bioaccumulation and antioxidant enzymes-oriented biomarkers on fish species from İzmir Bay where heavily affected from natural and anthropogenic pollutions. Samplings were carried out at Inner (İnciraltı) and Outer (Foça) regions of İzmir Bay. Results of metal analyses showed that both sides of İzmir Bay should be monitored regularly. According to the statistics, significant differences were mainly detected at tissues. Simultaneous investigations between metals and biomarkers were demonstrated that especially MDA contents are directly linked to metal bioaccumulation. In addition, Mn concentration were showed significant correlations for all biomarkers as expected. This study represented that pollutant dependent biomonitoring studies, it is important to investigate combined utilization of biomarkers and metal concentrations in order to determine the effects on organisms.

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AUTHORSHIP CONTRIBUTIONS

Mustafa Bilgin: Literature, conceptualization, manuscript writing, field research, laboratory experiments, data analysis. Esin Uluturhan-Suzer: Supervision, conceptualization, laboratory experiments, reviewing and editing. Enis darilmaz: Field research, laboratory experiments, reviewing and editing.

CONFLICT OF INTEREST

Authors would like to declare that no financial or personal conflict of interests in this study entitled “Preliminary studies of integration between metal bioaccumulation and antioxidant enzymes related biomarkers on fish species from İzmir Bay”.

ETHICS APPROVAL

In order for the experimental design to be carried out on a legal basis, the approval dated 03.05.2019 and numbered 13/2019 was obtained from the Multidiscipline Laboratory Animal Experiments Local Ethics Committee of Dokuz Eylül University.

DATA AVAILABILITY

Further questions about datasets, the corresponding author should be contacted.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Spatial variation in relationships of otolith measurements with body length of Prussian carp, *Carassius gibelio* (Bloch, 1782) collected from four lentic habitats in Samsun Province, Türkiye

Samsun İli (Türkiye)'ndeki dört lentik habitattan örneklenen gümüşi havuz balığının (*Carassius gibelio* (Bloch, 1782)) vücut uzunluğu ile otolit ölçümlerinin ilişkilerinde alansal varyasyon

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Abstract: This study aimed to disclose the spatial variability in the lagenar otolith (asteriscus) dimensions-total length relationships generated for four Prussian carp, *Carassius gibelio* (Bloch, 1782) populations (Altınkaya Dam Lake, Bafra Fish Lakes, Lake Ladik and Lake Simenit) in Samsun Province, Türkiye. Relationship between length and weight of the fish was also described for each population. Samples were obtained from commercial fishermen in different periods between February 2017 and March 2018. The length, height, and weight of each otolith were determined. A non-linear function was used to define allometric relationships between asteriscus measurements and body size. The slopes of the regressions acquired for the right and left otoliths at each sampling site did not show any significant difference. All of the relationships were found to be statistically significant (P<0.001). The r^2 values ranged from 0.61 to 0.95, and the mean values of the percent prediction error varied from 3.10 to 7.45. The ANCOVA test determined significant spatial differences in regression slopes for the three otolith variables. Our findings revealed that otolith development reflected somatic growth, but this varied by sampling area.

Keywords: Otolith biometry, asteriscus, length-weight relationship, *Carassius gibelio*

Öz: Bu çalışma, Samsun İli (Türkiye)'ndeki dört gümüşi havuz balığı, *Carassius gibelio* (Bloch, 1782) popülasyonu (Altınkaya Baraj Gölü, Bafra Balık Gölleri, Ladik Gölü ve Simenit Gölü) için elde edilen lagenar otolit (asteriskus) boyutları-toplam uzunluk ilişkilerindeki alansal değişkenliği ortaya koymayı amaçlamıştır. Her popülasyon için boy ve ağırlık ilişkisi de tanımlanmıştır. Örnekler, Şubat 2017 ile Mart 2018 arasında farklı dönemlerde ticari balıkçılardan temin edilmiştir. Her bir otolitin uzunluğu, yüksekliği ve ağırlığı belirlenmiştir. Otolit ve balık büyümesi arasındaki allometrik ilişkileri tanımlamak için üssel bir fonksiyon kullanılmıştır. Her bir örnekleme alanında sağ ve sol otolitler için elde edilen regresyonların eğimleri önemli bir farklılık göstermemiştir. Tüm ilişkiler istatistiksel olarak önemli bulunmuştur (P<0,001). Belirleme katsayıları (r^2) 0,61 ile 0,95 ve yüzde tahmin hatasının ortalama değerleri 3,10 ile 7,45 arasında değişmiştir. ANCOVA testi, üç otolit değişkeni için regresyon eğimlerinde önemli alansal farklılıklar tespit etmiştir. Bulgularımız otolit gelişiminin somatik büyümeyi yansıttığını, ancak bu durumun örnekleme alanına göre değişiklik gösterdiğini ortaya koymuştur.

Anahtar kelimeler: Otolit biyometrisi, asteriskus, boy-ağırlık ilişkisi, *Carassius gibelio*

INTRODUCTION

Otoliths are white stones located in the inner ears of fishes (Tuset et al., 2008). They provide a sense of balance to fish and also aid in hearing (Popper and Fay, 1993). Teleosts have three pairs of otoliths, known as the sagitta, lapillus, and asteriscus. These calcified structures differ markedly in size. The sagittae are the largest pair in most teleost fishes, whereas the asterisci are bigger than other otolith types in Cypriniform fishes (Assis, 2003; Schulz-Mirbach and Reichenbacher, 2006). Otoliths also exhibit significant intra- and interspecific variability in shape (Wright et al., 2002; Vignon and Morat, 2010; Yedier and Bostancı, 2022). There is always a strong correlation between otolith development and fish length, as otoliths tend to grow as the fish grows. In this case, it is possible to estimate the length of the fish from which the otolith was

acquired based on a measurement of otolith size (Campana, 2004). These estimates can be a reliable tool in studies on length-frequency distributions of fish populations, as well as in predator-prey studies with the aim to calculate the original size of consumed prey (Belfethi and Moulaï, 2022). The otolith-fish size relationships and their importance may vary between species or between different populations of the same species, as well as between different sizes of individuals of the same species (Bostancı et al., 2017; Saygın et al., 2017; Zengin Özpiçak et al., 2018; Emre, 2019; Saygın et al., 2020).

The Prussian carp, belongs to the Cyprinidae family, is regarded to be native from central-eastern Europe to Siberia (Perdikaris et al., 2012). It has been introduced outside of its natural range, into Europe, Asia and North America (Kottelat and Freyhof, 2007; Elgin et al., 2014). In Türkiye, the Prussian carp was first reported from Lake Gala in 1986 (Baran and Ongan, 1988). It has since spread to nearly all of Türkiye's lotic and lentic water bodies (Yerli et al., 2014; Tarkan et al., 2015; Çiçek et al., 2022). This species has become one of the most successful invasive fish beyond its natural distribution area due to its unique biological characteristics and extreme ecological tolerance (Tarkan et al., 2012; Fuad et al., 2021). The relationships between otolith parameters and body size of the Prussian carp were investigated in the Topçam Dam Lake (Bostancı, 2009) and Lake Ladik (Yılmaz et al., 2015) in Türkiye. However, no study has previously been carried out on otolith-fish size relationships of the Prussian carp inhabiting many freshwater environments in Samsun Province, northern Anatolia. Here, we examined whether there was a difference in otolith measurements-fish size relationships among the Prussian carp populations of four geographically distant lakes in Samsun Province, Türkiye. Additionally, the length-weight relationship of each population was also determined.

MATERIALS AND METHODS

The Prussian carp specimens were collected from four lentic ecosystems (Altınkaya Dam Lake, Bafra Fish Lakes, Lake Ladik, and Lake Simenit) in Samsun Province, Türkiye (Figure 1). The Altınkaya Dam Lake is 27 kilometers southwest of the Bafra district, on the Kızılırmak River. It has a surface area of 11830 hectares and a length of 70 kilometers (Yılmaz et al., 2007). The eastern part of the Kızılırmak Delta, which is located in the north of the Bafra district, is known as the Bafra Fish Lakes. It is made up of a series of different-sized lakes with lagoon characteristics (Gumus et al., 2007). Lake Ladik is 10 kilometers away from Ladik district. Its formation is tectonic, with a surface area of 1000 hectares and a maximum depth of 6 meters (Yılmaz et al., 2012). Lake Simenit is located within the Terme district's Gölyazı town. This lake with a surface area of 80 hectares and a maximum depth of 2 meters is the lagoon characteristic. It has a waterway on its south end that connects it to Lake Akgöl (Ersanlı and Gönülol, 2003).

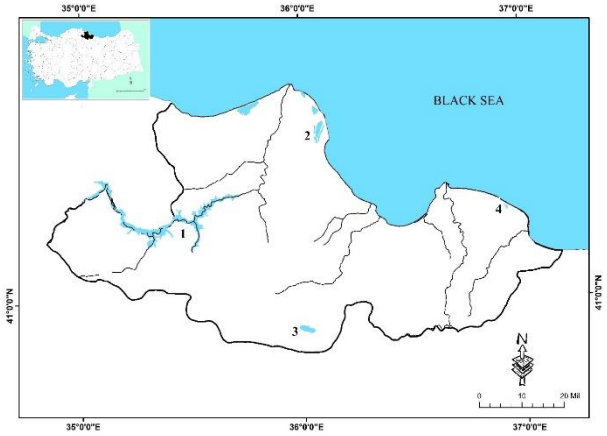

Figure 1. The sampling sites (1-Altınkaya Dam Lake, 2-Bafra Fish Lakes, 3-Lake Ladik, and 4-Lake Simenit)

Samplings were performed in different periods, from February 2017 to March 2018. Fish samples were obtained from commercial fishermen in these lakes. At the laboratory, fish body size (TL in cm), weight (W in g), and gender were determined and recorded. The lagenar otolith (asteriscus) pairs were removed, washed, dried, and stored in labeled plastic vials. Due to the low number of male individuals in all sampling areas, only female individuals were used in this study. The weight of each otolith (OW) was weighed with an accuracy of 0.01 mg. After that, the right and left otoliths were viewed under a binocular microscope (Leica S8APO) at 10x magnification. Two-dimensional digital images of the otoliths were taken with a digital camera (Leica DFC295). Otolith length (OL) and otolith height (OH) were measured with an accuracy of 0.001 mm using image analysis software (Leica Application Suite ver. 3.8). The OL and OH (Figure 2) were defined as the maximum distance between the anterior and posterior edges and between the dorsal and ventral borders, respectively (Yılmaz et al., 2015).

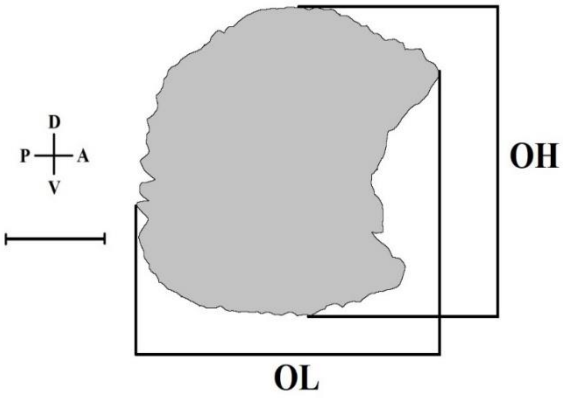

Figure 2. Distal view and measurement axes of the right asteriscus of Prussian carp. (OL-otolith length, OH-otolith height, D-dorsal, V-ventral, P-posterior, A-anterior, scale bar=2 mm)

The relationships of OL, OH, and OW versus TL were calculated using the simple non-linear regression model: $Y = aX^b$, where the coefficients *a* and *b* are constants of the regression (Lleonart et al., 2000). Three regressions were generated separately for the right and left otolith of each sampling site. Then, the slopes of the regressions were compared using the ANCOVA test (Zar, 1999). When no considerable differences were found, the H_0 hypothesis ($b_{right} = b_{left}$) was accepted and only one regression was presented for each variable by preferring the right otolith data. The *F*-test was used to confirm the regressions' significance. The deflection of each regression slope from isometry was controlled by a t-test (Zar, 1999). The statistical differences in regression slopes among the sampling areas were checked with the ANCOVA test (Zar, 1999).

The r^2 and the mean percent prediction error (PE%) were both used to evaluate the strength of each relationship. The mean PE% for a correlation is the average of the PE% values obtained for entire samples. The calculation of the PE% for an individual was performed by using the equation proposed by Smith (1980):

$$PE\% = \frac{\left|x_{observed} - x_{predicted}\right|}{x_{predicted}} \times 100$$

The dissimilarity between predicted and observed TL was analyzed for each measurement of the otolith by using a t-test or Mann-Whitney U test. The ANOVA test or Kruskal-Wallis test was used to compare the variations between mean PE% values of three parameters of the otolith at each sampling site. The variability in the mean PE% values of each otolith parameter among the sampling localities was also examined with the Kruskal-Wallis test (Zar, 1999).

The length-weight relationship (LWR) of female Prussian carp specimens from each sampling site was estimated through the formula $W = a\ TL^b$, where W is the body mass, TL is the body size, *a* and *b* are coefficients of the equation (Bagenal and Tesch, 1978). The *t*-test was used to see if the parameter *b* differed significantly from 3, indicating whether the growth was isometric ($b = 3$) or allometric ($b \neq 3$) (Zar, 1999).

RESULTS

A total of 288 female Prussian carp (61 from Altınkaya Dam Lake, 80 from Bafra Fish Lakes, 82 from Lake Ladik, and 65 from Lake Simenit) was used in this study. In the analysis of asteriscus dimensions against TL, no considerable differences were detected between the slopes of the relationships acquired for the right and left asteriscii in each sampling locality (ANCOVA test, TL-OL, F=0.289, P=0.592; TL-OH, F=0.717, P=0.399; TL-OW, F=0.133, P=0.716 for Altınkaya Dam Lake; TL-OL, F=0.302, P=0.584; TL-OH, F=0.006, P=0.937; TL-OW, F=0.004, P=0.948 for Bafra Fish Lakes; TL-OL, F=0.076, P=0.783; TL-OH, F=0.101, P=0.751; TL-OW, F=0.030, P=0.863 for Lake Ladik; TL-OL, F=0.270, P=0.604; TL-OH, F=0.358, P=0.551; TL-OW, F=0.004, P=0.952 for Lake Simenit). Therefore, the regressions generated from the right otoliths were chosen for the next analyses. The details of the dataset are summarized in Table 1.

Table 1. Descriptive statistics of Prussian carp specimens and their otoliths collected from four lentic habitats in Samsun Province, Türkiye (n, sample size; SD, standard deviation)

Locality	Measurement	*n*	Mean±SD	Range
	Fish Length (cm)	61	33.04±5.06	22.4–40.3
	Fish Weight (g)	61	703.66±288.95	193.02–1191.30
Altınkaya Dam Lake	Otolith Length (mm)	61	5.80±0.71	4.34–7.05
	Otolith Height (mm)	61	5.25±0.68	3.69–6.36
	Otolith Weight (mg)	61	36.04±12.26	12.98–58.08
	Fish Length (cm)	80	20.28±4.11	11.7–30.7
	Fish Weight (g)	80	179.64±105.83	16.69–520.51
Bafra Fish Lakes	Otolith Length (mm)	80	4.20±0.64	2.56–5.46
	Otolith Height (mm)	80	3.83±0.60	2.35–5.11
	Otolith Weight (mg)	80	14.36±5.87	3.61–14.36
	Fish Length (cm)	82	26.09±2.58	19.1–35.1
	Fish Weight (g)	82	379.56±131.46	136.77–859.77
Lake Ladik	Otolith Length (mm)	82	4.66±0.32	3.78–5.89
	Otolith Height (mm)	82	3.92±0.27	3.28–5.17
	Otolith Weight (mg)	82	16.11±3.20	9.82–33.24
	Fish Length (cm)	65	18.76±2.63	14.8–27.4
	Fish Weight (g)	65	131.42±58.52	67.01–342.24
Lake Simenit	Otolith Length (mm)	65	4.15±0.42	3.46–5.22
	Otolith Height (mm)	65	3.69±0.39	3.04–4.62
	Otolith Weight (mg)	65	13.74±3.68	7.62–22.84

All relationships were highly significant (P<0.001). The r^2 values varied from 0.61 to 0.95 and the regressions explained more than 70% of the variance in most cases (Table 2). The r^2 values of the regressions generated for the Altınkaya Dam Lake and Bafra Fish Lakes were higher than those of the other lakes. The otolith height, which accounted for 95% of the variability in Altnkaya Dam Lake, and the otolith weight, which accounted for 94% in Bafra Fish Lakes, were the measurements most strongly related to fish size. The otolith length was the best indicator of fish length for Lake Ladik and Lake Simenit, with 75% and 73% of the variability, respectively. The results of the ANCOVA test indicated significant differences among slopes of four sampling sites for TL-OL (F=5.017, P=0.002), TL-OH (F=8.124, P=0.000), and TL-OW (F=10.721, P=0.000) relationships. The slopes of three regressions were higher for the Altınkaya Dam Lake and Bafra Fish Lakes. Negative allometric growth was observed for two relationships in Altınkaya Dam Lake and Bafra Balık Lakes (*t*-test, TL-OL and TL-OH, P<0.001), and for three in Lake Ladik and Lake Simenit (*t*-test, TL-OL, TL-OH and TL-OW, P<0.001). However, the TL-OW relationship was positive allometric for the Altınkaya Dam Lake (*t*-test, P<0.005), and isometric for the Bafra Fish Lakes (*t*-test, P>0.05).

The mean PE% values ranged from 3.10 to 7.45 (Table 2). The values of the mean PE% of three otolith variables for the Altınkaya Dam Lake were lower than those of the other localities. No significant difference was determined between observed and predicted TL values in the Altınkaya Dam Lake (Mann-Whitney U test; OL, P=0.884; OH, P=0.965; OW, P=0.737), Bafra Fish Lakes (*t*-test; OL, P=0.961; OH, P=0.927; OW, P=0.886), Lake Ladik (Mann-Whitney U test; OL, P=0.914; OH, P=1.000; OW, P=0.908), and Lake Simenit (*t*-test; OL, P=0.949; OW, P=0.907; Mann-Whitney U test; OH, P=0.883). There were no significant differences in the mean PE% values of the otolith variables in all sampling sites (ANOVA test; F=1.05, P=0.352 for Altınkaya Dam Lake, Kruskal-Wallis test; H=0.629, P=0.730 for Bafra Fish Lake, and H=1.264, P=0.532 for Lake Simenit) except Lake Ladik (Kruskal-Wallis test; H=14.96, P=0.001). The Kruskal-Wallis test demonstrated that mean PE% values were different among sampling areas for OH (P=0.000) and OW (P=0.004), but not for OL (P=0.063).

Table 2. Estimated parameters of the relationships between otolith measurements and body length, and the mean percent prediction error value of each otolith variable of the Prussian carp collected from four sampling sites in Samsun Province, Türkiye (r^2, coefficient of determination; PE, prediction error)

			Observed TL	Predicted TL	PE%
Locality	**Relationship**	r^2	**(Mean±SD)**	**(Mean±SD)**	**(Mean±SD)**
Altınkaya Dam Lake	$OL=0.404TL^{0.762}$	0.93	34.04±5.06	33.15±5.30	3.72±2.53
	$OH=0.312TL^{0.808}$	0.95		33.03±5.32	3.10±2.28
	$OW=0.015TL^{2.210}$	0.94		33.35±5.39	3.35±2.27
Bafra Fish Lakes	$OL=0.447TL^{0.745}$	0.93	20.28±4.11	20.31±4.14	4.39±3.78
	$OH=0.402TL^{0.750}$	0.92		20.34±4.25	4.63±3.70
	$OW=0.033TL^{2.008}$	0.94		20.18±4.17	4.35±2.95
Lake Ladik	$OL=0.667TL^{0.597}$	0.75	26.09±2.58	26.05±3.00	4.48±3.35
	$OH=0.671TL^{0.542}$	0.61		26.12±3.46	6.74±4.11
	$OW=0.083TL^{1.611}$	0.74		26.21±3.10	4.81±3.05
Simenit Lake	$OL=0.657TL^{0.630}$	0.73	18.76±2.63	18.78±3.05	6.31±5.21
	$OH=0.591TL^{0.625}$	0.68		18.85±3.18	7.33±5.57
	$OW=0.151TL^{0.533}$	0.64		18.81±3.24	7.45±7.24

The length-weight relationship for female individuals of the Prussian carp was obtained as $W=0.012\ TL^{3.125}$ in the Altınkaya Dam Lake (*n*=61, SE (*b*)=0.048, r^2=0.97), $W=0.021\ TL^{2.975}$ in the Bafra Fish Lakes (*n*=80, SE (*b*)=0.065, r^2=0.96), $W=0.011\ TL^{3.200}$ in Lake Ladik (*n*=82, SE (*b*)=0.083, r^2=0.95), and $W=0.027\ TL^{2.876}$ in Lake Simenit (*n*=65, SE (*b*)=0.065, r^2=0.97). Positive allometric growth was detected in the Altınkaya Dam Lake (*t*-test, P=0.012) and Lake Ladik (*t*-test, P=0.018), whereas isometric growth was observed in the Bafra Fish Lakes (*t*-test, P=0.690) and Lake Simenit (*t*-test, P=0.061).

DISCUSSION

The results of our study indicated no statistical difference between the biometric relationships generated separately for the right and left otoliths. Therefore, the size of the Prussian carp can be back calculated using only the right or left otolith measurements. The literature reports that remarkable differences between right and left asteriscus parameters are mostly not found for many members of the family Cyprinidae (Saygın et al., 2017; Zengin Özpiçak et al., 2018; Emre, 2019; Saygın et al., 2020).

In contrast, Kontas and Bostanci (2015) detected the asymmetry between right and left asteriscus measurements of *Barbus tauricus* captured from the lower basin of the Melet River, Türkiye. Also, Jawad and Mahé (2022) indicated that there was a significant effect of the head side on the relationship of each variable of the lagenar otolith with the body length of common carp (*Cyprinus carpio*) sampled from three Iraqi rivers.

In this study, we detected the spatial differences in relationships between asteriscus descriptors and body length of *C. gibelio*. Similar observations have also been reported for many fish species (Tombari et al., 2011; Bostancı et al., 2017; Jawad et al., 2017; Saygın et al., 2017; Nguyen and Dinh, 2020; Dinh et al., 2021). These variations may mainly be due to differences in the abiotic factors (e.g., temperature, pH, salinity, diet, etc.) of the sampling sites. According to Campana (1990), fish-otolith regressions often differ among populations or fish species with varying development rates.

The present study suggested that the allometric relationships between asteriscus variables and fish growth are reliable for the original somatic size estimates of the Prussian carp due to their high r^2 and low mean percent prediction error values. However, the best indicator of fish size differed among populations studied. The OL was the best predictor of fish length for Lake Ladik and Lake Simenit populations, whereas the OH and OW were the best predictors of fish somatic growth for Altınkaya Dam Lake and Bafra Fish Lakes populations, respectively. Similar results have also been obtained in previous works on otolith biometry of some other cyprinid fishes. Saygın et al. (2017) reported that the best indicator for estimating the body size of *Alburnus tarichi* is otolith length in Lake Erçek and Lake Aygır, and otolith height in Lake Van and Lake Nazik. Zengin Özpiçak et al. (2018) found that asteriscus otolith weight for Terme Stream and Yedikır Dam Lake populations, and asteriscus otolith length for the populations of Abdal and Akçay streams of *Squalius cephalus* are better indicators of the fish size than other measurements of otoliths. Similarly, when the relationships between lagenar otolith dimensions and body size of *Barbus tauricus* caught from five streams in the Black Sea region of Türkiye were analyzed, the best fit was found between total length and otolith height in Akçay, Engiz, Karadere, and Değirmenağzı streams, and between total length and otolith height in Terme Stream (Ozpicak, 2020). In previous works regarding the species studied, the otolith weight in Topçam Dam Lake (Bostancı, 2009) and the otolith height in Lake Ladik (Yılmaz et al., 2015) were shown to be the variables most closely associated with fish size.

From the otolith measurements-fish size regressions established in this study, the body length of the Prussian carp can be reconstructed but not its somatic weight. For this reason, the length-weight relationship is also given for each population of the fish in question. The relationship between size and body weight of Prussian carp was previously studied in Bafra Fish Lakes (Bostancı et al., 2007) and Lake Ladik (Yazıcıoğlu et al., 2013). This research provides the first information on the length-weight relationship of Altınkaya Dam Lake and Lake Simenit populations.

In conclusion, our study confirmed that the lagenar otolith growth of the Prussian carp reflected its somatic development, but that differed among populations. It is advised that all regressions from the current study be used within the fish size and weight limits presented in Table 1. The outcomes of this study can be used to provide the information about size and mass of this prey fish in future studies on the trophic ecology of the potential predators of Prussian carp.

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AUTHORSHIP CONTRIBUTIONS

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

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Prediction models of dye adsorption by *Treptacantha barbata*

Treptacantha barbata'nın boya adsorpsiyonunun tahmin modellemeleri

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Abstract: This study's objective was to develop a model to determine dye adsorption efficiency of *Treptacantha barbata* (Stackhouse) Orellana& Sansón, 2019 (formerly *Cystoseira barbata* (Stackhouse) C. Agardh, 1820). During the experiments, treatment groups, such as initial dye Methylene Blue (MB) concentration (0.1-10.0 mg L^{-1}), contact time (5 to 1440 min) and adsorbent dosage (0.1-2 g) were applied. Scanning electron microscopy, energy dispersive X-ray, and Fourier Transform Infrared Spectroscopy were used to analyze the adsorbent. *T. barbata* was found to be quite successful in removing dye (69% -100%) for all experiments, and the *qe* values increased with the increased the initial dye concentration. Very rapid dye removal was detected during the first contact time, especially up to 15 min. Isotherms, kinetics, and regression models were applied to the batch experimental results. The results displayed that adsorption process was suitable with the Langmuir isotherm model (R^2: 0.97).

Keywords: Adsorption, isotherm models, regression, *Treptacantha barbata*

Öz: Bu çalışmanın amacı, *Treptacantha barbata* (Stackhouse) Orellana& Sansón, 2019 (önceki ismi ile *Cystoseira barbata* (Stackhouse) C. Agardh, 1820) boya adsorpsiyon etkinliğini araştırmak ve modellemektir. Deneyler, başlangıç Metilen Mavisi boya konsantrasyonu (0,1-10,0 mg L^{-1}), temas süresi (5- 1440 dakika) ve adsorban dozu (0,1-2 g) gibi parametrelere göre tasarlanmıştır. Adsorban, taramalı elektron mikroskobu-enerji dağılımlı X-ışını ve Fourier Dönüşümü Kızılötesi Spektroskopisi ile karakterize edilmiştir. *T. barbata* tüm deney gruplarında boya gideriminde (%69-100) oldukça başarılı bulunmuş ve *qe* değerleri başlangıç boya konsantrasyonundaki artışa paralel olarak artmıştır. İlk temas süresinde, özellikle 15 dakikaya kadar boyanın çok hızlı uzaklaştırıldığı tespit edilmiştir. Kesikli deneysel verilere izoterm, kinetik ve regresyon modelleri uygulanmıştır. Sonuçlar, adsorpsiyon işleminin Langmuir izoterm modeliyle (R^2: 0.97) iyi bir şekilde uyduğunu ortaya koymuştur.

Anahtar kelimeler: Adsorpsiyon, izoterm modelleri, regresyon, *Treptacantha barbata*

INTRODUCTION

Recently, the importance of sustainable and renewable systems has been increasing order to overcome the increasing environmental problems. Especially, the removal of contaminants from the environment has gained great importance, and different treatment methods such as ion exchange, membrane separation, electrochemical treatment, ozonation, chemical reduction, and microbial treatment have been developed for their removal (Jerold and Sivasubramanian 2016; Amin et al., 2020; Hamouda et al., 2020; Renita et al., 2021; Soto-Ramirez et al., 2021).

Owing to its affordable price and high effectiveness, the adsorption technique is more popular than other methods. (Marzbali et al., 2017; Jafari et al., 2020), versatility and reversibility (Hannachi and Hafidh, 2020), non-toxic and environmentally friendly properties (Radoor et al., 2020; Joshiba et al., 2021). The adsorption based on the process of pulling based liquid mixture in the environment to the solid surface by chemical or physical bonds (Al-Ghouti and Da'ana, 2020). The unbalanced forces of the atoms on adsorbent attract adsorbent in solution to the solid surface and balance the surface forces. In this way, the adsorption of the contaminant in the solution onto the solid surface occurs. In the adsorption process, biological agents such as algae and bacteria are used (living or dead forms) (Giannakoudakis et al., 2018; Majhi and Patra, 2020; Venkataraghavan et al., 2020), as well as biopolymers like alginate (Üçüncü Tunca et al., 2017; Vu et al., 2017; Xia et al., 2018) are developed as adsorbents. Research continues to find new, inexpensive, and efficient adsorbents for removing dye and metal ions from wastewater.

Marine macroalgae, also called seaweeds, are the primary producers in marine ecosystems. The ability of algae to absorb pollutants from the environment has enabled them to be accepted as pioneers (Priyadarshini et al., 2019) and they are

thought to exhibit highly dominant adsorption behavior (Jerold and Sivasubramanian 2016; El-Naggar et al., 2021). In addition to their efficient adsorption capabilities, they are preferred due to their non-toxic properties, widely distributed in nature (El Nemr et al., 2021a), eco-friendly and inexpensive production costs (El-Naggar et al., 2021). Macroalgae have important features like reuse of rich functional groups in the adsorption process. Hannachi and Hafidh (2020) revealed that the cell wall of brown macroalgae is generally consisting of sulfated fucoidans (polysaccharides), cellulose, alginates and researchers stated that this situation is important in binding the pollutants.

Macroalgae are considered suitable organisms for organic bio-sorption because they contain different anionic active regions that is hydroxyl, carboxyl, amine, and phosphate in structure of their cell walls (Daneshvar et al., 2017; El-Naggar et al., 2021). The sorption capacity of each macroalgae varies because these active regions differ depending on the macroalgae species. Simultaneously, the type of pollutant that macroalgae is exposed to can affect the success of removal. Researchers have looked at the ability of macroalgae to remove a variety of pollutants, containing heavy metals and dyes with various characteristics. (Essekri et al., 2020; Mahajan and Kaushal, 2020; Mahini et al., 2018; Omar et al., 2018; Filote et al., 2019; Lebron et al., 2021). These synthetic dyes, used in many sectors like cosmetics, food, paper, and textiles (Lyra et al., 2009; Mohammed and Mabrouk, 2020), pollute the aquatic environment and can form dangerous derivatives after being metabolized (Bouzikri et al., 2020). Furthermore, it is stated that the accumulation of these dyes in aquatic systems may reduce the amount of dissolved oxygen due to the reduction of light transmittance and this may cause the extinction of living things (Lebron et al., 2021). Methylene blue (MB), which was utilized as a contaminant in this work, is a cationic synthetic dye having toxic effects such as teratogenicity, mutagenicity, neurotoxicity, and nucleic acid damage (Ghosh et al., 2021). It is used for treating methemoglobinemia (Cefalu et al., 2020; Radoor et al., 2020), treating fish diseases in aquaculture (Xu et al., 2012; Lv et al., 2018; Xu et al., 2019) and as a colorant in the textile industry (Deng et al., 2011; Ait Ahsaine et al., 2018; Hasan et al., 2020).

In this research, the removal efficiency of *T. barbata* was evaluated for cationic MB dye, and the adsorption process was modeled using regression analysis. The impact of some variables such as, initial dye level (mg L^{-1}), contact time (min), and bio-sorbent mass (g) was investigated. The various kinetic models and equilibrium isotherm models were investigated to explain the mechanism of removal of MB. Fourier Transform Infrared Spectroscopy (FT-IR analysis) was used to determine the interaction between the functional groups of the macroalgae surface and dye molecules. Scanning electron microscopy–energy dispersive X-ray (SEM-EDX) was also used to investigate the morphology and surface characteristics.

MATERIAL AND METHODS

Chemicals and adsorbent preparation

Methylene Blue (MB) (molecular formula: $C_{16}H_{18}ClN_3S$ and molecular weight: 319.85 g mol^{-1}) was obtained from Pancreac®. A stock solution (1000 mg L^{-1}) of MB was prepared by dilution.

Treptacantha barbata (brown marine algae) biomasses were collected from the coast of the Black Sea (Ordu, Türkiye) (41° 12'61'' N; 37° 69' 28''E). The samples brought to the laboratory where washed to remove impurities. Macroalgae samples were first sun-dried for 96 hours and then in the oven at 60°C for 24 hours (Vijayaraghavan et al., 2016). Dry biomass was ground and sieved through a 941 µm mesh sieve.

Characterization of adsorbents

The FT-IR spectrometer (Bruker Tensor 27) was utilized to determine the functional groups on the *T. barbata* surface. The morphology and adsorbent surface composition was investigated using a scanning electron microscope (Hitachi SU 1510).

Sorption experiments

A series of batch tests with three replicates were performed to search *T. barbata 's* ability to adsorb MB. (T: 25 ± 2, pH:9.2-9.5). The adsorption process was studied with several initial dye concentrations (0.5, 1.0, 2.0, 5.0, and 10 mg L^{-1}), contact time (5 to 1440 min), and adsorbent dosage (0.1 to 2.0 g) to find the effect of sorption conditions on MB removal.

