Ege Journal of
Fisheries and Aquatic Sciences

www.egejfas.org

E-ISSN 2418-3140

EgeJFAS

# Su Ürünleri Dergisi

Volume 40 Number 1 2023

Ege University Faculty of Fisheries

# Authors Guidelines

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

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

## GENERAL INFORMATION

### Aim & Scope

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

The journal does not charge any submission and publication fees.

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

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

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

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

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

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

### Language

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

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

### Editorial Policy and Referee Process

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

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

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

### Article Types

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

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

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

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

#### Reports

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

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

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

## MANUSCRIPT SUBMISSION

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

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

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

#### While starting

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

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

To follow the status of the article;

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

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

#### Similarity Report

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

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

#### Cover Letter

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

#### Ethics in Publishing

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

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

## PREPARATION OF MANUSCRIPTS

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

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

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

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

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

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

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

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

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

### First Page

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

### Author Names and Affiliation

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

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

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

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

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

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

## Abstract

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

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

## Keywords

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

## Following pages

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

## Introduction

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

## Material and Methods

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

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

## Results

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

## Discussion

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

## Conclusions

This should briefly state the major findings of the study.

## Acknowledgements and Funding

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

Examples:

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

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

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

Financial support acknowledgwment should be written like the example given:

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

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

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

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

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

## Authorship Contributions

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

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

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

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

Full name/s: Conceptualization, Methodology, Software

Full name: Data curation, Writing- Original draft preparation

Full name/s: Visualization, Investigation

Full name/s: Supervision

Full name/s: Software, Validation

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

Full name/s: Writing- Reviewing and Editing

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

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

## Changes to Authorship

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

## Conflict of Interest Statement

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

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

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

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

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

## Ethics Approval

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

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

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

Examples:

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

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

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

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

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

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

## Retrospective Ethics Approval

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

## Data Availability

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

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

Examples:

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

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

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

Data availability: All relevant data is in the article.

## Scientific Style

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

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

### Equations and units

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

### Abbreviations

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

### Footnotes

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

## References

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

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

### In-Text Citation

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

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

For example: Kocataş, 1978, p. 3

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

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

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

**One author:**

Consider the following examples:

-…….(Kocataş, 1978)

- Kocataş (1978) states…….

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

**Two authors:**

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

Consider the following examples:

-…….(Geldiay and Ergen, 1972)

- Geldiay and Ergen (1972) states……

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

**More than two authors:**

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

See below examples:

-Geldiay et al. (1971) state…….

-……( Geldiay et al., 1971).

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

**Two or more works by different author:**

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

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

**Two or more works by the same author:**

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

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

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

For Example:

-Geldiay and Ergen, 1972a

-Geldiay and Ergen, 1972a, b

**No authors:**

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

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

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

**No publication date:**

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

Example: (Geldiay, n.d.).

**Citation to secondary sources:**

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

For Example:

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

**Personal communication and unpublished results:**

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

Example:

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

**General use of websites and software:**

It should be showed as below.

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

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

### In References

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

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

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

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

Hanging indent paragraph style should be used.

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

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

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

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

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

For example:

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

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

For example:

Kocataş, A. (1978)

Kocataş, A., & Ergen, Z. (1972).

Kocataş, A., & Geldiay, R. (1972)

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

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

**Journal Articles**

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

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

**Books**

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

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

**Chapter in books**

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

**E-books and chapter in e-books**

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

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

**Proceedings**

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

**Websites**

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

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

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

**Thesis**

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

### Tables and Figures

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

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

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

**Tables**

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

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

**Figures**

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

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

## Permissions

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

### Page Charges

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

### Plagiarism Detection

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

### Indexes

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

## COPYRIGHT NOTICE

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

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

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

Authors publishing in this journal accept the conditions described below.

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

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

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

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

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

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

## Submission checklist

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

## Important Note

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

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

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

**E- ISSN 2148-3140**

**Su Ürünleri Dergisi**
**Ege Journal of Fisheries and Aquatic Sciences**



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

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

**Yayımlanma Tarihi** **Publishing Date**
15 Mart March 15th, 2023

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

# Su Ürünleri Dergisi

Ege Journal of Fisheries and Aquatic Sciences

**Volume 40 Number 1** **ISSN 1300-1590 / E-ISSN 2418-3140**

## *İÇİNDEKİLER CONTENTS*

### ARAŞTIRMA MAKALELERİ RESEARCH ARTICLES



### KISA MAKALE SHORT COMMUNICATION



### DERLEME REVIEW



**Published by**

**Ege University Faculty of Fisheries, İzmir, Türkiye**

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 1-14 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.01

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Determination of risk perception in small-scale fishing and navigation

## Küçük ölçekli balıkçılıkta ve seyir sırasında risk algısının belirlenmesi

**Can Atacan[1] ● Faik Ozan Düzbastılar[2]***

[1] Ege University, Urla Maritime Vocational School, 35430, Urla, İzmir, Türkiye
[2] Ege University, Faculty of Fisheries, 35100, Bornova, İzmir, Türkiye

https://orcid.org/0000-0002-4490-7566
https://orcid.org/0000-0002-5376-7198

*Corresponding author: f.ozan.duzbastilar@ege.edu.tr

Received date: 27.07.2022 Accepted date: 03.10.2022

***How to cite this paper:***
Atacan, C. & Düzbastılar, F.O. (2023). Determination of risk perception in small-scale fishing and navigation. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 1-14. https://doi.org/10.12714/egejfas.40.1.01

**Abstract:** In this study, risk perception and the impact of various environmental factors on accidents involving fishing vessels in small-scale fishing vessels during navigation were examined. Thirty fishing vessel captains from Çeşmealtı and İskele fishing ports evaluated the risks of environmental conditions under different scenarios in the bridge navigation simulator based on the Fine-Kinney risk assessment method. Nonparametric (Mann-Whitney U-test) and parametric tests (Pearson correlation and independent-samples t-test) were performed to analyse other related parameters. The study also conducted a small questionnaire study that included questions such as the number of past accidents by fishermen and the number of engine rudder failures. According to the sum of the fishermen's assessments, reduced visibility was the highest factor increasing the probability and consequences of accidents in sea navigation, while they identified night and heavy weather conditions as the highest factor in port navigation. Fishermen also found navigating their ports safer than sea navigation. There is a significant, positive, and strong correlation between the number of fishermen's accidents and the number of engine rudder failures (p=0.047, r=0.714), the perception of accident probabilities in port navigating with restricted visibility (p=0.027, r=0.726) and in port navigation at night and in heavy weather (p=0.003, r=0.866). According to the results of the study, using the outcomes of the pre-fishing environmental risk assessment, the competent maritime authorities may be able to take effective measures to prevent the occurrence of serious marine casualties.

**Keywords:** Small-scale fishery, accident probability, accident consequence, risk perception, navigation

**Öz:** Bu çalışmada, küçük ölçekli balıkçı teknelerinde seyir sırasında karıştığı kazalardaki risk algısı ve çeşitli çevresel faktörlerin etkisi incelenmiştir. Çeşmealtı ve İskele balıkçı barınaklarından Otuz balıkçı gemisi kaptanı, Fine-Kinney risk değerlendirme metoduna dayanarak köprü üstü seyir simülatöründe farklı senaryolar altında çevresel koşulların risklerini değerlendirmişlerdir. Diğer verilerin analizi için de parametrik (Pearson korelasyon analizi ve t testi) ve parametrik olmayan (Mann-Whitney U testi) testler kullanılmıştır. Çalışmada ayrıca balıkçıların daha önceki deneyimlerinden kaza sayıları, makine dümen arızası gibi sorulardan oluşan bir anket çalışması da yapılmıştır. Balıkçıların değerlendirmeleri sonucu ortaya çıkan puanların toplamına göre deniz seyrinde kaza ihtimal ve neticesini arttıran en yüksek etken olarak kısıtlı görüşü belirlemişken liman seyrinde gece ve şiddetli hava koşulunu en yüksek etken olarak belirlemişlerdir. Ayrıca balıkçılar kendi limanlarında yaptıkları seyri deniz seyrine göre daha emniyetli bulmuşlardır. Balıkçıların yaşadıkları kaza sayısı ile makine-dümen arıza sayısı (p=0,047, r=0,714), liman seyrinde kısıtlı görüşte (p=0,027, r=0,726) ve şiddetli havada gece seyrinde kaza ihtimali algısı (p=0,003, r=0,866) arasında anlamlı ve pozitif ve güçlü ilişki vardır. Çalışmanın sonuçlarına göre, küçük ölçekli balıkçıların denize çıkmadan önce çevresel şartlara göre risk değerlendirmesi sonuçlarından yararlanılarak ilgili denizcilik makamlarının ciddi deniz kazalarının meydana gelmesini önlemek için etkili önlemler alması mümkün olabilir.

**Anahtar kelimeler:** Küçük ölçekli balıkçılık, kaza ihtimali, kaza neticesi, risk algısı, seyir

## INTRODUCTION

It is estimated that half a billion people worldwide make a living from artisanal (small-scale) fishing. The Food and Agriculture Organization of the United Nations reports that small-scale fishermen are responsible for 40% of the total catch and 68% of marine capture (FAO, 2021). Although small-scale fisheries depend on specific conditions such as developed or underdeveloped countries, it can be briefly defined as a type of traditional fishery for local consumption using small boats, low-tech gear, and deck equipment, mainly near shore (Halim et al., 2019; Smith and Basurto, 2019). Due to its ecological, economic, cultural, socio-political, and nutritional importance, the sustainability of small-scale fisheries is increasing worldwide (Halim et al., 2019; Smith and Basurto, 2019; FAO, 2021; Villasante et al., 2022). However, maritime and commercial fishing, in particular, is one of the most dangerous occupations in the world in terms of safety and has a high mortality rate (Jaremin and Kotulak, 2004; Jin and Thunberg, 2005; FAO, 2014).

The accident statistics of the European Maritime Safety Report show the danger in the fishing industry and endanger its sustainability (EMSA, 2022). For example, 50% of all accidents involving fishing vessels in EU countries are classified as serious or very serious. Although fishing accidents rank third among vessel types, when the severity of the accident is taken into account, they move up to second place. Especially in the case of serious accidents such as shipwreck and ship loss, fishing boats are far ahead of other types of ships (EMSA, 2022; Wang et al., 2021).

Maritime accidents have been an important subject of study in the literature for many years. In particular, some previous work has examined a significant association between fishing vessel accidents and boat length, boat age, weather, sea conditions, visibility, and time of day (Jin et al., 2001; Jin et al., 2002; Wu, 2008; Wu et al., 2009; Roberts et al., 2010; Kim and Kang, 2011; Jin, 2014; Pleskacz, 2015; Yıldırım and Başar, 2019). Most of the work in this research has focused on accident categories such as collision, stranding, sinking, fire-explosion, industrial accident, and man overboard (Wang et al., 2005; Bayar, 2010; Sur and Kim, 2020; Uğurlu et al., 2020). Much research has been done in the literature on human error concepts emphasized in accidents involving fishing vessels like other marine casualty studies (Kim and Kang, 2011; Jung, 2014; Won and Kim, 2019). One of the reasons for marine casualties is defined as the fact that fishermen at busy traffic separation lines follow different routes than other vessels, which increases the number of accidents (Oh et al., 2015). The other reasons like falling asleep on the bridge, lack of experience, and leaving the helm unattended can lead to consequences like sinking of the ship and death of the fishing vessel were mentioned by Soykan (2018). While not enough to reduce incidents at sea caused by human error, engineers over the past five decades have focused on technological improvements in hull design, ship propulsion, auxiliary deck equipment, navigation, and safety equipment (Formela et al., 2019; Hasanspahić et al., 2021).

Numerous studies have identified human error as the most important factor influencing accident occurrence (Roberts et al., 2010; Awal and Hasegawa, 2017; Kim and Na, 2017; Fan et al., 2020; Hasanspahić et al., 2021; Demirci et al., 2022). However, looking at these studies in general, environmental factors influence accident occurrence in almost all of them (Kim and Kang, 2011; Pleskacz, 2015). There is no specific study on how different environmental factors increase the probability of accidents or how they affect the risk of accidents. Previous studies on marine casualties have generally taken a reactive approach (Awal and Hasegawa, 2017) and sought a risk assessment based on previous casualties. A proactive approach in this area and a close examination of the risk development process can become more important to eliminate the risks (Psaraftis, 2002; Montewka et al., 2014; Haapasaari et al., 2015; Luo and Shin, 2019).

Many methods in the literature address the problem by identifying or preventing factors that influence marine casualties. Our potential solution is a proactive approach to risk assessment. The aim of this study was to fill the gap by proactively addressing this issue and identifying the risks of operating on small fishing vessels. Therefore, a risk assessment method for fishermen navigating in the different scenarios prepared in the bridge navigation simulator was used to determine the environmental conditions affecting the level of risk during navigation.

## MATERIAL AND METHODS

The study consists of (1) a questionnaire with demographic and occupational questions and their statistical analysis for a better understanding of the topic and (2) simulator experiments in different scenarios in which a risk assessment is made. The second goal was the principal objective of this research. 20 out of 27 fishermen from İskele Port and 10 out of 30 fishermen from Çeşmealtı (in total, 30 fishermen from two ports) participated in the study. The fishermen completed a questionnaire containing some information from their experience along with demographic questions and finally assessed the accident probability and consequences according to a risk assessment method based on the scenarios in the simulator.

The bridge operation simulator ARI (Applied Research International, Version 1.0), classified by DNV and GL (Det Norske Veritas and Germanischer Lloyd), was used for simulator experiments. The simulator is supported by a total of 21 computers. Navigation aids such as radar, ECDIS, and echo sounder are available in the simulator (Figure 1). The methodology imposed on the simulation allows, through the simultaneous application, to effectively estimate the probabilities and consequences of different random scenarios (Li et al., 2012). Three different scenarios (sea navigation, İskele port navigation, and Çeşmealtı port navigation) have been created in the bridge simulator to allow the fishermen to navigate in 30 minutes under different environmental conditions (current, night navigation, etc.) (Figure 1). Scenarios were prepared in the bridge simulator and the fishermen were asked to use the fishing boat (LOA 18 m, width: 4.5 m, draft: 1.5 m). Only active captains of fishing vessels working in İskele and Çeşmealtı fishing ports have been included in the scenarios. No restrictions such as age, gender, experience, and competence variables were set for the participants. The same scenario has been applied to everyone in sea navigation. However, for port navigation, fishermen from İskele and Çeşmealtı Port sailed into their own port. It was considered more appropriate to conduct the navigation in their own ports and evaluate their results, since fishing boats do not call at different ports like commercial boats.

The simulation experiments were performed in the following flow order: (1) The same parameters were applied to the environmental conditions (current, heavy weather, restricted visibility, etc.) for both port and sea navigation. Scenarios in the simulator were started in calm weather and sea conditions. (2) In the scenarios, the shipping traffic was prepared according to the customs of the region. Since sea navigation is located in the traffic separation scheme, shipping traffic from east to west and vice versa was prepared. All participants were instructed to cross the separation line in a northerly direction. (3) In the port navigation, a sailing boat has been placed on the breakwater and three fishing boats have been placed in the port area outside of the port. In this condition, all participants were instructed to proceed to port about 5 cables from the breakwater. (4) In the scenario, the current speed was set to 4 knots, the range for the restricted visibility was reduced to 100 meters and the wind force was

determined to be 5 Beaufort. (5) The fishermen were given sufficient time (At least 30 min.) during the simulation to adapt to the situation and provide healthy feedback. (6) Before the simulation experiments, they were informed about the test methodology and the Fine Kinney risk assessment with regard to accident probability, consequences, and frequency. (7) A maximum of four fishermen were employed in the simulator at the same time. (8) Fishermen initially navigated in the traffic separation scheme, where commercial ships, sailing boats, and fishing boats navigated in calm seas and calm weather. Then environmental conditions were changed in the simulation. After applying each environmental factor, it ceded its place to the new condition. (9) The scenario assumed a current of 4 knots that act laterally on the fishing boat and waited a while for the fishermen to notice the current. When fishermen feel the current effect, their feedback is collected and the risk perception and score are calculated. (10) Then the range for the restricted visibility was reduced to 100 meters in the experiment. Similarly, they were allowed to cruise for a while and their opinions were taken. (11) After that, night navigation was applied using the same procedure. (12) After the night's navigation, the fishing boat faced heavy weather from north winds (5 Beaufort). Again, feedback was received from the fishermen at the end of the scenario. (13) In the end, the combination of heavy weather and night navigation was applied simultaneously in the scenario. Then, the views of the fishermen were sought to carry out a risk assessment.

Occupational hazards (current, night navigation, reduced visibility, etc.) are identified before applying this risk assessment method. Work-related hazards identified by fishermen are collision, allision, grounding, flooding, and capsizing. Then the probability of occurrence of these hazards, the consequence values (*C-value*) (Table 1), and the frequency factor ($F_2$) (Table 2) are determined by the experts according to the environmental conditions. The risk score is obtained by multiplying the criteria defined by the experts for these three parameters.


**Figure 1.** ARI (Applied Research International, Version 1.0) bridge operation simulator and port navigation for İskele and Çeşmealtı fishing ports in the simulator

The probability and consequences of accidents were determined based on the personal assessments of the fishermen participating in the study. In determining the frequency factor, both the weather information in the region and the exposure to environmental and marine conditions were taken into account.

**Table 1.** Probability and consequence scales are rated by fishermen according to the Fine-Kinney risk assessment

| Probability (*P*) | | Consequence (*C*) | |
|---|---|---|---|
| ***P-value*** | ***Statement*** | ***C-value*** | ***Statement*** |
| 10 | Might well be expected | 100 | Catastrophic (many fatalities, or > $10^7$ damage) |
| 6 | Quite possible | 40 | Disaster (few fatality, or > $10^6$ damage) |
| 3 | Unusual but possible | 15 | Very serious (fatality, or > $10^5$ damage |
| 1 | Only remotely possible | 7 | Serious (serious injury, or > $10^4$ damage) |
| 0.5 | Conceivable but very unlikely | 3 | Important (disability, or > $10^3$ damage) |
| 0.2 | Practically impossible | 1 | Noticeable (minor first aid accident, or > $10^2$ damage) |
| 0.1 | Virtually impossible | | |

In the Fine-Kinney method, the frequency factor (*F*) shows the frequency of exposure of the worker to the working conditions. Frequency is a key factor in risk assessment. Therefore, this step is essential for making the method feasible for a valid risk assessment. According to the fishermen involved in the investigation, they cruise to fish several times a week. The frequency of occurrence of environmental conditions such as calm air-sea conditions, currents, restricted visibility, and heavy weather was determined using fisherman experience, related literature, and annual meteorological reports. It is considered that fishermen in İzmir Bay meet boats once a week; because the ships entering or leaving the ports operate in a traffic separation scheme and there are many fishing ports and marinas. Therefore, the calm air-sea condition state frequency expression was chosen from time to time (once a week) and the $F_2$ value was chosen as 3 from Table 2.

The current in the bay is caused by temperature-dependent density fluctuations and wind (Eronat, 2017). The literature states that the wind plays a crucial role in the current long-term winds for İzmir Bay. Accurate information about the current strength in İzmir Bay could not be obtained from the sources, but the fact that the winds act on the current means that the seasonal continuous surface currents and tidal currents are weak. Therefore, the frequency factor for the magnitude of the current was determined according to the wind. The frequency factor $F_2$ value for the wind variable was determined as 2 from the meteorological data and the assessments of the fishermen. A rare expression was chosen for the restricted visibility, corresponding to an $F_2$ value of 1, as fog/haze factor occurs several times a year in the region. The night-time condition occurs once a day, but since fishermen do not work at sea every day, the frequency of night navigation was chosen to be occasional (once a week), which corresponds to the $F_2$ value of 3. Since the occurrence of night and heavy weather conditions was correspondingly rare, the same value was selected for the night-heavy weather conditions with the frequency of heavy weather. The frequency values to be used to determine the risk score are listed in the $F_2$ values section of Table 2.

**Table 2.** Frequency table used in the risk assessment calculation ($F_1$: Fine-Kinney risk assessment frequency table. $F_2$: Values derived from fishermen's experience, previous studies, and local meteorological data)

| Environmental condition | Frequency (*F*) | | | |
|---|---|---|---|---|
| | $F_1$ value | Statement | $F_2$ value | Statement |
| Calm weather and sea | 10 | Continuous | 3 | Occasional (weekly) |
| Current* | 6 | Frequently (daily) | 2 | Unusual (monthly) |
| Restricted visibility** | 3 | Occasional (weekly) | 1 | Rare (a few per year) |
| Night navigation | 2 | Unusual (monthly) | 3 | Occasional (weekly) |
| Navigation in heavy weather*** | 1 | Rare (a few per year) | 2 | Unusual (monthly) |
| Night-heavy weather navigation*** | 0.5 | Very rare (yearly) | 2 | Unusual (monthly) |

*Current speed = 4 knot
**Visibility = 100 m
***Heavy weather = 5 Beaufort (NNE)

There are some well-established methods for determining risk assessment. Much research has been done on risk assessment at sea (Jin et al., 2002, Haapasaari et al., 2015, Awal and Hasegawa, 2017, Hasanspahić et al., 2021). Several methods for risk assessment have been reported in the literature. Considering the studies, risk matrix methods have been used quite frequently (Akyıldız, 2015, Gucma and Ślączka, 2018, Hsu et al., 2022). In this study, the Fine Kinney method, a qualitative and applied risk analysis method in occupational safety, was used to estimate the risk of sea and port navigation under different environmental conditions (restricted visibility, current, calm weather, sea, etc.). (Kinney and Wiruth, 1976). The Fine-Kinney method was first introduced in 1971 by William T. Fine (Fine, 1971). An improvement over this method was developed by Kinney and Wiruth (Kinney and Wiruth, 1976).

Although the Fine-Kinney method, which is similar to the risk matrix method, is not widely used in research in the maritime field, it has been used in research in various fields and has been found to provide more reliable and realistic results than the risk matrix method (Okumuş and Barlas, 2016; Bekdemir, 2019; Zaloğlu, 2019; Ölçücü and Ersöz Kaya, 2019; Usanmaz and Köse, 2020). The Fine-Kinney method differs from the risk matrix method in that it includes a wider range of parameters and the frequency factor. Offering the decision maker, a wider choice of options ensures that the risk assessment provides more reliable results. The fishermen chose the appropriate statement according to their knowledge during the simulator experiments as shown in Table 1.

After determining the probability (*P-value*) and consequence (*C-value*) values, the risk was calculated using Eq. (1). Correlation tests were performed to determine the significant differences between the fishermen's age, experience, length of the fishing boat, number of accidents, number of engine rudder failures, and accident probability and consequence values. The Mann-Whitney U test was used to determine the significant differences between two groups of fishermen from İskele and Çeşmealtı fishing ports, as well as accident probability and consequence values. The Mann-Whitney U test requires the presence of two independent variables (İskele and Çeşmealtı fishermen) and also dependent variables (probability and consequence ratings) that are not normally distributed. A secondary objective of this study was to obtain some of the occupational information presented in the Results section from the fishermen via questionnaire. An independent sample t-test was used for these data (boat size, experience, etc.) from the questionnaire. It was used to determine the significant differences between two groups of fishermen from İskele and Çeşmealtı fishing ports in terms of their experience, boat length, number of accidents, and engine-rudder failures. We use the above methods because they are simple and relatively efficient. All nonparametric and parametric tests were performed using the SPSS 22.0 software package. Figures 2, 3, 4, and 5 were drawn according to the fishermen's responses, and Figures 5, 6, 7, and 8 were drawn from the results of Eq. 1.

$$\text{Risk} = P \times C \times F_2 \qquad \text{Equation (1)}$$

## RESULTS

This study examined the probability and consequences of maritime accidents related to environmental factors. First, survey questions were prepared to uncover the profile information and experiences of the participants on a topic-specific basis. The parameters in the Fine-Kinney risk assessment method were added at the end of the prepared questionnaire and communicated to the decision-makers during the scenarios. Fishing captains took turns entering the simulator room and giving their evaluations. The results were analysed and the following findings were obtained. As the results are based on the opinions of active fishermen, it is believed that the risk perceived by fishermen during the fishing trip should be appropriately assessed.

A total of 20 fishermen from the İskele fishing port and 10 fishermen from the Çeşmealtı fishing port took part in our study. All fishermen who participated in the survey were male (30). Of these, 70% of the fishermen were married. The percentage of fishermen aged 46 years or older in the study was 63.3%. The educational structure of the participants was; Elementary education (46.7%), high school (33.3%), associate degree (10.0%), bachelor's degree (6.7%), and master's degree (3.3%). When assessing the competence of the participants, it was found that 3.3% (1) bosun, 30.0% (9) able seaman, 46.7% (14) seaman, 6.7% (2) fishing boat captain, 3.3% (1) were fishermen' crew, 3.3% (1) were watchkeeping officer, and 6.7% (2) were amateur seaman's certificate.

Regarding work experience in small-scale fisheries, it was found that 13.3% (4) had 1-10 years of experience, 30.0% (9) had 11-20 years of experience, 26.7% (8) had 21-30 years of experience, 23.3% (7) had 31-40 years of experience, and 6.7% (2) had more than 41 years of experience. The average experience of the fishermen was 27.8 years (s.e.=0.285) and 21.8 years (s.e.=0.268) for İskele and Çeşmealtı fishing ports, respectively (p=0.133). There was no significant difference between the fishermen's experience and their fishing ports (p=0.324) (Table 3).

**Table 3.** Independent samples t-Test results (grouping variable is fishing port)

| Test variables | | Levene's Test for Equality of Variances | | | | |
|---|---|---|---|---|---|---|
| | | F | Sig. | t | df | Sig. (2-tailed) |
| Experience | Equal variances assumed | 2.400 | 0.133 | 1.005 | 28.000 | 0.324 |
| | Equal variances not assumed | | | 1.148 | 25.427 | 0.262 |
| Boat size | Equal variances assumed | 2.687 | 0.112 | 0.409 | 28.000 | 0.685 |
| | Equal variances not assumed | | | 0.508 | 27.999 | 0.615 |
| Accident | Equal variances assumed | 3.892 | 0.080 | 1.833 | 9.000 | 0.100 |
| | Equal variances not assumed | | | 2.360 | 7.643 | 0.047 |
| Engine or rudder failures | Equal variances assumed | 0.342 | 0.564 | 0.768 | 26.000 | 0.449 |
| | Equal variances not assumed | | | 0.800 | 14.109 | 0.437 |

Most commercial fishing boats are typically shorter than 12 meters (60% 4-7 m and 30% 8-11 m) but only three boats (10%) were longer than 15 m. The average length of fishing boats was 9.31 m (s.e.=0.243) and 8.77 m (s.e.=0.166) in İskele and Çeşmealtı fishing ports, respectively (p=0.112). No significant differences were found between the size of fishing boats and their fishing ports (p=0.685) (Table 3). While the fishermen indicated that almost all fishermen (86.7% - 26 participants) used gillnets, the remaining fishermen used other fishing gear such as handline, longline, etc. In the study, fishermen's navigational experience was assessed using eight survey questions. 70% of the participants stated that they had never had a marine casualty. 30% of the fishermen who had accidents told us how often they had an accident (three people for 1-3 times, two people for 4-7 times, two people for 8-11 times, and two people for 14+). The average number of accidents that fishermen had was 5.5 (s.e.=0.633) and 0.5 (s.e.=0.250) for İskele and Çeşmealtı fishermen, respectively (p=0.080). It was found that there was no significant difference between the number of accidents and the associated fishing ports (p=0.100) (Table 3). All fishermen accepted that the perception of risk when fishing is higher than when navigating at sea or in port.

They specified the type of accidents they had as collision and allision (3 people), grounding (5 people), flooding (4 people), and capsizing (1 person). All fishermen except two fishermen reported suffering engine or rudder failure while working at sea. When the fishermen were asked how often they had engine or rudder failures, it was found that 35.7% (10) had 1-4 failures, 32.1% (9) had 5-8 failures, 7.1% (2) had 9-12 failures, 14.3% (4) had 13-16 failures, and 10.7% (3) had 17+ failures. The mean engine or rudder failures were 10.2 (s.e.=0.320) and 5.1 (s.e.=0.462) for İskele and Çeşmealtı fishermen, respectively (p=0.564). There was no significant difference between the number of any type of failures and their fishing ports (p=0.100) (Table 3).

According to the results of the correlation test, there is a significant (p=0.038), negative (-), and moderate (r=0.381; Pearson correlation) relationship between the experience of fishermen and the probability of an accident in port navigation with the current situation (Table 4). More experienced

fishermen said that under current conditions, port navigation is less likely to result in accidents than less experienced fishermen. No significant difference was found between fishermen's experience and length of boat used (p=0.361), number of accidents (p=0.850), number of engine and rudder failures (p=0.066), and probability and consequence values of accidents under other environmental conditions while navigating at sea and in port (all values greater than 0.05). No significant difference was found between the boat lengths reported by the fishermen and the number of accidents suffered (p=0.089), the number of engine and rudder failures (p=0.118), and the probability and consequence values of accidents under environmental conditions during navigation at sea and in port (all "p" values greater than 0.05).

**Table 4.** The results of the correlation test between experience and probability of an accident in port navigation in the current state

| | | Probability of accident in port navigation having the current condition |
|---|---|---|
| **Experience** | Pearson correlation | -0.381 |
| | Sig. (2-tailed) | 0.038 |
| | Number | 30 |

There is a significant, positive, and strong association between the number of accidents experienced by fishermen and the number of engine and rudder failures (p=0.047, r=0.714), the probability of an accident in port navigation with restricted visibility (p=0.027, r=0.726) and with the night-heavy weather conditions in port navigation (p=0.003, r=0.866) (Table 5).

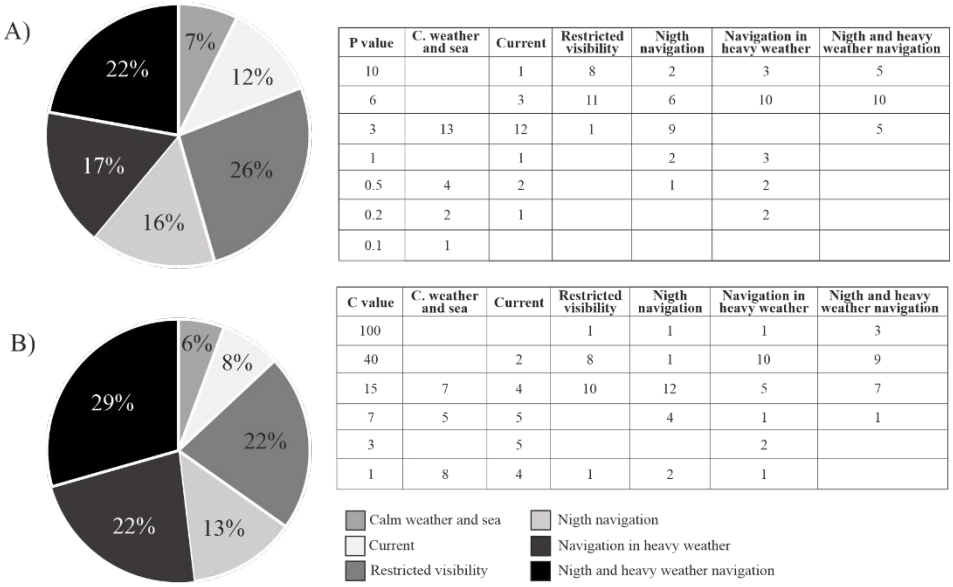
**Table 5.** The results of the correlation test between the number of accidents and other parameters show a significant connection

| | | Number of machine-rudder failures | Probability of accident in port navigation with restricted visibility | Probability of accident with the night-heavy weather condition in port navigation |
|---|---|---|---|---|
| **Number of accidents** | Pearson correlation | 0.714 | 0.726 | 0.866 |
| | Sig. (2-tailed) | 0.047 | 0.027 | 0.003 |
| | Number | 8 | 9 | 9 |

Fishermen from both fishing ports reported being at sea several times a week to fish throughout the year. Before the simulator experience, all participants foresaw stranding, collision, and allision as a risk of navigation at sea. In order to design a scenario on the ship bridge simulator, we were told that the prevailing wind direction is north.

According to the simulator experiments, the most important condition emerged as restricted visibility (26%), followed by night and weather navigation (22%), and heavy weather navigation for accident probability of fishermen from the İskele fishing port in sea navigation (Figure 2).

The most hazardous conditions were identified as night and weather navigation (29%), restricted visibility (22%), and heavy weather navigation (22%) for the consequences of marine casualties assessed by fishermen from İskele fishing port (Figure 2).

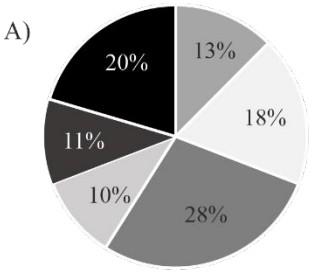



| P value | C. weather and sea | Current | Restricted visibility | Nigth navigation | Navigation in heavy weather | Nigth and heavy weather navigation |
|---|---|---|---|---|---|---|
| 10 | | 1 | 8 | 2 | 3 | 5 |
| 6 | | 3 | 11 | 6 | 10 | 10 |
| 3 | 13 | 12 | 1 | 9 | | 5 |
| 1 | | 1 | | 2 | 3 | |
| 0.5 | 4 | 2 | | 1 | 2 | |
| 0.2 | 2 | 1 | | | 2 | |
| 0.1 | 1 | | | | | |

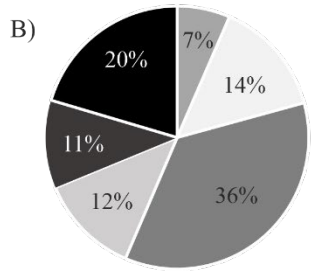
| C value | C. weather and sea | Current | Restricted visibility | Nigth navigation | Navigation in heavy weather | Nigth and heavy weather navigation |
|---|---|---|---|---|---|---|
| 100 | | | 1 | 1 | 1 | 3 |
| 40 | | 2 | 8 | 1 | 10 | 9 |
| 15 | 7 | 4 | 10 | 12 | 5 | 7 |
| 7 | 5 | 5 | | 4 | 1 | 1 |
| 3 | | 5 | | | 2 | |
| 1 | 8 | 4 | 1 | 2 | 1 | |

**Figure 2.** A) Percentages of probability and rating of fishermen from İskele fishing port in sea navigation. B) Percentages of consequences and ratings of fishermen from İskele fishing ports in sea navigation (*P-value*: value of the probability of accidents; *C-value*: value of the consequences of accidents)

The most dangerous factor, according to Çeşmealtı fishermen, was restricted visibility (28%), followed by night and weather navigation (20%), and current (18%) for accident probability in sea navigation (Figure 3). When assessing the accident consequence of fishermen from Çeşmealtı fishing port in the sea navigation, the factors with the highest percentages were restricted visibility (36%), night and weather navigation (20%), and current (14%) (Figure 3).



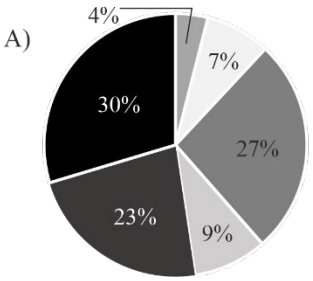

| P value | C. weather and sea | Current | Restricted visibility | Nigth navigation | Navigation in heavy weather | Nigth and heavy weather navigation |
|---|---|---|---|---|---|---|
| 10 | 2 | 3 | 7 | 1 | 1 | 4 |
| 6 | 2 | 4 | 3 | | 2 | 2 |
| 3 | 2 | 1 | | 7 | 3 | 4 |
| 1 | | | | | 1 | |
| 0.5 | 2 | 1 | | 2 | 1 | |
| 0.2 | | 1 | | | | |
| 0.1 | | | | | 2 | |



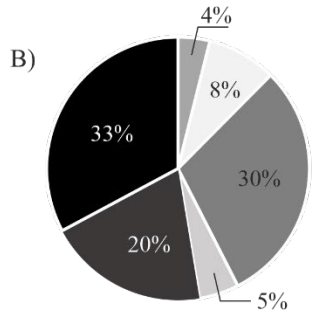

| C value | C. weather and sea | Current | Restricted visibility | Nigth navigation | Navigation in heavy weather | Nigth and heavy weather navigation |
|---|---|---|---|---|---|---|
| 100 | | 1 | 4 | | 1 | 2 |
| 40 | | 2 | 3 | 4 | 1 | 2 |
| 15 | 6 | 1 | 1 | 1 | 1 | 1 |
| 7 | 1 | 3 | 2 | 1 | | 1 |
| 3 | | | | 2 | 2 | 4 |
| 1 | 3 | 3 | | 2 | 5 | |


**Figure 3.** A) Percentages of probability and rating of fishermen from Çeşmealtı fishing port in sea navigation. B) Percentages of consequences and ratings of fishermen from Çeşmealtı fishing ports in sea navigation (*P-value*: value of the probability of accidents; *C-value*: value of the consequences of accidents)

In port navigation, night and weather navigation (30%), restricted visibility (27%), and heavy weather navigation (23%) were the most important factors for the accident probability of İskele fishermen (Figure 4). The most dangerous accident consequence condition, rated by fishermen from the same port, was night and weather navigation (33%), followed by restricted visibility (30%), and heavy weather condition (20%) in port navigation (Figure 4).




| P value | C. weather and sea | Current | Restricted visibility | Nigth navigation | Navigation in heavy weather | Nigth and heavy weather navigation |
|---|---|---|---|---|---|---|
| 10 | | | 8 | 1 | 1 | 5 |
| 6 | | 2 | 10 | | 8 | 7 |
| 3 | 4 | 3 | 8 | 5 | 10 | 8 |
| 1 | 1 | 8 | | 9 | 1 | |
| 0.5 | 1 | 3 | | 3 | | |
| 0.2 | 7 | 3 | | | | |
| 0.1 | 7 | 1 | | 2 | | |




| C value | C. weather and sea | Current | Restricted visibility | Nigth navigation | Navigation in heavy weather | Nigth and heavy weather navigation |
|---|---|---|---|---|---|---|
| 100 | | 1 | 1 | | 1 | 2 |
| 40 | 1 | | 5 | | 2 | 4 |
| 15 | | | 7 | 1 | 2 | 6 |
| 7 | | 1 | 7 | 3 | 12 | 6 |
| 3 | | 2 | | 11 | 1 | 1 |
| 1 | 19 | 16 | | 5 | 2 | 1 |

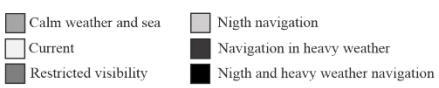
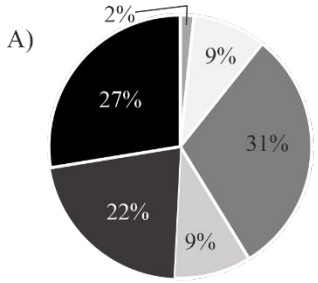
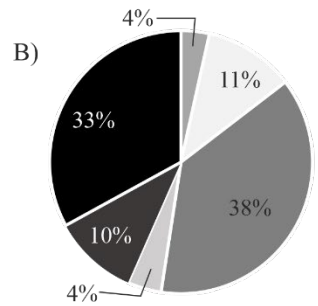



**Figure 4.** A) Percentages of probability and rating of fishermen from İskele fishing port in port navigation. B) Percentages of consequences and ratings of fishermen from İskele fishing ports in port navigation (*P-value*: value of the probability of accidents; *C-value*: value of the consequences of accidents)

In the Çeşmealtı fishing port, fishermen cited restricted visibility (31%) as the number one determinant of accident probability (Figure 5). In the same fishing port, they chose restricted visibility (38%) as the most dangerous factor influencing the consequences of the accident, followed by night and weather navigation (33%), and current (11%) in port navigation (Figure 5). Restricted visibility (mean=7.90) for the probability of accidents and heavy weather navigation at night (mean=37.03) for the consequence of the accident was identified as the most dangerous factor for all fishermen in sea navigation (Table 6). For port navigation, night-heavy weather navigation was the most important factor for both the accident probability (mean=5.56) and consequence (mean=19.70) for all fishermen (Table 6).




| P value | C. weather and sea | Current | Restricted visibility | Nigth navigation | Navigation in heavy weather | Nigth and heavy weather navigation |
|---|---|---|---|---|---|---|
| 10 | | | 3 | 1 | | |
| 6 | | 1 | 4 | | 4 | 7 |
| 3 | | 2 | | 2 | 5 | 3 |
| 1 | | 3 | 2 | | 1 | |
| 0.5 | 5 | 3 | 1 | 2 | | |
| 0.2 | 1 | 1 | | 2 | | |
| 0.1 | 4 | | | 3 | | |




| C value | C. weather and sea | Current | Restricted visibility | Nigth navigation | Navigation in heavy weather | Nigth and heavy weather navigation |
|---|---|---|---|---|---|---|
| 100 | | | | | | |
| 40 | | | 1 | | | 1 |
| 15 | | | 4 | | | 2 |
| 7 | | 3 | | | 2 | 2 |
| 3 | | 2 | 2 | 1 | 4 | 3 |
| 1 | 10 | 5 | 3 | 9 | 4 | 2 |


**Figure 5.** A) Percentages of probability and rating of fishermen from Çeşmealtı fishing port in port navigation. B) Percentages of consequences and ratings of fishermen from Çeşmealtı fishing ports in port navigation (*P-value*: value of the probability of accidents; *C-value*: value of the consequences of accidents)

**Table 6.** Results of the descriptive analysis of values related to the probability and consequences of the accident

| | | Probability (Mean) | | | Consequence (Mean) | | |
|---|---|---|---|---|---|---|---|
| | | İskele | Çeşmealtı | *Total* | İskele | Çeşmealtı | *Total* |
| ***Sea*** | Calm weather and sea | 2.2 | 5.90 | 3.43 | 7.40 | 10.80 | 8.53 |
| | Current | 3.56 | 5.77 | 4.29 | 9.15 | 21.90 | 13.40 |
| | Restricted visibility | 7.45 | 8.80 | 7.90 | 27.40 | 44.40 | 33.06 |
| | Night navigation | 4.28 | 3.70 | 4.08 | 16.70 | 5.00 | 12.80 |
| | Heavy weather | 4.97 | 3.52 | 4.48 | 30.70 | 13.1 | 24.83 |
| | Night-heavy weather navigation | 6.25 | 6.40 | 6.30 | 39.85 | 31.40 | 37.03 |
| ***Port*** | Calm weather and sea | 0.78 | 0.31 | 0.62 | 2.95 | 1.00 | 2.30 |
| | Current | 1.56 | 1.67 | 1.59 | 6.40 | 3.20 | 5.33 |
| | Restricted visibility | 5.20 | 5.60 | 5.33 | 22.00 | 10.90 | 18.30 |
| | Night navigation | 1.91 | 1.77 | 1.86 | 4.40 | 1.20 | 3.33 |
| | Heavy weather | 4.45 | 4.00 | 4.30 | 14.90 | 3.60 | 11.13 |
| | Night-heavy weather navigation | 5.80 | 5.10 | 5.56 | 24.80 | 9.50 | 19.70 |

Regarding sea navigation in calm weather and sea, the fishermen's mean accident probability values were 2.20 (s.e.=0.28) and 5.90 (s.e.=1.25) for İskele and Çeşmealtı, respectively. There was a significant difference in this condition between fishermen from two different ports (p=0.008) (Table 7). The results of the fishermen from Çeşmealtı fishing port (Figure 3) on the probability of sea navigation accidents in calm weather were higher than those of the fishermen from İskele port (Figure 2). In night navigation, the mean values for the consequences of accidents for fishermen were 16.70 (s.e.=4.77) and 5.00 (s.e.=1.26) for İskele and Çeşmealtı in the sea, respectively. There was a significant difference between the two fishing ports (Table 7) (p=0.007) in terms of the consequences of the accident for night navigation. The values of the İskele fishermen (Figure 2) for the accident consequence of sea navigation at night were found higher than the results of the Çeşmealtı fishermen (Figure 3). The average heavy weather sea navigating scores for accident consequences were 30.70 (s.e.=5.05) and 13.10 (s.e.=9.75) for İskele and Çeşmealtı, respectively. There was a significant difference in the consequences of the accident between the two fishing ports (p=0.003) (Table 7).

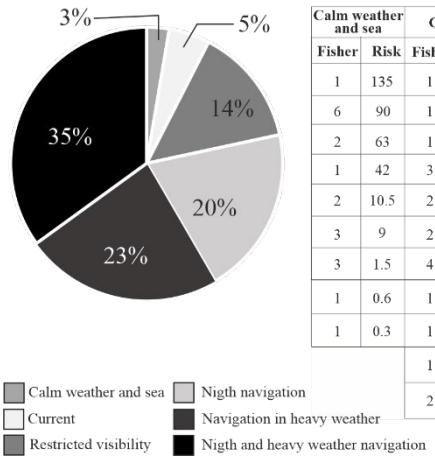
**Table 7.** The results of the Mann-Whitney U test

| | Sea navigation | | | Port navigation | | |
|---|---|---|---|---|---|---|
| | Probability | Consequence | | Consequence | | |
| | Calm weather and sea | Night navigation | Heavy weather | Night-heavy weather navigation | Heavy weather | Night navigation |
| Mann Whitney U | 45.000 | 41.000 | 35.000 | 53.5000 | 42.000 | 27.500 |
| Wilcoxon W | 255.000 | 96.000 | 90.000 | 108.500 | 97.000 | 82.500 |
| Z | -2.638 | -2.699 | -2.955 | -2.094 | -2.738 | -3.451 |
| Asymp. Sig. (2-tailed) | 0.008 | 0.007 | 0.003 | 0.036 | 0.006 | 0.001 |
| Exact Sig. [2*(1-tailed Sig.)] | 0.015[b] | 0.008[b] | 0.003[b] | 0.039[b] | 0.010[b] | 0.001[b] |

The results of the İskele fishermen (Figure 2) for the consequences of sea navigating accidents in heavy weather were higher than those of the Çeşmealtı fishermen (Figure 3). The mean values for night and heavy weather port navigation with regard to the consequences of the accident were 24.80 (s.e.=6.43) and 9.50 (s.e.=3.76) for İskele and Çeşmealtı, respectively. There was a significant difference between two fishing ports in the accident consequences of night and heavy weather port navigation (p=0.036) (Table 7). The results of the İskele fishermen were higher than those of the Çeşmealtı fishermen for the accident consequences of night and heavy weather port navigation (Figure 4 and 5). The mean heavy weather accident consequences for port navigation were 14.9 (s.e.=5.07) and 3.6 (s.e.=0.79) for İskele and Çeşmealtı, respectively. We found a significant difference in the consequences of port navigation accidents in heavy weather between two fishing ports (p=0.006) (Table 7). The İskele fishermen's score for the consequences of port navigating accidents in heavy weather was higher than the Çeşmealtı fishermen's scores (Figure 5 and 6).

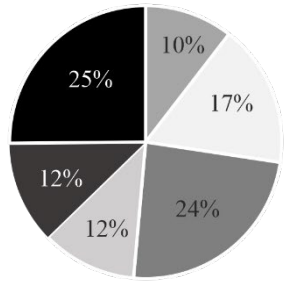
When assessing port navigation under all conditions, there was no significant difference in the probability of accidents between the two different fishing ports (Figure 5 and 6). The mean accident consequences in port navigating at night were 4.40 (s.e.=0.90) and 1.20 (s.e.=0.20) for İskele and Çeşmealtı fishing ports, respectively. There was a significant difference between the consequences of accidents in port navigation at night and their fishing ports (p=0.001) (Table 7). It has been established that İskele fishermen are more likely than Çeşmealtı fishermen to have accidents in port navigation at night (Figure 5 and 6).

If the risk assessment analysis includes the frequency factor ($F_2$), a statement can be made about the effects of the environmental conditions at sea and in port navigation on site. The results of the risk assessments of the fishermen's estimates from the İskele port showed that the most dangerous situation was night and heavy weather navigation (35%), followed by heavy weather navigation (23%), and night navigation (20%) for sea navigation in the Gulf of İzmir (Figure 6). These assessments by fishermen from Çeşmealtı fishing port showed that the most dangerous situation was night and heavy weather navigation (25%), followed by restricted visibility (24%), and current (17%) for sea navigation in the Gulf of İzmir (Figure 7). The results of the risk assessment of fishermen's decisions from İskele fishing port showed that the most dangerous situation was night and heavy weather navigation (45%), followed by navigation in heavy weather (24%), and restricted visibility (17%) for İskele port (Figure 8). The results from the fishermen from Çeşmealtı fishing port showed that the most hazardous situation was night and heavy weather navigation (46%), followed by restricted visibility (32%), and heavy weather navigation (12%) for Çeşmealtı fishing port (Figure 9).




| Calm weather and sea | | Current | | Restricted visibility | | Nigth navigation | | Navigation in heavy weather | | Nigth and heavy weather navigation | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk |
| 1 | 135 | 1 | 480 | 1 | 1000 | 1 | 3000 | 1 | 2000 | 3 | 2000 |
| 6 | 90 | 1 | 300 | 5 | 400 | 1 | 1200 | 2 | 800 | 2 | 800 |
| 2 | 63 | 1 | 240 | 3 | 240 | 5 | 270 | 8 | 480 | 6 | 480 |
| 1 | 42 | 3 | 90 | 2 | 150 | 7 | 135 | 2 | 180 | 1 | 240 |
| 2 | 10.5 | 2 | 84 | 7 | 90 | 1 | 126 | 1 | 15 | 4 | 180 |
| 3 | 9 | 2 | 42 | 1 | 45 | 1 | 63 | 1 | 14 | 3 | 90 |
| 3 | 1.5 | 4 | 18 | 1 | 42 | 1 | 21 | 4 | 6 | 1 | 42 |
| 1 | 0.6 | 1 | 6 | | | 1 | 9 | 1 | 1 | | |
| 1 | 0.3 | 1 | 4 | | | 1 | 3 | | | | |
| | | 1 | 2.8 | | | 1 | 1.5 | | | | |
| | | 2 | 1 | | | | | | | | |

**Figure 6.** Risk percentage and rating of fishermen from İskele fishing port in sea navigation




| Calm weather and sea | | Current | | Restricted visibility | | Nigth navigation | | Navigation in heavy weather | | Nigth and heavy weather navigation | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk |
| 4 | 450 | 1 | 2000 | 4 | 1000 | 1 | 1200 | 1 | 2000 | 2 | 2000 |
| 1 | 270 | 1 | 800 | 3 | 400 | 3 | 360 | 1 | 480 | 1 | 800 |
| 1 | 135 | 1 | 480 | 1 | 90 | 1 | 135 | 1 | 180 | 1 | 480 |
| 1 | 126 | 1 | 180 | 2 | 42 | 1 | 63 | 2 | 18 | 1 | 180 |
| 1 | 9 | 1 | 140 | | | 2 | 27 | 1 | 6 | 1 | 60 |
| 2 | 1.5 | 2 | 84 | | | 2 | 1.5 | 1 | 2 | 1 | 42 |
| | | 1 | 6 | | | | | 1 | 1 | 3 | 18 |
| | | 1 | 1 | | | | | 2 | 0.2 | | |
| | | 1 | 0.4 | | | | | | | | |

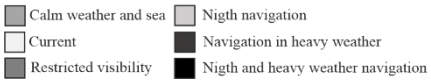
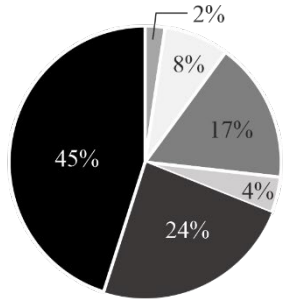



**Figure 7.** Risk percentage and rating of fishermen from Çeşmealtı fishing port in sea navigation




| Calm weather and sea | | Current | | Restricted visibility | | Nigth navigation | | Navigation in heavy weather | | Nigth and heavy weather navigation | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk |
| 1 | 360 | 1 | 1200 | 1 | 600 | 1 | 450 | 1 | 2000 | 2 | 2000 |
| 3 | 9 | 1 | 42 | 2 | 400 | 2 | 63 | 2 | 480 | 1 | 800 |
| 1 | 3 | 1 | 12 | 3 | 240 | 1 | 27 | 2 | 180 | 3 | 480 |
| 1 | 1.5 | 4 | 6 | 4 | 90 | 1 | 10.5 | 4 | 84 | 1 | 300 |
| 1 | 0.6 | 6 | 2 | 3 | 45 | 11 | 9 | 8 | 42 | 2 | 180 |
| 6 | 0.4 | 3 | 1 | 2 | 42 | 1 | 4.5 | 3 | 6 | 1 | 140 |
| 6 | 0.3 | 3 | 0.4 | 5 | 21 | 1 | 1.5 | | | 3 | 90 |
| 1 | 0.2 | 1 | 0.2 | | | 2 | 0.3 | | | 5 | 42 |
| | | | | | | | | | | 1 | 36 |
| | | | | | | | | | | 1 | 12 |

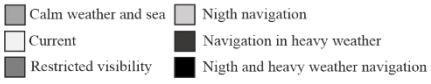
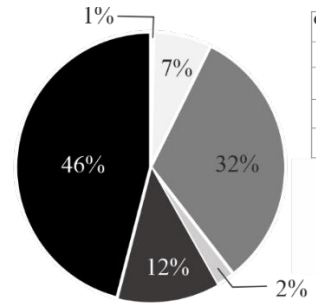



**Figure 8.** Risk percentage and rating of fishermen from İskele fishing port in port navigation




| Calm weather and sea | | Current | | Restricted visibility | | Nigth navigation | | Navigation in heavy weather | | Nigth and heavy weather navigation | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk | Fisher | Risk |
| 5 | 1.5 | 1 | 84 | 1 | 240 | 1 | 30 | 1 | 84 | 1 | 480 |
| 1 | 0.6 | 1 | 42 | 2 | 150 | 2 | 9 | 2 | 42 | 2 | 180 |
| 4 | 0.3 | 1 | 18 | 2 | 90 | 1 | 1.8 | 2 | 36 | 1 | 84 |
| | | 1 | 7 | 1 | 30 | 2 | 1.5 | 1 | 18 | 1 | 42 |
| | | 1 | 6 | 1 | 6 | 1 | 0.6 | 1 | 12 | 3 | 36 |
| | | 2 | 2 | 1 | 3 | 3 | 0.3 | 3 | 6 | 2 | 6 |
| | | 2 | 1 | 1 | 1 | | | | | | |
| | | 1 | 0.4 | 1 | 0.5 | | | | | | |


**Figure 9.** Risk percentage and rating of fishermen from Çeşmealtı fishing port in port navigation

## DISCUSSION

There is much research aimed at preventing accidents at sea, especially on commercial ships (Haapasaari et al., 2015; Awal and Hasegawa, 2017; Formela et al., 2019; Hasanspahić et al., 2021). Although it has a high economic value and the accidents have many and serious consequences, such studies on the fishing side have been found to be lacking. This study attempted to address the deficiency in this area. Measuring the perception of risk during navigation from the perspective of fishermen has introduced a new approach to the studies in this field. This study also aims to identify the key factors that increase risk perception and raise awareness of this issue. This is intended to help reduce accidents. Our results have been remarkably close to those expected. In this regard, the desired results have been achieved and the nature of environmental conditions arranged in the simulator scenarios. Much more can be revealed by changing the nature and severity of environmental conditions.

Simulators have been used for training and certification in Maritime Education and Training since their first appearance in the 1950s (Sellberg, 2017). For example, today simulators are used in several areas of the maritime industry; offshore operational training on ships and oil platforms, bridge operations, cargo handling, engine controls, crane operations, towing, and anchor handling. One of them, bridge simulators are real-time simulators that bring users a very realistic feeling. Developing new ship bridge software components based on many sophisticated and long phases to make them reliable and realistic is a very complex subject (Stratmann et al., 2018). Simulated reality provides a realistic practical training environment for participants to make situational decisions in a protected environment (Tichon & Burgess-Limerick, 2011). With simulators, the closer the simulation is to the real task, the more likely it is that skills will transfer from one context to another (Sellberg, 2017). Regardless of the quality of the simulators (high-fidelity or low-fidelity simulators) (Dahlstrom et al., 2009), realistic simulations depend on designing scenarios that match the capabilities of the simulator users (Saus et al., 2010) as fishermen correspond in our study. From another perspective, to improve safety and reduce the risk of fatal accidents, the computerized simulator experience with a realistic environment has become the method of choice in maritime education. High simulator realism means that the participants experience the training realistically from their areas of interest (Saus et al., 2010). In fact, during this study, it was observed that some fishermen lost their balance in the severe weather scenario. It is possible to create a realistic scenario by adding ships and buoys in the desired place in relation to the number and type of other ships included in the simulator. Almost the same equipment that is used on a real ship is used in simulators. While it feels realistic, it wouldn't be right to expect users to react the same way they would in a real emergency. The fact is that it is almost impossible to conduct the study without the simulator under the same environmental conditions and with the same procedures.

The study showed that changing weather and sea conditions had an impact on the probability and consequences of accidents at sea. Similarly, one study examined fishing boat accidents from a human factor perspective and found that environmental factors were the underlying causes of the accident (Yıldırım and Başar, 2019). The heavy weather conditions examined in the study were shown to be one of the most important restricted visibility factors increasing the probability and consequences of accidents (Table 6). The mean values reported by the fishermen showed that navigating in heavy weather was a factor that increased the probability and consequences of accidents more than navigating in calm weather, current and night hours (Table 6). Pleskacz (2015) found that limited space during severe weather, shallow waters, and limitations in the manoeuvrability of fishing gear are factors that increase the probability of accidents for fishermen. In this study, the limitation of visibility was identified as a very important element of meteorology that directly affects the safety of navigation. Jin (2014) also showed that heavy daytime weather conditions increase the severity of accidents. Wu (2008) and Wu et al. (2009) define that wave height and ice concentration are determinants of the severity and relative casualty rate of fishing boat casualties. Wang et al. (2021) reported that the severity of maritime accidents is positively correlated with distance from the port, strong wind, rough seas, strong currents, and/or good visibility. Similar to Wang et al. (2021), this study found that the above environmental factors increase the probability and consequences of accidents compared to calm weather and sea conditions (Table 6). In addition, this study gives an idea of the relative ranking of weather conditions that increase the probability and consequences of accidents (Figure 2, 3, 4, and 5).

The mean value of the accident probability and impact assessments by the fishermen according to various environmental and sea conditions showed that navigating in their own ports was safer than when the sea cruise was under the same environmental and sea conditions. In parallel with the results of this study in the in the European Maritime Safety Report, it was found that accidents and injuries mainly occur during the route legs of the journey, excluding arrival, departure, and other legs (EMSA, 2022). Similarly, Jin (2014) found that the probability of accidents and consequences is higher when the vessel is not close to shore.

There were slight differences in rating between fishermen working in different ports. Although the fishing ports of Çeşmealtı and İskele are structurally similar, the probability of exposure to the north wind applied in the scenario may not be the same for both ports due to their different orientations of construction. For this reason, it can be assumed that the directions in which the ports were built increase or decrease the probability of accidents, depending on the prevailing wind direction. Galor (2005) mentioned that to maintain ship movement safety, one of the critical design aspects was the depth of the port waters. In our study, fishermen did not mention draft problems when entering their fishing ports. Çınar

(2020) found that the Fine-Kinney risk assessment method can be crucial in port construction. In this study, it was found that increasing wind intensity significantly increased risk scores. In our study, high wind intensity increases accident probability, impact scores, and risk assessment compared to calm weather and rough seas.

In the studies by Wang et al. (2005) and Pleskacz (2015), found that accident probability and consequence values are higher during fishing than during navigation at sea. The fishermen explained that they were neglecting their lookout duties as they would be busy with their work while setting or hauling in their nets, increasing their risk value. Our findings parallel these studies in that fishing activity is more dangerous than navigating the fishing boat at sea or in port. Jin et al. (2001), Roberts et al. (2010), and Yıldırım and Başar (2019) analyzed previous accidents and found that collision accidents are more common in dark hours. In parallel with these studies, the fishermen participating in our investigation found that the risk level for navigating during the night hours is higher than navigating during the day and navigating in heavy weather at night compared to navigating in heavy weather during the day (Table 6). Jin (2014) emphasized that while the severity of damage to fishing vessels is inversely related to vessel length, it is positively related to capsizing, sinking, daily wind speed, boat age, and distance from shore. In this study, it was shown that boat lengths reported in the low range were unrelated to other parameters, including the number of accidents ($p=0.089$) and the probability of accidents and the assessment of consequences.