The desired dye concentrations were obtained from the stock solution and 100 mL of solution was added into 250 mL conical flasks. First, a calibration curve was formed and the test concentrations were determined accordingly (R^2 value: 0.9991).

The test flasks were located in a shaking incubator (WiseCube, Precise Shaking Incubator, BenchTop Type (WIS-20R) at 80 rpm during the experiment. According to the determined contact times, 2 mL samples from the supernatant part of the test groups were taken into centrifuge tubes. The suspended materials were centrifuged for 5 min at 1000 rpm and the number of MB concentrations were analyzed at 664 nm respectively utilizing a UV-Vis Spectrophotometer (Shimadzu-UVmini-1240).

Using the following equations, MB removal effectiveness and adsorption capacity were estimated. (Naushad et al., 2015; Zhang et al., 2016; Broujeni et al., 2021; Renita et al., 2021).

$$q_e = \frac{(C_0 - C_e)}{m} \times V$$

$$R\ \% = \frac{C_0 - C_e}{C_0} \times 100$$

Where q_e (mg g^{-1}) demonstrates the equilibrium adsorption capacity, R % is the removal efficiency of contaminant, C_0 and C_e (mg L^{-1}) are first and the equilibrium concentrations of MB, V (L) is the volume of test concentration, and m (g) is the dry weight of the adsorbent.

Adsorption isotherms

The adsorption isotherm models, such as Langmuir and Freundlich models were used to find the interactive mechanisms in the adsorption process. The adsorption isotherm equation of the Langmuir was applied in following equation: (Langmuir, 1916; Zhang et al., 2016; El Nemr et al., 2021b).

$$\frac{C_e}{q_e} = \frac{1}{q_{maxb}} + \frac{1}{q_{max}}$$

Where q_e (mg g^{-1}) represents dye level at the equilibrium on the adsorbent, C_e (mg L^{-1}) is the equilibrium concentration of dye in solution, b is the Langmuir constant, and q_{max} is the monolayer adsorption capacity (mg g^{-1}).

The Freundlich model applies to heterogeneous surfaces and uses the equation below: (Abdelhameed et al., 2020).

$$\ln q_e = \ln Kf + \frac{1}{n} \ln C_e$$

where K_f (L mg^{-1}) and *n* are constants that define the adsorption capacity and intensity, respectively. q_e and C_e are previously defined.

Statistical analysis

Regression analysis was applied to the prediction models of the adsorption data. The most suitable regression models for the data were selected and presented, considering the R^2 values. All statistical calculations were made using SPSS 21.0 software (IBM, Portsmouth, UK).

RESULTS AND DISCUSSION

Several physicochemical processes such as initial concentration, pH, time, etc. have an impact on the adsorption process (Broujeni et al., 2021). In this work, in which the adsorption efficiencies of MB concentrations by *T. barbata* were examined; the effects of contact time, initial dye concentration, and adsorbent concentration were studied. In the experiments, it was found that there was no dye precipitation in the test groups, and *T. barbata* was found to be quite successful in removing MB at tested concentrations. Adsorption isotherms were formed and dye removal estimation models were performed using regression analysis. Control groups were also studied under the same conditions as all test groups to determine the amount of precipitation. It was determined that the control group containing only adsorbent did not statistically affect the results of the experimental groups.

Characterization of adsorbent

The surface structural features of *T. barbata* were examined before and after the adsorption of MB using SEM. Samples were coated with gold. The surface images of the prepared samples were analyzed at different magnifications (x650, x1000, x4000). The control group and the samples exposed to dye molecules showed different surface properties. As seen in Figure 1a, the surface of raw macroalgae has a bumpy structure with prominent pits. However, the depths of the pits on the macroalgae surfaces were considerably decreased after the adsorption process (Figure 1b) suggesting that the biomass surfaces appear to be covered by methylene blue dye. These results were similar to those observed by Koyuncu and Kul (2020), who reported that non-living lichen (*Pseudevernia furfuracea* (L.) Zopf.) surfaces were covered with the adsorption of MB dye.

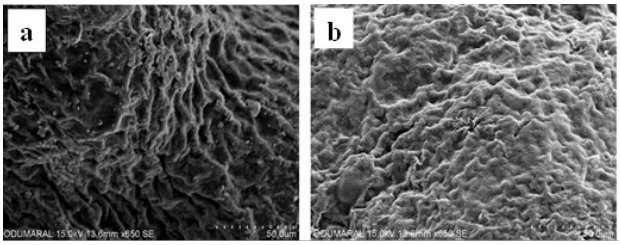

Figure 1. SEM images of *T. barbata* (a) before adsorption of MB, (b) after adsorption of MB

EDX analysis was used to investigate the distribution of various elements in the biomass. There are active negative functional groups on algae sorbents and they are suitable for the adsorption of molecules with cationic properties (Abdelhameed et al., 2020). EDX results indicated various elements containing carbon (C), oxygen (O), potassium (K^+), magnesium (Mg^{+2}), sulfur (S) and chlorine (Cl^-). S, Cl^-, Na, and P were eliminated after dye interactions between macroalgae and MB dye. These elements tend to create negative charges, and this decrease could be described by the interaction of the cationic dye with macroalgae (Figure 2). The FT-IR spectra of raw and MB biosorbed *T. barbata* were investigated to gain a better understanding of the intensity and kind of functional groups on the biomass surface. The FT-IR spectra of the raw *T. barbata* biomass with and without dye-loaded are demonstrated in Figure 3. Figure 3 shows the infrared spectrum of pure algae, which shows a variety of absorption peaks, suggesting its complexity. Various bands were detected at 3281 cm^{-1} (–OH or $–NH_2$), 2922 cm^{-1} (–CH or COOH), 1613 cm^{-1} (>C=O), 1414 cm^{-1} (aromatic ring), 1247 cm^{-1} (C–O) and 1024 cm^{-1} (C–O or >S=O) (El Jamal and Ncibi, 2012). Identical spectra were reported in the literature from other algae such as *C. barbatula*, and *C. baccata* (Caparkaya and Cavas 2008; Lodeiro et al., 2006). When the biomass was loaded with the dye ion, there were some changes in the distance between these peaks, leading to the conclusion that carboxyl groups were primarily involved in dye absorption.

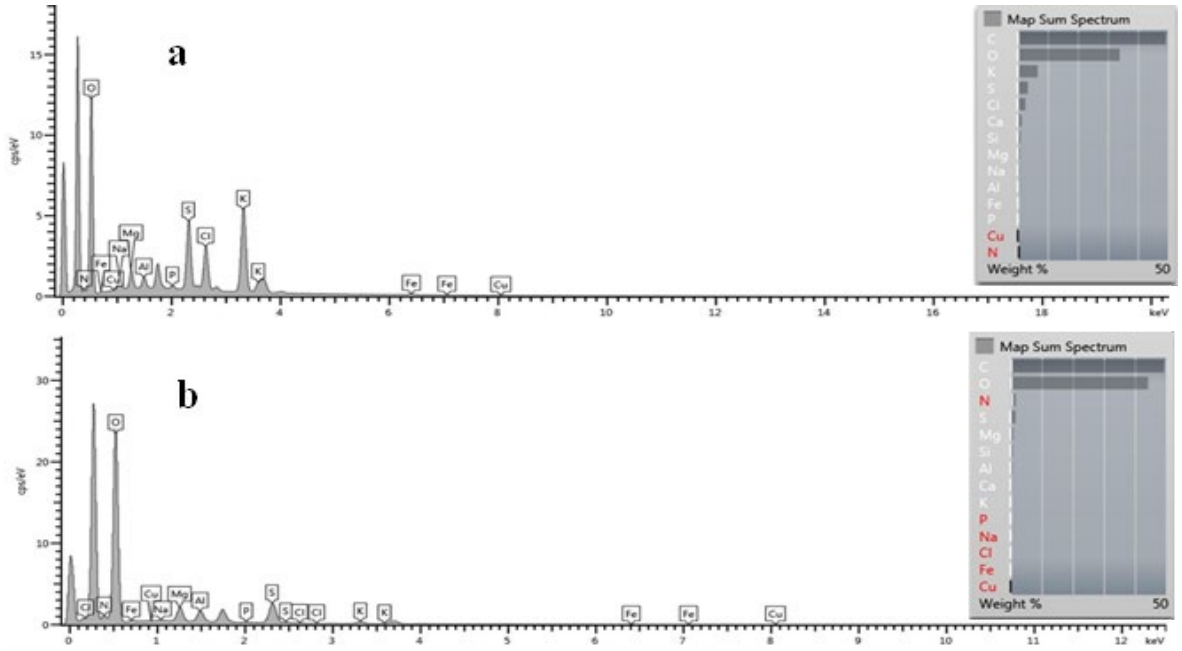

Figure 2. EDX spectrum of algal biomass (a) and MB+algal biomass (b)

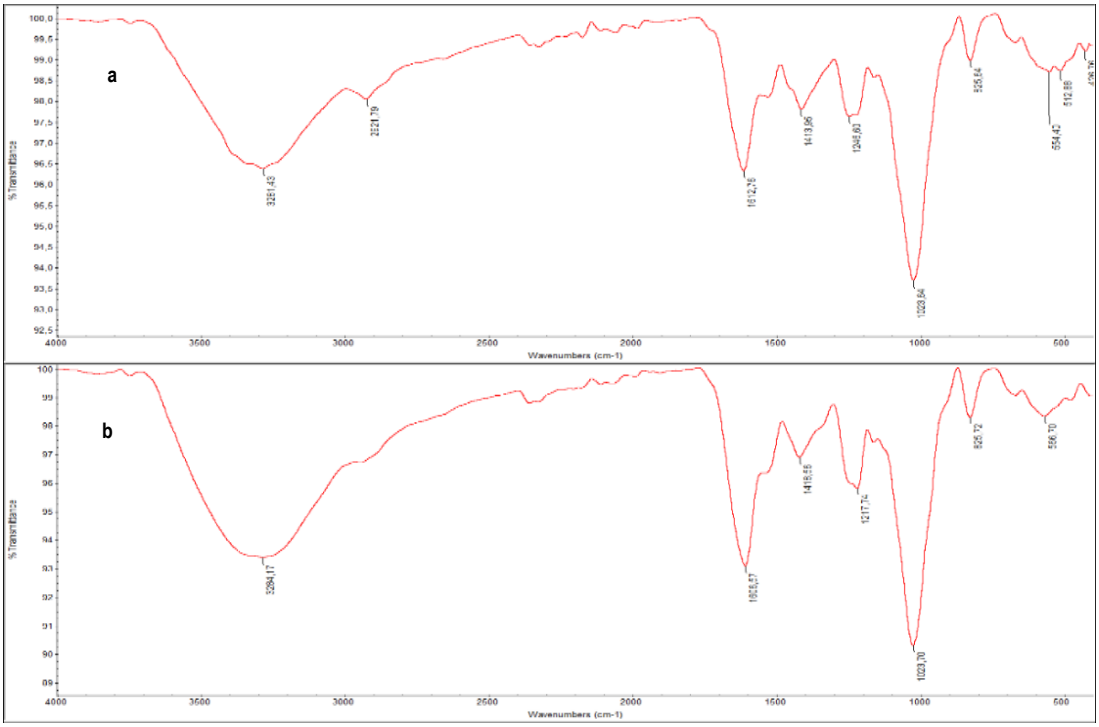

Figure 3. FT-IR spectra of algal biomass (a) and MB+algal biomass (b)

Prediction models

Regression analysis was done to compare the modeled adsorption in the medium and to observe any potential correlations or differences depending on the time. Time-dependent, adsorbent dosage-dependent and initial concentration-dependent estimation models were examined, and all regression models (linear, cubic, logarithmic, etc.) were applied. The most suitable models were selected on the basis of R^2 values ($R^2 > 0.8$).

Prediction models of initial concentration effect on adsorption

The adsorption efficiencies of *T. barbata* were investigated using five initial concentrations of MB dye (0.5, 1.0, 2.0, 5.0, and 10.0 mg L^{-1}) at room temperature (25 ± 2 °C), pH 9.2–9.5 (Figure 4). The effects of 9 different contact times (5, 15, 30, 45, 60, 120, 240, 480, and 1440 min) were also studied on MB adsorption by macroalgae for each concentration applied.

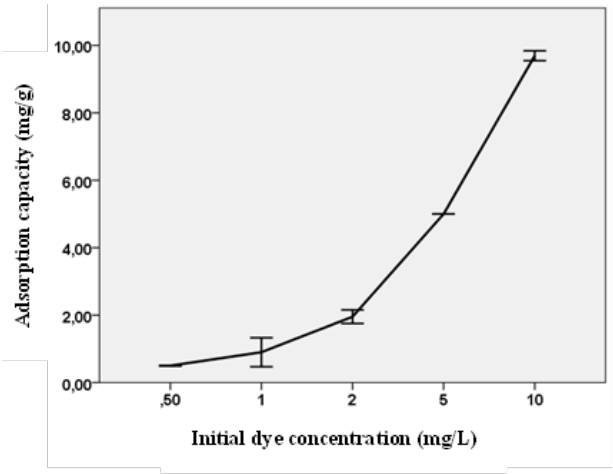

Figure 4. Effect of initial MB concentration on adsorption capacity (24 h)

The maximum dye adsorption capacity of macroalgae was found as 9.70 mg g^{-1} for the applied concentration. The *qe* values increased in parallel with the increment in the primary dye availability, as in many studies in the literature (Mahajan and Kaushal, 2020; El Nemr et al., 2021b). This indicates presence of an interaction between the applied cationic dye and macroalgae (Bouzikri et al., 2020). Increasing the amount of adsorbents in the medium also increases the possibility of adsorbents and sorbent binding or collision (Sun et al., 2019; Radwan et al., 2020). However, the increase in the concentration decreases the mass transfer resistance of the adsorptive, and therefore adsorptive uptake increases (Melo et al., 2018; Radwan et al., 2020).

Some studies have shown that metal adsorption rates increase owing to the increase in the initial level (Sun et al., 2019; Hamouda et al., 2020; Ghosh et al., 2021). It has also been determined that the removal efficiency of different dyes or metal ions, increases with increasing concentration, but this increase was determined to be in small quantities (Hamouda et al., 2020). In this study, it was seen that with the increase in the initial dye level, removal percentages gradually decreased, similar to some studies in Figure 5. (Renita et al., 2021; El Nemr et al., 2021b). This decrease tends to increase again after reaching a certain concentration. Furthermore, when the initial concentration-dependent removal efficiencies were investigated, it was determined that the highest adsorption rates were concentrated in the lower initial concentration groups (Figure 5).

Despite the high removal rates, a tendency to decrease in efficiency was observed in groups up to 2 mg L^{-1}. At low concentrations, the presence of more binding sites than the dye molecule in the solution may have increased the efficiency (Renita et al., 2021). The reason for this sharp decrease trend may be due to the fact that the dye molecules where the amount increases in solution cannot find enough surface area to bind (Husien et al., 2019). These results supported each other with the regression results. Figure 6 shows the regression models of the change in removal efficiencies relative to the initial concentration (R^2: 0.869-0.998 except at 120 min). It was determined that other groups, except for 120 min, 480 min, and 1440 min, formed quite similar trends. The decrease-increase movements stand out in the efficiency of removal depending on the initial concentration. In groups with similar trends, it is anticipated that the initial declining trend will tend to increase after about 3 mg L^{-1} and then decrease after 8 mg L^{-1}. In 120 min model, the R^2 value was determined as 0.542 and it showed a different lighter decrease-increase profile than the other groups. The 120 min regression modeling was not used in the article due to its low R^2 value (0.542). It is thought that the adsorption will reach equilibrium after 60 min, and it may have become more stable after 120 min.

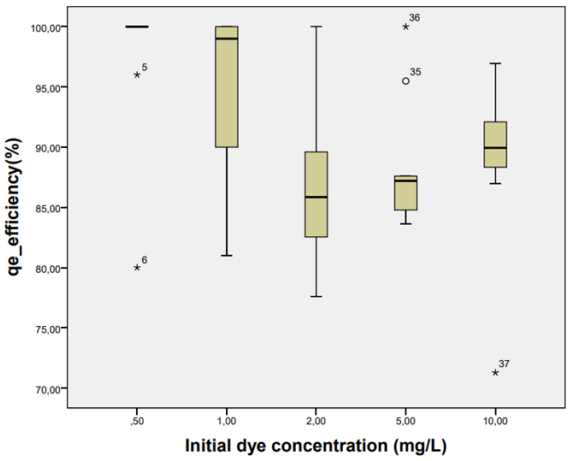

Figure 5. Effect of initial concentration on the MB adsorption efficiency by *T. barbata*

Prediction models of contact time on adsorption

Adsorption efficiencies of *T. barbata* according to the exposure time to the cationic dye MB were also investigated. The effect of contact time on the adsorption process was investigated at different time intervals (5 to 1440 min). Figure 7 illustrates the evolution of the adsorption of MB by *T. barbata* for different contact times. It shows that MB adsorption tends to increase with time, although it makes an insignificant number of increase-decrease movements, and it reaches equilibrium after 500 min. After equilibrium, it was determined that there was a very small amount or no adsorption. Studies have suggested that sorption efficiencies increase depending on the contact time until equilibrium (Nasoudari et al., 2020). Contact time is a factor significantly affecting adsorption rates (Hannachi and Hafidh, 2020). When evaluated according to the removal percentages, very rapid dye removal (71.28%-100%) was detected during the first contact time (especially up to 15 min, Figure 8).

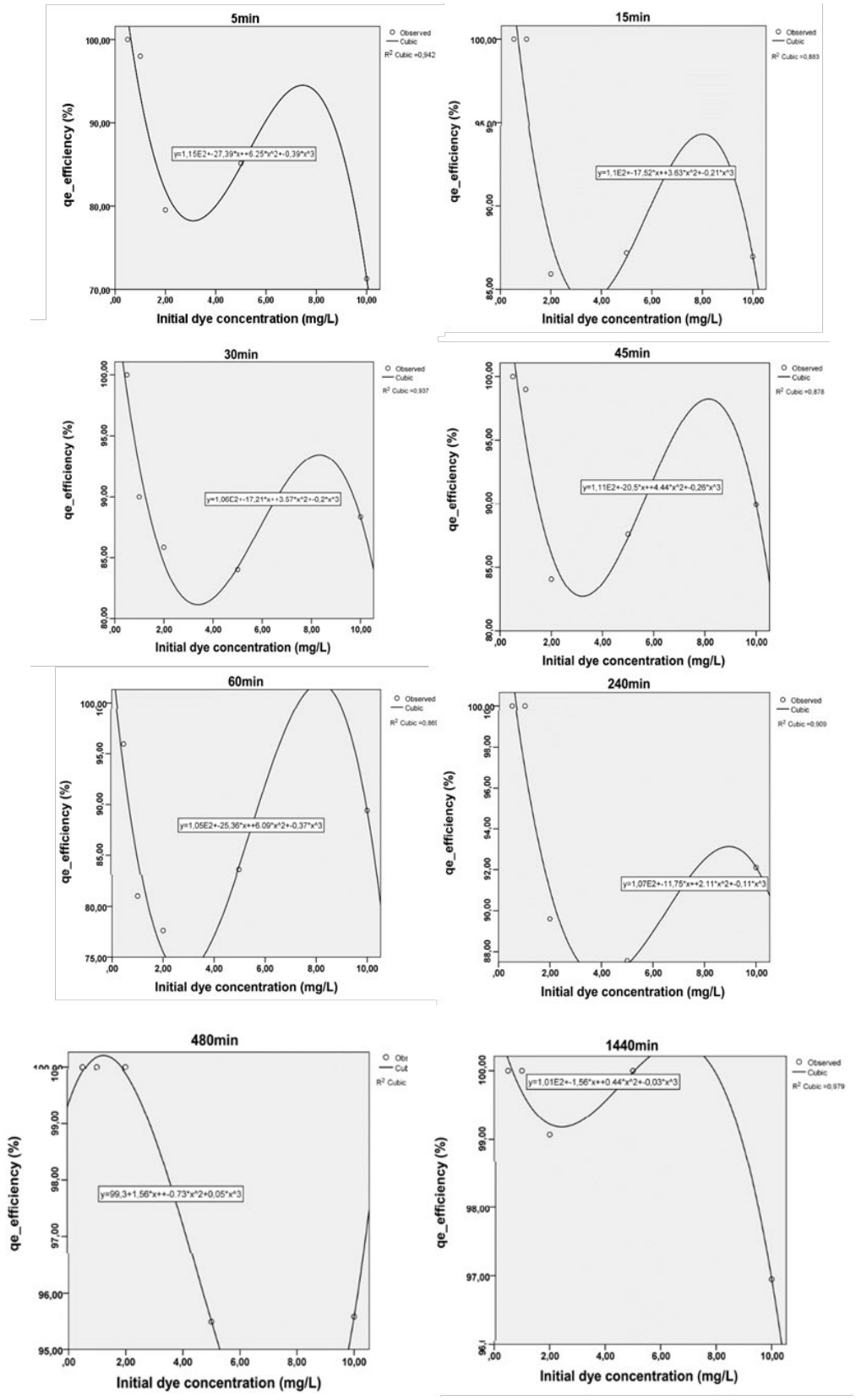

Figure 6. Prediction models of adsorption efficiency (initial dye concentration-dependent)

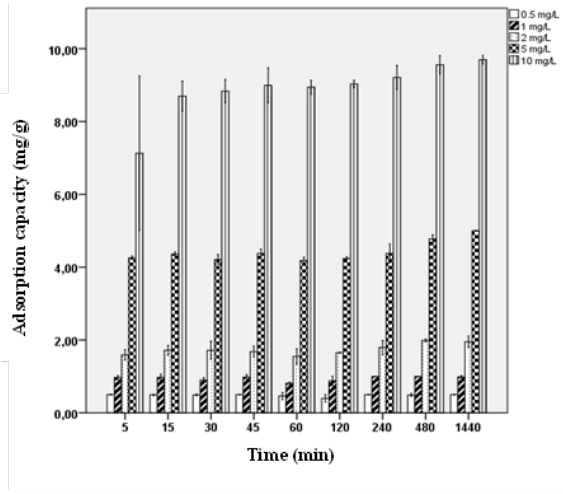

Figure 7. The effect of contact time on the MB adsorption capacity of *T. barbata*

As is known, there are some active binding sites on adsorbents (Abdelhameed et al., 2020). The reason for the rapid removal seen at the beginning of the experimental phase, may be due to the presence of these free active binding sites on the adsorbent (Ghosh et al., 2021; Mahajan and Kaushal 2020). It is thought that the reason why adsorption reached equilibrium may be the decrease in active binding sites on macroalgae over time (Sun et al., 2019) and the affinity of the adsorbant to the adsorbate may decrease. This saturation on the surface will decrease the driving force for removal (Mahajan and Kaushal 2020). Another reason may be the limitation of the mass transfer for the adsorbate molecules from the solution to the adsorbant surface (Broujeni et al., 2021). When all concentration groups were evaluated separately, the lowest adsorption percentages were 71.28% for 10 mg L^{-1} (5 min), 79.54% (5 min) and 77.61% (60 min) for 2 mg L^{-1}. Notably, the 1 mg L^{-1} groups showed a decrease-increase profile both positively and negatively over time and were more unstable than the other groups (Figure 8).

The lowest levels (0.5, 1.0 and 2.0 mg L^{-1}) showed similar time-dependent removal trend. In all groups, a decreasing trend was observed in the removal rates in 45 min. A smaller decrease-increase movement and a more stable profile were observed in the 5 and 10 mg L^{-1} groups. The time-dependent regression models were formed. However, due to the increase-decrease trend observed in all groups, a suitable model could not be created for our data. Although models with an R^2 value above 0.8 were formed for some groups, when the models were examined, it was seen that the resulting peaks did not explain our data properly.

Effect of adsorbent dosage

Macroalgae dosage was applied in amounts varying from 0.1 to 2.0 g and removal efficiencies were determined after 45 min and 24 h. The other parameters were kept constant (T: 25 ± 2, pH:11.0, dye concentration: 2 mg L^{-1}).

The weight of the biosorbent in the medium is directly related to availability of adsorption sites (Nasoudari et al., 2020) and affects the removal rates (Hannachi and Hafidh 2020). Some studies have determined an increase (El-Naggar et al., 2021; Radwan et al., 2020) or decrease (Tumin et al., 2008; Ghosh et al., 2021) in removal rates depending on the adsorbent dosage (Sun et al., 2019). The results may vary according to time, adsorbent, quantity, pH, T and the other parameters. In this study, both an increase and a decrease trend were observed (Figure 9). It is seen in the regression model that the removal rates increment with the increment in the amount of adsorbent, reaching their maximum value at about 1.5 g and then beginning to decrease slowly at 45 min. (Figure 9).

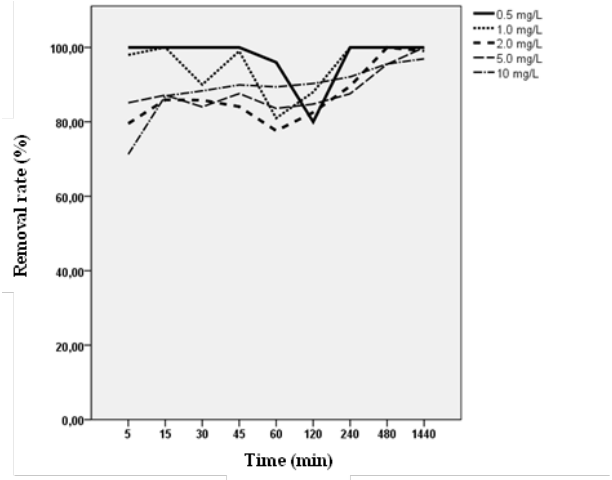

Figure 8. Time-dependent removal efficiencies of MB for all test groups

It is thought to be that the increase in the adsorbent in the medium also increases the adsorbent surface, thus providing more active binding sites for the dye (Sun et al., 2019; Melo et al., 2018; Radwan et al., 2020). The 24 h results showed an opposite trend of removal compared to the 45 min results. With the increase in the amount of the adsorbent, the removal efficiency tended to decrease, and it was observed that it reached equilibrium at a dose number of approximately 1.5 g. The possibility of aggregation will occur with increasing time in high adsorbent amounts (El-Naggar et al., 2021). In addition, it is thought that this situation will decrease the surface area and hence reduce the adsorption efficiency (Karthikeyan et al., 2007; Tural et al., 2017; Silva et al., 2019; Ghosh et al., 2021). This may explain the decrease in efficiency seen in the high number of adsorbents in our data. Parameters such as the surface area of the adsorbent, particle size, and temperature are among the factors affecting the adsorption efficiency. Under suitable conditions, the width of the surface area is directly proportional to the molecular retention capacity of the adsorbent surface (Weber, 1972; El-Naggar et al., 2021). In this context, with the addition of the adsorbent to the adsorption process in smaller pieces, the number of molecules to be held on the unit surface will increase. It is thought that the fact that macroalgae were used in small pieces in this study may have increased the efficiency of adsorption.

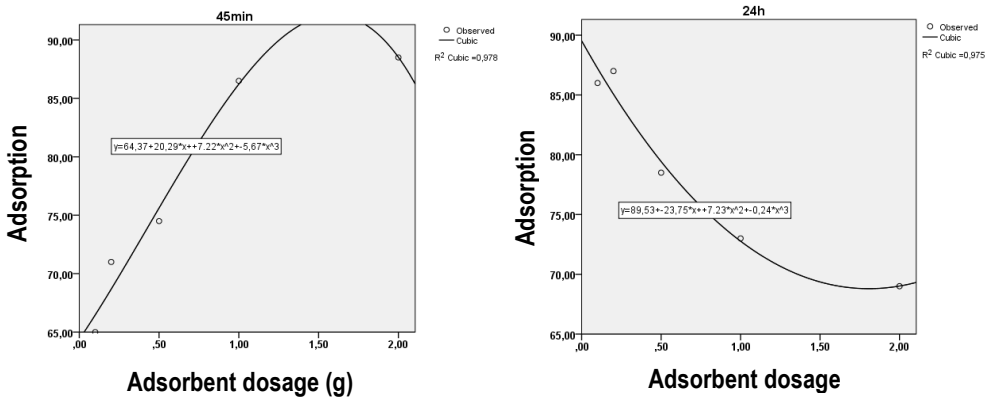

Figure 9. Prediction models (adsorbent dosage-dependent)

Adsorption isotherm study

The isotherm models of Langmuir and Freundlich were used to examine the removal process of MB dye by *T. barbata*. The corresponding parameters of the models and the fitted equilibrium data are shown in Table 1. Results indicated that the Langmuir model described adsorption more accurately than the Freundlich model, with $R^2 > 0.97$. The fitting outcome is in line with the previous classification, which found that the majority of adsorption systems with a H type and subclass 2 curves exhibit good convergence with the Langmuir model (Giles et al., 1960).

As indicated in Table 1, the maximum adsorption capacity (Q_m) of *T. barbata* for the adsorption of MB dye was 73.53 mg g^{-1}. The maximum adsorption levels of some adsorbents for the adsorption of MB dyes from hydrated solutions are shown in Table 2. The data shown in Table 2 indicates that the Q_m of MB dyes on *T. barbata* is comparable to or higher than some other adsorbents. The essential characteristics of the Langmuir isotherm model can be given regarding the separation factor, R_L, as in the equation given below: (Soltani et al., 2014)

$$R_L = \frac{1}{1 + C_o K_L}$$

where C_0 (mg L^{-1}) is the first metal ion density and K_L (L mg^{-1}) is the Langmuir constant. The values of R_L parameters are considered $R_L = 0,0 < R_L < 1$ and $R_L > 1$ which shows that adsorption is unchangeable, suitable, and unsuitable, respectively. The values of the R_L demonstrated in Table 1 indicate that R_L is from 0.580 to 0.933; therefore, the adsorption of MB dyes onto *T. barbata* would be suitable.

Table 1. Langmuir and Freundlich adsorption isotherm details for MB adsorption on *T. barbata*

Q_{exp} (mg g^{-1})	Isotherm models	Parameters	
92.41	Langmuir	R^2	0.97
		Q_{max} (mg g^{-1})	73.53
		K_L (L mg^{-1})	0.072
		R_L	0.580-0.933
	Freunlich	R^2	0.95
		K_F (L g^{-1})	6.92
		n	0.819

Table 2. Maximum adsorption capacity of some adsorbents for MB removal from aqueous solution

Adsorbent	Q_m (mg g^{-1})	Reference
Modified ball clay	100	Auta and Hameed, 2012
Iron−tannic acid (TA) complexes	67.41	Li et al., 2016
Halloysite nanotubes	40.82	Zhao and Liu, 2008
TA-modified Fe3O4 nanoparticles	90.90	Abkenar et al., 2015
Cellulose nanocrystals	118	Batmaz et al., 2014
Rice husk	8.07	Shih, 2012
Multi-walled carbon nanotubes	119	Shahryari et al., 2010
NaOH-treated waste activated sludge	53.7	Gobi et al., 2011
Natural Jordanian Tripoli	16.6	Alzaydien, 2009
T. barbata	73.53	This work

CONCLUSION

Removal efficiency of MB dye by *T. barbata* was evaluated according to dissimilar parameters (contact time, adsorbent dosage, and initial dye concentration). The process of dye adsorption was modeled using regression analysis and adsorption isotherms. The algal biomass was investigated by SEM-EDX and FT-IR before and after treatment. According to the results, very rapid dye removal (71.28%-100%) was detected during the first contact time (up to 15 min). The highest adsorption rates were determined in lower initial concentration groups because of the presence of more binding sites. The decrease-increase movements were observed in the removal efficiency depending on the initial concentration and the contact time until equilibrium was reached. Regression models showed that the removal rates increment with the increment in the amount of adsorbent, reaching the maximum value at about 1.5 g and then begin to decrease slowly. The Langmuir isotherm seemed to be the super fitted model for all the experimental data. These results indicated that the *T. barbata* surface had monolayer and homogenous properties. In conclusion, it was considered that *T. barbata* was a useful and efficient adsorbent for the removal process. It is thought that *T. barbata* may be preferred as an alternative sorbent to be used in treatment studies due to its advantages such as convenient adsorptive properties, being inexpensive, and being eco-friendly.

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AUTHORSHIP CONTRIBUTIONS

The experiments were designed by Esra Üçüncü Tunca. Laboratory studies were carried out by Pınar A. Şirin. Adsorption isotherm models were interpreted by Hasan Türe. The article was written by all researchers.

CONFLICT OF INTEREST

The authors no conflict of interest to declare.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Forecasting sea surface temperature with feed-forward artificial networks in combating the global climate change: The sample of Rize, Türkiye

Küresel iklim değişikliğiyle mücadelede deniz yüzeyi sıcaklığının ileri beslemeli yapay sinir ağları ile tahminlenmesi: Rize örneği, Türkiye

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Abstract: The increase of the world population, especially in the global competition, together with the increasing use of fossil fuel resources to meet energy needs, leads to more greenhouse gases (more than one CO_2, methane etc.) emissions and the global climate crisis. In this process, changes in meteorological events such as temperature, precipitation, and wind, attract attention moreover but when considered as a whole, we know that these negative changes in the ecosystem negatively affect many living groups. Sea Surface Temperature (SST) as measured meteorologically is the most important environmental parameter where these changes are monitored and observed. It draws attention to the fact that changes in SST are not limited to living organisms as habitats, but also catalyze many chain reactions, especially socio-economic impacts. Therefore, much of the work is devoted to forecasting studies to adapt to changing habitats and take the necessary precautions against potential risks. Feed-forward artificial neural networks have been commonly used to address these emerging needs. Artificial neural networks, which are a simple imitation of the human neurological system, have been used as an artificial intelligence method in forecasting problems due to their superior performance and not having the limitations of classical time series. In this study, the forecasting of the time series of monthly mean SST temperature obtained from Rize station between the years 2010 and 2020 is performed by using feed-forward artificial neural networks, and the forecasting performance of the corresponding time series is compared with many forecasting methods with different characteristics. The comparison of the methods used the mean square error and mean absolute percentage error criteria, which are commonly used in the forecasting literature. The analysis results showed that the analysis results obtained with the feed-forward artificial neural networks have the best prediction performance. As a result, it can be stated that the sea surface temperature can be forecasted with a very high accuracy using the feed-forward artificial neural networks.