Avoiding loss of life and property due to marine casualties as a result of various factors is a very important issue in maritime shipping both locally and globally. Therefore, dynamic studies like the ones we are conducting could provide important clues to reduce marine casualties involving fishermen. However, the authors are aware that there are several limitations of the study, e.g. work with commercial fishermen and are forced to stay in the simulator for at least 30 minutes. The other limitation is the number of participants contributing to the experiment, which can lead to low study reliability. Nevertheless, this is the first proactive study to determine risk perceptions in small-scale fisheries off Turkish coasts.

In this study, the problem of marine casualties was examined from the perspective of fishermen. The results obtained are parallel to previous similar studies. External environmental conditions such as restricted visibility and heavy weather increase the risk of accidents. For fishermen, night navigation and currents are not as dangerous conditions as heavy weather and restricted visibility. It was also found that fishermen are more likely to avoid sea navigation than port navigation.

Only a limited environmental variable such as current, weather, etc. could be tested with the bridge simulator. Sufficient observation time is required to obtain data in the studies conducted in the simulator room. The number of participants may seem small but including more than enough participants in the simulator can be one of the challenging aspects of the study as it increases observation time. Despite these limitations, the findings of this study are important because we could have obtained sufficient results close to real sea conditions. Nonetheless, further studies involving more participants and incorporating a greater variety of environmental factors can support this work. For example; some fishing techniques require night navigation and even turning off navigation lights and all other lights to prevent fish from escaping. Likewise, in the case of illegal fishing, fishermen turn off all their lanterns and lights so as not to be noticed. These two conditions can include additional scenarios in the bridge simulator for further study.

## CONCLUSION

These results demonstrate that simulator experiments can be a useful tool to prevent accidents at sea in terms of a proactive approach. Although an important part of the maritime causes of damage are accidents involving fishing vessels, investigating marine casualties on the fishing side is not enough. Available data are limited and no previous study has focused on measuring risk perception during navigation from the fishermen's perspective using the Fine-Kinney risk assessment method. Our results are relevant for both local and general maritime navigation. In this study, it can be assumed that environmental factors could increase the probability and consequence of accidents without considering regional differences. On the other hand, if the risk assessment is evaluated taking into account the frequency factor of the environmental conditions, the results we had are local.

If the risk rating is high as a result of the experimental study, fishermen should not go to sea without taking precautions. Fishermen should navigate at a safe cruising speed when visibility is limited. Before leaving the port, one should find out about the weather conditions at sea and if possible precautions do not reduce the probability of accidents, the cruise should be cancelled. Similar results mentioned above can be used to assist the relevant maritime authorities to take effective measures to prevent serious maritime accidents. For this reason, this study has significant potential for many future applications in risk assessment for maritime accidents.

## ACKNOWLEDGMENTS AND FUNDING

This study arose from the master's thesis of Can Atacan under the supervision of the corresponding author. The authors are grateful to the fishermen for their unconditional cooperation during the simulator experiments.

## AUTHORSHIP CONTRIBUTIONS

Can Atacan: Conceptualization, methodology, investigating, writing - original draft, writing - review & editing. F. Ozan Düzbastılar: Conceptualization, writing - original draft, writing - review & editing, visualization.

## CONFLICT OF INTEREST STATEMENT

The authors state that they are not aware of any competing financial or non-financial, professional, or personal conflicts that may have influenced this study.

## ETHICS APPROVAL

Ethical approval for this study was obtained from Ege University Science and Engineering Sciences Scientific Research and Publication Ethics Committee.

## DATA AVAILABILITY

All relevant data is in the article.

## REFERENCES


Akyıldız, H. (2015). Formal safety assessment of a fishing vessel. *GİDB Dergi*, 2, 31-46.

Awal, Z.I., & Hasegawa, K. (2017). A study on accident theories and application to maritime accidents. *Procedia Engineering*, *194*, 298-306. https://doi.org/10.1016/j.proeng.2017.08.149

Bayar, N. (2010). *Analysis of maritime traffic safety in İstanbul Strait using fuzzy AHP and FMEA methods based on risk*. Doctoral dissertation, Yıldız Teknik University, Turkey.

Bekdemir, E. (2019). *Application of Fine Kinney and 5x5 matrix risk analysis methods in building construction* (in Turkish with English abstract). Doctoral dissertation, İstanbul Aydın University, Turkey.

Çınar, F. (2020). *A model on risk analysis methods in ship handling during port manoeuvres*. Master's thesis, Piri Reis University, Turkey.

Dahlstrom, N., Dekker, S., Van Winsen, R. & Nyce, J. (2009) Fidelity and validity of simulator training. *Theoretical Issues in Ergonomics Science*, *10*(4):305-314. https://doi.org/10.1080/14639220802368864

Demirci, S. M. E., Canımoğlu, R., & Elçiçek, H. (2022). An evaluation of the effects of human factors on potential ship accidents under pilotage. *Marine Science and Technology Bulletin*, *11*(1), 76-87. https://doi.org/10.33714/masteb.1064311

EMSA (2022). *European Maritime Safety Report 2022*. Retrieved from https://emsa.europa.eu/publications/item/4735-emsafe-report.html

Eronat, C. (2017). An overview on İzmir Bay physical oceanography. *Ege Journal of Fisheries and Aquatic Sciences*, *34*(1), 1-9. https://doi.org/10.12714/egejfas.2017.34.1.01

Fan, S., Zhang, J., Blanco-Davis, E., Yang, Z., & Yan, X. (2020). Maritime accident prevention strategy formulation from a human factor perspective using Bayesian Networks and TOPSIS. *Ocean Engineering*, *210*(107544), 1-12. https://doi.org/10.1016/j.oceaneng.2020.107544

FAO (2014). *The State of World Fisheries and Aquaculture: Opportunities and challenges*. Retrieved from https://www.fao.org/3/i3720e/i3720e.pdf

FAO (2021). *FAO Yearbook. Fishery and Aquaculture Statistics*, 2019. Rome/Roma. https://doi.org/10.4060/cb7874t

Fine, W.T. (1971). Mathematical evaluations for controlling hazards. *Journal of Safety Research*, 3, 157-166.

Formela, K., Neumann, T., & Weintrit, A. (2019). Overview of definitions of maritime safety, safety at sea, navigational safety and safety in general. *TransNav, The International Journal on Marine Navigation and Safety of Sea Transportation*, *13*(2), 285-290. https://doi.org/10.12716/1001.13.02.03

Galor, W. (2005). The managing of the navigational safety of ships in port water areas. *WIT Transactions on the Built Environment*, 82, 151-160.

Gucma, S. & Ślączka, W. (2018). Comprehensive method of formal safety assessment of ship manoeuvring in waterways. *Scientific Journals of the Maritime University of Szczecin*, *54*(126), 110-119. https://doi.org/10.17402/292

Haapasaari, P., Helle, I., Lehikoinen, A., Lappalainen, J. & Kuikka, S. (2015). A proactive approach for maritime safety policy making for the Gulf of Finland: Seeking best practices. *Marine Policy*, *60*, 107-118. https://doi.org/10.1016/j.marpol.2015.06.003

Halim, A., Wiryawan, B., Loneragan, N. R., Hordyk, A., Sondita, M. F., White, A. T., Koeshendrajana, S., Ruchimat, T., Pomeroy, R. S., & Yuni, C. (2019). Developing a functional definition of small-scale fisheries in support of marine capture fisheries management in Indonesia. *Marine Policy*, *100*, 238-248. https://doi.org/10.1016/j.marpol.2018.11.044

Hasanspahić, N., Vujičić, S., Frančić, V., & Čampara, L. (2021). The role of the human factor in marine accidents. *Journal of Marine Science and Engineering*, *9*(3), 261. https://doi.org/10.3390/jmse9030261

Hsu, W.K.K., Chen, J.W., Huynh, N.T., & Lin, Y.Y. (2022). Risk assessment of navigation safety for ferries. *Journal of Marine Science and Engineering*, *10*(5) 700, 1-17. https://doi.org/10.3390/jmse10050700

Jaremin, B. & Kotulak, E. (2004). Mortality in the Polish small-scale fishing industry. *Occupational Medicine*, *54*(4), 258-260. https://doi.org/10.1093/occmed/kqh054

Jin, D. (2014). The determinants of fishing vessel accident severity. *Accident Analysis & Prevention*, 66, 1-7. https://doi.org/10.1016/j.aap.2014.01.001

Jin, D., Kite-Powell, H., & Talley, W. (2001). The safety of commercial fishing: Determinants of vessel total losses and injuries. *Journal of Safety Research*, *32*(2), 209-228. https://doi.org/10.1016/s0022-4375(01)00047-0

Jin, D., Kite-Powell, H. L., Thunberg, E., Solow, A. R., & Talley, W. K. (2002). A model of fishing vessel accident probability. *Journal of Safety Research*, *33*(4), 497-510. https://doi.org/10.1016/s0022-4375(02)00050-6

Jin, D., & Thunberg, E. (2005). An analysis of fishing vessel accidents in fishing areas off the northeastern United States. *Safety Science*, *43*(8), 523-540. https://doi.org/10.1016/j.ssci.2005.02.005

Jung, C. (2014). A study on the requirement to the fishing vessel for reducing the collision accidents. *Journal of the Korean Society of Marine Environment and Safety*, *20*(1), 18-25. https://doi.org/10.7837/kosomes.2014.20.1.018

Kim, H.T., & Na, S. (2017). Development of a human factors investigation and analysis model for use in maritime accidents: A case study of collision accident investigation. Journal of Navigation and Port Research, *41*(5), 303-318. https://doi.org/10.5394/KINPR.2017.41.5.303

Kim, S.K., & Kang, J.P. (2011). A Study on the relationships between the casualties of fishing boats and meteorological factors. *Journal of Fisheries and Marine Sciences Education*, *23*(3), 351-360.

Kinney, G.F., & Wiruth, A.D. (1976). Practical risk analysis for safety management, NWC Technical Publication 5865, Naval Weapons Center, China Lake CA, USA, 1976.

Li, S., Meng, Q., & Qu, X. (2012). An overview of maritime waterway quantitative risk assessment models. *Risk Analysis*, *32*(3), 496-512. https://doi.org/10.1111/j.1539-6924.2011.01697.x

Luo, M., & Shin, S. (2019). Half-century research developments in maritime accidents: Future directions. *Accident Analysis & Prevention*, *123*, 448-460. https://doi.org/10.1016/j.aap.2016.04.010

Montewka, J., Ehlers, S., Goerlandt, F., Hinz, T., Tabri, K., & Kujala, P. (2014). A framework for risk assessment for maritime transportation systems - A case study for open sea collisions involving RoPax vessels. *Reliability Engineering & System Safety*, *124*, 142-157. https://doi.org/10.1016/j.ress.2013.11.014

Oh, J., Kim, K., & Jeong, J. (2015). A study on the risk analysis based on the trajectory of fishing vessels in the VTS area. *International Journal of e-Navigation and Maritime Economy*, 2, 38-46. https://doi.org/10.1016/j.enavi.2015.06.004

Okumuş, D., & Barlas, B. (2016). *An applied comparison of 5x5 analysis matrix and Fine-Kinney methods in the shipbuilding industry* (in Turkish). *Gemi ve Deniz Teknolojisi,* 22 (Supp: 204-205):95-106.

Ölçücü, H., & Ersöz Kaya, İ. (2019). Comparative risk analysis and risk assessment of biological factors in hazardous waste disposal facilities (in Turkish with English abstract). *European Journal of Science and Technology*, 1375-1382. https://doi.org/10.31590/ejosat.668653

Pleskacz, K. (2015). The impact of hydro-meteorological conditions on the safety of fishing vessels. *Scientific Journals of the Maritime University of Szczecin*, *41*(113), 81-87.

Psaraftis, H.N. (2002). Maritime Safety: To be or not to be proactive. *WMU Journal of Maritime Affairs*, *1*, 3-16.

Roberts, S.E., Jaremin, B., & Marlow, P.B. (2010). Human and fishing vessel losses in sea accidents in the UK fishing industry from 1948 to 2008. *International Maritime Health*, *62*(3), 143-153.

Saus, E.R., Johnsen, B.H. & Eid, J. (2010). Perceived learning outcome: the relationship between experience, realism, and situation awareness during simulator training. *International Maritime Health*, *61*(4), 258-264.

Sellberg, C. (2017). Simulators in bridge operations training and assessment: a systematic review and qualitative synthesis. *WMU Journal of Maritime Affairs, 16*, 247-263. https://doi.org/10.1007/s13437-016-0114-8

Smith, H., & Basurto, X. (2019). Defining small-scale fisheries and examining the role of science in shaping perceptions of who and what counts: A systematic review. *Frontiers in Marine Science*, *6,* 236, 1-19. https://doi.org/10.3389/fmars.2019.00236

Soykan, O. (2018). Risk assessment in industrial fishing vessels by L type matrix method and its usability. *Ege Journal of Fisheries and Aquatic Sciences*, *35*(2), 207-217. https://doi.org/10.12714/egejfas.2018.35.2.15

Stratmann, T.C., Gruenefeld, U., Hahn, A., Boll, S., Stratmann, J., & Schweigert, S. (2018). Mobile bridge - A portable design simulator for ship bridge interfaces. *The International Journal on Marine Navigation and Safety of Sea Transportation*, *12*(4), 763-768. https://doi.org/10.12716/1001.12.04.16

Sur, J.M., & Kim, D.J. (2020). Comprehensive risk estimation of maritime accident using fuzzy evaluation method–Focusing on fishing vessel accident in Korean waters. *The Asian Journal of Shipping and Logistics*, *36*(3):127-135. https://doi.org/10.1016/j.ajsl.2019.12.013

Tichon, J. & Burgess-Limerick, R. (2011). A review of virtual reality as a medium for safety related training in mining. *Journal of Health & Safety Research & Practice*, *3*(1), 33-40.

Uğurlu, F., Yıldız, S., Boran, M., Uğurlu, Ö., & Wang, J. (2020). Analysis of fishing vessel accidents with Bayesian network and CHI-square methods. *Ocean Engineering*, *198*, 106956. https://doi.org/10.1016/j.oceaneng.2020.106956

Usanmaz, D. & Köse, E. (2020). Comparative statistical analysis of two different methods for risk assessment in chemical research laboratory (in Turkish with English abstract). *International Journal of Engineering Research and Development*, *12*(2):337-348. https://doi.org/10.29137/umagd.606402

Villasante, S., Gianelli, I., Castrejón, M., Nahuelhual, L., Ortega, L., Sumaila, R. & Defeo, O. (2022). Social-ecological shifts, traps and collapses in small-scale fisheries: Envisioning a way forward to transformative changes. *Marine Policy*, *136*(104933), 1-8. https://doi.org/10.1016/j.marpol.2021.104933

Wang, H., Liu, Z., Wang, X., Graham, T., & Wang, J. (2021). An analysis of factors affecting the severity of marine accidents. *Reliability Engineering & System Safety*, *210*, 107513. https://doi.org/10.1016/j.ress.2021.107513

Wang, J., Pillay, A., Kwon, Y.S., Wall, A.D. & Loughran, C.G. (2005). An analysis of fishing vessel accidents, *Accident Analysis & Prevention*, *37*(6):1019-1024.

Won, Y., & Kim, D. (2019). Risk analysis and selection of the main factors in fishing vessel accidents through a risk matrix. *Journal of the Korean Society of Marine Environment and Safety*, *25*(2), 139-150. https://doi.org/10.7837/kosomes.2019.25.2.139

Wu, Y. (2008). *Statistical evaluation of weather patterns on fishing vessel incidents in Atlantic Canada*. Doctoral dissertation, Dalhousie University, Canada.

Wu, Y., Pelot, R.P., & Hilliard, C. (2009). The influence of weather conditions on the relative incident rate of fishing vessels. *Risk Analysis*, *29*(7), 985-999. https://doi.org/10.1111/j.1539-6924.2009.01217.x

Yıldırım, U., & Başar, E. (2019). Analysis of collision accidents on fishing vessels through human factors analysis and classification system (HFACS) (in Turkish with English abstract), *Dokuz Eylül University Maritime Faculty Journal*, *11*(2):203-219. https://doi.org/10.18613/deudfd.659807

Zaloğlu, D.I. (2019). *Risk assessment with Fine Kinney Method in the fossil location in the scope of occupational health and safety* (in Turkish with English abstract). Master's thesis, Başkent University, Turkey.

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 15-19 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.02

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Infection of the swim bladder parasite *Anguillicoloides crassus* in the European eel (*Anguilla anguilla*) caught from the Gediz Delta (Aegean Sea)

## Gediz Deltası (Ege Denizi)'ndan avlanan Avrupa yılanbalığında (*Anguilla anguilla*) yüzme kesesi paraziti *Anguillicoloides crassus* enfeksiyonu

**Alp Salman**

Ege University, Faculty of Fisheries, Marine and Inland Water Sciences and the Technology Department, Bornova, İzmir, Türkiye https://orcid.org/0000-0002-2853-6507

*alp.salman@ege.edu.tr*

*Received date: 06.10.2022* *Accepted date: 17.11.2022*

***How to cite this paper:***
Salman, A. (2023). Infection of the swim bladder parasite *Anguillicoloides crassus* in the European eel (*Anguilla anguilla*) caught from the Gediz Delta (Aegean Sea). *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 15-19. https://doi.org/10.12714/egejfas.40.1.02

**Abstract:** The wild European eels (776 fish, mean total length of 547±91 mm, mean weight 340±198 g) were obtained monthly between October 2015-September 2016 in Gediz Delta (Izmir Bay, Aegean Sea). A total of 263 nematode parasites (*Anguillicoloides crassus*) were isolated from the swim bladders of 52 parasitized fish. Parasitological indices were found relatively low. The prevalence was 6.7% and the mean intensity was 5.05. The abundance of *A. crassus* was also calculated as 0.33 in all sampled eels.

**Keywords:** *Anguilla anguilla,* swimbladder parasites, *Anguillicoloides crassus*, Aegean Sea

**Öz:** Gediz Deltası'ndan (İzmir Körfezi, Ege Denizi) Ekim 2015-Eylül 2016 tarihleri arasında Avrupa yılan balıkları (776 balık, ortalama boy 547±91 mm, ortalama ağırlık 340±198 g) aylık olarak temin edilmiştir. Parazitli 52 balığın yüzme keselerinden toplam 263 nematod (*Anguillicoloides crassus*) izole edilmiştir. Parazitolojik indeksler nispeten düşük bulunmuştur. Görülme sıklığı; %6,7 ve ortalama yoğunluk 5,05 olarak belirlenmiştir. *A. crassus* bolluğu da örneklenen tüm yılan balıklarında 0,33 olarak hesaplanmıştır.

**Anahtar kelimeler:** *Anguilla anguilla*, yüzme kesesi paraziti, *Anguillicoloides crassus*, Ege Denizi

### INTRODUCTION

The European eel, *Anguilla anguilla* Linnaeus, 1758, is a catadromous species found in all European waters distributes from the Atlantic coast of Europe to northern Africa, the Black Sea and the Mediterranean (Bilecenoğlu et al., 2014). Over the past decades, the abundance of the eel population has decreased dramatically. Since 2008 it has been included in IUCN Red List of threatened species as critically endangered (Durif et al., 2011). The population of European eels is in decline due to several factors including parasitism (Moriarty and Dekker, 1997). For example, *Anguillicoloides crassus* Kuwahara, Niimi & Itagak, 1974 defined as a swim bladder parasite, was introduced accidentally into European waters with live *Anguilla japonica* from Asia in the early 1980s imported for consumption and aquaculture (Hafir-Mansouri et al., 2018). Palstra et al., (2007) suggested that this swim bladder parasite poses a serious threat to reproductive success as it causes damage to the swim bladder hence infected European eels cannot reach their spawning grounds during their breeding migration and they consider it therefore likely that *A. crassus* played a role in the current collapse of the European eel population.

Many studies have been carried out to determine the density of the swim bladder parasite *A. crassus*, which has entered the European eel population in the last two decades, according to abiotic factors in different regions of the Mediterranean (Aly et al., 2007; Quadroni et al., 2013; Koyuncu et al., 2017; Kantzoura et al., 2021) or in different ecosystems (Sauvaget et al., 2003; Loukili and Belghyti, 2007; Mayo-Hernandez et al., 2015; Bakaria et al., 2018; Giari et al., 2021), or to determine its life cycle (De Charleroy et al., 1990; Kirk, 2003; Rolbiecki, 2008) in the Mediterranean region. This study aims to determine the infection rates in the study area based on the prevalence data of *A. crassus* and to compare these values with different regions and habitats of the Mediterranean.

### MATERIALS AND METHODS

The Gediz Delta (30°38' N; 55°26' E) is located northeast of the İzmir Bay (Aegean Sea) and protected by Ramsar convention covers an area of 20.400 km$^2$. It is composed of four lagoons and the Gediz River. Eel specimens were obtained from the commercial fishers who are using fyke nets (12 mm mesh size) in the Gediz Delta: between October 2015 and September 2016 (Figure 1). Besides that, some of the physicochemical parameters such as oxygen, pH, salinity and temperature of the delta water were measured every month with portable WTW multi 3420 set G (Figure 2).

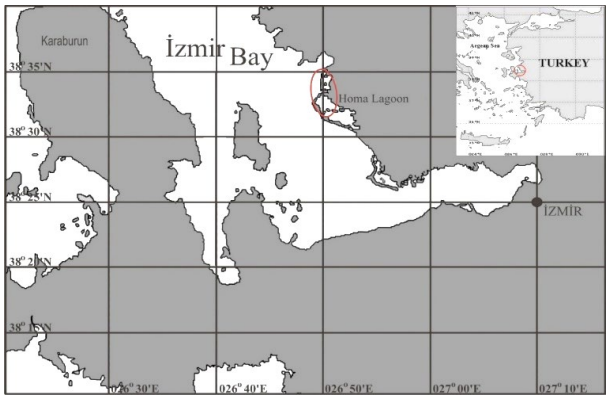



**Figure 1.** Sampling area

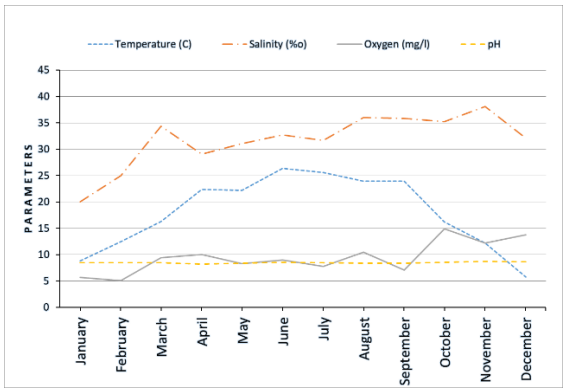



**Figure 2.** Monthly changes in some physicochemical parameters in the study area

A total of 776 wild European eels with a mean length of 547 ± 91 mm and mean weight of 340±198 g. which were obtained monthly between October 2015 to September 2016 from a local fisherman who has fished with fyke net in the Gediz delta of Izmir Bay. Collected samples were kept frozen until analysis. The swim bladder of each fish was removed and examined macroscopically. The presence of pre-adult and adult *A. crassus* was collected from the lumen of the eels by forceps. To identify the parasite, determined as *A. crassus*, we used the descriptions given by Moravec and Taraschewski (1988). Classical epidemiological parameters—i.e.

**Abundance:** abundance is the number of individuals of a particular parasite in/on a single host regardless of whether or not the host is infected [A: parasite abundance (A=Pn/N)].

**Mean intensity:** Mean intensity is the average intensity of a particular species of parasite among the infected members of a particular host species. In other words, it is the total number of parasites of a particular species found in a sample divided by the number of hosts infected with that parasite [Mi: mean intensity Mi=Pn/Ni)].

**Prevalence:** Prevalence is the number of hosts infected with 1 or more individuals of a particular parasite species divided by the number of hosts examined for that parasite species. It is commonly expressed as a percentage [P: Prevalence P=(Ni/N) x100)] (N: Number of samples; Ni: Number of infected eels; Pn: Total number of parasites) were used as defined by Bush et al., (1997).

## RESULTS

*Anguillicoloides crassus* nematode parasites were found in the swim bladder of 52 out of 776 individuals examined in the region. The total body length (LT) ranged from 303 to 852 mm, and their average length was calculated as 547±91 mm.

The total number of *A. crassus* in the examined parasitized individuals was 263, and a maximum of 25 (adults and pre-adults) recorded in a single eel were found in the swim bladder of a sampled fish. One of the parasitological indices the prevalence of *A. crassus* among all samples was, 7%, and the abundance was 0.33 nematodes per all sampled eel (Table 1). The minimum and maximum lengths of the parasitized individuals were 336 mm and 678 mm, respectively (Figure 3).

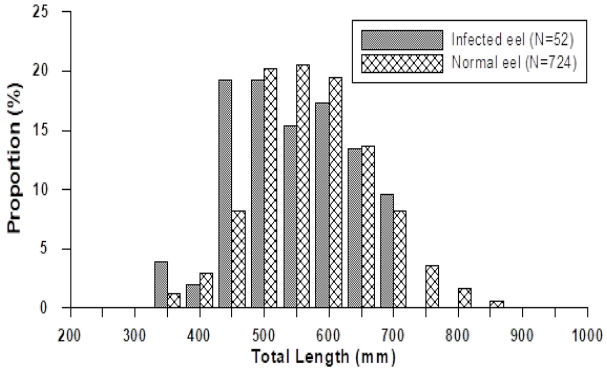



**Figure 3.** Length distribution of *Anguillicoloides crassus* Infected individuals within the sampled individuals

When the distribution of the parasites encountered according to the seasons is examined, it has been determined that the abundance is observed at the highest level in the summer season, and it decreases gradually in the autumn and winter seasons (Table 1).

**Table 1.** Seasonal variation of *Anguillicoloides crassus* in Gediz delta (N: Number of specimens; Ni: Number of infected eels; Pn: Total number of parasites; P: prevalence [P=(Ni/N) x100)]; A: Parasite abundance (A=Pn/N)], Mi: mean intensity (Mi=Pn/Ni))

| SEASONS | N | Ni | Pn | P | Mi | A |
|---|---|---|---|---|---|---|
| **Spring** | 191 | 14 | 65 | 7.3 | 4.64 | 0.34 |
| **Summer** | 176 | 19 | 133 | 10.7 | 7 | 0.97 |
| **Autumn** | 209 | 10 | 32 | 4.78 | 3.2 | 0.15 |
| **Winter** | 200 | 9 | 33 | 4.5 | 3.66 | 0.17 |
| **Total** | 776 | 52 | 263 | 6.7 | 5.05 | 0.33 |

## DISCUSSION

According to Moravec and Taraschewski (1988), *Anguillicoloides crassus*, which started to be found in Europe in the early 1980s, was first reported in Turkey by Genç et al., (2005) in the Ceyhan river. In the following years, different studies have been found in the river systems of the northeastern coasts of the Mediterranean (Genç et al., 2008; Koyuncu et al., 2017; İnnal et al., 2019). However, a limited number of studies have been reported on the Turkish coasts of the Aegean Sea, including Köyceğiz-Dalyan (Çolak et al., 2012), İzmir Bay Çamaltı (Gürkan et al., 2022) and Vistonis Lake (Macnamara et al., 2014; Kantzoura et al., 2021) on the Greek coasts. Among these studies, only the study in İzmir Bay Çamaltı region was carried out in a salty environment, and it was determined as the study with the lowest prevalence (2.24%), (Table 2). The prevalence value in this study conducted in the Gediz Delta was calculated as 6.7%. It has been observed that this value is quite low when compared to other parts of the Mediterranean such as 78% in the Seyhan river (Genç et al., 2005), 75.2% in Egypt (Aly et al., 2007), 61.7% in the eutrophic lakes of Greece (Macnamara et al., 2014) and 39.7% in Macedonia (Cakić et al., 2002).

Prevalence values vary considerably according to the salinity values of the environments where the samples are taken. Many researchers have reported that *A. crassus* is less common in saltwater environments than in fresh waters (Louklidi and Belghyti, 2007; Aly et al., 2007; Mayo-Hernandez et al., 2015; Bakaria et al., 2018; Hafir-Mansouri et al., 2018; Wariaghli and Yahyaoui, 2018; Oudjane, 2021), (Table 2).

**Table 2.** Occurrence of *Anguillicoloides crassus* according to different regions in the Mediterranean (N: Number; iN: Infected eel number; P: Prevalence; ‰S: Salinity; pN: Parasite number in a host; Mi: Mean intensity; Npt: Number of total parasites)

| Regions | (N) | (iN) | P (%) | ‰S | (pN) Min-max | Mi | (Npt) | Reference |
|---|---|---|---|---|---|---|---|---|
| Loukkos Est. | 60 | - | 51.6 | - | - | 1.9 | - | El-Hilali et al., 1996 (Morocco) |
| Sebou Estuary | 60 | - | 43.3 | - | - | 1.9 | - | |
| Ohrid Lake | 68 | 27 | 39.71 | - | 1-90 | 10.33 | - | Çakiç et al., 2002 (Macedonia) |
| Ceyhan River | 64 | 50 | 78.2 | - | 1-7 | 3.3 | - | Genç et al., 2005 (Türkiye) |
| Al-Salam Chnl | 186 | 154 | 82.8 | 0.2 | - | 7.48 | 1152 | Aly et al., 2007 (Egypt) |
| Al-Gohhr, Manzala Lake | 204 | 152 | 74.5 | 5.4 | - | 5.34 | 812 | |
| Al-Gameel, Port-Said | 106 | 64 | 60.4 | 39.8 | - | 4.20 | 269 | |
| Bahr el Bakar ShaderAzzam | 72 | 57 | 79.2 | 1.2 | - | 6.88 | 392 | |
| Total | 568 | 427 | 75.2 | - | - | 6.15 | 2625 | |
| Asi River | 18 | 11 | 61.1 | - | 1-18 | 4.55 | 50 | Genç et al., 2008 (Türkiye) |
| Salam Channel | 65 | 7 | 10.7 | - | - | 1.85 | - | Abdelmonem et al., 2009 (Egypt) |
| Tinga River, | 546 | - | 46.3 | 19 | - | 5.6 | - | Hizem-Hebbechi et al., 2012 (Tunisia) |
| Bizerte Lagoon | 103 | - | 15.5 | 28-38 | - | 1.6 | - | |
| El Kebir River- Skikda | 495 | - | 9.72 | - | - | 3.37 | | Rouag-Laouira et al., 2012 (Algeria) |
| Köyceğiz-Dalyan | 73 | 29 | 39.7 | 15 | 1-14 | 3.3 | 97 | Çolak et al., 2012 (Türkiye) |
| Camargue Lagoon, | 277 | - | 52.7 | - | 1-37 | 4.1 | - | Lefebvre et al., 2013 (France) |
| North Aegean Sea Vistonis estuarine system | 188 | - | 61.7 | - | 1-21 | 3.41 | - | Macnamara et al., 2014 (Greece) |
| Mar Menor Lagoon, | 189 | - | 3.0 | 43-46 | 0-10 | 1 | - | Mayo-Hernandez et al., 2015 (Spain) |
| Göksu River | 45 | 13 | 28.8 | - | - | 4.69 | 61 | Koyuncu et al., 2017 (Türkiye) |
| Seyhan River | 42 | 8 | 19.04 | - | - | 8.12 | 56 | |
| Ceyhan River | 42 | 8 | 19.04 | - | - | 3.12 | 25 | |
| Asi River | 41 | 18 | 43.9 | - | - | 3.44 | 62 | |
| Sebou Estuary | 1138 | | 66.7 | 13-33 | - | 2.93 | - | Wariaghli and Yahyaoui, 2018 (Morocco) |
| Loukkos Estuary | 100 | | 41.8 | 22-34 | 1-5 | - | - | |
| Golf of Bejaia | 87 | | 42.5 | - | - | 6.65 | 226 | Hafir-Mansouri et al., 2018 (Algeria) |
| Tonga Lake | - | 144 | 40.0 | - | - | 4.4 | 628 | Bakaria et al., 2018 (Algeria) |
| El Mellah Lagoon | - | 18 | 5.0 | 26-35 | - | 1.4 | 26 | |
| Manavgat River | 22 | 14 | 63.6 | - | | 5.7 | - | İnnal et al., 2019 (Türkiye) |
| Göksu River | 28 | 17 | 60.7 | - | 1-48 | 5.9 | - | |
| Seyhan River | 20 | 15 | 75 | - | | 6.7 | - | |
| Vistonis estr syst. | - | 26 | - | - | - | 2.31 | 268 | Kantzoura et al., 2021 (Greece) |
| Amvrakikos Gulf | - | 10 | - | - | - | 2.20 | - | |
| Mesolongi Lagoons | - | 34 | - | - | - | 3.88 | - | |
| Peloponnese Lagoons | - | 15 | - | - | - | 3.60 | - | |
| Comacchio Lagoon | 339 | | 5.6 | 35 | 34 | 1.68 | 32 | Giari et al., 2021 (Italy) |
| Gediz Estuary | 89 | 2 | 2.24 | 35 | 1-23 | 7.5 | 24 | Gürkan et al., 2022 (Türkiye) |
| Gediz Estuary | 776 | 52 | 6.7 | 32 | 1-25 | 5.05 | 263 | Present study (Türkiye) |

According to these results, we can say that when comparing values such as mean intensity or prevalence, comparing them not according to regions but according to the freshwater or saltwater environments in which the creature is distributed can yield more successful results.

When the *A.crassus* parasite in the European eel and the density of this parasite in the population are examined, many reasons are showing that it is less in salt water than in freshwater.

Some of those; are related to which planktonic crustacea species or small fish they prefer in their first feeding in the regions they come from (Tesch, 1977). Kirk et al., (2000a) reported that the extracellular (pseudocoelomic) fluid of *A. crassus* had a higher mortality rate due to the osmotic pressure difference in saline waters compared to the environment. In addition, this osmotic pressure difference also causes rapid degeneration in parasite larvae and eggs (Kirk et al., 2000b). Palstra et al., (2007) and Sjöberg et al., (2009) reported that *A. crassus* caused damage to the swim bladder of eels, affecting their reproductive migration and not reaching their breeding areas.

In this case, considering that the reproductive ability decreases in areas with high parasites, it can be said that the breeding probability of eels living in the Gediz Delta is quite high compared to other regions and they constitute relatively healthy individuals of the Mediterranean population.

## CONCLUSION

According to the data of all these studies, it turns out that eels in saltwater lagoons with low prevalence values are important in ensuring the future of the population in the Mediterranean. In addition, it is a fact that toxic substances carried to these areas by rivers (Aydın et al., 2017) as a result of human activities affect this species, which is already endangered, even more. Therefore, pollution protection and monitoring studies on such deltas are very important for the future of the European eel *Anguilla anguilla*.

## ACKNOWLEDGMENTS AND FUNDING

I would like to thank The Scientific and Technological Research Council of Turkey (TÜBİTAK) to fund this study with the TÜBİTAK project of 115Y399.

## CONFLICT OF INTEREST STATEMENT

The author declares that there is no known financial or personal conflict that may affect the research article.

## ETHICS APPROVAL

The author declares 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.

## REFERENCE

Abdelmonem, A.A., Metwally, M.M., & Hussein, H.S. (2009). Pathological studies on some parasitic diseases of eel (*Anguilla anguilla*). *Egyptian Journal of Comparative Pathology and Clinical Pathology*. *22*(3), 96-113.

Aly, S.G., El-Nobi G. Ahmed, & El-Dosoky, A.El-Sayed (2007). Occurrence of *Anguillicola crassus* (Nematoda, Dracunculidae) a parasite of European Eel (*Anguilla anguilla*) in Egypt. *Zagazig Veterinary Journal*, *35*(3), 104-114.

Aydın, G.Ş., Salman, A., Oral, R., Şimsek, K. & Koçbaş, F. (2017). Some heavy metal levels of water and sediments from North drainage channels and Lagoon areas (Homa and Kırdeniz) of Gediz Delta (Izmir Bay). *XIII. Ecology and Environment Congress 2017* (p. 338) Edirne, Türkiye, Proceedings Book.

Bakaria, F., Belhaoues, S., Djebbari, N., Tahri, M., Ladjama, I., & Bensaad, L. (2018). Metazoan Parasites and Health State of European Eel, *Anguilla anguilla* (Anguilliformes, Anguillidae), from Tonga Lake and El Mellah Lagoon in the Northeast of Algeria. *Vestnik Zoologii*, *52*(4), 279–288. https://doi.org/10.2478/vzoo-2018-0029

Bilecenoğlu, M., Kaya, M., Cihangir, B., & Çiçek, E. (2014). An updated checklist of the marine fishes of Turkey. *Turkish Journal of Zoology, 38*(6), 901-929. https://doi.org/10.3906/zoo-1405-60

Bush, A.O., Lafferty, K.D, Lotz, J.M. & Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology*, *83*(4), 575-583. PMid:9267395. https://doi.org/10.2307/3284227

Cakić, D.P., Stojanovski, S., Kulišić, Z., Hristovski, N., & Lenhardt, M. (2002). Pojava *Anguillicola crassus*, nematoda: Dracunculoidea, kod jegulje iz Ohridskog jezera, Makedonija. *Acta Veterinaria*, *52*, 163-168. https://doi.org/10.2298/AVB0203163C

Çolak, S.Ö., Soylu, E., Erdoğan, F., & Erdoğan, M. (2012). Metazoan parasites of European eel (*Anguilla anguilla*) from the Köyceğiz Dalyan Estuarine channel system, Turkey. *Bulletin of the European Association of Fish Pathologists*, *32*(5), 159-163.

De Charleroy, D., Grisez, L., Thomas, K., Belpaire, C., & Ollevier, F. (1990). The life cycle of *Anguillicola crassus*. *Diseases of Aquatic Organisms*, *8*(2), 77–84. https://doi.org/10.3354/dao008077

Durif, C.M.F., Gjosaeter, J., & Vollestad, L.A. (2011). Influence of oceanic factors on *Anguilla anguilla* (L.) over the twentieth century in coastal habitats of the Skagerrak, southern Norway. *Proceedings of the Royal Society of London. Series B. 278*, 464–473. https://doi.org/10.1098/rspb.2010.1547

El-Hilali, M., Yahyaoui, A., Sadak, A., Maachi, M., & Taghy, Z. (1996). Premières données épidémiologiques sur l'anguillicolose au Maroc. *Bulletin Français de la pêche et de la pisciculture, 340*, 57-60. https://doi.org/10.1051/kmae:1996005

Genç, E., Şahan, A., Altun, T., Cengizler, I., & Nevsat, E. (2005). Occurrence of the swim bladder parasite *Anguillicola crassus* (Nematoda, Dracunculoidea) in European eels (*Anguilla anguilla*) in Ceyhan River, Turkey. *Turkish Journal of Veterinary and Animal Sciences*, *29*, 661-663.

Genç, E., Sangun, M.K., Dural, M., Can, M.F., & Altunhan, C. (2008). Elements Concentrations in the Swimbladder Parasite *Anguillicola crassus* Nematoda and Its Host the European Eel, *Anguilla anguilla* from Asi River (Hatay/Turkey). *Environmental Monitoring Assessment, 141*, 59-65. https://doi.org/10.1007/s10661-007-9878-9

Giari, L., Castaldelli, G., Gavioli, A., Lanzoni, M., & Fano, E.A. (2021). Long-term ecological analysis of *Anguillicola crassus* occurrence and impact

on the European eel population in a Mediterranean lagoon (North Italy). *Estuarine, Coastal and Shelf Science*, *249*, 107117. https://doi.org/10.1016/j.ecss.2020.107117

Gürkan, Ş., Taylan, B., & Taşkavak, E. (2022). Determination of the swim bladder parasite *Anguillicola crassus* (Nematoda, Dracunculoidea) in the European Eel, *Anguilla anguilla* (Linnaeus, 1758) from the locality Çamalti Tuzla of Izmir Bay, Eastern Aegean Sea. *Turkish Journal of Agriculture - Food Science and Technology*, *10*(2), 276-279. https://doi.org/10.24925/turjaf.v10i2.276-279.4717

Hafir-Mansouri, D., Kadji-Djoudad, H., Ramdane, Z., Trilles, J.P., & Amara, R. (2018). *Anguillicola crassus* (Kuwahara, Niimi & Hagaki, 1974) infecting the European eel in Algeria. *Cahiers de Biologie Marine*, *59*, 19-24. https://doi.org/10.21411/CBM.A.62B9C0DE

Hizem-Hebbechi, B., Kraiem, M.M., & Elie, P. (2012). Etude de la contamination de l'anguille européenne (*Anguilla anguilla* L. 1758) par *Anguillicoloides crassus* dans quelques hydrosystèmes de la Tunisie septentrionale: analyse de son impact sur les paramètres de croissance. *Cybium*, *36*, 417-433

İnnal, D., Özmen, O., & Genç, E. (2019). Infection of European eel, *Anguilla anguilla* with the nematode *Anguillicoloides crassus* from some estuarine systems in Turkey. *Turkish Journal of Fisheries and Aquatic Sciences. 19*, 899–905. https://doi.org/10.4194/1303-2712-v19_11_01

Kantzoura, V., Sapounidis, A., Kouam, M.K., Kolygas, M.N., Krey, G., & Koutrakis, E. (2021). *Anguillicola (Anguillicoides) crassus*: Morphometric characteristics and pathogenicity in eels (*Anguilla anguilla*) in Greece. *Veterinary Parasitology: Regional Studies and Reports 25*, 100586. https://doi.org/10.1016/j.vprsr.2021.100586

Kirk, R.S., Lewis, J.W., & Kennedy, C.R. (2000a). Survival and transmission of *Anguillicola crassus* Kuwahara, Niimi & Itagaki, 1974 (Nematoda) in seawater eels. *Parasitology*,*120*, 289–295. https://doi.org/10.1017/s0031182099005399

Kirk, R.S, Kennedy, C.R., & Lewis, J.W. (2000b). Effect of salinity on hatching, survival and infectivity of *Anguillicola crassus* (Nematoda: Dracunculoidea) larvae. *Diseases of Aquatic Organisms 40*, 211–218. https://doi.org/10.3354/dao040211

Kirk, R.S. (2003). The impact of *Anguillicola crassus* on European eels. *Fisheries Management and Ecology*. *10*, 385–394. https://doi.org/10.1111/j.1365-2400.2003.00355.x

Koyuncu, C.E., Kaya, D., Özer, S., Barış, M., & Genç, E. (2017). Infection status of *Anguillicoloides crassus* in wild European eels (*Anguilla anguilla*) from Four Rivers of the Northeast Mediterranean Region, Turkey. *Acta Biologica Turcica*, *30*(4), 152-156.

Lefebvre, F., Fazio, G., Mounaix, B., & Crivelli, AJ. (2013). Is the continental life of the European eel *Anguilla anguilla* affected by the parasitic invader *Anguillicoloides crassus*? *Proceedings of the Royal Society B: Biological Sciences* 280, 20122916. https://doi.org/10.1098/rspb.2012.2916

Loukili, A., & Belghyti, D. (2007). The dynamics of the nematode *Anguillicola crassus*, Kuvahara 1974 in eel *Anguilla anguilla* (L. 1758) in the Sebou estuary (Morocco). *Parasitology Research. 100*, 683–686. https://doi.org/10.1007/s00436-006-0349-y

Macnamara, R., Koutrakis, E.T., Saounidis, A., Lachouvaris, D., Arapoglou, F., & McCarthy, T.K. (2014). Reproductive potential of silver European eels (*Anguilla anguilla*) migrating from Vistonis Lake (Northern Aegean Sea, Greece). *Mediterranean Marine Science*, *15*, 539-544. https://doi.org/10.12681/MMS.614

Mayo-Hernandez, E., Penalver, J., Garcıa-Ayala, A., Serrano, E., Munoz, P., & Ruiz de Ybanez, R. (2015). Richness and diversity of helminth species in eels from a hypersaline coastal lagoon, Mar Menor, south-east Spain. *Journal of Helminthology*, *89*, 345–351. https://doi.org/10.1017/S0022149X14000145

Moravec, F., & Taraschewski, H. (1988). Revision of the Genus *Anguillicola-yamaguti*, 1935 (Nematoda, Anguillicolidae) of the Swimbladder of eels, including descriptions of 2 new species, *Anguillicola-novaezelandiae* sp-n and *Anguillicola-papernai* sp-n. *Folia Parasitologica*. *35*, 125-46. PMID: 3169643

Moriarty, C., & Dekker, W. (1997). Management of the European eel. *Fisheries Bulletin* (Dublin), 15, 110 pp.

Oudjane, F. (2021). Parasitic infection by the nematode *Anguillicola crassus* (Kuwahara, Niimi et Itagaki, 1974) in the European eel *Anguilla anguilla* (Linnaeus, 1758) (Pisces Anguilliidae) of Lake Oubeïra (eastern Algeria). *Biodiversity Journal*, *12* (1), 255–260. https://doi.org/10.31396/Biodiv.Jour.2021.12.1.255.260

Palstra, A.P., Heppener, D.F.M., Van Ginneken, V.J.T., Székely, C., & van den Thillart, G.E.E.J.M. (2007). Swimming performance of silver eels is severely impaired by the swim bladder parasite *Anguillicola crassus*. *Journal of Experimental Marine Biology and Ecology 352*, 244-256. https://doi.org/10.1016/j.jembe.2007.08.003

Quadroni, S., Galassi, S., Capoccioni, F., Ciccotti, E., Grandi, G., De Leo, G.A., & Bettinetti, R. (2013). Contamination, parasitism and condition of *Anguilla anguilla* in three Italian stocks. *Ecotoxicology 22*, 94–108. https://doi.org/10.1007/s10646-012-1006-0

Rolbiecki, L. (2008). New data on the biology of the introduced exotic nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 in the eel *Anguilla anguilla* in Lake Wdzydze (Polish waters). *Oceanological & Hydrobiological Studies*, *37*(3), 37-48. https://doi.org/10.2478/v10009-008-0006-0

Rouag-Laouira, L. (2012). Rythme alimentaire, embonpoint et parasitisme à *Anguillicola crassus* (Kuwahara, Niimi et Itagaki, 1974) chez l'anguille européenne *Anguilla Anguilla* (L. 1758) de l'Oued El Kébir (Wilaya de Skikda), Mémoire de Magister, Université d'Annaba, 102 pp.

Sauvaget, B., Fatin, D., & Briand, C. (2003). Contamination par *Anguillicola crassus* de cinq populations d'anguilles (*Anguilla anguilla*) du littoral de Bretagne sud (France). *Bulletin français de la pêche et de la pisciculture*. *368*, 21–26. https://doi.org/10.1051/kmae:2003033

Sjöberg, N.B., Petersson, E., Wickström, H., & Hansson, S. (2009). Effects of the swim bladder parasite *Anguillicola crassus* on the migration of European silver eels *Anguilla anguilla* in the Baltic Sea. *Journal of Fish Biology*, *74*, 2158–2170. https://doi.org/10.1111/j.1095-8649.2009.02296.x

Tesch, F.W. (1977). *The eel: biology and management of Anguillid eels*. Chapman & Hall, London, 408pp.

Wariaghli, F., & Yahyaoui, A. (2018). *Anguillicoloides crassus* (Nematoda: Dracunculoidea) infection in eels in Moroccan estuaries. *AACL Bioflux 11*(1), 194-202.

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 20-26 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.03

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# A cost effective alternative method to ddRADseq library construction during size selection

## ddRADseq kütüphanesi oluşturma işlemi fragman seçiminde uygun fiyatlı bir alternatif yöntem

**Münevver Oral**

Faculty of Fisheries and Aquatic Science, Recep Tayyip Erdoğan University, Rize, 53100, Türkiye https://orcid.org/0000-0001-7318-6641

*munevver.oral@erdogan.edu.tr*

*Received date: 16.08.2022* *Accepted date: 30.11.2022*

***How to cite this paper:***
Oral, M. (2023). A cost effective alternative method to ddRADseq library construction during size selection. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 20-26. https://doi.org/10.12714/egejfas.40.1.03

**Abstract:** Next generation sequencing (NGS) technologies constitute the most powerful scientific advance of 21st century with a promise of fast and cost effective data generation in biology. Yet, up to date NGS studies remain often limited to laboratories with established resources. In the present study, we employed construction of ddRADseq library by using routine lab consumables (agarose gel electrophoresis: AGE thereafter) compared to high-tech NGS consumables (paramagnetic beads) during size selection. The ddRADseq library was constructed for sequencing size selected based on universally used paramagnetic beads, while remaining aliquot was used as a template to assess the feasibility of ddRADseq library construction using AGE for labs with limited resources. Both libraries were optimised for 15 PCR cycles indicating similarity in template intensity. Post-PCR quantification of the libraries was comparable (~10 ng.µL$^{-1}$). Size distribution assessment revealed a cleaner pick at the ddRADseq library size selected manually based on AGE. Similarly, intercalating agent of Qubit confirmed the quantity of libraries was similar (>3 ng.µL$^{-1}$). Although being more time consuming due to pre-electrophoresis preparations, serial wash and staining steps, ddRADseq library construction is achievable using routine lab consumables provided to supply the adaptors and PCR primers for the initial wet-lab work. These results manifest the feasibility of ddRADseq library generation for labs with limited resources.

**Keywords:** Library preparation, ddRADseq lab workflow, next generation sequencing

**Öz:** Yeni nesil dizileme (YND) teknolojileri, biyolojide hızlı ve uygun maliyetli veri üretimi vaadi ile 21. yüzyılın en güçlü bilimsel ilerlemesini oluşturmaktadır. Yine de, güncel YND çalışmaları genellikle yerleşik kaynaklara sahip laboratuvarlarla sınırlı kalmaktadır. Bu çalışmada, kütüphane fragman seçimi sırasında yüksek teknoloji ürünü YND sarf malzemelerine (paramanyetik boncuklar) kıyasla rutin laboratuvar sarf malzemelerinden (agaroz jel elektroforezi: buradan itibaren AGE) kullanarak ddRADseq kütüphaneleri oluşturuldu. Standart ddRADseq kütüphanesi, evrensel olarak kullanılan paramanyetik boncuklara dayalı olarak seçilen fragmanlarla oluşturulurken, kalan kısım, sınırlı kaynaklara sahip laboratuvarlar için AGE kullanılarak aynı fragman büyüklüğünde ddRADseq kütüphanesi yapılabilirliğini değerlendirmek için bir şablon olarak kullanıldı. Her iki kütüphane de kalıp DNA yoğunluğunda benzerlik gösteren 15 PCR döngüsü için optimize edilmiştir. Kütüphanelerin PCR sonrası yoğunlukları benzerlik gösterdi (~10 ng.µL$^{-1}$). Boyut dağılımı değerlendirmesi, AGE ile manuel olarak seçilen ddRADseq kütüphane boyutunda daha temiz bir seçim olduğunu ortaya çıkardı. Benzer şekilde, Qubit ölçümleri de kütüphane DNA miktarının yakın olduğunu ortaya koydu (>3 ng.µL$^{-1}$). Elektroforez öncesi hazırlıklar, seri yıkama ve boyama adımları nedeniyle daha fazla zaman almasına rağmen, ddRADseq kütüphane kurulum işlemi başlangıç için gerekli adaptör ve PCR primerlerinin sağlanması kaydıyla rutin laboratuvar sarf malzemeleri kullanılarak gerçekleştirilebilir. Bu sonuçlar, sınırlı kaynaklara sahip laboratuvarlar için ddRADseq kütüphanesi oluşturmanın uygulanabilirliğini ortaya koymaktadır.

**Anahtar kelimeler:** Kütüphane hazırlama, ddRADseq laboratuvar iş akışı, yeni nesil dizileme

### INTRODUCTION

The most recent breakthrough achieved in biological science is the development of next generation sequencing (NGS) technologies (Koboldt et al., 2013; McCombie et al., 2019; Hu et al., 2021). The ability of generating large number of genetic markers in relatively short period of time makes these technologies as a state of art methodology for genomic studies. NGS enables more individuals to be analysed at the same time by utilising high throughput sequencing, while massively parallel sequencing capacity multiplies the altitude of data generated with increasing accuracies as the depth of coverage rise. The validity of these technologies has widely been reviewed (MacLean et al., 2009; McCormack et al., 2013; Davey et al., 2011; Andrews et al., 2016; Fonseca et al., 2016; Tan et al., 2019). However, regardless of their potential, NGS technologies are still limited to established laboratories in developed, high-income countries with large research budgets, thus also having related resources in terms of infrastructure and human power. This is essential as all stages of NGS including pre-library trials, library construction, sequencing and bioinformatics analysis require a demanding workload that necessitates cross-disciplinary collaboration (Knapp et al., 2015). Some pre-library steps (e.g. *in silico* analysis, adapter design) do not require extensive resources while sequencing is often shipped to public or private providers for the service. The bioinformatics analysis (e.g. quality check and filtering of data generated, variant calling, assembly, and downstream

genomic analysis) have often been put forward as the most critical part of the NGS workload (e.g. Guo et al., 2014; Shafer et al., 2016; Paris et al., 2017), however, few attention has been paid to wet-lab procedures in order to reduce the production cost and then to produce reliable and comparable libraries. Library preparation involves a series of molecular techniques which can be summarized in four main steps: (I) fragmentation of the genome of interest using restriction enzyme(s), (II) ligation of adaptors carrying sequencing primers for bridge amplification during sequencing by synthesis, (III) size selection ensuring desired fragments are captured, and (IV) enrichment of the library through PCR.

In the present study, we carried out a wet-lab work by using routine molecular genetic lab consumables (AGE) *versus* expensive NGS lab consumables (paramagnetic beads) during the size selection step of ddRADseq (double digest restriction-site associated DNA sequencing) library construction. We assessed two metrics as proxies while defining successful ddRADseq library construction procedures: (I) the number of PCR cycles required for enrichment of the library and (II) the quantification of the libraries as well as the distribution of the fragment range.

## MATERIAL AND METHODS

### Sampling

All individuals (*Salmo* spp.) considered in this study were sampled in the wild using pulsed DC electroshocking equipment under the supervision of Prof. Dr. Davut Turan following the Local Ethics Committee of RTE University for the use of animals in scientific experiments with a permit reference number of 2019/13. Specimens were released to nature once fin clips were taken. Trout species included *Salmo chilo*, *S. labecula*, *S. opimus*, *S. kottelati*, *S. platycephalus* and *S. tigridis* (Turan et al., 2011, 2012). Tissue samples of 55 individuals were used as a template to construct ddRADseq libraries.

### Genomic DNA Extraction, Quantification and Quality Control (QC)

Genomic DNA was freshly extracted from individual fin clips using DNeasy® Blood & Tissue DNA extraction commercial kit (Qiagen, Valencia, CA), and performed on Qiacube DNA extraction robot (Qiagen, Valencia, CA). Following the manufacturer's guidelines, a RNase inhibition step was performed. These solutions constituted the stock DNA. The purity and the concentration of the extracted genomic DNA were initially assessed using NanoDrop spectrophotometry (2000C, Thermo Fisher Scientific, USA), while the integrity of the high molecular weight DNA was visualized on 0.8% agarose gel. Dilutions were made from stock DNA solutions down to 100 ng.µL$^{-1}$using double distilled water (dd$H_2O$). These served as working solutions throughout entire lab protocols. A final and more precise assessment of double stranded (ds)DNA concentration was carried out using Qubit fluorometer (Invitrogen, France) BR assay. Thus, based on Qubit concentrations, samples were diluted down to 50 ng.µL$^{-1}$ready to use in ddRADseq library construction.

### ddRADseq Library Construction

The ddRADseq library was generated originally by following Peterson et al. (2012) protocol with minor modifications detailed elsewhere (Palaiokostas et al., 2015; Leitwein et al., 2016; Oral et al., 2017). Each sample (200 ng DNA) was digested with two restriction enzymes, *Eco*RI-HF (G^AATTC) and *Msp*I (C^CGG) for 120 minutes at 37 °C in 25 µL reaction volume. The reactions were then treated with heat inactivation at 80 °C for 20 minutes. Some individuals were duplicated randomly in the library to reach 96 samples format so as to ensure higher coverage. P1 adaptor compatible with *Eco*RI and universally forked P2 adaptor compatible with *Msp*I were ligated to the digested DNA at 23°C room temperature for 120 minutes by adding 2 µL of each P1 and P2 adaptors (40 mM), 5 µL of ligase buffer, 1 µL of T4 DNA ligase (2K Units/µL) reaction volumes were made up to 25 µL using nuclease free water per sample (total reaction volume of 50 µL per sample). The ligation reaction was carried out in a thermal cycler, kept away from disruptions. Following heat inactivation at 65°C for 10 minutes, the plate carrying 96 samples was slowly cooled down to room temperature and pooled into a single ddRADseq library, labelled as *Pool*. The first purification was performed on ddRADseq library using 1X paramagnetic beads (Agencourt® AMPure® XP, Beckman Coulter, USA). Once purified, half of the reaction mix (25 µL) called DigLig (thereafter digested and ligated genomic DNA samples), was stored in -20 °C for *Pool* as a backup. Size selection (200-700 bp; see below for details) was carried out using paramagnetic beads (0.5-0.65X) by following manufacturer's guideline (the SPRI select User Guide, Beckman Coulter). The library was returned into individually labelled tube in 20 µL volume. Size selected library was then enriched by 15 cycles of PCR using 2x Phusion PCR master mix, 5 µL of library template, 2 µM of each PCR primer in 25 µL volume, reactions following cycling conditions of 98/65/72 °C for 10/30/45 seconds with a final extension at 72 °C for 5 minutes. PCR products were cleaned up using 0.75X paramagnetic beads to remove PCR primers. Purified and amplified PCR products were then quantified using both NanoDrop and Qubit. Library was assessed with Fragment Analyzer (Advanced Analytical Technologies, France) to determine DNA fragment size distribution. Based on this, one more round of 1X paramagnetic beads purification was carried out to remove primer dimers and small DNA fragments from the final library. After purification, the library was taken to the NGS sequence provider at minimum of 10 nM concentration in 20 µL volume. Library was sequenced (150 bp paired-end reads) on the Illumina HiSeq 2500 system.

### Size Selection Paramagnetic Beads *versus* Agarose Gel

The final library (*Pool*) sent for sequencing was size selected based on paramagnetic beads. For that, an initial volume of 50 µL was used. First, larger fragments longer than

700 bp were removed from the library by using volume of low beads to ligation volume ratio (0.50X), then supernatant carrying smaller fragments was eliminated by using higher beads to sample volume (0.65X) (SPRIselect User Guide, 2012).

Alternatively, remaining DigLig was size selected using AGE on the same gel. For that, 1.1% agarose gel was poured using freshly made/diluted 0.5X TAE buffer with no ethidium bromide (EtBr). Once set, the gel was left on the fridge submerged in 0.5X TAE buffer for 2 hours. Several combs (7) were taped using autoclave tape to form a high volume comb that can accommodate 65 µL volume (55 µL Library and 10 µL 6X DNA loading dye ensuring 1X concentration on the gel) for the library (see Figure 1). Markers were loaded to both sides of the library so as to indicate marker cut regions. The electrophoresis tank was filled up with freshly made 0.5X TAE buffer that was used for the gel preparation previously and the tank was located on ice ensuring chilled run. The electrophoresis system was pre-run at a lower voltage (10V/cm) to ensure electronic contacts were sound. First, 10µL marker (customised) to both side of library was loaded. Then, 65 µL library DNA loading dye homogenous mix was loaded to gel slowly ensuring enough time interval for library to equilibrate in the well. Electrophoresis was initiated in low voltages (10 V/20 V/30 V) and gradually increased up to 90 V to ensure no heating up in the tank buffer. The run was stopped when the loading dye migrated 3.5 cm from the origin (approx. 1 hr) (Figure 1A). The gel was placed on a glass and the library carrying fragment (ensuring loading dye band midway along the section) was safely separated from the gel using a sterile scalpel and stored in the fridge until required (Figure 1B-1C). Both sides of the gels were left behind (1-2 mm inside cut) so as to avoid the edge effect during electrophoresis. The remaining gel, carrying markers at both sides were stained in EtBr solution (2 µL of 5 mg/mL stock dissolved in 100 mL $ddH_2O$) for 5 minutes to visualise markers. Then, stained gel was washed with $ddH_2O$ before come in contact with library carrying gel fragment (Figure 1C). Desired fragment size was identified under UV trans-illuminator located in a dark room and a small cut was marked just beside the markers that were previously left for avoiding electrophoresis edge effect (Figure 1D; orange box). Once both gels put together a horizontal cut was made and library carrying desired fragment size was taken from the agarose gel (Figure 1D; green box). Size selected library was first weighted and evenly split between three Eppendorf tubes to be purified using a column based gel extraction kit (Qiagen, France). The temperature of elution buffer was increased to 50 °C on heated block so as to increase the binding capacity. The remaining gel was visualised to obtain the restriction pattern of the library (Figure 1D).