Keywords: Artificial neural network, sea surface temperature, global climate change, Rize

Öz: Küresel rekabet başta olmak üzere dünya nüfusundaki artış beraberinde enerji gereksinimlerini karşılamak adına fosil yakıt kaynaklarının kullanımının artmasına bu durum da sera gazlarının daha fazla salınmasına (daha fazla CO_2, metan, vb.) ve küresel iklim değişikliğine neden olmaktadır. Bu süreçte başta sıcaklık, yağış ve rüzgâr gibi meteorolojik olaylarda yaşanan değişikler dikkat çekmekle birlikte, bir bütün olarak değerlendirildiğinde ekosistemde meydana gelen bu olumsuz değişimlerin pek çok canlı grubunu da olumsuz etkilediği bilinmektedir. Deniz suyu sıcaklığı bu değişikliğin izlendiği ve gözlemlendiği en önemli çevresel parametre olup, deniz suyunda meydana gelen değişimlerin sadece habitat olarak yaşam faaliyetlerini sürdüren canlılarla sınırlı kalmayıp, aynı zamanda sosyoekonomik etkileri başta olmak üzere zincirleme birçok reaksiyonu katalize etmesiyle de dikkat çekmektedir. Dolayısıyla değişen yaşam koşullarına adaptasyon sağlamada ve olası risklere karşı gereken önlemin alınması noktasında öngörü çalışmalarına büyük iş düşmektedir. Son zamanlarda meydana gelen bu gereksinimleri karşılamak adına ileri beslemeli yapay sinir ağlarından sıkça faydalanılmaktadır. İnsan nörolojik sisteminin basit bir taklidi olan yapay sinir ağları, klasik zaman serisi yöntemlerinin sahip olduğu kısıtlamalara sahip olmaması ve üstün performansı ile öngörü problemlerinde araştırmacılar tarafından sıklıkla kullanılan bir yapay zekâ yöntemi olarak kullanılmaktadır. Bu çalışmada Rize istasyonundan 2010-2020 yılları arasında elde edilen aylık ortalama deniz suyu sıcaklığına ait zaman serisinin tahmini ileri beslemeli yapay sinir ağları ile birlikte gerçekleştirilmiş olup ilgili zaman serisinin tahmin performansı farklı özelliklere sahip birçok öngörü yöntemi ile de karşılaştırılmıştır. Yöntemlerin karşılaştırılmasında öngörü literatüründe sıklıkla kullanılan hata kareler ortalaması karekök ve ortalama mutlak yüzdelik hata kriterleri kullanılmış olup elde edilen analiz sonuçlarına göre ileri beslemeli yapay sinir ağları ile elde edilen analiz sonuçlarının en uygun öngörü performansına sahip olduğu görülmüştür. Sonuç olarak; ileri beslemeli yapay sinir ağları yönteminden faydalanılarak deniz suyu yüzey sıcaklığının çok yüksek doğruluk oranı ile tahmin edilebileceği ifade edilmektedir.

Anahtar kelimeler: Yapay sinir ağları, deniz yüzey sıcaklığı, küresel iklim değişikliği, Rize

INTRODUCTION

The unique feature of water, which is the dominant compound of the chemical structure and is the basis of life on Earth; is its atomic structure, bonds, and bonding abilities in different phases of water molecules. One of the most important

environmental factors determining the behaviour of not only biological life, but also the water in the biosphere is temperature. Especially in aquatic ecosystems, water temperature is important as it causes direct and/or indirect changes in many physical and chemical variables of seawater (Akbari et al., 2017). It is known that sudden and large-scale changes in temperature in marine ecosystems, regardless of their origin, can cause stresses that may result in death in biological life. Likewise, in species of economic importance such as fish; It has been found to have a direct effect on forage finding and utilization, metabolism and vitality, changes in migration routes, and spawning (Houlihan et al., 2001). On the other hand, especially within the scope of combating the global climate crisis, the Intergovernmental Panel on Climate Change (IPCC) states that the definition of seawater temperatures is one of the most important environmental parameters in studies on climate change (Houghton, 1996).

Marine areas are affected by many factors such as water temperature, atmospheric oscillations, precipitation, density changes, and current systems. In this direction, water temperature is among the most important variables monitored in many studies so far, especially in the sea, in different habitats such as rivers (Daigle et al., 2017) and lakes (Sharma et al., 2015). The data for detailed analysis of trends in the seawater temperature; climate change-induced negative or opportunities for the evaluation of different fields and sectors is important for hydrology, meteorology, agriculture, livestock, tourism, etc. Moreover, in the studies where the changes and trends in seawater temperatures were evaluated in detail, deficiencies were found by Şişman (2019). This study, it is aimed to estimate the seawater temperature by using foresight methods. This provides a great advantage both in the creation of action plans that contribute significantly to national and international institutions/organizations in the long-term struggle, especially in the global climate crisis scenarios, and in the determination of possible changes in biological life. For this purpose, the time series of the monthly average seawater temperature obtained from the Rize station between 2010 and 2020 is analysed. In the analysis phase, first of all, the structure of the relevant time series is examined and, other analysis methods suitable for the data structure are determined. The analytical performance of these determined analysis methods and the feed-forward artificial neural network method is compared with some error criteria frequently used in the forecasting literature.

MATERIAL AND METHODS

Study Area

In this study, long-term seawater surface temperature data measured by the Rize meteorological observation station located on the Black Sea coast are used. All data have been obtained from the Republic of Türkiye Ministry of Environment, Urbanization and Climate Change, Turkish State Meteorological Service.

The surface area of Rize, which is located in the Eastern Black Sea Region, is 3920 km² and the length of the coastline is 80 km (Ay and Duman, 2015). There are many rivers in this region due to the slope and flat areas, and these rivers carry their waters to the Black Sea (Tecer and Cerit, 2009).

Methods

The methods used in this paper are Seasonal Naive, ETS (error, trend, seasonal), SARIMA (autoregressive integrated moving average), and feed-forward artificial neural network methods. In the analysis phase, some functions in the "forecast" package in R software prepared by Hyndman and Khandakar (2008) and Hyndman et al. (2021) are used.

The Seasonal Naive Method

The logic of the seasonal naive method is similar to the naive method, but with the seasonal naive method, the forecast is taken as the last observed value of the same season of the year. The seasonal naive method is used in the analysis of time series containing seasonality. A forecast for the seasonal naive method can be obtained by Equation (1).

$$\hat{x}_{t+h} = x_{t+h-s(k+1)} \qquad (1)$$

In Equation (1), s is the seasonal period, k is the full part of $(h-1)/s$, and h is the number of forecast steps.

ETS Method

ETS (Error, Trend, Seasonal) method is a univariate analysis method used in the analysis of time series containing trend and/or seasonal components. The first letter of the ETS method indicates the error type ("A", "M" or "Z"); the second letter is the trend type (“N”,” A”,”M” or “Z”); the third letter represents the seasonality type (“N”,” A”,”M” or “Z”). From these expressions, “N” =none, “A” =addition, “M” =multiplicative and “Z” =auto. The ETS method automatically selects the best method among different combinations of error, trend, and seasonality types.

SARIMA

The SARIMA method, known as the seasonal ARIMA method, is used in the analysis of time series containing seasonality. The automatic SARIMA method proposed by Hyndman and Khandakar (2008) is an automatic analysis method whose purpose is to find the best model of the SARIMA model.

Feed Forward Artificial Neural Networks

Feed-forward artificial neural network (FF-ANN) is one of the most commonly used artificial neural network models. FF-ANN is a feed-forward artificial neural network that produces a series of outputs from a series of inputs. In FF-ANN, information is transferred from the input layer to the output layer through hidden layers. Each unit in a layer is linked to all units in the previous layer. Each link can have different weights. Although obtaining the weights in the network structure is known as an optimization problem, this process is called the

training of the network. Rumelhart et al. (1986) used the backpropagation algorithm to update the weights. The data passes from layer to layer in the network until it reaches the output layer, and there is no feedback between the layers. For this reason, these artificial neural networks are called feed-forward neural networks.

The architecture of FF-ANN with two hidden layers with m inputs is given in Figure 1. In Figure 1, the first layer is called the input layer, and the last layer is called the output layer. The layers between these two layers are also hidden layers.

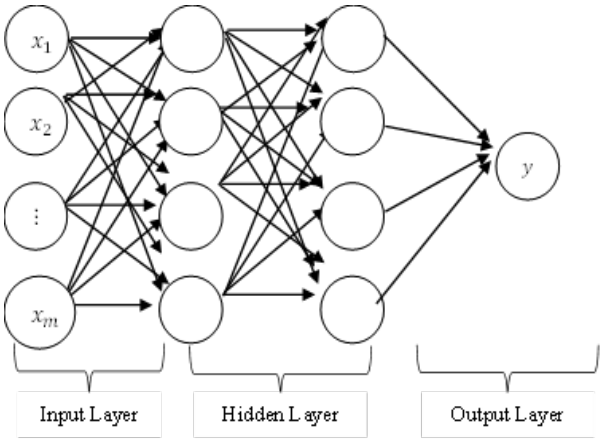

Figure 1. A two hidden layer architecture with m inputs

RESULTS

In this study, the time series of mean seawater temperature (RIAODSS) obtained monthly from Rize station between the years 2010 and 2020 are forecasted. The graph of the relevant time series is given in Figure 2.

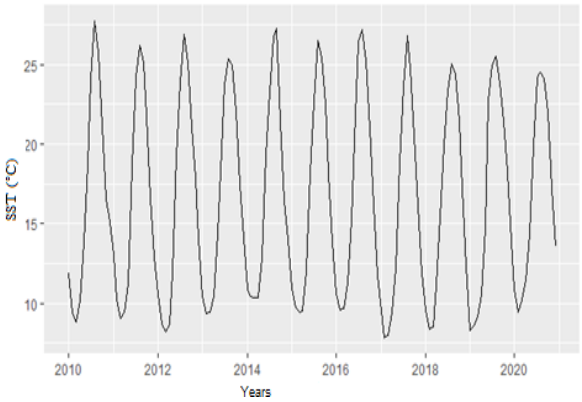

Figure 2. Average seawater temperature (SST) obtained monthly from Rize station between the years 2010-2020

When the time graph of "RIAODSS" given in Figure 2 is examined, at first glance, it is seen that there is seasonality in the data. To confirm the accuracy of this situation, the autocorrelation and partial autocorrelation graphs created for the "RIAODSS" time series are as in Figure 3 and Figure 4.

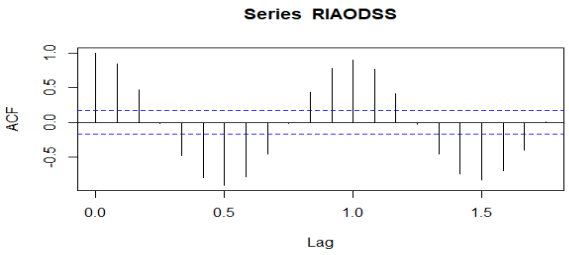

Figure 3. Autocorrelation plot for RIAODSS time series

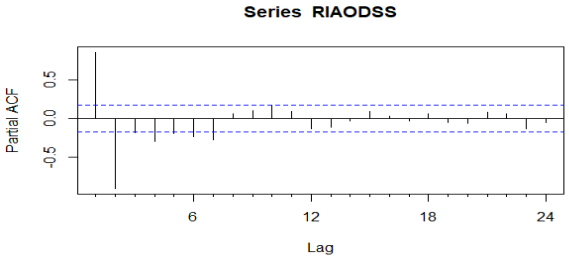

Figure 4. Partial autocorrelation plot for RIAODSS time series

When Figures 3 and 4 are examined, it can be stated that the RIAODSS time series has a dominant seasonal component. When the seasonality graph given in Figure 5 of the RIAODSS time series is examined, it is revealed that the relevant series has seasonality.

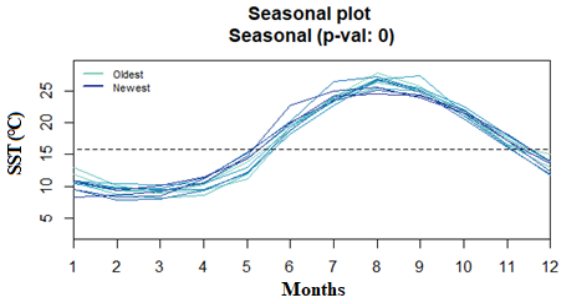

Figure 5. Seasonality graph of the RIAODSS time series

In this study, the RIAODSS time series is analyzed by Seasonal Naive, ETS, and SARIMA methods apart from the FF-ANN method. In the analysis phase, the last 12 observations (last one year) of the "RIAODSS" time series are taken as the test set. In the analysis of the RIAODSS time series with the FF-ANN method, the number of hidden layers is 1, the number of hidden layer elements is 2, the maximum number of iterations is 1000, and the training algorithm is the "Quickprop" algorithm. Two lagged variables (x_{t-1}, x_{t-12}) are used as the input of the network. The results for the root mean square error (RMSE) and mean absolute percentage error (MAPE) criteria values of the test set obtained from all methods are given in Table 1. The values of RMSE and MAPE error criteria are calculated by Equation (2) and Equation (3).

$$RMSE = \sqrt{\frac{\sum_{t=1}^{n}(x_t - \hat{x}_t)^2}{n}} \quad (2)$$

$$MAPE = \frac{1}{n}\sum_{t=1}^{n}\left|\frac{x_t - \hat{x}_t}{x_t}\right| \quad (3)$$

In Equation (2) and Equation (3), n, x_t and $\hat{x}_t$ are the number of learning samples, observed values, and predictive values respectively.

Table 1. RMSE and MAPE error criteria values obtained by all methods for the "RIAODSS" time series

Method	RMSE	MAPE
Seasonal Naive	1.29	7.01
ETS (M, N, M)	0.80	4.14
SARIMA	0.81	3.98
FF-ANN	1.21	0.90

When the analysis results given in Table 1 are examined, the best forecasts are obtained with a 0.90% error rate, especially considering the MAPE criterion, with the FF-ANN method. In addition, the graph of the test set of the RIAODSS time series and the forecasts obtained from the FF-ANN method are determined as in Figure 6.

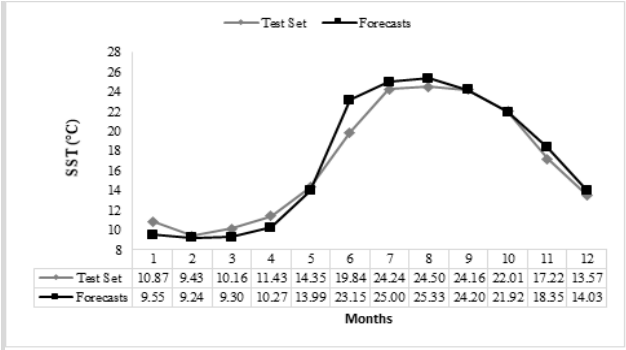

Figure 6. Graph of the test set of RIAODSS time series and the forecasts obtained from the FF-ANN method

Therefore, when the seawater temperatures in Figure 6 are examined, it has been noted that the forecasts and the observation values of the test set are quite compatible.

DISCUSSION

In this study, which is carried out for the first time in our country for the forecasting seawater surface temperatures in Rize province by the FF-ANN method, an accuracy rate of 99.10% is achieved. Although there is no similar study conducted for the current area in the literature, it has been determined that there is a general increase in seawater surface temperatures on the Black Sea coasts, and this trend has been more noticeable, especially in the last 10 years (Ağırbaş and Çakıroğlu, 2021). Similarly, it has been determined by the modeling results that seawater temperatures in the Aegean and Mediterranean coasts have been in a constant warming trend after 1970, and the most significant and significant increase has been recorded in the Iskenderun Bay from 2000 through 2009 (Şişman, 2019). Moreover, it is stated in many other studies that the average seawater temperatures in all our seas in our country tend to increase when the long-term trend is considered (Demircan et al., 2013; Dabanli et al., 2021; Kalıpcı et al., 2021).

In this study, which is carried out with quantitative data and a very high accuracy rate compared to the literature studies, it is revealed that the forecasting seawater surface temperatures in our country can be successfully performed with FF-ANN. With the results obtained in this direction, we provide an advantage in taking possible measures to the global climate crisis scenarios, and our national economic activities; A serious advantage can be gained in making plans for different fields and sectors such as agriculture, and animal husbandry and tourism, etc. Moreover, the determination and prediction of the quantitative change of variables that directly concern biological life, such as seawater temperature; will also help to understand the change of different species, especially fish of commercial importance, and therefore the change of biodiversity. Relatedly, it will provide a serious advantage in determining the dynamic change that commercial fishing activities will face in the short, medium, and long term, and in creating solutions for the problems.

CONCLUSION

As a result, with this study we have done, we can state that the determination of the seawater surface temperature (SST) with a very high accuracy rate, using the FF-ANN method, can be performed with different variables according to needs and purposes, and this will make significant contributions to future action plans.

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AUTHORSHIP CONTRIBUTIONS

All authors contributed equally.

CONFLICT OF INTEREST STATEMENT

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

ETHICS APPROVAL

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

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Development of prediction models to estimate the total number of mesophilic aerobic and lactic acid bacteria of squid rings that were cooked before marinating

Marinasyon öncesinde pişirme işlemi uygulanan kalamar halkalarının toplam mezofilik aerobik ve laktik asit bakteri sayılarını tahmin etmek için modellerin geliştirilmesi

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Abstract: This study was conducted in order to develop different statistical models for estimating the bacterial count of squid rings marinated with lemon juice and mineral water after cooking. The marination ratios and times were as follows: (10:90; 90:10; 50:50; 100:100/100 g squid ring) and (1, 3, 6, 12, 24, 48 and 72 h), respectively. The effects of marination ratios and times on the microbiological and sensory changes of the cooked squid rings were observed at 4°C. Pathogenic bacteria (*Vibrio* spp., *Staphylococcus aureus* and *Escherichia coli*) were not found in the cooked (C) and cooked marinated (CM) squid rings in the present study. The TMC (total mesophilic aerobic bacteria counts) of all groups were determined as consumable at 72 h, whereas the TMC of C and CM samples (C7, CM7, CM14, CM21, CM28) increased to 5.92, 5.83, 5.71, 5.57 and 5.42 log cfu/g, respectively. Regression models were created to estimate the TMC and lactic acid bacteria count (LBC) of cooked squid rings during the marination process at 4°C to determine the increasing rates of bacterial growth of samples. As a result of this study; when compared with Model I and Model II; both of them can be preferred for predicting the TMC of C and CM samples. The variability in the TMC of C and CM squid samples was obtained as 93% in Model I, whereas the variability in the TMC of these samples was observed as 91% in Model II. So, these two models performed well, and they can be used for predicting the TMC of C and CM samples. Additionally, Model III was also developed for estimating the prediction value of LBC of cooked squid samples during the marination process at 4°C. This model was also determined very good performance (86%) to estimate the predicting values of LBC and it can be very essential together used with Model I or Model II for marinated fishery products to estimate the real shelf-life.

Keywords: Marination, prediction models, cooked squid ring, bacteria counts, estimation

Öz: Bu çalışma, pişirme sonrasında limon ve maden suyu ile marine edilen kalamar halkalarının bakteri sayılarını tahmin etmede farklı istatistiksel modeller geliştirmek amacıyla yapılmıştır. Marinasyon oranları (10:90; 90:10; 50:50; 100:100/100 g kalamar halkası) ve süreleri (1, 3, 6, 12, 24, 48 ve 72 saat) sırasıyla bu şekildedir. Marinasyon oranlarının ve sürelerinin pişmiş kalamar halkalarının 4°C'de depolama aşamasında mikrobiyolojik ve duyusal değişimleri üzerindeki etkileri gözlenmiştir. Bu çalışma sonucunda pişirilmiş marine edilmiş (CM) ve pişirilmiş kalamar halkalarında (C) patojenik olan *Vibrio* spp., *Staphylococcus aureus* ve *Escherichia coli* türü patojenik bakterilere saptanmamıştır. C ve CM örneklerinin (C7, CM7, CM 14, CM21, CM28) toplam mezofil aerobik bakteri sayısı (TMC) 72. saatte sırasıyla 5.92, 5.83, 5.71, 5.57 ve 5.42 log kob/g'a yükselmiştir. Marinasyon işlemi esnasında örneklerin artan bakteri gelişim oranlarının belirlenmesi için; 4°C'de marine işlemi sırasında pişirilmiş kalamar halkalarının TMC ve laktik asit bakteri sayılarının (LBC) tahmini için regresyon modelleri geliştirilmiştir. Bu çalışmanın sonucunda; Model I ve Model II karşılaştırıldığında, C ve CM örneklerinin her ikisi içinde iki modelde TMC sayılarını tahmin etmek için tercih edilebilir olduğu görülmüştür. TMC'deki değişkenliğin %93'ü Model I ile %91'i Model II ile açıklanabilmektedir. Bu nedenle bu iki modelinde C ve ve hem CM örneklerinde TMC sayılarını tahmin etmede çok iyi performans gösterdiği ve kullanılabileceği belirlenmiştir. Buna ek olarak, pişirilmiş kalamar örneklerinin 4°C'de marinasyon işlemi esnasında LBC sayılarını tahmin etmek için Model III geliştirilmiştir. Bu modelinde LBC sayılarının tahmini değerlerinin belirlenmesi için çok iyi performans gösterdiği ve Model III'ün marine edilen su ürünleri için gerçek raf ömrünü tayin etmede Model I veya Model II ile birlikte kullanıldığında çok önemli olabileceği belirlenmiştir.

Anahtar kelimeler: Marinasyon, tahmin modelleri, pişmiş kalamar halkası, bakteri sayısı, tahmin

INTRODUCTION

In traditional microbiological methods, the quality and safety of foods have been studied by microbiologists using challenge tests, which are only relevant to the characteristics of food products tested and the conditions of the foods. In addition, these tests are also very slow and very expensive (Roberts, 1995). Nevertheless, predictive microbiology models

propose an alternative to the traditional microbiological evaluations of food quality and food safety. In this concept, the effect of distribution, processing, and storage conditions on the microbial growth of foods can be expressed by mathematical models (McMeekin et al., 1992). Most predictions can be generated by these models that the results observed in the challenge studies. In certain conditions; the predictions show the product to be safe or fail. Otherwise, the growth rate of microorganisms predicted from the models is faster, or the predicted time to the growth of microorganisms is shorter than the time that occurred in the food products (Hyytia et al., 1999). In addition to this, predictive modeling provides not only for determining the shelf-life of food products during storage, but also gives rise to predicting the shelf-life of foods during distribution and retail sales (McMeekin and Ross, 1996).

Shelf-life has a significant concept and important property of today's food products. The safety or quality of food products, if unchecked, can be hazardous and limited the shelf-lives of many food products (Man, 2004). Statistical forecasting models can be used to estimate the shelf-life of food products, and for hygienic assessment of food processing conditions that are normal or not according to the characteristics of storage and distribution of food products. It can also help determine distribution times so that the shelf-life of food products becomes adequate (Dalgaard, 2002). Additionally, predictive modeling provides to estimate the remaining shelf-life of foods accurately (McMeekin and Ross, 1996). In both cases, the shelf-life prediction models may contribute to reducing the losses of food products due to spoilage. In addition, shelf-life prediction is used in research and also education, where the applications of mathematical shelf-life models of food products (Dalgaard, 2002).

Predictive modeling such as the Weibull hazard method also is used for the determination of the shelf-life of many products (Keklik et al., 2017). This model is used especially for predicting the true shelf-life of refrigerated foods (Fu and Labuza, 1993). The growth of surviving bacteria after the processing of fishery products during storage time can be predicted by a modified Gompertz model and a polynomial expression (Tomac et al., 2013). Furthermore, Baranyi and Roberts equation can be used for determining the growth rates of microorganisms are modeled as the function of time. Arrhenius Equation the rapid and reliable method; can be also used for predicting the shelf-life of fishery products (Li et al., 2019). Additionally, Grey and Regression prediction models can be also used for estimating the aerobic bacteria counts on frozen squid rings (*Loligo vulgaris* Lamarck, 1798) during the thawing process (Kilinc et al., 2021). Effective applications are necessary for modeling the temperature dependency and the shelf-life of fishery products. This prediction model would be made by establishing a time correlation among measured sensory, microbial, chemical and biochemical changes for the conditions of interest (Taoukis et al., 1999). The use of predictive mathematical models helps to reduce the requirements of process modifications, storage trials, product reformulations, and challenge tests, which are labor-intensive, expensive, and time-consuming (Blackburn, 2000).

There have been made many studies about the predictive modeling for the shelf-life control of chilled fishery products under storage terms and predictive quality changes of fishery products (Dalgaard et al., 1997; Taoukis et al., 1999; Hyytia et al., 1999; Bulat et al., 2020; Kilinc et al., 2021; Wang et al., 2022). In these studies, models predicting the growth of spoilage organisms in fresh and processed fishery products have been conducted. Squid is considered a very healthy fishery product owing to it has high nutritional composition. Nevertheless, it can be easily spoiled by microbial contamination (Xuan et al., 2017). So, it is very important to extend the shelf-life of squid with the processing technologies.

Therefore, the purpose of this study was firstly to investigate the effects of the different marinating time and the ratios of the different marination solutions on the microbiological, sensory and pH changes of cooked squid rings. Secondly, another aim was to create prediction models to estimate the TMC and LBC of cooked squid rings during marination process as well as predicting the shelf-life of samples in the refrigerated storage.

MATERIAL AND METHODS

Material and the preparation of marinade formulations

In this study, frozen squid rings (*Loligo vulgaris* Lamarck, 1798) were taken from the seafood processing factory, which was located in Izmir province of Türkiye. The length and weight of the squid rings were approximately 5-10 cm and 10-15 g, respectively. Frozen squid samples were transported from the factory to the laboratory of Fish Processing Technology of Ege University Fisheries Faculty in approximately 20-30 minutes with a cold chain. Squid rings were washed with tap water before the first treatments. After that, the heat treatment was applied to the squid rings at 50˚C for 5 minutes in a water bath. Cooked squid rings were divided into groups. Some of which were marinated by using the different solutions at different times.

The groups of C and CM samples are shown in Table 1 and 2. The group of frozen squid rings (F), from groups C1 to C7 indicated as the groups of cooked squid rings, which were stored at 4°C. Subsequently, cooked squid samples were marinated by using the different ratios of lemon juice and mineral water with the same ratios of sugar and carbonate indicated as the group CM. The components of the marinating solution (lemon juice, mineral water, sugar, carbonate) were determined as a result of sensory evaluations. The pH value of the lemon juice used in the marination was determined as 2.3±0.03. The ratio of (marinade solution: squid ring) was (1:1) from the groups CM1 to CM21, whereas the ratio of (marinade solution: squid ring) was (2:1) from the groups CM22 to CM28. Total groups of the squid rings were stored at 4ºC up to the experiment times. The temperature of the marinating solution applied for each group is 4ºC.

Table 1. The groups of C and CM squid rings

Frozen squid rings (F) / Cooked (C)		1 h C1	3 h C2	6 h C3	12 h C4	24 h C5	48 h C6	72 h C7
Cooked Marinated (CM)	100 g squid ring +10 ml lemon juice + 90 ml mineral water+0.5 g sugar+0.5 g carbonate	CM1	CM2	CM3	CM4	CM5	CM6	CM7
	100 g squid ring +90 ml lemon juice + 10 ml mineral water+0.5 g sugar+0.5 g carbonate	CM8	CM9	CM10	CM11	CM12	CM13	CM14
	100 g squid ring+50 ml lemon juice + 50 ml mineral water+0.5 g sugar+0.5 g carbonate	CM15	CM16	CM17	CM18	CM19	CM20	CM21
	100 g squid ring+100 ml lemon juice + 100 ml mineral water+0.5 g sugar+0.5 g carbonate	CM22	CM23	CM24	CM25	CM26	CM27	CM28

Frozen squid rings: F; Cooked squid rings: C1-C7; Cooked marinated squid rings; CM1-CM28

Table 2. The prediction groups of cooked squid rings during predictive marination process for determining the estimation values of bacteria counts according to the regression models

Frozen squid rings (F) / Cooked (C)		75 h C8	80 h C9	85 h C10	90 h C11	100 h C12	120 h C13	150 h C14
Cooked Marinated (CM)	100 g squid ring +10 ml lemon juice + 90 ml mineral water+0.5 g sugar+0.5 g carbonate	CM29	CM30	CM31	CM32	CM33	CM34	CM35
	100 g squid ring +90 ml lemon juice + 10 ml mineral water+0.5 g sugar+0.5 g carbonate	CM36	CM37	CM38	CM39	CM40	CM41	CM42
	100 g squid ring+50 ml lemon juice + 50 ml mineral water+0.5 g sugar+0.5 g carbonate	CM43	CM44	CM45	CM46	CM47	CM48	CM49
	100 g squid ring+100 ml lemon juice + 100 ml mineral water+0.5 g sugar+0.5 g carbonate	CM50	CM51	CM52	CM53	CM54	CM55	CM56

Cooked; C8-C14, Cooked marinated squid rings; CM29-CM56

The methods of the analysis

pH analysis

Cooked (C) and cooked marinated (CM) squid rings (10 g) were calculated for pH analysis and then samples were put into the 10 ml of the distilled water to be dissolved in it. The pH values of squid ring groups were performed by using a pH meter (HANNA-HI 2211 model, Leighton Buzzard, England). For the determination of pH values of samples, analysis were performed in triplicate for each group (C and CM) according to the method (Bongiorno et al., 2018).

Sensory analysis

The sensory analysis had a hedonic scale (changed from 9; extremely liked to 1; extremely disliked) was used the determine the characteristics of color, texture, odor, and general acceptability of C and CM (Tomac et al., 2017). Randomly chosen 3 pieces of the groups (approximately 10-15 g of each) were served to each panelist to evaluate the sensory characteristics of the groups. A total of ten panelists, whose very experience in evaluating sensory characteristics of fish products in Ege University Fisheries Faculty, were joined to the sensory analysis for giving a numerical score to C and CM squid samples.

Microbiological analysis

For microbiological analysis of C and CM squid rings, firstly the dilutions were prepared according to the aseptic conditions. The sample of 10 g of was calculated and then put into 90 ml of 0.1% peptone water (Merck, 1.07228.0500, Darmstadt, Germany). Secondly, other decimal dilutions were prepared from the first dilution. After that, squid rings were homogenized by using a stomacher (IUL, Barcelona, Spain) for 60s.

For total psychrotrophic bacteria counts (TPC) and TMC, the Plate Count Agar (PCA, Merck, 1.05463.0500) was used as a medium for the pour plate method (Harrigan and McCance, 1976). After the inoculation stage, the inoculated petri dishes were incubated at 30˚C for 24-48 h in the incubator (EN500, Nevü, Ankara, Türkiye) for determining the TMC of the samples. For determining the TPC of squid samples, this time inoculated petri dishes were incubated in the refrigerator and were adjusted at 7˚C for 10 days (Harrigan and McCance, 1976).

Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck, 1.16000.0500) was used as the medium for determining the yeast and mold counts (YMC) by using The Pour plate method. Accordingly, the inoculated petri dishes were incubated at 25°C for 3-5 days for enumerating the YMC (Anonymous, 2000) and Baird Parker Agar (BPA, Merck 1.05406) with egg yolk tellurite emulsion was used the determine the *Staphylococcus aureus* count (SAC). According to the spread plate method, 0.1 ml of inoculum was taken and inoculated onto the BPA. After the inoculation, the inoculum was spread onto this medium. After the inoculated petri dishes were incubated at 37°C'de for 30 h for SAC in an incubator (EN500, Nevü, Ankara, Türkiye) according to the method (Mossel and Moreno, 1985). The spread plate method was also used for determining the *Vibrio* bacteria count (VBC). 0.1 ml of inoculum was taken and then inoculated onto the TCBS Agar (Triosulfate Citrate Bile Sucrose). Then, inoculation was the TCBS Agar inoculated petri dishes were incubated at 20°C for 3-5 days for VBC (Serratore et al., 1999).

The medium of MRS Agar (De Man, Rogosa Sharpe, Merck, 1.10660.0500) was used for determining the LBC by using the double agar layer method and was incubated for 2-3 days at 30°C (Baumgart et al., 1986). In addition, the double agar layer method with the Violet Red Bile Agar (VRB Agar, Merck, 1.10275.0500) was used for determining the Enterobacteriaceae counts (EBC) for C and CM squid samples and were incubated at 30°C for 25h (Harrigan and McCance, 1976). For determining the coliform bacteria counts (CBC) of C and CM squid rings, the double agar layer method was used, and the Violet red bile agar (VRB agar, Merck, 1.10275.0500) was used as the medium. Subsequently, the inoculated petri dishes were incubated at 30°C for 24 h for determining the CBC of C and CM squid rings.