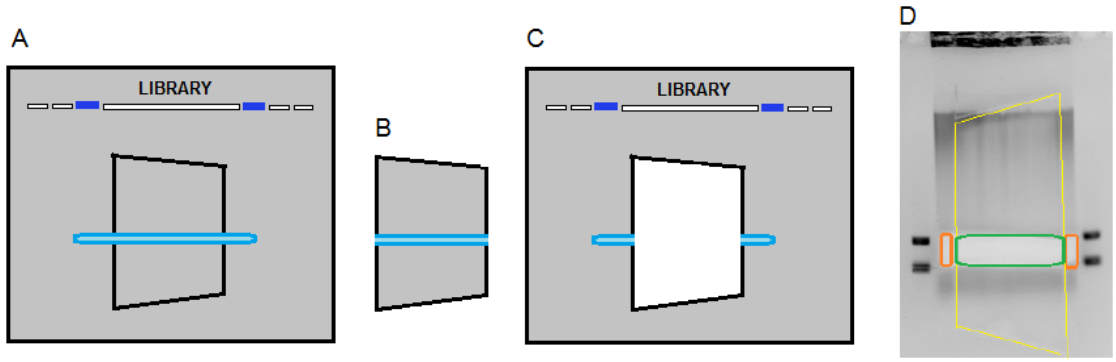



**Figure 1.** Schematic display of agarose gel based size selection of ddRADseq library. **A.** Electrophoresis was terminated once DNA loading dye migrated 3.5 cm from the origin; **B.** Library carrying gel was cut and stored at +4 °C; **C.** the remaining gel carrying markers was stained in EtBr solution, washed and a small cut was made under UV light indicating desired fragment size; **D.** ddRAD library gel processed throughout, yellow box: smear indicating restriction profile of the library as a positive control; orange box: indicative cut side of desired fragment size; green box: the fragment of interest ddRAD library

## RESULTS

### Quantification of ddRADseq libraries

One ddRADseq library, *Pool*, was constructed using the protocol size selected based on paramagnetic beads. The sequencing results and the downstream bioinformatics analysis of this library are discussed elsewhere (Oral et al., in preparation). Then, one more ddRADseq library was constructed, size selected based on agarose gel by using the remaining aliquot of DigLig genomic DNA of the same samples. This library was named as *AG_Pool* and was successfully extracted from the agarose gel. Size selection gel utilised was thicker (>6 mm) than usual to ensure that the gel could compensate serial washing and staining during process. This is particularly the case for the size selection of multiple libraries. As a rule of thumb, a single gel in larger volume with gaps between the pools needs to be used so as to minimise any variation during electrophoresis. As temperature plays significant role during electrophoresis, the lower voltage was used so as to avoid warming up the buffer which leads to smiling effect on the gel as the heat increases. Therefore, the rationale behind the cold run was to minimise the diffusion of small fragments in the gel and obtain more precise sample fraction. The gel slice for *AG_Pool* weighted as 0,75 g and the

library was eluted in 50 µL volume (2x25 µL) using heated elution buffer.

Table 1 shows the quantification results of ddRADseq libraries generated. Size selected and PCR enriched ddRADseq libraries were initially quantified using NanoDrop. As the concentrations of the libraries were higher than 5 ng/µL (Table 1), 1:1 dilution was made using dd$H_2O$ before checking the distribution of the DNA fragments with Fragment Analyzer. Therefore, Qubit accurately binds to dsDNA fragments resulted in lower concentrations for ddRADseq libraries produced following dilution. Similar library concentrations detected both in NanoDrop and Qubit were indicative of minimal variation achieved among the libraries generated regardless of the size selection method. Based on the intensity of libraries and concentrations, the number of PCR cycles could be decreased down to 12 or 13 cycles, which would also aid limiting the PCR duplicates.

**Table 1.** Quantification of the ddRADseq libraries

| | PCR cycles | NanoDrop (ng/µL) | Qubit BR assay (ng/µL) | Average library size (bp) | Lib concentratio (nM) |
|---|---|---|---|---|---|
| *Pool** | 15 | 9.92 | 3.1 | 396 | 11.86 |
| *AG_Pool*#* | 15 | 9.79 | 3.1 | 329 | 14.27 |

*: Paramagnetic beads based size selected and PCR enriched ddRADseq library
#: Agarose gel based size selected and PCR enriched ddRADseq library

### Fragment distribution of ddRADseq libraries

In agreement with the quantifications of libraries by NanoDrop and Qubit, the Fragment Analyzer showed a similar distribution in the structure of the diagrams. The average sizes of the libraries were detected as 396 bp and 329 bp, respectively, in *Pool* and AG_*Pool* (Figure 2A and B), confirming that the desired fragment size of interest have been captured in both libraries. In this study, it should be further noticed that agarose gel-based size selection resulted in smaller size fragment distribution which was desired as dealing with larger fragments is more challenging in size selection with paramagnetic beads. A small pick observed consistently in *Pool* and AG_*Pool* is expected in species with duplicated genomes. These are likely results of repetitive regions represent redundant copies post-whole genome duplication of Salmonidae (e.g. Glasauer & Neuhauss, 2014). An additional round of 1X paramagnetic beads clean-up was carried out based on fragment analyser results so as to remove small fragments detected at 44 bp and 45 bp length in the diagrams, respectively (Figure 2A and B). Although detected in lower concentrations, if prominent, these can decrease the sequencing yield by competing to bind flow cell thus hamper proper cluster generation derived from adaptor ligated samples. The removal of this fragments following final 1X paramagnetic beads clean-up was confirmed by the results provided by the sequence provider prior sequencing.

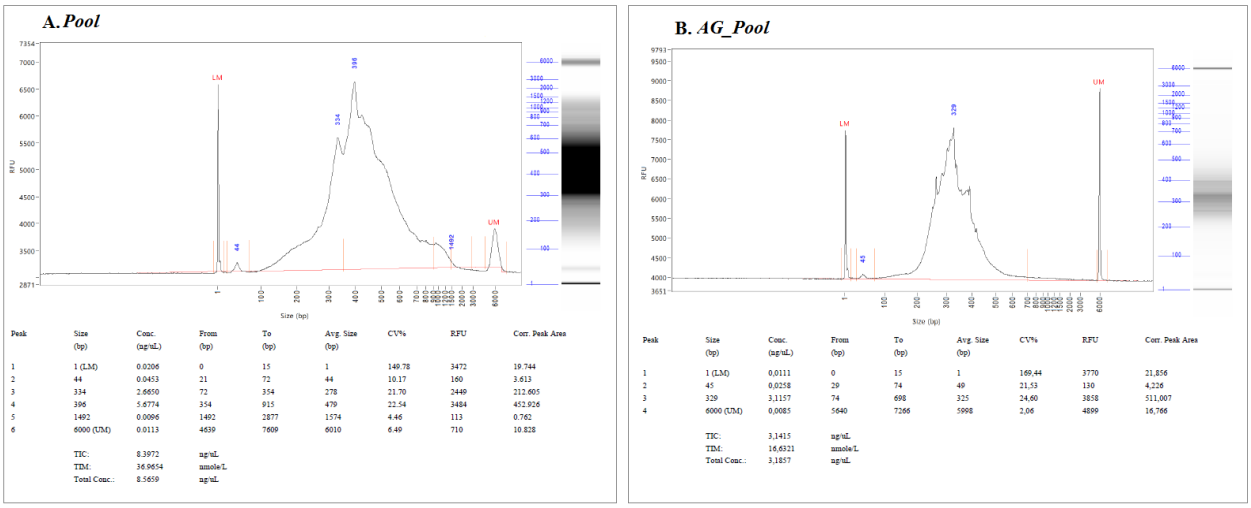



**Figure 2.** Distribution of the library fragments in ddRADseq libraries size selected based on (**A**) paramagnetic beads, *Pool* and (**B**) agarose gel electrophoresis *AG_Pool*

## DISCUSSION

In the present study, we provided experimental data on the feasibility of ddRADseq library construction using cost effective routine molecular lab consumables. The rationale behind developing a cost-effective methodological improvement was motivated by the fact that feasibility of scaling up conventional molecular genetic laboratories to adapt working with cutting edge technologies such as ddRADseq.

PCR enrichment of the final library is essential step so as to ensure amplification of desired size range in the final library (Peterson et al., 2012). In standard ddRADseq libraries 12 to 18 PCR cycles are routinely used (Peterson et al., 2012; Capblancq et al., 2015; Palaiokostas et al. 2015; Yang et al., 2016; Burns et al., 2017; Oral et al., 2017; Cumer et al., 2021). The higher the PCR cycles increases the risk of PCR duplicates, while the lower PCR cycles camouflage the existing

diversity. Cumer et al. (2021) investigated the effect of some wet-lab procedures including DNA quantity and PCR cycles thus detected lower individual heterozygosity in 10 PCR cycles compared to optimal range of 15 PCR cycles both at the inter-species level for the animal model (in the butterfly species complex of *Coenonympha*) and at the intraspecific level for the plant model (in European/common beech, *Fagus sylvatica*) (Figure 2). Such debate indicates the significance of the trade-off exist between a satisfactory coverage and limitation of errors originates from the excess amount of PCR cycles (Hohenlohe et al., 2012). Alternatively, a series of test PCR can be set up for future ddRADseq library construction in half reaction volume (12.5 µL) by visualising the desired PCR cycle of the library on the agarose gel. These steps should be investigated in a case by case study.

Fragment Analyser results indicated similar size fragment ddRADseq libraries were successfully produced (Figure 2). While evaluated with another and less precise technology, a similar fragment size distribution (range: 300-350 bp) was also observed in ddRADseq libraries of Leitwein et al. (2016) on another trout species (*S. trutta*) (M. Leitwein & B. Guinand, *personal communication*).

Taken all together, agarose gel-based size selection provides a cost-effective alternative to expensive paramagnetic beads-based size selection. Agarose (0.70 € for 1 gr) is almost one quarter of the price compared to the paramagnetic beads (2.61 Euro for 100 µL) for the required amount. Additionally, given the availability of agarose as a routine molecular lab consumable and the experience that comes with it favours agarose as an economic alternative for any sized laboratory with limited research fund. Yang et al. (2016) suggested using conventional low melting agarose for size selection as opposed to the expensive automatized alternative of pippin prep in an experimental study to provide an alternative method for ddRADseq library construction for a wider community working on angiosperm plants. Similarly, final quality control of the ddRADseq library can efficiently be carried out on agarose gel to detect the average size of the library using an appropriate marker (e.g. 1kb GeneRuler). Based on the gel image, visualized on short (loading dye 1.5 cm away from the origin) and long run (loading dye 3.0 cm away from the origin) the minimum, maximum, mean and median size of the library fragment can be detected and this would be used for calculating the average size of the library. Final ddRADseq library concertation in nM can be then calculated using the following formula (provided from Illumina.com support web page) while gel image is sent to sequencing provider:

$$concentration\ in\ nM = \frac{\left(concentration\ in \frac{ng}{\mu l}\right)}{\left(660 \frac{g}{mol} \times average\ library\ size\ in\ bp\right)} \times 10^6$$

The performance of our protocols was only evaluated based upon wet lab trials and experimental results. The biggest limitation of our study is to confirm the protocols by sequencing and provide the results from the data analysis (e.g. alignment rate to target reference genome, available SNP markers, basic statistics on sequence depth and coverage etc). However, the fact that all libraries provided sufficient requirements in terms of desired size range, concentration and available volume, we may anticipate the sequencing results would be of high quality and comparable between the two protocols (standard paramagnetic beads versus low-cost agarose gel electrophoresis).

In the present study, we provided experimental data on the feasibility of ddRADseq library construction using cost effective routine molecular lab consumables. As in all NGS experiments, the key to success is the availability of high molecular weight, intact genomic DNA and accurate quantification of dsDNA. Once this is achieved by using NanoDrop, Qubit and gel image, assuring a major band on the gel, sequencing produces sufficient amount of data as clearly demonstrated by Yang et al. (2016). In a recent study by Cumer et al. (2021) library construction pre-sequencing parameters including DNA quantity, number of PCR cycles during ddRADseq library preparation have shown to possess significant impact on the number of recovered reads and SNPs as well as on the number of unique alleles and individual heterozygosity. Additionally, same authors indicated the high reproducibility of the method provided to optimise the wet-lab procedures carefully. Furthermore, given the applicability of the protocol for any molecular laboratory, this study should motivate researchers in labs with limited resources to employ ddRADseq library construction provided to find partners that can supply adaptors and PCR primers for a start-up. Then, the cost only involves the investment of consumables for library preparation and sequencing. In the present study, expensive consumables were replaced by conventional alternatives where possible hence this protocol requires minimum technical investment for costly laboratory equipment and infrastructure. Therefore, taken all together, we anticipate our approach is applicable for any molecular laboratory with limited access to research funding thus the related human power.

## CONCLUSION

In an effort to optimise the ddRADseq library construction for wider community, here we assessed the feasibility of size selection from agarose gel as opposed to paramagnetic beads. PCR conditions during enrichment of the libraries were identical for both groups, amplifying desired fragment size intensity. Besides, quantifications of the libraries based on NanoDrop and Qubit showed similar concentrations between two groups. Although agarose gel size selection was more laborious, this method produced a better fragment size distribution. Thus, given the availability of agarose and the experiences in electrophoresis this can be of a low-cost alternative to the high-tech paramagnetic beads during size selection of ddRADseq library construction for molecular laboratories with limited resources.

## ACKNOWLEDGEMENTS AND FUNDING

Münevver Oral has received funding from Montpellier University of Excellence (MUSE) an Initiative for Science, Innovation, Territories, and Economy (I-SITE) of the French Investment for the Future Program under Explore#2 international mobility grant. Entire wet-lab work involved in the manuscript was performed at the genomics facility of ISEM the LabEx CeMEB (Centre Méditerranéen pour l'Environnement et la Biodiversité, Montpellier). This study was designed based on the experience of the author gathered working under guidelines of John B. Taggart, Stirling, Scotland during her PhD thesis. Author would like to thank Prof. Dr. Davut Turan for his help and guideline during fieldwork.

## CONFLICTS OF INTEREST

The author declares no conflicts of interest.

## DATA AVAILABILITY

Data generated within the course of the present study will be provided upon request to corresponding author.

## ETHICS APPROVAL

This study was carried out with the approval of Local Ethics Committee of Recep Tayyip Erdoğan University for the use of animal in scientific experiments (Permit Number: 2019-13 and Date: 18.06.2019).

## REFERENCES

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P.A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, *17*(2), 81–92. https://doi.org/10.1038/nrg.2015.28

Burns, M., Starrett, J., Derkarabetian, S., Richart, C. H., Cabrero, A., & Hedin, M. (2017). Comparative performance of double-digest RAD sequencing across divergent arachnid lineages. *Molecular Ecology Resources*, *17*(3), 418–430. https://doi.org/10.1111/1755-0998.12575

Capblancq, T., Després, L., Rioux, D., & Mavárez, J. (2015). Hybridization promotes speciation in *Coenonympha* butterflies. *Molecular Ecology*, *24*(24). https://doi.org/10.1111/mec.13479

Cumer, T., Pouchon, C., Boyer, F., Yannic, G., Rioux, D., and Bonin, A., & Capblancq, T. (2021). Double-digest RAD-sequencing: do pre- and post-sequencing protocol parameters impact biological results*? Molecular Genetics and Genomics*, *296*, 457–471. https://doi.org/10.1007/s00438-020-01756-9

Davey, J. W., Hohenlohe, P. A, Etter, P. D., Boones, J.Q., Catchen, J.M., & Blaxter, M.L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nature Reviews Genetics*, *12*(7), 499–510. https://doi.org/10.1038/nrg3012

Fonseca, R.R., Albrechtsen, A., Themudo, G.E., Madriagal, J.R., Sibbesen, J.A., Maretty, L., Mendoza, M.L., Campos, P.F., Heller, R, & Pereira, R.J. (2016). Next-generation biology: Sequencing and data analysis approaches for non-model organisms. *Marine Genomics*, *30*, 3-13. https://doi.org/10.1016/j.margen.2016.04.012

Glasauer, S. M. K. & Neuhauss, S. C. F. (2014). Whole-genome duplication in teleost fishes and its evolutionary consequences. *Molecular Genetics and Genomics*, *289*(6), 1045–60. https://doi.org/10.1007/s00438-014-0889-2

Guo, Y., Ye, F., Sheng, Y., Sheng, Q., Clark, T., & Samuels, D.C. (2014). Three-stage quality control strategies for DNA re-sequencing data. *Briefings in Bioinformatics*, *15*(6), 879–889. https://doi.org/10.1093/bib/bbt069

Hohenlohe, P. A., Catchen, J., & Cresko, W. A. (2012). Population Genomic Analysis of Model and Nonmodel Organisms Using Sequenced RAD Tags. In *Data Production and Analysis in Population Genomics* (pp. 235–260). Humana Press, Totowa, NJ. https://doi.org/10.1007/978-1-61779-870-2_14

Hu, T., Chitnis, N., Monos, D., & Dinh, A. (2021). Next-generation sequencing technologies: An overview. *Human Immunology 82*; 801–811.

Illumina.com. (Accession date: 20.05.2022; 9:00). Converting ng/µl to nM when calculating dsDNA library concentration. (https://emea.support.illumina.com/bulletins/2016/11/converting-ngl-to-nm-when-calculating-dsdna-library-concentration-.html?langsel)

Knapp, B., Bardenet, R., Bernabeu, M.O., & Deane, C.M. (2015). Ten simple rules for a successful cross-disciplinary collaboration. *Plos Computational Biology*, *11*(4), e1004214. https://doi.org/10.1371/journal.pcbi.1004214

Koboldt, D.C., Steinberg, K.M., Larson, D.E., Wilson, R.K., & Mardis, E.R. (2013). The next-generation sequencing revolution and its impact on genomics. *Cell*, *155*:27–38. https://doi.org/10.1016/j.cell.2013.09.006

Leitwein, M., Gagnaire, P. A., Desmarais, E., Guendouz, S., Rohmer, M., Berrebi, P., & Guinand, B. (2016). Genome-wide nucleotide diversity of hatchery-reared Atlantic and Mediterranean strains of brown trout *Salmo trutta* compared to wild Mediterranean populations. *Journal of Fish Biology, 89*, 2717–2734. https://doi.org/10.1111/jfb.13131

MacLean, D., Jonathan D.G.J., & Studholme, D. J. (2009). Application of next-generation sequencing technologies to microbial genetics. *Nature Reviews Microbiology*, 7, 287-296. https://doi.org/10.1038/nrmicro2088

McCombie, W.R., McPherson, J.D., & Mardis, E.R. (2019). Next Generation Sequencing Technologies, *Cold Spring Harb Perspect Med* 2019;9:a036798. https://doi.org/10.1101/cshperspect.a036798

McCormack, J.E., Hird, S.M., Zellmer, A. J., Carstens, B.C., & Brumfield, R.T. (2013). Applications of next-generation sequencing to phylogeography and phylogenetics. *Molecular Phylogenetics and Evolution*, *66*(2), 526-538. https://doi.org/10.1016/j.ympev.2011.12.007

Oral, M., Colléter, J., Bekaert, M., Taggart, J.B., Palaiokostas, C., McAndrews, B.J., Vandeputte, M., Chatain, B., Kuhl, H., Reinhard, R., Peruzzi, S., & Penman, D.J. (2017). Gene-centromere mapping in meiotic gynogenetic European seabass. *BMC Genomics, 18*, 449. https://doi.org/10.1186/s12864-017-3826-z

Palaiokostas, C., Bekaert, M., Khan, M. G. Q., Taggart, J.B., Gharbi, K., McAndrew, B.J., & Penman, D.J. (2015). A novel sex-determining QTL in Nile tilapia (*Oreochromis niloticus*). *BMC Genomics*, *16*(1), 171. https://doi.org/10.1186/s12864-015-1383-x

Paris, J. R., Stevens, J. R., & Catchen, J. M. (2017). Lost in parameter space: A road map for stacks. *Methods in Ecology and Evolution*, *8*(10), 1360-1373. https://doi.org/10.1111/2041-210X.12775

Peterson, B.K., Weber, J., Kay, E.H., Fisher, H.S., & Hoekstra, H.E. (2012). Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. *Plos One*, 7(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Shafer, A. B. A., Peart, C. R., Tusso, S., Maayan, I., Brelsford, A., Wheat, C.W., & Wolf, J.B.W. (2016). Bioinformatic processing of RAD-seq data dramatically impacts downstream population genetic inference. *Methods in Ecology and Evolution*, *8*(8), 907–917. https://doi.org/10.1111/2041-210X.12700

SPRIselect User Guide. (Accession date: 17.06.2022; 14:20). *Size selection based of paramagnetic beads*. p. 1-8. (https://research.fhcrc.org/content/dam/stripe/hahn/methods/mol_biol/SPRIselect%20User%20Guide.pdf)

Tan, M.P., Wong, L.L., Razali, S.A. Aleng, N.A., Nor, S.A.M., Sung, Y.Y., Peer, Y.V., Sorgeloos, P., & Daniel, M.D. (2019). Applications of Next-Generation Sequencing Technologies and Computational Tools in Molecular Evolution and Aquatic Animals Conservation Studies: A Short

Review. *Evolutionary Bioinformatics*, *15*, 1-5. https://doi.org/10.1177/1176934319892284

Turan, D., Kottelat, M., & Bektaş, Y. (2011). *Salmo tigridis*, a new species of trout from Tigris River, Turkey (Teleostei: Salmonidae). *Zootaxa 2993*, 23–33. https://doi.org/10.11646/zootaxa.2993.1.2

Turan, D., Kottelat, M., & Engin, S. (2012). The trouts of the Mediterranean drainages of southern Anatolia, Turkey, with description of three new species (Teleostei: Salmonidae). *Ichthyological Exploration of Freshwaters*, *23*, 219–236.

Yang, G.Q., Chen, Y.M., Wang, J.P., Guo, C., Zhao, L., Wang, X.Y., Guo, Y., Li, L., Li, D.Z., & Guo, Z.H. (2016). Development of a universal and simplified ddRAD library preparation approach for SNP discovery and genotyping in angiosperm plants. *Plant Methods*, *12*, 39. https://doi.org/10.1186/s13007-016-0139-1

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 27-34 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.04

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Evaluation of lettuce (*Lactuca sativa* L.) in aquaponic system in terms of food safety

## Marul bitkisinin (*Lactuca sativa* L.) akuaponik sistemde gıda güvenliği açısından değerlendirilmesi

**Gökhan Tunçelli[1]* ● İdil Can Tunçelli[2] ● Devrim Memiş[3]**

[1] Department of Aquaculture, Faculty of Aquatic Sciences, İstanbul University, 34134, İstanbul, Türkiye https://orcid.org/0000-0003-1708-7272
[2] Department of Seafood Processing Technology, Faculty of Aquatic Sciences, İstanbul University, 34134, İstanbul, Türkiye https://orcid.org/0000-0002-9999-6658
[3] Department of Aquaculture, Faculty of Aquatic Sciences, İstanbul University, 34134, İstanbul, Türkiye https://orcid.org/0000-0001-7378-0165

*Corresponding author: gokhan.tuncelli@istanbul.edu.tr
Received date: 05.08.2022 Accepted date: 25.11.2022

***How to cite this paper:***
Tunçelli, G., Can Tunçelli, İ., & Memiş, D. (2023). Evaluation of lettuce *(Lactuca sativa* L.) in aquaponic system in terms of food safety. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 27-34. https://doi.org/10.12714/egejfas.40.1.04

**Abstract:** We determined the number of coliform bacteria, *Escherichia coli*, yeast, and molds that may occur in the system, and the quality of lettuce grown in aquaponics for consumers from sensory, colour, and texture points of view. The amount of yeast and mold in the plant growing medium (hydroton) and water was 4.67 log CFU/cm$^2$ and 2.25 log CFU/mL at the end of the six-week experiment, respectively. The number of coliform bacteria and *E. coli* in the growing medium and in the system water was found to be 2.57 log CFU/cm$^2$ and 3.46 log CFU/mL for coliform, 0.75 log CFU/cm$^2$ 0.31 log CFU/mL for *E. coli*, respectively. Organisms that pose a risk to food safety, accumulate in the culture media. After the harvest, lettuce cultured in the aquaponic system (AP) was compared with the lettuce cultured in soil (SC). According to the results, AP lettuce was found to have darker colors (Lightness: 56.4 AP, 49.09 SC, $p<0.05$), harder (Hardness: 209.3 AP, 153.7 SC, $p<0.05$), and slightly appetizing (Sensory analysis overall liking: 8.4 AP, 7.7 SC) than SC. In conclution, aquaponic systems are much more preferable in terms of sensory quality and consumer preferences than soil-based production systems.

**Keywords:** Aquaponics, lettuce quality, coliform bacteria, *Escherichia coli,* yeast and molds

**Öz:** Bu çalışmada, akuaponik sistemde üretilen marul bitkisinin duyusal tüketicii tercihi, renk ve doku kalitesi açısından değerlendirilmiş ve sistemde oluşabilecek koliform bakteri, *Escherichia coli*, maya ve küf miktarları belirlenmiştir. Bitki yetiştirme ortamındaki (hidroton) ve sudaki maya-küf miktarları, altı haftalık deneyin sonunda sırasıyla 4,67 log KOB/cm$^2$ ve 2,25 log KOB/mL olarak tespit edilmiştir. Hidrotonda ve sistem suyundaki koliform bakteri miktarı sırasıyla 2,57 log KOB/cm$^2$ ve 3,46 log KOB/mL, *E. coli* miktarı ise sırasıyla 0,75 log KOB/cm$^2$ ve 0,31 log KOB/mL olarak bulunmuştur. Gıda güvenliği açısından risk oluşturan mikroorganizmalar yetiştiricilik ortamında birikebilmektedir. Akuaponik sistemde (AP) yetiştirilen marul hasat edildikten sonra topraklı tarımda (TT) üretilen marul ile karşılaştırılmıştır. Sonuçlara göre AP marulun renkleri TT'den daha koyu (Parlaklık: 56,4 AP, 49,09 TT, $p<0,05$), daha sert (Sertlik: 209,3 AP, 153,7 TT, $p<0,05$) ve duyusal analize göre daha iştah açıcı (Genel duyusal beğenisi: 8,4 AP, 7,7 TT) bulunmuştur. Sonuç olarak akuaponik sistemler duyusal kalite ve tüketici tercihleri açısından topraklı üretim sistemlerine göre daha çok tercih edilmiştir.

**Anahtar kelimeler:** Akuaponik, marul kalitesi, koliform bakteri, *Escherichia coli,* maya ve küf

## INTRODUCTION

Aquaponics is a combination of recirculating aquaculture and soilless agriculture. These systems are more advantageous than traditional agricultural techniques in water consumption, land use, soil salinization, yield, plant growth rate, chemical fertilizer requirement, and pesticide and herbicide usage factors. Although aquaponics has gained a lot of popularity in recent years, there is a lack of information in the field of food safety as it is a newly developing system (Hollyer et al., 2009).

The aquaponic system is an ecosystem of fish, plants, and bacteria which include both autotrophic and heterotrophic bacteria. Bacteria are essential to maintaining an aquaponic ecosystem (Blancheton et al., 2013; Eck et al., 2019; Schmautz et al., 2017). The successful administration of aquaponics depends on the complex microbial ecosystem it contains. Thanks to this microbial ecosystem, mineralization of nutrients required for plant production and biological cleaning of water are provided. However, while some species of these microorganisms in the system are beneficial, others may be harmful to human health.

There is a health risk of soil-borne agricultural pests, bacteria, and fungi in traditional soil farming. Although the risk of pathogen in aquaponic systems is less than in conventional agriculture (Somerville et al., 2014), it still exists (Yavuzcan Yildiz et al., 2017; Willmon, 2018). In aquaponic systems, pathogenic bacteria (e.g. *E. coli*) can enter the system in various ways due to the soil used in the germination stage of the plants used, the water added to the system daily, fish feed (Petreska et al., 2013), the digestive system of the fish or the non-sterile environment of the system.

Leafy vegetables have been increasing in popularity in aquaponics in recent years due to their great nutritional value and ease of use due to being a ready-to-eat product. However, due to pathogen contamination, leafy vegetables have caused numerous foodborne disease outbreaks (Hilborn et al., 1999; Taylor et al., 2013). In recent years, the number of outbreaks linked to *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* spp. has increased (Deering et al., 2012; Strawn et al., 2013), of course, detection methods and back-tracking procedures (Brashears et al., 1999; Abadias et al., 2012), which have largely developed after an outbreak, also contributed to the detection of this increase. Fresh fruit and vegetables are responsible for approximately 48% of foodborne outbreaks (Hoagland et al., 2018). Additionally, aggressive processing methods like heat processing, acid treatment, etc. can not be used on fresh vegetables like lettuce without quality loss. Therefore, reducing the risk of foodborne pathogen contamination before harvest or until it reaches the consumer is a critical step.

No food outbreak has been encountered yet from a product grown in aquaponic systems (Kasozi et al., 2021). However, since this risk is always present, it is essential to examine the microbiological risks in aquaponic systems and to set appropriate standards. The risk to human health should be negligible.

There are several pieces of research on food safety in commercial aquaponic systems that aim to determine the levels of microorganisms in water and products (Chalmers, 2004; González-Alanis et al., 2011; Fox et al., 2022). *E. coli*, a coliform bacteria, is one of the most prominent. *E. coli* is a bacterium that is frequently employed as an indicator of fecal contamination and microbiological water quality in the formulation of regulatory standards based on human health.

In addition to bacterial load in terms of food safety, post-harvest quality is also an important parameter for the marketability of aquaponic products. Features such as color, size, appearance, and texture, which affect consumers' preferences, are strongly related to the sensory properties of lettuce (Schröder, 2003). According to Holmes et al. (2019), overall flavor and overall texture were stronger predictors of marketability than bitterness and crispness. This situation suggests that broader sensory categories, rather than narrower categories, may better capture human sensory perceptions of lettuce.

The purpose of this study was to determine the presence and amount of coliform bacteria, *E. coli*, yeast, and molds that may occur in the system when the wastewater of fish farming is given directly to the plant roots without passing through any disinfection system (UV, ozone, etc.) in a decoupled aquaponics system. Hence, we determine the texture, color, and sensory effects of the quality of lettuce grown in aquaponics for consumers and compared aquaponics lettuce with soil-grown lettuce.

## MATERIAL AND METHODS

### Growing conditions of the aquaponics system

This study was performed in decoupled aquaponics systems in Sapanca, Turkey. The aquaponic system is composed of a recirculating aquaculture system (RAS) and a hydroponic (HP) unit. There was four aquaponics and each AP include three fish tanks (400 L * 3), a sump (400 L), mechanical (Eheim Classic 1500 XL, Germany) and biological filtration units (500 L), a chiller (Teco TK-2000, Italy), and a blower (Aquaticlife PG-370, USA) in the RAS, while two plant beds (1.3 $m^2$ * 2) and a sump (400 L) were in the HP (Figure 1).

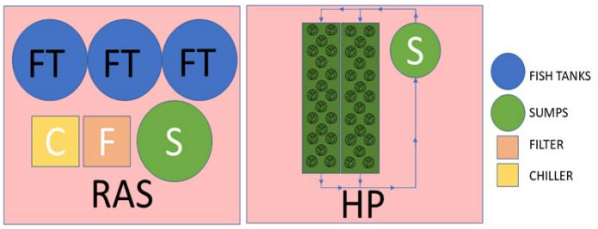



**Figure 1.** System design of the aquaponics

Lettuce (*Lactuca sativa* L. var. elmaria) seed germination was carried out in the soil. When they grew as 10-days-seedling, their roots were gently washed off with sytem water and transferred to the HP unit. The seedlings planted on pots with filled brand new hydrotons (Canna Aqua Clay Pebbles Hydroton 8-16 mm, Australia). The whole system had 240 cultivars (25 heads/$m^2$) and 432 rainbow trout (2.5 kg/$m^3$) (*Oncorhynchus mykiss* Walbaum 1792) juveniles. During the study, dissolved oxygen (9.94 mg/L), pH (8.3), temperature (15.7 °C), and electrical conductivity (257 µS/cm) parameters were followed daily and all values were kept at optimum conditions according to Somerville et al. (2014). The soil-cultured lettuce used in this study was obtained from a greenhouse in Istanbul. The harvest period of lettuce is 65 days.

### Microbiological analysis

Water samples from the aquaponic system were collected in sterile glass bottles (1 L-Duran Shott bottles). Hydroton samples were collected under aseptic conditions using sterile gloves and immediately placed in sterile stomacher bags. Samples were kept in styrofoam box with ice gel packs during transport to the microbiology laboratory until bacterial enumeration on the same day. All microbiological analyzes were performed aseptically in a sterile laminar airflow cabin (CRYSTE, Korea).

The surface area of hydroton is determined by Nuevaespana and Matias (2022) and it was 450 $m^2/m^3$. Hydroton samples (n~10) were placed in a sterile bag and diluted with maximum recovery diluent (MRD) at a 1:10 ratio before being blended with a stomacher. The water and hydroton samples were diluted serially. Spread plating was done in triplicate for each dilution sample.

### Total coliform and *Escherichia coli*

Total coliform and generic *Escherichia coli* counts were according to Feng et al. (2022). One milliliter of the sample was spread plated on VRB agar with MUG plates (VRB (Violet Red Bile Lactose)-MUG agar Merck 1.04030). All spread plates were incubated at 37 °C for 18-24 hours before counting typical pink colonies to determine the total coliform count. To define generic *Escherichia coli*, colonies that fluoresced under UV light were counted. The colonies were then recorded for analysis.

### Yeast-Mold

The yeast and mold were counted using Dichloran Rose Bengal Chloramphenicol Agar (DRBC) (Merck, 1.00466). All spread plates were incubated for 5 days at 25°C (Tournas et al., 2022).

### Sensory analysis

Sensory quality was determined as described by Martínez-Sánchez et al. (2011), by a ten-member trained panelist. Samples were evaluated by changes in visual quality, flavour, odour, texture, and browning of the leaf edge/surface. Samples of lettuce were properly washed with sterile Milli-Q water before sensory evaluation, dried with paper towels, and kept in the refrigerator (at 4 °C) in zipper-sealed plastic bags until analysis. Prior to the sensory panel, a 3-digit numerical code was randomly issued to each sample. Individual leaves that did not contain the innermost or outermost leaves made up the sample parts. Consumers were asked to score sensory quality using a 9-point hedonic scale, where 9 = excellent, 5 = limit of marketability and 1 = inedible.

### Color analysis

The color measurements of aquaponics grown and soil culture lettuce samples were determined with the Konica Minolta Chromometer (model CR 400; Minolta, Osaka, Japan). After the calibration using a white reference tile (CR-A44; L* = 94.93, a* = −0.13, b* = 2.55, and C* = 2.55), the lightness (L* value), the color (a*: + a, red; – a, green, b*: + b, yellow; – b, blue) and Munsell Hue (H) (GY; Green Yellow), Munsell Value (V) and Munsell Chroma (C) were measured three times on the samples at 3 different locations (E: Edge, M: Middle, S: Stem) on each leaf (n=10). The results are presented as the mean ± SD for the triplicate samples (Gerdes and Santos Valdez, 1991).

### Texture analysis

Texture profile analysis (TPA) on aquaponics grown and soil culture lettuce samples were determined by using a texture analyser (Brookfield CT3 Texture Analyzer, USA), equipped with a blade set probe, described by Back et al. (2014). Three stacked samples (3 by 3 cm) were placed onto the press holder, and a blade was moved down at 2 mm/s. TexturePro CT software (version 1.2, Brookfield Engineering Laboratories, Inc.) was used for the tabulation of TPA values (hardness, springiness, cohesiveness, chewiness, average peak load). The test had the following parameters: a pretest speed of 2 mm/s, a test speed of 1 mm/s, and a post-test speed of 1 mm/s. A measure of hardness was defined as the maximum force necessary to shear the samples. All experiments were performed three times, with independently-prepared samples (n=3) from three different parts of leaves (E: Edge, M: Middle, S: Stem) as in Figure 2.

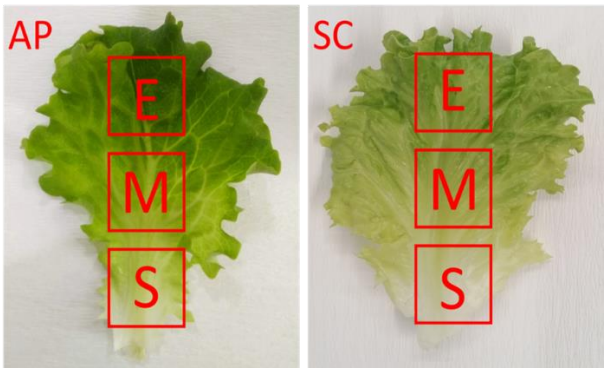



**Figure 2.** Different sampling locations on aquaponics grown (left) and soil culture lettuce (right) leaves (AP: Aquaponics grown lettuce, SC: Soil culture lettuce, E: Edge, M: Middle, S: Stem)

### Statistical analysis

The data were analyzed using IBM Statistical Package for the Social Sciences 28 software (USA). All data are given as mean values ± standard deviation (SD). The hydroton and water samples were examined at two-week intervals for a total of 6 weeks. Significant differences between groups on yeast and mold data were determined by ANOVA (post-hoc Tukey and Duncan) test, on coliform bacteria was determined by a tailed-independent Student's T-test. To identify significant differences between aquaponics and soil-cultured lettuce samples in terms of sensory, color, and texture analyses, a tailed-independent Student's T-test analysis was used. A 95 percent level of confidence was used for all statistical analyses ($p < 0.05$).

## RESULTS

Yeast and mold results in the first week, third week, and sixth week were determined as 3.31±0.8, 2.29±0.55, and 2.25±0.51 log CFU/ml in water samples and 2.41±0.25, 3.57±0.58, 4.68±0.63 CFU log/cm$^2$ in hydroton samples, respectively. While there was no difference between hydroton and water in the first week, there was a significant difference in the third and sixth weeks. Throughout the study, there is a decreasing trend ($R^2$ = 0.78) in water samples while an increasing trend ($R^2$ = 0.99) in hydroton samples (Figure 3).

Although the mean number of coliform bacteria was higher in water samples (3.46±0.65 log CFU/ml) compared to hydroton samples (2.57±1.53 log CFU/cm$^2$), a statistical difference was not detected between the groups (Figure 4).

The total *E. coli* count is given in Figure 5. Results show that growing media had more colonies than water. However, there were no significant differences between groups.

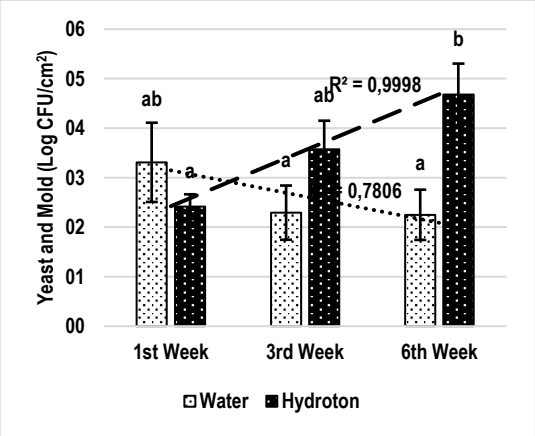



**Figure 3.** Mean Log count of yeast and mould colonies on water (log CFU/ml) and hydroton samples (log CFU/cm$^2$) (mean values ± SD, *n* = 4, Different letters on the top of SD bars indicate significant differences between mean values, Tukey's HSD multiple comparison method, $P < 0.05$)

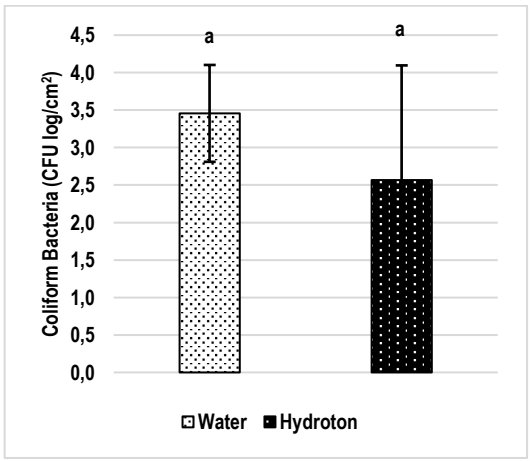



**Figure 4.** Coliform bacteria count of hydroton (log CFU/cm$^2$) and water samples (log CFU/ml) (mean values ± SD, *n* = 4, Different letters of the top on SD bars indicate significant differences between mean values, Student's T-Test, P < 0.05)

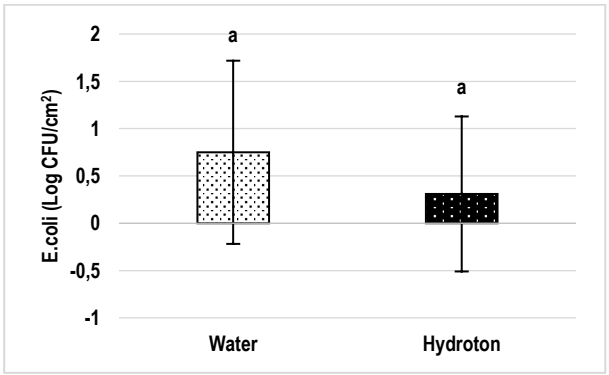



**Figure 5.** *E. coli* count on water (log CFU/ml) and hydroton (log CFU/cm$^2$) media at the end of the study

According to the overall liking of consumers, aquaponic lettuce has a mean value of 8.4 out of 10, while lettuce grown in soil has a mean value of 7. Lettuces that are cultivated in the aquaponics system had slightly higher quality than those cultivated in soil but the differences were not significant. The results from the sensory panel such as visual, flavour, texture, odour parameters are found as similar to overall liking results as given in Figure 6.

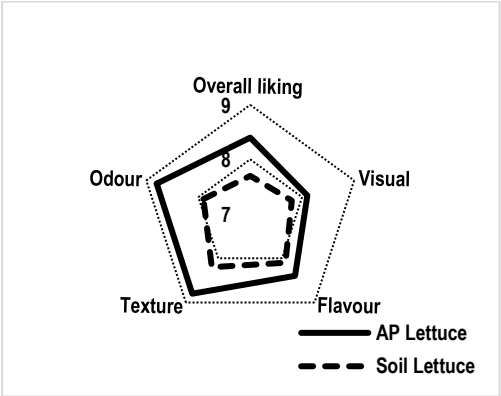



**Figure 6.** Sensory panel evaluations of aquaponics-grown lettuce and soil-grown lettuce (Mean ± standard deviation, AP: Aquaponics grown lettuce, SC: Soil culture lettuce, E: Edge, M: Middle, S: Stem)

The leaves of lettuce grown in aquaponics and lettuce grown in soil were examined in three regions (Edge, Middle, Stem) (Figure 2) and compared in terms of hardness, springiness, and cohesiveness (Figure 7).

The hardness value at the edge of AP (347.67 g) lettuce leaves was found to be statistically significantly higher than SC (261 g). The amount of hardness in the middle of the leaf was measured as 190.8 g for AP and 152.33 g for SC, while the amounts in the stem were measured as 89.5 g and 47.8 g. According to these results, it was determined that AP leaves had higher hardness values than SC. In addition, it was observed that the hardness values decreased from the edge of the leaves to the stem (Figure 7).

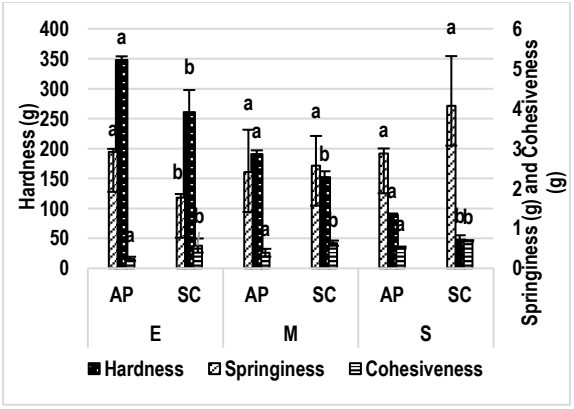



**Figure 7.** Hardness (left side), Springiness and Cohesiveness (right side) parameters of aquaponics grown lettuce and soil-grown lettuce (Mean ± standard deviation, AP: Aquaponics grown lettuce, SC: Soil culture lettuce, E: Edge, M: Middle S: Stem)

Color parameters such as L*, a*, b*, Munsell hue, value and chroma are given in Figure 8. AP lettuce varied from green-yellow and had a Munsell hue between 5.2-5.9. Conversely, SC lettuce was between 5.1 and 5.8.

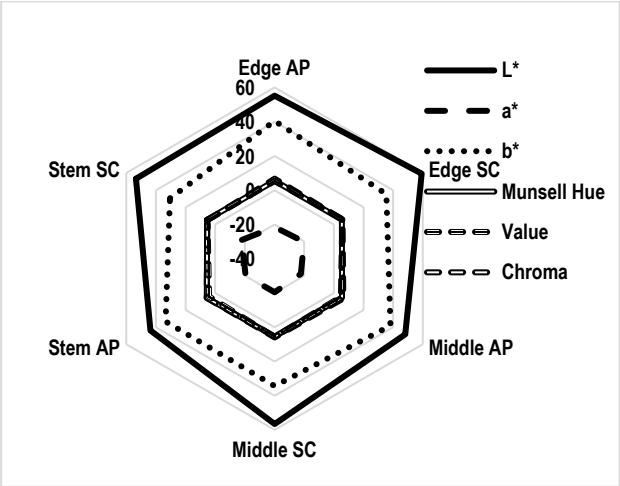



**Figure 8.** Color and texture parameters of aquaponics-grown lettuce and soil-grown lettuce (Mean ± standard deviation, AP: Aquaponics grown lettuce, SC: Soil culture lettuce, E: Edge, M: Middle S: Stem)

## DISCUSSION

The microbial profiles and counts (yeast and mold, coliforms, *E. coli*) for soil cultured and aquaponics-grown lettuce were determined by the current study. Yeast, mold, and coliform bacteria were detected in all 480 samples taken from the water and hydroton of the aquaponics system. Our findings are consistent with Sirsat and Neal (2013), Moriarty et al., (2018), and Weller et al. (2020) who observed coliforms or yeast and mold in all samples collected regularly from aquaponic and hydroponic systems. In our study, there was more yeast and mold in the hydrotons where the plants are attached by their roots compared to the water in the system. This may be due to microorganisms in the root environment being dependent on root exudates (Khalil, 2018). There was no difference in coliform bacteria amount between hydroton and water samples. *E. coli* was found in 50% of the four aquaponic systems constructed. According to Sirsat and Neal (2013), soil culture lettuce contained 2 to 3.5 log CFU/g *E. coli*. The amount of yeast and mold in the water, which was 3.31 ± 0.8 log CFU/g in the first week, was similar to the hydroton results of 2.41 ± 0.25 log CFU/g. The yeast and mold results in the third and sixth weeks were 2.29 ± 0.55 log CFU/g and 3.57 ± 0.58 log cfu/g, respectively, while the yeast and mold values in the hydroton were 2.25 ± 0.8 log CFU/g and ± 0.8 log CFU/g, respectively. Similar results in fungal flora of aquaponic system water between 2.8-3.4 log CFU/mL were observed by Khalil (2018). Therefore, the amount of yeast and mold on the hydroton to which the roots of the plants are attached showed an increasing trend between the third and sixth weeks compared to the first week ($R^2$=0.99), and a decrease ($R^2$=0.78) was observed in the water as time passed. This is thought to be because hydrotons create a suitable environment for yeast and mold organisms, like as nitrifying bacteria need surface area to form colonies (Pedersen et al., 2017).

Sirsat and Neal (2013) made a comparative microbial analysis of lettuce cultured with different techniques such as conventional, organic, bagged, farmers' market, and aquaponics and found lettuce from farmers' markets contained 2 to 3.5 log CFU/g *E. coli*. Organic and conventional lettuce contained about 2 log CFU of *E. coli* per gram of lettuce. According to Tyson et al., 2012, the population level of the coliform is 2.2 ± 0.2 log CFU/mL in aquaponics. Our findings show that aquaponic production has lower levels of coliform, mold, and yeast contamination compared to other production systems (Sirsat and Neal, 2013), and these findings are in agreement with the results of previous studies (Tyson et al., 2012; Elumalai et al., 2017).

Market pricing and consumer choice for lettuce are determined by internal attributes such as functional nutritional value and texture, as well as external qualities like color and size (Mampholo et al., 2016). In this study aquaponics grown lettuce scored higher in terms of sensory properties than those grown in soil. Similar results for soilless red-leafed lettuce cultures were reported by Selma et al. (2012), however, there are contrasting data for green leaf lettuce in the same study. For the textural properties, Fontana et al. (2018) reported that the hydroponic lettuces were significantly better accepted in relation to organic samples. The sensory approval of lettuce grown in conventional, hydroponic, and organic growing systems were tested using the hedonic scale by Fávaro-Trindade et al. (2007), and similarly, the authors did not observe any significant differences. Aquaponics products could have an odour because of the off-flavors (Atique et al., 2022). However, consumers stated that the lettuce grown in aquaponics did not have a fishy odor, which may have been the reason why they were not preferred aquaponics products by consumers.

Physiological properties such as color, size and texture are known to be affected by the conditions and environment in which lettuce is grown (Lei and Engeseth, 2021). There is variation in our TPA results as some lettuce pieces contain lettuce hearts and others are much leafier as in Predmore et al. (2015). Ibrahim and Zuki (2012) found that lettuce grown hydroponically had a much higher tensile strength than lettuce grown aquaponic and then planted in the ground. This showed that lettuce crispness values were also higher when grown hydroponically. None of the measured force values were significantly different from the soil-grown lettuce samples. However, this may be due to the small sample size of $n$ = 3 as in Schnabel et al. (2021). Also, Lei and Engeseth (2021) stated that when compared to lettuce grown in soil, lettuce grown hydroponically has softer leaves and tighter midrib, which may be due to lignin in the cell wall. In accordance with this our values show that leaf tissue became softer from S and E to M.

In our study, edge, middle, stem of lettuce samples from SC had a higher L* level than AP. In general AP groups had a greener color in leaves than SC lettuces, which could be

related to concentrations of chlorophyll in the leaves. Soil and aquaponics nutrients could be the reason for color differentiation. According to Ibrahim and Zuki (2012), regardless of plant growing technique, there was no significant difference in plant color. Matthew T et al. (2011) compared the visual quality of different lettuce varieties grown under hydroponic, conventional and organic conditions, and no difference was detected between groups. Similar results were observed by Lei and Engeseth (2021) who compared hydroponics vs soil-grown lettuce. On the contrary, Fontana et al. (2018) stated that hydroponically grown lettuce was lighter in color compared to organic and conventional ones. Outer leaves of green cultivars have lower (more negative) a* values due to higher chlorophyll content and darker green hue, according to Ozgen and Sekerci (2011). Our study showed that AP had slightly higher a* value than SC. Color results show that it can be affected by environmental conditions and phytochemical processes such as secondary metabolites (Ozgen and Sekerci, 2011).

## CONCLUSION

In conclusion, it has been determined that there is a risk of pathogens in the edible parts of the plants produced in aquaponic systems, the media used in the media bed technique poses a greater risk than the water, and the lettuce grown in aquaponics is preferred by the consumers because has stiffer leaves, darker green color and better quality for the consumer than the lettuce produced in traditional soil agriculture. Since organisms at risk accumulate in the culture media, it is necessary to compare the 'media bed technique' with the 'nutrient film technique' or 'deep water culture technique' in future studies. Increasing awareness of the state of scientific issues regarding indicator organisms in an aquaponic setting enables the consumer to make informed decisions. Thus, an increase in food safety in products produced through aquaponics is ensured. Determining the color, texture, and sensory profile of aquaponics products can be important tools in deciding under what conditions the crop will be grown and marketed. Food preferences are impacted by sensory perceptions. It is critical for producers to carry out research on this subject in order to determine which cultivar will be grown to meet market demands throughout the year. Studies on the quality and consumption risks of the products produced in the aquaponic system should be done more comprehensively.

## ACKNOWLEDGEMENTS AND FUNDING

We thank the Sapanca Inland Fisheries Production Research and Application Unit staff. This work was supported by the Scientific Research Projects Coordination Unit of Istanbul University. Project numbers FDK-2020-37081 and FBG-2018-31504.

## AUTHORSHIP CONTRIBUTIONS

Gökhan Tunçelli and Devrim Memiş: Conceptualization, methodology. Gökhan Tunçelli: Data curation, writing- original draft preparation. Gökhan Tunçelli and İdil Can Tunçelli: Visualization, investigation. Devrim Memiş: Supervision, project administration, resources, funding acquisition. Gökhan Tunçelli, İdil Can Tunçelli and Devrim Memiş: Writing-reviewing and editing.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare that are relevant to the content of this article.

## ETHICS APPROVAL

This study was approved by the local ethics committee for animal experiments of Istanbul University, Istanbul, Turkey (Approval No. 2019/10).

## DATA AVAILABILITY

Not applicable.

## REFERENCES

Abadias, M., Alegre, I., Oliveira, M., Altisent, R., & Viñas, I. (2012). Growth potential of *Escherichia coli* O157:H7 on fresh-cut fruits (melon and pineapple) and vegetables (carrot and escarole) stored under different conditions. *Food Control*, *27*, 37–44. https://doi.org/10.1016/j.foodcont.2012.02.032

Atique, F., Lindholm-Lehto, P., & Pirhonen, J. (2022). Is Aquaponics Beneficial in Terms of Fish and Plant Growth and Water Quality in Comparison to Separate Recirculating Aquaculture and Hydroponic Systems?. *Water*, *14*(9), 1447. https://doi.org/10.3390/w14091447

Back, K.H., Ha, J.W., & Kang, D.H. (2014). Effect of hydrogen peroxide vapor treatment for inactivating Salmonella Typhimurium, *Escherichia coli* O157: H7 and *Listeria monocytogenes* on organic fresh lettuce. *Food Control*, *44*, 78-85. https://doi.org/10.1016/j.foodcont.2014.03.046

Blancheton J.P., Attramadal K.J.K., Michaud L., D'Orbcastel E.R., & Vadstein O. (2013). Insight into bacterial population in aquaculture systems and its implication. *Aquaculture Engineering 53*, 30–39. https://doi.org/10.1016/j.aquaeng.2012.11.009

Brashears, M.M., & Durre, W.A. (1999). Antagonistic Action of *Lactobacillus lactis* toward *Salmonella* spp. and *Escherichia coli* O157:H7 during Growth and Refrigerated Storage. *Journal of Food Protection*, *62*, 1336–1340. https://doi.org/10.4315/0362-028X-62.11.1336

Chalmers, G.A. (2004). Aquaponics and Food Safety Aquaponics and Food Safety. http://www.byap.backyardmagazines.com/Travis/Aquaponics-and-Food-Safety.pdf. (accessed 08.04.2022)

Deering, A.J., Mauer, L.J., & Pruitt, R.E. (2012). Internalization of *E. coli* O157:H7 and *Salmonella* spp. in plants: A review. *Food Research International*, *45*, 567–575. https://doi.org/10.1016/j.foodres.2011.06.058

Eck, M., Sare, A.R., Massart, S., Schmautz, Z., Junge, R., Smits, T.H.M., & Jijakli, M.H. (2019). Exploring bacterial communities in aquaponic systems. *Water, 11*, 260. https://doi.org/10.3390/w11020260

Elumalai, S.D., Shaw, A.M., Pattillo, D.A., Currey, C.J., Rosentrater, K.A., & Xie, K. (2017). Influence of UV treatment on the food safety status of a model aquaponic system. *Water*, *9*(1), 27. https://doi.org/10.3390/w9010027

Fávaro-Trindade, C.S., de Carvalho Balieiro, J.C., Dias, P.F., Amaral Sanino, F., & Boschini, C. (2007). Effects of culture, pH and fat concentration on melting rate and sensory characteristics of probiotic fermented yellow

mombin (*Spondias mombin* L) ice creams. *Food Science and Technology International*, *13*(4), 285-291. https://doi.org/10.1177/1082013207082387

Feng, P., Weagant, S., Grant, M., & Burkhardt, W. (2022, August 4). *Enumeration of Escherichia coli and the Coliform Bacteria*. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria

Fontana, L., Rossi, C.A., Hubinger, S.Z., Ferreira, M.D., Spoto, M.H., Sala, F.C., & Verruma-Bernardi, M.R. (2018). Physicochemical characterization and sensory evaluation of lettuce cultivated in three growing systems. *Horticultura Brasileira*, *36*, 20-26. https://doi.org/10.1590/S0102-053620180104

Fox, B.K., Tamaru, C.S., Hollyer, J., Castro, L.F., Fonseca, J.M., Jay-Russell, M., & Low, T. (2022, August 4). *A preliminary study of microbial water quality related to food safety in recirculating aquaponic fish and vegetable production systems*. https://scholarspace.manoa.hawaii.edu/server/api/core/bitstreams/b92b8b32-2edc-468b-b4b3-80450c22ecc3/content

Gerdes D.L., & Santos Valdez C. (1991). Modified atmosphere packaging of commercial Pacific red snapper (*Sebastes entomelas*, *Sebastes flavidus* or *Sebastes godei). Lebensmittel-Wissenschaft und –Technologie*, *24*, 256–258.

González-Alanis, P., Gutierrez-Olguín, J.I., Castro-Segura, I., Ezqueda-Palacios, H., Acosta, M.H., Gojon-Báez, H.H., Aguirre-Guzmán, G., Guzmán-Saénz, F.M., & Fitzsimmons, K.M. (2011). Food Safety Study of Leafy Green Irrigated with Tilapia Farm Effluents in Tamaulipas. In *Better Science, Better Fish, Better Life: Proceedings of the Ninth International Symposium on Tilapia in Aquaculture* (pp. 121-122).

Hilborn, E.D., Mermin, J.H., Mshar, P.A., Hadler, J.L., Voetsch, A., Wojtkunski, C., Swartz M., Mshar, R., Lambert-Fair, M.A., Farrar, J.A., Glynn, M.K., & Slutsker, L. (1999). A multistate outbreak of *Escherichia coli* O157: H7 infections associated with consumption of mesclun lettuce. *Archives of Internal Medicine*, *159*(15), 1758-1764. https://doi.org/10.1001/archinte.159.15.1758

Hoagland, L., Ximenes, E., Ku, S., Ladisch, M. (2018). Foodborne pathogens in horticultural production systems: ecology and mitigation. *Scientia Horticulturae*, *236*, 192–206. https://doi.org/10.1016/j.scienta.2018.03.040

Hollyer, J., Tamaru, C., Riggs, A., Klinger-Bowen, R., Howerton, R., Okimoto, D., Castro, L., Ron, T., Fox, B.K., Troegner, V., & Martinez, G. (2009). On-Farm Food Safety: Aquaponics. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa Food Safety and Technology FST-38: 1–7. http://byap.backyardmagazines.com/Travis/Safety.pdf (accessed 07.04.2022)

Holmes, S.C., Wells, D.E., Pickens, J.M., & Kemble, J.M. (2019). Selection of heat tolerant lettuce (*Lactuca sativa* L.) cultivars grown in deep water culture and their marketability. *Horticulturae*, *5*(3), 50. https://doi.org/10.3390/horticulturae5030050

Ibrahim, R., & Zuki, W.A.M. (2012). The Physico-Chemical Properties Of Lettuce (Lactuca Sativa 'Grand Rapid') Grown Under Different Planting Methods. In H. Abdullah, M.N. Latifah (Eds.) VII International Postharvest Symposium 2012 (pp. 201-206). Kuala Lumpur, Malaysia: Proceedings Book.

Kasozi, N., Abraham, B., Kaiser, H., & Wilhelmi, B. (2021). The complex microbiome in aquaponics: significance of the bacterial ecosystem. *Annals of Microbiology*, *71*(1), 1-13. https://doi.org/10.1186/s13213-020-01613-5

Khalil, S. (2018). Growth performance, nutrients and microbial dynamic in aquaponics systems as affected by water temperature. *European Journal of Horticultural Science*, *83*(6), 388-394. https://doi.org/10.17660/eJHS.2018/83.6.7

Lei, C., & Engeseth, N.J. (2021). Comparison of growth characteristics, functional qualities, and texture of hydroponically grown and soil-grown lettuce. *Lebensmittel-Wissenschaft & Technologie*, *150*, 111931. https://doi.org/10.1016/j.lwt.2021.111931

Mampholo, B.M., Maboko, M.M., Soundy, P., & Sivakumar, D. (2016). Phytochemicals and overall quality of leafy lettuce (*Lactuca sativa* L.) varieties grown in closed hydroponic system. *Journal of Food Quality*, *39*(6), 805–815. https://doi.org/10.1111/jfq.12234

Martínez-Sánchez, A., Tudela, J.A., Luna, C., Allende, A., & Gil, M.I. (2011). Low oxygen levels and light exposure affect quality of fresh-cut Romaine lettuce. *Postharvest Biology and Technology*, *59*(1), 34-42. https://doi.org/j.postharvbio.2010.07.005

Matthew T.M., Fannie, Z., Yukiko K.N., & Stanley T.O. (2011). Comparison between hydroponically and conventionally and organically grown lettuces for taste, odor, visual quality and texture: A pilot study. *Food and Nutrition Sciences*, *2*(2), 4534. https://doi.org/10.4236/fns.2011.22017

Moriarty, M.J., Semmens, K., Bissonnette, G.K., & Jaczynski, J. (2018). Inactivation with UV-radiation and internalization assessment of coliforms and *Escherichia coli* in aquaponically grown lettuce. *Lebensmittel-Wissenschaft & Technologie*, *89*, 624-630. https://doi.org/10.1016/j.lwt.2017.11.038

Nuevaespana, J., & Matias, J.R. (2022, August 4). *Comparison of the physical profile of Klayton and LECA as media for aquaponics*. https://www.researchgate.net/profile/Jonathan-Matias/publication/261914267_Comparison_of_the_physical_profile_of_Klayton_and_LECA_as_media_for_aquaponics/links/0c960535f358c6f169000000/Comparison-of-the-physical-profile-of-Klayton-and-LECA-as-media-for-aquaponics.pdf

Ozgen, S., & Sekerci, S. (2011). Effect of leaf position on the distribution of phytochemicals and antioxidant capacity among green and red lettuce cultivars. *Spanish Journal of Agricultural Research*, 9(3), 801-809. https://doi.org/10.5424/sjar/20110903-472-10

Pedersen, P. B., von Ahnen, M., Fernandes, P., Naas, C., Pedersen, L. F., & Dalsgaard, J. (2017). Particle surface area and bacterial activity in recirculating aquaculture systems. *Aquacultural Engineering*, *78*, 18-23. https://doi.org/10.1016/j.aquaeng.2017.04.005

Petreska M., Ziberoski J. and Zekiri M., (2013). Fish feed microbiological status. *Journal of Hygienic Engineering and Design*, *4*, 16-19.

Predmore, A., Sanglay, G., Li, J., & Lee, K. (2015). Control of human norovirus surrogates in fresh foods by gaseous ozone and a proposed mechanism of inactivation. *Food Microbiology*, *50*, 118-125. https://doi.org/10.1016/j.fm.2015.04.004

Schmautz Z, Graber A, Jaenicke S, Goesmann A, Junge R, Smits THM (2017). Microbial diversity in different compartments of an aquaponics system. *Archives Microbiology*,*199*:613–620. https://doi.org/10.1007/s00203-016-1334-1

Schnabel, U., Handorf, O., Stachowiak, J., Boehm, D., Weit, C., Weihe, T., Thomas, W., Schäfer, J., Below, H., Bourke, P., & Ehlbeck, J. (2021). Plasma-functionalized water: from bench to prototype for fresh-cut lettuce. *Food Engineering Reviews*, *13*(1), 115-135. https://doi.org/10.1007/s12393-020-09238-9

Schröder, M.J. (2003). *Origins and Nature of Sensory and other Performance Attributes in Foods*. In Food Quality and Consumer Value (pp. 137-165). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-07283-7_6

Selma, M.V., Luna, M.C., Martínez-Sánchez, A., Tudela, J.A., Beltrán, D., Baixauli, C., & Gil, M.I. (2012). Sensory quality, bioactive constituents and microbiological quality of green and red fresh-cut lettuces (*Lactuca sativa* L.) are influenced by soil and soilless agricultural production systems. *Postharvest Biology and Technology*, *63*(1), 16-24. https://doi.org/10.1016/j.postharvbio.2011.08.002

Sirsat, S.A., & Neal, J.A. (2013). Microbial profile of soil-free versus in-soil grown lettuce and intervention methodologies to combat pathogen surrogates and spoilage microorganisms on lettuce. *Foods*, *2*(4), 488-498. https://doi.org/10.3390/foods2040488

Somerville, C., Cohen, M., Pantanella, E., Stankus, A., & Lovatelli, A., (2014). *Small-scale aquaponic food production: integrated fish and plant farming*. In: FAO fisheries and aquaculture technical paper food and agriculture organization of the United Nations, Rome, Italy, p 262.

Strawn, L.K., Gröhn, Y.T., Warchocki, S., Worobo, R.W., Bihn, E.A., & Wiedmann, M. (2013). Risk factors associated with salmonella and listeria monocytogenes contamination of produce fields. *Applied Environmental Microbiology*, *79*, 7618–7627. https://doi.org/10.1128/AEM.02831-13

Taylor, E.V., Nguyen, T.A., Machesky, K.D., Koch, E., Sotir, M.J., Bohm, S. R., Folster J.P., Bokanyı, R., Kupper, A., Bıdol, S.A., Emanuel, A., Arends, K.D., Johnson, S.A., Dunn, J., Stroıka, S., Patel, M.K., & Williams, I.

(2013). Multistate outbreak of Escherichia coli O145 infections associated with romaine lettuce consumption, 2010. *Journal of Food Protection*, *76*(6), 939-944. https://doi.org/10.4315/0362-028X.JFP-12-503

Tournas, V., Stack, M.E., Mislivec, P.B., Koch, H.A., & Bandler, R. (2022, August 4). *Bacteriological Analytical Manual Chapter 18: Yeasts, Molds and Mycotoxins*. https://www.fda.gov/food/laboratory-methods-food/bam-chapter-18-yeasts-molds-and-mycotoxins

Tyson, R.V., Danyluk, M.D., Simonne, E.H., & Treadwell, D.D. (2012). Aquaponics—Sustainable vegetable and fish co-production. *Proceedings of the Florida State Horticultural Society*, *125*, 381-385.

Weller, D.L., Saylor, L., & Turkon, P. (2020). Total coliform and generic E. coli levels, and Salmonella presence in eight experimental aquaponics and hydroponics systems: A brief report highlighting exploratory data. *Horticulturae*, *6*(3), 42. https://doi.org/10.3390/horticulturae6030042

Willmon, E. (2018). *Microbial Quality of Aquaculture Water Used for Produce Irrigation*. Master's thesis. The Graduate Faculty of Auburn University.

Yavuzcan Yildiz, H., Robaina, L., Pirhonen, J., Mente, E., Domínguez, D., & Parisi, G. (2017). Fish welfare in aquaponic systems: its relation to water quality with an emphasis on feed and faeces-a review. *Water*, *9*(1), 13. https://doi.org/10.3390/w9010013

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 35-42 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.05

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Determination of anti-cancer and antioxidant properties of protein extracts obtained from aquatic *Helophorus* (Coleoptera: Helophoridae) insects

## Sucul *Helophorus* (Coleoptera: Helophoridae) böceklerinden elde edilen protein ekstraktlarının anti-kanser ve antioksidan özelliklerinin belirlenmesi

**Tuba Elhazar[1] ● Bülent Kaya[2] ● Fatma Caf[3*]**

[1] Faculty of Sciences and Arts, Biology Department, University of Bingöl, 12000, Türkiye
[2] Faculty of Sciences and Arts, Molecular Biology and Genetics Department, University of Bingöl, 12000, Türkiye
[3] School of Food, Agriculture and Livestock, University of Bingöl, 12000, Türkiye

https://orcid.org/0000-0003-3632-1159
https://orcid.org/0000-0002-1216-6441
https://orcid.org/0000-0002-0363-4848

*Corresponding author: fcaf@bingol.edu.tr

Received date: 02.09.2022 Accepted date: 13.01.2023

**How to cite this paper:**
Elhazar, T., Kaya, B., & Caf, F. (2023). Determination of anti-cancer and antioxidant properties of protein extracts obtained from aquatic *Helophorus* (Coleoptera: Helophoridae) insects. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 35-42. https://doi.org/10.12714/egejfas.40.1.05

**Abstract:** In this study, protein extraction was performed from the aquatic insect *Helophorus aquaticus* (Linnaeus, 1758) and *Helophorus syriacus* (Kuwert, 1885) species belonging to the genus *Helophorus* (Coleoptera: Helophoridae). Then, these protein extracts were studied *in vitro*. These species were collected from the shallow parts of various streams, springs, creeks, ponds and hot springs from Bingöl city centre and its districts between May to June 2017. The protein amount was determined 34.78 mg/mL in the *H. aquaticus* and 35.14 mg/mL in the *H. syriacus*, after that he antioxidant capacity of protein extracts was examined. Metal chelating activity was determined as 90-88.5% and DPPH removal activity 53.19-61.7% for *H. aquaticus* and *H. syriacus* respectively. Protein samples belonging to both species were tested for cell vitality with WST-1 in PC-3 (prostate cancer) cells with *in vitro* cell culture. Upon examination of the test results, it has been found out that protein extracts from both of the studied species caused a decrease in cell inhibition. The highest cell inhibition was observed in samples with 1000 µg/mL insect protein extract added. In this study, protein expression providing apoptosis was examined with the Western blot technique after the effective dose was established. By looking at the proteins of Cyt-C and Caspase 3 with the Western blot technique, the efficacy of the protein extracts from both species was demonstrated effectively for the *in vitro* PC-3 line in non-apoptosis cell death. As a result of the study, insect proteins were shown to support the production of proteins that ensure cell death with the western blot technique.

**Keywords:** *Helophorus*, protein, Cyt-C, Caspase-3, antioxidant

**Öz:** Bu çalışmada, *Helophorus* (Coleoptera: Helophoridae) cinsine ait sucul böcek *Helophorus aquaticus* ve *Helophorus syriacus* türlerinden protein ekstraksiyonu yapılmıştır. Böcek türleri 2017 Mayıs-Haziran ayları arasında ve Bingöl il merkezi ve ilçelerinden çeşitli akarsu, kaynak, dere, birikinti ve sıcak su gözelerinin sığ kesimlerinden toplandı. Protein miktarı *H. aquaticus*'da 34,78 mg/mL, *H. syriacus*'da 35,14 mg/mL olarak belirlenmiştir. Daha sonra elde edilen protein ekstraktlarının antioksidan kapasitesi incelendi. Metal şelatlama aktivitesi *H. aquaticus* ve *H. syriacus* için sırasıyla %90-88,5, DPPH uzaklaştırma aktivitesi %53,19-61,7 olarak belirlendi. Her iki türe ait protein numuneleri, *in vitro* hücre kültürü ile PC-3 (prostat kanseri) hücrelerinde WST-1 ile hücre canlılığı açısından test edildi. Test sonuçlarının incelenmesi üzerine, çalışılan türlerin her ikisinden alınan protein ekstraktları, hücre inhibisyonunda bir azalmaya neden oldu. En yüksek hücre inhibisyonu, 1000 µg/mL böcek proteini ekstresi eklenen numunelerde gözlendi. Bu çalışmada, etkin doz belirlendikten sonra Western blot tekniği ile apoptozu sağlayan protein ekspresyonu incelenmiştir. Western blot tekniği ile Cyt-C ve Caspase-3 proteinlerine bakılarak, apoptoz dışı hücre ölümünde *in vitro* PC-3 hattı için her iki türden protein ekstraktlarının etkinliği etkili bir şekilde gösterildi. Çalışma sonucunda böcek proteinlerinin western blot tekniği ile hücre ölümünü sağlayan proteinlerin üretimini desteklediği gösterildi.

**Anahtar kelimeler:** *Helophorus*, protein, Cyt-C, Kaspaz-3, antioksidan

## INTRODUCTION

Insects represent 55% of the biological diversity on the earth (Chernysh et al., 2002). Members of this highly diversified group have spread to almost all ecosystems around the world. Insects' ability to thrive in various ecosystems is due to their extremely strong adaptability. Insects have a very important place in terms of ecological and economic life of people and other living groups (Koç et al., 2011).

Coleoptera, containing 170 families, is the largest group of insects and is represented by more than 350,000 species worldwide. Globally, Hydrophilinae, which is sub-coleoptera is represented by approximately 57 genera and 1784 species, mostly consisting of species living in wetlands (Mart, 2009; Fikáček et al., 2010). Hydrophilidae is distributed in Europe, Asia and North Africa with a total of 34 genera. In Türkiye, 19 genera, 95 species and 4 subspecies belonging to two subfamilies have been identified living in certain regions (Polat et al., 2021). Helophoridae is a family of aquatic beetle (Coleoptera) with a single genus (*Helophorus*) and an average of 200 species (Darılmaz, 2010). *Helophorus* species, in general aquatic or semi-aquatic, spread over a wide area from the peaks of high mountains all the way to sea level. Many species prefer stagnant and shallow waters rich in organic matter as their habitat, for example, the edges of small puddles. Furthermore, sandy or muddy areas between water and soil, as well as very vital areas with moss or other

vegetation elements, can also be selected as habitats by the aforementioned species (Yılmaz, 2011).

Insects are healthy and sustainable sources of high-quality protein. Entomophagia is encountered in more than 90 developing countries (Defoliart, 1995). Many insect species are used in traditional and folk medicine in various parts of the world. (Koç et al., 2019).

Some edible insects contain pharmacologically active substances, while others contain toxic metabolites produced for self-defence or other purposes, and still others contain chemical compounds such as alkaloids. Other insects can separate secondary metabolites from host plants for their own defence mechanisms and store them by converting to substances such as aristocolitic acid and glucosinolates (Duffey, 1980; Berenbaum, 1993; Blum, 1994).

Use of insects and insect-derived from products for therapeutic purposes is called "entomotherapy" (Costa-Neto, 2002). Entomotherapy is practiced in traditional folk medicine by various societies in numerous parts of the world. It prefers bees and wasps, ants, grasshoppers, termites, crickets, cockroaches, dung beetles and caterpillars. These are used to treat a variety of diseases, including upset stomach, skin diseases, epilepsy, asthma, bronchitis, rheumatism, and infertility (Costa-Neto, 2002). Most multi-protein complexes are key regulators in the cellular process. The sizes of these complexes can vary from only two or three components to multimeric complexes (Charbonnier et al., 2008; Doucet and Hetzer 2010; Riccio, 2010). Recombinant protein technologies form the fundamentals of not only a lot of research but also biological drugs (McKenzie and Abbott, 2018). Recombinant proteins represent the largest class of new therapeutic products developed by the biopharmaceutical industry (Stuible et al., 2018). Various studies showed that antioxidant and anti-inflammatory peptides have protective effects against reactive oxygen species and may contribute to a significant reduction in the level of oxidative stress, which is the main risk factor for diseases in civilization (Torres-Fuentes et al., 2011; Karaś et al., 2015).

The most widely consumed insect groups globally are in the order of Coleoptrea with a rate of 31%. The species belonging to the Helophoridae family which are used in traditional medicine and widely consumed in Central Asian and African countries have spread over a wide geography. These beetle species are potential sources of antioxidants like plants, seafood, and mushrooms, but are relatively unexplored (Van Huis, 2013). More taxonomic studies on these insect groups are being conducted in Türkiye. At the same point, important unsaturated fatty acid groups (palmitoleic acid and vaccenic acid) such as omega-7 were determined in these aquatic insects (Caf et al., 2020). In insect organisms, these bioactive compounds play a vital role against oxidative damage (Suh et al., 2010). Insects can also quickly resolve microbial infections by producing most immune-induced molecules, including antibacterial or antifungal peptides and polypeptides (Chernysh et al., 2002). Zielińska et al. (2018) concluded that edible insects were a valuable source of bioactive peptides with antioxidant and anti-inflammatory properties, and demonstrated that peptide fractions isolated from edible insect hydrolysates had high antioxidant and lipoxygenase and cyclooxygenase-2 inhibitory activities. Moreover, they reported that the heat treatment process had a significant effect on improving these properties, and twelve antioxidant and anti-inflammatory peptides were also identified in their study.

This study was conducted due to the lack of previous research on the antioxidant and anti-cancer properties of protein extracts from helophorid species. This was supported by the perception that these protein extracts might exhibit antioxidant and anticancer properties owing to the belief that these insects had high antimicrobial efficiencies due to their living habitat of aquatic ecosystems.

## MATERIALS AND METHODS

### Chemicals

Trypsin-EDTA, trypan-blue, WST-1 viability and spreading agent, penicillin streptomycin solution, foetal bovine serum (FBS), SDS, 10% trichloroacetic acid (TCA), Dulbecco's Modified Eagle Media (DMEM), 70% and 95% ethyl alcohol, methanol, dimethyl sulfoxide (DMSO), phosphate buffer saline (PBS), antibody (GABDH, Caspase-3 and CYT-C), sample buffer, running buffer, transfer buffer, marker, 12% gel, RIPA lysis buffer, nitrocellulose membrane, blotting paper, skimmed milk powder, secondary antibody, ECL, peroxide solution, TBS, Tween20, Ac./Bis, 10% APS, TEMED, Tris-Cl, NaCl, KCl. Thoma slide, 25 cm and 75 cm flasks, serological pipettes of various sizes, 96-well plates, 15 mL centrifuge tubes, 50 mL centrifuge tubes, Eppendorf tubes of various sizes, automatic pipettes of various sizes, and homogenizer (Cell disruption tube).

The materials used comprised the following items and trans blot was completed; microbiological safety cabin, heated water bath, automatic pipettes, freezer, mortar, vortex, sonicator, precision scales, refrigerator (+ 4 ºC), autoclave, pure water device, oven 5% $CO_2$ incubator, vertical and horizontal electrophoresis and gel imaging.

### Preparation of Protein Extract

Insect species were collected from Bingöl province and identified by Prof. Dr. Abdullah Mart. *Helophorus syriacus* and *Helophorus aquaticus* species stored at -80 °C were weighed to 2 g, and were thoroughly disintegrated in a homogenizer by adding 4 mL 10% TCA after being placed in flacons. Subsequently, another 1 mL of 10% TCA was added, and cooled in a cooled centrifuge for 50 minutes at 4,000 RPM. The supernatant portion was removed and the pellet portion was rotated at 3,500 RPM for 10 minutes with 3 repetitions, washed with 5 mL of 95% ethyl alcohol and kept in an oven at 37 °C for drying. The dried sample was properly crushed in a

mortar and 2 mL of purified water was poured on it. The sample was then incubated in an oven at 37 °C for 15 minutes and centrifuged at 4,500 RPM for 15 minutes. Then 100 µL of the sample was used for Bradford. At this point, 2 mL of 95% ethyl alcohol was added to the remaining sample, and this was left at 37 °C for 1 night. The following day, it was centrifuged at 4,500 RPM for 1 hour and left at 37 °C to completely removed alcohol and dried the protein extracts. The precipitate, which alcohol was removed, was poured into a tube with a thin spatula and pulverized into powder (Coşkun et al., 2009).

**Total Protein Determination with Bradford Method**

This method (5-100 µL) is based on the interaction of basic and acidic groups from proteins to form organic dyes. The protein's amino acid composition is important in the formation of blue colour. The dye has a strong affinity for basic amino acids like arginine and certain aromatic amino acids. The main phenomenon in this method is that while the dye has the highest absorbance at 465 nm under normal conditions, it has the highest absorbance at 595 nm wavelength when bound to protein. A standard calibration graph was prepared with bovine serum albumin (BSA) solution (Figure 1).

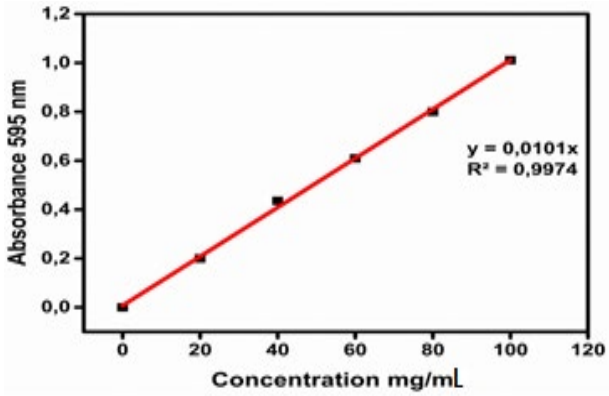



**Figure 2.** Bradford standard calibration chart for protein determination

The previously prepared insect samples, stored at +4 °C, were measured with the UV-VIS spectrophotometer (Shimadzu/Jasco V650). Protein amounts of the samples were calculated by placing the read absorbances in the regression equation. The absorbance value read for *Helophorus syriacus* at 595 nm was 0.3478. The total protein amount was 34.78 mg/mL after incorporating the absorbance into the regression equation. At 595 nm, the absorbance value for *Helophorus aquaticus* was 0.3514. Using the regression equation and the absorbance, the total protein amount was calculated to be 35.14 mg/mL.

**Determination of DPPH Activity**

First, 2.7 mL of methanolic solution containing DPPH radical prepared to 6x10 $^{-5}$ mol per litre was mixed into the insect protein extract (0.3 mL) prepared at a concentration of 1 mg/mL. This mixture was strongly mixed and kept in a dark place for 60 minutes. Removal of DPPH radical activity was determined by measuring the absorption with the spectrophotometer at 517 nm. Efforts to remove this radical were performed by following the method proposed by various researchers (Hatano et al., 1988). BHT was used as a positive control for the DPPH test.

**Determination of Metal Chelating Activity**

The metal chelating activity was examined through the iron chelating feature. Determination of the properties according to this method in brief, began by adding 1.6 mL of deionized water and 0.05 mL of 2 mM $FeCl_2$ to each 0.5 mL extract. After 30 seconds, 0.1 mL of 5 mM ferrozine was added. Ferrozine became very soluble in water after reactions with bivalent iron. Subsequently, the absorbance of the $Fe^{2+}$ ferrozine complex was measured at 562 nm for 10 minutes at room temperature. Here, EDTA was used as a standard chelator, and the chelating activity was expressed according to the EDTA standard. Thus, the chelating activity of iron from the extract was calculated using the formula below (Wenli et al., 2004).

% Chelation Rate = $(A_0 - A_1)/ A_0 \times 100$;

$\mathbf{A_1}$ is the absorbance value measured in the presence of the extract, and $\mathbf{A_0}$ is the control or blind absorbance.

**Cell Vitality Analysis with WST-1**

In this study, the PC-3 cell line was utilized. The PC-3 cell line was procured from the Molecular Biology and Genetics department of Bingol University. Firstly, 500 mL of DMEM, 50 mL of foetal bovine serum, and 5 mL of penicillin-streptomycin medium were mixed in the medium container to grow these cells. PC-3 cells stored at -80 °C were propagated in DMEM growth medium, passaged at 37 °C in a 5% $CO_2$ oven every 2 or 3 days. The proliferated cells were examined with a reverse microscope in a 96-well plate. After establishing that the cells were proliferating sufficiently, insect protein extract concentration in the range of 5-1000 µg/mL was prepared and added to each well with 3 repetitions and incubated for 48 hours. PC-3 cells were seeded in 96 wells and incubated. Insect protein extracts (3.125, 6.25, 12.5, 25, 50 and 100 mg/mL) were diluted with cell culture medium, treated with cells and incubated for 48 hours. Only medium + cells were used as the control group. After 48 hours, 15 µl of WST-1 was added to each well. Cells were incubated at 37 °C for 4 hours in an incubator with 5% $CO_2$. After 4 hours of incubation, the 96-well plate was placed on an ELISA reader and absorbance values for each well were recorded at 450-630 nm.

Colour formation of cells and dead cells in the WST-1 toxicity test were spectrophotometrically measured at 450 nm, and the presence of inactive WST-1 was measured at 630 nm. Absorbance values were plotted on a graph.

### Analysis of Target Proteins with the Western Blot Technique

PC-3 cells were grown in 75 $cm^2$ flasks to reach 3-4x$10^6$, and treated with protein extracts obtained from the insects at a concentration of 1 mg/mL, after which they were prepared for protein isolation by washing with PBS and centrifuging. Around 3-4x$10^6$ cells were homogenized in a cold environment with the aid of a protein isolation kit at a ratio of 1:5 (w/v). In order to prevent the proteins from degrading due to protease activity, both protease inhibitor cocktail (PIC) and PMSF were used during homogenization processes and all processes were performed on ice. The supernatants were placed in microcentrifuge tubes after centrifuging the homogenates in a cooled centrifuge at 14.000 RPM for 20 minutes at +4 °C were placed in microcentrifuge tubes. The Bradford method was used to determine the amount of protein in each sample. Samples were then stored at –80 °C, until Western blotting experiments were carried out. Protein lysates from the cell culture were processed with the 12% SDS-PAGE (Sodium dodecyl sulphate polyacrylamide gel electrophoresis) technique in concentration gel; then, caspase 3, cytochrome C and GABDH were used as housekeeping and transferred to the PVDF membrane. The blotting sequence with 5% BSA for 1 hour began. Subsequently, the bands of the proteins fixed on the membrane were incubated for 3 hours with suitable primary antibodies and washed with TBST (Tris Buffer Saline, 0.1% Tween 20 ) for 5x5 minutes, and incubated for 1.5 hours with the secondary suitable for the primary. Then, washing was done with 5x5 min TBS-T. The membrane was then incubated with ECL buffer for about 3-4 minutes and thanks to the radiation on the membrane, protein bands were fixed on X-Ray films in the medical X-Ray image stabilization device. Then, the synthesis quantity of these tapes was calculated using computerized software (Image Lab, Bio Rad). The calculation method normalized the target genes with the housekeeping used in the gene GABDH and changed the percentage based on the control.

## RESULTS AND DISCUSSION

Natural antioxidant endogenous compounds play an important role in most living organisms' innate host defence mechanisms of, including plants, insects, amphibians and mammals (Koczulla et al., 2003). Insects are a significant source of potential be considered as a serious antioxidant and antimicrobial agents because they contain more antimicrobial and antioxidant compounds depending on the environment in which they live.

Due to the rapid resistance of microorganisms to existing antibiotics, insects are being considered as a potential source of new antibiotic agents. (Mittapalli et al., 2007). Compounds with antioxidant effects can be found in edible insect species, or antioxidant effects can be achieved with bioactive compounds obtained from these sources, for oxidant compounds, which human beings face more due to factors such as industrial development of and rapid eating habits, There are studies to determine the antioxidant activities of insect proteins (Liu et al., 2012; Suh et al., 2010; Zielińska et al., 2017; Zielińska et al., 2018). Peptide fractions formed as a result of enzymatic hydrolysis of edible insect species reduced the concentration of free radicals that cause oxidative stress. DPPH and metal chelation are assays commonly used to determine antioxidant activity. Figures 2 and 3 show the DPPH and metal chelation results for insect protein.

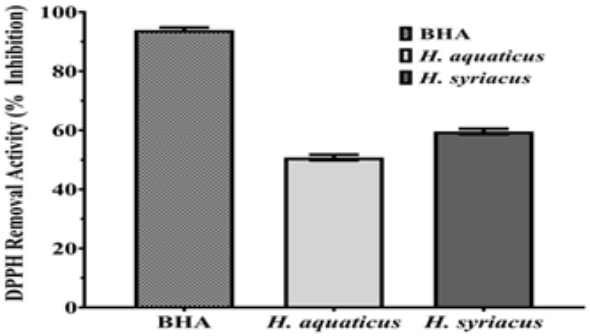



**Figure 2.** DPPH removal activity of insect protein

When DPPH removal activity is examined and species are compared according to the absorbance value of DPPH, it was close to 62% for *H. syriacus* and 48% for *H. aquatic*. This result shows that the protein extracts from the species have the capacity to remove DPPH oxidant**.** In a study investigating the *in vitro* antioxidant effect of water and oil-soluble extracts from edible insects, grasshopper, silkworm and cricket had antioxidant capacity 5 times higher than fresh orange juice (Di Mattia et al., 2019). The DPPH activity of *Allomyrina dichotoma* insect larvae was examined in different solvent media and the best activity was obtained in ethanol medium (Suh et al., 2010).

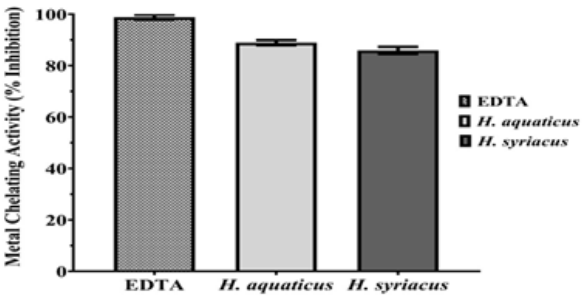



**Figure 3.** Metal chelating activity

Insects are constanty exposed to microbial infections, pathogens and oxidative stress on a daily basis. Antioxidant and antimicrobial products, in particular, play important roles in defence mechanisms in order to protect themselves against environmental attacks (Bulet et al., 2004). One of the ways to determine the antioxidant property of insects is with the metal chelating property. In this study, Metal chelating activity was examined through the iron-chelating feature. When metal

chelating activity is evaluated, it has been seen that around 90% of metal ions in the environment were removed with the metal chelation activity of *H. aquaticus* while *H. syriacus* removed about 89%. Studies on iron-metal chelation of the beetles extracts and insect protein lysates are limited. In a study determining the metal chelating activity of proteins from three edible insect species, insect proteins had iron chelating properties (Zieliǹska et al., 2018). In another study, silkworm protein hydrolysates were reported to have high iron chelation capacity ($IC_{50}$ 2.03 mg/mL) (Wu et al., 2011). These beetles groups lived in swamps have developed their defence systems. The redox properties of phenolics and polyphenolic compounds are primarily responsible for their antioxidant activities (Gil et al., 2000). It is thought that proteins in insects may be responsible for the antioxidant activity of phenolic and polyphenolic compounds. Because of phenols are one of the important compounds as antioxidant (Suh et al., 2010).

Studies show that insect protein extracts can inhibit or promote cell proliferation. Protein hydrolysates can be administered as bioactive components that suppress inflammation, regulate the synthesis of extracellular matrix proteins, or stimulate the proliferation of skin cells (Zielińska et al., 2015). Peptides with antimicrobial effect are also isolated by hydrolysis of proteins contained in edible insects (Jantzen da Silva Lucas et al., 2020). As shown in Figure 4, the addition of insect hydrolysates is observed to significantly inhibit PC-3 cell proliferation. This effect occurs depending on the type of insect protein hydrolysate samples and their handling. Both insect protein hydrolysates showed the highest cytotoxic effect at 1000 µg/mL. In the study of cytotoxicity for human skin fibroblast, both stimulant and inhibitory effects were observed in a study with different insect groups. The presence of insect hydrolysates from *T. molitor* and G. *sigillatus* stimulated the growth of human skin fibroblasts, while *S. gregaria* protein hydrolysate showed cytotoxic effects (Zielińska et al., 2015). The highest inhibition by *H. aquaticus* and *H. syriacus* was seen at 1000 µg/mL. Subsequently, inhibition was observed at concentrations of 500 µg/mL, 250 µg/mL, and 62.5 µg/mL, in order. As a result of this study, 1000 µg/mL was determined to be the most effective concentration when all studies were compared.

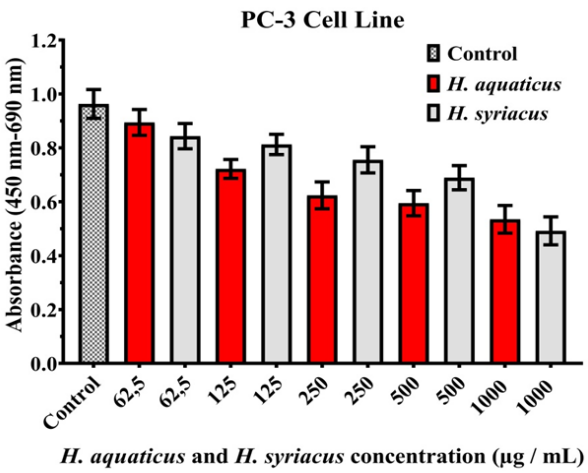



**Figure 4.** WST-1 viability test analysis showing the effect of different concentrations of *H. aquaticus* and *H. syriacus* protein extracts on cell inhibition. No agent was applied to control cells and only growth medium was added. Statistical analysis was performed with one-way ANOVA using the Dunnett's multiple comparison test as a post-test in the confidence interval of $p < 0.01$. Values are expressed as mean ± SEM (n = 3)

### Analysis of Target Proteins with the Western Blot Technique

The antioxidant mechanisms influenced by peptide fractions formed as a result of insect protein hydrolysis are still unknown. The Western Blot technique was used to examine protein products that cause cell death. In this study, the amount of protein was compared in *H. aquaticus* and *H. syriacus* in accordance with the control group. GAPDH was utilized as housekeeping protein. Compared to this protein, Cyt-C and Cas-3 ratios were evaluated, which provide information about cell inhibition and cell death. As stated in Figure 5, Cyt-C levels were determined in both types compared to the control group. Cas-3 activity was also determined in both types.

The tendency for apoptosis in cells is followed by the infiltration of cytochrome-c into the cytoplasm by disruption of mitochondrial outer membrane permeability (MOMP), followed by activation of caspases. Caspases mediate the formation of apoptosis, but inhibiting caspases does not solve the problem of cell survival after the formation of MOMPs. In this case, "caspase-independent cell death" (CICD) occurs in cells.

Therefore, the disruption of mitochondrial outer membrane permeability, namely MOMP, and the release of Cyt-C may represent the main entry point to cell death. Increases in the amount of Cyt-C and leakage from mitochondria cause the cell to enter the apoptosis pathway (Colell et al., 2007).

Caspases are the most critical mediates in the formation of apoptosis. Within the caspase family, caspase-3 is the protease within the caspase family that catalyses apoptosis by stimulating the specific cleavage of multiple important cellular proteins and activates the most fundamental step of apoptosis, which is frequently activated. However, when the mechanism of apoptosis is studied, the specific requirements of a member of the caspase family are not known as a definitive diagnosis until now. Looking at the pathways leading to caspase-3 activity, they were identified as dependent on or independent of mitochondrial cytochrome-c release and caspase-9 function. Caspase-3 is important in other apoptotic scenarios, as it is required for normal brain development and exhibits remarkable activity specific to cell type, tissue, or death stimulus. However, caspase-3 is required in the presence of typical markers of apoptosis. It is essential for the condensation of apoptotic chromatin and

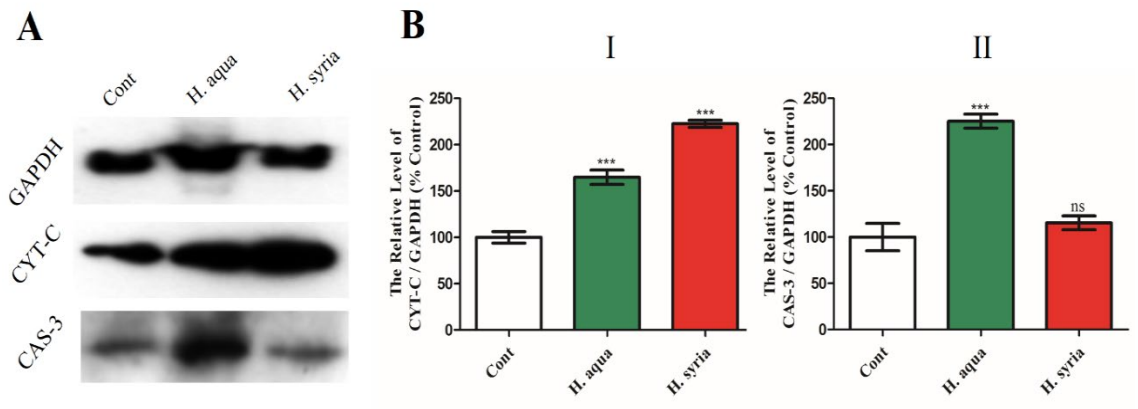



**Figure 5.** Effects of protein extracts on apoptosis-related protein expression in PC-3 cells. **(A)** Cytochrome-*c* (15 kDa) and caspase-3 protein levels were measured after western blotting. GAPDH was used as the loading control. **(B)** Data are shown as mean ± SEM (n=3). $p$ <0.01(**) Control vs protein extracts.

subsequent DNA fragmentation in most cell types. In the light of these evaluations, caspase-3 is a necessity for its presence in the cell and subsequent formation of apoptotic bodies and the formation of pathways (Porter and Jänicke, 1999).

As seen from the graph, the ratio of Cyt-C to GAPDH is higher compared to the control group for *H. aquaticus*. This difference indicates that Cyt-C is released in high amounts when compared to the control group (i.e. cells replicated under the same conditions but without insect protein added). When we look at Cyt-C/GAPDH rate, it has been seen that there is a high Cyt-C release. Hence, it has induced the death of cell because of increasing the permeability of mitochondrial outer membranes and explained the setting of apoptosis. When the GAPDH ratio of the caspases is examined, it was also higher than the control group. This difference indicates that the amount of caspase-3 is higher than the control group. This excess shows the high amount and presence of precursor caspase-3 activity that leads to cell death; thus, showing that *H. aquaticus* proteins direct the cell toward cell death (Colell et al., 2007).

In *H. syriacus*, the ratio of Cyt-C to GAPDH is approximately 2.25 times higher than that of the control Cyt-C to GAPDH. This difference indicates that Cyt-C is released more and is more effective for *H. aquaticus* compared to the control group (i.e., cells replicated at the same time under the same conditions without insect protein added). However, when evaluated in terms of caspase-3compared to *H. aquaticus*, the amount of Caspase-3 was lower compared to the cell vitality test, and a different pathway supports cell death. As there is no study planned around the project, DNA fragmentation should be supported by methods such as real time PCR for definitive proof. However, the budget and scope of this study did not cover this.

These results show that when the data for both types studied are evaluated, the WST-1 cell vitality test showed that PC-3 cancer cell line had a higher level of cellular death than the control group. Attempts were made to explain how cell death occurs via the western blot test. The cause of cell death was also observed at the protein level by examining Cyt-C and Caspase-3 activities. In this study, when *H. aquaticus* and *H. syriacus*, which live in the aquatic environment, are regarded as the content of antioxidants, they have high antioxidant capacity. Similarly, they displayed activity on PC-3 (human prostate cancer) *in vitro* cell vitality, and the vitality of cancer cells diminished with the increased concentrations and increasing activity (62.5-1000 µg/mL). *H. aquaticus* and *H. syriacus* extracts may play an important role in ROS scavenging against oxidative stress. Given the high consumer demand for beneficial health effects, these groups of insects can be used to develop functional food, health promoting and pharmaceutical agents.

## CONCLUSION

It is important for sustainability of the ecological system that insects consume much less water and produce fewer greenhouse gases compared to other animals. Insects are economical sources that can be used as an alternative to animal protein, especially when produced on an industrial scale. At the same time, as a good source of protein and antioxidants, they may be an alternative food that can eliminate anxiety about food shortages in the future.

This study is a first step towards determining the protein content of *Helophorus* (Coleoptera: Helophoridae), which is abundant in Türkiye, as an alternative to prevent anxiety about finding nutrients that may occur in the future. These proteins are also antioxidant and have a positive effect on cancer cells. Investigation of PC-3 cell line activity, antioxidant activity and apoptosis function of protein extracts from *H. syriacus*, and *H. aquaticus* may be an important step in this area. Further molecular and *in vivo* studies are needed on this beetle extracts.

**Acknowledgements and Funding**

This study was supported by the project numbered BAP-FEF.2017.00.003 of Bingöl University Scientific Research Projects Unit (BÜBAP). We thank Abdullah Mart for the identification of the species. We would also like to thank Ekrem Darendelioğlu for his help and laboratory for the Western Blot part of the study.

**Authorship Contributions**

This study is a part of Master Thesis by Tuba ELHAZAR (2019). Tuba Elhazar carried out the collection and storage conditions of the samples from the field for this study. Tuba Elhazar and Bülent Kaya performed the anticancer, antioxidant, and Western Blot. The evaluation of the study's data and the article's writing was done by Bülent Kaya and Fatma Caf.

**Conflict Of Interest**

There is no conflict of interest in this study

**Ethical Approval**

No specific ethical approval was required for this study.

**Data Availability**

All relevant data is inside the article

## REFERENCES

Berenbaum, M.R. (1993). Sequestered plant toxins and insect palatability. *The Food Insects Newsletter*, *6*(3), 6-9.

Blum, M.S., 1994. The limits of entomophagy: a discretionary gourmand in a world of toxic Insects. *The Food Insects Newsletter, 7*(1), 6-11.

Bulet, P., Stöcklin, R., & Menin. L. (2004). Anti-microbial peptides: from invertebrates to vertebrates. *Immunological Reviews, 198*, 169-184. https://doi.org/10.1111/j.0105-2896.2004.0124.x

Caf, F., Yildiz, G., Özdemir, N.S., & Mart A. (2020). A chemotaxonomic approach to fatty acid composition of the genera *Helochares* Mulsant, 1844 and *Coelostoma* Brullé, 1835 (Coleoptera: Hydrophilidae). *Turkish Journal of Entomology, 44*(3), 399-412. https://doi.org/10.16970/entoted.657190

Chernysh, S., Kim, S.I., Bekker, G., Pleskach, V.A., Filatova, N.A., Anikin, V.B., & Bulet, P. (2002). Antiviral and antitumor peptides from insects. *Proceedings of the National Academy of Sciences, 99*(20), 12628-12632. https://doi.org/10.1073/pnas.192301899

Charbonnier, S., Gallego, O., & Gavin, A.C. (2008). The social network of a cell: Recent advances in interactome mapping. *Biotechnology Annual Review*, *14*, 1-28. https://doi.org/10.1016/S1387-2656(08)00001-X

Colell, A., Ricci, J.E., Tait S., Milasta, S., Maurer, U., Bouchier-Hayes, L., Fitzgerald, P., Guio-Carrion, A., Waterhouse, N.J., Li, C.W., Mari, B., Barbry, P., Newmeyer, D.D., Beere, H.M., & Green D.R. (2007). GAPDH and autophagy preserve survival after apoptotic cytochrome c release in the absence of caspase activation. *Cell, 129*(5), 983-997. https://doi.org/10.1016/j.cell.2007.03.045

Costa-Neto, E.M. (2002). The use of insects folk medicine in the State of Bahai, Northeastern Brazil, with notes on insects reported elsewhere in Brazilian folk medicine. *Human Ecology, 30*(2), 254-263. https://doi.org/10.1023/A:1015696830997

Coşkun, M., Kayis, T., Ozalp, P., Kocalar, K., Tatlicioğlu, C. I., & Emre, I. (2009). The effects of a meridic diet on the sex ratio of offspring, on glycogen and protein content, and on productivity and longevity of adult *Pimpla turionellae* (Hymenoptera: Ichneumonidae) for five generations. *Belgian Journal of Zoology*, *139*(2), 103-108.

Darılmaz, M. (2010). Investigation of Inner West Anatolia Aquatic Coleoptera Fauna. Doctoral dissertation, Gazi University Institute of Science and Technology, Turkey (in Turkish)

da Silva Lucas, A. J., de Oliveira, L. M., da Rocha, M., & Prentice, C. (2020). Edible insects: an alternative of nutritional, functional and bioactive compounds. *Food Chemistry*, *311*, 126022. https://doi.org/10.1016/j.foodchem.2019.126022

Defoliart, G.R. (1995). Edible insects as minilivestock. *Biodiversity and Conservation*, *4*, 306-321. https://doi.org/10.1007/BF00055976

Di Mattia, C., Battista, N., Sacchetti, G., & Serafini, M. (2019). Antioxidant activities in vitro of water and liposoluble extracts obtained by different species of edible insects and invertebrates. *Frontiers in Nutrition*, *6*, (106). https://doi.org/10.3389/fnut.2019.00106

Doucet, C.M., & Hetzer, M.W. (2010). Nuclear pore biogenesis into an intact nuclear envelope. *Choromosoma*, *119*, 469-477. https://doi.org/10.1007/s00412-010-0289-2

Duffey, S.S. (1980). Sequestration of plant natural products by insects. *Annual Review of Entomology*, *25*, 447-477. https://doi.org/10.1146/annurev.en.25.010180.002311

Fikáček M., Schmied H., & Prokop, J. (2010). Fossil hydrophilid beetles (Coleoptera: Hydrophilidae) of the Late Oligocene Rott Formation (Germany). *Acta Geologica Sinica 84* (4), 732-750. https://doi.org/10.1111/j.1755-6724.2010.00239.x

Gil, M.I., Tomás-Barberán, F.A., Hess-Pierce, B., Holcroft, D.M., & Kader, A.A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry 48*, 4581-4589. https://doi.org/10.1021/jf000404a

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects, *Chemical and Pharmaceutical Bulletin, 36*(6), 2090-2097. https://doi.org/10.1248/cpb.36.2090

Jantzen da Silva Lucas, A., Menegon de Oliveira, L., da Rocha, M., & Prentice, C. (2020). Edible insects: An alternative of nutritional, functional and bioactive compounds. *Food Chemistry, 311*, 126022. https://doi.org/10.1016/j.foodchem.2019.126022

Karaś, M., Baraniak, B., Rybczynska, K., Gminski, J., Gawel- Bezben, K., & Jakubczyk, A. (2015). The influence of heat treatment of chickpea seeds on antioxidant and fibroblast growth-stimulating activity of peptide fractions obtained from proteins digested under simulated gastrointestinal conditions. *International Journal of Food Science & Technology*, *50*, 2097-2103. https://doi.org/10.1111/ijfs.12872

Koczulla, A.R., & Bals, R. (2003). Antimicrobial peptides-current status and therapeutic potentials. *Drugs, 63*, 389-406. https://doi.org/10.2165/00003495-200363040-00005

Koç, K. (2011). Investigation of In vitro Genotoxic and Oxidative Effects of Some Edible Insects, Master Thesis, Atatürk University, Institute of Science, Erzurum (in Turkish)

Koç, K., İncekara, Ü., Türkez, H., & Çelik, K. (2019). *In vitro* Assessment of Genotoxic And Oxidative Effects Potentials Of Edible Bamboo Worms And Weaver Ants. *Munis Entomology and Zoology Journal*, *14*(2), 496-501.

Liu S., Sun J., Yu L., Zhang C., Bi J., Zhu F., Qu M., & Yang Q. (2012). Antioxidant activity and phenolic compounds of *Holotrichia parallela* Motschulsky extracts. *Food Chemistry 134*, 1885-1891. https://doi.org/10.1016/j.foodchem.2012.03.091

Mart, A. (2009). Water scavenger beetles (Coleoptera: Hydrophilidae) provinces of Central Black Sea region of Turkey. *Journal of The Entomological Reserch Society*, *11*(1), 47-70.

Mittapalli, O., Neal, J.J., & Shukle, R.H. (2007). Antioxidant defense response in a galling insect. *Proceedings of the National Academy of Sciences, 104*(6), 1889-1894. https://doi.org/10.1073/pnas.0604722104

McKenzie, E.A., & Abbott, W.M. (2018). Expression of recombinant proteins in insect and mammalian cells. *Methods, 147*, 40-49. https://doi.org/10.1016/j.ymeth.2018.05.013

Polat, A., Darılmaz, M.C. & İncekara, Ü. (2021). An annotated checklist of the Hydrophiloidea (Coleoptera) of Turkey. *Munis Entomology & Zoology, 16* (1), 151-178.

Porter, A.G., & Jänicke, R.U. (1999). Emerging roles of caspase-3 in apoptosis. *Cell Death and Differentiation*, *6*, 99-145. https://doi.org/10.1038/sj.cdd.4400476

Riccio, A. (2010). Dynamic epigenetic regulation in neurons: enzymes, stimuli and signaling pathways. *Nature neuroscience*, *13*, 1330-1337. https://doi.org/10.1038/nn.2671

Stuible, M., Burlacu, A., Perret, S., Brochu, D., Paul-Roc, B., Baardsnes, J., Loignon, M., Grazzini, E., & Durocher, Y. (2018). Optimization of a high-cell-density polyethylenimine trasfection method for rapid protein production in CHO-EBNA1 cells. *Journal of Biotechnology, 281*, 39-47. https://doi.org/10.1016/j.jbiotec.2018.06.307

Suh, H. J., Kim, S. R., Lee, K. S., Park, S., & Kang, S.C. (2010). Antioxidant activity of various solvent extracts from *Allomyrina dichotoma* (Arthropoda: Insecta) larvae. *Journal of Photochemistry and Photobiology B: Biology*, *99*(2), 67-73. https://doi.org/10.1016/j.jphotobiol.2010.02.005

Torres-Fuentes, C., Alaiz, M., & Vioque, J. (2011). Affinity purification and characterisation of chelating peptides from chickpea protein hydrolysates. *Food Chemistry*, *129*, 485-490. https://doi.org/10.1016/j.foodchem.2011.04.103

Yılmaz, A. (2011). Faunistic and Systematic Investigation of Helophoridae, Hydrophilidae (Coleoptera) Species in Ista Province, Master Thesis, Süleyman Demirel University, Institute of Science, Isparta (in Turkish)

Van Huis, A. (2013). Potential of insects as food and feed in assuring food security. Annual review of entomology, 58, 563-583. https://doi.org/10.1146/annurev-ento-120811-153704

Wenli, Y., Yaping, Z., & Bo, S, (2004). The radical scavenging activities of *radix puerariae* isoflavonoids: A chemiluminescence study. *Food Chemistry*, *86*(4), 525-529. https://doi.org/10.1016/j.foodchem.2003.09.005

Wu, Q.Y., Jia, J.Q., Tan, G.X., Xu, J.L., & Gui, Z.Z. (2011). Physicochemical properties of silkworm larvae protein isolate and gastrointestinal hydrolysate bioactivities. *African Journal of Biotechnology*, *10*, 6145-6153.

Zielińska, E., Baraniak, B., Karas, M., Rybczynska, K., & Jakubczyk, A. (2015). Selected species of edible insects as a source of nutrient composition. *Food Research International*, *77*, 460-466. https://doi.org/10.1016/j.foodres.2015.09.008

Zielińska, E., Karaś, M., & Jakubczyk, A. (2017). Antioxidant activity of predigested protein obtained from a range of farmed edible insects. *International Journal of Food Science & Technology, 52*, 306-312. https://doi.org/10.1111/ijfs.13282

Zielińska, E., Karaś, M., & Baraniak, B. (2018). Comparison of functional properties of edible insects and protein preparations thereof. *LWT - Food Science and Technology*, *91*, 168-174. https://doi.org/10.1016/j.lwt.2018.01.058

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 43-50 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.06

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# A preliminary investigation of the effects of surface waters of the Bakırçay River on the growth of green algae *Scenedesmus dimorphus*

## Bakırçay nehri yüzey sularının *Scenedesmus dimorphus* yeşil algi gelişimi üzerine etkileri hakkında bir ön çalışma

**Meltem Boyacıoğlu[1]* ● Cem Aygen[2] ● Muhammet Ali Karaaslan[3] ● Didem Özdemir Mis[4] ● Özlem Çakal Arslan[5]**

[1] Ege Üniversitesi, Su Ürünleri Fakültesi, Temel Bilimler Bölümü, 35100 Bornova, İzmir, Türkiye https://orcid.org/0000-0002-9871-6132
[2] Ege Üniversitesi, Su Ürünleri Fakültesi, Temel Bilimler Bölümü, 35100 Bornova, İzmir, Türkiye https://orcid.org/0000-0002-6351-0409
[3] Ege Üniversitesi, Su Ürünleri Fakültesi, Temel Bilimler Bölümü, 35100 Bornova, İzmir, Türkiye https://orcid.org/0000-0003-3737-2361
[4] Ege Üniversitesi, Su Ürünleri Fakültesi, Temel Bilimler Bölümü, 35100 Bornova, İzmir, Türkiye https://orcid.org/0000-0002-9962-6577
[5] Ege Üniversitesi, Su Ürünleri Fakültesi, Temel Bilimler Bölümü, 35100 Bornova, İzmir, Türkiye https://orcid.org/0000-0002-3883-9317

*Corresponding author: meltem.boyacioglu@ege.edu.tr
Received date: 04.07.2022 Accepted date: 26.01.2023

***How to cite this paper:***
Boyacıoğlu, M., Aygen, C., Karaaslan, M.A., Özdemir Mis, D., & Çakal Arslan, Ö. (2023). A preliminary investigation of the effects of surface waters of the Bakırçay River on the growth of green algae *Scenedesmus dimorphus*. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 43-50. https://doi.org/10.12714/egejfas.40.1.06

**Abstract:** Bakırçay River, one of the important rivers of the Aegean region, has a length of 129 km and an approximate catchment area of 3160 km$^2$. Bakırçay River, which carries agricultural drainage and polluted wastewater with high nitrogen and phosphorus content, is polluted due to domestic and agricultural resources. The water of the Bakırçay River is used for irrigation in agriculture and fishing from the dam lakes on the river for feeding purposes also negatively affects the health of the people in the vicinity. In the Bakırçay River, there is widespread pollution caused by mining areas as well as domestic, industrial and agricultural pollution. For this purpose, the "Algal Growth Inhibition Test, OECD 201" was applied to the water samples obtained from 10 stations on the Bakırçay River. *Scenedesmus dimorphus* (Turpin) Kützing, 1834 green algae culture, accepted as the primary trophic level representative for the " Algal Growth Inhibition Test, OECD 201" test, one of the short-term phytotoxicity test methods, was used in this test. Water samples were tested at five different dilutions (40, 60, 80, 100 %). To determine the effects on the growth of *Scenedesmus dimorphus*, the cells were counted by fluorimeter (Turner design) daily at the same time and the test duration was 72 hours. In conclusion, the highest level of toxicity was found at Stations 1, 9, 10,13,14, and 15. The reason for this was that there was a coal facility near Station 9 and that all branches of the Bakırçay River joined near Station15 and then drained into Çandarlı Bay.

**Keywords:** Bakırçay River, algal growth inhibition test, pollution, freshwater, *Scenedesmus dimorphus*

**Öz:** Ege bölgesinin önemli akarsularından biri olan Bakırçay'ın uzunluğu 129 km ve yaklaşık havza alanı 3160 km$^2$'dir. Tarımsal drenaj ve yüksek azot ve fosfor içerikli kirli atık suları taşıyan Bakırçay, evsel ve tarımsal atıklara bağlı olarak sürekli kirlilik yükü altında kalmaktadır. Bakırçay Deresi'nin suyu tarımsal olarak sulama amacıyla kullanılmakta ve Bakırçay'ın üzerindeki baraj göllerinden beslenme amacıyla balık avlanması Bakırçay çevresinde yaşayan halkın sağlığını olumsuz etkileyerek halk sağlığı açısından risk oluşturmaktadır. Bakırçay'da evsel, endüstriyel ve tarımsal kirliliğin yanı sıra maden alanlarının neden olduğu yaygın kirlilik de söz konusudur. Bu amaçla, Bakırçay Nehri üzerinde bulunan 10 istasyondan temin edilen su örneklerine "Algal Büyüme İnhibisyon Testi, OECD 201" uygulanmıştır. Kısa zamanlı fitotoksisite test yöntemlerinden olan "Algal Büyüme İnhibisyon Testi, OECD 201" testi için birincil trofik seviye temsilcisi olarak kabul edilen *Scenedesmus dimorphus* (Turpin) Kützing, 1834 yeşil alg kültürü bu testte kullanılmıştır. Su örnekleri beş farklı dilusyonda (% 40, 60, 80 ve 100) test edilmişlerdir. *Scenedesmus dimorphus*'un üremesi üzerindeki etkileri belirlemek için hücreler 72 saat boyunca her gün aynı saatte florometre (Turner tasarımı) kullanılarak sayılmıştır. Sonuç olarak en yüksek toksisite düzeyleri İstasyon 1, 9, 10, 13,14 ve15' te saptanmış olup bunun sebebi 9 nolu istasyonun yakınlarda bir kömür tesisinin bulunması ve Bakırçay Nehrinin bütün kollarının 15 nolu istasyonda birleştikten sonra Çandarlı Körfezi'ne dökülmesidir.

**Anahtar kelimeler:** Bakırçay, alg büyüme inhibisyon testi, kirlilik, tatlısu, *Scenedesmus dimorphus*

## INTRODUCTION

Bakırçay River is among most the important sub-basins in the North Aegean Basin. Its length is about 129 kilometres. Joining with its most crucial branch Yağcılar Brook near Kınık County, Bakırçay River goes through Bergama and then drains to Aegean Sea near Çandarlı County. Contained in the basin are Manisa and Izmir Cities. Being one of the most important rivers in North Aegean Basin (see Figure 1), the river is under the pressure of domestic and agricultural pollution. Coal mining in Soma County of Manisa may be considered the most important industrial activity for environmental pollution. Soma County Harbors Management of Aegean Lignite Business and many private mining plants. Olive and several fruits and vegetables are widely cultivated in the basin. Quarries and tomato paste factories exist in Bergama County. Soma County

hosts a thermal power plant. Based on the results of water quality for the summer season of 2015, the river appears to have *Class IV* water quality in the context of the Regulation on Management of Superficial Water Quality. The most polluted locations were found in Soma County (ÇŞB, 2016).

Our country is not rich enough in soil and water sources. Soil and water pollution represents a significant environmental problem with a steadily increasing extent. Soil and water are the most important strategic sources in the 21st century during which famine and hunger threaten the world. Agricultural pollutants, industrial and domestic wastes, excessive use, and lack of planning water sources complicate solutions to maintain and sustain the ecosystem (Tomar, 2009).

The pollutants are considered to be PAH, pesticides and domestic wastes. Carcinogenic and mutagenic substances from industrial and agricultural activities reach the lakes and the seas via terrestrial drainage and rivers. It has been reported that a remarkable increase in the number of fish with several tumours might have been caused by pollution, caused by the mutagenic and carcinogenic xenobiotics in the environment (De Flora et al., 1991).

Recently, water sources have been of steadily increasing importance all around the world. In near-east region including Turkey, this is of much more interest; additionally, the region's potential for water sources is low. Rapidly increasing population, technological advances, and increasing quality of life on the region's countries of the region, on the other hand, significantly increase need for water. In the context of technical and economic conditions, annually consumable -under and above-ground water potential of our country is average 112 billions $m^3$ (Tomar, 2009). It appears possible that Turkey will be one of the countries experiencing water scarcity. Thus, it is hard to claim that Turkey is rich in water. The usage of water potential in our country has reached 40% of economically consumable water potential (Tomar, 2009).

Investigating the effects of environmental pollutants is crucial for ecosystem health. It can be done in many ways such as determining the number of pollutants or detecting their effects on organisms by bioassays. Algae play critical roles in ecosystems; thus, they have been used widely to improve health status of many ecosystems worldwide. The effects of the chemicals can also be detected by bioassays using the algae. For a number of researchers all around the world, algal growth inhibition test is the most commonly used and standardized method for this type of bioassay (OECD, 2011). Algae have been reported to be equally or more sensitive organisms than animals and have been used very commonly in toxicity assays (Ferreira and Graca, 2002).

Currently, aquatic toxicity test data are routinely used to evaluate the risks associated with discharge of effluents into water bodies and sediments (Üstün, 2011). Algal bioassay is a test method increasingly used to detect the toxic potential of chemicals, xenobiotics, and most environmental samples from several sources of discharge. Toxicity Test Methods for freshwater algae are intended to be used on environmental chemicals, industrial and municipal effluents, drugs for human and animal use, freshwater dredge material, contaminated sediment/elutriates, hazardous chemical wastes, and groundwater contamination. Examples of the contaminants with recently determined toxicity on the algae include several dyes, chemicals for fire control, fuel oil, oil refinery chemicals, manure runoff, metals, herbicides/pesticides, PCBs, waste dump leachates, sediment, wastewaters, and landfill leachate (Hoffman et al., 2003).

Cheung et al. (1993) investigated toxic effect of the landfill leachate on microalgae and found important results. According to the results there were differential sensitivities to leachate exhibited by the tested algal species. Susceptibility to leachates in terms of cell number was in the ascending order of *Chlorella pyrenoidosa, Scenedesmus* sp., *Chlorella vulgaris* and *Dunaliella tertiolecta*.

Several toxicity tests were conducted with freshwater microalgae species including *Selanastrum capricornatum* (Blaise et al.,1986); *Scenedesmus subspicatus* Chodat (OECD., 1984); Scenedesmus quadricauda (Turp.) Breb (EPA, 1985); *Chlorella vulgaris* Beij., *Scenedesmus* sp. (Cheung et al., 1993). This test is also an easy method with its advantages including saving time, being economic, and its easy implementation. These tests usually use *Scenedesmus subspicatus, Scenedesmus (=Desmodesmus) dimorphus, Chlorella spp. and other species (Selenastrum capricornatum, S. quadricauda* (Turp.) Breb. *Scenedesmus sp. Navicula pelliculosa, Anabaena flos-aquae, Synechococcus leopoliensis* (Hoffman et al., 2003).