For determining the fecal coliform bacteria count (FCB) of C and CM squid rings, the Brilliant Green Lactose Broth (BGLB) was used as the medium. Afterward, the inoculated tubes were incubated at 44-45°C for 48 h according to the method of ICMSF (1986). For defining the *E. coli* (EC) of squid samples, the Eosin Methylene Blue Lactose Sucrose Agar (EMB, Merck, 1.01347.0500) was used as the medium. In this method, the EMB Agar spread by using the spread plate method was inoculated for *E. coli* at 37°C for 24 h (Mossel ve Moreno, 1985). On each analysis day in triplicate, were done for all microbiological analysis. On each experiment day, three repetitions were performed from each C and CM squid sample.

Statistical methods

Statistical Package Program for the Social Sciences Version 25.0 software (IBM SPSS Statistics 25) was used to determine the statistical changes between the groups according to the marination times of cooked squid rings during the same period and in the same groups. The differences in the results of all analysis were evaluated statistically. The ANOVA test (One-way analysis of variance) was carried out for examining the significant difference ($p>0.05$) according to the method by Montgomery and Runger (2003). In this ANOVA test, the normality assumption was checked using the Kolmogorov-Smirnov test, while the homogeneity assumption was checked using the Levene test. Kruskal-Wallis nonparametric test was used to determine the significant difference ($p>0.05$) between the groups according to sensory analysis. Moreover, the non-parametric Friedman S test and two dependent sample sign tests were also carried out using the method (Gamgam and Altunkaynak, 2017). Regression models were also used estimate the values of TMC and LBC of C squid rings with respect to the method (Montgomery and Runger, 2003).

In this study, various regression models were created to determine the factors affecting the TMC values in C and CM squid rings. In these regression models, TMC was the dependent variable, lemon juice (LJ), mineral water (MW), and marination time (T) were the independent variables. In the present study, the lemon juice and mineral water variables were 4 levels (10, 90, 50, 100 ml), whereas the T variable was 7 levels (1, 3, 6, 12, 24, 48, 72 h). The model equation containing the quadratic effect of independent variables and interaction terms with appropriate factor levels is given below:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

In this equation, Y dependent variable (TMC), X_i's independent variables (lemon juice, mineral water, and T), β_0 constant terms, β_i's linear effects of the independent variables, β_{ii}'s were the quadratic effects of the independent variables, and β_{ij}'s were the interaction effects of independent variables on the dependent variable.

Mean Absolute Error (MAE), Mean Square Error (MSE), and Mean Square Error (RMSE) performance measurements are used to measure the prediction errors of the models. These measures are calculated as follows:

$$MAE = \frac{\sum_{i=1}^{n} |(y_i - \hat{y}_i)|}{n}, \quad MSE = \frac{\sum_{i=1}^{n} |(y_i - \hat{y}_i)|^2}{n},$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} |y_i - \hat{y}_i|^2}{n}},$$

where n is the sample size, y_i denotes the actual values, and $\hat{y}_i$ denotes the predicted value obtained from the models for i=1,2,3,…,n, respectively (Sharma, 2019).

RESULTS

pH changes and the microbiological of all C and CM sample groups during refrigerated storage are shown in Table 3.

Table 3. Changes in microbial load and pH values of C and CM squid rings during refrigerated storage

Groups	TPC (log cfu/g)	TMC (log cfu/g)	LBC (log cfu/g)	YMC (log cfu/g)	Pathogenic bacteria	pH
F	<1^{A}	<1^{A}	<1^{A}	<1^{A}	Negative	7.06±0.02^{A}
C1	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	7.54±0.10^{B1}
C2	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	7.58±0.18^{C1}
C3	<1^{A1}	2.44±0.00^{B2}	<1^{A1}	<1^{A1}	Negative	7.65±0.02^{D1}
C4	<1^{A1}	2.78±0.03^{C2}	<1^{A1}	<1^{A1}	Negative	7.66±0.01^{D1}
C5	<1^{A1}	3.54±0.09^{D1}	<1^{A1}	<1^{A1}	Negative	7.65±0.07^{D1}
C6	<1^{A1}	4.57±0.68^{E1}	<1^{A1}	<1^{A1}	Negative	7.66±0.01^{D1}
C7	1.51±0.04^{B2}	5.92±0.07^{F1}	<1^{A1}	<1^{A1}	Negative	7.70±0.13^{E1}
CM1	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	6.90±0.06^{A2}
CM2	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	6.75±0.15^{B2}
CM3	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	6.87±0.09^{C2}
CM4	<1^{A1}	1.54±0.04^{B3}	<1^{A1}	<1^{A1}	Negative	6.54±0.03^{D2}
CM5	<1^{A1}	1.85±0.07^{C2}	<1^{A1}	<1^{A1}	Negative	6.17±0.16^{E2}
CM6	1.51±0.03^{B2}	3.41±0.55^{D2}	1.69±0.10 ^{B2}	<1^{A1}	Negative	6.45±0.07^{F2}
CM7	1.56±0.04 ^{C3}	5.83±0.05^{E2}	2.15±0.26^{C2}	1.51±0.03^{B2}	Negative	6.78±0.04^{G2}
CM8	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.83±0.02^{A3}
CM9	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.43±0.08^{B3}
CM10	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.82±0.05^{C3}
CM11	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.97±0.06^{D3}
CM12	<1^{A1}	1.61±0.05^{B3}	1.52±0.04^{B2}	<1^{A1}	Negative	3.67±0.01^{E3}
CM13	<1^{A1}	4.93±0.25^{C3}	3.03±0.24^{C3}	<1^{A1}	Negative	3.59±0.04^{F3}
CM14	1.49±0.01^{B2}	5.71±0.17^{D3}	3.67±0.05^{D3}	<1^{A1}	Negative	3.66±0.03^{G3}
CM15	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	4.11±0.03^{A4}
CM16	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	4.25±0.12^{B4}
CM17	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	3.92±0.05^{C4}
CM18	<1^{A1}	<1^{A1}	<1^{A1}	<1^{A1}	Negative	4.32±0.04^{D4}
CM19	<1^{A1}	1.54±0.05^{B4}	1.51±0,05 ^{B2}	<1^{A1}	Negative	3.84±0.08^{E4}
CM20	<1^{A1}	5.05±0.23^{C4}	2.76±0,29 ^{C4}	<1^{A1}	Negative	3.52±0.02^{F4}
CM21	<1 ^{A1}	5.57±0.03^{D4}	3.49±0,04^{D4}	<1^{A1}	Negative	3.92±0.03^{C4}
CM22	<1^{A1}	<1^{A1}	<1^{A}	<1^{A1}	Negative	4.00±0.06^{A5}
CM23	<1^{A1}	<1^{A1}	<1^{A}	<1^{A1}	Negative	3.78±0.01^{B5}
CM24	<1^{A1}	<1^{A1}	<1^{A}	<1^{A1}	Negative	3.53±0.04^{C5}
CM25	<1^{A1}	<1^{A1}	<1^{A}	<1^{A1}	Negative	3.56±0,01^{C5}
CM26	<1^{A1}	1.62±0.05^{B5}	1.59±0.03^{B3}	<1^{A1}	Negative	3.32±0.04^{D5}
CM27	<1^{A1}	4.46±0.05^{C5}	2.38±0.04^{C5}	<1^{A1}	Negative	3.61±0.01^{E5}
CM28	<1^{A1}	5.42±0.05^{D5}	3.45±0.08^{D4}	<1^{A1}	Negative	3.52±0.03^{C5}

Frozen squid rings: F; Cooked squid rings: C1-C8; Cooked marinated squid rings; CM9-CM36; TPC: Total Psychrotrophic bacteria count; TMC: Total mesophilic bacteria count; LBC: Lactic acid bacteria count; YMC: Yeast-Mould Count, Negative: Pathogenic bacteria (*S. aureus, Vibrio spp., E. coli*) were not detected. n=3; The microbiological and pH results were presented as the average value of the three analysis. The results are given as mean value (X) ± Standard deviation (SD). The different capital letters A-G in the same column indicate the difference ($p<0.05$) according to the marinating time in the same groups. The different numbers $^{1-5}$ in the same column indicate the difference ($p<0.05$) between the groups in the same marination time.

In the present study, the LBC of CM squid rings reached to 2.15, 3.67, 3.49, and 3.45 log cfu/g at the end of 72 h of marinating, of the groups CM7, CM14, CM21, and CM28, respectively. *Staphylococcus aureus, Vibrio* spp. and *Escherichia coli* were absent in the C and CM samples. The pH values of cooked squid rings increased from 7.54 (C1) to 7.70 (C7) at the end of the storage at 4°C. The marinating process has reduced the pH values of the cooked squid rings. Additionally, the higher the lemon juice concentration used in the marination process, the more caused the decrease in the pH values of cooked squid rings. At the end of the 72 h of marination process, the pH values of cooked squid rings decreased to 6.78, 3.66, 3.92, and 3.52 for the groups CM7, CM14, CM21, and CM28, respectively.

The sensory data of the C and CM squid rings are given in Table 4. In the study, both cooking and marination treatments caused to increase in the shelf-life of squid rings. In addition to this, TMC and LBC of all groups were increased during the marinating process. However, all the groups were determined as consumable in terms of microbiological as well as sensory evaluations. All groups remained below the TMC upper limit for processed fishery products (6 log cfu/g) set by the ICMSF (1986). Therefore, regression models were developed for estimating the TMC and LBC of cooked squid rings during

marination at 4°C. According to these created prediction models, the bacterial counts of cooked squid rings can be easily estimated during the marination process in the different solutions and marination times. In the light of the similar studies given, three models have been created in this study as follows: Model I and Model II were developed for predicting the values of TMC for C and CM squid rings, whereas Model III was developed for estimating the prediction value of LBC of cooked squid samples during marination process.

Table 4. Sensory changes of C and CM squid rings during refrigerated storage

Groups	Colour	Odour	Texture	General Acceptability
F	8.5±1.08^{A}	8.3±0.95^{A}	7.0±0.67^{A}	8.3±0.67^{A}
C1	8.1±1.19^{B1}	7.6±0.97^{B1}	8.2±0.79^{B1}	8.2±0.79^{B1}
C2	8.0±1.15^{B1}	7.5±0.85^{BC1}	8.2±0.79^{B1}	8.2±0.79^{B1}
C3	7.7±0.82^{C1}	7.4±0.70^{C1}	7.3±0.82^{C1}	7.3±0.82^{C1}
C4	6.8±0.79^{D1}	6.1±0.87^{D1}	6.7±0.67^{D1}	6.5±0.53^{D1}
C5	6.1±0.74^{E1}	5.3±0.67^{E1}	6.2±0.63^{E1}	5.8±0.63^{E1}
C6	5.5±0.97^{F1}	4.5±0.53^{F1}	6.1±0.74^{EF1}	4.9±0.74^{F1}
C7	4.9±0.74^{G1}	4.3±0.67^{G1}	6.0±0.82^{F1}	4.4±0.52^{G1}
CM1	7.6±0.52^{A2}	7.5±0.53^{A1}	7.3±0.48^{A2}	7.5±0.53^{A2}
CM2	7.3±0.48^{B2}	7.0±0.67^{B2}	6.8±0.42^{B2}	7.1±0.74^{B2}
CM3	6.8±0.63^{C2}	6.4±0.52^{C2}	5.8±0.63^{C2}	6.8±0.79^{C2}
CM4	6.4±0.52^{D2}	5.3±0.67^{D2}	5.5±0.53^{D2}	6.0±0.82^{D2}
CM5	6.1±0.32^{E2}	4.9±0.57^{E2}	4.8±0.63^{E1}	5.1±0.88^{E2}
CM6	5.6±0.52^{F2}	4.5±0.53^{F1}	4.5±0.53^{F2}	4.8±0.79^{F1}
CM7	5.4±0.52^{G2}	4.1±0.57^{G2}	4.1±0.57^{G2}	4.5±0.53^{G}
CM8	8.4±0.52^{A3}	8.4±0.52^{A2}	8.1±0.88^{A1}	7.8±0.63^{A3}
CM9	8.3±0.48^{A3}	8.3±0.48^{A3}	7.8±0.79^{B3}	7.4±0.52^{B3}
CM10	7.7±0.67^{B3}	7.6±0.51^{B3}	7.2±0.79^{C1}	7.1±0.74^{C3}
CM11	6.7±0.48^{C3}	7.2±0.42^{C3}	6.2±0.63^{D3}	5.8±0.42^{D3}
CM12	5.9±0.57^{D3}	6.1±0.57^{D3}	5.2±0.63^{E2}	5.3±0.48^{E3}
CM13	5.4±0.52^{E3}	5.1±0.74^{E2}	4.4±0.52^{F3}	4.7±0.48^{F1}
CM14	4.5±0.53^{F3}	4.4±0.52^{F1}	4.1±0.57^{G2}	4.3±0.48^{G1}
CM15	8.5±0.53^{A3}	8.5±0.53^{A2}	7.3±0.48^{A2}	8.0±0.82^{A4}
CM16	7.7±0.67^{B4}	8.4±0.52^{A3}	6.9±0.32^{B4}	7.6±0.70^{B4}
CM17	7.0±0.82^{C4}	7.9±0.32^{B4}	6.2±0.42^{C3}	7.3±0.48^{C4}
CM18	6.5±0.71^{D4}	7.5±0.53^{C4}	5.6±0.52^{D4}	6.1±0.74^{D4}
CM19	5.1±0.74^{E4}	6.1±0.74^{D4}	5.1±2.57^{E2}	5.4±0.84^{E3}
CM20	4.5±0.53^{F4}	4.6±0.52^{E3}	4.4±0.70^{F3}	4.9±0.87^{F1}
CM21	4.4±0.52^{F4}	4.2±0.63^{F2}	4.1±0.57^{G2}	4.4±0.52^{G1}
CM22	7.8±0.79^{A5}	8.0±0.82^{A3}	7.9±0.74^{A3}	8.0±0.82^{A4}
CM23	7.4±0.52^{B2}	7.5±0.53^{B4}	7.5±0.75^{B5}	7.5±0.71^{B4}
CM24	7.6±0.70^{C3}	6.5±0.85^{C5}	7.1±0.74^{C4}	7.0±0.67^{C3}
CM25	6.9±0.74^{D5}	5.8±0.63^{D5}	6.1±0.57^{D5}	6.0±0.82^{D2}
CM26	6.6±0.70^{E5}	5.3±0.48^{E5}	5.5±0.53^{E3}	5.6±0.52^{E4}
CM27	6.0±0.67^{F5}	4.9±0.57^{F4}	4.6±0.70^{F4}	4.7±0.48^{F1}
CM28	5.5±1.08^{G5}	4.7±0.48^{G3}	4.3±0.48^{G3}	4.4±0.52^{G1}

Frozen squid rings: F; Cooked squid rings: C1-C7; Cooked marinated squid rings; CM1-CM28; TPC: Total Psychrotrophic bacteria count; TMC: Total mesophilic bacteria count; LBC: Lactic acid bacteria count; YMC: Yeast-Mould Count, n=3; The microbiological and pH results were presented as the average value of the three analysis. The results are shown as mean value (X) ± standard deviation (SD). The different capital letters A-G in the same column indicate the difference ($p<0.05$) according to the marinating time in the same groups. Dec Decimals 1-5 in the same column indicate the difference ($p<0.05$) between groups during the same storage period.

First of all, correlations between dependent variable TMC and independent variables time, LJ, MW were investigated. The correlation between TMC and time was obtained as 0.945 (it is significant at the 0.01 level), and the correlation between LJ and MW was obtained as 0.22 (it is significant at the 0.05 level). In order to estimate the model given by equation (1), the result model equation obtained by using the stepwise method was given below:

Model I: $\hat{Y} = 0{,}660 + 0{,}1046T - 0{,}0004T^2 - 0{,}0146LJ - 0{,}0099MW + 0{,}0001LJxMW + 0{,}0001TxLJ$

All coefficients of Model I was significant (p-value <0.05). According to the results, TMC was significantly (p–value <0.01) affected by both the linear (T, positively) and quadratic (T^2, negatively) effects of the T. We could say that the TMC was significantly (p–value <0.01) affected linearly (negatively) both lemon juice and mineral water. Moreover, the interaction of the lemon juice and the T affected the TMC positively and significantly (p–value <0.015), and the interaction of the mineral water and the lemon juice affected the TMC positively and significantly (p–value <0.01), the adjusted r^2 value of this model was calculated as 0.931. With the estimation model obtained, 93% of the variability in the TMC could be explained. In other words, there was a strong and significant effect of the quadratic effect of T and the rates of marinating on the TMC values of CM squid samples.

The basic principle of regression analysis is that the model is simple. Therefore, another estimation equation, which is simpler than the estimation equation given by Model I, but whose $AdjR^2$ value (0.913) is close to Model I, is obtained and is given below:

Model II: $\hat{Y} = 0{,}46354 + 0{,}0833T - 0{,}0060LJ - 0{,}0045MW$

Although Model II included only linear effects of independent variables, it explained 91% of the variability in the dependent variable. This value (%91) was determined very close to the value in Model I (%93). In order to make a comparison, for different values of independent variables, the actual (observed) values of TMC and the estimated values obtained from Model I and Model II are given in Table 5. In this table, the estimated values obtained from Model I and Model II according to different times and formulations (for different values of LJ and MW) are given in Table 5. According to the results, the TMC value for $LJ = 0$ and $MW = 0$ reached the unacceptable limit for consumption earlier than the other content values.

Table 5. The actual (observed) values of TMC (log cfu/g) and the estimated values of TMC (log cfu/g) of samples obtained from Model I and Model II

Time	Groups	LJ	MW	The observed TMC(log	The estimated TMC (log cfu/g) from Model I	The estimated TMC
1 h	C1	0	0	0	0.7638	0.5468
	CM1	10	90	0	-0.1719	0.0797
	CM8	90	10	0	-0.5403	-0.0365
	CM15	50	50	0	-0.1856	0.0216
	CM22	100	100	0	-0.6019	-0.5037
3 h	C2	0	0	0	0,9697	0.7134
	CM2	10	90	0	0,0368	0.2462
	CM9	90	10	0	-0.3094	0.1300
	CM16	50	50	0	0.0343	0.1881
	CM23	100	100	0	-0.3682	-0.3371
6 h	C3	0	0	2.44	1.2726	0.9632
	CM3	10	90	0	0.3438	0.4960
	CM10	90	10	0	0.0310	0.3798
	CM17	50	50	0	0.3580	0.4379
	CM24	100	100	0	-0.0237	-0.0873
12 h	C4	0	0	2.78	1.8567	1.4628
	CM4	10	90	1.54	0.9363	0.9957
	CM11	90	10	0	0.6900	0.8795
	CM18	50	50	0	0.9837	0.9376
	CM25	100	100	0	0.6436	0.4123
24 h	C5	0	0	3.54	2.9382	2.4621
	CM5	10	90	1.85	2.0344	1.9949
	CM12	90	10	1.61	1.9214	1.8787
	CM19	50	50	1.54	2.1485	1.9368
	CM26	100	100	1.62	1.8917	1.4116
48 h	C6	0	0	4.57	4.7547	4.4606
	CM6	10	90	3.41	3.8842	3.9935
	CM13	90	10	4.93	4.0377	3.8773
	CM20	50	50	5.05	4.1315	3.9354
	CM27	100	100	4.46	4.0413	3.4101
72 h	C7	0	0	5.92	6.1090	6.4592
	CM7	10	90	5.83	5.2719	5.9920
	CM14	90	10	5.71	5.6918	5.8758
	CM21	50	50	5.57	5.6524	5.9339
	CM28	100	100	5.42	5.7287	5.4087

Cooked squid rings: C1-C7; Cooked marinated squid rings; CM1-CM28; LJ: Limon juice; MW: Mineral water; TMC: Total mesophilic bacteria count.

The performance measures are based on the differences between the actual values and the predicted values, that is, errors. Therefore, the model with lower errors should give more accurate estimates (Table 6).

Table 6. The estimation errors of the models

	Model I	Model II
MAE	0.4632	0.4965
MSE	0.3139	0.4044
RMSE	0.5603	0.6359

Mean Squared Error (MSE), Mean Absolute Error (MAE), and Root-Mean Square Error (RMSE)

According to this table (Table 6), both models were determined to be performed well in terms of being used for prediction. Moreover, TMC is only not a good indicator for determining the shelf-life of fermented food products such as marinated fishery products. This bacteria count should be considered with the LBC, which consists of the main microbial flora of fermented food products (Unluturk and Turantas, 2003). For this reason, Model III was developed for estimating the LBC of cooked marinated squid rings. The adjusted r^2 value of this model was calculated as 0.864.

Model III: $\hat{Y} = -0{,}1631 + 0{,}0004TLJ + 0{,}0154T + 0{,}00008TMW$

The estimated values of TMC (log cfu/g) of samples from Model I and Model II are given in Table 7. In addition to this, the estimated values of LBC (log cfu/g) of samples from Model III are also in given Table 7.

Table 7. The estimated values of TMC (log cfu/g) of samples from Model I, Model II and the estimated values of LBC (log cfu/g) of samples from Model III

Time	Groups	LJ	MW	$\hat{Y}_{MI}$	$\hat{Y}_{MII}$	$\hat{Y}_{MIII}$
75h	C8	0	0	6.2550	6.7110	0.9919
	CM29	10	90	5.3830	6.2460	1.8319
	CM36	90	10	5.6070	6.1260	3.7519
	CM43	50	50	5.6550	6.1860	2.7919
	CM50	100	100	5.5550	5.6610	4.5919
80h	C9	0	0	6.4680	7.1275	1.0689
	CM30	10	90	5.6010	6.6625	1.9649
	CM37	90	10	5.8650	6.5425	4.0129
	CM44	50	50	5.8930	6.6025	2.9889
	CM51	100	100	5.8180	6.0775	4.9089
85h	C10	0	0	6.6610	7.5440	1.1459
	CM31	10	90	5.7990	7.0790	2.0979
	CM38	90	10	6.1030	6.9590	4.2739
	CM45	50	50	6.1110	7.0190	3.1859
	CM52	100	100	6.0610	6.4940	5.2259
90h	C11	0	0	6.8340	7.9605	1.2229
	CM32	10	90	5.9770	7.4955	2.2309
	CM39	90	10	6.3210	7.3755	4.5349
	CM46	50	50	6.3090	7.4355	3.3829
	CM53	100	100	6.2840	6.9105	5.5429
100h	C12	0	0	7.1200	8.79354	1.3769
	CM33	10	90	6.2730	8.32854	2.4969
	CM40	90	10	6.6970	8.20854	5.0569
	CM47	50	50	6.6450	8.26854	3.7769
	CM54	100	100	6.6700	7.74354	6.1769
120h	C13	0	0	7.4520	10.45954	1.6849
	CM34	10	90	6.6250	9.99454	3.0289
	CM41	90	10	7.2090	9.87454	6.1009
	CM48	50	50	7.0770	9.93454	4.5649
	CM55	100	100	7.2020	9.40954	7.4449
150h	C14	0	0	7.3500	12.95854	2.1469
	CM35	10	90	6.5530	12.49354	3.8269
	CM42	90	10	7.3770	12.37354	7.6669
	CM49	50	50	7.1250	12.43354	5.7469
	CM56	100	100	7.4000	11.90854	9.3469

The predictive groups of cooked; C37-C40, Cooked marinated squid rings; CM41-CM56 LJ: Limon juice; MW: Mineral water; TMC: Total mesophilicbacteriacount;$\hat{Y}_{MI}$:The estimated TMC (logcfu/g) fromModel I; $\hat{Y}_{MII}$: The estimated TMC (log cfu/g) from Model II; $\hat{Y}_{MIII}$: The estimated LBC (log cfu/g) from Model III.

After 120 h of the predictive marination process, the predictive value of TMC of cooked squid rings increased to 6.62, 7.20, 7.07, and 7.20 log cfu/g, respectively for the groups CM34, CM41, CM48, and CM55 according to Model I.

Additionally, at that time the predictive value of LBC of samples increased to 3.02, 6.10, 4.56, and 7.44 log cfu/g, respectively for the groups CM34, CM41, CM48, and CM55 according to the Model III.

DISCUSSION

Squid meat is known to be sensitive to heat treatment due to its unique structure, which gives traditional squid products a harsh and harsh taste (Hu et al., 2014). This special structure of squid meat directly affects the acceptance of consumers (Jun-Hui et al., 2020). Therefore, this study, it was aimed to soften the squid rings by cooking and marinating them. Reported that cooking at lower temperatures (55°C) significantly decreased cooking loss, physicochemical and textural attributes of squid meat, when compared with cooking at a higher temperature of 77°C. In addition, it is noted that cooking temperature of 55°C, squid meat has both sufficient juiciness and a pleasant texture (Schmidt et al., 2021). The maximum softening level was reported and softening is achieved by incubating squid mantle rings at 45°C and pH 2 (Collignan and Montet, 1998).

Many studies have been conducted about the processing technologies that are applied to squid for decreasing the TMC. The TMC of squid treated with slightly acidic electrolyzed water ice significantly inhibited this value up to 1.46±0.10 log cfu/g, and also observed relatively slow microbial production during storage (Xuan et al., 2017). In a reported study that high-pressure processing at 300 MPa after 20 minutes reduced the number of TMC in squid by 1.26 log cfu/g (Gou et al., 2010). In addition to this, gamma irradiation was also reported by Tomac et al. (2017), reducing bacterial loads of highly perishable squid rings and resulting in significantly extended shelf-life under cooling conditions. Reported in another study that heat treatment was lethal to some types of microorganisms in seafood products (Zavadlav et al., 2020). In addition to this, each type of microorganism had its own special heat tolerance.

In the present study, the TMC of cooked squid rings began to increase after 6h stored at 4°C. The growth of most of the bacteria species could be inhibited by the heat and marination treatments. However, acid tolerance lactic acid bacteria began to grow in acidic conditions in CM squid products. The numbers of all C and CM sample groups of microorganisms were determined under the detectable limit at the beginning of the marination process (Table 3). However, all the groups of microorganisms began to increase during the marination process at 4°C. The number of TMC began to increase in CM4 after 12 h of the marination process, whereas the TMC of groups CM12, CM19, and CM26 increased after 24 h of the marination process. The population of TMC in groups C7, CM7, CM14, CM21, and CM28 significantly increased up to 5.92, 5.83, 5.71, 5.57, and 5.42 log cfu/g, respectively, then 72 h of the refrigerated storage. However, according to the ICMSF (1986), all groups C and CM did not exceed the microbiological consumption limit specified as 6.0 log cfu/g for processed aquaculture after 72 h of storage at 4°C.

Andrighetto et al. (2009) reported that LBC was found to be high in the Italian seafood salads discussed in this study and increased the cfu/g values by 8.0 log at the end of the shelf-life of the seafood salad. The results of the study correlated well with the literature studies provided (Andrighetto et al., 2009), stating that the increase in the marinating conditions of lactic acid bacteria is in accordance with the increased storage time. However, pathogenic bacteria *S. aureus, Vibrio spp.* and *E.coli* were not found in the C and CM squid rings in the present study.

The authors reported that longer cooking times could be caused to increase the pH values of cooked squid mantle during refrigerated storage (Stanley and Hultin, 1982). In the present study; after marinating process the decreasing of pH values of cooked squid samples are confirmed by these studies (Kilinc and Çaklı, 2004; Szymczak et al., 2020).

All the groups of C and CM squid rings were still not exceeded the limit of consumption after 72 h of storage at 4°C (Tomac et al., 2017). At that time the general acceptability results of the groups C7, CM7, CM14, CM21, and CM28 were determined as 4.4, 4.5, 4.3, 4.4, and 4.4, respectively. In one report, the panelists noted that the most important sensory quality of cuttlefish is the texture, which is the primary quality factor, and their most common complaint is a rubbery texture or stiffness. However, the longest cooking time (32 minutes) produced squid with the best sensory quality (Stanley and Hultin, 1982). In addition to this, taking into consideration of the processing technologies, they could be induced an increase in the shelf-lives of fishery products (Keklik et al., 2017). In another report, the higher pressurization was reported to be caused to extend the longest shelf-life of squid samples (Paarup et al., 2002). These results of all the studies given were found to be very similar to the findings of this study that cooking and marinating caused an increase in the shelf-life of squid rings. Andrighetto et al. (2009) reported that LAB was highly present in the Italian marinated seafood salads and it increased to a value of 8.0 log cfu/g at the end of the shelf-life of the seafood salad. In the present study, the predictive LBC of the group CM56 during the marination process stored at 4°C exceeded this value after 150 h according to the predictive regression model III.

CONCLUSION

As a result, three models were created for estimating the TMC and LBC of C and CM squid rings during marination at 4ºC. For marinated fishery products; Model III was also developed for estimating the value of LBC of samples. Therefore, Model III can be very essential together used with Model I or Model II because of observing the increasing LAB of

cooked squid rings during marination. These created models can be preferred not only for estimating bacterial counts of fishery products during marination process but also for deciding the shelf-life of marinated and fermented fishery products.

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AUTHOR CONTRIBUTIONS

All authors contributed equally.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

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

DATA AVAILABILITY

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

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Biology, digestive enzymes and organosomatic indices of *Chrysichthys nigrodigitatus* (Lacépède, 1803) from Oyan Dam, Southwestern Nigeria

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Abstract: Some aspects of the biology, digestive enzymes and organosomatic indices of *Chrysichthys nigrodigitatus* purchased from fishermen in Oyan dam was investigated. This study was aimed at providing information on the composition of food materials found in the gut and specific activities of selected enzymes as it affects the domestication of the species. 100 specimens of the species were examined for stomach contents, length-weight relationship, digestive enzyme assay and organosomatic indices, using standard methods. Food items observed were detritus (4%), fish part (12%), Insecta (13%). Sand was observed to be 11% of total stomach volume. The logarithmic equation for length-weight relationship $lnW = 2.68lnl - 3.79$ indicated that an increase in length led to a corresponding increase in weight with 'R' = 0.611, calculated 'r'=0.78, 'a'=2.68 and 'b'=3.79 indicating positive allometric growth pattern. Amylase exhibited high activity in the stomach, while lipase and proteinase in the stomach and posterior intestine. Specific activities of digestive enzymes showed significant differences (p<0.05). Viscerosomatic (2.92± 0.25), hepatosomatic (2.27± 0.22) indices and Fulton condition factor (1.93± 0.06) were recorded. Feed items present in the species confirm its overlapping feeding habit, indicating that the species is an omnivorous detritivore. This was also depicted in the activities of the different digestive enzymes.

Keywords: *Chrysichthys nigrodigitatus*, digestive enzymes, Oyan Dam, length- weight relationship, food item, produce

INTRODUCTION

Fish species of the Claroteidae family has wide commercial value in the Nigeria context (Akinsanya et al., 2007). Of this family, the genus *Chrysichthys* is well commercialized and known as "Inanga" by the people of Ibibio, "Warushe" to the Hausa's, Igangan to the Yoruba's Nigeria. The common silver catfish - *Chrysichthys nigrodigitatus* Lacépède, 1803 is native to Africa and widely distributed in Nigeria's fresh water.

Growth as a function of animal feeding portrays the different metabolic interactions and adjustments that occur within the animal. These are sustained by the nutrient availability and nutritional status of the animal. Studies have shown that the potential of fish to digest its food is highly variable and it's influenced by species, food preference, style of feeding, size, age and temperature (Garcia-Carreno et al., 2002; Gioda et al., 2017). Many problems associated with fish feeding is due to the lack of knowledge of fish species digestive processes (Rønnestad et al., 2013). Investigating the digestive enzymes of a species relate the feeding habits of that fish species to the enzymes that are present in the fish gut. This provides a clue to explaining the digestive processes that take place once food is consumed by the species (Fagbenro et al., 2001).

Several studies have been conducted on the food and feeding habits (Atobatele and Ugwumba, 2011; Kuton and Akinsanya, 2016), length–weight relationship (Kareem et al., 2015; Uneke, 2015) of the species. For successful domestication and culture of any fish species, there is need to investigate the biology of the species. However, there is a paucity of data on the activity of the digestive enzymes of this fish species. This study was aimed at providing information on the composition of food materials found in the gut and specific activities of selected enzymes that break down nutrients in the diets of the species obtained from catches of fishermen in the study area.

MATERIALS AND METHODS

Study Area

The government of Nigeria under the management of the Ogun-Osun River Basin Development Authority (Figure 1) owns Oyan Dam. Its longitudinal and latitudinal location is 3° 16° East and 7° 15° North with an elevation of 43.3 m above sea level on the confluence of Oyan and Ofiki rivers (Ofoezie et al., 1991; O-ORBDA, 1998). The catchment area of the dam is about 9,000 km² within the southern climatic belt of Nigeria. The reservoir is a home for booming fishing activities and fishermen.

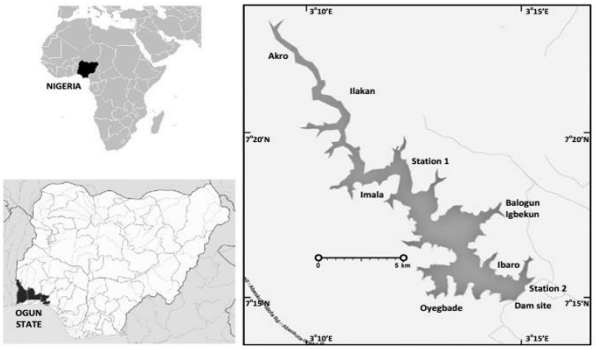

Figure 1. Map showing Oyan Dam (area of study), Ogun State, Nigeria

Collection of Samples

The sampling stations used for the study were the Imala and Ibaro, which are known for their thriving fishing activities (Ikenweiwe et al., 2007). Sampling was carried out every month for five months. On a monthly basis, ten fish samples were purchased from fishermen using gill nets from each location. Conveyance of samples was by the use of an ice chest to the biology laboratory, Department of Zoology, Federal University of Agriculture, Abeokuta for fresh examination.

Samples identification and laboratory Procedure

Species identification was by the guides, as described by Olaosebikan and Raji (2004). The morphometric measure of length was to the nearest 0.1 cm while weight was to the nearest 0.1 g. Standard measuring and digital balance (Camry electronic weighing scale, Model EK3250. Zhongshan Camry Electronic Co, Ltd., Zhongshan, Guangdong, China) were used for length and weight. A pair of stainless–steel scissors and forceps were used to make a longitudinal incision from the anus to the mouth thereby exposing the internal organs of the samples. This were carefully removed using a pair of throngs.

Stomach Fullness Classification

Classification of stomach contents into different categories was as described by Ugwumba and Ugwumba (2007). Categories includes:

0/4 = empty stomach

1/4 = one quarter full stomach

2/4 = half full stomach

3/4 = three quarter full stomach

4/4 = full stomach

Identification of food items in stomach

The stomachs of the experimental fish were cut open and the contents emptied into different petri dishes. Ten percent of normal saline was poured into the petri dish to dislodge clogged particles. Food materials were then graded into various groups and observed under Biobase Optical microscope (BMP-107B). Identification of food items to the species level was done using the key guides described by Mellanby (1965) and Frid (2002). Frequency of occurrence and numerical methods of stomach contents analysis was then used to analyze stomach contents, as described by Adeosun et al. (2017).

For frequency of occurrence, the formula used was:

$$\%\ \text{Occurrence of particular food item} = \frac{\text{No. of point of the particular food item}}{\text{Total number of points of all food}} \times 100$$

Also, according to the description in the Bowen guide for stomach contents identification, the number of stomachs in which the'particular' food particle is found is expressed as a percentage of the total stomachs with food examined.

$$\%\ \text{Occurrence of the i food item in the sample} = \frac{\text{No. of stomachs in which the i item is found}}{\text{No. of stomachs with food in the sample}} \times 100$$

For numerical analysis of stomach content, the expression below was used.

$$\%\ \text{Number of a food item} = \frac{\text{Total number of the particular food item}}{\text{Total number of all food items}} \times 100$$

Length-Weight Relationship

Length-Weight Relationship **(**LWR) was determined as described by Bagenal (1978), using the equation:

Weight of fish = constant 'a' x Length of fish x constant 'b'

$$(W = aL^b) \quad [1]$$

Where: *b* is the slope and *a* is the intercept on the length axis.

Logarithmic transformation of equation 1 gives the straight-line relationship depicted by the equation 2 below:

$$\text{LogW} = \text{Log}a + b\text{LogL} \quad [2]$$

Condition factor (K)

The state of wellness of the samples was calculated using the formula of Froese (2006) as described by Jin et al. (2015).

$$K = 100\ X \frac{W\ (g)}{L^3\ (cm)}$$

W = Weight of fish

L^3= Cube root of standard length of fish

After determining the Fulton's condition factor of the samples, the liver and visceral were removed carefully and weighed using Camry electronic weighing scale (Model EK3250. 5kg). Viscerosomatic index was used as a method of determining the growth performance in fish feeding. The

mathematical equation below was used to calculate organosomatic indices:

$$\text{OSI} = \frac{\text{Weight of organ (g)}}{\text{weight of fish (g)}} \text{ x } 100$$

Gut Enzymes Analysis

Total amylase (α and b) activity

One milliliter of 1% soluble starch in 1/10M sodium acetate buffer pH 5.0 was added to 1 ml of supernatant and the mixture was incubated for 1 hour at 27°C. Two milliliters of DNSA reagent were added to the resultant mixture to terminate enzyme action and the resulting coloured solution allowed to boil for 5 minutes. The solution was then made to 10 ml mark with distilled water and cooled under running tap water. The volume of reducing sugar formed was determined by reading the optical density of the solution at 540 nm against the blank and calculated from a standard curve of Maltose (Swain and Dekker, 1966).

α-Amylase activity

From the supernatant obtained from the total amylase test, 5 ml was heated for 15 minutes at 70°C. After heating, 1 ml of the extract was then incubated in 1 ml of 1% soluble starch in 1/10M sodium acetate buffer solution (pH 5.0) for 1 h at 27°C. At the end, incubation of specific enzyme activity was stopped by the addition of 2 ml DNSA reagent to the reaction mixture. The quantity of reducing sugar formed was then determined, using the same procedure for total amylase activity.

Proteinase activity

The enzyme extract preparation method used for proteinase activity was as for total amylase activity, except that 20 ml of 0.05 M sodium phosphate pH 6.0 was the extracting buffer. The Lowry protein assay method for determining proteinase activity in the enzyme extract was adopted (Lowry et al., 1951). The optical density of the solution was measured at 700 nm against a blank similarly treated (1 ml boiled enzyme extract). Proteinase activity was then calculated using a standard curve of various concentrations of tyrosine (Somkuti and Babel, 1967).

Lipase activity

The method described by Yong and Wood (1977) was adopted for this analysis. Three milliliters of the enzyme extract supernatant was mixed with an emulsion solution that comprised of 100 ml glycerol tributyrate, 4 mg sodium taurocholate, 20 ml 0.1M sodium acetate buffer (pH 5.0) and 10 ml 2.2% $CaCl_2$. The resulting mixture was then incubated for one hour at 40°C. After incubation, 20 ml of absolute ethanol was added and the ensuing solution was titrated against 0.02 M NaOH using a phenolphthalein indicator (0.1 g in 50 ml absolute ethanol and 5 ml water). A blank that contained 3 ml of boiled enzyme extract was similarly treated as the enzyme extract.

Statistical Analysis

All data were subjected to statistical analysis using the Statistical Package for Social Science (SPSS 2007) software. The coefficient of regression was used to assess food preference and style of feeding of the species.

RESULTS

Stomach content

Stomach fullness values for *C. nigrodigitatus* sampled revealed that of the 100 fish specimen sampled, 21% of the stomach were observed to be full while 15% of the stomachs were empty (Figure 2). Insects and scales were found in the majority of the stomach of *C. nigrodigitatus* with food materials. Fish crumbs and nematode were also found in the majority of the guts. Other food items observed were plants, Crustaceans, Rotifers, Arachnida, Mollusca, and detritus (Figure 3)

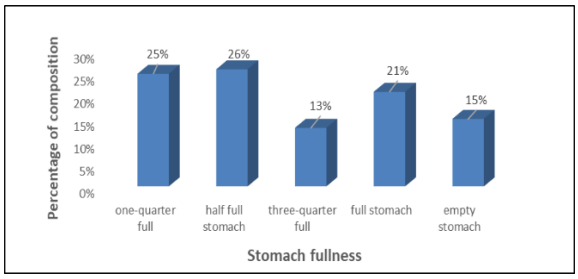

Figure 2. Graphical illustration of stomach fullness

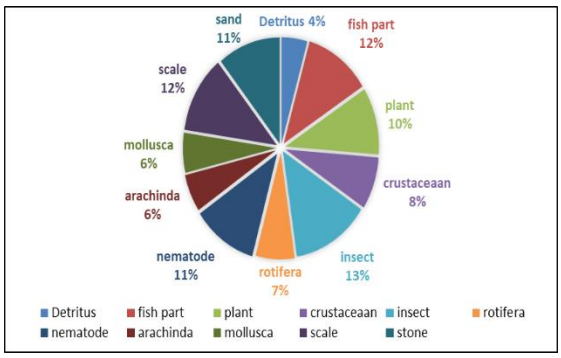

Figure 3. Food items in the stomachs of *Chrysichthys nigrodigitatus*

Length-weight relationship (LWR)

LWR of the species is depicted (Figure 4). The linear equation i.e. $W = 2.68lnl - 3.79$ and the regression parameters are significant F 1.98=153.96 (p<0.05) and correlation (r=0.78). Regression co-efficient R^2 = 0.611.

Organosomatic indices and condition factor

The organosomatic indices and condition factor of *C. nigrodigitatus* are as presented in Table 1. The confidence interval estimation at 99% confidence level using appropriate statistical tool showed that the hepatosomatic (liver somatic) index (HSI) of the sampled specimen was between 2.05 and 2.49 and viscerosomatic index (VSI) between 2.67 and 3.17 with 'K' between 1.87 and 1.99.

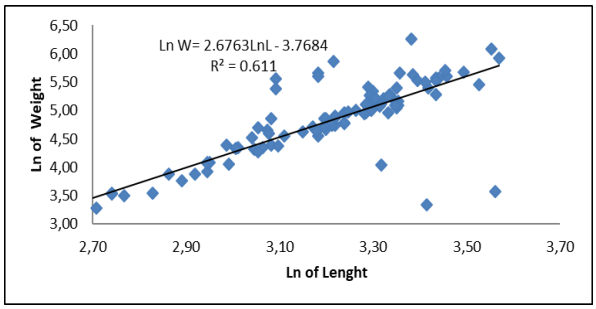

Figure 4. Length-weight relationship of *C. nigrodigitatus*

Table 1. Organosomatic indices and condition factor of *Chrysichthys nigrodigitatus*

Parameters (%)	Mean ± SE	P-value
Liver somatic index	2.27± 0.22	<0.01
Viscerosomatic index	2.92± 0.25	<0.01
Condition Factor	1.93± 0.06	<0.01

Activity of digestive enzymes in the gut of *Chrysichthys nigrodigitatus*

Enzymes activities in the guts are presented in Table 2. Various segments of the guts, showed varying enzymes activity strength. The highest amylase activity was observed in the stomach and was significantly different (p<0.05). Lipase and proteinase activity was lowest in the anterior intestine and increased through to the posterior intestine.

Table 2. Specific activity of digestive enzymes (U/g) in the gut

Digestive enzyme	Anterior intestine	Stomach	Small intestine	Posterior intestine
Amylase	2.29±1.28^{b}	15.69±0.97^{a}	10.54±1.06^{c}	.07±0.99^{d}
Lipase	5.84±1.31^{c}	20.29±1.33	22.62±1.39^{ab}	3.49±3.23^{a}
Proteinase	.57±0.56^{b}	4.15±0.57^{b}	7.01±1.04^{a}	.62±0.96^{a}

Rows with Means ± S.D with superscripts at variance implies statistical difference at 95% probability

DISCUSSION

The composition of the stomach content of the experimental fish is in agreement with the findings of previous studies on this species (Yem et al., 2009; Lawal et al., 2010; Thomas and Ogomade 2019). The study however did not agree with the result presented by Dada and Araoye (2008) who documented higher dominance of plant materials in the stomach of the species from Asa Dam. This could be a result of the low and high abundance of the various food items in the different areas. The prevalence of insect parts observed in this study agreed with the finding of Atobatele and Ugwumba (2011) who posited that *C. nigrodigitatus* consumed more insects as it increased in size with a decrease in preference for the crustacean. However, the study of Udosen and Rufus (2018) reported a higher percentage of phytoplankton in the stomach of the species from Uta-Ewa Creek in the South-South, Nigeria confirming the opportunistic feeding behaviour of the species. The study also confirms the overlapping and omnivorous feeding nature of the species and corroborates the findings of previous researchers (Atobatele and Ugwumba, 2011; Uneke, 2014, Udosen and Rufus, 2018) and could be due to the trophic flexibility and opportunistic feeding behaviour of the species.

The viscerosomatic index, which indicates the growth performance of fish during fish feeding was higher than one. This indicated that the species feeding in the environment during the study was optimal. The high value obtained for the hepatosomatic index also revealed that the species in the study location were in good health condition. This was seen in the value obtained for the Fulton condition factor indicating that the environment is conducive for the survival of the species. The present finding is similar to that documented by Kumar et al. (2010); Lawal et al. (2010). Thomas and Ogomade (2019) also documented similar condition factors for the species. The findings however differ from that of Amoah et al. (2008). The species exhibited a positive allometric growth pattern and corroborated the earlier study by Yem et al. (2009) but disagreed with that of Offem et al. (2008) and Lawal et al. (2010). The correlation value indicated that standard length increased or decreased with a corresponding increase or decrease in weight of *C. nigrodigitatus* in the course of the study which could be linked to food availability and good environmental condition for the survival of the fish species.

The strong specific activity of the amylase enzyme observed in the stomach starting from the anterior intestine is an indication that the breakdown of carbohydrate foods in the fish commenced from the anterior segment (oesophagus) and got stronger in the stomach. This could indicate that the digestion of carbohydrate foods was concluded in these parts of the alimentary canal (AC). A similar result was presented in the study of Odedeyi and Fagbenro, (2010).

The high activity of the lipase enzyme in the AC, especially in the stomach to the posterior intestine could also indicate that lipid metabolism was stronger in the stomach region down to the posterior end of the intestine. A similar observation has been reported by Fagbenro et al., (2000) in *Heterotis niloticus* Cuvier 1829. The omnivorous feeding habit of the species affects the activities of the different enzymes. In another study by Fagbenro et al. (2001) they needed high activity level of proteinase and trypsin in the posterior end of the intestine of *Malapterurus electricus* (Gmelin 1789) which was supported by the findings of this study. The occurrence of proteinase and lipase in the gut of *C. nigrodigitatus* could be associated with the omnivore detritivorous food habits of the species as posited by Tramati et al. (2005).

Chrysichthys nigrodigitatus is an omnivorous detritivore as it exhibited an overlapping feeding habit. The omnivorous feeding habit of the species also affected the activities of the different digestive enzymes. The study provided useful information on the use of the viscerosomatic index as a key for determining the growth performance of fish to fish feeding. The study also concludes that the study area was conducive for the growth and survival of the species.

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AUTHORSHIP CONTRIBUTION

Oghenebrorhie Mavis Oghenochuko, Festus Idowu Adeosun: Conceptualization, idea, design. Fiyinfoluwa Georgina Leramo, Olamide Modinat Adeosun, Paul Bangura: Data curation. Fiyinfoluwa Georgina Leramo: Writing- Original draft preparation. Olamide Modinat Adeosun, Paul Bangura: Revised first draft. Oghenebrorhie Mavis Oghenochuko, Festus Idowu Adeosun: Revised final draft. Oghenebrorhie Mavis Oghenochuko, Festus Idowu Adeosun: 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 freshly moribund fish were used for the study.

DATA AVAILABILITY

All relevant data is in the article.

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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Estimating length-weight, length-length relationships, and condition factor of eight fish species, a case study of Bashar River, Tigris drainage (Iran)

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Abstract: This study investigate investigated the length-weight, length-length relationships and condition factor of eight fish species collected from Bashar River, Tigris drainage by sampling 341 specimens of *Alburnus sellal* (Heckel, 1843), *Barbus karunensis* (Khaefi, Esmaeili, Geiger & Eagderi, 2017), *Capoeta aculeate* (Valenciennes, 1844), *Capoeta coadi* (Alwan, Zareian & Esmaeili, 2016), *Chondrostoma regium* (Heckel, 1843), *Garra gymnothorax* (Berg, 1949), *Glyptothorax galaxias* (Mousavi-Sabet, Eagderi, Vatandoust & Freyhof, 2021) and *Turcinoemacheilus hafezi* (Golzarianpour, Abdoli, Patimar & Freyhof, 2013). The results showed that the b parameter was form 2.41 (in *C. aculeate*) to 3.88 (in *A. sellal*) and condition factors ranged from 0.74 (in *A. sellal* and *B. karunensis*) to 1.35 (in *C. aculeate*). The coefficient of determination (r^2) in the length-weight and length-length relationships was > 0.83. In conclusion, allometric growth patterns for *A. sellal*, *B. karunensis*, *G. gymnothorax*, and *G. galaxias* were positive while for *C. aculata*, *C. regium* and *T. hafezi*, and *C. coadi* presented negative patterns. This study represents the first data for *G. galaxias*.

Keywords: Length-weight relationship, Bashar River, Iranian inland waters, LLRs, fishery management

INTRODUCTION

Studies of the length-weight relationship (LWR) are vital tools to describe several biological aspects of fishes as well as condition of populations in their habitat (Froese, 2006; Mouludi–Saleh and Eagderi, 2019; Eagderi et al., 2020). The length-weight relationship can be used for (a) conversion of growth-in-length equations to growth-in-weight stock assessment models; (b) biomass estimation from length-frequency data; (c) calculating the total weight of fish caught from length-frequency data; (d) investigating the changes in health status of fish species (compared to past or future samples at the same place and the same sampling season); (e) determining the relative condition factor of small fish compared to those of large fish and (f) for between-region life histories comparisons of certain fish species (Moradinasab et al., 2012). Standing stock, yield, and biomass are frequently estimated from length-frequency data converted with length-weight relationships (LWRs) and length–length relationships (LLRs) are useful for the standardization of length type when data are summarized (Simon & Mazlan, 2008). The condition factor of a fish is an index that reflects the interplay of physical and biological factors and fluctuations in the physiological status of fishes and may vary among fish species in different locations (Getso et al., 2017).

The Bashar River is one of the biggest tributaries of the Tigris-Euphrates basin in Kohkiluyeh and Boyer-Ahmad Province with rich fish diversity, being located in southwest, as the sub-basins of the Karun River, which originates from the Sepidan Mountains in Fars province. It crosses the Pataveh region, joins the Khersan River and finally flows into the Karun River (Jamali et al., 2015; Mortazavi and Hatami, 2018). This river is being exploited for various agricultural, industrial, and recreational activities, thereby greatly contributing to the economy of Yasuj city. Although, the Bashar River has been influenced and polluted by sugar factory, hospitals, agricultural farms, municipal surface runoff and wastewater treatment plants (Boustani and Hojati, 2010; Rahimi et al., 2011). Due to the threats to the aquatic environment of this ecosystem, the abundance and diversity change every year, so sustainable monitoring has become important for sound management.

This study aimed to investigate the parameters of the LWR, LLR, and condition factor of *Alburnus sellal* (Heckel, 1843), *Barbus karunensis* (Khaefi, Esmaeili, Geiger & Eagderi, 2017), *Capoeta aculeata* (Valenciennes, 1844), *C. coadi* (Alwan, Zareian & Esmaeili, 2016), *Chondrostoma regium* (Heckel, 1843), *Garra gymnothorax* (Berg, 1949), *Glyptothorax galaxias* (Mousavi-Sabet, Eagderi, Vatandoust & Freyhof, 2021) and *Turcinoemacheilus hafezi* (Golzarianpour, Abdoli, Patimar & Freyhof, 2013) from Bashar River. The results of the present study may be useful for biology and stock assessment of endemic species. In addition, given that two species, i.e. *T. hafezi* and *G. galaxias* have not been previously investigated, so this study can be beneficial for biological investigations in future studies.

Materials and Methods

During the summer of 2021, some 341 specimens were captured from 16 sampling sites (covering an area of 100 m^2 at each sampling) of the Bashar River, Kohgiluyeh and Boyer-Ahmad Province, Iran (Figure 1), by various types of fishing gear, electrofishing devices (Table 1).

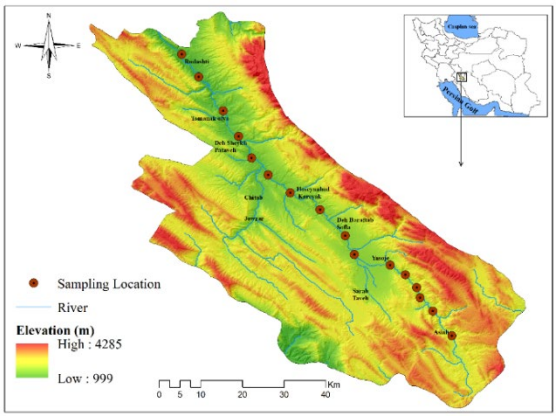

Figure1. Collecting stations of samples in the Bashar River, Kohkiluyeh and Boyer-Ahmad Province, Iran

Table 1. Geographical coordinates of the sampling site stations

Stations	Geographical coordinates	
1	30°32'37.30"N	51°42'38.25"E
2	30°33'29.74"N	51°41'43.82"E
3	30°33'57.64"N	51°37'32.27"E
4	30°34'27.89"N	51°41'07.17"E
5	30°38'13.75"N	51°37'31.41"E
6	30°38'56.86"N	51°36'52.33"E
7	30°40'10.40"N	51°32'06.19"E
8	30°40'36.08"N	51°31'59.63"E
9	30°46'01.12"N	51°27'36.81"E
10	30°46'30.17"N	51°07'36.16"E
11	30°51'20.51"N	51°20'33.42"E
12	30°51'47.15"N	51°20'13.38"E
13	30°55'26.49"N	51°17'13.19"E
14	30°58'31.91"N	51°15'10.58"E
15	30°01'55.42"N	51°13'15.48"E
16	31°02'25.53"N	51°13'02.58"E

All the procedures were performed based on the approved protocol guidelines and procedures employed by the Iranian Environmental organization. The species were identified according to Coad (2016) and Esmaeili et al. (2018).

Some morphometric characteristics were measured in the field including total length (TL), standard length (SL), and fork length (FL), using digital calipers to the nearest 0.01 cm. The specimens were weighed (W) to the nearest 0.01 g using an electronic balance. The length-weight relationship (LWR) was calculated using below mathematical equations (Froese, 2006):

$$W=aL^b$$

Where W= whole body weight (g), L= the total length (cm), *a* = intercept, and b = the slope of the regression line. The log-log plots of the length-weight pairs were used to find outliers (Froese et al., 2011). The following relationships were found using linear regression analysis: (a) FL versus TL; (b) SL versus TL as FL= a + bTL and SL = a + bTL, respectively (Mouludi-Saleh and Keivany, 2018). Also, the following formula was used to calculate the condition factor (Fulton, 1904; Nikmehr et al., 2021):

$$K= (W/L^3) \times 100$$

Where W= total body weight (g), L= total length (cm), and the scaling factor of 100 were used to bring the K close to the unit. To estimate whether the b-value is significantly different from the expected or theoretical value of 3 (i.e. b = 3), a student's t-test (ts) was performed. All analyses were performed in Excel v2019 and PAST v2.17b.

Results and Discussion

To the best of our knowledge, this is the first study to evaluate the length-weight relationships and the condition factors of the eight studied species in the study area. The descriptive statistics i.e. ranges of the total length (TL) and weight (W), calculated length-weight relationship parameters including a, b, r^2, and condition factor (K) of the studied species are presented in Table 2. Our finding indicated that b-value, coefficient of determination (r^2) and mean condition factor ranged 2.41-3.88, 0.83-0.95 and 0.66-1.35, respectively.

The values of a, b, and r^2 of the species and related statistics for LLRs are shown in Table 3. The b value for FL-TL ranged 0.884-0.978 and for SL-FL 0.875-0.977, respectively.

A total of 341 specimens, representing eight fish species, were analyzed and the first LWRs data are reported for *G. galaxias* and other studied species belonging to new localities. In LWRs parameter, the b value ranges between 2.5 and 3.5 (Froese, 2006) or 2-4 (Tesch, 1971). In this study, the b-values of the studied fish species were within the expected ranges. For example, the LWRs for *A. sellal* was $W= 0.0009L^{3.88}$ (r^2 = 0.93), for *C. aculeate* was $W= 0.06L^{2.41}$ (r^2 = 0.87) and for *T. hafezi* was $W= 0.007L^{2.9}$ (r^2 = 0.95).

Table 2. LWR data, regression parameters, 95% confidence limit (CL) and condition factor (K) for eight fish species in Bashar River of Iran during the summer season of 2021

		Total length (cm)	Total weight (g)		Regression parameters						Condition factor (Mean ± SD)	P	t-test	Growth pattern
Species	N	Min- Max	Min	Max	a	a 95% CL	*b*	b 95% CL	±SE	r^2				
A. sellal	43	6.99-15.03	1.57	31.4	0.0009	0.0004-0.0016	3.88	3.67-3.92	0.116	0.92	0.74±0.17	<0.05	51.86	positive allometric
B. karunensis	23	5.76-15.18	1.03	29.4	0.002	0.0005-0.014	3.40	2.75-3.62	0.165	0.83	0.74±0.22	<0.05	12.62	positive allometric
C. aculeate	47	10.86-20.12	15.04	93.5	0.060	0.03-0.11	2.41	2.19-2.67	0.012	0.87	1.35±0.25	<0.05	-22.11	negative allometric
C. coadi	83	4.26-23.59	1.11	144.3	0.018	0.017-0.03	2.69	2.48-2.86	0.232	0.87	0.90±0.34	>0.05	-0.248	isometric
C. regium	21	7.68-14.79	1.64	16.84	0.006	0.0023-0.02	2.86	2.39-3.28	0.133	0.90	0.48±0.12	<0.05	-23.2	negative allometric
G. gymnothorax	89	5.27-13.78	1.05	31.3	0.004	0.002-0.007	3.37	3.15-3.64	0.123	0.90	1.00±0.25	<0.05	25.43	positive allometric
G. galaxias	23	5.62-14.49	0.69	32.3	0.004	0.0005-0.015	3.34	2.8-4.15	0.118	0.90	0.90±0.20	<0.05	15.34	positive allometric
T. hafezi	12	3.69-5.28	0.35	0.9	0.007	0.005-0.013	2.90	2.51-3.12	0.128	0.95	0.66±.05	<0.05	2.21	negative allometric

N: number of species; Min: minimum; Max: maximum; a: intercept; b: slope; r^2: coefficient of determination

Table 3. Length–length relationships of TL, FL, and SL for eight species in Bashar River of Iran during 2021

Species	Total length	Fork length	Standard length	Equation	*b*	*a*	r^2
A. sellal	10.96±1.88	10.11±1.72	9.00±1.66	FL= a+b×TL	0.917	0.056	0.99
				SL= a+b×FL	0.924	-0.342	0.99
B. karunensis	10.76±2.41	10.12±2.14	9.09±2.19	FL= a+b×TL	0.923	0.177	0.99
				SL= a+b×FL	0.956	-0.579	0.99
C. aculeate	13.73±2.35	12.56±2.17	11.20±1.91	FL= a+b×TL	0.922	-0.102	0.99
				SL= a+b×FL	0.908	-0.208	0.98
C. coadi	13.19±4.30	12.08±3.95	10.61±3.46	FL= a+b×TL	0.917	-0.012	0.99
				SL= a+b×FL	0.875	-0.034	0.99
C. regium	11.06±2.73	10.01±2.46	8.73±2.23	FL= a+b×TL	0.903	-0.027	0.99
				SL= a+b×FL	0.903	-0.313	0.99
G. gymnothorax	8.53±1.88	8.05±1.79	7.15±1.71	FL= a+b×TL	0.952	-0.078	0.99
				SL= a+b×FL	0.947	-0.467	0.98
G. galaxias	10.66±2.24	9.75±1.98	8.95±1.94	FL= a+b×TL	0.884	0.324	0.99
				SL= a+b×FL	0.977	-0.581	0.99
T. hafezi	4.32±0.53	4.18±0.52	3.70±0.47	FL= a+b×TL	0.978	-0.050	0.99
				SL= a+b×FL	0.898	-0.057	0.99

The b-value, a, and r^2 parameters of the studied species in the previous studies are presented in Table 4. For example, the b-value for *A. sellal* reported to be 2.95 (Zare-Shahraki et al., 2020), Zamani-Faradonbe et al. (2018) reported a b-value of 3.11 for *G. gymnothorax* in Iranian basins, almost similar to our results i.e., 3.11. In Beheshtabad River (Tigris basin, Iran) b-value for *C. coadi* had been reported to be 2.91 (Keivany and Siami, 2020) and Zare-Shahraki et al. (2020) reported a b-value of 2.97 for *B. karunensis* from the Karun River system, southwestern Iran.

Table 4. Length-weight relationship parameters and condition factor (K) data about studied species in previous studies in Iranian Inland waters

Species	N	Sampling Site	*LW* parameters				References
			a	b	r^2	K	
A. sellal	1435	Karun River system	0.009	2.95	0.97	-	Zare-Shahraki et al. 2020
B. karunensis	25	Karun River system	0.01	2.97	0.98	-	Zare-Shahraki et al. 2020
C. coadi	1084	Karun River system	0.014	2.92	0.99	-	Zare-Shahraki et al. 2020
C. coadi	32	Iranian inland waters	0.018	2.80	0.99	-	Zareian et al. 2018
C. coadi	426	Beheshtabad River	0.02	2.91	0.97	-	Keivany and Siami, 2020
C. regium	135	Iranian inland waters	0.009	3.03	0.99	1.01	Abbasi et al. 2019
C. regium	-	Zayandeh River	0.009	3.21	0.97	-	Kashkooli et al. 2018
C. regium	335	Bibi-Sayyedan River	0.007	3.08	0.98	-	Keivany et al. 2016
C. regium	335	Beheshtabad River	0.008	3.10	0.94	-	Keivany et al. 2015
C. aculeata	50	Gamasiab River	0.06	2.92	0.99	0.85	Radkhah and Nowferesti, 2016
G. gymnothorax	45	Iranian basins	0.008	3.11	0.99	-	Zamani-Faradonbe et al. 2018

N: number of species

The reported LWR parameters for *C. regium* in different rivers of Iran ranged from 3 to 3.21 which was higher than those of the current study (Table 4). However, no LWRs and condition factor data were available for *G. galaxy* from the Iranian inland waters for comparison purposes.

The LWR data of fish species is a critical index to estimate population and biomass dynamics that play a key role in fisheries management evaluation including storage population, age at maturity, life period, and mortality (Jafari-Patcan et al., 2018; Sorosh Hadad et al., 2018). In addition, the length-weight relationships are not constant over the year and biological factors, seasons, ecological properties of the study areas, sex, gonad maturity, stomach fullness, health, and even sampling can affect the LWR parameters (Tesch, 1971; Bagenal and Tesch, 1978; Froese, 2006; Kamal et al., 2009; Suiçmez et al., 2011; Jalili et al. 2015), and also environmental conditions of habitats such as temperature and photoperiod (Keivany and Soofiani, 2004; Hasankhani et al., 2013).

Generally, a b value >3 indicate positive allometric growth, though the value can vary between 2.5 and 4, depending on the changes in fish shape, age, season, dietary behavior, competition, feeding, habitat geographical location and growth (Özcan, 2011; Suiçmez et al., 2011; Esmaeili et al., 2014). In our study, there were positive allometric growth (A+) patterns for *A. sellal* (b= 3.88), *B. karunensis* (b = 3.40), *G. gymnothorax* (b = 3.37), and *G. galaxias* (b = 3.34), and negative values for *C. aculata* (b = 2.41), *C. regium* (b = 2.86) and *T. hafezi* (b = 2.90), while *C. coadi* had isometric growth pattern.

The condition factor index indicated the relationship between biological and non-biological factors on fish physiology that can be used to compare various populations in different conditions and life cycles (Bagenal and Tesch, 1978;

Tran et al., 2021). With regards to the importance of growth studies in effective management and conservation of fish populations in this aquatic ecosystem, which is affected by environmental and human pollution, the present study may help to design and perform better conservational plans and studies for endemic species (González Acosta et al., 2004; Kashkooli et al., 2018). Condition factors (K) > 1 (*C. aculeate* and *G. gymnothorax*) can indicate a proper condition in their habitats (Radkhah and Eagderi, 2015) for these species inhabiting the Bashar River. Variation of the condition of factor is related to environmental parameters, such as seasonal changes of the gonads and nutritional conditions (Biswas, 1993; Muchlisin et al., 2010; Tran et al., 2021).

This study presented the first basic information on length-weight relationship (LWRs), length–length relationships (LLRs), and Condition factors (K) data for eight fish species from the Bashar River, therefore, these results may be useful for future fisheries management, ecological investigations, conservation and fish population dynamic studies.

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Authorship Contributions

All authors certify that they have taken part sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Material preparation and investigation were performed by Saeid Shahbazi Naserabad. Data analysis was done by Saeid Shahbazi Naserabad and Hadi Poorbagher. Data curation was done by Soheil Eagderi. The writing/editing was carried out by Hadi Poorbagher, Soheil Eagderi and Saeid Shahbazi Naserabad. Hadi Poorbagher and Soheil Eagderi supervised and Project administration of this study. Also, all authors have read and approved the article.

Conflicts of interest/Competing interests

There is no conflict of interest to declare.

Ethics approval

The authors confirm that all procedures performed in their study involving animals were in accordance with the ethical standards. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All experiments were performed following the protocol approved by the committee of ethics of the faculty of sciences of the University of Tehran (85A1672; 20 May 2021).

Data availability

The datasets in this study are available from the corresponding author on reasonable request. All data and materials are available for publication.

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CASE REPORT **OLGU SUNUMU**

Abnormal hermaphroditic shark *Squalus blainville* (Risso, 1826) (Chondrichthyes: Squalidae) from the Aegean Sea

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Abstract: Eighty-six specimens of longnose spurdog *Squalus blainville* were examined for determination of some bio-ecogical features. During the study, one of the specimens was determined as a hermaphrodite with a clasper and yolk sac formation. This very rare case in elasmobranch is a report of hermaphroditism recorded from the Aegean Sea.