The present study aimed to assess the effects of surface waters of the Bakırçay River on the growth of green algae *Scenedesmus dimorphus* as the representative of the first trophic level by Algal growth inhibition test (OECD, 2011).

## MATERIALS AND METHODS

Water samples were collected from 15 stations located on the Bakırçay River between 17 and 19 April 2018 within the context "Limnofauna of Bakırçay River Basin (16/SÜF/038)", (Figure 1, Table 1). In our study, Algal Inhibition Assay was performed on the water samples collected from Stations 1, 2, 5, 6, 8, 9,10,13, 14, to 15. Briefly, all samples were transferred to the laboratory under ice-cold conditions as soon as possible. Prior to the experiment, all water samples were filtered using filters of 0.45 µm and 0.22 µm and then added to the media directly. The samples from ten stations on the Bakırçay River were tested in set of four dilutions (40, 60, 80, and 100 % (v:v)). Control series including only the test medium were also prepared. All of the test series were replicated three times in accordance with by the guideline "Freshwater Algal and *Cyanobacteria* Growth Inhibition Test" (OECD, 2011).

Freshwater algae species *Scenedesmus dimorphus* (UTEX1237) was cultured in the stock solution of OECD TG 201 medium (enhancement medium) in sterilized glass balloon

after it was obtained in pure form. Four days before the test, a pre-culture was set up and incubated at 21±2°C and pH 8,1. Cell count was set by means of culture medium so that the cell count of *Scenedesmus dimorphus* would be 2-5 x$10^3$ cell/ml from the stock culture solution of 4-7 days. Test tubes were held on a shaker at 100 rpm under constant illumination at approximately 2000 lux. The final volume was 6 milliliters in the test tubes. The cells were counted by fluorimeter (Turner design) daily at the same hour of the day and the test duration was 72 hours. The relative inhibition of growth rate was determined as the reduced cell number of the treated samples relative to the controls. $K_2HPO_4$ solution was used as positive control. Controls were studied in 6 replicates and then their average was calculated.

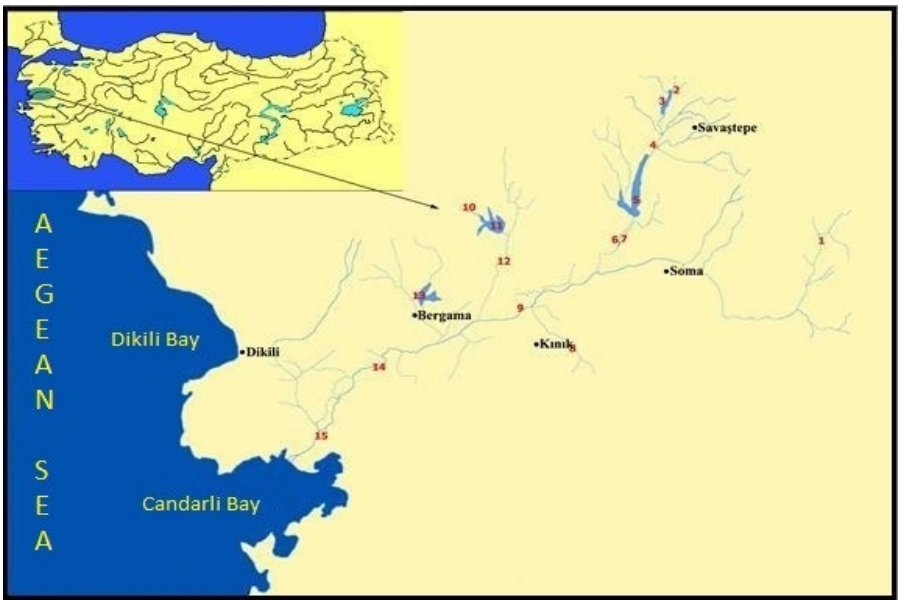



**Figure 1**. Sampling Sites

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

| St. | Location | Coordinates |
|---|---|---|
| 1 | Koca Creek(Kırkağaç-Manisa) | 39°13'39"N - 27°51'30"E |
| 2 | Kuzulu Dede Creek (Savaştepe-Balıkesir) | 39°26'21"N - 27°23'34"E |
| 3 | Sarıbeyler Dam Lake (Savaştepe-Balıkesir) | 39°24'42"N - 27°36'38"E |
| 4 | Büyük Creek (Savaştepe-Balıkesir) | 39°21'22"N - 27°35'39"E |
| 5 | Sevişler Dam Lake (Soma-Manisa) | 39°15'59"N - 27°33'23"E |
| 6 | Yağcılı Creek (Soma-Manisa) | 39°11'17"N - 27°29'16"E |
| 7 | Bakırçay River (Soma-Manisa) | 39°10'41"N - 27°28'55"E |
| 8 | Karadere Creek (Kınık-İzmir) | 39°05'23"N - 27°25'19"E |
| 9 | Bakırçay River (Kınık-İzmir) | 39°06'44"N - 27°16'26"E |
| 10 | Çınarlıdere Creek (Bergama-İzmir) | 39°15'30"N - 27°18'53"E |
| 11 | Çaltıkoru Dam Lake (Bergama-İzmir) | 39°14'15"N - 27°18'15"E |
| 12 | İlyasdere Creek (Bergama-İzmir) | 39°12'59"N - 27°20'02"E |
| 13 | Kestel Dam Lake (Bergama-İzmir) | 39°08'33"N - 27°11'49"E |
| 14 | Bakırçay River (Bergama-İzmir) | 39°03'11"N - 27°06'40"E |
| 15 | Bakırçay River (Dikili-İzmir) | 38°57'09"N - 27°00'35"E |

### Algal growth inhibition test

The green algae *Scenedesmus dimorphus* (Turpin) Kützing 1834: 608, was used as the test organism. The stock algal culture was maintained in an algal medium according to OECD 201. The pH of the medium after equilibration with air was approximately 8. An Algal Bioassay was carried out according to the OECD 201 Standard for algal growth inhibition assay (OECD 2011; Katalay et al., 2012). The endpoints were evaluated based on cell count data and calculated growth rate (0 to 72h) as described in the standard protocols (OECD, 2011) from the mean cell counts of each test series.

The average specific growth rate (μ) for exponentially growing cultures was calculated as follows:

**μ0- j=** lnxj- lnx0/tj-t0 (day-1)

**μ0- j:** growth rate,

**X0:** nominal number of cells / m at time $t_0$,

**Xj:** measured number of cells/ml at $t_j$,

**Tj:** time of first measurement of after beginning of test.

The percentage of inhibition of the cell growth (Ir %) of each test substance concentration is calculated as the difference between the control growth curve (μc) and the growth curve at each test substance concentration (μt) as:

**Ir % =** μc -μr /μc x 100.

Inhibition percentage were estimated by comparing the growth with the controls

**Ir %:** Percent inhibition in average specific growth rate;

**μC:** Mean value for average specific growth rate (μ) in the control group;

**µT:** Average specific growth rate for the treatment replicate.

The percentage of growth inhibition was calculated by probit analyses. The Statistica-6.0 software was used for probit analysis and the statistical significance of the data on growth rates was compared with controls using t-test.

## RESULTS

Results of the Algal Growth Inhibition Test we performed on superficial water samples from the Bakırçay River using *S. dimorphus* are given as exponential and bar graphics in the Figure 2, 3 and 4. Table 2 shows a comparison of each station with control using Student's T-test. All stations were statistically significantly different from the control group ($p < 0.05$).

The highest level of toxicity was found at Stations 1, 9, 10, 13, 14 and 15 (Figure 2, 3 and 4). For this reason, there was a coal facility near to a Station 9 and that all branches of the Bakırçay River joins near Station15 and then drains into the Çandarlı Bay as one river. Along the river, there are many active agricultural zones, domestic wastes and many wastewater discharges drain into the river. Wastewaters originating from these areas reach to the Bakırçay River and finally discharge to the Çandarlı Bay (Aliağa-İzmir) (Ortabük, 2007).

No inhibition was observed on Stations 2, 5, 6, and 8 (Figure 2, 3); in contrast, hormesis was observed on dilutions of the samples from these stations due to increased concentration (the biological response to low exposures to toxins and other stressors is generally favorable. In toxicology, hormesis is a dose-response phenomenon to xenobiotics or other stressors characterized by a low-dose stimulation, with zero dose and high-dose inhibition).

**Table 2.** Comparison of each station with control ($p < 0.05$)

| Stations | P values |
|---|---|
| St.1 | 0.00145 |
| St.2 | 0.03630 |
| St.5 | 0.02540 |
| St.6 | 0.00108 |
| St.8 | 0.03464 |
| St.9 | 0.00526 |
| St.10 | 0.00688 |
| St.13 | 0.00365 |
| St.14 | 0.01426 |
| St.15 | 0.00945 |

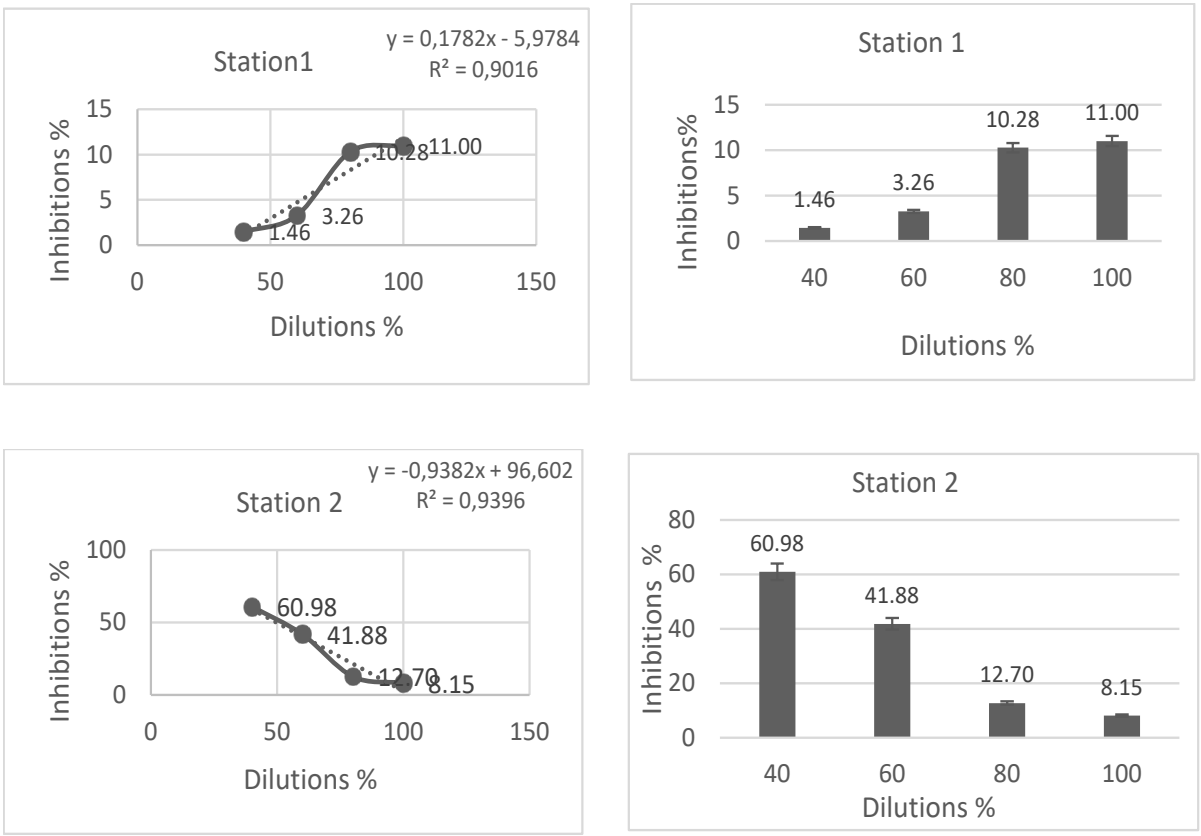



**Figure 2.** Growth rate of *S. dimorphus* at 72h exposure period

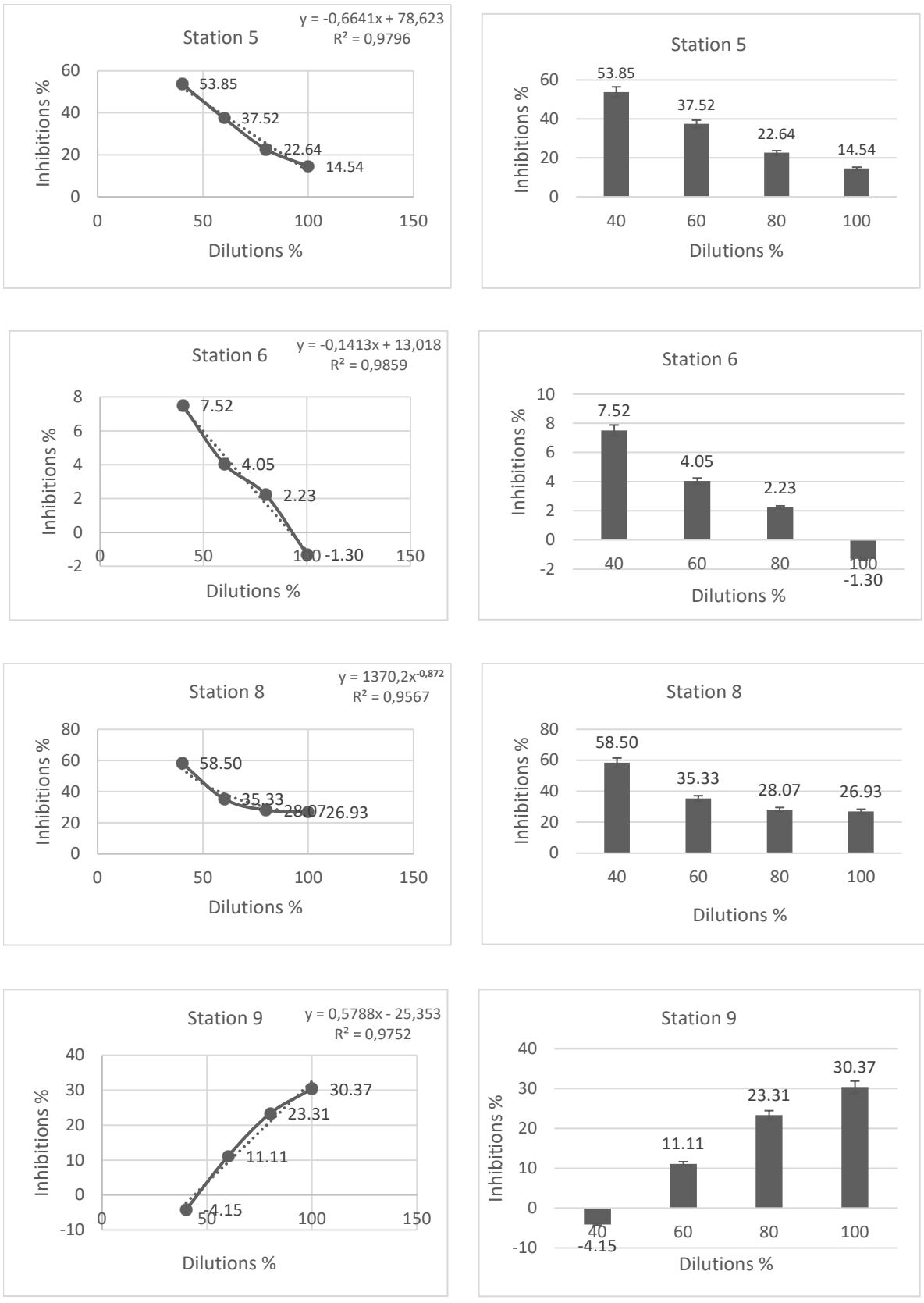



**Figure 3**. Growth rate of *S. dimorphus* at 72h exposure period

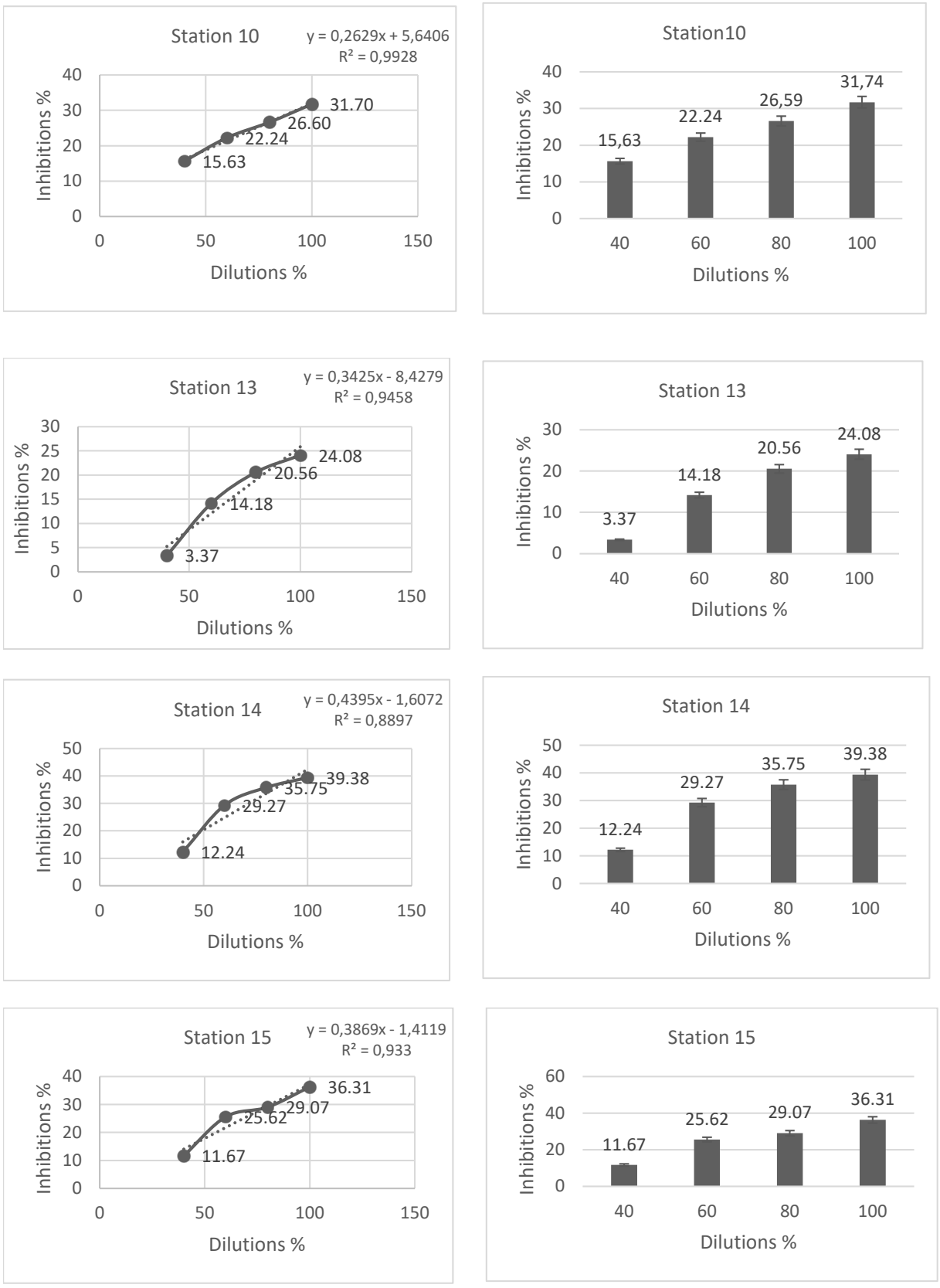



**Figure 4.** Growth rate of *S. dimorphus* at 72h exposure period

## DISCUSSION

Mixing of the drainage waters polluted by the fertilizers and drugs used for agricultural activities with erosion material carried by rainfall increases the pollution burden in the Bakırçay River. Furthermore, water from olive cultivation activities in the region creates a significant problem in the river because Çandarlı Bay is surrounded by crowded settlements, agricultural fields, and industrial areas, Bakırçay River carries wastewater from these regions to Çandarlı Bay (Kaymakçı Başaran, 2004).

Gündoğdu and Turhan (2004) reported that pollution in the Bakırçay Basin was mainly originated from water from processing and cooling activities in Soma Thermal Power Plant, domestic wastes, industrial activities, mining and agricultural activities, and that Bakırçay River had water quality of Class IV according to the Water Quality Control Regulations. According to Kaymakçı Başaran (2004) one of the most important threats to the water quality of Bakırçay River is the presence of the Soma Thermal Power Plant. Additionally, drainage of the wastewater from olive oil plants and other industrial facilities in the region and increased drainage of the fertilizers and drugs used in agricultural activities causes an increased pollution burden in the river. According to Tomar (2009), on the other hand, widespread pollution caused by mine areas exists on the Bakırçay Basin in addition to domestic, industrial, and agricultural pollution.

Department of Laboratory Measurement and Monitoring on the General Directorate of EIA Permission and Surveillance of Ministry of Environment and Urbinazation has been performing surveys on the physicochemical parameters and heavy metals since 2015. In the comparison of summertime results from 2011 to 2015, a decrease was observed in many parameters while an increase was observed in Pb, Cu, total Cr, and Zn parameters. The river was usually detected annually to have Class IV water quality. Being one of the most important rivers of the North Ege Basin, Bakırçay River is under the pressure of domestic pollution (ÇŞB, 2016). Wastes from Soma Thermal Power Plant located on the Bakırçay Basin and domestic wastes have been increasing due to population growth in the region are discharged into the Bakırçay River and its branches with inadequate or no treatment (Kaymakçı Başaran, 2004).

Many studies have been performed on Bakırçay Basin especially focusing on water quality and criteria but no studies exist involving biological tests for the toxic pollutants. Considering the fact that the toxic and mutagenic pollutants are discharged combinedly to the environment without being treated or they pollute the environment indirectly, it will be of great importance to know existence of these pollutants or to gain insight on their concentration in the environment. Thus, biological tests such as the "Algal Growth Inhibition" test are becoming important.

De Liguoro et al. (2010) used *S. dimorphus* as test organism in their study on aquatic toxicity level of Sulfaquinoxalines (SQOs) and Sulfaguanidines (SGDs) and obtained important results.

Arensberg et al. (1995) conducted a study which was similar to ours. A simple mini scale (approx. 1- 2.5 ml) toxicity test procedure with the freshwater green algae *Selenastrum capricornatum* described. The procedure fulfils the validity criteria of the IS0 (International Association for Standardization) standard test protocol. Practically identical concentration-response curves were obtained with the IS0 standard test and the minitest for potassium dichromate and 3,5dichlorophenol (Arensberg et al., 1995).

Katalay et al. (2012) used standard test protocol (Algal Growth Inhibition test, OECD 201) with freshwater algae *Desmodesmus (= Scenedesmus) subspicatus* to determine short-term toxicity and tested water and sediment samples using several dilutions.

Another study was performed on sediment and water samples from Gölcük Lake; in that study, *S. dimorphus* was used in "Algal Growth Inhibition Assay" (OECD, 2011) to determine phytotoxic effect (Boyacıoğlu et al., 2017).

Based on the "Algal Growth Inhibition" test we performed on S. *dimorphus* in the water samples from ten locations on the Bakırçay River, the most toxic and growth-inhibiting samples were those from Soma and those located on the main line draining into Çandarlı Bay.

## CONCLUSION

In conclusion, toxicity was detected at Stations 1, 9, 10, 13, 14 and 15 according to the phytotoxicity assay we conducted on ten stations on the Bakırçay River. Most importantly, a phytotoxicity test was done on the Bakırçay River for the first time.

We believe that the present study will be a basement for future studies and guide the multidisciplinary studies. We also hope that many establishments and units will be informed on results of the present study and maybe action will be taken to make necessary measures as a consequence of publication of this study.

## ACKNOWLEDGEMENTS AND FUNDING

This study was carried out by examining the water samples obtained from the project numbered 16/SÜF/038 which was supported by Ege University Scientific Research Projects Coordination (BAP).

## AUTHORSHIP CONTRIBUTIONS

Meltem Boyacıoğlu: Conceptualization, methodology, resources, investigation, writing-reviewing and editing. Cem Aygen: Conceptualization, funding acquisition, project administration, investigation, formal analysis, visualization. Muhammet Ali Karaaslan: Conceptualization, methodology, investigation. Didem Özdemir Mis: Resources, investigation. Özlem Çakal Arslan: 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.

## REFERENCES


Arensberg, P., Hemmingsen, V.H., & Nyholm, N. (1995). A mini scale algal toxicity test. *Chemosphere*, *30* (11), 2103-2115. https://doi.org/10.1016/0045-6535(95)00090-U

Blaise, C., Legault, R., Bermingham, N., van Coillie, R., & Vasseur, P. (1986). A simple microplate algal assay technique for aquatic toxicity assessment. *Toxicity Assessment*, *1*(3), 261-281. https://doi.org/10.1002/tox.2540010302

Boyacıoğlu, M., Parlak, H., Çakal Arslan, Ö., Karaaslan, M.A., Tez, S., Gülsever, G., & Nalbantlar, B. (2017). Phytotoxicity of sediment from Gölcük Lake (İzmir, Turkey) on *Desmodesmus* (=*Scenedesmus*) *dimorphus*. *Ege Journal of Fisheries and Aquatic Sciences*, *34*(2), 145-150. https://doi.org/10.12714/egejfas.2017.34.2.05

Cheung, K.C., Chu, L.M., & Wong, M.H. (1993). Toxic effect of landfill leachate on microalgae, *Water Air Soil Pollution, 69*(3-4)), 337–349. https://doi.org/10,1007/BF00478169

ÇŞB (2016). Domestic And Industrial Pollution Monitoring Program (EKIP) 2015 Water Quality Monitoring Final Report for Ergene, Gediz, Northern Aegean (Bakırçay), Küçük Menderes, Susurluk and Sakarya Basins. *General Directorate of Permission and Inspection, Ministry of Environment and Urbanization, Ankara (in Turkish).*

De Flora, S., Bagnasco, M., & Zanacchi, P. (1991). Genotoxic, carcinogenic hazards in the marine environmental, with special reference to the Mediterranean Sea. *Mutation Research*, *258*(3), 285-320. https://doi.org/10.1016/0165-1110(91)90013-1

De Liguoro, M., Di Leva, V., Gallina, G., Faccio, E., Pinto, G., & Pollio, A. (2010). Evaluation of the aquatic toxicity of two veterinary sulfonamides using five test organisms. *Chemosphere 81*(6), 788–793. https://doi.org/10.1016/j.chemosphere.2010.07.003

EPA. (1985). U.S. Environmental protection agency, environmental effects test guidelines, federal register, 50, 39321.

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

Gündoğdu, V. & Turhan, D., (2004). Study on the pollution of Bakırçay River Basin. *Journal of Science and Engineering of Faculty of Engineering of Dokuz Eylül University*. 6(3), 65-83 (In Turkish with English abstract).

Hoffman, D.J., Rattner, B.A., Burton Jr., G.A. & Cairns Jr.J. (2003). Handbook of Ecotoxicology. (2nd ed.) CRC Press. https://doi.org/10.1201/9781420032505

Katalay, S., Boyacıoğlu, M., Çakal Arslan, Ö., Parlak H., & Karaaslan, M.A. (2012). Phytotoxicity of water and sediment from Nif Brook (İzmir, Turkey) on green algae *Desmodesmus* (=*Scenedesmus*) *subspicatus. Ekoloji. 21*(83), 25-31. https://doi.org/10.5053/ekoloji.2012.833

Kaymakçı Başaran, A. (2004*)*. Pollution parameters of Bakırçay Deltaic Zone and Its Interactions with Çandarlı Bay. *Doctoral Dissertation, Ege University, Turkiye* (in Turkish with English abstract).

Kützing, F.T. (1834 '1833'). Synopsis Diatomearum oder versuch einer systematischen zusammenstellung der Diatomeen. Linnaea (8):529-620, pls XIII-XIX [79 figs]. *Desmodesmus* (=*Scenedesmus*) *dimorphus* (Turpin) Kützing 1834: 608. *http://www.algaebase.org/search/species/detail/?Species_id=58629:(24.01.2023)*.

OECD. (2011). Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris. https://doi.org/10.1787/9789264069923-en

Ortabük, F. (2007). The determination of heavy metals and radioactive element concentrations of Bakırcay River Basin and the scrutiny of their causes through factor analysis methods. *Master Thesis. Institute of Nuclear Sciences, Graduate School of Natural and Applied Sciences, Ege University Türkiye.* (in Turkish with English abstract)

Tomar, A. (2009). Soil and Water Pollution and protecting the water basins. *TMMOB Izmir City Symposium* (pp:333-345) *(*in Turkish*).*

Üstün, G.E. (2011). The assessment of heavy metal contamination in the waters of the Nilufer stream in Bursa. *Ekoloji, 20*(81), 61-66. https://doi.org/10.5053/ekoloji.2011.819

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 51-55 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.07

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Cytogenetic characteristics of endemic *Squalius cappadocicus* Özuluğ and Freyhof, 2011 in Türkiye

## Türkiye'ye endemik *Squalius cappadocicus* Özuluğ and Freyhof, 2011'un sitogenetik özellikleri

**Ahmed Sadeq Jaber Doori[1] ● Atilla Arslan[2]***

[1] Graduate School of Natural Applied Sciences, Selçuk University, 42030 Konya, Türkiye
[2] Department of Biology, Faculty of Science, Selçuk University, 42030 Konya, Türkiye

https://orcid.org/0000-0002-7452-657X
https://orcid.org/0000-0002-4766-4969

*Corresponding author: aarslan@selcuk.edu.tr

Received date: 05.01.2023 Accepted date: 02.02.2023

**How to cite this paper:**
Doori, A.S.J., & Arslan, A. (2023). Cytogenetic characteristics of endemic *Squalius cappadocicus* Özuluğ and Freyhof, 2011 in Türkiye. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 51-55. https://doi.org/10.12714/egejfas.40.1.07

**Abstract:** In this study, a detailed chromosome analysis of the endemic Cappadocian Chub, *Squalius cappadocicus* in Melendiz Stream (Aksaray) was performed. The standard Giemsa staining, C-banding (CBG and CB-DAPI), and Ag-NOR technique were applied. The diploid chromosome number was 50; its karyotype formula was 14M + 16Sm + 10St + 10A. Heteromorphic sex chromosomes weren't detected in the karyotype of the studied specimens. The number of all chromosomal arms (NF) was 90. In the standard C-banded and CB-DAPI karyotype of the species, dark C-bands were observed in the centromeric region of some bi-armed and acrocentric chromosomes, while slightly centromeric or pericentromeric C-bands were detected in some chromosomes. Three different active Ag-NORs, which were hemizygous, were detected in all samples examined. Two of these active NORs were detected in the bi-armed and the other in the acrocentric chromosome short arm. The Ag-NOR number of this species was evaluated as a feature that distinguishes it from other *Squalius* species in Türkiye.

**Keywords:** Cappadocian Chub, Chromosome, C-banding, CB-DAPI, Ag-NOR

**Öz:** Bu çalışmada, Melendiz Çayı'nda (Aksaray) endemik Kapadokya Kefali, *Squalius cappadocicus*'un detaylı kromozom analizi yapıldı. Standart Giemsa boyama, C-bantlama (CBG ve CB-DAPI) ve Ag-NOR tekniği uygulandı. Diploid kromozom sayısı 50'dir; karyotip formülü 14M + 16Sm + 10St + 10A'dır. İncelenen örneklerin karyotipinde heteromorfik cinsiyet kromozomları saptanmadı. Tüm kromozom kollarının (NF) sayısı 90'dı. Türün standart C-bantlı ve CB-DAPI karyotipinde, bazı çift kollu ve akrosentrik kromozomların sentromer bölgesinde koyu renkli C-bantları gözlenirken, bazı kromozomlarda sentromerik veya perisentromerik hafif C-bantlar tespit edildi. İncelenen tüm örneklerde hemizigot olan üç farklı aktif Ag-NOR tespit edildi. Bu aktif NOR'lardan ikisi iki kollu, diğeri akrosentrik kromozom kısa kolunda tespit edildi. Bu türün Ag-NOR sayısı, onu Türkiye'deki diğer *Squalius* türlerinden ayıran bir özellik olarak değerlendirilmiştir.

**Anahtar kelimeler:** Kapadokya Kefali, Kromozom, C-bandlama, CB-DAPI, Ag-NOR

## INTRODUCTION

The Forty-five *Squalius* species in the world are distributed in the Western Palearctic. There are 21 species in total, 15 of which are endemic in Türkiye. These species are distributed in rivers, lakes and dam lakes in different regions of Türkiye (Çiçek et al., 2018). *Squalius cappadocicus* was first described by Özuluğ and Freyhof (2011) in the Melendiz River in the Tuz Lake basin. The karyological studies on *Squalius* species in the world are very few. The cytogenetic features of *S. cephalus* have been investigated by different researchers both in Europe and Türkiye (Wolf et al., 1969; Cataudella et al., 1977; Sofradzija, 1977; Bianco et al., 2004; Boroń et al., 2009; Pekol and Arslan, 2014; Kılıç and Şişman, 2016). The karyological characteristics of four *Squalius* species (*S. lucumonis, S. squalus, S. aradensis* and *S. torgalensis*) in Europe were determined by Rossi et al. (2012) and Nabais et al. (2013). Chromosome structures of only *S. orientalis*, *S. seyhanensis, S. carinus, S. fellowesii, S. anatolicus* and *S. recurvirostris* among 15 endemic species distributed in Türkiye were investigated by different researchers (Kılıç Demirok, 2000; Ünal and Gaffaroğlu, 2016; Karasu Ayata, 2020; Ünal Karakuş and Gaffaroğlu, 2021; Doori and Arslan, 2022). However, until now, no karyological studies have been conducted on *S. cappadocicus*, which spreads in Aksaray and its surroundings. The aim of this article is to perform a chromosomal banding analysis of the karyotype of *S. cappadocicus* using C-banding and Ag-NOR staining, and to compare the findings with those obtained in other previous studies.

## MATERIALS AND METHODS

The specimens of *S. cappadocicus* were caught from the Melendiz Stream in Ihlara (Aksaray) (Figure 1) by the appropriate method. The captured samples were brought to the laboratory and rested. The karyology of the samples was done according to the method of Bertollo et al. (2015). Some of the chromosome slides were traditionally stained with Giemsa. Constitutive heterochromatin and nucleolus organizer regions (NORs) were detected by applying C-banding (CBG and CB-DAPI banding) (Sumner, 1972) and Ag-NOR staining (Howell

and Black, 1980). Well-spread metaphases of each staining were photographed under the microscope and karyotyped. Chromosomes were defined according to Levan et al. (1964).

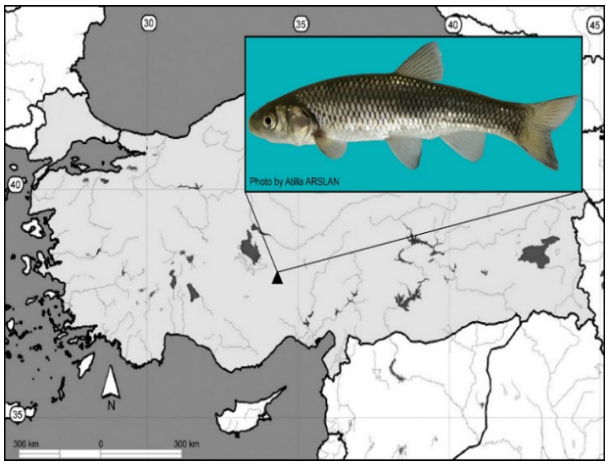



**Figure 1.** Collecting site in Stream Melendiz at Ihlara from Aksaray

## RESULTS

In this study, the diploid chromosome number of *S. cappadocicus* was determined as 2n = 50. The karyotype consists of seven pairs of metacentric (nos. 1-7), six pairs of submetacentric (nos. 8-13), seven pairs of subtelocentric (nos. 14-20) and five pairs of acrocentric (nos. 21-25) autosomal chromosomes (NF = 90) (Figure 2). The heteromorphic sex chromosome of the species was not detected in the karyotype. The standard C-banded and CB-DAPI karyotype of Cappadocian chub was shown in Figure 3, and Figure 4. Dark C-bands in the centromeric region of some bi-armed and acrocentric chromosomes were observed. In addition, slightly centromeric or pericentromeric C-bands were determined in some chromosomes. Three active Ag-NORs were detected in all specimens examined. All three of these NORs are hemizygous. Two of these active NORs were identified in the short arm of one of the homologues of the bi-armed chromosome pair 10 and 11. The other active NOR was found in the short arm of one of the homologues of the acrocentric chromosome pair no. 23 (Figure 5).

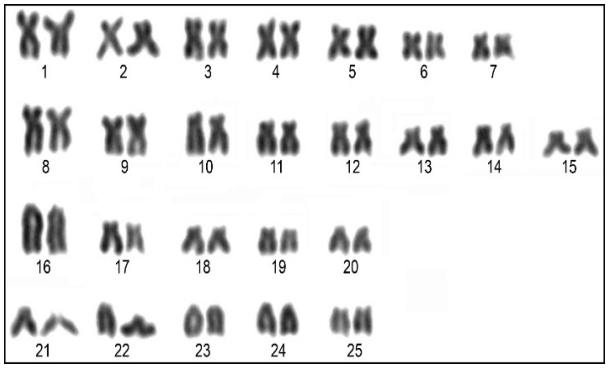


**Figure 2.** Standard Giemsa staining karyotype of *Squalius cappadocicus*

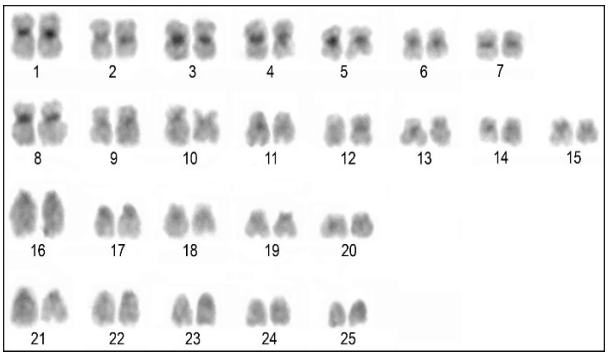


**Figure 3.** C-banded (CBG) karyotype of *Squalius cappadocicus*

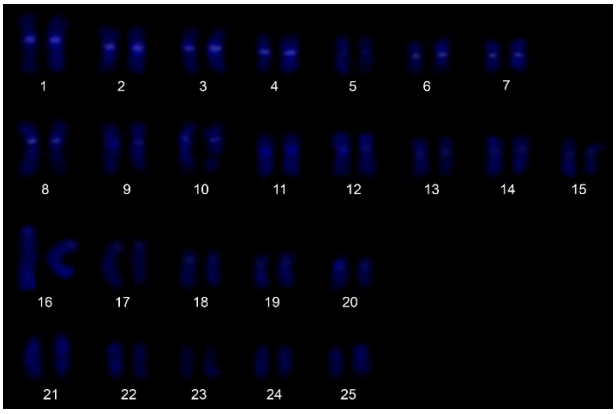


**Figure 4.** C-banded (CB-DAPI) karyotype of *Squalius cappadocicus*

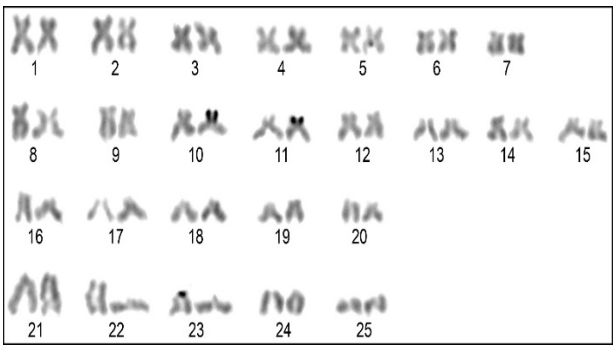


**Figure 5.** Silver-stained karyotype of *Squalius cappadocicus*

## DISCUSSION

After *S. cappadocicus* was described by Özuluğ and Freyhof (2011) from the Melendiz River in the Tuz Lake basin, the cytogenetic features of this species were investigated for the first time in this study. In all examined individuals, the diploid chromosome number (2n) was 50 and the fundamental number of autosomal arms (NF) was 90. Sex chromosomes could not be detected in male and female individuals. The karyological characteristics of eight endemic *Squalius* species in Türkiye have been studied by different researchers and this information is summarized in Table 1.

Among these endemic species, sex chromosomes were detected in *S. recurvirostris*, which is distributed in the Konya

Ilgın basin (Doori and Arslan, 2022). ZZ-ZW sexual chromosome system was detected in Polish samples of *S. cephalus* distributed in Europe Vujosevic et al. (1983). Although very rarely, sex chromosomes have been detected in some cytogenetic studies on fish. It has been determined that some of them have ZZ-ZW and some have XX-XY sex chromosomes system (Doori and Arslan, 2022).

In general, the diploid chromosome number (2n) in leuciscin species (Leuciscidae) is 50. However, some species have different diploid chromosome numbers. In the studies carried out so far, 50 chromosomes have been identified in the chromosome set of all *Squalius* species. However, the number of bi-armed and acrocentric chromosomes in the set of these studied species differs (Table 1).

**Table 1.** Chromosomal records of *Squalius* species. NF–fundamental number of chromosomal arms, Z and W sex chromosomes

| Species | Locality | 2n | Karyotype | Z | W | NF | Reference |
|---|---|---|---|---|---|---|---|
| *S. cephalus* | Germany | 50 | 38M/Sm + 12St/A | - | - | - | Wolf et al. (1969) |
| *S. cephalus* | Italy | 50 | 16M + 12Sm + 12St + 10A | - | - | 90 | Cataudella (1977) |
| *S. cephalus* | Italy | 50 | 16M + 26Sm + 8St/A | - | - | - | Bianco et al. (2004) |
| *S. cephalus* | Czechia | 50 | 34M/Sm + 16A | - | - | 84 | Sofradzija (1977) |
| *S. cephalus* | Poland | 50 | 10M + 22Sm + 10St + 10A | - | - | 90 | Boron et al. (2009) |
| *S. cephalus* | Türkiye | 50 | 18M + 12Sm + 20St/A | - | - | - | Pekol and Arslan (2014) |
| *S. cephalus* | Türkiye | 50 | 10M + 22Sm + 10St + 8A | - | - | 92 | Kılıç and Şişman (2016) |
| *S. lucumonis* | Italy | 50 | 16M + 26Sm + 8St/A | - | - | - | Rossi et al. (2012) |
| *S. squalus* | Italy | 50 | 16M + 26Sm + 8St/A | - | - | - | Rossi et al. (2012) |
| *S. aradensis* | Portugal | 50 | 10M + 36Sm + 4St/A | - | - | - | Nabais et al. (2013) |
| *S. torgalensis* | Portugal | 50 | 10M + 36Sm + 4St/A | - | - | - | Nabais et al. (2013) |
| *S. orientalis* | Türkiye | 50 | 14M + 20Sm + 16St/A | - | - | - | Kılıç Demirok (2000) |
| *S. seyhanensis* | Türkiye | 50 | 16M + 28Sm + 6St/A | - | - | - | Ünal and Gaffaroğlu (2016) |
| *S. carinus* | Türkiye | 50 | 24M + 20Sm + 6St/A | - | - | - | Karasu Ayata (2020) |
| *S. fellowesii* | Türkiye | 50 | 20M + 20Sm + 10St/A | - | - | - | Karasu Ayata (2020) |
| *S. anatolicus* | Türkiye | 50 | 14M + 26Sm + 10St/A | - | - | - | Ünal Karakuş and Gaffaroğlu (2021) |
| *S. recurvirostris* | Türkiye | 50 | 12M + 18Sm + 10St + 8A | A | St | 90 | Doori and Arslan (2022) |
| *S. cappadocicus* | Türkiye | 50 | 14M + 16Sm + 10St + 10A | - | - | 90 | This study |

Since most of the researchers evaluated submetacentric and acrocentric chromosomes together, the NF value of the studied species was not clear. This is due to the shortness of the chromosomes and the inability to differentiate between submetacentric and acrocentric chromosomes. Leuciscin species have two characteristic features within the chromosome set.

First, there are more metacentric/submetacentric chromosomes, and second, the largest chromosome pair is subtelocentric/acrocentric (Ráb et al., 2008). It was observed that these two features were preserved in both *Squalius* species studied so far and *S. cappadocicus*. The chromosome morphologies of *S. cappadocicus* within the set are quite similar to *S. recurvirostris* distributed in the Ilgın basin. It differs from *S. anatolicus* in the Beyşehir basin in terms of submetacentric, subtelocentric and acrocentric chromosome numbers. The morphology of the chromosomes of *S. cappadocicus* is different from that of *S. orientalis*, *S. seyhanensis*, *S. carinus* and *S. fellowesii* distributed in different regions of Türkiye. This difference can be explained by the different arrangements that occur in the chromosomes.

Heterochromatin blocks (C bands) are used for the comparison of species in karyological studies due to their structural properties. For this reason, the chromosomes of species can be studied in detail with the C-banding method. This method is an important technique in terms of chromosomal identification of species and determination of sex chromosomes of a species (Arslan and Arslan, 2007). Recently, DAPI fluorescent stain is also used instead of Giemsa in the C-banding method. In this study, C-bands of this species were detected using both Giemsa and DAPI. Similar bands were observed in both stainings. The distribution of constitutive heterochromatin (C-band) in the chromosomes of some Leuciscine species has been investigated by different researchers. There are differences in the heterochromatin distribution in the chromosomes of these studied species. According to Boroń et al. (2009), all the chromosomes of *Leuciscus leuciscus*, *L. idus*, and *S. cephalus* have centromeric C-band in different rivers in Poland. The constitutive heterochromatin analysis of some *Squalius* species was performed in Türkiye (Karasu Ayata, 2020; Ünal Karakuş and Gaffaroğlu, 2021; Doori and Arslan, 2022). In these species, C-bands have been found in different regions of some bi-armed and acrocentric chromosomes. Centromeric, pericentromeric and paracentromeric C-bands have been detected in *S. seyhanensis* (Ünal and Gaffaroğlu, 2016). In the centromeric regions of some bi-armed and acrocentric chromosomes of Akşehir Chub *S. recurvirostris* had dark C bands. Also, the some chromosomes of this species had centromeric or pericentromeric slightly C bands, while the sex chromosomes were C-negative (Doori and Arslan, 2022). Similar heterochromatin bands were observed in the chromosomes of *S. carinus*, *S. fellowesii* and *S. anatolicus*, which were distributed in Afyon, Denizli, Beyşehir (Karasu Ayata, 2020; Ünal Karakuş and Gaffaroğlu, 2021). It has been determined that there are terminal bands in some of the chromosomes of *S. anatolicus* (Ünal Karakuş and Gaffaroğlu, 2021). Although

there are some differences, C-heterochromatin amount and distribution of chromosomes of *S. cappadocicus* are similar to that of other *Squalius* species studied in Türkiye.

Ag-NOR technique has been used in some cytotaxonomic studies. Recently, it has been used frequently in the cytogenetic studies of fish. The number of Ag-NORs detected in populations of *L. leuciscus* in Poland varies. Active NORs were localized in the long arm of the largest metacentric pair and the short arm of a submetacentric chromosome pair in some populations. It has been reported to be localized in only one pair of metacentric or one pair of subtelocentrics in different populations (Boroń et al., 2009). Hemizygous three different active Ag-NORs of *S. cappadocicus* were detected in all samples examined. One of these active NORs (no. 10) was considered a characteristic pleisomorphic NOR. The active NOR on acrocentric chromosome 23 was detected for the first time in *Squalius* species in Türkiye. The localization of active NORs detected in two different bi-armed chromosomes in *S. recurvirostris* in Türkiye was determined by Doori and Arslan (2022). The short arms of a pair of submetacentric chromosomes of European specimens of *S. cephalus* have active NOR positively associated with $CMA_3$ (Bianco et al., 2004). Boroń et al. (2009) reported a 28S rDNA probe localized on a pair of subtelocentric chromosomes and NOR positively associated with $CMA_3$ in Poland specimens of the same species. One NOR-bearing chromosome pair (with NORs located on the short arms) observed in the karyotype of *S. cephalus* is the most common NOR phenotype among Leuciscinae (Collares-Pereira et al., 1998). This NOR in *S. cephalus* is a highly plesiomorphic character. The active NOR of *S. cephalus* in Kastamonu is also on the short arm of a pair of subtelocentric chromosomes (Pekol and Arslan, 2014), and this NOR phenotype is pleisomorphic for *Squalius* species. NORs in *S. recurvirostris* distributed in the Ilgın basin are on two pairs of submetacentric chromosomes. One of them is localized on the short arm and the other on the long arm. They stated that the NOR on the short arm in this species is pleisomorphic (Doori and Arslan, 2022). Active NOR on the long arm of any chromosome in the chromosome set was detected only in *L. leuciscus* and *S. recurvirostris*. *S. seyhanensis*, *S. carinus*, *S. fellowesii* and *S. anatolicus*, whose cytogenetics have been studied in Türkiye, also have pleisomorphic active NOR on the short arm of a pair of submetacentric chromosomes (Ünal and Gaffaroğlu, 2016; Karasu Ayata, 2020; Ünal Karakuş and Gaffaroğlu, 2021).

## CONCLUSION

As a result, the current cytogenetic results of *Squalius* species are examined, it is seen that there are variations in terms of chromosome morphology, heterochromatin distribution and Ag-NOR number. Especially the change in the Ag-NOR number is quite remarkable. Both the high number of NORs and the presence of NOR in the acrocentric chromosome in *S. cappadocicus* can be considered as distinguishing this species from other *Squalius* species in Türkiye.

## ACKNOWLEDGEMENTS AND FUNDING

This article is based on Ahmed Sadeq Jaber DOORI's Ph.D. summarized from a part of his thesis. This study was financed with a grants from the Scientific Research Projects Coordination Board of Selçuk University (Project number: 21211009).

## AUTHORSHIP CONTRIBUTIONS

The contribution of the authors is equal.

## ETHICS APPROVAL

The samples in this study were collected with the permission of the TR Ministry of Forestry and Water Affairs (Permit No: E-21264211-288.04-3435924). This permission replaces the local ethics committee permission in accordance with 8/L of the regulation "On Working Procedures and Principles of Animal Experiments Ethics Committees" prepared by the Ministry of Forestry and Water Affairs and published in the Official Gazette on February 15, 2014.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

## DATA AVAILABILITY

All relevant data is inside the article.

## REFERENCES

Arslan, A., & Arslan, E. (2007). Importance of C-banding (Constitutive Heterochromatin) in karyosystematic (in Turkish with English abstract). *Selçuk Üniversitesi Fen Fakültesi Fen Dergisi, 2*(29), 121-126.

Bertollo, L., Cioffi, M., & Moreira-Filho, O. (2015). Direct chromosome preparation from freshwater teleost fishes. In C. Ozouf-Costaz, E. Pisano, F. Foresti, & L. Almeida Toledo (Eds.), *Fish Cytogenetic Techniques (Chondrichthyans and Teleosts)* (pp. 21-26). United States: CRC Press.

Bianco, P. G., Aprea, G., Balletto, E., Capriglione, T., Fulgione, D., & Odierna, G. (2004). The karyology of the cyprinid genera *Scardinius* and *Rutilus* in Southern Europe. *Ichthyological Research, 51*, 274-278. https://doi.org/10.1007/s10228-004-0221-y

Boroń, A., Porycka, K., Ito, D., Abe, S., & Kirtiklis, L. (2009). Comparative molecular cytogenetic analysis of three *Leuciscus* species (Pisces, Cyprinidae) using chromosome banding and FISH with rDNA. *Genetica, 135*, 199-207. https://doi.org/10.1007/s10709-008-9269-3

Cataudella, S., Sola, L., Muratori, R.A., & Capanna, E. (1977). The chromosomes of 11 species of Cyprinidae and one Cobitidae from Italy, with some remarks on the problem of polyploidy in the Cypriniformes. *Genetica, 47*(3), 161-171. https://doi.org/10.1007/BF00123236

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

Collares-Pereira, M.J., Próspero, M., Biléu, R., & Rodrigues, E. (1998). *Leuciscus* (Pisces, Cyprinidae) karyotypes: transect of Portuguese populations. *Genetics and Molecular Biology, 21*(1), 63-69. https://doi.org/10.1590/S1415-47571998000100011

Doori, A.S.J., & Arslan, A. (2022). Karyotypes and ZZ/ZW sex chromosome system of endemic *Squalius recurvirostris* (Leuciscinae, Cyprinidae) in Turkey. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 25*(4), 649-657. https://doi.org/10.18016/ksutarimdoga.vi.915278

Howell, W.M., & Black, D. A. (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia*, *36*, 1014-1015. https://doi.org/10.1007/BF01953855

Karasu Ayata, M. (2020). Comparative cytogenetics of two *Squalius* Bonaparte, 1837 species (Cypriniformes: Leuciscidae). *Iranian Journal of Science and Technology, Transactions A: Science, 44*, 355-360. https://doi.org/10.1007/s40995-020-00836-0

Kılıç, D., & Şişman, T. (2016). Karyotype analysis of chub, *Squalius cephalus* (Linnaeus, 1758)(Teleostei: Cyprinidae) from Karasu River, Erzurum, Turkey. *Caspian Journal of Environmental Sciences, 14*(2), 95-103.

Kılıç Demirok, N. (2000). *The Studies on the chromosomes of the some cyprinid species and subspecion of the tigris river systems.* Doctoral dissertation, Dicle University, Turkey. (in Turkish wish English abstract)

Levan, A., Fredga, K., & Sandberg, A.A. (1964). Nomenclature for centromeric position on chromosomes. *Hereditas, 52*(2), 201-220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Nabais, C., Rampin, M., & Collares-Pereira, M.J. (2013). Comparative cytogenetics of two endangered leuciscine fish, *Squalius aradensis* and *S. torgalensis* (Teleostei, Cyprinidae), from the Iberian Peninsula. *Comparative Cytogenetics*, *7*(1), 33-42. https://doi.org/10.3897/CompCytogen.v7i1.4672

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

Pekol, S. & Arslan, O. (2014). Evaluation of *Squalius cephalus* (L., 1758) in terms of nor phenotype and aquatic environment ecotoxicological studies (Kastamonu Beyler Dam Population). *Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi, 2*, 23-28. (in Turkish with English abstract)

Ráb, P., Rábová, M., Pereira, C.S., Collares-Pereira, M.J., & Pelikánová, Š. (2008). Chromosome studies of European cyprinid fishes: interspecific homology of leuciscine cytotaxonomic marker—the largest subtelocentric chromosome pair as revealed by cross-species painting. *Chromosome Research, 16*, 863-873. https://doi.org/10.1007/s10577-008-1245-3

Rossi, A.R., Milana, V., Hett, A. K., & Tancioni, L. (2012). Molecular cytogenetic analysis of the Appenine endemic cyprinid fish *Squalius lucumonis* and three other Italian leuciscines using chromosome banding and FISH with rDNA probes. *Genetica, 140*(10), 469-476. https://doi.org/10.1007/s10709-012-9695-0

Sofradzija, A. (1977). Kariologia i citotaksonomija vrsta roda *Leuciscus* iź voda Bosne i Hercegovine. *Godišňjak Biološkog Instituta Univerziteta u Sarajevu Posebno izdanje, 30*, 113-211.

Sumner, A. (1972). A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research*, *75*, 304-306. https://doi.org/10.1016/0014-4827(72)90558-7

Ünal, S., & Gaffaroğlu, M. (2016). Karyology of six cyprinid fishes from Seyhan and Ceyhan rivers in Anatolia. *Caryologia, 69*(4), 362-369. https://doi.org/10.1080/00087114.2016.1247328

Ünal Karakuş, S. & Gaffaroğlu, M. (2021). Karyotype, C-band and NOR phenotype of Anatolian endemic fish *Squalius anatolicus* (Bogutskaya, 1997) (Teleostei, Leuciscidae). *Ege Journal of Fisheries and Aquatic Sciences*, *38*(3), 311-315. https://doi.org/10.12714/egejfas.38.3.07

Vujosevic, M., Zivkovic, S., Rimsa, D., Jurisic, S., & Cakic, P. (1983). The chromosomes of 9 fish species from Dunav basin in Yugoslavia. *Ichthyologia, 15*(2), 29-40.

Wolf, U., Ritter, H., Atkin, N., & Ohno, S. (1969). Polyploidization in the fish family Cyprinidae, order Cypriniformes. *Humangenetik, 7*, 240-244. https://doi.org/10.1007/BF00273173

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 56-61 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.08

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Selectivity of monofilament gillnets with different mesh sizes used in fishing of pearl mullet, (*Alburnus tarichi* (Güldenstädt, 1814)) in Lake Van

## Van Gölü İnci Kefali (*Alburnus tarichi* (Güldenstädt,1814)) avcılığında kullanılan farklı ağ göz açıklığına sahip monofilament sade uzatma ağlarının seçiciliği

**Mustafa Akkuş[1]* ● Adem Sezai Bozaoğlu[2] ● Ahmet Raif Eryaşar[3]**

[1] Faculty of Fisheries, Van Yüzüncü Yıl University, 65080, Van, Türkiye https://orcid.org/0000-0002-8900-9495
[2] Faculty of Fisheries, Van Yüzüncü Yıl University, 65080, Van, Türkiye https://orcid.org/0000-0003-4078-5159
[3] Vocational School of Technical Sciences, Recep Tayyip Erdoğan University, 53100, Rize, Türkiye https://orcid.org/0000-0001-7656-6113

*Corresponding author: makkus@yyu.edu.tr

Received date: 11.11.2022 Accepted date: 10.02.2023

***How to cite this paper:***
Akkuş, M., Bozaoğlu, A.S., & Eryaşar, A.R. (2023). Selectivity of monofilament gillnets with different mesh sizes used in fishing of pearl mullet, (*Alburnus tarichi* ( Güldenstädt, 1814)) in Lake Van. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 56-61. https://doi.org/10.12714/egejfas.40.1.08

**Abstract:** This study was carried out between September 2020 and March 2021 to determine the selectivity of monofilament gillnets used in pearl mullet, *Alburnus tarichi* (Güldenstädt, 1814), fishing in Lake Van. The SELECT method was applied to determine the selectivity. The best model for the selectivity of monofilament gillnets used in pearl mullet fishing was determined as the log-normal model. The optimum catch lengths of monofilament gillnets with 20, 22 and 24 mm mesh sizes were calculated as 21.69 cm, 23.84 cm and 26.01 cm, respectively. Catch per unit effort was determined as 10.03 kg/90 m/day at 20 mm, 15.91 kg/90 m/day at 22 mm and 12.99 kg/90 m/day at 24 mm. The average catch per unit effort was determined as 151 unit/90 m/day at 20 mm, 151 unit/90 m/day at 22 mm, and 110 unit/90 m/day at 24 mm. According to the results of optimum catch length and daily average catch per unit effort, using monofilament gillnets with 22 mm mesh size was more convenient for sustainable pearl mullet fishing in Lake Van.

**Keywords:** Pearl mullet, gillnet, selectivity, Lake Van

**Öz:** Bu çalışma, Van Gölü inci kefali, *Alburnus tarichi* (Güldenstädt, 1814), avcılığında kullanılan monofilament sade uzatma ağlarının seçiciliğinin belirlenmesi amacıyla 2020 Eylül ve 2021 Mart tarihleri arasında gerçekleştirilmiştir. Seçiciliğin belirlenmesinde SELECT metod kullanılmıştır. İnci kefali avcılığında kullanılan monofilament galsama ağlarının seçiciliği için en iyi modelin log-normal modeli olduğu belirlenmiştir. 20, 22 ve 24 mm ağ göz genişliğine sahip monofilament sade uzatma ağlarının optimum yakalama boyları sırasıyla 21,69 cm, 23,84 cm ve 26,01 cm olarak hesaplanmıştır. Birim av verimi 20 mm de 10,03 kg/90 m/gün, 22 mm de 15,91 kg/90 m/gün ve 24 mm de 12,99 kg/90 m/gün olarak tespit edilmiştir. Adet olarak ortalama birim av verimi 20 mm de 151 adet/90 m/gün, 22 mm de 151 adet/90 m/gün ve 24 mm de 110 adet/90 m/gün olarak belirlenmiştir. Optimum yakalama boyu ve günlük ortalama birim av verimi sonuçlarına göre, Van Gölü'nde sürdürülebilir inci kefali avcılığı için 22 mm ağ göz genişliğindeki monofilament galsama uzatma ağlarının kullanılmasının daha uygun olduğu belirlenmiştir.