Keywords: Abnormality, reproduction biology, longnose spurdog, demersal shark

INTRODUCTION

Chondrichthyes have wide of reproductive type, including oviparity, aplacental and placental viviparity, with several variations while all species are considered gonochoristic. Hermaphroditism is defined by Atz (1964) as the existence of both sexes in a single specimen and is very rare in elasmobranchs. At the same time, examples of hermaphroditism have been observed in different elasmobranch species [longhead catshark *Apristurus longicephalus* (Iglesias et al., 2005), bigeye houndshark *Iago omanensis* (Barnes et al., 2018), portjackson shark *Heterodontus portusjacksoni* (Jones et al., 2005), brown lantern shark *Etmopterus unicolor* (Yano and Tanaka, 1989), southern lantern shark *Etmopterus granulosus* (Irvine et al., 2002), velvet belly *Etmopterus spinax* (Costa et al., 2013), lesser spotted dogfish *Scyliorhinus canicula* (Murray and Baker, 1924), blue shark *Prionace glauca* (Pratt, 1979), blacktip shark *Carcharhinus limbatus* (Hendon et al., 2013), pelagic stingray *Pteroplatytrygon violacea* (Ribeiro-Prado et al., 2009), brazilian guitarfish *Rhinobatos horkelii* (Gianeti and Vooren, 2007), multispine skate *Bathyraja multispinis* (Scenna et al., 2007), Black Dogfish, *Centroscyllium fabricii* (Yano, 1995), Blackmouth Catshark, *Galeus melastomus* (D'Iglio et al., 2022), longnose spiny dogfish *Squalus blainvillei* (Kousteni and Megalofonou, 2010)].

The longnose spurdog *Squalus blainvillei* (Risso, 1826) is a benthic shark with aplacental viviparous positioned at the top of the trophic web that is widely distributed in the Eastern and Western Atlantic, Mediterranean (Anastasopoulou et al., 2017; Serena, 2005; Dunn et al., 2013). Squalids are not the target species in Mediterranean fisheries and are often considered by-catch (Serena et al., 2009).

The present paper details a very rare occurrence of abnormal hermaphroditism in *Squalus blainville* specimen from the Aegean Sea.

MATERIAL AND METHODS

The eighty-six specimens of *S. blainville* were collected from the fish market in İzmir on July 2019 for bio-ecological studies. These specimens, which were transferred to Izmir Fish Market, were caught by bottom trawl. One of these specimens showed abnormalities in the reproductive system with having both clasper and female specific yolk sac (Figure 1). The total length, total body weight and lengths of clasper (from the anterior end of the cloaca to the distal tip of the clasper) of the abnormal hermaphrodite specimen were recorded. This sample was stored in 4% formalin solution after photographing. The collected specimen was deposited in the Fish Collection Centre of İzmir Kâtip Çelebi University (IKC.PIS.1259).

Figure 1. Abnormal Hermaphroditic *S. blainville* of 47 cm TL, Ys, Yolk sac; C, claspers

RESULT AND DISCUSSION

The specimen with 47 cm total length was detected as a male with claspers due to external morphologic examination. However, when it was dissected, a female gonad formation with yolk sac had been found which signs of hermaphroditism (Figure 1). There are two yolk sacs with a diameter of 2.3-2.7 cm.

In cartilaginous fish, the claspers, which are unique to males, are structures derived from the pelvic fin that allow the transfer of sperm to the female. Unmatured males, the clasper length is shorter than the pelvic fin, but exceeds the pelvic fin length in mature males (D'Iglio et al., 2022). It was found that the claspers of the hermaphrodite specimen were less developed than other same size mature males (Table 1).

Table 1. Comparisons of measurements between recorded abnormal hermaphrodite specimens and twelve mature males of *Squalus blainville*

	Hermaphrodite	Mature males	
		Mean	Range
Total length (mm)	470	470.4	420-530
Total weight (g)	486	475.91	348-684
Clasper length (mm)	15.76	43.31	30.98-55.72

Similarly, Iglesias et al. (2005) and Pratt (1979) reported that the clasper length of the hermaphrodite female specimens is smaller than gonochoristic males *Apristurus longicephalus* and *Prionace glauca,* respectively and Iglesias et al. (2005) used the definition of "functional female" for these hermaphrodite specimens. In addition, Barnes et al. (2018) has pointed out the simultaneous clasper formation in the presence of developed female sexual organs for the hermaphrodite *Iago omanensis* species, which is in concordance with our findings. Differently from this, Kouesteni and Megalofonou (2010) reported a *S. blainville* specimen with an internal male reproductive organ without claspers. Yano (1995) detected abnormal hermaphroditism in only 4 of the 2600 *E. fabricii* samples examined. Until now, the case of hermaphroditism has been reported in seven species of Squalidae (Irvine et al., 2002; Kouesteni and Megalofonou, 2010) and mostly seen in Squaliformes among Chondrichthyes (Gianeti and Vooren, 2007). Hermaphroditism is described as by abnormal and normal hermaphrodite by Atz (1964) and Iglesias et al (2005), while 'pseudo-hermaphrodite' and 'true hermaphrodite' terms are described by Irvine et al. (2002). Considering these, our findings are consistent with the definition of abnormal or pseudo-hermaphrodite.

The reasons for hermaphroditism in chondrichthyans are complex and difficult to explain (Atz, 1964). Hermaphroditism in sharks may be a sign of developmental anomaly, but it is known that anthropogenic contamination (man-made chemicals, including organochlorine pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, surfactants and plasticisers) affects the reproductive behaviour of marine organisms (Colborn and Clement, 1992; Sumpter et al., 1996; Devlin and Nagahama, 2002; De Metrio et al., 2003). In addition, compounds containing even in low concentrations synthetic chemicals such as birth control pills are known to cause sex change in wild fish populations (Rana et al., 2015).

The Mediterranean elasmobranch community is thought to suffer from strong depletion because of overfishing. Besides, considering the possibility of the increasing number of effects, such as anthropogenic contamination that are aforementioned, would make elasmobranch populations more fragile.

Spurdogs are benthic species mostly living on the soft bottom in inshore and offshore waters and are likely to accumulate pollution. In conclusion, these abnormalities in the reproductive biology of demersal sharks, such as *S. blainville* should be subject to further investigations.

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AUTHORSHIP CONTRIBUTIONS

Uğur Özden: Sampling, writing – original draft preparation. Erhan Irmak: Sampling, methodology, conceptualisation, reviewing, editing.

CONFLICTS OF INTEREST

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

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

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

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DERLEME **REVIEW**

Sıfır atığa doğru: Su ürünleri yetiştiriciliğinde sürdürülebilir atık yönetimi

Towards zero waste: Sustainable waste management in aquaculture

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Öz: Su ürünleri yetiştiriciliğine artan gereksinime paralel olarak artan üretim miktarı, işletmelerin atık miktarında da doğrusal bir artışa neden olmaktadır. Bu durum, yoğun su ürünleri yetiştiriciliğinde atık suların etkin bir şekilde arıtımı konusunu öne çıkarmaktadır. Günümüzde su ürünleri yetiştiriciliği kaynaklı atık suların arıtımında, geleneksel fiziksel ve kimyasal yöntemlerin yerine, ekosisteme duyarlı ve besin zincirinin farklı seviyelerinde üretimi devreye sokan sistemler daha fazla benimsenmeye başlanmıştır. Atık su içinde bulunan besin elementlerinin farklı bir gıda üretimiyle döngüye katılması, sıfır atık yaklaşımının esasını oluşturmaktadır. Su ürünleri yetiştiriciliği yapan işletmelerin çıkış sularını kullanan entegre üretim sistemleri (örneğin; akuaponik) atık içinde bulunan besin elementlerinin tekrar geri kazanımını sağlamaktadır. Entegre sistemler, su ürünleri atık sularının arıtımında hem atık içinde besin elementlerinin biyomasa dönüşümünü sağlamakta hem de çevreyle uyumlu arıtım yöntemi olarak değer kazanmaktadır. Su ürünleri yetiştiriciliği çıkış suları, entegre sistemler yardımıyla balık yanında ikili (örneğin; balık+midye) ya da üçlü (örneğin; balık+midye+yosun) yetiştiricilikte kullanılabilmektedir. Su ürünleri yetiştiriciliği çıkış sularının yapay sulak alanlarda ya da bitki lagünlerinde belli besin elementlerinin azaltılması amacıyla kullanılması da sürdürülebilir yetiştiricilik ve atık su arıtımı için uygun yaklaşımlar arasındadır.

Bu derleme çalışmasında, su ürünleri yetiştiriciliğinden gelen atık suların arıtımına yönelik mevcut ve ekolojik bazda yenilikçi teknolojiler ele alınmış; sürdürülebilir su ürünleri yetiştiriciliği ve çevreyle uyumlu atık arıtımı için yapay sulak alanlar ve entegre multi-trofik yetiştiricilik sistemlerinin pratikte kullanımının artırılması gereği vurgulanmıştır.

Anahtar kelimeler: Su ürünleri yetiştiriciliği, atık su arıtımı, akuaponik, entegre multi-trofik sistemler

Abstract: Increases in aquaculture production due to higher demand for aquatic foods result in an increase in the amount of aquaculture wastewater. This situation highlights the need for the effective treatment of wastewater in sustainable aquaculture. Today, instead of traditional physical and chemical methods in the treatment of wastewater originating from aquaculture, ecosystem-sensitive and by-product-oriented systems have begun to be adopted. The main principle of the zero-waste approach is the recycling of the nutrients in the wastewater to produce another food. In this new innovative approach, the production of other organisms from the different trophic levels using the wastewater of aquaculture in the integrated multi-trophic systems (such as aquaponics) is possible to recycle the nutrients. It has been considered the integrated multi-trophic systems (IMTA) more valuable as these systems can be used both in environment-friendly wastewater treatment and in the conversion of nutrients in wastewater to biomass. The nutrients such as nitrogen and phosphorus in aquaculture wastewater can be utilized to produce two organisms (i.e. fish+mussel) or three organisms (i.e. fish+mussel+seaweed) through IMTA. Aquaculture wastewater can be used to reduce the nutrients in constructed wetlands and plant lagoons representing the reasonable approach for sustainable aquaculture and wastewater treatment.

Here, the innovative approach to sustainable aquaculture wastewater treatment was reviewed for the current and innovative technologies. It was emphasized that the need for environment-friendly wastewater treatment Technologies such as aquaponics, enlargement of constructed wetlands, or increase in using the integrated multi-trophic production systems (IMTA) in practice are recommended for sustainable aquaculture.

Keywords: Aquaculture, wastewater treatment, aquaponics, integrated multi-trophic systems

GİRİŞ

Su ürünleri yetiştiriciliğinde 'suyun kendisinin üretim ortamı' olması nedeniyle yeterli ve sağlıklı suyun muhafazası, diğer tarımsal faaliyet alanlarından daha fazla anlam taşımaktadır. Sürdürülebilir su ürünleri yetiştiriciliği, öncelikle su kaynaklarının akılcı kullanımına ihtiyaç duymaktadır (Yavuzcan vd., 2020). İklim değişikliğinin beklenen etkileri, ekonomik belirsizlikler ve doğal kaynaklar üzerindeki baskı gibi olumsuz koşullar altında, artan dünya nüfusunun nasıl besleneceği önemli ve global bir sorun olarak çözüm beklemektedir. Bu çerçevede insan tüketimine sunulacak su ürünleri yetiştiriciliğinin stratejik önemi daha iyi kavranabilir. Yetiştiricilik yoluyla elde edilen su ürünleri gıda güvencesine ve sağlıklı beslenmeye sağlayacağı katkılar bağlamında vazgeçilmez bir gıda üretim kolu olarak varlığını büyüyerek devam ettirecektir. Bu bağlamda, su ürünleri yetiştiriciliğindeki artış, çıkış sularının askıda katı madde (AKM), toplam azot (TN) ve toplam fosfor (TP) yükünde de artışla sonuçlanmaktadır. Karada kurulu su ürünleri işletmesi çıkış sularının, sucul ekosisteme olan etkileri arasında, alıcı ortamın su-sediment kalitesinin bozulması yanında bentik organizma

ve floranın bozulması ile sonuçlanan ötrofikasyon önemli bir yer tutmaktadır. Özellikle artan yoğun yetiştiricilik faaliyetleri sonucuna paralel olarak, işletmelerin çıkış suları yönetiminde uygulanmakta olan birtakım uygulamalar ve/veya arıtım teknolojileri de sürdürülebilir arıtım teknolojilerine doğru evrilmektedir.

Geleneksel atık su arıtımında kullanılan fiziksel, kimyasal ve biyolojik yöntemler, su ürünleri yetiştiricilik sistemlerine de uygulanmaktadır. Bu bağlamda, son yıllarda çıkış suyu yerine 'atık su' terimi de sıkça kullanılır hale gelmiştir. Atık sularının arıtımında, farklı ülkelerde farklı arıtım teknikleri kullanılmakta ve bazı yasal düzenlemelerle de izlenmektedir. Biyolojik yöntemler ucuz ve çevre dostu yöntemler olarak ön plana çıkmaktadır (Adekanmi vd., 2020; Sikder vd., 2016). Günümüzde sürdürülebilir su ürünleri yetiştiriciliği ve bu yetiştiricilik faaliyetinden kaynaklanan atıkların sürdürülebilir arıtımı için yenilikçi yaklaşımlar çerçevesinde atık içinde bulunan besin elementlerinin döngüsel olarak bitkiler aracılığıyla yararlı besin elementi formuna dönüştürülmesi ve sonuçta sıfır atığa ulaşma anlayışı benimsenmektedir. Atık içinde bulunan besin elementlerinin besin zincirinin farklı seviyelerindeki organizmaların üretiminde kullanımı entegre multi-trofik sistemler olarak ilgi görmektedir. Bu sistemlere en güncel örnek akuaponik ve özellikle deniz için entegre multi-trofik sistem (IMTA) verilebilir. Belirtilen teknolojiler yeni olmasına rağmen çıkış sularındaki besin elementi yükünün kontrolünde başka bir deyişle besin maddelerinin uzaklaştırılmasında yapay sulak alanların kullanımı da çevreyle uyumlu entegre sistemlerin farklı bir şeklidir (Cripps ve Bergheim, 2000; Hernandez vd., 2004; Sindilariu, 2007; Tom vd., 2021).

Bu çalışmada, sürdürülebilirlik ekseninde su ürünleri atıklarının arıtımına ilişkin güncel uygulamalar ile doğayla uyumlu, sıfır atık yaklaşımı çerçevesinde döngüsel yetiştiricilik sistemlerinin atık su arıtımında kullanılabilirliği anlatılmıştır. Özellikle atıklarda bulunan değerli besin elementlerinin yeniden üretim döngüsüne katılmasına ilişkin yenilikçi yaklaşımlar vurgulanmıştır.

SU ÜRÜNLERİ YETİŞTİRİCİLİĞİ ATIK SULARININ İÇERİĞİ VE MEVCUT ARITIM UYGULAMALARI

Atık sulardaki biyo-katılar

Su ürünleri yetiştiriciliğinde atık suların ana bileşenlerini yemler, kimyasallar ve patojenlerin de dahil olduğu tüm mikroorganizmalar oluşturmaktadır. Atıklar katı ve çözünmüş formları kapsamakta; katı atıklar yem, tüketilmemiş yem ve dışkılardan kaynaklanmaktadır. Katı atıkların bir bölümü askıda kalırken bir kısmı çökmektedir. Atık sulardaki çözünmüş formlar ise besin elementlerini kapsamakta olup, azot (N) ve fosforun (P) kaynağını genellikle tüketilmeyen yemler ve balık dışkısıdır (Ahmad vd., 2022).

Genel olarak, bir uygulamaya tabi tutulmamış çıkış sularındaki askıda katı madde konsantrasyonları, yetiştiricilik sisteminin yönetimine bağlı olarak değişiklik göstermektedir. Katı maddelerin uzaklaştırılması işlemi sedimentasyon, kum veya mekanik filtrasyon şeklinde gerçekleştirilirken, sualtı biyofiltreler, damlatmalı filtreler, dönen biyolojik kontaktörler ve akışkanlı yatak reaktörleri de organik maddenin oksidasyonu, nitrifikasyon veya denitrifikasyon amacıyla uygulanmaktadır (Sindilariu, 2007). Adı geçen separatör ve damlatmalı filtrelerin etkinliği askıda katı madde için %75, sualtı filtrelerin ise %84 olarak bildirilmiştir (Tablo 1). Damlatmalı filtre ve ozonlamanın birlikte kullanıldığı sistemlerde ise, amonyum azotu, nitrit – azotu ve nitrat azotu için etkinliğin sırasıyla (%) 31; 13,2 ve 50 olduğu belirtilmiştir (Jegatheesan vd., 2011).

Tablo 1. Su ürünleri yetiştiriciliği atık sularında farklı filtre sistemlerinin etkinliği (Jegatheesan vd., 2011)

Table 1. The efficiency of different filtering methods of aquaculture wastewater (Jegatheesan vd., 2011)

Arıtım yöntemi	İşlem	Etkinlik (%)
Separatör ve damlatmalı filtre	Nitrifikasyon ve askıda katı madde uzaklaştırma	Askıda katı madde:75 KOİ: 2-25
Damlatmalı filtre	Nitrifikasyon- denitrikasyon	90
Akışkan yataklı filtre	Nitrifikasyon- denitrikasyon	-
Damlatmalı filtre	Nitrifikasyon-denitrifikasyon	-
Sualtı filtre	Denitrifikasyon	84
Damlatmalı filtre ve ozonlama	Azot uzaklaştırma	NH_4-N$^+$=31 NO_2^--N=13.2 NO_3^--N=50

Atık sularda bulunan besin elementleri

Çıkış sularındaki besin elementi (azot, fosfor gibi) yükü, yetiştiricilik sistemine bağlı değişim göstererek alıcı ortamlarda olumsuz etkilere yol açabilen özellikle ötrofikasyonu tetikleyen bir unsur olarak karşımıza çıkmaktadır. Kafeslerde balık yetiştiriciliğinde, yem dönüşüm oranı 1.5 ve N-P düzeyi %7.2-%0.9 olan yemler kullanıldığında; ton/balık başına sırasıyla; 61 çözünmüş –N, 2.2 kg çözünmüş –P ve 17 kg partiküler-N, 7.3 kg partiküler-P'un alıcı ortama bırakıldığı (Ackefors ve Enell, 1990) tespit edilmiştir. Kesikköprü Baraj Gölü'nde geleneksel pelet yem kullanılarak yapılan gökkuşağı alabalığı yetiştiriciliğinde, azot yükü 56.00-62.92 kg/ton balık ve fosfor yükü 10.66-12.17 kg/ton balık; ekstrude yemin kullanıldığı kafeslerde ise azot yükü 33.47-25.97/ton balık ve fosfor yükü 7.32-7.96 kg/ton balık olarak tahmin edilmiştir. Özellikle azot yükündeki önemli düzeydeki azalma, ekstrude yemin sindirilebilirliğinin de yüksek olması bağlamında çevresel etki bakımından daha olumlu olduğu vurgulanmıştır (Aşır ve Pulatsü, 2008). Çıkış suyunda katı maddelerin genel olarak, %7–%32'si toplam azot (TN), %30–%84'ü toplam fosfor (TP) olarak bildirilmiştir. Besin elementleri ile ilgili kritik nokta,

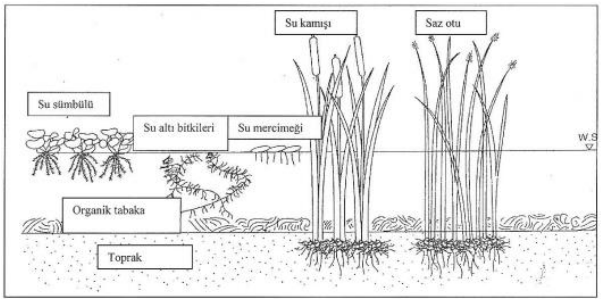
çözünmüş haldeki besin elementlerinin, partikül ayırma teknikleri ile uzaklaştırılamamasıdır (Cripps ve Bergheim, 2000).

Çıkış sularındaki katı madde ve besin elementi miktarı, yem kaynaklı atıklar ile doğrusal bir artış göstermektedir ve doğal olarak yemde bulunan besin elementlerinin miktarı ve kompozisyonu da etkilidir (Pulatsü ve Kaya, 2016). Örneğin, yüksek enerjili ekstrude yemlerin kullanımı, azotlu atıkların azaltılması için yemdeki sindirilebilir proteinin artırılması ve yeterli düzeyde sindirilebilir fosfor içeriğinin ayarlanması gibi girişimlerle çıkış sularının kalitesi iyileştirilebilmektedir (Cho ve Bureau, 2001).

SU ÜRÜNLERİ YETİŞTİRİCİLİĞİ ATIK SULARINDA ÇEVREYLE UYUMLU ARITIM UYGULAMALARI

Yapay sulak alanlar

Antropojenik kaynaklı ötrofikasyonu önlemede kullanılan yapay sulak alanların işlevleri, su ürünleri işletmeleri çıkış suyunun yönetiminde de benzer olup ana prensip, sudaki besin elementlerinin bitkiler tarafından kullanılmasıdır (Şekil 1). Çeşitli biyotik ve abiyotik prosesler, sulak alanlardaki kirleticilerin uzaklaştırılmasını sağlamaktadır. Nitrifikasyon ve denitrifikasyon gibi mikrobiyal mineralizasyon ve transformasyon süreçleri ile bitkiler tarafından alım işlemi temel biyotik prosesler iken, kimyasal çökme, sedimentasyon ve substrat adsorpsiyonu abiyotik proseslerdir.



Şekil 1. Yapay sulak alan kesiti (Ayaz vd., 2011)

Figure 1. Constructed wetland section (Ayaz et al., 2011)

Çıkış sularının sulak alanlara iletilmeden önce birincil çöktürme sisteminden geçirilmeleri zorunluluğu ve nispeten geniş bir araziye ihtiyaç duymaları gibi bazı dezavantajları olsa da, düşük yatırım maliyeti- enerji tüketimi ve bakım ihtiyaçları, görsel zenginlik, doğal yaşam habitatına destek sağlaması gibi avantajları da söz konusudur. Özellikle sulak alanların büyüklüğünün belirlenmesi önem taşımakta olup, kirlenmiş işletme çıkış sularının arıtımı için havuz alanından 0.7-2.7 kat daha fazla bir sulak alan büyüklüğüne ihtiyaç duyulduğu belirtilmiştir (Lin vd., 2002). Çıkış suları ile besin elementi yükleme oranı 30 kg/m2 yıl olduğunda, askıda katı maddenin %95, azotun %80 ve fosforun %90 oranında uzaklaştırıldığı (Miller ve Simmens, 2002) ifade edilmiştir. Sindilariu vd. (2007) tarafından, toplam askıda katı madde için yüzey-altı akışlı sulak alanlarda, standart mekanik arıtım etkinliğinin mikro-eleme (ayırma) uygulaması ile benzer olduğu belirtilmiştir. Hidrolik yükleme hızı 1.8–13.5 cm/gün olan bir sulak alanda ortalama uzaklaştırma etkinliği; NH4–N için %86–98, NO2–N için >%99.8, NO3–N için %2–99 ve toplam inorganik azot (TİN) için %95–98 olarak bildirilmiştir. Sulak alan, kimyasal oksijen ihtiyacı-KOİ (%25–%55), askıda katılar (%47–%86) ve klorofil-a (%76–%95) gibi parametrelerin uzaklaştırılmasında oldukça etkin olmuştur (Lin vd., 2002). Sindilariu vd. (2009), yapay sulak alanların toplam fosfor, biyokimyasal oksijen ihtiyacı, kimyasal oksijen ihtiyacı, toplam askıda madde ve toplam amonyak azotu için %35'den fazla arıtım etkinliğine sahip olduğunu belirtmişlerdir. Jegatheesan vd. (2011) yapay sulak alanların NH4-N arıtımında %57-66, NO2-N arıtımında %83-94 ve NO3-N arıtımında %68 düzeyinde etkili olabileceğini saptamışlardır. Tom vd. (2021) ise azot bileşikleri içeren çıkış sularının arıtımı için önerilen yapay sulak alanların etkinliğinin oldukça yüksek olduğunu (NH4-N: %86-98 ve NO2-N: %99) belirtmişlerdir.

Su ürünleri işletmeleri çıkış sularının arıtımı için kullanılan filtrelerin ve sedimentasyon havuzlarının çözünmüş besin elementleri ve tuzlar açısından yeterli etkiye sahip olmadığı bilinmektedir (Lymbery vd., 2007). Farklı konsantrasyonlarda toplam azot, toplam fosfor ve sodyum klorür bulunan gökkuşağı alabalığı işletmeleri çıkış sularını, Juncus krausii (sulak alanlarda yetişen bir bitki) barındıran yüzey-altı akışlı yapay sulak alana bırakılan bir çalışmada, yaklaşık bir ay sonra, toplam azot, toplam fosfor ve sodyum klorür miktarları sırasıyla %69, %88.5 ve %54.8 oranında azalmış ancak *J.kraussii*'nin büyümesi, tuzluluk konsantrasyonundan olumsuz etkilendiğinden yüksek tuzluluğa tolerans gösteren daha farklı türlerin kullanımı önerilmiştir. Ayrıca tuzluluk artışı toplam azotun uzaklaştırılmasında etkili bulunmamasına karşın toplam fosfor uzaklaştırma etkinliğini azaltmaktadır (Lymbery vd., 2007).

Danimarka gibi bazı Avrupa ülkelerinde su ürünleri yetiştiricilik ünitelerinin çıkış noktalarında bitki lagünleri oluşturulmaktadır. Bitki lagünlerinde üretilen bitkilerin ticari bir değeri yoktur. Bitki lagünleri, zemininde yabani bitkilerin gelişebileceği toprak tabanlı havuzları, kanalları ifade etmektedir. Bitki lagünleri, nitratın azota dönüşümü ve bitki biyoması için fosfor alımı açısından önemlidir. Fakat bitki lagünlerinin dibinde ya da dibe yakın kısmında anaerobik şartlar oluştuğunda amonyağın nitrata dönüşümü yeterince etkin olmamaktadır (Jokumsen ve Svendsen, 2010). Su ürünleri işletmeleri çıkış sularının yapay sulak alanlardan veya bitki lagünlerinden geçirildikten sonra doğal sulara ulaşması çevreyle uyumlu bir teknik olarak yaygınlaştırılabilir.

Akuaponik sistemler

Akuaponik, hidroponik ile kapalı devre yetiştiricilik sistemi (RAS) elementlerini bir araya getiren bir sistemdir. Geleneksel hidroponik sistemde (topraksız bitki üretimi) bitkilerin gerekli besin elementlerini alabilmesi için mineral gübre gereklidir. Akuaponik sistemde ise, içinde balık bulunan, bitki gelişimi için yeterli düzeyde besin maddesince zengin balık dışkısı içeren su kullanılır (Yavuzcan Yildiz vd., 2017). Bu kombinasyonun

temel avantajı, bol besin elementi bulunan suyu, geleneksel su ürünleri yetiştiriciliğinde olduğu gibi periyodik olarak temiz su ile değiştirme zorunluluğunun olmamasıdır. Temel ilke olarak, sistemde bulunan bitkilerin gelişiminde yararlanılan besin elementlerin en az %50'sinin balık atığı ya da tüketilmemiş yemden gelmesi durumunda üretim sistemi "entegre multi-trofik su ürünleri yetiştiricilik sistemi" veya "akuaponik" olarak sınıflandırılabilmektedir (Palm vd., 2019). Akuaponik sistem, balık, mikroorganizma ve bitkiler arasında bir simbiyozis oluşumuna zemin sağlar. Bu simbiyozis, su ve besin elementlerinin geri kazanımlı kullanımı ile sürdürülebilirlik olgusuna katkı sağlar (Şekil 2). Bu sinerjistik etkileşim içinde su ürünleri yetiştiriciliğinin olumsuz ekolojik etkisinden ileri gelen zayıflık, güçlü yöne dönüşür. Akuaponikte oluşturulan kombinasyon ile besin elementi içeren atık çıktısı minimize edilir (Goddek vd., 2015). Ekolojik kapsamda akuaponik sistemler sürdürülebilir gıda üretimine ve yetiştiricilikten çıkan atıkları azaltma konusuna iyi bir örnek teşkil etmektedir (Turcios ve Papenbrock, 2014).

Şekil 2. Akuaponik sistem (Palm vd., 2019)

Figure 2. Aquaponic system (Palm et al., 2019)

Bitkiler gelişimleri için C, H, O, N, P, K, Ca, ve Mg gibi makro besin elementlerine ve Fe, Cl, Mn, Zn ve Cu gibi mikro besin elementlerine ihtiyaç duyarlar. Hidroponik sistemlerde bu elementlerin oranı iyi bilinmektedir ve C, H, ve O hariç iyonik formda sisteme eklenmektedir. Akuaponik sistemde balık dışkısının dönüşümünü sağlayacak mikroorganizma topluluklarına da gereksinim bulunmaktadır. Akuaponik sistemde birkaç farklı besin elementi döngüsünden söz edilebilir, fakat en fazla çalışılmış ve anlaşılmış olan, biyofiltre seviyesinde olan azot döngüsüdür. Bu döngüde, azot üç farklı forma girebilir: amonyak (NH3), nitrit (NO2) ve nitrat (NO3). Amonyak, balıktan gelen ana boşaltım ürünüdür. Hem iyonize olmamış amonyak hem de nitrit çok düşük dozlarda bile suyun pH'sına bağlı olarak balık için toksik olabilir. Nitrifikasyon sürecinde bazı ototrofik bakteriler (öncelikle Nitroso-bacter, örneğin: *Nitrosomonas* sp.) amonyağı nitrite ve nitriti nitrata (öncelikle *Nitro-bacter*, örneğin: *Nitrospira sp.*, ve *Nitrobacter sp.*) yükseltgenmektedir (Sanchez, 2014; Thorarinsdottir, 2015). Azot dönüşümü sudan amonyağın eliminasyonunu sağlar. Nitrat, belirtilen biyodönüşümün bitki için yararlı olabilecek son ürünüdür. Nitrat, çok yüksek dozlar söz konusu olmadığı sürece balık için toksik değildir. Akuaponikte azotlu ürünler bitki faktörüyle birlikte ele alınır (Sanchez, 2014; Yavuzcan Yildiz vd., 2017). Kapalı devre yetiştiricilik sistemlerinde (RAS) biriken atığın yok edilmesi de önemli bir sorundur. Resirküle/dolaşımlı sistemler, çevreye bırakılan atığın daha az hacimde olduğu sistemler olarak ön plana çıkarılmaktadır. Bu sistemlerde atık hacminin az olmasına karşın birim deşarj hacmine düşen kirlilik yükü (organik madde, çözünmüş besin elementleri) daha fazladır. Bu tip bir konsantre deşarj çevre için daha tehlikelidir. Akuaponik sistemlerde ise bu besleyici elementlerin önemli bir kısmı bitki tarafından kullanılır, böylelikle çevreye bırakılan atık su çok daha az besin elementi içerir. Lennard ve Leonard (2006), akuaponik sistem (çakıltaşı sistemi) kullanıldığında, sadece balık (Tatlısu morinası, *Maccullochella peelii*) üreten sistemlere göre nitrat birikiminin %91 civarında azaldığını kanıtlamışlardır (Tablo 2). Ancak, akuaponik sistem tiplerine (çakıl gibi maddelerin kullanıldığı dolgulu basit teknik, besin film tekniği ya da sal tekniği gibi) bağlı olarak besin elementi uzaklaştırma başarısı da değişmektedir.

Tablo 2. Akuaponik ve sadece balık üretilen sistemde nitrat birikimi (Lennard ve Leonard, 2006)

Table 2. Nitrate deposition in aquaponic system and only-fish culture system (Lennard and Leonard, 2006)

Parametre	Sadece balık (tatlısu morinası) yetiştiriciliği	Akuaponik (çakıl taşı dolgulu, basit teknik)
Balık yem değerlendirme katsayısı	1.01±0.08	1.07±0.07
Marul ürünü (kg/m^2)	-	5.05±0.25
NO_3 birikimi (mg/l)	51.23±0.58	4.63±2.85
NO_3 uzaklaştırma (%)	-	90.9

Akuaponik sistemde bulunan çözünmüş besin maddeleri bitkiler tarafından kullanıldığından biyolojik ve kimyasal filtrasyon ihtiyacı azalır. Bu çerçevede, su kalite yönetiminin farklı bir boyutta incelenmesi gerekir. Balık dışkısından gelen çözünmüş halde bulunan besin elementlerinin bitki gelişimi için kullanılabilirliği ve bitki biyomasını artırdığı, bağlantılı olarak gıda üretiminde oluşan çevresel etkinin azaltılabileceği bilinmektedir. Akuaponik balık ve bitkinin birlikte üretildiği entegre bir sistem olduğundan balık yetiştiriciliğinden gelen besin elementi miktarı ile bitkinin alacağı besin elementlerinin dengelenmesi bakımından balık türü ile bitki türü uyumu önemlidir. Akuaponik sistemde 1 kg balık yetiştiriciliği için harcanan yeme karşılık çıkış suyunda bulunan besin elementleriyle 7 kg bitki bioması üretilebilmektedir (Wilson, 2005).