**Anahtar kelimeler:** İnci kefali, monofilament uzatma ağ, seçicilik, Van Gölü

## INTRODUCTION

Lake Van, the largest lake in Türkiye with a surface area of 3547 km$^2$, has a maximum depth of 450 meters and an average of 171 meters. It has extreme water quality characteristics with a pH of 9.5 and a salinity of 21.28‰ (Sarı, 1997). The pearl mullet, *Alburnus tarichi* (Güldenstädt, 1814), which is well adapted to the salty and sodic waters of the lake, is the only species caught in the lake. A total of 33140 tons of fish were caught in the inland waters of Türkiye in 2021 and pearl mullet caught from Lake Van constitutes approximately 1/3 of this figure with 9925 tons (TUIK, 2022). Fishing is one of the major sources of income today (Aura et al., 2018).

Advances in technology and increased catch pressure on fish stocks have led to the overexploitation of many fish stocks in Türkiye and around the globe (Williams, 1998; Mullon et al., 2005; Kılıç, 2014). Regarding sustainable use of fish stocks, it is essential to consider the biological data of fish stocks as well as the features of fishing gear used in fishing. The selectivity of the gillnet is crucial in the sustainable use of fish stocks. Selectivity in gillnets is associated with the shape, size, and behavioral characteristics of the fish, and the colour, hanging ratio and rigging factor of the net. (Rosman and Maugeri, 1980; İlkyaz, 2005).

In the Lake Van Basin, studies to improve the gillnet selectivity were aimed at increasing the mesh size, using different twine thicknesses, or testing different rigging properties (Çetinkaya et al., 1995; Sarı and Tokaç, 2000). Since *A. tarichi* is the only commercial species fished in Lake Van, the contribution of aforementioned studies, which have generally given positive outcomes, has been through the elimination of small individuals rather than species selectivity. It was stated that the minimum mesh size should not be less than 20 mm and the catchable fish length should not be less

than 18 cm for a sustainable pearl mullet fishery in Lake Van (Sarı, 1997). Increases in the average fish size of the pearl mullet population have been determined in recent years (Bozaoğlu et al., 2019). This change in fish size over time is expected to affect selectivity. There is a need to examine the current situation in terms of selectivity. To our knowledge, there is no current study on the selectivity of monofilament gillnets used in pearl mullet fishing. Therefore, the selectivity of monofilament gillnets with different mesh sizes was evaluated in the present study.

## MATERIAL AND METHODS

The present study was conducted with 12 fishing operations in Lake Van between September 2020 and March 2021 (Figure 1). In the study, gill nets with 20–22–24 mm nominal mesh sizes were investigated. The technical plan of used nets was given in Figures 2, 3, and 4. Fishing was carried out the coast of Edremit in Lake Van. The nets were deployed at 9:00 a.m. and collected at the same time the following day. The ground of the study area is sandy.

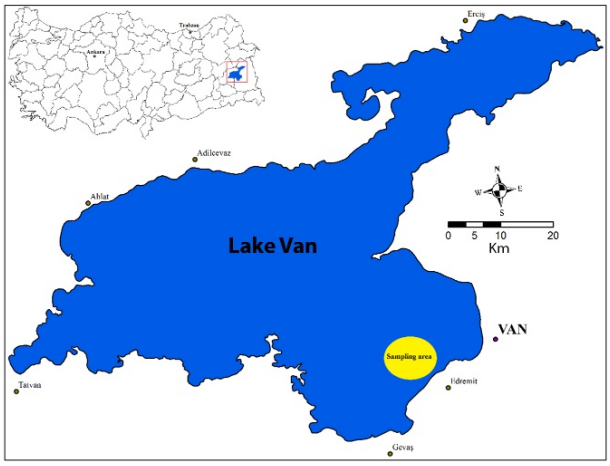



**Figure 1.** Lake Van and the sampling area

### Selectivity Estimation

The SELECT (Share Each Length's Catch Total) method was applied to estimate the selectivity (Millar, 1992; Millar and Holst, 1997; Millar and Fryer, 1999). In this method, as shown in the equation below, the number of fish with *l* length caught in the gillnet with *j* mesh size has an *nlj* Poisson distribution.

$$nlj \approx nlj \approx \text{Pois}\ (pj\ \lambda l\ rj(l))$$

In the equation, *nlj* is the number of fish of length *l* caught in a mesh, *pj* (*l*) is the relative fishing density, and λl is the abundance of fish of length *l* that contacts the gillnet and the number of fish of length *l* caught in the gillnet with *J* mesh of the Poisson distribution is *pj (l) λl*.

The distribution *rj*(*l*) generates the selectivity curve for the j mesh. The log-likelihood function of *nlj* in the equation is calculated as shown below:

$$\sum_{l}\sum_{j}\{n_1\ log[p_j\lambda_i r_j(l)] - p_j\lambda_i r_j(l)\}$$

PASGEAR II software programme (version 2.5; Kolding and Skalevik, 2011) was used in the analysis of the data obtained in the present study. The software makes calculations using 5 different models (normal location, normal scale, log-normal, gamma and bi-modal) (Millar and Fryer, 1999). Among these models, the model with the least deviation was determined as the most suitable model.

Normal location;

$$exp\left(-\frac{(L-k\cdot m_j)^2}{2\sigma^2}\right)$$

Normal scale;

$$exp\left(-\frac{(L-k\cdot m_j)^2}{2k_2^2 m_j^2}\right)$$

Log-normal;

$$\frac{1}{L}exp\left(\mu + log\left(\frac{m_i}{m_1}\right) - \frac{\sigma^2}{2} - \frac{\left(log(L) - \mu - log\left(\frac{m_i}{m_1}\right)\right)^2}{2\sigma^2}\right)$$

Gamma;

$$\left(\frac{L}{(\alpha-1).k\cdot m_j}\right)^{\alpha-1} exp\left(\alpha - 1 - \frac{L}{k\cdot m_j}\right)$$

Bi-modal;

$$exp\left(-\frac{(L-k_1\cdot m_j)^2}{2k_2^2\cdot mj^2}\right) + c\cdot exp\left(-\frac{(L-k_3 m_j)^2}{2k_4^2\cdot m_j^2}\right)$$

### Calculating Catch Per Unit Effort (CPUE) and YPUE (number of weight)

CPUE and YPUE of gillnets with different mesh sizes was calculated. The CPUE and YPUE for each gillnet group was calculated using the following formula (Godøy et al., 2003).

$$YPUE_i = \frac{ci}{ni\ si} \qquad CPUE_i = \frac{ci}{ni\ si}$$

ci =i. represents the total number of individuals (CPUE) and total weight (YPUE) caught with the tested gillnet group

ni =i. represents the number of gillnet in the gillnet group

si = i. represents the number of days in the gillnet group

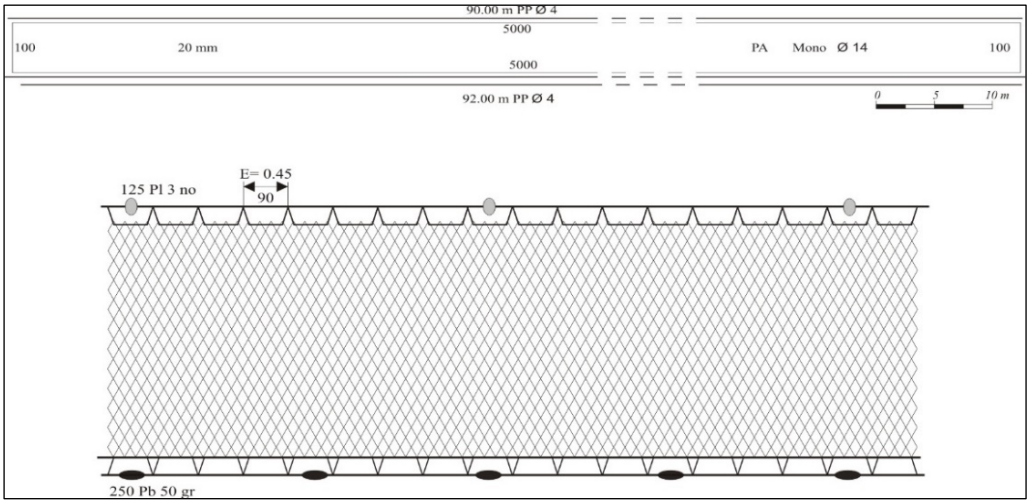



**Figure 2.** Technical plan of monofilament gillnet with 20 mm mesh size

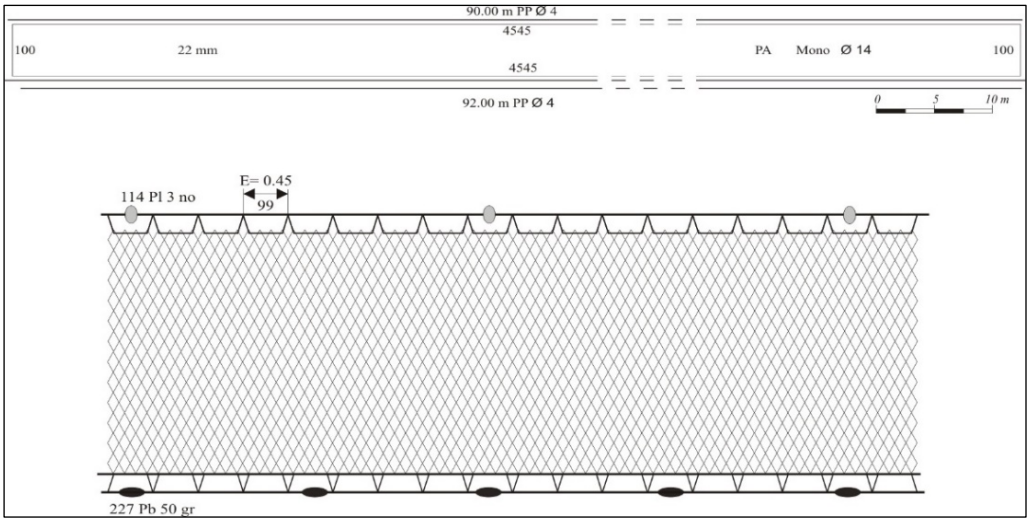



**Figure 3.** Technical plan of monofilament gillnet with 22 mm mesh size

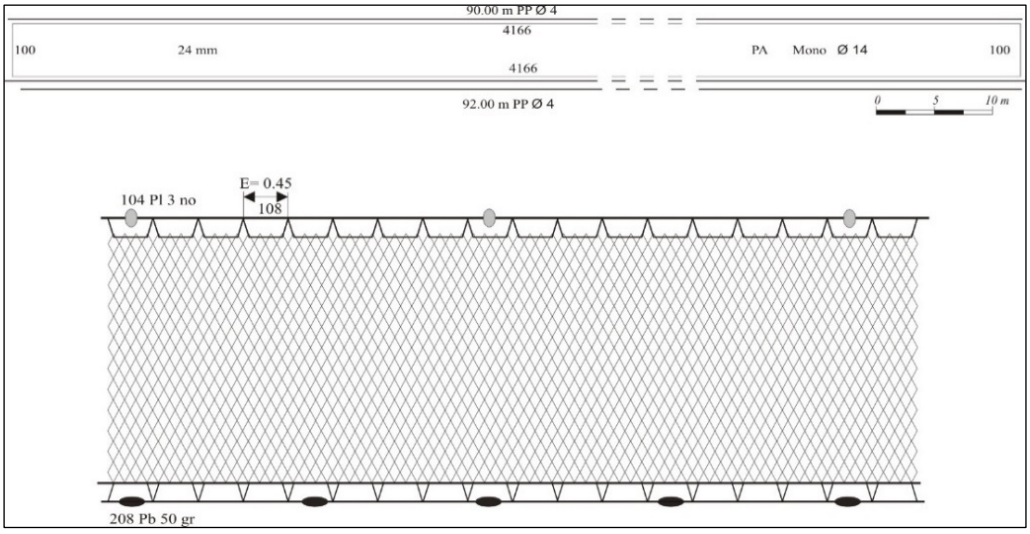



**Figure 4.** Technical plan of monofilament gillnet with 24 mm mesh size

## RESULTS

A total of 4943 pearl mullets (470.3 kg in total) were sampled during the study. Catch data of tested gill nets were given in Table 1.

**Table 1.** Maximum and minimum length-weight values of fish caught using the gillnets with 20 mm, 22 mm and 24 mm mesh sizes

| Mesh size (mm) | Number of fish | Total weight (kg) | Minimum length (cm) | Maximum length (cm) | Average length (cm) |
|---|---|---|---|---|---|
| 20 | 1810 | 123.5 | 17 | 25 | 19.78 ± 1.32 |
| 22 | 1811 | 190.9 | 19 | 27 | 22.50 ± 1.36 |
| 24 | 1322 | 155.9 | 19 | 28 | 23.23 ± 1.39 |

According to the SELECT method, the most suitable method was determined as the Log-normal model with the lowest deviation (Table 2). In the study, spread values and optimum catch lengths for gillnets with several mesh sizes were given in Table 3 and selectivity curves were shown in Figure 5. According to mesh sizes, the optimum catch length was found as 21.69 cm for 20 mm mesh size, 23.84 cm for 22 mm mesh size, and 26.01 cm for 24 mm mesh size (Table 3).

**Table 2.**The selectivity parameter values of the pearl mullet

| Model | Parameters | Deviance | p-value | Degree of freedom |
|---|---|---|---|---|
| Normal location | (k, σ) = (1.078, 2.903) | 311.059 | 0,00000 | 28 |
| Normal scale | (k1, k2) = (1.090, 0.129) | 325.096 | 0,00000 | 28 |
| Log-normal | (μ1, σ) = (3.090, 0.126) | 304.825 | 0,00000 | 28 |
| Gamma | (k, α) = (0.016, 66.975) | 309.868 | 0,00000 | 28 |
| Bi-modal | (k1, k2, k3, k4, w) = (0.469, -0.005, -1.090, 0.129, 846.348 | 325.096 | 0,00000 | 25 |

**Table 3.** Optimum catch length and spread values for the most suitable model (Log-normal)

| Mesh size (mm) | Optimum catch length (cm) | Spread value (cm) |
|---|---|---|
| 20 | 21.69 | 2.69 |
| 22 | 23.84 | 2.96 |
| 24 | 26.01 | 3.22 |

### YPUE and CPUE

YPUE and CPUE values for the number and weight of captured fish with tested gill nets were calculated. According to the results, the YPUE in weight was determined as 10.03 kg/90 m/day at 20 mm mesh size, 15.91 kg/90 m/day at 22 mm mesh size and 12.99 kg/90 m/day at 24 mm mesh size. The CPUE in number/fish was designated as 151 units/90 m/day at 20 mm mesh size, 151 units/90 m/day at 22 mm mesh size and 110 units/90 m/day at 24 mm mesh size.

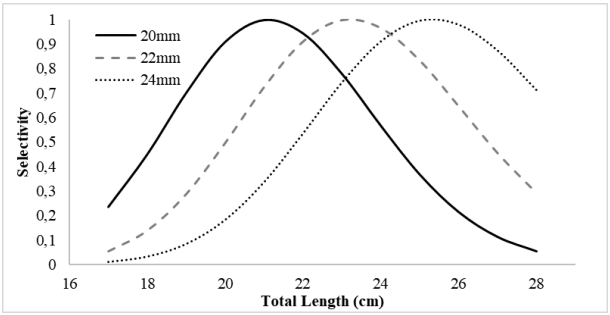



**Figure 5.** Selectivity curves for mesh sizes

## DISCUSSION

Although the natural habitat of pearl mullet is Lake Van, it is indeed a migratory species that migrate from the lake to the streams in flocks during its reproduction period (April-July). Therefore, Pearl mullet fishing is prohibited between April 15 and July 15 in the Lake Van Basin (BSGM, 2020). The distribution of pearl mullet differs according to season. When the lake temperature is high, the fish prefer to live close to the shores between of 3–20 meters, whereas they can be found up to 140 meters in depth during winter months. Since the pearl mullet lives in the deep parts of the lake during the winter months, November, December, January and February, fishing is performed using the multifilament trammel nets. On the other hand, in the summer season fishing is carried out using monofilament gillnets in the areas close to the surface and the coast.

One of the main causes of loss in fish stocks is non-selective fishing gears (Alverson et al., 1994). For the sustainable use of fish stocks, the nets used in fishing should have an accurate mesh size and selectivity that does not harm the stock (Regier and Robson, 1966). Since the selectivity properties of monofilament gillnets used in Lake Van are undetermined, this may put the sustainable use of pearl mullet stock at risk. There is no current study on the selectivity of monofilament gillnets in Lake Van. For this reason, this study was carried out to investigate the effect of different mesh sizes on the selectivity of monofilament gillnets to ensure the sustainability of pearl mullet fishing in Lake Van.

This study determined the optimum catch length as 21.69 cm for 20 mm mesh size, 23.84 cm for 22 mm mesh size, and 26.01 cm for 24 mm mesh size (Table 3). The result of the study on the selectivity of monofilament gillnets in Lake Van conducted by Özdemir (2000) showed that the optimum catch length varied between 14.8–17.1 cm using 18 mm mesh size, and 16.4–19.1 cm using 20 mm mesh size. In another study, Çetinkaya et al. (1995) used 17 mm, 20 mm and 24 mm mesh sizes to determine the catch efficiency and selectivity of multifilament trammel nets used in pearl mullet fishing. The average lengths of the fish caught in the related study were determined as 15.6 cm, 20.2 cm and 20.8 cm, respectively

(Çetinkaya et al., 1995). The values obtained in the current study are higher than the values acquired from the previous studies conducted in Lake Van. Avşar (2005) stated, a decrease in the size of the fish that constitute the stock is the most obvious indicator of overfishing. In recent years, it was reported that the average length of the pearl mullet has increased with the effective conservation measures over the years in Lake Van (Bozaoğlu et al., 2019). This situation was interpreted that the difference being due to the increase in the size of the pearl mullet because of the conservation efforts.

The optimum catch length calculated from all gillnet groups was determined higher than the first reproduction length of pearl mullet. Therefore, it is not anticipated that the monofilament gillnets used in Lake Van will cause any damage to the pearl mullet stock of Lake Van. However, the results of the present study showed that the average catch per unit effort for 22 mm mesh size was higher than gill net groups. Sarı (1997) reported that the average catch per unit effort was 3.538 kg/90 m/day for the 1994-1995 fishing season and 2.677 kg/90 m/day for the 1995-1996 fishing season in Lake Van. According to another report conducted by Çetinkaya et al. (1995), the results were reported as 881.4-4747.6 g/90 m/24 hours at 17 mm mesh size, 680-7608.3 g/90 m/24 hours at 22 mm mesh size, and 1684.3-2367.7 g/90 m/24 hours at 24 mm mesh size. There were notable differences between the average catch per unit effort obtained in the current study and previous reports. The most important reason for this remarkable difference is the structure of the mesh material used in these studies. While other studies used multifilament trammel nets, monofilament gillnets were utilized in the present study. As it is well known, it has been reported that the selectivity of gillnets is better than that of trammel nets (Sürer and Kuşat, 2013).

## CONCLUSION

Lake Van is the biggest fishing area in the inland water of Turkey. There are more than 100 fishing boats in the lake. The use of gillnets with appropriate mesh size in the lake where intensive fishing activities are carried out is important for fisheries management and sustainability.

Consequently, according to the data obtained in this study, for sustainable and efficient use of stocks, we recommend using 22 mm and above for monofilament gillnets in pearl mullet fishing in Lake Van.

## ACKNOWLEDGMENTS AND FUNDING

This study was financially supported by Van Yüzüncü Yıl University Scientific Research Projects Coordination Unit (FHD-2020-8664).

## AUTHORS CONTRIBUTION

Mustafa Akkuş: Data acquisition, statistical analysis, writing – review & editing. Ahmet Raif Eryaşar: Hardware-software implementation, statistical analysis, validation, editing. Adem Sezai Bozaoğlu: Data acquisition, writing, review and editing.

## STATEMENT OF CONFLICT OF INTEREST

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

## ETHICS APPROVAL

The research was approved by Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee in terms of sampling and use of experimental animals with decision number 2/2 at the meeting held on 03.10.2019. All researchers declare that all trials were conducted in accordance with ethical values.

## DATA AVAILABILITY

The corresponding author should be contacted for questions about datasets.

## REFERENCES

Alverson, D.L., Freeberg, M.H., Pope, H., & Murawski, S.A. (1994). A Global assessment of fisheries by-catch and discards. FAO Fish Technical Paper No: 339, 233p.

Aura, C.M., Nyamweya, C.S., Njiru, J.M., Musa, S., Ogari, Z., May, L., & Wakwabi, E. (2018). Exploring the demarcation requirements of fish breeding and nursery sites to balance the exploitation, management and conservation needs of Lake Victoria ecosystem. *Fisheries Management and Ecology*, 1-9. https://doi.org/10.1111/fme.12311

Avşar, D. (2005). *Fisheries Biology and Population Dynamics*. Nobel Yayınevi, 332 s. (in Turkish)

Bozaoğlu, A.S., Akkuş, M., & Yeşil, A. (2019). Pearl Mullet (*Alburnus tarichi* (Guldenstaedtii, 1814)) Fishing with trammel nets in Lake Van. *Commagene Journal of Biology, 3*(1): 27-31. https://doi.org/10.31594/commagene.547234

BSGM, (2020). Regulation for Commercial Fisheries in Seas and Inland Waters for 2020–2024 Fishing Period. Number 5/1 (No: 2020/20). Ankara: General Directorate of Fisheries and Aquaculture (BSGM), Republic of Turkey Ministry of Food Agriculture and Livestock; 69 pp. (in Turkish)

Çetinkaya, O., Sarı, M., & Arabacı, M. (1995). A Preliminary study on catch composition and selectivity of the trammel net used in fishing of *Chalcalburnus tarichi* (Pallas 1811) in Lake Van (Türkiye). *Su Ürünleri Dergisi*, 12 (1-2), 1-13. (in Turkish with English abstract)

Godøy, H., Furevik, D., & Løkkeborg, S. (2003). Reduced bycatch of red king crab (Paralithodes camtschaticus) in the gillnet fishery for cod (Gadus morhua) in northern Norway. *Fisheries Research*, *62*(3), 377-384. https://doi.org/10.1016/S0165-7836(02)00281-3

İlkyaz, A.T. (2005). Determination of gillnet size selectivity by the direct estimation method. Ege University Graduate School of Natural and Applied Sciences, PhD Thesis, 131 p.

Kılıç, S. (2014). A new concept "precautionary fisheries management" for the management of fish stocks in Turkey seas. *Yunus Araştırma Bülteni*, (*4*): 85-97.

Kolding, J., & Skalevik, A. (2011). PasGear 2. A database package for experimental or artisanal fishery data. Version 2.5. Retrieved from http://www.imr.no/forskning/utviklingssamarbeid/eaf_nansen_programmet/pasgear_2/en

Millar, R.B. (1992). Estimating the size-selectivity of fishing gear by conditioning on the total catch. *Journal of the American Statistical*

*Association*, *87*, 962- 968. https://doi.org/10.1080/01621459.1992.10476250

Millar, R.B., & Fryer R.J. (1999). Estimating the size-selection curves of towed gears, traps, nets and hooks. *Reviews in Fish Biology and Fisheries*, *9*, 89-116. https://doi.org/10.1023/A:1008838220001

Millar, R.B., & Holst, R. (1997). Estimation of gillnet and hook selectivity using Log-linear Models. *ICES Journal of Marine Science*, *54*, 471-477. https://doi.org/10.1006/jmsc.1996.0196

Mullon, C., Freon, P., & Cury, P. (2005). The dynamics of collapse in world fisheries. *Fish and Fisheries*, *6*(2), 111-120. https://doi.org/10.1111/j.1467-2979.2005.00181.x

Özdemir, H. (2000). A study on the catch yield and selectivity of monofilament gillnets used in the fishing of İnci Kefali (*Chalcalburnus tarichi* Pallas, 1811) in Lake Van. Master Thesis, Yüzüncü Yıl University Department of Fisheries and Processing Technology, Van, 68 p.

Regier, H.A. & Robson, D.S. (1966). Selectivity of gill nets, especially to lake whitefish. Journal Fisheries Research Board Canada. *23,* 423-454p. https://doi.org/10.1139/f66-034

Rosman, I. & Maugeri, S. (1980). Fishing with bottom gillnets, FAO training series 3, Roma, 39p.

Sarı, M. (1997) The Stock Assessment of *Chalcalburnus tarichi*, Pallas 1811 in The Lake of Van and The Determination of The Basis Fishery Management. PhD Thesis. Ege University Department of Fisheries and Processing Technology. İzmir, 150p. (in Turkish).

Sarı, M., & Tokaç, A. (2000). Comparison of the catching efficiency of the two different trammel nets used in fishing of *Chalcarburnus tarichi*, Pallas (1811). *Ege Journal of Fisheries and Aquatic Sciences*, *17*(3-4):27-33

Sürer, M.İ., & Kuşat, M. (2013). Comparative of catching and economic efficiency of monofilament and multifilament gill nets in Eğirdir Lake. *Suleyman Demirel University Journal of Natural and Applied Science*, *17*(1), 43-48.

TUIK (2022). Fishery Statistics of Turkey. https://data.tuik.gov.tr/Bulten/Index?p=Su-Urunleri - 2021-45745 (07.08.2022).

Williams, N. (1998). Overfishing Disrupts Entire Ecosystems. *Sciences*, *279*, 809-809. https://doi.org/10.1126/science.279.5352.809

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 62-68 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.09

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Karyotypic analysis of *Chondrostoma regium* (Teleostei: Leuciscidae) distributed in the Karasu River (Erzurum)

## Karasu ırmağında (Erzurum) yayılış gösteren *Chondrostoma regium*'un (Teleostei: Leuciscidae) karyotipik analizleri

**Büşra Yamaç[1] ● Turgay Şişman[2*]**

[1] Department of Biology, Science Faculty, Atatürk University, 25240, Erzurum, Türkiye https://orcid.org/0000-0002-0469-3321
[2] Department of Molecular Biology and Genetics, Science Faculty, Atatürk University, 25240, Erzurum, Türkiye https://orcid.org/0000-0002-1098-1741

*Corresponding author: tsisman@atauni.edu.tr
Received date: 21.10.2022 Accepted date: 13.02.2023

**How to cite this paper:**
Yamaç, B., & Şişman, T. (2023). Karyotypic analysis of *Chondrostoma regium* (Teleostei: Leuciscidae) distributed in the Karasu River (Erzurum). *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 62-68. https://doi.org/10.12714/egejfas.40.1.09

**Abstract:** In this study, the karyotypic characteristics of *Chondrostoma regium* (Heckel, 1843) have been investigated. Fish samples were caught from the Karasu River (Euphrates River Basin) with fishing net. The live fish were transported to the laboratory and kept in aerated aquaria before the analyses. The karyotype analysis was performed in fish kidney and gill epithelium cells. It was determined that *C. regium* had 2n=50 chromosomes. In detail, the karyotype formula of *C. regium* was determined as 9 metacentric, 7 submetacentric, 1 subtelocentric and 8 telocentric chromosome pairs (18M+14SM+2ST+16T), and fundamental arm number was calculated as 82. Constitutive heterochromatin regions were determined on telomeres of the chromosomes. Nuclear orgnizer regions were detected on 21st chromosome. Karyotype symmetry/asymmetry index was calculated as 2.32. The karyotypes of gill and kidney cells were the same. No sex chromosomes were cytologically detected.

**Keywords:** *Chondrostoma regium*, fish cytotaxonomy, chromosome banding

**Öz:** Bu çalışmada *Chondrostoma regium*'un (Heckel, 1843) karyotipik özellikleri araştırılmıştır. Balık numuneleri Karasu Irmağından (Fırat Nehri havzası) serpme ağlarla yakalanmıştır. Analizlerden önce canlı balıklar laboratuvara taşınmış ve havalandırmalı akvaryumlarda tutulmuştur. Karyotip analizi balıkların böbrek ve solungaç epitel hücrelerinde yapılmıştır. *C. regium*'un 2n=50 kromozoma sahip olduğu belirlenmiştir. Ayrıntılı olarak *C. regium*'un karyotip formülü 9 metasentrik, 7 submetasentrik, 1 subtelosentrik ve 8 telosentrik kromozom çifti (18M+14SM+2ST+16T) olarak belirlenmiş olup, temel kol sayısı 82 olarak hesaplanmıştır Kromozomların telomerlerinde konstitütif heterokromatin bölgeleri belirlenmiştir. 21. kromozom üzerinde nüklear organizer bölge tespit edilmiştir. Karyotip simetri/asimetri indeksi 2,32 olarak bulunmuştur. Solungaç ve böbrek hücrelerinin karyotipi aynı olup sitolojik olarak hiçbir eşey kromozomu tespit edilmemiştir.

**Anahtar kelimeler:** *Chondrostoma regium*, balık sitotaksonomisi, kromozom bantlama

## INTRODUCTION

It is known that 391 fish species are living in freshwaters (Saygun, 2021) and 512 fish species in the seas (Bilecenoğlu et al., 2014) in Turkey. According to the cytotaxonomic studies, it is seen that the karyotype of approximately 89% of these species has not been determined. From a methodological point of view, although molecular cytogenetic methods have become widespread in the world, traditional cytotaxonomic methods (Giemsa, GTG-, AgNOR-, C-, Q- and RE-banding made by air drying and culture methods) are used even in the latest studies (Araya-Jaime et al., 2020; Goes et al., 2020; Moreva, 2020). Finally, there are studies in Turkey in the field of cytogenetics that examine only some studies on carp (Saygun, 2021). However, no study has yet been conducted on the karyotype of *Chondrostoma regium*.

*Chondrostoma* is a genus of the family Leuciscidae and this genus consists of about 26 species (Freyhof, 2014). A total of thirteen *Chondrostoma* species (*C. beysehirense, C. ceyhanensis, C. colchicum, C. cyri, C. holmwoodii, C. meandrense, C. nasus, C. kinzelbachi, C. regium, C. smyrnae C. toros, C. turnai* and *C. vardarense*) are reported to be found in various regions of Turkey (Küçük et al., 2007; Özcan, 2008; İlhan, 2009; Freyhof and Özuluğ, 2009; Kuru et al., 2014; Çiçek et al., 2015; Küçük et al., 2017; Güçlü et al., 2018; Çiftçi et al., 2020; Küçük et al., 2021; Küçük et al., 2023).

The genus *Chondrostoma* also disributed in the Caspian Sea, Isfahan, Tigris-Euphrates and Kor River basins. Four *Chondrostoma* species (*C. cyri, C. esmaeilii, C. regium* and *C. orientale*) are in Iranian inland waters (Eagderi et al., 2017). There are also records of *C. regium* being found in Greater Zab River, west of the Erbil city, Iraq (Al-Marjan, 2016). Various studies have been conducted on the general biology, taxonomy, karyology and morphology of the species in Iran (Esmaeili et al., 2010; Jouladeh-Roudbar, 2014). Karyotype analyzes of *Chondrostoma* species were also described from various geographical regions of Europe. For example, karyotype analysis of *Iberochondrostoma lusitanicum* (formerly *Chondrostoma lusitanicum*) in Portugal has been reported by Collares-Pereira and Ráb (1999). Chromosome studies of *C.*

*nasus* were performed by Ráb et al. (2008) and Boroń et al. (2020). Karyotype analyzes of *Chondrostoma* species were also defined from different regions of Turkey (Arslan and Gündoğdu, 2016). There are only two karyotype studies on *C. regium*. One of these studies was reported from Iran (Esmaeili et al., 2010) and the other from Turkey (Kaya, 2009). Moreover, nucleolar organizer region and constituve heterochromatin characteristics in the *C. regium* were not reported. In this study, karyotypic properties of *C. regium* were determined by using C-banding, Ag-NOR and giemsa staining techniques. Chromosomal banding findings were compared with those obtained from previous studies on *Chondrostoma* species.

## MATERIAL AND METHODS

*Chondrostoma regium* specimens (12♀♀, 10♂♂) (Figure 1) were caught from Karasu River, Erzurum province in eastern Turkey. The fish were brought alive to the laboratory and placed in well-aerated aquaria.

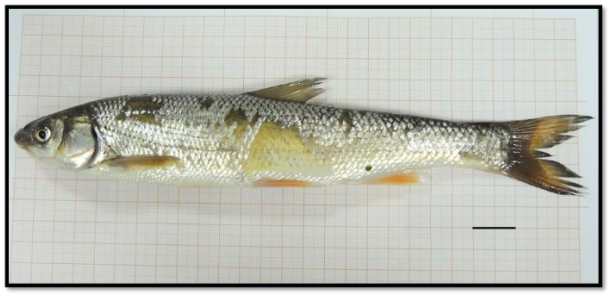


**Figure 1.** *Chondrostoma regium* Karasu River (Erzurum), 236 mm SL

For karyotypic analysis, Collares-Pereira's (1992) air drying technique was modified and applied. On the other hand, C-banding technique of Sumner (1972) was performed to detect constitutive heterochromatin regions in chromosomes. Ag-NOR staining was performed to detect nucleolar organizer regions (NOR) in chromosomes. For this, the method of Howell and Black (1980) was used. The preparations prepared in two ways were examined under a Leica DM750 light microscope and a computer-aided microscope unit with a Leica ICC50 HD camera at x100 magnification, and the best metaphase plaque spreads were photographed. At least 10 preparations from each tissue of each of the fish caught in the study (Denton, 1973) were evaluated. Karyotypes were prepared by classifying them according to their chromosome lengths. For this, chromosome arms were measured. Arm measurements were made using the Leica LAS EZ 3.0 image analysis program. The arm ratio (AR), centromeric index (CI) and relative arm length (RAL) of each chromosome were calculated. This process was performed in accordance the method of Levan et al. (1964). Karyotype symmetry and asymmetry were also calculated according to Eroğlu (2015).

## RESULTS

Total 580 and 644 metaphase plaques were counted in the gill and kidney tissues taken from female and male *C. regium* species. The most repeated value was found to be 2n=50 in both tissues. In the karyogram prepared from the metaphase plaques, it was determined that 9 pairs of chromosomes were metacentric (M), 7 pairs were submetacentric (SM), 1 pair were subtelocentric (ST) and 8 pairs were telocentric (T) (18M+14SM+2ST+16T) (Figure 2).

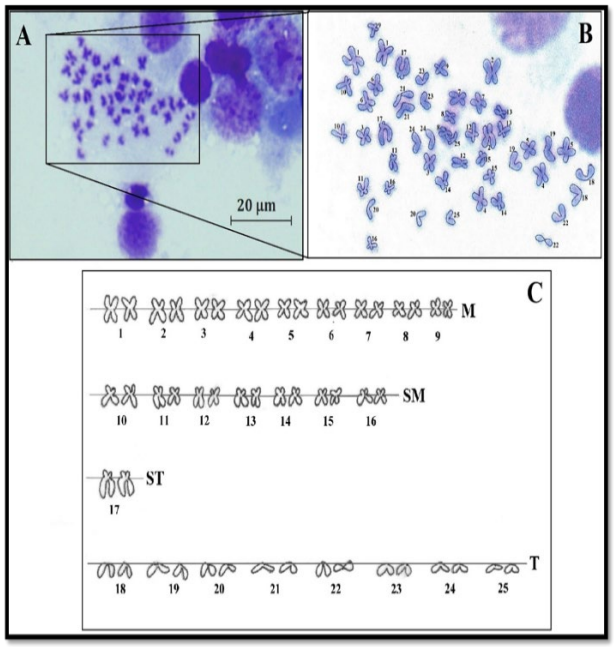



**Figure 2.** A) Metaphase spreads and standard giemsa staining of *Chondrostoma regium*. B) Chromosomes determined by image processing, C) Karyotype: 18M+14SM+2ST+16T (M: metacentric, SM: submetacentric, ST: subtelocentric, T: telocentric)

Chromosome arm lengths were found to vary between 0.9 and 3.9 µm, the longest chromosome was metacentric, the shortest was telocentric, and the Fundamental Arm Number (FN) was 82 (Table 1).

The ideogram prepared according to the measurements was shown in Figure 3. No sex chromosomes were detected in this species. The chromosomal symmetry/asymmetry index value was calculated as 2.32 ($S/A_I$= (1×18) + (2×14) + (3×2) + (4×16) / 50), and it was understood that the karyotype type of *C. regium* was between symmetric and asymmetric.

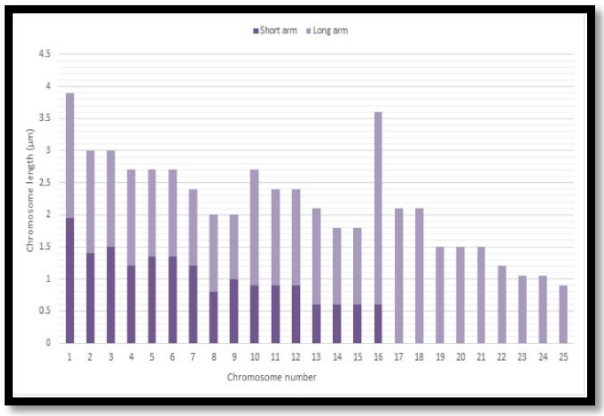



**Figure 3.** Haploid ideogram of *Chondrostoma regium*

**Table 1.** Measurements (in µm) and classification of the chromosomes of *Chondrostoma regium* (M: metacentric, SM: submetacentric, ST: subtelocentric, T: telocentric)

| Chromosome number | Short arm | Long arm | Chromosome length | Arm ratio | Centromeric index | Relative arm length (%) | Chromosome type | Arm number |
|---|---|---|---|---|---|---|---|---|
| 1 | 1.95 | 1.95 | 3.9 | 1.0 | 50 | 7.27 | M | 4 |
| 2 | 1.4 | 1.6 | 3.0 | 1.14 | 46.7 | 5.59 | M | 4 |
| 3 | 1.5 | 1.5 | 3.0 | 1.0 | 50 | 5.59 | M | 4 |
| 4 | 1.2 | 1.5 | 2.7 | 1.25 | 44.44 | 5.03 | M | 4 |
| 5 | 1.35 | 1.35 | 2.7 | 1.0 | 50 | 5.03 | M | 4 |
| 6 | 1.35 | 1.35 | 2.7 | 1.0 | 50 | 5.03 | M | 4 |
| 7 | 1.2 | 12 | 2.4 | 1.0 | 50 | 4.47 | M | 4 |
| 8 | 1.1 | 1.1 | 2.2 | 1.0 | 50 | 3.94 | M | 4 |
| 9 | 1.0 | 1.0 | 2.0 | 1.0 | 50 | 3.73 | M | 4 |
| 10 | 0.9 | 1.8 | 2.7 | 2.0 | 33.33 | 5.03 | SM | 4 |
| 11 | 0.9 | 1.5 | 2.4 | 1.66 | 37.5 | 4.47 | SM | 4 |
| 12 | 0.9 | 1.5 | 2.4 | 1.66 | 37.5 | 4.47 | SM | 4 |
| 13 | 0.8 | 1.5 | 2.3 | 1.88 | 34.78 | 4.12 | SM | 4 |
| 14 | 0.6 | 1.5 | 2.1 | 2.5 | 28,57 | 3.91 | SM | 4 |
| 15 | 0.6 | 1.2 | 1.8 | 2.0 | 33.33 | 3.35 | SM | 4 |
| 16 | 0.6 | 1.2 | 1.8 | 2.0 | 33.33 | 3.35 | SM | 4 |
| 17 | 0.6 | 3.0 | 3.6 | 5.0 | 16.66 | 6.71 | ST | 4 |
| 18 | 0 | 2.1 | 2.1 | 0 | 0 | 3.91 | T | 2 |
| 19 | 0 | 2.1 | 2.1 | 0 | 0 | 3.91 | T | 2 |
| 20 | 0 | 1.5 | 1.5 | 0 | 0 | 2.79 | T | 2 |
| 21 | 0 | 1.5 | 1.5 | 0 | 0 | 2.79 | T | 2 |
| 22 | 0 | 1.5 | 1.5 | 0 | 0 | 2.79 | T | 2 |
| 23 | 0 | 1.2 | 1.2 | 0 | 0 | 2.23 | T | 2 |
| 24 | 0 | 1.05 | 1.05 | 0 | 0 | 1.95 | T | 2 |
| 25 | 0 | 0.9 | 0.9 | 0 | 0 | 1.67 | T | 2 |
| **Total** | 16.85 | 37.25 | 55.8 | - | - | - | - | 82 |

C-Banding method was also used to determine the karyotypic features in detail. In the analysis, it was determined that the constitutive heterochormatine regions of *C. regium* were located at the ends of the 1st and 6th chromosome pairs, and the 13th and 16th chromosome pairs were localized at the end of the long arm (Figure 4). One active Ag-NORs was detected in the species. NORs were observed in the telomeres of the long arms of 21st chromosome (Figure 5).

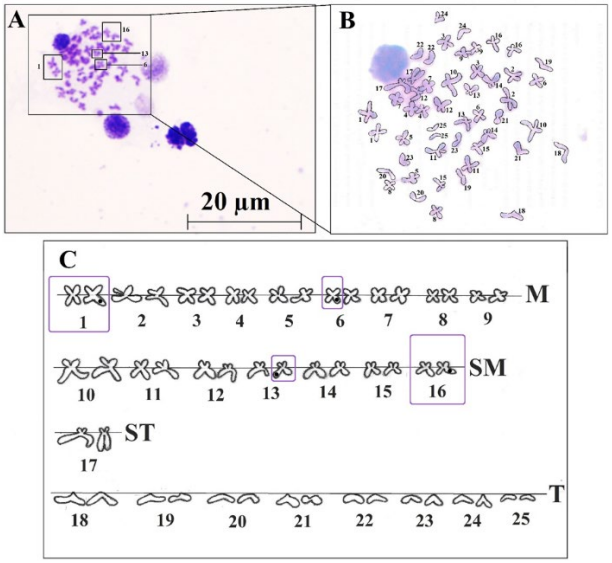



**Figure 4.** A) C-banded metaphase plate of *Chondrostoma regium*. B) Chromosomes determined by image processing. C) Karyotype. Heterochromatin regions were shown in the square

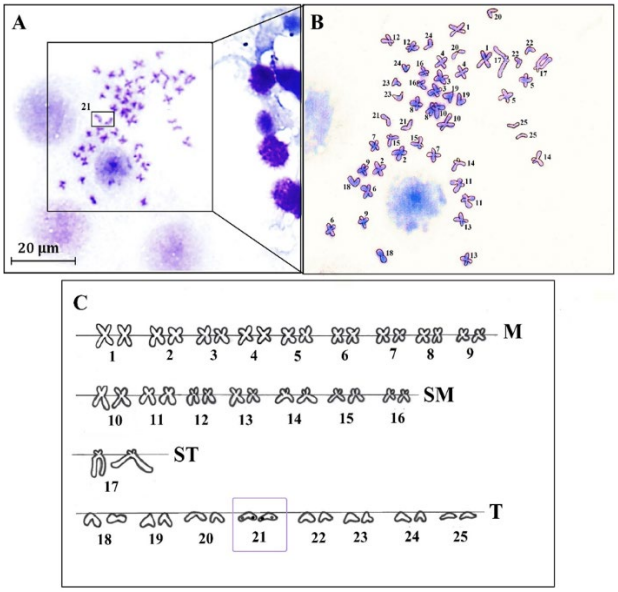



**Figure 5.** A) Ag-NOR stained metaphase plate showing NOR regions of *Chondrostoma regium*. B) Chromosomes determined by image processing. C) Karyotype. NOR region was shown in the square

## DISCUSSION

Since the 1960s, significant contributions have been made to the fields of genetics, taxonomy and environmental toxicology by performing karyological analyzes of fish species belonging to Teleostei (Cucchi and Baruffaldi, 1990). Karyotype provides basic information about chromosome

morphology, size and number, and is used to distinguish controversial species and to construct evolutionary family trees (Tan et al., 2004). In addition, fish karyotype studies provide important datas for polyploidy, gynogenesis, androgenesis, and the generation of new aqua-hybrid species (Dai and Han, 2018). In recent years, an increase in the number of cytogenetic studies applied to fish has been observed. The karyotypes of 3.425 fish species or subspecies are known (Arai, 2011). Leuciscidae is one of the richest and most important fish family. The species of the family have a wide living space in Turkey. In the study, karyotypic analysis of *C. regium*, which is found in the native fauna of the Karasu River (Erzurum, Turkey) and belongs to the Leuciscidae family, was carried out.

There is no cytogenetic study of *C. regium* in the inland waters of Eastern Anatolia to date. In the current study, the chromosome number of *C. regium* inhabiting in Karasu River was determined as 2n=50. As a result of the karyotype analysis, it was determined that there were 9 metacentrics, 7 submetacentrics, 1 subtelocentric and 8 telocentrics chromosome pairs (18M+14SM+2ST+16T) in the species. The arm number was found to be FN=82. Except for this study, there is no previous karyotype study of this species in our country. In the world, only the karyotype of *C. regium* from Iran's Fahlian River has been reported (Esmaeili et al., 2010). In the study, the diploid chromosome number of this species was determined as 2n=52. The karyotype consists of 21 submetacentric and 5 subtelocentric chromosome pairs (42SM+10ST). The FN was determined as 58. Cytologically, no heteromorphic sex chromosomes were found. When the study was compared with the current study results, it was understood that the Karasu river fish specimens was not karyotypically similar to the Persian fish specimens. In addition, it was concluded that the number of arms in Persian fish was calculated incorrectly. Because if even the number of arms of subtelocentric chromosomes is not taken into account, there must be at least 84 arms in 42 submetacentric chromosome sets. Therefore, there is a significant difference between the two species. The difference in normal comparison is due to the separate habitats.

Results of the current study are partially in agreement with the results obtained from other studies with *Chondrostoma* genus (Table 2). There are differences in the number of chromosome arms in the comparative karyotype, which is parallel to the current study in terms of chromosome number. The reason for the difference in karyotype may also be population-related conditions other than the reason mentioned above. Interspecific polymorphism or species at the sampling site, or even inter-population differences can lead to this condition. In addition, other factors such as chromosome losses during preparation, abnormal positions of fixed cells, chromosomes from nearby cells, unidentified microarms in chromosomes, insufficient sample number, errors in chromosome arm measurement, and incorrect determination of chromosome type may cause differences between studies (Arai, 2011; Khosravanizadeh et al., 2011). Therefore, giemsa stained karyotype studies should be supported by other cytogenetic techniques. For this reason, C-banding method was used in the research. C-bands are constitutive regions of heterochromatin containing repetitive DNA sequences that are transcriptionally inactive (Gold et al., 1986). C-banding studies are few in Cyprinidae. The characteristic C-band patterns are mostly specific to Teleostei. Heterochromatin difference can be used in the chromosome evolution of Cyprinidae and the differentiation of species (Gaffaroğlu and Yüksel, 2009). In our study, constitutive heterochromatin was detected in four pairs of chromosomes. To date, no C-banding studies on *C. regium* have been found in the literature.

**Table 2.** Karyotypic features of *Chondrostoma* species living in Turkey and the World-wide

| Species | Locality | Chromosome numbers (2n) – FN- symmetry/asymmetry index | Chromosome formula | C-band | NOR | Reference |
|---|---|---|---|---|---|---|
| *C. regium* | Karasu River (Erzurum) | 50 – 82 – 2.4 S/A | 18M+12SM+2ST+18T | 1., 6., 13., 16. | 21. | This study |
| *C. regium* | Göksu River (Mersin) | 50 – 86 – 2.24 S/A | 22M+8SM+6ST+14T | - | - | Kaya, 2009 |
| *C. regium* | Fahlian River (Iran) | 52 – 58 – 2.19 S/A | 42SM+10ST | - | - | Esmaeili et al., 2010 |
| *C. beysehirense* | Lake Beyşehir (Konya) | 50 – 92 – 2.14 S/A | 20M+22SM/ST+8T | 1., 3., 4., 7., 8., 15. | 6. | Arslan and Gündoğdu, 2016 |
| *C. meandrense* | Menderes River (Aydın) | 52 – 82 – 2.61 S/A | 18M+6SM+6ST+22T | - | - | Uysal, 2011 |
| *C. nasus* | Drian River (Bosnia) | 50 – 88 – 2.28 S/A | 38M/SM+12T | - | - | Barshiene, 1977 |
| *C. knerii* | Neretva River (Yugoslavia) | 50 – 86 – 2.56 S/A | 36M/SM+14T | - | - | Berberović et al., 1970 |
| *C. prespense* | Lake Prespa (Italy) | 50 – 92 – 2.0 S | 16M+26SM+8T | - | - | Bianco et al., 2004 |
| *C. soetta* | Po River (Italy) | 50 – 94 – 2.2 S/A | 16M+14SM+14ST+6T | - | - | Cataudella et al., 1977 |
| *C. phoxinus* | Neretva River (Yugoslavia) | 50 – 86 – 2.56 S/A | 36M/SM+14ST/T | - | - | Berberović et al., 1970 |

NORs are regions where nucleoli are in contact on chromosomes and contain genes that transcribe rRNA. Systematic and phylogenetic comparisons between species can be made with chromosomal NOR information (Amemiya and Gold, 1988). The number and location of NORs are also used as chromosomal markers in fish systematics (Pereira et al., 2012; Rossi et al., 2012; Nabais et al., 2013). NORs are usually located at the end of the short arm of submetacentric chromosomes (Gromicho and Collares-Pereira, 2007). Rarely, it can be observed at the end of the long arm of telocentric chromosomes (Rab, et al., 1990). Changes in the NOR region and activity can be observed between and within the species (Ulupınar and Alaş, 2002). These differences are due to the differentiated activation of rDNA cistrons, unequal amplification including rDNA segments, and paracentric inversion of chromosomal segments carrying rDNA cistrons (Porto-Foresti

et al., 2007). In our study, NOR was observed on the one telocentric chromosome (21st chromosome). The NOR placement is consistent with the literature. There are no NOR banding studies with *C. regium*. In a study with another *Chondrostoma* species, active NOR regions were observed in the short arm of the sixth submetacentric chromosome pair of *Chondrostoma beysehirense* in Beyşehir Lake (Gündoğdu, 2016). In the *Chondrostoma prespense* species, two pairs of NOR regions were determined on the chromosomes (Bianco et al., 2004). When all these results are compared, the studies partially agree with our research findings (Table 2).

Kaya (2009) reported that the karyotype of *C. regium* caught from Göksu River (Mersin, Turkey) was 2n=50 with 11 pairs of metacentric, 4 pairs of submetacentric, 3 pairs of subtelocentric and 7 pairs of telocentric chromosomes (22M+8SM+6ST+14T) and FN=86. Although the Göksu River fish specimens was later identified to be *C. toros*, we can say that the species are similar to each other in terms of chromosome type and chromosome arm numbers.

When Table 2 was examined, the most important common point of *Chondrostoma* from Iran to Central Europe was that the number of chromosomes was 2n=50 and the chromosomal symmetry/asymmetry values were between 2.00 – 2.61 and all have a symmetrical/asymmetrical chromosome structure, except one (*C. prespense*). However, there are some differences in the chromosome structure. These differences are usually due to submetacentric and subtelocentric chromosomes. Scientists accept that fish species with more telocentric chromosomes are primitive, while species with metacentric and submetacentric chromosomes are more complex (Geng et al., 2013; Şahin, 2015). Accordingly, as can be seen from Table 2, M and SM chromosomes are frequently seen in *Chondrostoma* species. The data emerging in many studies support the hypothesis that this genus was complex, as M and SM type chromosomes are more than single-armed chromosomes (T) in *Chondrostoma* genomes. Since there are 30 double-armed and 18 single-armed chromosomes in the karyotype (18M+14SM+2ST+16T) of *C. regium* determined in the present study, it can be said that this species is also complex. Likewise, when we consider double-armed chromosomes as M/SM, the sum of M/SM (average 34.5) is more than T (mean 12.2) in all *Chondrostoma* species, as indicated in Table 2. In this study, the sum of M and SM chromosome numbers of *C. regium* was determined as 30. This shows that Karasu River (Erzurum) *C. regium* specimens relatively have the general karyotype features of the genus *Chondrostoma*.

Fundamental arm numbers (FN) vary depending on the karyotype. In some cases, it is also used as an indicator of differentiation between species. Although FN values of chromosomes vary between 58 and 94 in *Chondrostoma*, it was seen that this value mostly varies between 80-86. In the present study, FN = 82 and remained within the most repetitive value range (Table 2). On the other hand, the number and structure of chromosomes in *Chondrostoma* species vary slightly. Even the same researchers working in the same region at different times for a single species report variations in their results. This is an unstable situation that can be seen in the cytogenetic results of fish (Şahin, 2015). It is estimated that studies using molecular cytogenetic methods such as FISH (fluorescence in situ hybridization) will become widespread rapidly and reduce the confusion on this subject.

## CONCLUSION

There is no karyotype studies on *Chondrostome regium*, which is distributed in the Euphrates River and its tributaries. In this respect, the work is a first for the Euphrates and Karasu Rivers. Karyotypic analysis of two other fish species (*Squalius cephalus* and *Alburnus mossulensis*) in the parts of the same river within the borders of Erzurum province was previously performed by Kılıç and Şişman (2016) and Şişman et al. (2016), and this is the third study. Thus, the faunistic structure of the Karasu River was clarified a little more. Moreover, the analyses of C-positive regions and NORs were added to the karyotype in this study for the first time. We think that revealing the genome characteristics of fish species living in the Karasu River by using molecular staining (FISH, $CMA_3$ etc.) techniques will give clearer results.

## ACKNOWLEDGEMENTS AND FUNDING

This research was supported by Atatürk University Research Fund (BAP: 2012/477). This study is prepared from Büşra Yamaç's master thesis.

## AUTHORSHIP CONTRIBUTIONS

Turgay Şişman collected the fish samples, have edited the graphics and figures of the manuscript, and designed and wrote the manuscript. Büşra Yamaç performed the experimental work. All authors approved the submission and publication of this manuscript.

## CONFLICTS OF INTEREST

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

## ETHICS APPROVAL

All necessary legal permissions were obtained before starting the study. The necessary permissions for this were: Atatürk University Animal Experiments Local Ethics Committee Ethics Committee Permission (ATA0107100-1332, 06.09.2011), Research Permit from the Ministry of Agriculture and Rural Affairs (67852565/140-0303-863, 03.04.2013), Research Permit from the Ministry of Environment and Forestry (72784983-48804-63471, 03.04.2013).

## DATA AVAILABILITY

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

## REFERENCES


Al-Marjan, K.S.N. (2016). Seasonal variations and prevalence of infections of some species of ectoparasites affecting freshwater fish, *Chondrostoma regium* from Greater Zab river, Kurdistan region, Iraq. *PolyTechnic*, *6*(1), 310-315.

Amemiya, C.T., & Gold, J.R. (1988). Chromosomal NORs as taxonomic and systematic characters in North American cyprinid fishes. *Genetica*, *76*(2), 81-90. https://doi.org/10.1007/BF00058806

Arai, R. (2011). Fish Karyotypes: A check list. Springer e–book. Tokyo Berlin Heidelberg New York. https://doi.org/10.1007/978-4-431-53877-6

Araya-Jaime, C., Palma-Rojas, C., Von Brand, E., & Silva, A. (2020). Cytogenetic characterization, rDNA mapping and quantification of the nuclear DNA content in *Seriolella violacea* Guichenot, 1848 (Perciformes, Centrolophidae). *Comparative Cytogenetics*, *14*(3), 319. https://doi.org/10.3897/CompCytogen.v14i3.53087

Arslan, A., & Gündoğdu, H. (2016). Cytogenetic studies on the endemic Beyşehir nase, *Chondrostoma beysehirense* (Bogutskaya, 1997) in Turkey. *Caryologia*, *69*(2), 116-120. https://doi.org/10.1080/00087114.2015.1109958

Barshiene, J.V. (1977). Karyotype studies in the under mouth *Chondrostoma nasus*. *Citologia, 19*, 390–392. (*in Russian*)

Berberović, L.J., Hadžiselimović, R. & Sofradžija, A. (1970). Comparative review on the basic data on the chromosome complements of *Chondrostoma phoxinus* Heckel and *Chondrostoma knerii* Heckel. *Ichthyologia, 2*(1)*,* 25–30. (in Serbo-Croatian)

Bianco, P.G., Aprea, G., Balletto, E., Capriglione, T., Fulgione, D., & Odierna, G. (2004). The karyology of the cyprinid genera *Scardinius* and *Rutilus* in southern Europe. *Ichthyological Research*, *51*(3), 274-278. https://doi.org/10.1007/s10228-004-0247-1

Bilecenoğlu, M., Kaya, M., Cihangir, B., & Çiçek, E. (2014). An updated checklist of the marine fishes of Turkey. *Turkish Journal of Zoology*, *38*(6), 901-929. https://doi.org/10.3906/zoo-1405-60

Boroń, A., Grabowska, A., Spóz, A., & Przybył, A. (2020). B chromosomes and cytogenetic characteristics of the common nase *Chondrostoma nasus* (Linnaeus, 1758). *Genes*, *11*(11), 1317. https://doi.org/10.3390/genes11111317

Cataudella, S., Sola, L., Accame Muratori, R., & Capanna, E. (1977). The chromosomes of 11 species of Cyprinidae and one Cobitidae from Italy, with some remarks on the problem of polyploidy in the Cypriniformes. *Genetica*, *47*(3), 161-171. https://doi.org/10.1007/BF00123236

Collares-Pereira, M.J. (1992). In vivo direct chromosome preparation (protocol for air drying technique). Paper presented at the First International Workshop on Fish Cytogenetic Techniques. 14–24 September, Concarneau, France.

Collares-Pereira, M.J., & Ráb, P. (1999). NOR polymorphism in the Iberian species *Chondrostoma lusitanicum* (Pisces: Cyprinidae) re-examination by FISH. *Genetica*, *105*(3), 301-303. https://doi.org/10.1023/A:1003885922023

Cucchi, C., & Baruffaldi, A. (1990). A new method for karyological studies in teleost fishes. *Journal of Fish Biology*, *37*(1), 71-75. https://doi.org/10.1111/j.1095-8649.1990.tb05928.x

Çiçek, E., Birecikligil, S.S., & Fricke, R. (2015). Freshwater fishes of Turkey: a revised and updated annotated checklist. *Biharean Biologist*, *9* (2), 141-157.

Çiftçi, Y., Gül Mutlu, A., Güçlü, S.S., Turan, D., & Küçük, F. (2020). Phylogeography of the genus *Chondrostoma* Agassiz, 1835 (Teleostei: Leuciscidae) in Anatolia, as inferred from mitochondrial DNA analysis. *Zoology in the Middle East*, *66*(3), 206-221. https://doi.org/10.1080/09397140.2020.1788255

Dai, Y., & Han, H. (2018). Karyological analysis of two species in the subfamily Schizothoracinae (Cypriniformes: Cyprinidae) from China, with notes on karyotype evolution in Schizothoracinae. *Turkish Journal of Fisheries and Aquatic Sciences*, *18*(1), 175-186. https://doi.org/10.4194/1303-2712-v18_1_20

Denton, T.E. (1973). Fish chromosome methodology. Charles C. Thomas Publisher, Springfifield, USA.

Eagderi, S., Jouladeh-Roudbar, A., Birecikligil, S. S., Çiçek, E., & Coad, B.W. (2017). *Chondrostoma esmaeilii*, a new cyprinid species from the Tigris river drainage in Iran (Teleostei: Cyprinidae). *Vertebrate Zoology, 67*(2), 125-132.

Eroğlu, H.E. (2015). Which chromosomes are subtelocentric or acrocentric? A new karyotype symmetry/asymmetry index. *Caryologia*, *68*(3), 239-245. https://doi.org/10.1080/00087114.2015.1032614

Esmaeili, H.R., Zareian, H., Gholamhosseini, A., Ebrahimi, M., Gholami, Z., Teimori, A., & Ansari, T. H. (2010). Karyotype analysis of the king nase fish, *Chondrostoma regium* (Heckel, 1843) (Actinopterygii: Cyprinidae) from Iran. *Turkish Journal of Fisheries and Aquatic Sciences*, *10*(4). https://doi.org/10.4194/trjfas.2010.0406

Freyhof, J., & Özuluğ, M. (2009). *Pseudophoxinus evliyae*, a new species of spring minnow from Western Anatolia with remarks on the distribution of *P. ninae* and the systematic position of *P. fahirae* (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters*, *20*, 309-318.