Su ürünleri yetiştiriciliğinde kullanılmış çıkış suyundan inorganik azot ve fosfat uzaklaştırılmasında, akuaponik performansı, sistemde su ıspanağı (*Ipomoea aquatica*) ve yeşil hardal (*Brassica juncea*) bitkilerinin üretimi için incelenmiştir. Su ıspanağı üretilmesi durumunda toplam amonyak azotu (NH3-N), nitrit azotu (NO2-N), nitrat azotu (NO3-N) ve ortofosfatın (PO4) sırasıyla %78.32–85.48, %82.93–92.22, %79.17–87.10, ve %75.36–84.94 oranında, yeşil hardal otu üretimi durumunda ise %69.0–75.85, %72.49–79.34, %66.67–80.65 ve %66.79–77.87 oranında azaldığı saptanmıştır. Akuaponik sistemlerde, kökleri daha fazla tutunma alanı sağlayan, atık suyu yeterli süre tutabilen, askıdaki maddelerin çökmesini sağlayan, kirleticinin adsorbsiyonu, alımı ve asimilasyonu için uygun yüzey alanı sağlayan bitkilerin inorganik azot ve fosfat uzaklaştırmada daha başarılı olduğu bildirilmiştir. Bu koşula su ıspanağı iyi bir örnek teşkil etmektedir (Enduta vd., 2011). Cohen vd. (2018), tilapia ve marulun akuaponik ortamda birlikte yetiştiriciliği ile geleneksel olarak marulun serada ve tilapyanın da havuzda ayrı ayrı üretildiği koşullar için teorik bir modelleme çalışması yapmışlardır. Bu modellemede 5 ton marul ve 1 ton tilapya üretildiği varsayılmıştır. Sonuçlara göre akuaponik üretimle su kullanımının, ötrofikasyon riskinin ve bölgesel çapta ekolojik ayak izinin, ayrı ayrı yapılan tarımsal üretime kıyasla azalacağı öngörülmüştür. Benzer şekilde Greenfeld vd. (2022) tarafından yapılan analizlerde 54 ton marul ve 0.75 ton akvaryum balığın akuaponikte üretilmesi durumunda çevresel etkinin ayrı ayrı yapılan üretime göre yarı yarıya azalacağı öngörülmektedir.

Akuaponik sistemlerde azot transformasyonunda bitkinin türü de önemlidir. Bitki kökünün yüzey alanının geniş olması nitrifikasyonda görev alan bakterilerin gelişimi için daha uygundur. Örneğin, domates ile lahana bitkisinin azot kullanma etkinliği karşılaştırıldığında domatesin köklerinin daha geniş olması nedeniyle daha başarılı olduğu tespit edilmiştir. Bunun nedeni domatesin kök yüzey alanında daha bol nitrifikasyon bakterisi bulunmasıdır (Hu vd., 2015). Karada kurulu deniz çiftliklerinde ise yüksek düzeyde tuza dayanıklı bitkiler sulak alanlarda biyofiltre olarak fayda sağlayabilmektedir (Buhmann ve Papenbrock, 2013).

Denizlerde özellikle kafeslerde su ürünleri yetiştiriciliğinin artması doğal olarak sürdürülebilirlik hakkında çok yönlü sorunları gündeme getirmektedir, bu sorunların başında da besin elementlerince zengin olan kafeslerde kullanılmış olan suların ekosisteme olan etkileri gelmektedir. Entegre multi-trofik su ürünleri yetiştiriciliği son zamanlarda hız kazandığından su ürünleri yetiştiriciliği çıkış sularının fitoremediasyonunda halofit bitkilerin kullanımı, özellikle akuaponik sistemler içinde kullanımı öne çıkmaktadır.

Alglerin kullanımı

Alglerin entegre edildiği atık su arıtımı, kimyasal arıtım gibi birtakım olumsuz durumlara yol açmayan ve toksik olmayan bir yöntem olup, atık suların arıtımında ucuz ve çevre dostu yöntemlerden biridir. Bu *amaçla Olithodiscus, Chlorella, Tetraselmis, Chaetocerous, Pyramimonas, Scenedesmus, Phormidium, Botryococcus, Chlamydomonas ve Spirulina*'nın atıkların uzaklaştırılmasında etkin ve umut verici olarak kullanıldığı bildirilmiştir (Sikder vd., 2016; Adekanmi vd., 2020). Adekanmi vd. (2020), *Coelastrum* morum ile muamele edilmiş atık suya ilişkin biyolojik arıtımın, bu sudaki besin elementi ve diğer kirleticileri uzaklaştırmada kullanılabileceğine işaret etmişlerdir. Bununla birlikte bitkili ve bitkisiz ortama bırakılan balıkçılık işletmesi atık sularında, bitkisiz ortamda yalnızca elektrik iletkenliği ve pH değerlerinde bir düşme olduğu, *Azolla caroliniana* ve *Salvinia auriculata*'nın bulunduğu bitkili ortamda ise çıkış suyu kalitesinin iyileştirilmesinde belirtilen bitkilerin düşük bir etkiye sahip olduğu bildirilmiştir (Toledo ve Penha, 2011).

Mekanik sedimantasyon ve biyolojik arıtımın birlikte çıkış suları arıtımında kullanıldığı havuzlarda, arıtım verimlilikleri askıda katı madde için % 54, toplam fosfor için % 35 olarak belirtilmiştir (Sindilariu, 2007). Jusoh vd. (2019), su ürünleri işletmeleri çıkış sularının arıtımı için bir arada kullandıkları fiziksel ve biyolojik yöntemleri –yeşil teknoloji- olarak ifade etmişlerdir. Besin elementlerince zengin çıkış sularının temel etkisinin ötrofikasyon üzerinde olduğunu, bu sorunun ise besin elementlerini kullanan mikroalglerin arıtımdan sonra hasat edilmesi ile aşılabileceğini bildirmişlerdir.

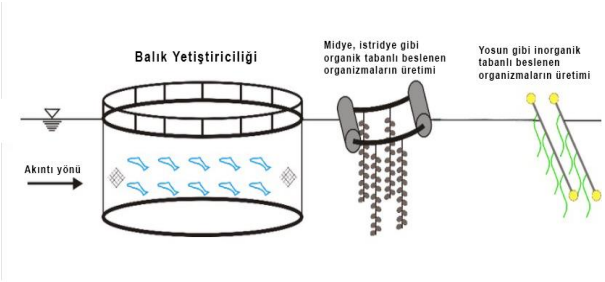
Entegre multi-trofik sistemler (IMTA)

Entegre multi-trofik sistemler, temel olarak iki ya da daha fazla sayıda ürünün bütünleşik bir ortamda birlikte üretilmesini ifade eder. Su ürünleri yetiştiriciliği alanında entegre multi-trofik sistemler, balık ürününün yanı sıra bitkisel üretimin de sağlandığı *akuaponik* ve özellikle denizlerde balık, midye ve yosun gibi farklı trofik seviyelerdeki ürünlerin üretildiği *entegre multi-trofik su ürünleri yetiştiriciliği* (IMTA) olarak karşımıza çıkmaktadır. Entegre multi-trofik sistemlerin su ürünleri yetiştiriciliği atık sularının zararlı etkilerinin azaltılmasında kullanılabilme potansiyeli günümüz koşullarında yoğun ilgi görmektedir.

Entegre multi-trofik yetiştiriciliğin temel prensibi, su ürünleri yetiştiriciliğinden gelen atık suda bulunan organik partikül ve çözünmüş besin elementlerinin trofik zincirin daha alt seviyelerinde bulunan türler tarafından alınmasıdır. Akuaponik sistemde olduğu gibi burada da simbiyotik ilişki söz konusudur. IMTA'de farklı trofik seviyelerdeki çeşitli organizmaların yetiştiriciliği ile doğal ekosistem taklit edilmiş olmaktadır. IMTA'nın dayanağı esasen ekosistem taklididir (Chopin, 2013). Basit olarak, IMTA; balık, kabuklu (midye, istiridye gibi) ve deniz yosunlarının aynı alan içinde üretilmesini ifade eder. Burada balık atıkları içinde bulunan inorganik besin elementlerinden yosun, fazlaca bulunan organik besin elementlerinden ise midye, istiridye gibi süzerek beslenen organizmalar yararlanır (Şekil 3).

Sistem kafes çiftliklerinden gelen partikül organik ve inorganik besinsel atıkların geri dönüşümü için üretime alınan balıkların yanı sıra atıkların uzaklaştırılması için fayda sağlayan diğer türlerin (süspanse tüketiciler, makroalgler gibi)

bir arada yetiştiriciliğini sağlama prensibine dayalıdır. Böylece tek bir türün yetiştirilmesine ve ihtiyaçlarına odaklanmak yerine, besin zincirinin farklı seviyelerinden tamamlayıcı türlerin üretim kombinasyonuyla doğal ekosistemi taklit ederek geri dönüşüm kavramına dayalı bir sistem oluşturulur.



Şekil 3. Entegre Multi-trofik Yetiştiricilik Sisteminde (IMTA) üretilen organizmaların yerleşimi (Angel ve Freeman, 2009)

Figure 3. Configuration of the Organisms in Integrated Multi-Trophic System (Angel and Freeman, 2009).

Entegre multi-trofik sistemler (IMTA) hem çevreye olan olumsuz etkinin azaltılmasına hem de ürün seçenekleri sınırlı bir alanın üretim kapasitesini artırmaya imkân sağlamasıyla çevresel ve ekonomik yönden avantaj sağlayarak sürdürülebilir su ürünleri yetiştiriciliğine katkıda bulunur (Sarıipek ve Karayücel, 2015). IMTA gerçekte çok yeni bir uygulama değildir çünkü Asya ülkelerinde yüzyıllardır "polikültür" olarak uygulanmaktadır. Fakat su ürünleri yetiştiriciliğinden kaynaklanan kirlilik, hastalık ve ekonomik sıkıntılar, polikültür uygulamasının özellikle batıda yeniden gözden geçirilmesini gerekli kılmıştır. IMTA'nın, yenilikçi bir atık yönetim modeli ve yetiştiricilik verimini arttırıcı gıda üretim sistemi olduğu dikkate alınmaktadır (Çantaş ve Yıldırım, 2019). Ferreira vd. (2012) tarafından yapılan çalışmada, off-shore çipura (*Sparus aurata*) ve Pasifik istiridyesinin (*Crassostrea gigas*), ayrı ayrı ve IMTA kapsamında üretilmesi durumunda çevresel çıktılar karşılaştırılmıştır. Tek başına off-shore çipura yetiştiriciliğine göre sisteme istiridye ekleyerek IMTA'ye dönüştürülmesi durumunda organik birikimde bariz bir azalma meydana gelmiştir. Reid vd. (2013) tarafından yapılan araştırmalar, IMTA'nın çevreye olan etkileri ile ilgili olumlu sonuçları net olarak ortaya koymaktadır. Atlantik salmonu (*Salmo salar*) kafeslerinin yakınında kelp (*Alaria esculenta* ve *Saccharina latissima*) yetiştirilmesi durumunda her bir kg kelpin 2.3-4.4 kg çözünmüş azotu uzaklaştırdığı saptanmıştır. IMTA, uygulamada bazı teknik kritik noktalar içermektedir. Örneğin, IMTA sisteminde deniz hıyarı (*Holothuria scabra*) ile *Sciaenops ocellatus* türü balıkların birlikte yetiştiriciliğinde, partikül olarak bulunan balık dışkısının %100 uzaklaştırılabilmesi için her bir kg balık ürününe karşı 1.3 kg deniz hıyarı bulunması gerekliliğidir. Ancak deniz hıyarının stok yoğunluğu az da olsa atık yükünün ve olumsuz bentik etkinin azaltılmasında kesinlikle yararlı etkilerinin bulunduğu bildirilmiştir (Chary vd., 2020). Doğu Akdeniz'de su ürünleri (çipura/levrek) yetiştiriciliği alanı olarak belirlenen alanlarda (Yunanistan) balık çiftliklerinden gelen atıkların artışıyla dinoflagellat artışı olduğu, bu artışın besin ağının değişimini gösterdiği Tsagaraki vd. (2011) tarafından ortaya konulmuştur. Çevreye etkinin en aza indirilmesini sağlayan ve farklı trofik seviyelerde ekolojik işlevi olan yetiştiricilik sistemlerinin su ürünleri üretim çiftliklerine entegrasyonu en fazla faydayı sağlayacak yenilikçi yaklaşım olarak dikkate alınmaktadır. Karada ve su içinde kurulacak IMTA sistemlerinin önümüzdeki 30 yılda özellikle kirliliğe karşı tercih edilen ve kullanımı yaygınlaşacak bir yetiştiricilik sistemi olacağı öngörülmektedir (Costa-Pierce, 2013).

SONUÇ

Su ürünleri yetiştiriciliğinden kaynaklanan atık suların arıtımı, su ürünleri yetiştiriciliğinin sürdürülebilirliği ile ilgili kritik faktörlerin başında gelmektedir. Bu kritik konu kapsamında çevreyle uyumlu ancak döngüsel olmayan biyoreaktör gibi biyolojik arıtım uygulamaları ile adsorpsiyon, ileri oksidasyon prosesleri, membran teknolojileri gibi fizikokimyasal arıtım metotları da etkin arıtım teknikleri olarak önerilmektedir (Ahmad vd., 2022). Yetiştiricilik atık sularının arıtımında, yine çevreye en düşük düzeyde etki gösteren, ancak maliyeti yüksek, düşük-basınçlı polieterfülfon membranlar (Nora'aini vd., 2005), ters osmoz membran (Cao vd., 2007), potasyum ferrata dayalı arıtım uygulamaları (Tanveer vd., 2016) mevcuttur ancak, henüz pratik anlamda geniş çapta uygulama alanları yetersizdir. Bu tekniklerin kullanımını sınırlayıcı ana faktör maliyetinin yüksek oluşudur. Buna karşın çevreyle uyumlu ve döngüsel teknikler (akuaponik, IMTA gibi) aynı zamanda atık bünyesinde bulunan besin elementlerinin yeni bir ürüne dönüşümünü sağlayan arıtım yöntemleri uygulama ve verimlilik açısından daha değerlidir. Bu bağlamda, işletmelerin yetiştiricilik kapasitesine bağlı olarak artış gösteren çıkış sularının arıtımında; alıcı ortamın sağlıklı bir şekilde muhafaza edildiği, karasal ya da sucul bitki ile birlikte su ürünleri yetiştiricilik ağırlıklı ve ekosisteme duyarlılığı yüksek yaklaşımlar hız kazanmıştır. Bu noktada aşağıdaki bazı çıkarımların dikkate alınmasının uygun olacağı öngörülmektedir:

1. Su ürünleri yetiştiriciliği faaliyetlerinin konumlandırılması, inşası, işletilmesi ve işlenmesi hakkında ayrıntılı rehberlik sağlayan- İyi Yönetim Uygulamaları (İYU)- çevresel etki düzeyini azaltma adına su ürünleri işletmeleri çıkış sularının arıtımında da uygulanmalıdır.

2. Sıfır atık yaklaşımı kapsamında azot, fosfor gibi atık su içindeki elementler olmaktan öte, gıda ürününe dönüşecek değerli besin elementleri olarak düşünülmelidir.

3. Yapay sulak alanların atık sudaki nitrit azotunun tamamına yakınının uzaklaştırabiliyor olması arıtımda başarıyla kullanılabileceğinin bir göstergesi olarak dikkate alınmalıdır.

4. Farklı trofik seviyelerden yararlanmayı ve aynı zamanda farklı organizma ve besinlerin de üretimini sağlayan akuaponik gibi entegre sistemlerde su ürünleri işletmeleri çıkış sularının kullanımı su ürünleri yetiştiriciliğinin sürdürülebilirliğini destekleyen atık arıtım yöntemleri bu bağlamda değerlendirilebilir.

5. Kafeslerde açık denizde yapılan geleneksel su ürünleri yetiştiriciliğine IMTA gibi sistemlerin eklenmesi ile hem su

ürünleri yetiştiriciliğinden gelen organik yükün azaltılmış olması hem de farklı organizmaların yetiştirilmesi şeklinde iki yönlü yarar sağlanması yine çevreyle uyumlu, sürdürülebilir su ürünleri yetiştiriciliği için önem taşımaktadır. Entegre gıda üretim sistemleri (akuaponik, IMTA gibi) yenilikçi yaklaşımlar olarak son yıllarda ivme kazanmakla birlikte, bu sistemlerin uygulamaya aktarılması için gerekli planlamalara öncelik verilmelidir.

6. Atık su içinde bulunan besin elementlerinin, çevreyle uyumlu atık su arıtım sistemleri yardımıyla biyomasa dönüştürülmesi yalnızca çevresel sürdürülebilirlik açısından değil aynı zamanda mali anlamda karlı bir yetiştiricilik modeli olarak değerlendirmeye alınmalıdır.

7. Bu çerçevede, yetiştiricilik kaynaklı atık sularının döngüsel yetiştiricilikte kullanılarak gıda ürününe dönüştüğü sistem ve yöntemler, sürdürülebilir su ürünlerinin temel prensiplerinden biri olarak benimsenmelidir.

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VERİ KULLANILABİLİRLİĞİ

Makalede kullanılan verilerle ilgili sorular için, sorumlu yazar ile iletişime geçilmelidir.

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DERLEME REVIEW

Dünyada ve Türkiye'de havza yönetimleri

Water basin managements in the World and Türkiye

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Öz: Çevre ekonomisinin önemli bir yönünü oluşturan su ve havza yönetimi, içme ve kullanma amaçları yanı sıra enerji üretimi, kalkınma ve ekosistem bütünlüğü yönleriyle de önemlidir. Küresel ısınma ve kıtlığın etkileriyle "Su Çerçeve Direktifi" kapsamında su kaynaklarının korunması, endüstriyel amaçlı su temini, su kalitesinin artırılması ve havza yönetimi hususları Türkiye'de gündeme gelmiştir. Bu çalışmada, dünyadaki havza yönetimi örnekleri incelenerek Türkiye'deki mevcut havza yönetimi anlayışıyla karşılaştırılmıştır.

Anahtar kelimeler: Çevre kirliliği, su yönetimi, havza yönetimi, su çerçeve direktifi

Abstract: Water and basin management, which is an important aspect of the environmental economy, is significant in terms of energy production, development, and ecosystem integrity as well as drinking and usage purposes. Besides global warming and famine effects, the protection of water resources, industrial water supply, water quality improvement, and basin management issues have been emphasized in Türkiye within the "Water Framework Directive" scope. In this study, the examples of basin management in the world were investigated and compared their approaches with the current understanding of basin management in Türkiye.

Keywords: Environmental pollution, water management, basin management, water framework directive

GİRİŞ

Su, insanlar ve diğer tüm canlıların yaşamlarını sürdürmeleri amacıyla ihtiyaç duyulan vazgeçilmez bir kaynaktır. Sürdürülebilir kalkınmanın sağlanması için su kaynaklarının korunması ve ihtiyaca göre kullanılması, arazi kullanımında planlama yapılması, bütüncül, sistematik ve kapsamlı politikaların ortaya konulması gerekmektedir. Dolayısıyla su kaynaklarının ekonomik, çevresel ve sosyal kullanımlar açısından ortak kullanımı için havza bazında planlama ve yönetimin sağlanması şarttır (Öztürk vd., 2014). Su kaynakları yönetiminin havza düzeyinde incelenmesi 1950'li yıllarda başlamıştır. Sanayi toplumunun yükselişi ile arazi kullanımı, su tüketimi ve planlama arasındaki ilişkiler belirgin hale gelmiştir. 2000 yılında AB Su Çerçeve Direktifi sayesinde nehirlerde havza yönetimi önem kazanmıştır (Öztürk, 2011).

Günümüzde su kaynaklarının yönetiminin ölçek bazında incelenmesi sonucunda farklılıklar ortaya çıkmıştır. Geçmişteki sorunların niteliği yerellik arz ederken, günümüzde alanların değişkenlikleri önem kazanmıştır. Kısacası, su kaynaklarına ilişkin büyüklüklerin belirli bir noktada zamana göre değişimini incelemek artık yetersiz kalmakta, alansal değişikliklerin de göz önünde bulundurulması gerekmektedir (Harmancıoğlu vd., 2002). Bu nedenle kaynak yönetiminin doğal kaynaklarla bütünleşmiş ve havza bazında ele alınması önem kazanmıştır.

Havzalar, genel olarak, "doğal sınırları içinde, iklim, jeoloji, topoğrafya, toprak, flora ve faunanın sular ile etkileşim içinde olduğu, suyun ayırım çizgisinden denize aktığı noktaya, kapalı havzalarda ise suyun toplandığı nihai noktaya göre suyun toplanma alanı" şeklinde tanımlanabilir (T.C. Orman ve Su İşleri Bakanlığı, 2014). Bu alanlarda havzaların belirli işlevleri bulunmakta ve bu işlevlerin toplumsal fayda sağlayacak şekilde sürdürülebilir olarak kullanımı için sistematik bir havza yönetimi gerekmektedir (Kahraman vd., 2018).

Bu çalışmada, dünyadaki havza yönetimi örnekleri incelenerek, Türkiye'deki mevcut havza yönetimi ile karşılaştırma yapılması amaçlanmıştır.

Su ve Havza Yönetimi Anlayışının Gelişimi

Avrupa Kömür ve Çelik Topluluğu ile 1951'de temelleri atılan ve 1991 Maastricht Antlaşması ile kurulan Avrupa Birliği'nde su kaynaklarının korunması ve yönetimine ilişkin mevzuat AB mevzuatı içerisinde çok önemli bir yer tutmakta olup bu alanda yirmiyi aşkın direktif bulunmaktadır. Bu direktifler arasında en önemlisi ise 23 Ekim 2000 tarihli ve

2000/60/EC sayılı "Su Çerçeve Direktifi"dir. Su Çerçeve Direktifi (SÇD)'nin asıl amacı iç yüzeysel suların, geçiş sularının, kıyı sularının ve yeraltı sularının korunması için bir çerçeve oluşturmaktır (Akkaya vd., 2006). Direktifin 7. maddesi, içme suyu temini için kullanılan sularla ilgilidir. Üye devletler, içme suyu üretiminde gerekli arıtma seviyesini azaltmak ve su kalitesinde bozulmayı önlemek amacıyla tanımlanan su kaynakları için gerekli korumayı sağlamaktadırlar (Official Journal, 2000).

SÇD, ortak bir yaklaşımla yeraltı ve yüzey sularının korunmasını ve çevresel düzenlemeler yapılmasını sağlamaktadır. Direktif, ortak bir çerçeve ve çevresel hedefler sunarken, ayrıca aktörlere, ulusal, bölgesel ve havza ölçeğinde bu amaçlara ulaşmada farklı yollar izleme özgürlüğünü de sunmaktadır. Bu direktif, özellikle her nehir havzası bölgesi için yapılması gereken stratejik yönetim planlarını talep etmektedir. Bu planlar, nehir havza yönetim planları olarak da bilinmektedir (Evrim-Maden, 2015).

AB uyum sürecinde SÇD'nin uygulanması açısından Türkiye'de su çerçeve mevzuatının benimsenmesi, özellikle su kalitesi müktesebatı uyumlaştırmayla ilgili mevzuatın uygulanmaya başlaması, nehir havzalarını koruma eylem planlarının oluşturulması hedeflenmiştir. Ayrıca, Avrupa Komisyonu, Türkiye'ye ilişkin ilerleme raporlarında sınır aşan sularla ilgili olarak diğer üye ülkelerle iş birliği yapılmasını vurgulamaktadır (Tuğaç, 2013). 2000 yılında SÇD'nin yayınlanmasının ardından "Implementation of the Water Framework Directive in Turkey Project (MATO1/ TR/9/3)" projesi ve daha sonra Türkiye'de Su Sektöründe Kapasitenin Güçlendirilmesi Projesi (Project No: TR 06 03) ortaya konmuştur. İlk proje, Hollanda'dan gelen uzmanlar tarafından MATRA programı (Türkiye-Hollanda hükümeti ikili işbirliği programı) kapsamında yürütülürken, ikinci proje ise 2007-2009 yılları arasında Türkiye Çevre ve Orman Bakanlığı, Hollanda Tarım, Orman ve Gıda Kalitesi Bakanlığı, İngiltere Çevre Ajansı ve Slovakya Su Araştırma Enstitüsü işbirliğiyle yürütülmüştür (Moroğlu vd., 2008).

Türkiye'de tarihsel açıdan su politikalarının önceliğini su kaynaklarını geliştirmek oluşturmuştur. Ayrıca Türkiye'nin içme suyu, enerji ve tarımsal su ihtiyaçlarını karşılamak bakımından su kaynaklarının gelişimi faaliyetleri de tamamlanmamıştır (Sümer, 2013). Bu nedenle su yönetimi açısından gelişmekte olan ülke konumundaki Türkiye'nin SÇD'ye uyumu, Avrupa ülkelerine kıyasla daha yavaş ilerlemektedir. Türkiye'nin sınır aşan sulara sahip olması ve SÇD'ye göre nehir havzalarının temel birimler olarak kabul edilmesi, ülkemizin uygulamada karşılaştığı diğer sorunlar arasındadır.

SÇD ile gelen yeniliklerden en önemlisi nehir havzası yönetimidir. Nehir havzaları doğal sınırlara göre ayrılarak yönetilmekte ve farklı bölgeler ya da ülkelerin ortak çalışmalarını gerektirmektedir. SÇD'ye göre Türkiye'de nehir havza planlaması tüm havzaların özelliklerinin analizi, koruma önlemlerinin tespiti ve havza yönetim planlarının oluşturulması öngörülmüştür (Aydın-Coşkun, 2010). SÇD'ye uyum sağlamak için 2012 yılında hazırlanan Su Kanun Tasarısı'nın yalnızca genel sorunlara değinmesi nedeniyle güncellemeye ihtiyaç duymaktadır. Burada su yönetiminde kurumlar arası işbirliği ve koordinasyonun yetersizliği en büyük sorun olarak göze çarpmaktadır. Türkiye Cumhuriyeti Tarım ve Orman Bakanlığı bünyesindeki DSİ'nin yanı sıra SÇD'nin etkisiyle Su Yönetimi Genel Müdürlüğü (SYGM) ve Su Enstitüsü kurulmuştur. SYGM, su yönetiminde AB uyum çalışmaları ile ilgilenirken, Su Enstitüsü su politikalarının geliştirilmesini, küresel su stratejileri ve araştırmaları ortaya koymayı amaçlamaktadır (Bulut vd., 2019).

Havza Yönetiminde Etkili Kurumsal Yapılar

Havza yönetimine katılım, dünya çapında çevre yönetimine kesinlik kazandırmıştır. Tanımlar farklılık gösterse de bu bağlamdaki katılımcı yönetişim; devlet ve devlet dışı aktörlerin, bu aktörlerin geçmişte rutin olarak bu tür kararlara dahil olmadığı politika oluşturma sürecine katılımını ifade etmektedir (Benson vd., 2014). Su yönetiminde yer alan katılımcılar genellikle paydaş olarak nitelendirilirken, bu paydaşların kamu kurum ve kuruluşlarının yanı sıra pek çok farklı organizasyondan meydana geldiği bilinmektedir. Bu katılım yapısı, ülkeler ve bölgeler arasında farklılıklar göstermektedir.

Son yıllarda benimsenen bütünleşmiş ve katılımcı havza yönetimi yaklaşımı, kamu kurumları ve yerel topluluklar arasında yeni kurumsal düzenlemelere yol açmıştır. Kurumların entegrasyonu ve işbirliği kapsamında yerel yönetim, teknik ajanslar ve toplum kuruluşları arasındaki etkileşim dikkatli bir şekilde tanımlanmalı, yönetilmeli ve her düzeyde kapasite geliştirme esas alınmalıdır. Yerel düzeydeki katılımcı yaklaşımlar, teknik işlevlerin insan merkeziyetçiliğini gerektirmektedir (Darghouth vd., 2008). Entegre ve sürdürülebilir su kaynakları yönetimini uygulayan ülkelerde, su yönetiminden sorumlu devlet idareleri nehir havzaları düzeyinde organize olan, çeşitli sektörlerden kullanıcıların yanı sıra ilgili idareler ve yerel makamlardır. "Suyu kullanıcı/kirleten öder" ilkesinin uygulanmasıyla elde edilen belirli bütçe kaynaklarına sahiptirler (Neveu, 2003).

Havza yönetiminde, Dünya Bankası, Uluslararası Tarımsal Kalkınma Fonu (IFAD), Gıda ve Tarım Örgütü (FAO) ve Uluslararası Su Yönetimi Enstitüsü (IWMI) gibi uluslararası kuruluşlar tarafından üretilen çözümler ön plana çıkmaktadır (Erdoğan, 2016). Su kaynaklarının yönetimi ve geliştirilmesi, Dünya Bankası'nın sürdürülebilir ekonomik büyüme ve yoksulluğun azaltılmasına yönelik stratejik hedefleri için kritik öneme sahiptir. Dünya Bankası'nın misyonu, yoksulluğun azaltılmasıdır. Dünya Bankası su kaynakları yönetimini yoksul insanlara doğrudan fayda sağlayacak şekilde iyileştirmek, yoksullar da dahil olmak üzere herkese fayda sağlayan su hizmetlerinin performansını iyileştirmek, su ve sanitasyon, sulama ve hidroelektrik hizmetleri sağlamaktadır (The World Bank, 2004).

Birleşmiş Milletlerin uzmanlaşmış bir kuruluşu olan IFAD, gelişmekte olan ülkelerde kırsal yoksulluğu ortadan kaldırmayı hedeflemektedir. IFAD, gıda güvenliğinin artırılması ve daha iyi

geçim kaynakları sağlamaya yönelik toprak ve su yönetimindeki değişikliklere ilişkin faaliyetler sürdürmektedir (Erdoğan, 2016). FAO, gıda güvenliğini sağlamak yanı sıra aktif ve sağlıklı bir yaşam sürdürülmesi amacıyla yeterli ve yüksek kaliteli gıdaya düzenli şekilde erişim sunmak için faaliyetler yürütmektedir (FAO, 2017). Ayrıca FAO, üye ülkelere sulama sistemlerinin tasarlanması ve uygulanmasının yanında su tasarrufu için teknik yardım sunarken, arıtılmış atık suyun tarımda yeniden kullanılması için çalışmalar yürütmektedir (Yılmaz, 2021). IWMI gıda, geçim ve çevreye yönelik toprak ve su kaynakları yönetimini geliştirmek yönünde faaliyetler sürdürmektedir. IWMI'nın temel faaliyet alanları su durumu ve erişim, verimli su kullanımı, su kalitesi, sağlık ve çevre, su ve toplumdur (Kanber, 2015).

STK'ların küresel, ulusal ve yerel çevre yönetişimi üzerindeki etkisi ve kapasitesi 1990'lardan itibaren nicelik ve nitelik olarak artmıştır. Bu argümanların birkaç nedeni bulunmaktadır; örneğin, Gemmill ve Bamidele-Izu (2002) STK'ların çevresel yönetişime katılımının bilgi alışverişi, politika geliştirme ve uygulama, değerlendirme, izleme ve çevresel adaletin sağlanması gibi ciddi teşvikler sağladığını iddia etmektedirler. STK'ların çıkar grupları arasındaki anlaşmazlık nedeniyle çevre koruma politikalarının yapımında ciddi ikilemlere yol açması eleştirilse de demokratik değerlerin kurumsallaşması, neden-sonuç çıkmazının ortadan kaldırılması, geliştirilmesi ve uygulanması nedeniyle kamu politikalarının etkin bir şekilde uygulanması için sivil toplumun katılımı gerekli görülmektedir (Bernauer vd., 2016).

Havza su kaynaklarının yapısı fark etmeksizin, bunları yönetmek için bir havza organizasyonu oluşturulmalıdır. Bu organizasyona başkanlık edecek bir havza heyeti, komisyonu veya havza konseyi oluşturulmalıdır. Havza komitesinin temel rolü, duruma göre farklı işlevlerle havzada su kaynakları yönetimine ilişkin kararlar almaktır. Havza heyeti veya komitesi, havzadaki su kaynakları yönetimine paydaşların ve kullanıcı temsilcilerinin etkin ve resmi katılımını geliştirmek açısından ayrıcalıklı bir oluşumdur. (INBO, 2018). Havza organizasyonları yasal karar alma ve/veya danışma organları, yönetim organları, kalkınma kurumları ve düzenleyici kurumlar gibi farklı şekillerde kurulabilmektedir. Bu organizasyonlar genellikle diğer devlet kurumları ve idari organlarla birlikte çalışmaktadırlar (INBO, 2009).

Diğer havza yönetimi paydaşları arasında ajanslar, özel sektör, meslek odaları ve basın gibi paydaşlar yer almaktadır. Ajanslar, nehir havzaları düzeyinde entegre ve sürdürülebilir su kaynakları yönetimiyle ilgili faaliyetleri destekleyen ikili ve çok taraflı işbirliği ajanslarıdır (Neveu, 2003). Özel sektör, havza organizasyonlarının yayılmasında yer alan önemli bir politika girişimini temsil etmektedir. Özel sektör genellikle suyun özelleştirilmesi ve tam fiyatlandırma gibi ekonomik çıkarlara fayda sağlamak için geliştirilen söylemlerle ilişkilendirilmiştir. Ulus ötesi temsilciler olarak nitelendirilen çevre ve mühendislik danışmanları, su altyapısı geliştirme ve planlamasındaki faaliyetlerin yaygınlaştırılması ve SÇD uygulamaları açısından önemli bir rol oynamaktadırlar (Mukhtarov vd., 2013).