Freyhof, J. (2014). *Chondrostoma beysehirense*. *The IUCN Red List of Threatened Species* 2014: e.T61366A19010470. https://dx.doi.org/10.2305/IUCN.UK.2014-1.RLTS.T61366A19010470.en. (Accessed on 19 January 2022).

Gaffaroğlu, M., & Yüksel, E. (2009). Constitutive heterochromatin in *Acanthobrama marmid* and *Cyprinion macrostomus* (Osteichthyes, Cyprinidae). Kafkas Üniversitesi Veteriner Fakültesi Dergisi, *15*, 169-172. https://doi.org/10.9775/kvfd.2008.72-A

Geng, L.W., Xu, W., Jiang, H.F., & Tong, G.X. (2013). Karyotype analysis of *Barbus capito* (Güldentstädt, 1773) using curve measurement software. *Journal of Applied Ichthyology*, *29*(4), 922-924. https://doi.org/10.1111/jai.12142

Goes, C.A.G., Daniel, S.N., Piva, L. H., Yasui, G.S., Artoni, R.F., Hashimoto, D.T., & Porto-Foresti, F. (2020). Cytogenetic markers as a tool for characterization of hybrids of *Astyanax* Baird & Girard, 1854 and *Hyphessobrycon* Eigenmann, 1907. *Comparative Cytogenetics*, *14*(2), 231–242. https://doi.org/10.3897/CompCytogen.v14i2.49513

Gold, J.R., Amemiya, C.T., & Ellison, J.R. (1986). Chromosomal heterochromatin differentiation in North American cyprinid fishes. *Caryologia*, *51*, 557-566. https://doi.org/10.1508/cytologia.51.557

Gromicho, M., & Collares-Pereira, M.J. (2007). The evolutionary role of hybridization and polyploidy in an Iberian cyprinid fish-A cytogenetic review. In E. Pisano, C. Ozouf-Costaz, F. Foresti and B. G. Kapoor (Eds.). *Fish cytogenetics*. Science Publishers, USA, pp. 41-67. https://doi.org/10.1201/b10746-3

Güçlü, S. S., Küçük, F., Turan, D., Çiftçi, Y., & Mutlu, A. G. (2018). A new *Chondrostoma* species from the Büyük Menderes River Basin, Turkey (Teleostei: Cyprinidae). *Zoology in the Middle East*, *64*(4), 315-321. https://doi.org/10.1080/09397140.2018.1511293

Gündoğdu, H. (2016). Cytogenetic studies on endemic Beyşehir squash, *Chondrostoma beysehirense* (Bogutskaya, 1997). Master thesis. Selçuk University, Turkey.

Howell, W.T., & Black, D.A. (1980). Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1-step method. *Experientia*, *36*(8), 1014-1015. https://doi.org/10.1007/BF01953855

Jouladeh-Roudbar, A. (2014): Morphological and molecular studyof the genus *Chondrostoma* Agassiz, 1832 in Iran. Sari University of Agricultural Sciences and Natural Resources, Sari, Iran. IX+76pp.

İlhan, A. (2009). Threatened fishes of the world: *Chondrostoma beysehirense* Bogutskaya, 1997 (Cyprinidae). *Environmental biology of fishes*, *86*(4), 483-484. https://doi.org/10.1007/s10641-009-9557-5

Kaya, F. (2009). *Investigation of karyology of some economical fish living in Göksu River*. Doctoral dissertation, Mersin University, Turkey.

Khosravanizadeh, A., Pourkazemi, M., & Nowruz Fashkhami, M.R. (2011). Karyology study on Bleak (*Alburnus alburnus*) from the South Caspian Sea region. *Caspian Journal of Environmental Sciences*, *9*(1), 27-36.

Kılıç, D., & Şişman, T. 2016. Karyotype analysis of chub, *Squalius cephalus* (Linnaeus, 1758) (Teleostei: Cyprinidae) from Karasu River, Erzurum, Turkey. *Caspian Journal of Environmental Sciences*, *14*, 95-103.

Kuru, M., Yerli, S.V., Mangıt, F., Ünlü, E., & Alp, A. (2014). Fish biodiversity in Turkish inland waters. Journal of Academic Documents for Fisheries and Aquaculture, *1*(3), 93-120. (in Turkish)

Küçük, F., Gümüş, E., Gülle, İ., & Güçlü, S. S. (2007). The fish fauna of the Göksu River (Türkiye): taxonomic and zoogeographic features. *Turkish Journal of Fisheries and Aquatic Sciences*, *7*(1), 53-63.

Küçük, F., Turan, D., Güçlü, S.S., Mutlu, A. G., & Çiftci, Y. (2017). Two new species of Chondrostoma Agassiz, 1832 (Teleostei: Cyprinidae) from the Ceyhan, Seyhan and Göksu rivers in the East Mediterranean region of Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*, *17*(4), 795-803. https://doi.org/10.4194/1303-2712-v17_4_15

Küçük, F., Çiftci, Y., Güçlü, S. S., & Turan, D. (2021). *Chondrostoma smyrnae*, a new nase from the Tahtalı reservoir drainage in the Aegean Sea basin (Teleostei, Leuciscidae). *Zoosystematics and Evolution*, *97*(1), 235-248. https://doi.org/10.3897/zse.97.63691

Küçük, F., Çiftçi, Y., Güçlü, S.S., Mutlu, A.G., & Turan, D. (2023) Taxonomic review of the *Chondrostoma* (Teleostei, Leuciscidae) species from inland waters of Turkey: an integrative approach. Zoosystematics and Evolution *99*(1): 1-13. https://doi.org/10.3897/zse.99.91275

Levan, A., Fredga, K., & Sandberg, A.A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, *52*, 201-220. https://doi.org/10.1111/j.1601-5223.1964.tb01953.x

Moreva, I.N. (2020). The karyotype of the brightbelly sculpin *Microcottus sellaris* (Gilbert, 1896) (Cottidae: Myoxocephalinae). *Russian Journal of Marine Biology*, *46*(1), 29-33. https://doi.org/10.1134/S1063074020010058

Nabais, C., Rampin, M. & Collares-Pereira, M.J. (2013). Comparative cytogenetics of two endangered leuciscine fish, *Squalius aradensis* and *S. torgalensis* (Teleostei, Cyprinidae), from the Iberian Peninsula. *Comparative Cytogenetics*, *7*(1), 33-42. https://doi.org/10.3897/CompCytogen.v7i1.4672

Özcan, G. (2008). Threatened fishes of the world: *Chondrostoma meandrense* Elvira, 1987 (Cyprinidae). *Environmental biology of fishes*, *83*(3), 297-298. https://doi.org/10.1007/s10641-008-9335-9

Pereira, C.S.A., Ráb, P., & Collares-Pereira, M. J. (2012). Chromosomes of European cyprinid fishes: comparative cytogenetics and chromosomal characteristics of ribosomal DNAs in nine *Iberian chondrostomine* species (Leuciscinae). *Genetica*, *140*(10), 485-495.

Porto-Foresti, F., Oliveira, C., Tabata, Y.A., Rigolino, M.G. & Foresti, F. (2007). NOR markers in the identification and management of cultured fish species: the case of rainbow trout stocks reared in Brazil. In E. Pisano, C. Ozouf-Costaz, F. Foresti and B. G. Kapoor (Eds.). *Fish cytogenetics*. Science Publishers, USA. https://doi.org/10.1201/b10746-12

Ráb, P., Roth, P. & Arefjev, V.A. (1990). Chromosome studies of European Leuciscine fishes (Pisces, Cyprinidae) karyotype of *Aspius aspius*. *Caryologia*, *43*(3-4), 249-255. https://doi.org/10.1080/00087114.1990.10797003

Ráb, P., Rábová, M., Pereira, C.S., Collares-Pereira, M.J., & Pelikánová, Š. (2008). Chromosome studies of European cyprinid fishes: interspecific homology of leuciscine cytotaxonomic marker—the largest subtelocentric chromosome pair as revealed by cross-species painting. *Chromosome Research*, *16*(6), 863-873. https://doi.org/10.1007/s10577-008-1245-3

Rossi, A.R., Milana, V., Hett, A.K. & Tancioni, L. (2012). Molecular cytogenetic analysis of the Appenine endemic cyprinid fish *Squalius lucumonis* and three other Italian leuciscines using chromosome banding and FISH with rDNA probes. *Genetica*, *140*, 469-476. https://doi.org/10.1007/s10709-012-9695-0

Saygun, S. (2021). Cytogenetical studies in Turkey: Fish (Vertebrata, Pisces). *Turkish Journal of Bioscience and Collection*, *5*, 83-107.

Sumner, A.T. (1972). A simple technique for demonstrating centromeric heterochromatin. *Experimental Cell Research*, *75*, 304-306. https://doi.org/10.1016/0014-4827(72)90558-7

Şahin, T.A. (2015). Karyotype analysis of *Barbus Tauricus* Kessler, 1877 (Pisces; Cypriniformes) in Ilıca stream (Fatsa/Ordu). Master Thesis, Ordu University, Turkey.

Şişman, T., Şanlı, F., Tepe, Y. & Kılıç, D. (2016). Karyotype characteristics of *Chalcalburnus mossulensis* (Heckel, 1843) fish from Erzurum Karasu River. *Yunus Araştırma Bülteni*, *4*, 281-292.

Tan, X., Qin, J. G., Chen, B., Chen, L., & Li, X. (2004). Karyological analyses on redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquaculture*, *234*(1-4), 65-76. https://doi.org/10.1016/j.aquaculture.2003.12.020

Ulupınar, M. & Alaş, A. (2002). Balık Sitogenetiği ve Laboratuvar Teknikleri. (1. Baskı). Isparta/Türkiye: Tuğra Matbaası.

Uysal, U.E. (2011). Karyotype analysis of *Chondrostoma meandrense* (Elvira, 1987) and *Acanthobrama mirabilis* (Ladiges, 1960) (Cyprinidae) caught from the Büyük Menderes River. Master thesis, Adnan Menderes University, Turkey.

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 69-76 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.10

ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

# Farklı su sıcaklıklarındaki pullu sazan (*Cyprinus carpio* Linnaeus, 1758)' da polenin antioksidan etkisinin araştırılması

# Investigation of antioxidant effect of pollen in scaly carp (*Cyprinus carpio* Linnaeus, 1758) in different water temperature

**Merve Taşkan[1] ● Muhammet Enis Yonar[2]***

[1] Fırat Üniversitesi Su Ürünleri Fakültesi, 23119, Elazığ, Türkiye
[2] Fırat Üniversitesi Su Ürünleri Fakültesi, 23119, Elazığ,Türkiye

https://orcid.org/0000-0002-5105-7010
https://orcid.org/0000-0001-9519-4247

*Corresponding author: meyonar@gmail.com

*Received date: 16.05.2022* *Accepted date: 18.07.2022*

***How to cite this paper:***
Taşkan, M., & Yonar, E. M. (2023). Investigation of Antioxidant Effect of Pollen in Scaly Carp (*Cyprinus carpio* Linnaeus, 1758) in Different Water Temperature. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 69-76. https://doi.org/10.12714/egejfas.40.1.10

**Öz:** Bu çalışmada farklı su sıcaklıklarında yemlerine polen ilave edilmiş pullu sazanda bazı immunolojik ve antioksidan parametrelerdeki değişimlerin araştırılması amaçlandı. Balıklar su sıcaklığı 18 ºC, 23 ºC ve 28 ºC' ye ayarlanmış akvaryumlara stoklandı. Balıklara % 2,5 oranında polen içeren yemler 14 gün süreyle verildi. Balıklardan alınan kan ve doku örneklerinde immunolojik ve oksidan/antioksidan parametreler analiz edildi.

Kontrol grubu (23 ºC) ile kıyaslandığında, 18 ºC' deki grubun NBT aktivitesinde istatistiksel olarak önemli bir artış belirlendi. 28 ºC' deki grubun NBT aktivitesinde belirlenen azalma istatistiksel olarak önemsiz bulundu. 18 ºC' deki grubun total protein ve total immunoglobulin düzeyleri azalırken, 28 ºC' deki grupta her iki parametre için belirlenen artış önemsiz bulundu. Kontrol grubu (23 ºC)' na kıyasla 18 ºC ve 28 ºC sıcaklıkta tutulan ve polen uygulanan grupların NBT aktivitesi ile total protein ve total immunoglobulin düzeylerinin kontrol grubundan istatistiksel olarak önemli herhangi bir farklılık göstermediği belirlendi. Kontrol grubu (23 ºC)' na kıyasla, 18 ºC ve 28 ºC' deki grupların doku MDA düzeyleri arttı. Sıcaklıktaki değişimle eşzamanlı olarak polen uygulanan grupların MDA düzeyleri 18 ºC ve 28 ºC' deki gruplardan daha düşüktü. Kontrol grubu (23 ºC) ile kıyaslandığında, 18 ºC ve 28 ºC' deki grupların doku GSH düzeyleri ve GST aktiviteleri azaldı. Sıcaklıktaki değişimle eşzamanlı olarak polen uygulanan gruplarda GSH düzeyleri ve GST aktiviteleri 18 ºC ve 28 ºC' deki gruplardan daha yüksekti. Sonuç olarak balıklarda sıcaklık farklılıklarından kaynaklanan stres polenle önlenebilir.

**Anahtar Kelimeler:** Antioksidanlar, bağışıklık, balık, oksidatif stres, sıcaklık

**Abstract:** In this study, it was aimed to investigate changes in some immunological and antioxidant parameters in scaly carp (*Cyprinus carpio*) added pollen to their feed in different water temperatures. Fish were stocked to glass aquariums adjusted to 18 °C, 23 °C and 28 °C water temperature. Fish were given diets containing % 2,5 pollen for 14 days. Blood and tissue samples were analysed to determine the immunological parameters and oxidant/antioxidant status.

When compared to the control group (23 ºC), a statistically significant increase in the NBT activity of the groups at 18 ºC was observed. Decrease in the NBT activity of the group at 28 ° C was statistically insignificant. The total protein and total immunoglobulin levels of the group at 18 ° C were decreased, while increase in both parameters at 28 ° C was not significant. When compared to the control group (23 ºC), the NBT activity, the total protein and total immunoglobulin levels in the groups that maintained at the same temperature with the control group (23 ° C) and applied pollen did not show any statistically significant difference. The tissue MDA levels were increased in the groups at 18 °C and 28 °C when compared to the control group (23 ° C). The tissue MDA levels of the groups treated pollen simultaneously with the change in temperature were lower than the groups at 18 °C and 28 °C. The tissue GSH levels and GST activities were decreased in the groups at 18 °C and 28 °C when compared to the control group (23 ° C). The tissue GSH levels and GST activities of the groups treated pollen simultaneously with the change in temperature were higher than the groups at 18 °C and 28 °C. In conclusion, stress caused by temperature differences in fish may be prevented by pollen.

**Keywords:** Antioxidants, immunity, fish, oxidative stress, temperature

## GİRİŞ

Balık yetiştiriciliği, dünyada hızla gelişen ve önem kazanan bir endüstri kolu haline gelmiştir. Ancak dışarıdan alınan pestisitler, ağır metaller, sıcaklık değişimleri, diyet tipleri, oksijen miktarı, parazitler ve farklı çevresel koşullar gibi faktörler, balıkları fizyolojik açıdan olumsuz olarak etkilemekte dolayısıyla da ekonomik kayıplara yol açmaktadır. Bu faktörler içerisinde en önemlilerden biri olan su sıcaklığı; balıkların yaşamını, büyümesini, beslenmesini ve diğer birçok fizyolojik fonksiyonlarını etkileyen en önemli faktörlerden biridir (Lou vd., 2011). Küresel ısınmanın bir sonucu olarak, kronik sıcaklık artışları ve aşırı sıcak hava dalgası gibi olaylar dünyada balıkları da içine alan farklı hayvan popülasyonlarını etkilemektedir. Soğukkanlı canlılar olarak balıkların küresel ısınmaya karşı özellikle savunmasız olmaları kaçınılmaz bir sonuçtur. Kronik sıcaklık artışı, balıkların yaşamlarını tehlikeye atabilecek ek stres faktörleriyle başa çıkma yeteneklerini değiştirebilen fizyolojik bir yük oluşturmaktadır. Ayrıca hızlı sıcaklık artışlarının balıklarda akut stres cevabını indüklediği bilinmektedir. Soğukkanlı canlılar olarak balıklarda vücut sıcaklığı, fizyolojileri, metabolizmaları ve davranışları üzerinde derin etkileri olan yaşadıkları su sıcaklıklarına eşittir (Alfonso vd., 2020).

Balıklarda immun sistem çevresel faktörlerden oldukça fazla etkilenmektedir. Bu çevresel faktörlerin başında da su sıcaklığı gelmektedir (Buchtíková vd., 2011). Diğer taraftan yüksek sıcaklık (Parihar ve Dubey, 1995; Heise vd., 2006; Luschchak ve Bagnyukova, 2007) ve düşük sıcaklık (Malek vd., 2004; Martinez-Álvarez vd., 2005) balıklarda oksidatif strese yol açmaktadır. Yüksek sıcaklık oksijen ihtiyacını artırmakta bu artış da balıklarda oksidatif strese neden olmaktadır. Diğer taraftan düşük sıcaklık antioksidan sistemi zayıflatarak veya radikal oluşumuna yol açarak oksidatif stresi indüklemektedir (Lushchak, 2011).

Küçük hücrelerden oluşmuş, bitkinin kalıtsal özelliklerini taşıyan, bitkilerin çiçeklenme dönemleri boyunca görülen, çiçeklerde erkek üreme organlarının başçık bölümünde bulunan tozlara polen denilmektedir (Çankaya ve Korkmaz, 2008). Antibakteriyel, antifungal, antikarsinojenik, antioksidan ve immunomodulatör özellikleri yapılan çalışmalarla kanıtlanan polene verilen önem, içerdiği besin maddelerinin zenginliği nedeniyle son zamanlarda daha fazla artmıştır (Yang vd., 2007; Eraslan vd., 2009; Xu vd., 2009; Abbass vd., 2012). Protein ve karbonhidrat polenin yapısında yüksek oranda bulunmakla birlikte yağ asitleri, vitaminler, mineral maddeler, enzimler ve aminoasitler yapılan analizler sonucunda belirlenen diğer maddelerdir. Bununla birlikte karotenoid, steroid ve flavonoidlerin polendeki varlığı da ispatlamıştır (Abbass vd., 2012).

Bu çalışmada farklı sıcaklıklarda tutularak ısı stresi oluşturulmuş pullu sazanda immunolojik ve antioksidan parametreler kullanılarak arı poleninin koruyucu etkisini araştırmak, ayrıca immunostimulan ve antioksidan özellikteki arı poleninin balıklarda kullanılabilirliğini belirlemek amaçlanmıştır.

## MATERYAL VE METOT

Çalışmada, DSİ 9. Bölge Müdürlüğü (Keban, Elazığ)' den canlı olarak temin edilen ve yaklaşık ağırlığı 40 ± 5 g olan 0+ yaş grubundaki pullu sazan (*Cyprinus carpio*) örnekleri kullanıldı. Çalışma 3 tekrarlı olarak yürütüldü. Her bir tekrar için 6 akvaryum ve 60 balık olmak üzere toplamda 18 akvaryum ve 180 balık kullanıldı (Her bir tekrar için 6 akvaryum olmak üzere toplamda 18 akvaryum ve her bir tekrar için 60 balık olmak üzere toplamda 180 balık). Balıklar 33 x 100 x 60 cm ebatlarında ve su sıcaklığı ayarlanabilir ısıtıcılarla 23 °C' ye ayarlanmış 6 farklı cam akvaryuma her birine 10 adet olacak şekilde yerleştirildi. Eş zamanlı olarak her bir tekrar için 6 akvaryumda bu işlem yapıldı. Balıkların 15 gün süreyle bu ortama adaptasyonu sağlandı.

15 günlük adaptasyon süresinden sonra, yine eşzamanlı olarak her bir tekrar için balıkların yerleştirildiği akvaryumlardan 2' sinin sıcaklığı 18 °C' ye düşürülürken, diğer ikisinin ise 28 °C' ye yükseltildi. Bu işlem her iki saatte bir sıcaklığın 1 °C azaltılması/arttırılması şeklinde yapıldı. 2 akvaryumun sıcaklığı ise 23 °C' de sabit tutuldu. Böylece 18, 23 ve 28 °C su sıcaklığına sahip aşağıdaki gruplar oluşturularak polen içeren yemlerle balıklar beslendi.

**Grup 1 (18 ºC):** 18 ºC sıcaklıkta tutulan ve polen uygulanmayan grup,

**Grup 2 (23 ºC-Kontrol Grubu):** 23 ºC sıcaklıkta tutulan ve polen uygulanmayan grup,

**Grup 3 (28 ºC):** 28 ºC sıcaklıkta tutulan ve polen uygulanmayan grup,

**Grup 4 (18 ºC + Po):** 18 ºC sıcaklıkta tutulan ve % 2,5 oranında polen uygulanan grup,

**Grup 5 (23 ºC + Po):** 23 ºC sıcaklıkta tutulan ve % 2,5 oranında polen uygulanan grup,

**Grup 6 (28 ºC + Po):** 28 ºC sıcaklıkta tutulan ve % 2,5 oranında polen uygulanan grup.

Sazanlar için optimum su sıcaklığı 22-24 °C olduğu için (Çelikkale, 1994), 23 ºC sıcaklıkta tutulan ve polen içermeyen yemlerle beslenen balıklar kontrol grubu olarak seçildi. Çalışma sırasında su sıcaklığının ayarlanan seviyelerde sabit kalarak süreklilik göstermesi için günde 4 defa ölçüm yapıldı. Çalışmanın 3., 7. ve 10. günlerinde akvaryum sularının 2/3' ü sifonlama yapılarak değiştirildi. Eksilen sular daha önceden sıcaklıkları 18, 23 ve 28 ºC' ye ayarlanmış sularla tamamlandı.

Polen örnekleri Yalova ilindeki arıcılardan temin edildi ve palinolojik olarak identifiye edildi. Yapılan analiz sonucunda çalışmada kullanılan polenin miks polen (*Castanea sativa*, Fabaceae, Asteraceca, Trifolium spp) olduğu belirlendi. Polenin metanolik ekstrasyonunun analizi neticesinde total fenolik madde miktarı 1719,20 ± 45,74 mg GAE/100 g (GAE: Gallic acid equivalents) ve antioksidan aktivite 33,65 ± 2,57 mg AAE/g (AAE: Ascorbic acid equivalents) olarak belirlendi.

Polenin uygulanan dozu (% 2,5 w/w) El-Asely vd. (2014)' e göre belirlendi. Polen içeren yemlerin hazırlanması için önce polen örnekleri % 2,5 oranında tartılıp 1 L su içerisinde çözüldü. Daha sonra özel bir firmadan alınarak toz haline getirilmiş pelet yemler (Ecobio; % 45 ham protein, % 20 ham yağ, % 11 kül, % 3 lif, % 8,5 nem, % 12,5 azotsuz öz madde, 5124 kcal) polen içeren 1 L' lik su ile hamur haline getirildi. Bu karışım kıyma makinesinden geçirildi ve tekrar pelet haline dönüştürüldü. Hazırlanan peletler tepsilere yerleştirilip yem fırınında kurutuldu. Yemler, kullanılana kadar oluşabilecek herhangi bir oksidasyonu önlemek için renkli cam şişelerde 4 °C' de muhafaza edildi (Harlioğlu vd., 2012; El-Asely vd., 2014; Mişe Yonar vd., 2020).

14 günlük uygulamadan sonra 15. günde kan örnekleri, benzocain (25 mg/L) ile anestezi edilen balıklardan kavdal pedünkül bölgesinin kesilmesiyle EDTA'lı tüplere alındı. Kan örneklerinin alınmasından sonra klinik muayenesi yapılan balıklar otopsi edildi (Arda vd., 2017) ve karaciğer, böbrek ve solungaç örnekleri çıkarıldı. Doku örnekleri folyolara sarılarak -20 °C' deki derin dondurucuya bırakıldı (Mişe Yonar vd., 2011; Sakin vd., 2012).

EDTA' lı tüplere alınan kan örneklerinde plazmalar çıkarılmadan önce oksidatif radikal üretimi

(nitrobluetetrazolium-NBT aktivitesi) belirlendi (Siwicki vd., 1994). Daha sonra plazma elde etmek için kan örnekleri 3500 rpm' de 10 dakika santrifüj edildi. Plazmada total protein (TP) ve total immunoglobulin (TI) düzeyleri Siwicki vd. (1994) tarafından açıklanan yöntemle ölçüldü.

Antioksidan parametrelerin belirlenmesi için homojenatlar hazırlandı. Karaciğer, böbrek ve solungaç örneklerinden homojenatların hazırlanması için Sakin vd. (2011) ve Mişe Yonar vd. (2017a)' in bildirdiği yöntemler kullanıldı. Homojenizasyon işleminin sonunda elde edilen süpernatantlarda lipit peroksidasyonun bir göstergesi olarak malondialdehit (MDA) düzeyi (Placer vd. 1966), antioksidan durumun belirlenmesi için ise redükte glutatyon (GSH) düzeyi (Ellman, 1959) ve glutatyon S-transferaz (GST) aktivitesi (Habig vd., 1974) ölçüldü.

GST spesifik enzim aktivitesi ile MDA ve GSH düzeylerini hesaplamak için ölçülen doku protein düzeyleri Lowry vd. (1951)' nin tarif ettiği yönteme göre belirlendi.

Sonuçların istatistiksel analizleri SPSS 22.0 istatistik programı kullanılarak gerçekleştirildi. Kontrol ve deneysel grupların immunolojik ve antioksidan parametrelerinde oluşan değişimlerin belirlenmesi için tek yönlü varyans analizi (ONEWAY – ANOVA) kullanıldı. Gruplar arasındaki farklılıklar ise Least Significant Difference (LSD) test edildi. Sonuçlar ortalama ± standart hata olarak gösterildi.

## BULGULAR

Çalışmaya başlamadan önceki 15 günlük adaptasyon sürecinde balıklarda herhangi bir ölüm olayına rastlanmadı. Adaptasyon ve deneme süresi boyunca balıkların yem alımlarında herhangi bir olumsuzluk yaşanmadı. Deneme süresi boyunca kontrol ve deneme grubu balıklarında ölüm gözlenmedi.

Kontrol ve deneme gruplarının NBT aktivitesi ile TP ve TI düzeylerindeki değişimler Tablo 1' de gösterilmiştir.

**Tablo 1.** Deneme gruplarının NBT aktivitesi (mg/mL) ile TP ve TI düzeyleri (mg/mL)

**Table 1.** NBT activity (mg/mL) and TP and TI (mg/mL) levels of the experimental groups

| Gruplar | NBT | TP | TI |
|---|---|---|---|
| **18 ºC** | 1,72 ± 0,17$^{c}$ | 25,59 ± 4,13$^{a}$ | 10,41 ± 2,41$^{a}$ |
| **23 ºC (Kontrol)** | 1,34 ± 0,11$^{a}$ | 31,24 ± 3,15$^{b}$ | 13,11 ± 3,62$^{b}$ |
| **28 ºC** | 1,30 ± 0,09$^{a}$ | 32,08 ± 5,37$^{b}$ | 14,09 ± 4,06$^{b}$ |
| **18 ºC + Po** | 1,32 ± 0,10$^{a}$ | 30,86 ± 5,19$^{b}$ | 12,84 ± 2,33$^{b}$ |
| **23 ºC + Po** | 1,61 ± 0,14$^{b}$ | 37,15 ± 6,11$^{c}$ | 19,71 ± 3,20$^{c}$ |
| **28 ºC + Po** | 1,33 ± 0,12$^{a}$ | 32,27 ± 4,32$^{b}$ | 13,40 ± 3,34$^{b}$ |

$^{a,b,c,d,e}$ Aynı sütundaki farklı harfler istatistiksel farkı göstermektedir ($p<0.05$).

Kontrol grubu (23 ºC) ile aynı sıcaklıkta tutulan ve polen uygulanan grubun (23 ºC + Po) NBT aktivitesinde belirlenen artış istatistiksel olarak önemli bulundu ($p < 0,05$). Kontrol grubuna (23 ºC) göre, 18 ºC sıcaklıkta tutulan fakat polen uygulanmayan grubun (18 ºC) NBT aktivitesinde belirlenen artış istatistiksel olarak önemli bulunurken ($p < 0,05$), 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan grubun (28 ºC) NBT aktivitesinde belirlenen azalma önemsiz bulundu ($p > 0,05$). Ancak 18 ºC sıcaklıkta tutulan ve polen uygulanan (18 ºC + Po) grup ile 28 ºC sıcaklıkta tutulan ve polen uygulanan (28 ºC + Po) grubun NBT değerlerinin kontrol grubundan (23 ºC) istatistiksel olarak önemli herhangi bir farklılık göstermediği saptandı ($p > 0,05$).

Kontrol grubu (23 ºC) ile aynı sıcaklıkta tutulan ve polen uygulanan grubun (23 ºC + Po) TP ve TI düzeylerinin arttığı ve bu artışın istatistiksel olarak önemli olduğu belirlendi ($p < 0,05$). Kontrol grubuna (23 ºC) göre 18 ºC sıcaklıkta tutulan fakat polen uygulanmayan grubun (18 ºC) TP ve TI düzeylerinde belirlenen azalma istatistiksel olarak önemli bulunurken ($p < 0,05$), 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan grubun (28 ºC) TP ve TI düzeylerinde belirlenen artış önemsiz bulundu ($p > 0,05$). Ancak 18 ºC sıcaklıkta tutulan ve polen uygulanan grup (18 ºC + Po) ile 28 ºC sıcaklıkta tutulan ve polen uygulanan grubun (28 ºC + Po) TP ve TI düzeylerinin kontrol grubuna (23 ºC) kıyasla istatistiksel olarak önemli herhangi bir farklılık göstermediği saptandı ($p > 0,05$).

Kontrol ve deneme gruplarının karaciğer, böbrek ve solungaç MDA düzeylerindeki değişimler Tablo 2' de gösterilmiştir.

**Tablo 2.** Deneme gruplarının doku MDA düzeyleri (nmol/g protein)

**Table 2.** Tissue MDA levels (nmol/g protein) of the experimental groups

| Gruplar | Karaciğer | Böbrek | Solungaç |
|---|---|---|---|
| **18 ºC** | 2,91 ± 1,09$^{e}$ | 1,56 ± 0,76$^{c}$ | 2,52 ± 1,14$^{e}$ |
| **23 ºC (Kontrol)** | 1,49 ± 0,71$^{b}$ | 1,50 ± 0,57$^{b,c}$ | 1,97 ± 0,89$^{b}$ |
| **28 ºC** | 3,45 ± 1,25$^{f}$ | 2,67 ± 1,10$^{e}$ | 3,92 ± 0,99$^{f}$ |
| **18 ºC + Po** | 1,69 ± 0,86$^{c}$ | 1,43 ± 0,57$^{b}$ | 2,19 ± 0,86$^{c}$ |
| **23 ºC + Po** | 1,24 ± 0,54$^{a}$ | 1,35 ± 0,69$^{a}$ | 1,72 ± 0,65$^{a}$ |
| **28 ºC + Po** | 2,33 ± 1,22$^{d}$ | 1,93 ± 0,67$^{d}$ | 2,37 ± 0,84$^{d}$ |

$^{a,b,c,d,e}$ Aynı sütundaki farklı harfler istatistiksel farkı göstermektedir ($p<0.05$)

Kontrol grubu (23 ºC) ile kıyaslandığında, kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan grubun (23 ºC + Po) karaciğer, böbrek ve solungaç MDA düzeylerinde belirlenen düşüş istatistiksel olarak anlamlı ($p < 0,05$) bulundu.

Kontrol grubuna (23 ºC) kıyasla, 18 ºC sıcaklıkta tutulan fakat polen uygulanmayan grupta (18 ºC) karaciğer ve solungaç MDA düzeylerinin istatistiksel olarak önemli düzeyde yükseldiği ($p < 0,05$) saptandı. Bu grubun böbrek MDA düzeyinde belirlenen artış ise istatistiksel olarak önemsiz bulundu ($p > 0,05$). Yine kontrol grubuyla kıyaslandığında 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan grubun (28 ºC) karaciğer, böbrek ve solungaç MDA düzeylerinin istatistiksel olarak önemli düzeyde arttığı ($p < 0,05$) belirlendi.

18 ºC ve 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan

gruplarla kıyaslandığında 18 ºC sıcaklıkta tutulan ve polen uygulanan grup (18 ºC + Po) ile 28 ºC sıcaklıkta tutulan ve polen uygulanan (28 ºC + Po) grubun karaciğer, böbrek ve solungaç MDA düzeylerinin istatistiksel olarak düştüğü ($p < 0,05$) tespit edildi. Fakat her iki grubun (18 ºC + Po ve 28 ºC + Po) karaciğer ve solungaç MDA düzeyleri düşmesine rağmen yine de kontrol grubundan yüksek bulunurken, 18 ºC + Po grubunun böbrek MDA düzeyi kontrol grubundan düşük bulundu.

Kontrol ve deneme gruplarının karaciğer, böbrek ve solungaç GSH düzeylerindeki değişimler Tablo 3' de gösterilmiştir.

Kontrol grubu (23 ºC) ile kıyaslandığında, kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan grubun (23 ºC + Po) karaciğer, böbrek ve solungaç GSH düzeylerinde belirlenen artış istatistiksel olarak önemli ($p < 0,05$) bulundu.

Kontrol grubuna (23 ºC) kıyasla, 18 ºC ve 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan gruplarda karaciğer, böbrek ve solungaç GSH düzeylerinin istatistiksel olarak önemli düzeyde azaldığı ($p < 0,05$) belirlendi.

18 ºC ve 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan gruplarla kıyaslandığında 18 ºC sıcaklıkta tutulan ve polen uygulanan grup (18 ºC + Po) ile 28 ºC sıcaklıkta tutulan ve polen uygulanan (28 ºC + Po) grubun karaciğer, böbrek ve solungaç GSH düzeylerinin istatistiksel olarak arttığı ($p < 0,05$) tespit edildi. Fakat her iki grubun (18 ºC + Po ve 28 ºC + Po) karaciğer ve böbrek GSH düzeyleri artmasına rağmen yine de kontrol grubundan düşük bulunurken, solungaç dokusunun GSH düzeyi kontrol grubundan yüksek bulundu.

**Tablo 3.** Deneme gruplarının doku GSH düzeyleri (µmol/g protein)

**Table 3.** Tissue GSH levels (nmol/g protein) of the experimental groups

| Gruplar | Karaciğer | Böbrek | Solungaç |
|---|---|---|---|
| **18 ºC** | 29,95 ± 7,14$^{b}$ | 33,75 ± 7,01$^{b}$ | 11,98 ± 3,87$^{b}$ |
| **23 ºC (Kontrol)** | 40,21 ± 8,91$^{d}$ | 45,85 ± 7,94$^{e}$ | 14,69 ± 2,21$^{c}$ |
| **28 ºC** | 23,75 ± 7,31$^{a}$ | 26,94 ± 6,19$^{a}$ | 10,53 ± 3,41$^{a}$ |
| **18 ºC + Po** | 38,81 ± 7,28$^{d}$ | 40,97 ± 7,55$^{d}$ | 19,04 ± 3,57$^{e}$ |
| **23 ºC + Po** | 52,31 ± 9,71$^{e}$ | 54,96 ± 6,75$^{f}$ | 23,67 ± 3,61$^{f}$ |
| **28 ºC + Po** | 35,59 ± 6,78$^{c}$ | 37,58 ± 7,89$^{c}$ | 17,53 ± 3,93$^{d}$ |

$^{a,b,c,d,e}$ Aynı sütundaki farklı harfler istatistiksel farkı göstermektedir ($p<0.05$).

Kontrol ve deneme gruplarının karaciğer, böbrek ve solungaç GST aktivitelerindeki değişimler Tablo 4' de verilmiştir.

Kontrol grubu (23 ºC) ile kıyaslandığında, kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan grupta (23 ºC + Po) karaciğer, böbrek ve solungaç GST aktivitelerinin istatistiksel olarak önemli düzeyde arttığı ($p < 0,05$) tespit edildi.

Kontrol grubuna (23 ºC) kıyasla, 18 ºC ve 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan gruplarda karaciğer, böbrek ve solungaç GST aktivitelerinin istatistiksel olarak önemli düzeyde azaldığı ($p < 0,05$) belirlendi.

18 ºC ve 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan gruplarla kıyaslandığında, 18 ºC sıcaklıkta tutulan ve polen uygulanan (18 ºC + Po) grup ile 28 ºC sıcaklıkta tutulan ve polen uygulanan (28 ºC + Po) grubun karaciğer, böbrek ve solungaç GST aktivitelerinin istatistiksel olarak önemli düzeyde arttığı ($p < 0,05$), fakat yine de kontrol grubundan düşük olduğu tespit edildi.

**Tablo 4.** Deneme gruplarının doku GST aktiviteleri (µmol/dakika/mg protein)

**Table 4.** Tissue GST activities (µmol/dakika/mg protein) of the experimental groups

| Gruplar | Karaciğer | Böbrek | Solungaç |
|---|---|---|---|
| **18 ºC** | 6,43 ± 19,62$^{a}$ | 8,71 ± 16,30$^{b}$ | 05,06 ± 15,92$^{b}$ |
| **23 ºC (Kontrol)** | 25,61 ± 17,21$^{d}$ | 30,96 ± 18,91$^{d}$ | 19,81 ± 17,53$^{c}$ |
| **28 ºC** | 3,78 ± 18,44$^{a}$ | 2,59 ± 15,02$^{a}$ | 9,53 ± 18,74$^{a}$ |
| **18 ºC + Po** | 18,48 ± 18,11$^{c}$ | 18,58 ± 15,57$^{c}$ | 16,48 ± 11,43$^{c}$ |
| **23 ºC + Po** | 37,29 ± 23,87$^{e}$ | 46,49 ± 20,10$^{e}$ | 32,69 ± 17,35$^{d}$ |
| **28 ºC + Po** | 05,73 ± 17,64$^{b}$ | 02,47 ± 17,85$^{b}$ | 15,73 ± 16,74$^{c}$ |

$^{a,b,c,d,e}$ Aynı sütundaki farklı harfler istatistiksel farkı göstermektedir ($p<0.05$).

## TARTIŞMA VE SONUÇ

Balıklarda nonspesifik immun direncin en önemli basamağını oluşturan fagositoz kemotaksis, opsonizasyon, absorpsiyon, intraselüler yıkım ve sindirim gibi birçok safhada gerçekleşmektedir. Fagositozda birinci derecede görev alan makrofajlar ve polimorfnükleer lökositler (özellikle nötrofiller) respiratory burst (solunum patlaması) sırasında reaktif oksijen türleri üretirler. Nötrofillerin oksidatif radikal üretimi yani bir başka ifadeyle ürettikleri reaktif oksijen türlerinin miktarı, nötrofillerin fagositik aktivitesinin belirlenmesi için kullanılmaktadır. Bunun için de en çok kullanılan yöntemlerin başında NBT testi gelmektedir (Siwicki ve Studnicka, 1987). Tilapia (*Oreochromis niloticus*)' larda yapılmış bir çalışmada balıkların fagositik hücre (nötrofil ve monositler) sayısının polen uygulamasıyla arttığı belirlenmiştir. Benzer şekilde Mişe Yonar vd. (2014) tarafından yapılan çalışmada, güçlü bir antioksidan ve immunostimulan olan ayrıca yapısındaki bileşiklerle polene benzerlik gösteren propolisin uygulandığı sazanlarda NBT aktivitesinin yükseldiği tespit edilmiştir. Bu çalışmada da kontrol grubu (23 ºC) balıklarıyla kıyaslandığında kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan, diğer bir ifadeyle sıcaklık değişimlerinden bağımsız olarak yalnız polen uygulanan gruptaki (23 ºC + Po) balıklarda, NBT aktivitesinin yükseldiği tespit edilmiş, elde edilen bu sonuç yukarıdaki çalışmalardan elde edilen sonuçlarla paralellik göstermiştir.

Diğer taraftan Ndong vd. (2007) 27 ºC' ye adapte edilmiş tilapia (*Oreochromis mossambicus*)' larda sıcaklığın 19 ve 23 ºC' ye düşürülmesi ve yine sıcaklığın 31 ve 35 ºC' ye

yükseltilmesi sonucunda spesifik ve nonspesifik bağışıklık parametrelerinde meydana gelen değişimleri araştırmışlardır. 12, 24, 48 ve 96 saat sonunda balıklardan alınan örneklerde, NBT testi kullanılarak belirlenen respiratory burst aktivitesinin 24, 48 ve 96 saatlerde, fagositik aktivitenin ise 12. saatten itibaren hem düşük hem de yüksek sıcaklıklarda tutulan balıklarda azaldığı belirlenmiştir. Yine bu çalışmada 48. saatten sonra düşük sıcaklıklarda tutulan balıkların fagositik aktivitesinin normal seviyeye geldiği fakat yüksek sıcaklıklarda tutulan balıklarda böyle bir sonucun gözlemlenmediği ifade edilmiştir. Bu araştırmacının (Ndong vd., 2007) elde ettiği bulguların aksine Nikoskelainen vd. (2004), gökkuşağı alabalığında respiratory burst aktivitesinin sıcaklıkla arttığını, Dittmar vd. (2014) ise 13, 18 ve 24 ºC' de stoklanmış *Gasterosteus aculeatus* türü balıklarda respiratory burst aktivitesinin ve lenfosit proliferasyonunun düşük sıcaklıkta arttığını, yüksek sıcaklıkta azaldığını bildirmişlerdir. Bu çalışmada 18 ºC sıcaklıkta tutulan fakat polen uygulanmayan balıkların NBT aktivitesi istatistiksel olarak önemli düzeyde artmış, 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan balıklarda ise önemsiz bir şekilde azalmıştır. Diğer çalışmalardan elde edilen bulgularla bu çalışmadan elde edilen bulgular arasındaki farklılığın nedeni balığın türü, sıcaklık değerlerindeki farklılık ve sıcaklığın uygulanma süresi olabilir.

Toplam plazma proteini nonspesifik immun sistemin (Jeney vd., 1997), immunoglobulinler ise spesifik bağışıklığın en önemli humoral unsuru olarak kabul edilmektedir (Siwicki vd., 1994). Memelilerde beş farklı immunoglobulin sınıfı bulunurken (Diker, 1998), balıklarda bunlardan sadece IgM' nin varlığı kesin olarak belirlenmiştir (Darson, 1981). Bu çalışmada kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan balıklarda, TP ve TI düzeylerinin yükseldiği gözlemlenmiştir. Benzer sonuçlar başka bir araştırmacı tarafından da ifade edilmiş, farklı oranlarda polen içeren yemlerin uygulandığı tilapia (*Oreochromis niloticus*)' ların total plazma protein düzeyinde belirlenen artış istatistiksel olarak önemli bulunmuştur (El-Asely vd., 2014).

Ayrıca bu çalışmada 18 ºC sıcaklıkta tutulan fakat polen uygulanmayan balıkların TP ve TI düzeyleri istatistiksel olarak önemli düzeyde azalmış, 28 ºC sıcaklıkta tutulan fakat polen uygulanmayan balıklarda ise önemsiz bir şekilde artmıştır. Balıklarda bağışıklığın humoral faktörlerinin yüksek sıcaklıkla arttığı belirtilmiştir (Ndong vd., 2007). Langston vd. (2002), Nikoskelainen vd. (2004) ve Holland vd. (2002) lizozim, C-raktif protein ve komplement aktivitesi gibi humoral faktörlerin balıklarda artan sıcaklıkla yükseldiğini ifade ederken, buna karşılık olarak lizozim aktivitesinin düşük sıcaklığa transfer edilen çipuralarda azaldığı belirtilmiştir (Tort vd., 1998; Tort vd., 2004). Genel bir fenomen olarak poikloterm bir canlı olan balıklarda su sıcaklığı ve antikor düzeyi arasında doğrusal bir ilişki bulunmaktadır. Bu fenomen *Dicentrarchus labrax*, *Oreochromis aureus*, *Paralichthys olivaceus*, *Hippoglossus hippoglossus*, *Pecoglossus altivelis*, *Oreochromis niloticus*, *Gadus morhua* ve *Scophthalmus maximus* türlerini de içeren birçok balık türünde kanıtlanmıştır (Makrinos ve Bowden, 2016). Bununla birlikte balıklarda nonspesifik bağışıklık düşük sıcaklıkta daha fazla aktivite gösterirken, spesifik bağışıklık ise yüksek sıcaklıkta daha fazla aktive olmaktadır (Makrinos ve Bowden, 2016). Yukarıda ifade edilen çalışmalarla uyumlu bir şekilde bu çalışmada da düşük sıcaklıkla TP ve TI düzeyi azalmıştır. Fakat yüksek sıcaklığın bu parametrelere istatistiksel olarak herhangi bir etkisi belirlenememiştir.

Bu çalışmadan elde edilen sonuçlara bakılarak, sazanlarda etkili bir immun fonksiyon için sıcaklığın optimal sınırlar içinde olması gerektiği ve bu sıcaklığın 23 ºC olduğu, düşük ve yüksek sıcaklığın Makrinos ve Bowden (2016) tarafından bildirilen çalışmayla uyumlu olarak immun parametrelerde dalgalanmalara yol açtığı görülmektedir. Bununla birlikte düşük ve yüksek sıcaklıklarla birlikte polen uygulanan 18 ºC + Po ve 23 ºC + Po grubu balıklarda, polen uygulamasının bir sonucu olarak sıcaklık farklılıklarının yol açtığı immun parametrelerdeki dalgalanmalar engellenebilir. Diğer taraftan kontrol grubu (23 ºC) balıklarıyla kıyaslandığında kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan, diğer bir ifadeyle sıcaklık değişimlerinden bağımsız olarak yalnız polen uygulanan gruptaki (23 ºC + Po) balıklarda incelenen tüm immun parametreler yükseldiği için polenin sazanlarda bir immunostimulan olarak kullanılabileceği söylenebilir.

Oksijenden tek elektron indirgenmesi sonucu oluşan serbest radikaller proteinlerin, lipitlerin, karbohidratların ve nükleik asitlerin yıkımına sebep olabilirler. Bununla birlikte DNA' ya zarar verme, enzimlerin aktivasyonunu ve membran geçirgenliğini bozarma diğer olumsuz etkileridir. Doymamış yağ asitlerinin oksidatif yıkımıyla meydana gelen lipid peroksidasyon sonucu açığa çıkan aldehitlerden biri olan MDA, hücrelerde oluşan oksidatif zararın en önemli göstergelerinden birisidir (Bragadottir, 2001; Morales vd., 2004; Mişe Yonar 2017b). Bu çalışmada da karaciğer, böbrek ve solungaç dokusunda, farklı su sıcaklıkları uygulamasıyla meydana gelen oksidatif stresin belirlenmesi için MDA düzeyindeki değişimler araştırılmıştır.

Dastan vd. (2017) 96 saat süreyle 0,5, 2,5, 5, 10, 20 ve 30 ppm konsantrasyonlarındaki polenin, alabalıklarda karaciğer MDA düzeyini tüm deneysel gruplarda, dalak ve kalp MDA düzeyini ise 0,5 ppm grubu dışındaki tüm deneysel gruplarda düşürdüğünü tespit etmişlerdir. Bu araştırmacıların (Dastan vd., 2017) elde ettiği sonucun aksine Ferreira vd. (2012) bir fungusit olan tebukonazolün etkisindeki *Rhamdia quelen* türü balıklarda, yalnız polenin uygulandığı grubun karaciğer, böbrek ve beyin MDA düzeylerinde istatistiksel herhangi bir farklılık belirlememişlerdir. Bu çalışmada da Dastan vd. (2017) tarafından elde edilen sonuçlara paralel olarak, kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan gruptaki (23 ºC + Po) balıkların karaciğer, böbrek ve solungaç MDA düzeylerinin kontrol grubuna göre azaldığı belirlenmiştir.

Su sıcaklığının balıklarda oluşturduğu stres konusunda bazı çalışmalar yapılmıştır. Örneğin; Kontrol (24 °C) grubuna göre 20 °C ve 28 °C' lik sıcaklıkların uygulandığı pullu sazanın karaciğer ve böbrek dokusundaki MDA düzeyinin önemli

düzeyde arttığı ifade edilmiştir (Mişe Yonar vd., 2013). Benzer şekilde Vinagre vd. (2012), 16 ºC' ye adapte ettikleri deniz levrekleri (*Dicentrarchus labrax*)' nde su sıcaklığının 18, 24 ve 28 °C' ye yükseltilmesiyle kas MDA düzeyinin yükseldiğini bildirmişlerdir. Roche ve Bogé (1996) aynı balık türünde oksidatif stres üzerine sıcaklığın etkisini araştırdıkları çalışmalarında, lipit peroksidasyon ve katalaz aktivitesinin termal stresle arttığını bulmuşlardır. Hwang ve Lin (2002) tarafından yapılan bir çalışmada da 25 °C ve 35 °C' de 10 hafta için ayrı ayrı kültür edilen sazanlarda hepatopankreas ve kas dokusundaki TBARS (thiobarbituric acid reactive substances) düzeyinin 35 °C' de tutulan balıklarda 25 °C' de tutulanlara kıyasla arttığı, bu balıklara vitamin C uygulamasıyla bu olumsuzluğun giderildiği belirlenmiştir. Diğer taraftan düşük sıcaklığın balıklarda oluşturduğu oksidatif stres de araştırılmıştır. Örneğin 11 ºC' den 8 ºC' ye sıcaklığın düşürülmesiyle strese sokulmuş salmonların karaciğer, böbrek ve beyin dokularındaki lipid peroksidasyon düzeyleri araştırılmış, 48. saatten itibaren MDA düzeyindeki artış en yüksek böbrekte en az karaciğerde belirlenmiştir. Bunun nedeni de karaciğerde vitamin E düzeyinin yüksek, börekte ise düşük olmasına bağlanmıştır (Welker ve Congleton, 2004). Bu çalışmada da kontrol grubu (23 ºC)' na kıyasla düşük (18 ºC) ve yüksek (28 ºC) sıcaklıkta tutulan fakat polen uygulanmayan gruplarda MDA düzeyi istatistiksel olarak artmış ve yukarıdaki araştırmacıların bulgularıyla paralel bulunmuştur. Fakat düşük (18 ºC) ve yüksek (23 ºC) sıcaklıklarda tutulan balıklara kıyasla, düşük ve yüksek sıcaklıklarda tutulan ve polen uygulanan (18 ºC + Po ve 28 ºC + Po) balıklarda MDA düzeyinin azaldığı belirlenmiştir. Polenin bu etkisi onun güçlü radikal temizleyici fonksiyonuyla (Eraslan vd., 2009) ve yapısında bulunan fenolik maddelerle (Leja vd., 2007) açıklanabilir.

Balıklarda antioksidanlar diğer yüksek omurgalılarda olduğu gibi enzimatik ve non-enzimatik olarak sınıflandırılırlar. Balıklarda bulunan antioksidan enzimler, süperoksit radikali ($O_2^{\cdot-}$)' ni temizleyen süperoksid dismutaz (SOD), hidrojen peroksit ($H_2O_2$)' i temizleyen katalaz (CAT), $H_2O_2$ ve lipid hidroperoksitleri yok eden glutatyon peroksidaz (GSH-Px) ile glutatyona bağlı diğer enzimlerdir (Belló vd., 2000; Mourente vd., 2002; Puangkaew vd., 2005).

Serbest radikaller ve peroksitlerle reaksiyona girerek hücreleri oksidatif stresten koruyan tripeptit karakterdeki GSH, çok önemli bir antioksidan olup non-enzimatik ve endojen özelliktedir. Protein yapısındaki sülfhidril gruplarını indirgenmiş halde tutan GSH böylece çoğu protein ve enzimin inaktive olmasını önler (Hayes ve McLellan 1999). Diğer taraftan GSH ile elektrofilik gruplar arasındaki konjugasyonu katalizleyen GST, ksenobiyotik ve endojen bileşiklerin detoksifikasyonu ve biyotransformasyonunda görevli faz II enzim ailesinin bir üyesidir (Hamed vd., 2003).

Bu çalışmada kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan balıklarda (23 ºC + Po), GSH düzeyinin ve GST aktivitesinin kontrol grubuna (23 ºC) göre arttığı belirlenmiştir. Aynı gruptaki balıkların MDA düzeyindeki düşüşle de ilişkili olarak antioksidan parametrelerde belirlenen bu artış polenin güçlü bir antioksidan olduğunu ve antioksidan kapasiteyi arttırdığını göstermektedir (Eraslan vd., 2009). Dastan vd. (2017) tarafından yapılan bir çalışmada, alabalıkların karaciğer, dalak ve kalp dokusundaki total serbest sülfirid grup düzeylerinin polenin uygulamasıyla arttığı, polenin toplam antioksidan kapasiteyi (TAS) artırarak total oksidan kapasiteyi (TOS) düşürdüğü belirlenmiştir. Ferreira vd. (2012) ise *Rhamdia quelen* türü balıklarda, polen uygulamasıyla karaciğer GST aktivitesinin arttığı, böbrek GST aktivitesinin düştüğü, beyin dokusundaki GST aktivitesinin ise etkilenmediğini ifade etmişlerdir. Yukarıda adı geçen araştırmacıların elde ettiği sonuçlar bu çalışmadan elde edilen sonuçlarla uyumlu bulunmuştur.

Diğer taraftan bu çalışmada düşük (18 ºC) ve yüksek (28 ºC) sıcaklıklarda tutulan fakat polen uygulanmayan balıkların karaciğer, böbrek ve solungacındaki GSH düzeyi, kontrol grubu (23 ºC)' na göre düşük bulunmuştur. Hwang ve Lin (2002) 25 °C su sıcaklığında tutulan sazanlara kıyasla 35 °C' de tutulanların hepatopankreas ve kas dokusundaki GSH düzeylerinin azaldığını ifade etmişlerdir. Kısa süreli yüksek sıcaklık uygulanan *Heteropneustes fossilis* türü balıklarda solungaç GSH-Px aktivitesi ve GSH düzeyi azalmıştır (Parihar vd., 1997). Benzer şekilde yine düşük (18 ºC) ve yüksek (28 ºC) sıcaklıklarda tutulan ancak polen uygulanmayan grupların karaciğer, böbrek ve solungacındaki GST aktivitesinin bu çalışmada azaldığı belirlenmiştir. *Paralichthys olivaceus* türü balıkların karaciğerindeki süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktivitesinin kontrol grubuna (25 °C) göre 28, 30 ve 32°C' deki deneme gruplarında uygulamanın 13. ve 19. günlerinde düştüğü belirlenmiştir (Lou vd., 2011). Kaur vd. (2005) 3 saat için sıcaklığın 12 ºC arttırılmasıyla 32 ºC' de strese sokulmuş *Channa punctata* türü balıklarda karaciğer, böbrek ve solungaç GST enzim aktivitesinin kontrol grubu (20 ºC)' na göre azaldığını göstermişlerdir. Fakat bu çalışmada düşük ve yüksek sıcaklıklarda tutulan ancak polen uygulanan balıkların (18 ºC + Po ve 28 ºC + Po) karaciğer, böbrek ve solungacındaki GSH düzeyi ve GST aktivitesinin polen uygulanmayanlara (18 ºC ve 28 ºC) kıyasla arttığı bulunmuştur. Bunun nedeni kontrol grubuyla aynı sıcaklıkta tutulan ve polen uygulanan grupta da (23 ºC + Po) gösterildiği gibi polenin serbest radikalleri temizlemesi ve antioksidan kapasiteyi arttırmasının bir sonucu olabilir.

Bu çalışmadan elde edilen sonuçlara göre, balıkların özellikle optimum aralıklar dışındaki çevresel sıcaklığa oldukça duyarlı olduğu, immun sistem fonksiyonlarının ve antioksidanların etkilerinin su sıcaklığıyla değiştiği söylenebilir. Polenin su sıcaklığının neden olduğu immun ve antioksidan parametreler üzerindeki olumsuz etkileri önleyebileceği, polenin balıklarda bir immunostimulan ve antioksidan olarak kullanılabileceği sonucuna varılabilir. Fakat farklı balık türlerinde, farklı sıcaklıklarda, farklı doz ve sürelerde polen uygulamalarının sonuçlarına ihtiyaç olduğu görülmektedir.

## TEŞEKKÜR VE MADDİ DESTEK

Bu çalışma; Yüksek Mühendis Merve TAŞKAN' ın yüksek lisans tezinden özetlenmiş ve Fırat Üniversitesi Bilimsel Araştırma Projeleri (FÜBAP) Yönetim Birimi tarafından SÜF.16.07. nolu proje olarak desteklenmiştir. Polen örneklerinin palinolojik olarak identifikasyonu için Erciyes Üniversitesi Seyrani Ziraat Fakültesi' nden Prof. Dr. Sibel SİLİCİ' ye teşekkür ederiz.


## YAZARLIK KATKISI

Merve TAŞKAN: Tasarım, denemenin yürütülmesi, örneklerin laboratuvarda analizi, yazma. M. Enis YONAR: Tasarım, gözden geçirme, düzenleme.

## ÇIKAR/REKABET ÇATIŞMASI BEYANI

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

## ETİK ONAY

Bu çalışma Fırat Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu Başkanlığı tarafından onaylanmıştır. (Protokol No: 2015/112, Karar No: 203).

## VERİ KULLANILABİLİRLİĞİ

Çalışma sırasında oluşturulan ve/veya analiz edilen veri setleri, editör veya hakemler tarafından talep edildiği takdirde ilgili yazar tarafından sağlanacaktır.

## REFERENCES


Abbass, A.A., El-Asely, A.M., & Kandiel, M.M.M. (2012). Effects of dietary propolis and pollen on growth performance, fecundity and some hematological parameters of *Oreochromis niloticus*. *Turkish Journal of Fisheries and Aquatic Sciences*, *12*, 851-859. https://doi.org/10.4194/1303-2712-v12_4_13

Alfonso, S., Gesto, M., & Sadoul, B. (2020). Temperature increase and its effects on fish stress physiology in the context of global warming. *Journal of Fish Biology*, *98*, 1496-1508. https://doi.org/10.1111/jfb.14599

Arda, M., Seçer, S., & Sarıeyyüpoğlu, M. (2017). *Fish Diseases*. (*Balık Hastalıkları*). Ankara: Medisan Yayınevi (in Turkish).

Belló, A.R.R., Fortes, E., Belló-Klein, A., Belló, A.A., Llesuy, S.F., Robaldo, R.B., & Bianchini, A. (2000). Lipid Peroksidataion induced by *Clinostomum detruncatum* in muscle of the freshwater fish *Rhamdia quelen*. *Diseases of Aquatic Organisms*, *42*, 233-236. https://doi.org/10.3354/dao042233

Bragadottir, M. 2001. Endogenous antioxidants in fish. The Degree of Master of Science in food science, Department of Food Science, University of Iceland.

Buchtíková, S., Šimková, A., Rohlenová, K., Flajšhans, M., Lojek, A., Lilius, E.M., & Hyršl, P. (2011). The seasonal changes in innate immunity of the common carp (*Cyprinus carpio*). *Aquaculture*, *318*, 169-175. https://doi.org/10.1016/j.aquaculture.2011.05.013

Çankaya, N., & Korkmaz, A. (2008). *Pollen*. (*Polen*). Samsun: Samsun İl Tarım Müdürlüğü Çiftçi Eğitimi ve Yayım Şubesi Yayını (in Turkish)

Çelikkale, M.S. (1994). *Freshwater Fish Culture*. (*İç Su Balıkları Yetiştiriciliği*). Trabzon: Karadeniz Teknik Üniversitesi Yayınları, (in Turkish).

Darson, M. (1981). Role and characterization of fish antibody. *Developmental Biological Standardisation*, *49*, 307-319.

Dastan, S.D., Gulhan, M.F., Selamoglu, Z., & Dastan, T. (2017). The determination of different effective concentration of ethanolic extract of bee pollen on biochemical analysis in liver, spleen and heart tissues of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792). *Iranian Journal of Fisheries Sciences*, *16*(1), 326-340.

Diker, S. (1998). *Immunology*. (*İmmunoloji*). Ankara: Medisan Yayınevi.