Dünya'da ve Türkiye'de Havza Yönetimi Örnekleri

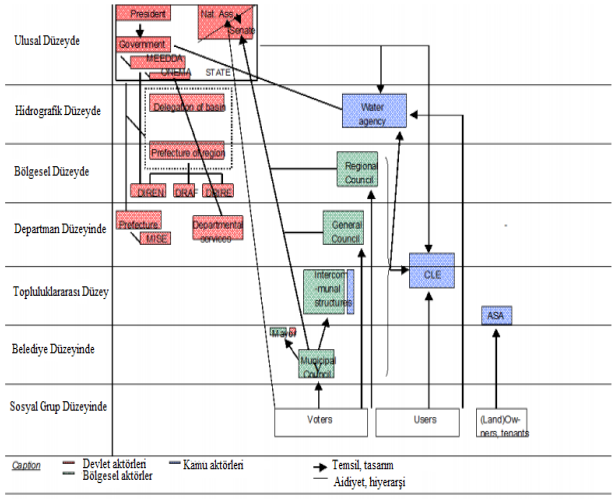
Dünyada Havza Yönetimi

Dünyada su yönetimiyle ilgili örnekler incelendiğinde, ön plana çıkan belli başlı ülkeler bulunmaktadır. Bu çalışmada su yönetiminde ele alınan örnekler arasında Fransa, İspanya, İngiltere, Çin ve İsrail yer almaktadır. Bu ülkeler, literatürde öncelikli örnek model olarak gösterilen ülkelerdir. Öte yandan Fransa'nın idari yapılanmasının Türkiye'ye benzer olması, İspanya ve Türkiye'de havza sayılarının benzerliği, İngiltere'de kamusal hizmetlerin özel sektör eliyle yürütülmesi, Çin'in dünyanın en büyük ekonomilerinden biri olması bu ülkelerin seçilmesinde rol oynamıştır.

Fransa

Günümüzde Fransa'nın su kaynakları yönetimi altı temel ilkeye dayanmaktadır. Bunlar nehir havzaları düzeyinde merkezi olmayan yönetim, entegre yaklaşım, kurumlar arası diyalog organizasyonu ve eylem koordinasyonu, finansal kaynak seferberliği, çok yıllık planlama ve belediye içme suyu temini ile sanitasyon hizmetlerinin yönetimi için kamu-özel operatör sorumluluklarının belirgin dağıtımıdır (Noël, 2009).

Fransa'da havza yönetiminde kamu paydaşları, özel ve ortak paydaşlar, karmaşık ve çok yönlü etkileşimlerle kamusal faaliyetler için karar verme sürecine dâhil olmaktadır. Fransa'da su yönetişim ağına dâhil olan ana paydaşlar Şekil 1'de sunulmuştur.



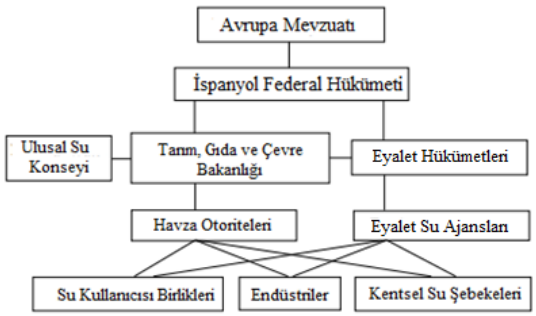
Kısaltmalar (Şekil 1): CLE (Commission Locale de l'Eau)-Yerel Su Komisyonu; ASA (Associations Syndicales Autorisées)-Özel Mülk Sahipleri Dernekleri; MISE (Mission inter-services pour l'eau)-Hizmet içi su görevleri; ONEMA (Office national de l'eau et des milieux aquatiques)-Tatlı Sular Ulusal Ofisi; DIREN (directions régionales de l'environnement)-Çevre Bölge Müdürlükleri; DRIRE (des directions régionales de l'industrie, de la recherche et de l'environnement)-Sanayi, Araştırma ve Çevre Bölge Müdürlükleri; MEEDDAT (ministère de l'Écologie, de l'Energie, du développement durable et de l'Aménagement du territoire): Ekoloji, Enerji, Sürdürülebilir Kalkınma ve Planlama Bakanlığı.

Şekil 1. Fransa'da su politikalarında rol oynayan aktörler (Richard vd., 2010).

Figure 1. Actors play a role in water policies in France (Richard et al., 2010).

İspanya

Su yönetiminin kurumsal organizasyonu (Şekil 2), Avrupa, İspanya federal ve eyalet hükümet seviyeleri arasındaki etkileşimden kaynaklanmaktadır (Varela-Ortega vd., 2010). Avrupa hükümeti, üye ülkeler tarafından geliştirilen genel su politikaları oluşturmaktadır. İspanya'daki su idaresi, federal ve eyalet hükümetleri arasında bölünmüştür. Federal yönetim, birden fazla eyaleti kapsayan nehir havzalarını ve eyaletlerin kendi eyalet sınırları içindeki havzaları yönetmesini üstlenmektedir (Albiac vd., 2012).



Şekil 2. İspanya'da Su Yönetiminin Kurumsal Organizasyonu (Albiac vd., 2012).

Figure 2. Institutional Organization of Water Management in Spain. (Albiac et al., 2012).

1985 yılında yürürlüğe giren İspanya Su Yasasını ve bu yasanın 1999 reformunu pekiştiren 2001 Su Yasası ve tüzükleri, Nehir Havzası Yetkililerinin örgütsel yapısını belirlemektedir. Söz konusu mevzuata göre otoritelerin rolü havza yönetim planlarını geliştirmek, izlemek ve revize etmek, su ve ilgili kamu kaynaklarını yönetmek ve misyonlarını geliştirmek için gerekli kamu altyapı çalışmalarını geliştirmek hedeflenmektedir (Varela-Ortega vd., 2010). Yönetim Konseyi (Junta de Gobierno), kurum için eylem planlarını ve bütçeleri onaylamaktan sorumludur. Kullanıcı Meclisi (Asamblea de Usuarios), havza genelinde hidrolik işlerin ve su kaynaklarının yönetimini koordine etmektedir. Kullanıcı Yönetim Konseyleri (Juntas de Explotación), yalnızca kullanımların özellikle birbiriyle ilişkili olduğu havza bölümlerinde Kullanıcı Meclisi ile aynı sorumluluklara sahiptir. Baraj Tahliye Komisyonu (Comisión de Desembalses), havzadaki çeşitli barajlardan su tahliye rejimleri sunmaktan sorumludur. Kullanıcı dernekleri, Tarım ve Sanayi Bakanlıkları ve Ulusal Elektrik Konsorsiyumu temsilcilerinden oluşmaktadır. Bayındırlık Konseyleri (Juntas de Obras), yapım işlerindeki ilerleme hakkında bilgilendirmek amacıyla önerilen bir hidrolik altyapının gelecekteki kullanıcılarının talebi üzerine oluşturulmaktadır. Nehir Havzası Su Konseyi (Consejo del Agua), havza yetkililerinin planlama koludur. Görevleri Havza Hidrolojik Planını ve periyodik gözden geçirmelerini tartışmak ve onaylamaktır. Anlatılan temel kurumlar dışında su yönetiminde merkezi düzeyde rol oynayan diğer organizasyonlar SEPRONA (çevre koruma için sivil muhafızlar-ulusal polis), Tarım Bakanlığı Planlama ve Kırsal Kalkınma Genel Müdürlüğü, Ulusal Coğrafya Enstitüsü, İspanya Jeo-Madencilik Teknoloji Enstitüsü, Hidrografik Araştırmalar Merkezi ve Tragsatee, sulama birlikleri, belediyeler ve kamu-özel ortaklıklarıdır (Maestu vd., 2003).

İngiltere

2000'li yılların başından itibaren Birleşik Krallıkta su kalitesine yönelik birçok iyileştirme, çevresel planlama ve düzenlemeye yönelik bir nehir havzası yönetimi yaklaşımı izlemiştir. Bu yaklaşım, su ortamını ve toplumun gereksinim duyduğu su kullanımlarını korumak için gerekli kalite hedeflerini belirlemeye odaklanmıştır. Kalite hedefleri içme suyu kaynaklarının korunmasını, rekreasyonel kullanımları, endüstriyel ve tarımsal su çıkarma ve deşarjı kapsamaktadır. Suyun çıkarılması ve arıtılmış atık suların deşarjı için gerekli izinler, nehir havzasının ihtiyaçlarını yansıtacak şekilde belirlenmektedir (Griffiths, 2002).

Çevre Ajansı, İngiltere Hükümeti tarafından desteklenen ve İngiltere'de çevreyi koruma ve iyileştirme sorumluluğuna sahip, bakanlığa bağlı olmayan bir kamu kuruluşudur. Çevre Ajansı, Su Çerçeve Direktifinin uygulanması için yetkili makamdır. Çevre Ajansı, önemli paydaş kuruluşların İngiltere ve Galler'deki çevresel baskılardaki olası değişiklikleri keşfetmelerine yardımcı olmak için temel sosyo-ekonomik senaryolar üreterek, strateji ve politikaların gözden geçirilmesini sağlamaktadır. (Henriques vd., 2015)

Su şirketleri veya Liman İdaresi gibi bazı kuruluşlar, su kaynaklarını yönetme sorumluluklarını paylaşmaktadırlar. Diğer kuruluşlar ise, su çıkarma veya rekreasyon için ya da su kaynaklarının durumundan etkilendikleri için su ortamının kullanıcıları olarak çıkarlara sahiptirler. İngiltere'de su ve havza yönetiminde paydaşlarla çalışmalar planlanırken paydaş düzenleyiciler, profesyonel paydaş kuruluşları, yerel paydaş kuruluşları ve halk olmak üzere dört farklı paydaş kategorisi ortaya atılmıştır (Tablo 1). Bu kategorilerdeki paydaşlar, kapsamlı ve karmaşık bir organizasyon veya birey gruplarıyla su yönetimi planlaması yapılmasına yardımcı olmaktadır (Orr vd., 2007).

Çin

Çin'in merkezi hükümeti, Çin'deki su kaynaklarını düzenlemekte ve yönetmektedir. 1970'lerden günümüze kadar toprak ve su koruma, içme suyu standartları, tarımsal sulama, atık su deşarjı ve çevre koruma dâhil olmak üzere Çin'in su kaynaklarını yönetmek ve korumak için bir dizi ulusal düzenleme oluşturulmuştur. 1950'ler ve 1970'ler arasında küçük ve orta ölçekli barajların inşası ve sulanan arazilerin hızla genişletilmesi teşvik edilmiştir. 1980'lerin başından 20. yüzyılın sonuna kadar suyun korunması, ulusal ekonominin hızlı büyümesini desteklemek için önemli bir temel ve koruma olarak kabul edilmiştir (Liu vd., 2013).

Tablo 1: İngiltere'de nehir havzası planlaması ve yönetiminde paydaşlar (Orr vd., 2007).

Table 1: Stakeholders in river basin planning and management in the UK. (Orr et al., 2007)

Paydaşlar	Tanım	Açıklama
Eş-dağıtımcılar	Ajanslar ve kurumlar	Nehir Havzası Yönetim Planlarını sunmak için gereken temel önlemleri uygulamak için yasal yetkiye sahip tüm kuruluşlar.
Profesyonel paydaş kuruluşları	Meslek kuruluşları	Kamu ve özel sektör kuruluşları, profesyonel gönüllü kuruluşlar ve STK'lar. Bu kategori akademisyenleri, endüstriyi, sigortacılığı, işletmeyi ve ücretli profesyonel kadrosu olan koruma kuruluşlarını içerebilir. Su kaynağı kullanan ve faaliyetleri üzerinde etkisi olan tüm meslek kuruluşları veya profesyonel kapasitede hareket eden bireyler.
Yerel paydaş kuruluşları	Yerel gruplar: bölgesel/yerel düzeyde faaliyet gösteren profesyonel olmayan organize kuruluşlar	Bölge sakinleri ve sosyal yardım dernekleri, bölge eylem grupları gibi yerel merkezli topluluklar ve çiftçiler, balıkçılar, kuş gözlemcileri gibi çıkar odaklı gruplar
Vatandaşlar	Meslek grupları yerine kendilerini temsil eden bireyler	Bireysel sakinler, kullanıcılar, bölgedeki işçiler, işletme sahipleri, arazi sahipleri, çiftçiler, dışarıdan gelen ziyaretçiler.

1990 ve 2010 yılları arasında Çin, "hard path" yaklaşımından "soft path" yaklaşımına geçiş yapmıştır. Su yönetiminde 1990 yılından önce Çin'in su tasarrufuna ilişkin tercih ettiği "hard-path" yaklaşımı, büyük altyapı inşaatları ve su teminindeki artışın ön planda olduğu uygulamaları kapsamaktadır. Bu dönemde suyun korunması, ulusal ekonominin hızlı büyümesini desteklemek için önemli bir strateji olarak kabul edilmiştir (Liu vd., 2013). Gleick (2002) tarafından tanımlanan "Soft path" kavramının "hard path" karşısında farklı ve daha geniş hedefleri vardır. "Soft path" yaklaşımı su kaynaklarını yönetmek ve su kullanım verimliliğini artırmak için ekonomik ve kurumsal önlemleri vurgulamaktadır. Soft path yaklaşımı, sonsuz yeni tedarik kaynakları aramak yerine, su kullanımının genel verimliliğini artırmaya çalışmaktadır. Yerel ve topluluk ölçeğinde su kullanıcılarıyla birlikte çalışmakta ve suyun sağladığı besin döngüsü, taşkın koruması, su habitatı, atık seyreltme ve uzaklaştırma gibi kritik ekolojik hizmetleri korumayı amaçlamaktadır. Bu geçişin temel nedeni, ülkedeki artan su kıtlığı ve su kirliliği olmuştur. Bu dönemde Su ve Toprağın Korunması Kanunu (1991), Su Kirliliğinin Önlenmesi ve Kontrolü Hakkında Kanun (1996), Taşkın Kontrolü Kanunu (1997), Su Kanunu (2002 revize edilmiştir) gibi su ile ilgili birçok kanun ve yönetmelik çıkarılmıştır.

Çin'in su tasarrufu, sürekli ekonomik kalkınmayı destekleme talebini karşılamak için giderek daha zor hale gelmiştir (Liu vd., 2013). Bu nedenle Çin hükümeti 2011 yılında su kaynaklarının yönetme konusunda bir güncelleme yapmıştır. Çin, nehirlerinin ve göllerinin kaynaklarını kullanmak ve ülkeyi sel ve kuraklık tehditlerinden koruyabilecek bir su sistemi kurmak için gelecek on yıl içinde (2010-2020) su tasarrufuna 618,8 milyar ABD doları yatırım yapmayı planlamıştır (Liu ve Yang, 2012).

Çin'de ülkenin su kaynaklarının yönetiminde yaklaşık 20 merkezi hükümet kurumu yer almaktadır. Temel kurumlar arasında büyük su işlerinin yapımını, bakımını ve su kaynaklarının tahsisini denetleyen Su Kaynakları Bakanlığı, ülkenin ekosistemlerinin ve su kaynaklarının kalitesinin yönetiminden ve korunmasından sorumlu olan Ekoloji ve Çevre Bakanlığı, tarımsal sulama ve üretim ile kırsal kalkınmayı yöneten Tarım ve Kırsal Kalkınma Bakanlığı bulunmaktadır. Ancak bu kurumların çoğu zaman belirsiz ve birbiriyle benzer sorumlulukları vardır ve koordinasyondan yoksundurlar. Bu durum işlem maliyetlerinin artmasına, politika geliştirme ve uygulamada gecikmelere yol açmaktadır (He vd., 2020).

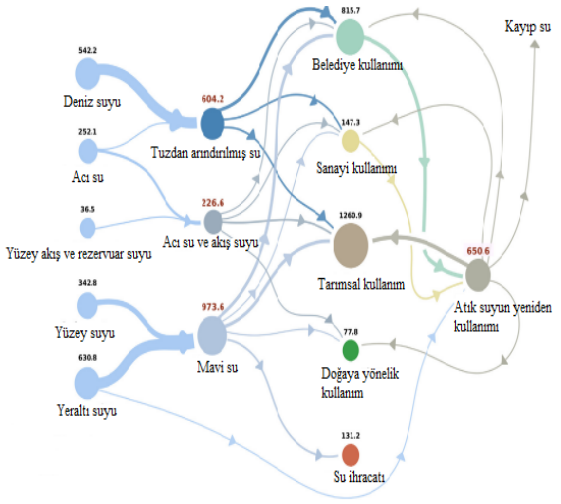
İsrail

İsrail'de önemli su reformlarına yönelik atılımlar, birkaç büyük kuraklık sonrasında su sektöründeki değişimin büyük ölçüde su krizinden kaynaklanmasına dikkat çekmiştir. 1998 yılında meydana gelen kuraklık, ilk kez İsrail'in büyük şehirlerinde kıtlık ve yetersiz su dağıtımıyla sonuçlanmış ve hükümetin geniş kapsamlı kurumsal reformlara başlaması için yeterli kamuoyu tepkisine yol açmıştır. 2001 yılında belediye su hizmetlerinin korunmasını gerektiren bir yasa çıkarılmıştır. 2002 yılında su sektörü için bir Parlamento Soruşturma Komisyonu kurulmuştur.

İsrail'de su reformunun dönüm noktası, 2007 yılında İsrail Su Otoritesinin (IWA) içme suyu ve sanitasyon, sulama, su kaynakları yönetimi için planlama ve düzenleme sorumluluklarını birleştiren özerk bir devlet kurumu olarak kurulmasıdır. Bu kurum, İsrail'in politika belirlemeden sorumlu olan siyasiler ile su sektörünü yöneten profesyoneller arasında bir çizgi çizmesini sağlamıştır. Su ve kanalizasyon sektörlerindeki tüm düzenleyici kurumlar, birkaç yıl içinde kademeli olarak IWA'ya devredilmiştir (Marin vd., 2017).

İsrail devletinde su yönetimine ilişkin yasal ve kurumsal teşkilatlanmada Enerji ve Su Bakanlığı, İsrail Su Otoritesi, Mekorot Su Şirketi, belediyeler ve bölgesel su kuruluşları, drenaj ve havza otoriteleri bulunmaktadır. Enerji ve Su Bakanlığı ulusal çapta su politikaları ortaya koyarken, İsrail Su Otoritesi tüm su kullanımları için planlama, kaynak tahsisi ve tarife düzenlemelerinden sorumludur. Mekorot Su Şirketi yine ulusal çapta faaliyet gösteren ve kullanma suyunun üretimi ve dağıtımından sorumlu olan kuruluştur. Bu şirketin akiferlerden yer altı suyunun çekilmesi ve su tesisleri, deniz suyunun tuzunun arındırılması, ulusal su ağının yönetimi gibi görevleri mevcuttur. Belediyeler ve bölgesel kuruluşlar, evsel ve endüstriyel su kullanıcılarına içme suyu dağıtımı, kanalizasyon toplama ve atık su arıtma yükümlülüklerine sahiptir (Marin vd., 2017).

Şekil 3'teki gibi İsrail'in su tedariki, suyun kullanım alanlarına göre değişmektedir. Mavi su (yeraltı suyu ve yüzey tatlı suyu), farklı kullanım kategorilerinin su temininde önemli bir rol oynamasına rağmen, herhangi bir birincil kullanım kategorisinin ana su kaynağı değildir. Tarım sektörü, yeniden kullanılmış atık suların %45'ini, mavi suların %32'sini ve acı suların %13'ünü kullanmaktadır. Tuzdan arındırılmış deniz suyu (%52) ve mavi su (%44) belediye sektöründe egemen olan su türüdür. Sanayi sektörü acı su (%38), mavi su (%35) ve tuzdan arındırılmış deniz suyu (%25) kullanmaktadır. Endüstriyel acı su kullanımının yaklaşık yarısının, tuzdan arındırma tesislerinden kaynaklandığı varsayılmaktadır. Doğaya yönelik kullanımlardaki su ise, mavi ve acı su karışımı ve ayrıca nehirlere arıtılmış atık su deşarjıdır. İhraç edilen su, yalnızca mavi sudan oluşmaktadır (Fridman vd., 2021).



Şekil 3: İsrail'de su kaynaklarından sektörlere doğru su akış şeması (Fridman vd., 2021)

Figure 3: Water flow chart from water sources to sectors in Israel. (Fridman et al, 2021)

İsrail'in su yönetiminde başarılı olduğu diğer bir husus, suyun geri dönüşümü ve yeniden kullanımıdır. Su tasarrufu ve geri dönüşüm yoluyla çevresel ihtiyaçları karşılayabilir ve yine de sürdürülebilir kalkınma ve uygulanabilir bir ekonomiye sahip olunabilir (Anderson, 2003).

Türkiye'de Havza Yönetimi

Cumhuriyet döneminde 1925 yılında "Su İdarelerinin Taksimat, Teşkilat ve Vezaifi Hakkında Talimat" adlı Kanun yürürlüğe girmiştir. Ancak su yönetiminde kurumsallaşma çabaları ve finansman bulma ihtiyacı 1939 yılından sonra hızlanmıştır. 1932-1939 yılları arası ekonomide devlet müdahalesinin yoğun olduğu devletçilik anlayışını yansıtmıştır (Yıldız, 2014).

1930'lu yıllardan başlayarak yabancı işletmelerin imtiyazlarının alınarak bunların belediyelere verilmesi ve şehir hizmetlerini yürütmek amacıyla kurulan şirketlerin belediyeler bünyesinde işletmelere dönüştürülmesi sağlanmıştır. 1980'li yıllara kadar bu belediyeleştirme politikası, su yönetiminde kamusal örgütlenmenin şekillenmesinde rol oynamıştır (Yıldız, 2014).

1950'lerde Türkiye'deki su kaynaklarının yönetiminden sorumlu olan kurum, Bayındırlık Bakanlığı'na bağlı Sular Umum Müdürlüğü'dür. Havza planlaması ve su yönetimine ilişkin yaklaşım, 1954'te "DSİ Umum Müdürlüğü Teşkilat ve Vazifeleri Hakkında Kanun" ile başlamıştır. Bu faaliyetlerle ilgili teknik kurumların başında Çevre ve Şehircilik Bakanlığına bağlı İller Bankası, Enerji ve Tabi Kaynaklar Bakanlığına bağlı Elektrik İşler Etüt İdaresi (Yenilenebilir Enerji İşleri Genel Müdürlüğü), İl Özel İdaresi Yerel Yönetimi ile Orman ve Su Bakanlığı (T.C. Tarım ve Orman Bakanlığı)'na bağlı DSİ'dir. Yasal düzenlemelerle ilişkili izleyici ve denetleyici kurumların en önemlileri Orman ve Su Bakanlığı, Sağlık Bakanlığı ve Kalkınma Bakanlığı'dır (Öztürk, 2011).

Ülkemizde havza yönetim çalışmaları, hidrolojik havzaların alt havzalara bölünmesi suretiyle gerçekleştirilmektedir. Su kaynaklarının mevcut su kalitesinin iyileştirilmesi ve korunması kapsamında AB Su Çerçeve Direktifi oluşturulmuş, ayrıca bu direktife bağlı olarak havza yönetimi yaklaşımı ile Havza Koruma Eylem Planları 2014 yılında hazırlanmaya başlanmış ve 2014-2023 yılı Ulusal Havza Yönetim Strateji (UHYS) vizyon hedefleri oluşturulmuştur (HKEPHP, 2014). Türkiye'de bu kapsam dahilinde 25 hidrolojik havza belirlenmiş, bu havzalara ve alt havzalarına yönelik olarak bütünleşik havza yönetimi için Havza Koruma Eylem Planları hazırlanmıştır (Memişoğlu vd., 2017).

Türkiye'de havza yönetimine ilişkin ilk kapsamlı düzenleme, 17/10/2012 tarihli ve 28444 sayılı Resmî Gazete'de yayımlanan "Havza Yönetim Planlarının Hazırlanması, Uygulanması ve Takibi Yönetmeliği'dir. Bu yönetmelik; yer altı ve yer üstü sularına bütüncül yaklaşımla havza temelinde fiziksel, kimyasal ve ekolojik kalite ölçütlerinin ve miktarının iyi olmasının sürdürülebilirliğini sağlamak, bozulan kaliteyi iyi hale getirmek, ihtiyaçları belirleyerek uygun kaynak tahsisi yapmak, ulusal su planı ve havza temelli yönetim planları hazırlamak, uygulamak ve takibini yapmak konularında usul ve esasları düzenlemektedir.

T.C. Tarım ve Orman Bakanlığı Su Yönetimi Genel Müdürlüğü tarafından hazırlanan 18 Ocak 2019 tarihli ve 30659 sayılı Resmî Gazete'de yayımlanan "Havza Yönetimi Merkez Kurulu, Havza Yönetim Heyetleri ve İl Su Yönetimi Koordinasyon Kurullarının Teşekkülü, Görevleri, Çalışma Usul ve Esaslarına Dair Tebliğ" yürürlüğe girmiştir. Tebliğ kapsamında havzaların yönetim planlarının hazırlanması, uygulanması ve uygulamaların takip edilmesi hedeflenmiştir. Bu süreçte kurumlar arası koordinasyonu sağlamak için Havza Yönetimi Merkez Kurulu, Havza Yönetim Heyetleri ve İl Su Yönetimi Koordinasyon Kurullarının kurularak faaliyet göstermesi esasları düzenlenmiştir.

SONUÇ

AB üye devletleri su politikası ve yönetiminde reform yapmak amacıyla 2000 yılında Su Çerçeve Direktifini

hazırlayarak Entegre Nehir Havzası Yönetimi paradigmasının uygulanması için önemli bir yapılanma adımı atmışlardır. AB su politikaları, 1970'li yıllardan 80'lerin sonuna kadar su kalitesi standartlarına ve belirli su kaynaklarının korunmasına öncelik vererek halk sağlığına odaklanmıştır. 1990'lı yıllarda ise AB su mevzuatı, kentsel atık sulardan ve tarımsal atıklardan kaynaklanan kirliliği ele almıştır. Ancak Avrupa'da su yönetimine ilişkin sorunlarda entegrasyon, koordinasyon ve sistem düzeyinde karar verme ihtiyacı giderek daha açık hale gelmiş ve Avrupa'nın çevre standartlarına ulaşmak için yeni düzenleyici tedbirlerin alınması ihtiyacı doğmuştur. Böylece birçok ülke tarafından kabul edilen Su Çerçeve Direktifinin temelleri atılmıştır. Bu direktif aracılığıyla dünyadaki su yönetiminin entegre su yönetimi ve paydaşların katılımı ile yürütülmesi hedeflenmiştir. Havza bazında su yönetimiyle gerek ajanslar gerek yerel yönetimin sürece katılımı sağlanmış ve küresel anlamda da uluslararası örgütlerin çevreyi korumaya yönelik projeleri ve işbirlikleri artmıştır.

Dünyada su yönetimi açısından ülkelere bakıldığında, her ülkenin idari yapılanmasına uygun gerek merkezden gerek yerelden örgütler oluşturulmuştur. Türkiye'ye benzer bir kamu örgütlenmesine sahip Fransa'da su yönetimi çoğunlukta devlet eliyle yürütülmektedir. Kamunun yanında entegre su yönetimine özel ve ortak paydaşlar dahildir. Belediyeler, su ajansları, ONEMA ve bazı yerel siyasi gruplar su yönetiminde etkili diğer paydaşlardır. Diğer örneklerde İspanya'da yerel yönetimler, İsrail'de merkez kontrolünden özel şirket otoritesi, Çin'de aşırı merkezi yönetim, İngiltere'de hükümet tarafından desteklenen Çevre Ajansı gibi yapılar etkindir. İngiltere'de ajanslar ve kurumlar yanı sıra meslek kuruluşları, profesyonel olmayan organize kuruluşlar ve yerel düzeyde vatandaşların da su yönetimi sürecinde rol oynamaları, merkezden yerele entegre havza yönetiminin iyi örnekleri arasındadır.

Türkiye'de su yönetimiyle ilgili çalışmalar 2. Dünya Savaşı sonrasında hız kazansa da 1954 yılında DSİ kurulduktan sonra kurumsal merkezi bir yapılanma ilk kez ortaya çıkmıştır. GAP İdaresi'nin 1989 yılında Güney Doğu Anadolu bölgesine özgü faaliyetlere başlamasına rağmen tamamen merkeze bağlı hareket ettiği gözlemlenmiştir. Türkiye'de de havza yönetiminde Avrupa Su Çerçeve Direktifinin yerel ve bölgesel yapılanma gerekliliğinden yola çıkılarak 2012 yılında mevzuatta düzenlemeler yapılmıştır. 2012 yılında yürürlüğe giren Havza Yönetim Planlarının Hazırlanması, Uygulanması ve Takibi Yönetmeliği ile Havza Yönetimi Merkez Kurulu, Havza Yönetim Heyeti ve İl Su Yönetimi Koordinasyon Kurulu gibi yapılanmalar ortaya konulmuştur.

Yönetmelikte yeraltı ve yerüstü sularının kalitesinin iyileştirilmesi ve havza yönetim planları yapılmasının öngörülmesi, Türkiye'nin Su Çerçeve Direktifine uyum sağlama girişimleridir. 2019 yılında çıkarılan tebliğ ile bu heyetlerin çalışma esasları detaylı biçimde düzenlenmiştir. Dünyada hükümetlerin yanı sıra su otoriteleri ve havza organizasyonları etkin olduğu görülürken, Türkiye'de bunlara karşılık gelen kuruluşların henüz çok yeni olduğu söylenebilir. Avrupa'da yerel ve bölgesel otoriteler daha çok aaktifken, Türkiye'de yalnızca kamu önderliğinde su yönetim faaliyetleri yürütülmektedir.

Türkiye'de su yönetimine ilişkin mevzuatın yapılanması için Avrupa'ya benzer biçimde toplu bir çerçeve kanun hazırlığının tamamlanması gereklidir. Özellikle havza yönetimiyle ilgili kurumsal yapının dağınık olması, Su Çerçeve Direktifinin başarılı şekilde uygulanmasında önemli bir engeldir. Su kanalizasyon idareleri (belediyeler), DSİ, Havza Yönetimi Merkez Kurulu, Havza Yönetim Heyeti ve İl Su Yönetimi Koordinasyon Kurulu gibi havza yönetimleri, GAP İdaresi, Tekirdağ Ergene Derin Deniz Deşarj (TEDDD AŞ) gibi anonim şirketlerinin ve halkın tümünün katılımını sağlayacak entegre havza yönetimi planlarına, koordinasyona ve denetime ihtiyaç duyulmaktadır. Bu hususta yeni havza yönetimlerinin bu eksikliği gidermesi açısından fayda sağlayacağı düşünülmektedir. Bu yönetimlerde dünyadaki uygulamalara benzer şekilde yerelden planlamanın ve yerelin görüşünün alınmasının, su yönetimine bölge paydaşlarının ilgisini artıracaktır. Bunun sonucunda daha düzgün bir çevresel planlamayı sağlayacaktır.

Avrupa'da olduğu gibi Türkiye'de belirtilen eksikliklerin giderilmesi sürecinin başlangıcında su ve havza yönetiminin karmaşık yapısı, teknik ve organizasyonel zorluklar gibi sorunlarla karşılaşılacağı öngörülmektedir. Ayrıca nehir havzası yönetiminin ülke genelinde farklılıklar gösteren mevcut su yönetişim yapıları bağlamında uygulanarak, ilgili devlet kurumlarının faaliyete geçmesi açısından önemli ölçüde zaman ve çaba harcanması beklenmektedir. Öte yandan getirilecek toplu mevzuatın uygulanması, önceki yönetim yaklaşımlarının hem baskın değerleri hem de çıkarları nedeniyle önemli bir dirençle karşılaşabilir. Havza yönetiminin tüm aşamalarında aşırı merkezi karar verme ve değişime direnç, yerleşik kurumlardan ve yönetişim rejimlerinden geçişi zorlaştırmaktadır. Bu nedenle Türkiye'de bölgesel ve yerel havza yönetimlerinin başarılı olmaları amacıyla gelecekte tüm paydaşları kapsayan bir çerçeve mevzuat oluşturulmalı, merkezi yapılar dışında havza organizasyonları, ajanslar, meslek grupları ve halk katılımı sağlanmalı, nehir havzası yönetim planları ortaya konularak uygulanmalıdır.

TEŞEKKÜR

Makale, Fatih Kızıltoprak'ın "Yeni Kurumsalcılık Yaklaşımına Göre Sanayi Atık Sularının Arıtılmasında Yeni Yönetim Modeli Analizi: TEDDD AŞ Örneği" isimli doktora tezinin bir kısmınıdır. Makalenin son aşamasında değerli katkılarını sunan Prof. Dr. Halil Şen'e teşekkürlerimi sunarım.

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Tüm yazarlar çalışma fikrine ve tasarımına katkıda bulunmuştur. Makale konusu Fatih Kızıltoprak tarafından, literatür taraması, materyal hazırlama ve veri analizini Fatih Kızıltoprak ve Cemile Ündücü Arıkoğlu tarafından gerçekleştirilmiştir. Makalenin yazılması ve düzenlenmesi Fatih Kızıltoprak ve Cemile Ündücü Arıkoğlu tarafından yapılmıştır ve tüm yazarlar makaleyi okumuş ve onaylamıştır.

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

ETİK ONAY

Bu makalede, insan ve hayvan ile ilgili bir çalışma içermemektedir. Dolayısıyla herhangi bir kurumdan etik onay alınmasına gerek görülmemiştir.

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

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