Dittmar, J., Janssen, H., Kuske, A., Kurtz, J., & Scharsack, J.P. (2014). Heat and immunity: an experimental heat wave alters immune functions in three-spined sticklebacks (*Gasterosteus aculeatus*). *Journal of Animal Ecology*, *83*, 744-757. https://doi.org/10.1111/1365-2656.12175

El-Asely, A.M., Abbass, A.A., & Austin, B. (2014). Honey bee pollen improves growth, immunity and protection of Nile tilapia (*Oreochromis niloticus*) against infection with *Aeromonas hydrophila*. *Fish and Shellfish Immunology*, *40*, 500-506. https://doi.org/10.1016/j.fsi.2014.07.017

Ellman, G.L. (1959). Tissue sulphydryl groups. *Archives of Biochemistry and Biophysics, 82*, 70-77. https://doi.org/10.1016/0003-9861(59)90090-6

Eraslan, G., Kanbur, M., & Silici, S., 2009. Effect of carbaryl on some biochemical changes in rats: The ameliorative effect of bee pollen, *Food and Chemical Toxicology*, *47*, 86-91. https://doi.org/10.1016/j.fct.2008.10.013

Ferreira, D., Unfer, T.C., Rocha, H.C., Kreutz, L.C., Gessi Koakoski, G., & Barcellos, L.J.G. (2012). Antioxidant activity of bee products added to water in tebuconazole-exposed fish. *Neotropical Ichthyology*, *10*(1), 215-220. https://doi.org/10.1590/S1679-62252012000100021

Habig, W.H., Pabst, M.J., & Jakoby, W.B. (1974). Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *The Journal of Biological Chemistry, 249* (22), 7130-7139.

Hamed, R.R., Farid, N.M., Elowa, S.H.E., & Abdalla, A.M. (2003). Glutathione related enzyme levels of freshwater fish as bioindicators of pollution. *The Environmentalist, 23*, 313–322. https://doi.org/10.1023/B:ENVR.0000031409.09024.cc

Harlioğlu, M.M., Çakmak, M.N., Köprücü, K., Aksu, Ö., Harlioğlu, A.G., Mişe Yonar, S., Çakmak Duran, T., Özcan, S., & Gündoğdu, H. (2012). The effect of dietary n-3 series fatty acids on the number of pleopadal egg and stage 1 juvenile in freshwater crayfish, *Astacus leptodactylus* Eschscholtz. *Aquaculture Research*, *44*, 860-868. https://doi.org/10.1111/j.1365-2109.2012.03090.x

Hayes, J.D., & McLellan, L.I. (1999). Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stres. *Free Radical Research, 31*, 273-300. https://doi.org/10.1080/10715769900300851

Heise, K., Puntarulo, S., Nikinmaa, M., Abele, D., & Pörtner, H.O. (2006). Oxidative stress during stressful heat exposure and recovery in the North Sea eelpout *Zoarces viviparus* L.. *Journal of Experimental Biology, 209*, 353-363. https://doi.org/10.1242/jeb.01977

Holland, M.C.H., & Lambris, J.D. (2002). The complement system in teleosts. *Fish and Shellfish Immunology*, *12*, 399-420. https://doi.org/10.1006/fsim.2001.0408

Hwang, D.F., & Lin, T.K. (2002). Effect of temperature on dietary vitamin C requirement and lipid in common carp. *Comparative Biochemistry and Physiology B, 131*(1), 1-7. https://doi.org/10.1016/s1096-4959(01)00449-3

Jeney, G., Galeotti, M., Volpatti, D., Jeney, Z., & Anderson, D. P., 1997. Prevention of stress in rainbow trout (*Oncorhynchus mykiss*) fed diets containing different doses of glucan. *Aquaculture*, *154*, 1-15. https://doi.org/10.1016/S0044-8486(97)00042-2

Kaur, M., Atif, F., Ali, M., Rehman, H., & Raisuddin, S., 2005. Heat stress-induced alterations of antioxidants in the freshwater fish Channa punctata Bloch. *Journal of Fish Biology*, *67*, 1653-1665. https://doi.org/10.1111/j.1095-8649.2005.00872.x

Langston, A.L., Hoare, R., Stefansson, M., Fitzgerald, R., Wergeland, H., & Mulcahy, M. (2002). The effect of temperature on non-specific defence parameters of three strains of juvenile Atlantic halibut (*Hippoglossus hippoglossus* L.). *Fish and Shellfish Immunology*, *12*, 61-76. https://doi.org/10.1006/fsim.2001.0354

Leja, M., Mareczek, A., Wyżgolik, G., Klepacz-Baniak, J., & Czekońska, K. (2007). Antioxidative properties of bee pollen in selected plant species, *Food Chemistry*, *100*(1), 237-240. https://doi.org/10.1016/j.foodchem.2005.09.047

Lou, B., Xu, D., Xu, H., Zhan, W., Mao, G., & Shi, H. (2011). Effect of high water temperature on growth, survival and antioxidant enzyme activities in the Japanese flounder *Paralichthys olivaceus*. *African Journal of Agricultural Research, 6*(12), 2875-2882.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., & Randal, R.J. (1951). Protein measurement with Folin phenol reagent. *Journal of Biochemistry*, *193*, 265-275. https://doi.org/10.1016/S0021-9258(19)52451-6

Lushchak, V.I. (2011). Environmentally induced oxidative stress in aquatic animals, *Aquatic Toxicology*, *101*(1), 13-30. https://doi.org/10.1016/j.aquatox.2010.10.006

Lushchak, V.I., & Bagnyukova, T.V., (2007). Hypoxia induces oxidative stress in tissues of a goby, the rotan *Perccottus glenii*. *Comparative Biochemistry and Physiology B*, *148*(4), 390-397. https://doi.org/10.1016/j.cbpb.2007.07.007

Makrinos, D.L., & Bowden, T.J. (2016). Natural environmental impacts on teleost immune function. *Fish and Shellfish Immunology*, *53*, 50-57. https://doi.org/10.1016/j.fsi.2016.03.008

Malek, R.L., Sajadi, H., Abraham, J., Grundy, M.A., & Gerhard, G.S. (2004). The effects of temperature reduction on gene expression and oxidative stress in skeletal muscle from adult zebrafish. *Comparative Biochemistry and Physiology C*, *138*(3), 363-373. https://doi.org/10.1016/j.cca.2004.08.014

Martinez-Álvarez, R.M., Morales, A.E., & Sanz, A. (2005). Antioxidant defenses in fish: Biotic and abiotic factors. *Fish Biology and Fisheries*, *15*, 75-88.

Mişe Yonar, S., Sakin, F., Yonar, M.E., Ispir, U., & Kirici, M. (2011). Oxidative Stress Biomarkers of Exposure to Deltamethrin in Rainbow Trout Fry (*Oncorhynchus mykiss*). *Fresenius Environmental Bulletin*, *20*(8), 1931-1935.

Mişe Yonar, S., Ural, MŞ., Silici, S., & Yonar, M.E. (2014). Malathion-induced changes in the haematological profile, the immune response, and the oxidative/antioxidant status of *Cyprinus carpio carpio*: Protective role of propolis. *Ecotoxicology and Environmental Safety*, *102*, 202-209. https://doi.org/10.1016/j.ecoenv.2014.01.007

Mişe Yonar, S., Yonar, M.E., Sağlam, N., & S. Silici, S. (2013). Farklı su sıcaklıklarında tutulmuş pullu sazan (*Cyprinus carpio carpio* linnaeus, 1758)'nın karaciğer ve böbreğindeki bazı antioksidan parametreler üzerine propolisin etkisi. *Menba, Kastomunu Üniversitesi Su Ürünleri Fakültesi Dergisi*, *1*, 11-16.

Mişe Yonar, S., Yonar, M.E., & Ural, M.Ş. (2017a). Antioxidant effect of curcumin against exposure to malathion in *Cyprinus carpio*. *Cellular and Molecular Biology*, *63*(3), 68-72. https://doi.org/10.14715/cmb/2017.63.3.13

Mişe Yonar, S., Köprücü, K., Yonar, M.E., & Silici, S. (2017b). Effects of dietary propolis on the number and size of pleopadal egg, oxidative stress and antioxidant status of freshwater crayfish (*Astacus leptodactylus* Eschscholtz). *Animal Reproduction Science*, *184*, 149-159. https://doi.org/10.1016/j.anireprosci.2017.07.010

Mişe Yonar, S., Yonar, M.E., Pala, A., Sağlam, N., & Sakin, F. (2020). Effect of trichlorfon on some haematological and biochemical changes in *Cyprinus carpio*: The ameliorative effect of lycopene. *Aquaculture Reports*, *16*, 100246. https://doi.org/10.1016/j.aqrep.2019.100246

Morales, A.E., Pèrez-Jimènez, A., Hidalgo, M.C., Abellán, E., & Gabriel C.G. (2004). Oxidative stres and antioxidant defenses after prolonged starvation in *Dentex dentex* liver. *Comporative Biochemistry and Physiology C*, *139*(1-3), 153-161. https://doi.org/10.1016/j.cca.2004.10.008

Mourente, G., Diaz-Salvago, E., Bell, J.G., & Tocher, D.R. (2002). Increased activities of hepatic antioxidant defence enzymes in juvenile gilthead sea bream (*Sparus aurata* L.) fed dietary oxidised oil: attenuation by dietary vitamin E. *Aquaculture, 214*, 343-361. https://doi.org/10.1016/S0044-8486(02)00064-9

Ndong, D., Chen, Y., Lin, Y., Vaseeharan, B., & Chen, J. (2007). The immune response of tilapia *Oreochromis mossambicus* and its susceptibility to *Streptococcus iniae* under stress in low and high temperatures. *Fish and Shellfish Immunology*, *22*, 686-694. https://doi.org/10.1016/j.fsi.2006.08.015

Nikoskelainen, S., Bylund, G., & Lilius, E.M. (2004). Effect of environmental temperature on rainbow trout (*Oncorhynchus mykiss*) innate immunity. *Development and Comparative Immunology*, *28*, 581-592. https://doi.org/10.1016/j.dci.2003.10.003

Parihar, M.S., & Dubey, A.K. (1995). Lipid peroxidation and ascorbic acid status in respiratory organs of male and female freshwater catfish *Heteropneustes fossilis* exposed to temperature increase. *Comparative Biochemistry and Physiology C*, *112*(3), 309-313. https://doi.org/10.1016/0742-8413(95)02025-x

Parihar, M.S. Javeri, T., Hemnani, T., Dubey, A.K., & Prakash, P. (1997). Responses of superoxide dismutase, glutathione peroxidase and reduced glutathione antioxidant defenses in gills of the fresh water catfish (*Heteropneustes fossilis*) to short-term elevated temperature. *Journal of Thermal Biology*, *22*, 151-156. https://doi.org/10.1016/S0306-4565(97)00006-5

Placer, Z.A. Cushman, L., & Johnson, B.C. (1966). Estimation of products of lipid peroxidation (malonyl dialdehyde) in biological fluids. *Analytical Biochemistry*, *16*, 359-364. https://doi.org/10.1016/0003-2697(66)90167-9

Puangkaew, J., Kiron, V., Satoh, S., & Watanabe, T. (2005). Antioxidant defense of rainbow trout (*Oncorhynchus mykiss*) in relation to dietary n-3 highly unsaturated fatty acids and vitamin E contents. *Comparative Biochemistry and Phsiology C, 140*, 187-196. https://doi.org/10.1016/j.cca.2005.01.016

Roche, H., & Boge, G. (1996). Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. *Marine Environmental Research*, *41*, 27-43. https://doi.org/10.1016/0141-1136(95)00015-1

Sakin, F., Ispir, Ü., Mişe Yonar, S., Yonar, M.E., & Taysi, R. (2011). Effect of short-term cypermethrin exposure on oxidant-antioxidant balance in the whole body of rainbow trout fry (*Oncorhynchus mykiss*). *Fresenius Environmental Bulletin*, *20*(10a), 2806-2809.

Sakin, F., Mişe Yonar, S., Yonar, M.E., & Saglam, N. (2012). Changes in selected immunological parameters and oxidative stress responses in different organs of *Oncorhynchus mykiss* exposed to ivermectin. *Revista de Chimie*, *63*(10), 989-995.

Siwicki, A., & Studnicka, M. (1987). The phagocytic ability of neutrophils and serum lysozyme activity in experimentally infected carp *Cyprinus carpio* L. *Journal of Fish Biology*, *31*(A), 57-60. https://doi.org/10.1111/j.1095-8649.1987.tb05293.x

Siwicki, A.K., Anderson, D.P., & Rumsey, G.L. (1994). Dietary intake of immunostimulants by rainbow trout affects non-specific immunity and protection against furunculosis. *Veterinary Immunology and Immunopathology*, *41*, 125–139. https://doi.org/10.1016/0165-2427(94)90062-0

Tort, L., Rotllant, J., & Roviva, L. (1998). Immunological suppression in gilthead sea bream *Sparus aurata* of the north-west Mediterranean at low temperatures. *Comparative Biochemistry and Physiology Part A*, *120*, 175-179. https://doi.org/10.1016/S1095-6433(98)10027-2

Tort, L., Rotllant, J., Liarte, C., Acerete, L., Hernández, A., Ceulemans, S., Coutteau, P., & Padros, F. (2004). Effect of temperature decrease on feeding rates, immune indicators and histopathological changes of gilthead sea bream *Sparus aurata* fed with an experimental diet. *Aquaculture*, *229*, 55-65. https://doi.org/10.1016/S0044-8486(03)00403-4

Vinagre, C., Madeira, D., Narciso, L., Cabral, H.N., & Diniz, M. (2012). Effect of temperature on oxidative stress in fish: Lipid peroxidation and catalase activity in the muscle of juvenile seabass, *Dicentrarchus labrax*. *Ecological Indicators, 23*, 274-279. https://doi.org/10.1016/j.ecolind.2012.04.009

Welker, T.L., & Congleton, J.L. (2004). Oxidative stress in juvenile chinook salmon, *Oncorhynchus tshawytscha* (Walbaum). *Aquaculture Research, 35*, 881-887. https://doi.org/10.1111/j.1365-2109.2004.01080.x

Xu, X., Sun, L., Dong, J., & Zhang, H. (2009). Breaking the cells of rape bee pollen and consecutvive extraction of functional oil with superficial carbon oxide, *Innovative Food Science and Emerging Technologies*, *10*, 42-46. https://doi.org/10.1016/j.ifset.2008.08.004

Yang, X., Guo, D., Zhang, J., & Wu, M. (2007). Characterization and anti-tumor activity of pollen polysaccharide. *International Immunopharmacology*, *7*(3), 401-408. https://doi.org/10.1016/j.intimp.2006.11.001

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 77-80 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.11

SHORT COMMUNICATION KISA MAKALE

# *Circulus* (Mollusca-Gastropoda) species of the Turkish coasts with a note on the presence of *Circulus octoliratus* (Carpenter, 1856)

## Türkiye kıyılarının *Circulus* (Mollusca-Gastropoda) türleri ve *Circulus octoliratus* (Carpenter, 1856)'un bulunuşu hakkında bir bilgi notu

**Bilal Öztürk[1]* ● Banu Bitlis[2] ● Neslihan Türkçü[3]**

[1] 832/1 Sokak No. 13/3, Bornova, İzmir, Türkiye. https://orcid.org/0000-0002-5638-2496
[2] Dokuz Eylul University, Institute of Marine Sciences and Technology, 35430 İnciralti, İzmir, Türkiye https://orcid.org/0000-0002-6542-8388
[3] Ege University, Faculty of Fisheries, Department of Hydrobiology, 35100 Bornova, İzmir, Türkiye https://orcid.org/0000-0003-4440-5600

*Corresponding author: ozturkbilal1955@gmail.com
Received date: 06.09.2022 Accepted date: 24.11.2022

**How to cite this paper:**
Öztürk, B., Bitlis, B., & Türkçü, N. (2023). *Circulus* (Mollusca-Gastropoda) species of the Turkish coasts with a note on the presence of *Circulus octoliratus* (Carpenter, 1856). *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 77-80. https://doi.org/10.12714/egejfas.40.1.11

**Abstract:** The present study is dealing with three *Circulus* species recorded along the Turkish coasts (İskenderun Bay, Levantine coast of Türkiye). Of the identified species, *Circulus novemcarinatus* and *Circulus octoliratus* are non-indigenous species originated outside the Mediterranean Sea, whereas *Circulus striatus* is a native one distributed in the eastern Atlantic Ocean and Mediterranean Sea. While *Circulus novemcarinatus* was recorded at depths between 9 and 60 m, *Circulus octoliratus* and *Circulus striatus* were encountered at shallow depths (5-11.7 m, respectively). Within the present study some morphological and distributional characteristics of the investigated species are described.

**Keywords:** *Circulus*, Gastropoda, morphology, distribution, İskenderun Bay, Türkiye

**Öz:** Bu çalışmada Türkiye kıyılarından (İskenderun Körfezi) örneklenen *Circulus* türleri konu edilmektedir. Tayin edilen türlerden *Circulus novemcarinatus* ve *Circulus octoliratus* yabancı kökenli türler olup, *Circulus striatus* ise yerli türdür ve Doğu Atlantik Okyanusu-Akdeniz dağılımlıdır. *Circulus novemcarinatus* 9-60 m arasındaki derinliklerde bulunmuş olmasına karşın, *Circulus octoliratus* ve *Circulus striatus* sığ derinliklerden örneklenmişlerdir (sırasıyla 5 ve 11,7 m). Bu çalışmada, incelenen türlerin bazı morfolojik ve dağılım özellikleri ele alınmıştır.

**Anahtar kelimeler:** *Circulus*, Gastropoda, morfoloji, dağılım, İskenderun Körfezi, Türkiye

### INTRODUCTION

The genus *Circulus* was established as a subgenus of *Trochus* with type species *Delphinula duminyi* Requien, 1848 (=*Valvata striata* Philippi, 1836) (Jeffreys, 1865). The representatives of the genus are rather small molluscs having a shell with smooth multispiral protoconch and a circular, flat teleoconch with a wide and deep umbilicus. On the whorls there are spiral cords only, of which some are prominent and forming keels. The genus was described in detail by Oliver and Rolán (2011).

In the studies carried out in the past (Fretter and Graham, 1962; Oliver and Rolán, 2011), the genus *Circulus* was investigated within the family Tornidae (subfamily Circulinae) than it was moved in the family Vitrinellidae (WoRMS, 2022), which taxon was upgraded to family rank in the work by Bouchet et al. (2017).

The present study is focusing on the morphologic and distributional features of three *Circulus* species recorded along the Turkish coasts.

### MATERIAL AND METHODS

In the past two decades, various cruises and research projects were performed in different areas along the Turkish coasts and a large amount of benthic materials were collected. Among the sampled materials from İskenderun Bay (Levantine coast of Türkiye) some *Circulus* specimens were found, which are dealing with herein (Figure 1).

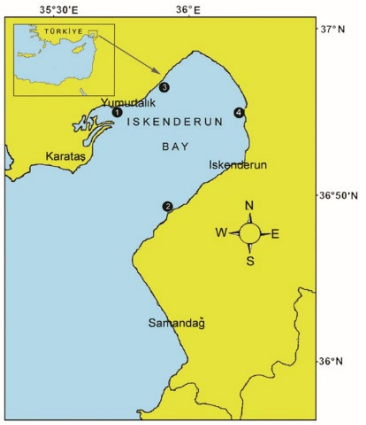



**Figure 1.** Map of the area where the investigated specimens were sampled

The material was sampled with a Van Veen Grab at depths between 5 and 60 m on soft bottoms. The specimens belonging to different species were deposited in the museum of the Faculty of Fisheries at Ege University (ESFM), (İzmir-Türkiye).

## RESULTS AND DISCUSSION

### SYSTEMATICS

Order: Littorinimorpha Golikov and Starobogatov, 1975

Family: Vitrinellidae Bush, 1897

Genus: *Circulus* Jeffreys, 1865

***Circulus novemcarinatus*** (Melvill, 1906) (Figure 2)

*Cyclostrema novemcarinatum*; Melvill, 1906: 22, pl. 3, fig. 3, 3a (*original description*).

*Circulus novemcarinatus*; Janssen et al., 2011: 421, pl. 18, fig.1.

*Lodderia novemcarinata*; Bosch et al., 1995: 38, fig. 64.

*Lodderia novemcarinata*; Öztürk et al., 2015: 207, fig. 2.

**Materials:** Sta. 1, 11.07.2010, 9 m, sandy mud, 2 spm (ESFM-GAS/2010-48); Sta. 1, 02.07.2014, 9 m, sandy mud, 3 spm (ESFM-GAS/2014-7); Sta. 2, 07.09.2019, 60 m, mud with shell fragments, 2 spm (ESFM-GAS/2019-14).

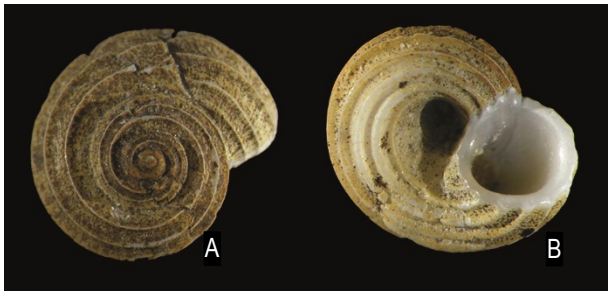



**Figure 2.** *Circulus novemcarinatus*: dorsal and ventral views of a specimen ($d_A$=$d_B$=5.1 mm, sandy mud, 9 m)

The type locality of the species is Gulf of Oman (Melvill, 1906) and since 2010 *Circulus novemcarinatus* is known to be distributed also in the Mediterranean Sea (Öztürk et al., 2015). Among the *Circulus* species encountered along the Turkish coasts, *C. novemcarinatus* is characteristic with its shell much stronger and more acute spiral carinae (keel), and more evident growth striae between the spiral ribs. The first record of the species from the Mediterranean Sea is based on the work by Öztürk et al. (2015) where the species was investigated in detail.

*Circulus novemcarinatus* is a non-indigenous species distributed in Gulf of Oman, Persian Gulf, Arabian Sea and Red Sea (Bosch et al., 1995; Janssen et al., 2011) and Mediterranean Sea (Öztürk et al., 2015). The species is considered among the established alien taxa being sampled multiple times.

***Circulus octoliratus*** (Carpenter, 1856) (Figure 3)

*Cyclostrema octolirata* Carpenter, 1856: 169, with type locality Red Sea.

*Circulus octoliratus* (Carpenter, 1856); Janssen et al., 2011: 422, pl. 18, fig. 2.

*Circulus octoliratus* (Carpenter, 1856); Ovalis and Mifsud, 2019: 267-270, figs. 1 A-C.

**Material:** Sta. 3, 11.07.2018, 7.3 m, sand, 1 spm (36°51'22"N-35°54'57"E) (ESFM-GAS/2018-13).

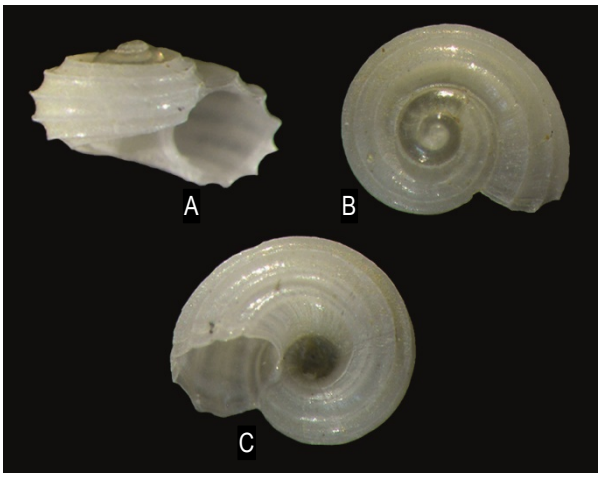



**Figure 3.** *Circulus octoliratus*: different views of the sampled specimen (A=B=C; h=0.5 mm; d=1.5 mm)

The type locality of the species is the Red Sea and it was described as *Cyclostrema octolirata* by Carpenter (1856). Then, Janssen et al. (2011) also recorded it from the same locality and indicated that the species was common in several localities in the Red Sea. Janssen et al. (2011), also mention of some specimens from Philippinnes identical to *C. octoliratus*. The species most probably entered into the Mediterranean via the Suez Canal, and was first recorded from Taşucu in August 2018 (Turkish Levantine coast) and published by Ovalis and Mifsud (2019). During marine sediment surveys carried out near Haydar Aliev pipeline terminal jetty at Ceyhan (İskenderun Bay, Levantine Sea) in July 2018, a single specimen of the species was found in a sandy biotope at a depth of 7.3 m.

*Circulus octoliratus* is characteristic with a shell consisting nearly of body whorl. Protoconch multispiral and smooth. The transition scar to the teleoconch is evident. Teleoconch circular and flat, with a wide and deep umbilicus. On the last whorl there are eight spiral cords of which the subsutural one overlaps the suture. Inside of umbilicus longitudinally striated. White in colour.

*Circulus octoliratus* is distributed in the Red Sea and Philippines (Janssen et al., 2011). In the Mediterranean, the

first recorded locality of *Circulus octoliratus* should be İskenderun Bay (July 2018) instead of Taşucu (August 2018).

***Circulus striatus*** (Philippi, 1836) (Figure 4)

*Delphinula duminyi*; Requien, 1848: 64.

*Circulus costulatus*; Locard, 1889: 283-307.

*Skeneia striatula*; Weinkauff,1862: 301-371, pl.13, figs 7-9.

**Materials:** Sta. 4, 14.09.2005, 5 m, sand, 1 spm (ESFM-GAS/2005-150); Sta. 3, 04.08.2013, 7.3 m, sand, 1 spm (ESFM-GAS/2013-211); Sta. 3, 02.07.2014, 11.7 m, sandy mud, 1 spm (ESFM-GAS/2014-134).

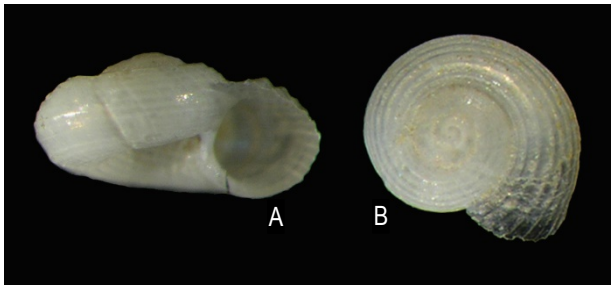



**Figure 4.** *Circulus striatus*: ventral and dorsal views of the recorded specimen (A=B; h=0.7 mm; d=1.3 mm)

Shell flat with about 3.5-4.0 convex teleoconch whorls. Body whorl is about 90 % of the shell. Spire small and looks like a bulge on the last whorl. Protoconch multispiral, smooth, with no ornamentation and its end is less obvious. On the whorls there are spiral cords not forming keels and they are as width as the half of the interspaces. Umbilical side is almost smooth or with less evident spiral cords. Aperture circular. Umbilicus wide and deep with four or five umbilical cords. Operculum corneous and rounded. The species was investigated in detail by Oliver and Rolan (2011).

*Circulus striatus* is distinguished from the other congeneric species distributed in the Mediterranean by having spiral cords, not keels. The species is distributed in the eastern Atlantic Ocean and Mediterranean Sea (Oliver and Rolan, 2011). Along the Turkish coasts it was previously recorded from the Levantine Sea (Bitlis et al., 2012), Aegean Sea (Demir, 2003) and Sea of Marmara (Oberling, 1969-1971).

In the world ocean, although is known to be distributed 233 *Circulus* species, only three species were reported from the Mediterranean Sea up to date, of which *Circulus novemcarinatus* and *Circulus octoliratus* are alien species originated outside the Mediterranean Sea. *C. novemcarinatus* is considered among the established species, whereas *Circulus octoliratus*, after a fresh shell recorded by Ovalis and Mifsud (2019) in Taşucu, some empty shells were also found on Israeli coast by Edelman-Furstenberg et al. (2020). With record of an alive specimen from İskenderun Bay, the species can be considered among the established alien species.

## CONCLUSION

Consequently, in the last decades, although, a large quantity of soft benthic material was studied from the Turkish coasts and the selecting process have been made under stereomicroscope, a few *Circulus* specimens were found only. This fact suggests that, in addition to their very small size, the population density of the genus representatives may be also low, especially in the eastern Mediterranean basin.

## ACKNOWLEDGEMENTS AND FUNDING

Thanks go to the anonymous referees for their contribution during the reviewing process of the manuscript. No funding was received for the present study.

## AUTHOR CONTRIBUTIONS

Bilal Öztürk: Designing of the study, identification of the investigated species, writing of the draft, submission, writing-review and editing. Banu Bitlis: Designing of the study, sorting the materials into taxonomic groups and checking the original draft. Neslihan Türkçü: Sorting the materials into taxonomic groups and checking the original draft.

## CONFLICTS OF INTEREST

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

## ETHICS APPROVAL

No specific ethical approval was necessary for the study.

## DATA AVAILABILITY

For any questions the corresponding author should be contacted.

## REFERENCES

Bitlis, B., Öztürk, B., Doğan, A., & Önen, M. (2012). Mollusc fauna of Iskenderun Bay with a checklist of the region. *Turkish Journal of Fisheries and Aquatic Sciences*, *12*(1), 171-184. https://doi.org/10.4194/1303-2712-v12_1_20

Bosch, D. T., Dance, S. P., Moolenbeek, R. G., & Oliver, P. G. (1995). Seashells of eastern Arabia. Dubai, Motivate publishing. 296 p.

Bouchet, P., Rocroi, J.P., Hausdorf, B., Kaim, A., Kano, Y., Nützel, A., Parkhaev, P., Schrödl, M. & Strong, E.E. (2017). Revised classification, nomenclator and typification of gastropod and monoplacophoran families. *Malacologia*, *61*(1-2),1-526. https://doi.org/10.4002/040.061.0201

Carpenter, P.P. (1856). *Description of new species and varieties of Calyptraeidae, Trochidae, and Pyramidellidae, principally in the collections of H. Cuming*. In Proceedings of the Zoological Society of London, 166-171.

Demir, M. (2003). Shells of Mollusca collected from the seas of Turkey. *Turkish Journal of Zoology*, *27*(2), 101-140.

Edelman-Furstenberg, Y., Kidwell, S. M., & de Stigter, H. C. (2020). Mixing depths and sediment accumulation rates on an arid tropical shelf based on fine-fraction 210Pb analysis. *Marine Geology*, *425*, 106198. https://doi.org/10.1016/j.margeo.2020.106198

Fretter, V., & Graham, A. (1962). *British prosobranch molluscs. Their functional anatomy and ecology. British prosobranch molluscs. Their functional anatomy and ecology. Ray Society, London, XVI*, 755.

Janssen, R., Zuschin, M., & Baal, C. (2011). Gastropods and their habitats from the northern Red Sea (Egypt: Safaga): Part 2: Caenogastropoda: Sorbeoconcha and Littorinimorpha. *Annalen des Naturhistorischen*

*Museums in Wien. Serie A für Mineralogie und Petrographie, Geologie und Paläontologie, Anthropologie und Prähistorie*, *113*, 373-509.

Jeffreys, J.G. (1865). *British Conchology: or, an Account of the Mollusca Which Now Inhabit the British Isles and the Surrounding Seas. Marine shells, comprising the remaining Conchifera, the Solenoconchia, and Gasteropoda as far as Littorina*. London, *3*, 393 p., 8 pls.

Locard, A. (1889). Matériaux pour servir l'histoire de la malacologie française. VIII. Note sur les espèces françaises appartenant au genre *Circulus*. *Bulletin de la Société Malacologique de France*, *6*, 283-307.

Melvil, J.C. (1906). A revision of the species of Cyclostrematidae and Liotiidae occurring in the Persian Gulf and North Arabian Sea. *Journal of Molluscan Studies, 7*(1), 20-28, pl. 3.

Oberling, J. J. (1969-1971). On the littoral Mollusca of the Sea of Marmara. *Separatdruck Aus Dem jahrbuch Des Naturhistoristorischen Museums Des Stadt*, *4*, 183-218.

Oliver, J.D., & Rolán, E. (2011). The family Tornidae (Gastropoda, Rissooidea) in the East Atlantic, 2. *Circulinae. Iberus*, *29*, 9-33.

Ovalis, P., & Mifsud, C. (2019). *Circulus octoliratus* (Carpenter, 1856) and *Phosinella digera* (Laseron, 1956): two new non-indigenous gastropod species for the Mediterranean Sea. *Iberus*, *37*(2): 267-270.

Öztürk, B., Recevik, M., & Geyran, K. (2015). New alien molluscs in the Mediterranean Sea. *Cahiers de Biologie Marine*, *56*(3), 205-212.

Requien, E. (1848). Catalogue des Coquilles de l'Île de Corse. Seguin, Avignon v-xii, 13-109.

Weinkauff, H.C. (1862). Catalogue des coquilles marines recueillies sur les côtes de l'Algérie. *Journal de Conchyliologie*, 10, 301-371 pl. 13 fig. 7-9.
WoRMS (2022). World Register of Marine Species. https://www.marinespecies.org/aphia.php?p=taxlist

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 40(1), 81-89 (2023)

Su Ürünleri Dergisi (2023)
DOI: 10.12714/egejfas.40.1.12

**DERLEME** **REVIEW**

# Metal nanopartiküllerin mikroalgler aracılığı ile yeşil sentezi

## Green synthesis of metal nanoparticles by microalgae

**Tuğçe Mutaf[1] ● Gülizar Çalışkan[2] ● Suphi Şurişvan Öncel[3] ● Murat Elibol[4*]**

[1] Biyomühendislik Bölümü, Mühendislik Fakültesi, Manisa Celal Bayar Üniversitesi, Manisa, Türkiye https://orcid.org/0000-0002-4195-4607
[2] Genetik ve Biyomühendislik Bölümü, Mühendislik Fakültesi, İzmir Ekonomi Üniversitesi, İzmir, Türkiye https://orcid.org/0000-0001-6221-9495
[3] Biyomühendislik Bölümü, Mühendislik Fakültesi, Ege Üniversitesi, İzmir, Türkiye https://orcid.org/0000-0003-2817-2296
[4] Biyomühendislik Bölümü, Mühendislik Fakültesi, Ege Üniversitesi, İzmir, Türkiye https://orcid.org/0000-0002-6756-6290

*Corresponding author: murat.elibol@ege.edu.tr

*Received date: 03.02.2022* *Accepted date: 09.06.2022*

***How to cite this paper:***
Mutaf, M., Çalışkan, G., Öncel, S.Ş., & Elibol, M. (2023). Green synthesis of metal nanoparticles by microalgae. *Ege Journal of Fisheries and Aquatic Sciences, 40*(1), 81-89. https://doi.org/10.12714/egejfas.40.1.12

**Öz:** Yeşil sentez olarak adlandırılan, nanopartiküllerin biyolojik kaynaklar aracılığı ile sentezlenmesine olan ilgi son yıllarda artış göstermiştir. Bunun temel nedeni geleneksel yöntemler olan fiziksel ve kimyasal yöntemlerde indirgeyici ve stabilize edici ajanlar olarak yüksek miktarlarda toksik kimyasala ihtiyaç duyuluyor olmasıdır. Daha çevre dostu ve insan sağlığı için tehdit oluşturmayan bitki, fungus, bakteri, alg gibi organizmalar yeşil nanopartikül sentezi için alternatif kaynaklardır. Sucul mikroorganizmalar olan mikroalgler üretmiş oldukları proteinler, vitaminler, pigmentler, yağ asitleri, hücre içi- hücre dışı polisakkaritler gibi fonksiyonel özelliğe sahip metabolitler sayesinde uzun yıllardır gıda, kozmetik ve ilaç endüstrilerinde formülasyonlara eklenmektedir. Bunların yanı sıra, son yıllarda yapılan çalışmalarla nanopartikül sentezinde de yüksek potansiyele sahip oldukları görülmüştür. Özellikle metal iyonlarının depolanmasını ve detoksifikasyonunu yapabildiklerinden ve metal iyonlarını elementel hale indirgeyen hücre içi ve hücre dışı metabolitlerce zengin olduklarından, metal nanopartiküllerin sentezi için yüksek potansiyele sahiptirler. Son yıllarda mikroalglerden nanopartikül sentezine odaklanmış olan yayın sayısı artmış ve pek çok mikroalg türünün gümüş, altın, titanyum, çinko, demir vb. metal nanopartikülleri hücre içi ve hücre dışı yollarla sentezleme potansiyeli araştırılmıştır.

Bu derleme makale kapsamında, nanopartikül sentezi için çalışılmış olan mikroalg ve siyanobakteri türleri, kullanılan farklı sentez yöntemleri, nanopartiküllerin sentez mekanizması, temel karakterizasyon yöntemleri ve yeşil sentezle üretilen nanopartiküllerin antimikrobiyal aktivitelerine odaklanılmıştır.

**Anahtar kelimeler:** Nanoteknoloji, metal nanopartikül, yeşil sentez, mikroalg, antimikrobiyal aktivite

**Abstract:** Green synthesis of metal nanoparticles through biological resources has attracted attention in recent years. The main reason for that, a lot of toxic chemicals as reducing and stabilizing agents are used in physical and chemical methods which are known as conventional methods. Organisms such as plants, fungi, bacteria, and algae are alternative sources for green nanoparticle synthesis because of their more eco-friendly nature and not be a threat to human health. Microalgae as aquatic microorganisms have been added into the formulations of food, cosmetics, and pharmaceutical for many years, due to their high value-added metabolites such as proteins, vitamins, pigments, fatty acids, intracellular and extracellular polysaccharides. In addition, microalgae have a high potential in biogenic nanoparticle synthesis because of their metal ions accumulation capability, phytoremediation potential, and rich in intracellular and extracellular metabolites that will reduce metal ions to elemental state. In recent years, the number of studies, focused on silver, gold, titanium, zinc, iron, etc. nanoparticle synthesis from many microalgae species by intracellular and extracellular pathways has increased.

This review article aims to provide a brief outline of microalgae and cyanobacteria species studied in the context of nanoparticle synthesis, different approaches for nanoparticle synthesis from microalgae, the mechanism of nanoparticle synthesis, and basic characterization principles and antimicrobial activities of nanoparticles produced by green synthesis.

**Keywords:** Nanotechnology, metal nanoparticle, green synthesis, microalgae, antimicrobial activity

### GİRİŞ

Nano kelimesi köken olarak Yunanca cüce anlamında kullanılan "nanos" kelimesinden türemiştir ve günümüzde metrenin milyarda birini ifade etmektedir. Bilimsel bir yaklaşım olarak ise nanoteknoloji, 1 nm ile 100 nm aralığında boyutlara sahip ve her biri kendine has karakteristik özellikler gösteren parçacıkların sentezini ve bu nano parçacıkların farklı disiplinlerde kullanım potansiyelini araştıran bir mühendislik yaklaşımıdır (Hulla vd., 2015; Narayanan ve Sakthivel, 2010). Malzemelerde boyut küçüldükçe yüzey alanı ve yüzey enerjisi arttığından, nanometre boyutlarında donma noktası, kaynama noktası, renk, iletkenlik, kimyasal reaksiyon verme eğilimi gibi fizikokimyasal özellikler değişmektedir. Nanomalzemelerin her biri sahip olduğu özgün boyut, şekil ve yüzey plazmon rezonansına göre farklı özellikler göstermekte, böylece biyoteknoloji, uzay teknolojileri, malzeme teknolojisi, biyomedikal, gıda, kozmetik, tekstil, çevre, tarım, tıp gibi disiplinlerde geniş bir kullanım alanına sahip olmaktadırlar (Saifuddin vd., 2009; Rai ve Posten, 2013; Pantidos ve Horsfall, 2014; Shah vd., 2015; Singh vd., 2016). Nanomalzemeler kapsamında sınıflandırılan metal nanopartiküller de endüstriyel alanlardan sağlık bilimlerine geniş bir yelpazede yenilikçi çözümler getirmeleri ile günden güne daha fazla ilgi görmekte ve bilimsel çalışmalar ışığında geliştirilen ticari ürünler son yıllarda artış göstermektedir. Gıda endüstrisinde; raf ömrünü uzatmak ve tüketime kadar olan süreçte gıdanın kalite ve güvenilirliğini korumak amacıyla

ambalajlama teknolojisinde nanomalzemeler kullanılmaktadır. Ambalaj içerisinde hedeflenen gaz ve nem konsantrasyonunun kontrolü için nanosensörlerin geliştirilmesi, ürünün tüketime kadar olan sürecinde mikrobiyal güvenilirliğini korumak amacıyla ambalaj malzemesinin antimikrobiyal gümüş nanopartiküller ile kaplanması ve tat-koku bileşenlerinin korunarak duyusal özelliklerin muhafazası için gıda bileşenlerinin enkapsüle edilmesi gıda endüstrisine yönelik ticari potansiyeli araştırılan yaklaşımlardır (Khezri vd., 2016). Kozmetik formülasyonlarda kullanılan neozomlar, lipozomlar, miseller, polimerik ve lipid bazlı nanopartiküller, karbon nanotüpler ve metal nanopartiküller etken maddelerin enkapsülasyonu veya sahip olduğu fonksiyonel özellikten dolayı (antimikrobiyal, antioksidan vb.) son zamanlarda sıklıkla kullanılmaktadır (Mu ve Sprando, 2010). Kozmetik formülasyonlarda etken madde olarak kullanılan ve stabilitesi düşük olan vitaminler, yağ asitleri, antioksidanlar nanomalzemeler içerisine enkapsüle edildiğinde stabiliteleri ve cilde emilimleri arttığından ürünü ticari açıdan iyileştirmektedir. Bunun yanı sıra titanyum dioksit ($TiO_2$) ve çinko oksit (ZnO) nanopartiküller UV koruma özelliklerinden dolayı uzun yıllardır nano boyutlarda kozmetik ürünlere eklenmektedir. Nanomalzemelerin kozmetik pazarına girişi ilk olarak 1986 yılında L'oreal tarafından losyon ve jel krem formülasyonlarına niozomların eklenmesi, Christian Dior tarafından ise lipozomların ilave edilmesiyle başlamıştır (Mu ve Sprando, 2010). Biyomedikal çalışmalarda ise nanopartiküller ilaç taşıma sistemlerinde nano-taşıyıcılar olarak görev yapmakta, etken maddenin nano-taşıyıcıya bağlanarak veya hapsedilerek hedeflenen bölgeye ulaşması amaçlanmaktadır. Erken tanı ajanı veya tümör hedefleme ajanı olarak başta kanser olmak üzere çeşitli rahatsızlıkların tanı ve tedavisinde başarılı sonuçlar vermektedirler (Singh, 2017; Tüylek, 2017). 2012 yılında yayınlanan bir çalışmada kloroplast metabolitleri aracılığıyla sentezlenen nano ölçekli altın-gümüş kompozitinin elektrokimyasal sensörlerde kullanıldığında düşük konsantrasyonlardaki 2-bütanon molekülünü tespit edebildiği ve kanserin erken teşhisini mümkün kıldığı belirtilmiştir (Zhang vd., 2012). Tekstil endüstrisinde ise fonksiyonel tekstillerin geliştirilmesi için nanoteknolojiden yararlanılmaktadır. Özellikle UV koruma sağlayan, antimikrobiyal özellikli, kolay temizlenebilir, hidrofobik kumaşların üretimi için nanomalzemeler oldukça yüksek potansiyele sahiptir. $TiO_2$ ve ZnO nanopartiküller nano boyutlarda sentezlendiğinde UV absorplama özelliği gösterdiğinden yeni nesil tekstil ürünlerinde kullanılmaktadır (Singh, 2017; Wong vd., 2006).

**Nanopartiküllerin geleneksel yaklaşımla sentezlenmesi**

Sentezlenen nanopartiküllerin şekil, boyut, kristalizasyon gibi morfolojik özellikleri fizikokimyasal özelliklerini de etkilemektedir. Özellikle ticari kullanımlarında istenen özellikleri sergileyebilmesi için nanomalzemeler uygulama alanına göre farklı yaklaşımlarla sentezlenmektedir (Pal vd., 2011; Raab vd., 2011).

Nanopartiküllerin fiziksel ve kimyasal yöntemlerle sentezlenmesi uzun yıllardır bilinen ve uygulanan metotlar olduğundan geleneksel yöntemler olarak adlandırılmaktadır. Geleneksel sentez yöntemleri yukarıdan aşağıya (top-down) ve aşağıdan yukarıya (bottom-up) olmak üzere iki temel yaklaşımda incelenmektedir. Yukarıdan aşağıya sentez yönteminde, mekanik öğütme ile makro boyutlardaki malzemeler öğütülmekte ve nano boyutlarda malzemeler elde edilebilmektedir. Aşağıdan yukarıya yaklaşımında ise gaz yoğunlaştırma, sol-jel tekniği, vakum uygulama, hidroliz gibi kimyasal yöntemler kullanılmaktadır (Raab vd., 2011). Fiziksel ve kimyasal yöntemler kullanılarak nanopartiküllerin büyük ölçeklerde düşük maliyetli üretimi mümkün olmaktadır, bu nedenle bilimsel araştırmalar ve endüstriyel uygulamalarda sıklıkla tercih edilmektedirler.

Bu avantajlarının yanı sıra, geleneksel yöntemlerde metal iyonlarının indirgenmesi, nanopartikül oluşum sonrası yüzey modifikasyonu ve stabilitenin korunması için indirgeyici ve stabilize edici kimyasallar kullanılmaktadır. Kullanılan toksik kimyasallar karsinojenik ve alerjenik olduğundan üretim sonrasında nanopartiküllerin özellikle biyoteknolojik kullanım alanlarını kısıtlamaktadır. Bunun yanı sıra ticari üretimlerin atığı olarak açığa çıkan yüksek hacimlerdeki kimyasallar ekosisteme zarar vermektedir. Bu durum canlı populasyonu ve biyoçeşitlilik için büyük bir tehlikeyi beraberinde getirecektir (Narayanan ve Sakthivel, 2010; Pantidos ve Horsfall, 2014; Shah vd., 2015; Thakkar vd., 2010).

**Nanopartiküllerin yeşil sentez yaklaşımıyla sentezlenmesi**

Geleneksel yöntemlerde karşılaşılan kısıtlamalar ve son yıllarda insan sağlığı ve çevre konularında artan bilinç, nanopartiküllerin üretimi için daha sürdürülebilir metotların geliştirilmesini beraberinde getirmiş ve sürdürülebilirlik hedefini karşılayan "yeşil nanoteknoloji" günümüzde oldukça ilgi çeken bir konuma gelmiştir. Yeşil nanoteknoloji, metal iyonlarının biyolojik organizmaların hücre içi-hücre dışı metabolitleri aracılığıyla indirgenmesi ve biyolojik polisakkaritlerle kaplanarak stabilizasyonlarının sağlanmasını hedefleyen bir yaklaşımdır (Singh vd., 2016). Nanopartiküllerin yeşil sentezi için bitkiler, funguslar, bakteriler, virüsler, mikroalgler, aktinomisetler gibi biyolojik organizmaların hücre içi metabolitlerini içeren ham ekstreleri, veya saflaştırılmış enzim, pigment, polisakkarit gibi biyomolekülleri, indirgeyici ve stabilize edici ajan olarak kullanılabilmektedir (Shah vd., 2015; Sharma vd., 2016; Singh vd., 2016).

Biyolojik organizmaların çoğunluğu sahip oldukları redüktaz enzimleri sayesinde metal tuzlarının hücre içerisinde depolanmasını ve detoksifikasyonunu gerçekleştirebilmektedir (Singh vd., 2016). Bu özellikleri ile hücre içine aldıkları metal iyonlarını indirgeyerek metal nanopartiküllere dönüştürebilmektedirler. Ayrıca redüktaz enzimlerini içeren ham ekstre ile veya saflaştırılmış redüktaz enzimleri ile hücre dışı ortamda da metal iyonlarının metal nanopartiküllere indirgenebilmesi mümkün olmaktadır.

Geleneksel yöntemlere alternatif olarak, biyolojik sentez yaklaşımında indirgeyici, stabilize edici kimyasallara gerek duyulmadan nanopartikül sentezi mümkün olmaktadır.

Böylece daha yüksek saflıkta, kimyasal kalıntı içermeyen, insan sağlığı ve çevre ile biyouyumlu, daha sürdürülebilir nanomalzemeler üretilebilmektedir.

**Mikroalgler aracılığı ile yeşil nanopartikül sentezi**

Genel terminolojide algler olarak tanımlanan makroalgler, mikroalgler ve siyanobakteriler tatlı, tuzlu ve sodalı su kaynaklarında ve toprakta tek hücre formunda veya koloniler halinde yaşayan fotosentetik canlılardır. Makroalgler çok hücreli, mikroalgler tek hücreli ökaryotik organizmalarken, siyanobakteriler prokaryotik mikroorganizmalardır. Habitatları gereği dönemsel olarak yüksek sıcaklık, yüksek UV radyasyonu, yüksek tuzluluk gibi ekstrem çevre şartlarına maruz kaldıklarından kendilerini koruyabilmek için çeşitli hücre içi ve hücre dışı metabolit sentezlemektedirler (Borowitzka, 2013). Sentezledikleri polisakkaritler, yağ asitleri, vitaminler, pigmentler (karotenoidler, fikobiliproteinler, klorofil pigmentleri), fenolik bileşenler vb. sayesinde zengin bir hücresel içeriğe sahip olduklarından son yıllarda alglere olan ilgi yoğunlaşmış, pek çok ticari alanda farklı amaçlarla kullanılmaya başlanmıştır. Özellikle gıda, tarım ve kozmetik sektörlerinde mikroalgler ve metabolitlerine olan ilgi günden güne artış göstermekte, doğala olan yönelim ve artan tüketici bilinci ile mikroalg türevli ticari ürün pazarı da büyümektedir.

Ticari potansiyellerinin yanı sıra yeşil nanoteknoloji kapsamında da alglere olan ilgi son 10 yılda artmıştır. Mikroalg ve siyanobakterilerin metal nanopartiküllerin biyojenik sentezini araştıran bilimsel rapor sayısında özellikle 2010 yılından sonra artış görülmektedir. Mikroalgler ve siyanobakteriler, fitoşelatinler ve metallotioneinler gibi hücre içi metal bağlayıcı peptidlerinde ve polifosfat yapılarında metal iyonlarını depolama ve detoksifiye etme özelliğine sahiptirler. Enzimatik detoksifikasyon, metal bağlayıcı proteinlerin sentezi, metallerin çözünmeyen kompleksler haline getirilerek çöktürülmesi gibi yöntemler ile mikroalglerin büyük çoğunluğu ağır metallere karşı tolerans gösterebilmektedirler (Önem, 2016).

Sahip oldukları detoksifikasyon mekanizmaları sayesinde de mikroalgler metal iyonlarının metal nanopartiküllere indirgenmesinde başarılı sonuçlar vermektedir (Bkz. Tablo 1). Yayınlanan raporlarda çoğunlukla çalışılmış olan türler, *Amphora* sp., *Euglena gracilis* Klebs, 1883, *Euglena deses* Ehrenberg, 1834, *Chlorealla* sp., *Botryococcus braunii*, *Chlamydomonas* sp., *Synechocystis* sp., *Synechococcus* sp., *Anabaena* sp., *Spirulina platensis*'tir (Dahoumane vd., 2016).

Tablo 1 incelendiğinde, literatürde çalışılan metal iyonlarının çoğunlukla gümüş iyonları olduğu görülmektedir. Bunun yanı sıra bakır, altın ve titanyum metal nanopartiküllerin sentezlenmesine yönelik çalışmalar da bulunmaktadır. Gümüş nanopartiküllere olan ilginin antimikrobiyal aktivite potansiyelinin yüksek olmasından kaynaklandığı düşünülmektedir. Gümüş iyonlarının mikroorganizmalar üzerine olan antimikrobiyal aktivitesi uzun yıllardır bilinmektedir. Yapılan çalışmalarda gümüş nanopartiküllerin de yüksek antimikrobiyal etkiye sahip olduğu görülmektedir. Bunun yanı sıra Caliskan vd. (2022) yayınladıkları çalışmada titanyum nanopartiküllerin de antimikrobiyal, antistatik ve antikarsinojen aktivitesi olduğu belirtilmiştir. Zayadi ve Bakar (2020) ise *Chlorella* sp. ve *Spirulina* sp. türlerinin kuru biyokütleleri ile altın nanopartiküllerin sentezlenme potansiyelini araştırmış ve 14 nm boyutunda küresel nanopartiküller sentezlemişlerdir.

Sentezlenen nanopartiküllerin fonksiyonel özelliklerini araştırarak antioksidan ve katalitik aktivite gösterdiğini belirtmişlerdir. Farklı türler kullanılarak farklı metal nanopartiküllerin sentezlendiği çalışmalar incelendiğinde, büyük çoğunluğunun üretim optimizasyonu yapmamış olduğu, belirlenen tek bir koşulda nanopartikülleri sentezlemiş olduğu ve sentezlenen nanopartiküllerin küresel morfolojiye sahip olduğu görülmektedir.

Morfolojinin fonksiyonel aktivite üzerinde etkili olduğu bilindiğinden, yapılan çalışmalarda sıcaklık, süre, pH gibi farklı üretim parametrelerinin optimize edilmesiyle farklı morfoloji ve boyutlarda nanopartiküllerin sentezlenerek çok farklı uygulama alanlarına hizmet edebileceği düşünülmektedir.

Şekil 1'de gösterildiği üzere mikroalgal metabolitlerin indirgeyici ve stabilize edici potansiyellerini araştıran yayınlarda nanopartiküllerin oluşumu için farklı yaklaşımlar denenmektedir. Temel olarak hücre içi ve hücre dışı nanopartikül sentezi olmak üzere iki yaklaşım bulunmaktadır:

***Hücre içi üretim:***

**a**. Hasat edilmiş, yaş veya kuru biyokütlenin kullanıldığı, metal iyonlarının bütünsel hücre içerisine alınarak, hücre içi metabolitler aracılığıyla indirgenmesi ve metal nanopartiküllerin oluşumu

**b.** Büyümenin devam ettiği kültüre metal iyonlarının ilave edilerek, iyonların hücre içinde depolanmasının sağlanması ve kültürle eş zamanlı nanopartikül oluşumu

***Hücre dışı üretim:***

**a**.Süpernatantın içerdiği hücre dışı metabolitler aracılığı metal iyonlarının metal nanopartiküllere indirgenmesi

**b.**Hasat sonrası ekstraksiyon yapılarak ham ekstrenin içerdiği metabolitler aracılığı ile metal iyonlarının metal nanopartiküllere indirgenmesi

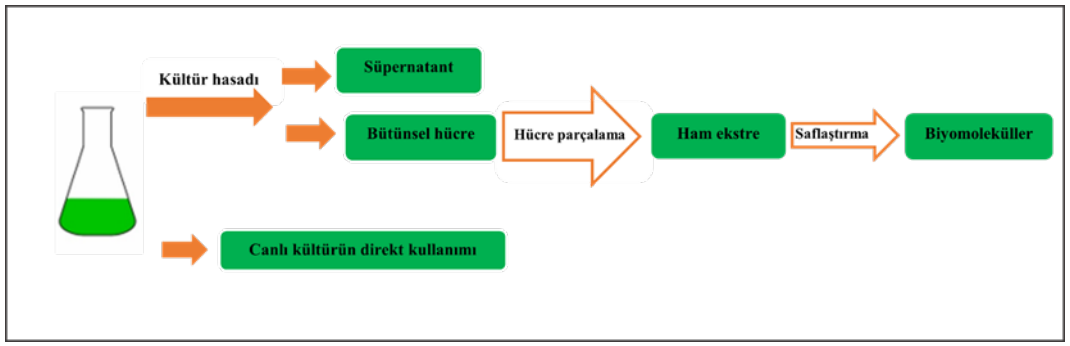
**c.**Ham ekstre içerisinden saflaştırılmış biyomolekülün indirgeyici özelliği ile metal nanopartiküllerin sentezi

Pek çok mikroalg ve siyanobakteri türü kültürleri sırasında dış ortama hücre dışı metabolitler salgılayabildiği için hücre içi nanopartikül sentezinin yanı sıra hücre dışı ortamda da metal iyonlarının indirgenmesi ve nanopartiküllerin sentezi mümkün olmaktadır (Dahoumane vd., 2016; Sharma vd., 2016).

**Tablo 1.** Mikroalgler aracılığı ile sentezlenen metal nanopartiküller

**Table 1**. Metal nanoparticles synthesized by microalgae

| Mikroalg | Nanopartikül | Sentez yöntemi | Boyut | Morfoloji | Fonksiyonel özellikler | Referans |
|---|---|---|---|---|---|---|
| *Chlorella vulgaris* | AgCl | Süpernatant | 9.8 nm | Küresel | Antimikrobiyal Aktivite | Silva Ferreira vd., 2017 |
| *Laurencia catarinensis* | AgNP | Kuru biyokütle-ham ekstre | 39 -77nm | Küresel, üçgen, dikdörtgen, altıgen | - | Abdel-Raouf vd., 2018 |
| *Desmodesmus abundans, Spirulina platensis* | AgNP | Yaş biyokütle, yaş biyokütle +süpernatant, süpernatant | 18-127 nm | - | - | Mora-Godinez vd., 2022 |
| *Trichodesmium erythraeum* | AgNP | Ham ekstre | 26.5 nm | Kübik | Antibakteriyel aktivite, antioksidan aktivite, antikarsinojen etki | Sathishkumar vd., 2019 |
| *Anabaena cylindrica* | CuO | Ham ekstre | 3.6 nm | - | Antimikrobiyal Aktivite | Bhattacharyaa vd., 2019 |
| *Chlorella* sp. | AgNP | Ham ekstre | 85 nm | Küresel | Hücrelerde biyohidrojen üretimini artırma potansiyeli | Yildirim vd., 2021 |
| *Chlorella* sp. *Spirulina* sp. | AuNP | Kuru biyokütle | 14 nm | Küresel | Antioksidan aktivite, katalitik aktivite | Zayadi vd., 2020 |
| *Chlorella vulgaris, Botryococcus braunii, Spirulina platensis, Amphora* sp. *Nitzschia* sp. | AgNP | Ham ekstre | 55 nm | Küresel | Fotokatalitik renk giderme aktivitesi | Rajkumar vd., 2021 |
| *Phaeodactylum tricornutum* | TiNP | Süpernatant | 50 nm | Küresel | Antimikrobiyal aktivite, antistatik aktivite, antikarsinojen etki | Caliskan vd., 2022 |




**Şekil 1**. Mikroalglerden farklı yaklaşımlarla nanopartikül sentezi

**Figure 1**. Various nanoparticle synthesis mechanisms via microalgae

## Mikroalgal nanopartiküllerin sentez yöntemleri

### *Hücre içi nanopartikül sentezi*

Hücre içi nanopartikül sentezi için iki yaklaşım bulunmaktadır. İlk yaklaşım logaritmik büyüme fazında bulunan kültür içerisine metal tuz çözeltisinin eklenmesi ve kültürle eş zamanlı olarak nanopartikül sentezinin gerçekleşmesidir (Dahoumane vd., 2016). Bu sentez yönteminde metal çözeltisinin konsantrasyonu önemli bir parametredir, çünkü kullanılan metallerin büyük çoğunluğu ağır metaller olduğundan hücreler üzerinde toksik etki yaratacak kadar yüksek konsantrasyonlar başarılı sonuç verememektedir. Bu nedenle metal tuz çözeltisinin konsantrasyonunun optimizasyonu gerekmektedir. Metal çözeltisinin konsantrasyonunun yanı sıra metal çözeltisi hacminin kullanılan mikroalgal kültürün hacmine olan oranı, pH, inkübasyon süresi, inkübasyon boyunca karıştırma hızı da sentezlenen nanopartiküllerin aglomerasyon eğilimini, nanopartiküllerin boyutunu ve fonksiyonel özelliklerini etkileyen diğer parametrelerdir (Caliskan vd., 2022). Literatürde nanopartikül sentez mekanizmasının tamamen aydınlatılabilmesi için her bir metale yönelik sentez parametrelerinin göz önüne alınması, optimizasyon çalışmaları yapılarak çalışma özelinde hangi koşullarda en başarılı sentezin gerçekleşebildiği tespit edilmelidir.

Bir diğer hücre içi sentez yaklaşımında ise, istenen biyokütle miktarı elde edildiğinde kültür hasat edilmekte ve hasat sonrası biyokütle yaş olarak veya kurutularak metal tuz çözeltisiyle süspanse edilmektedir. Oluşan süspansiyonun kontrollü koşullarda inkübasyonu ile metal iyonlarının hücre membranından içeriye girerek elementel hale indirgenmesi ve nanopartiküllerin sentez basamağının hücre içinde gerçekleşmesi sağlanmaktadır (Dahoumane vd., 2016; Wishkerman ve Arad, 2017). Her iki yaklaşım da araştırılmış ve mikroalg türlerinin hücre içi metabolitlerinin farklı metal iyonlarını indirgeme potansiyelleri incelenmiştir.

Wishkerman ve Arad (2017), *Phaeodactylum tricornutum* Bohlin, 1897 mikrolagini kullanarak gümüş nanopartiküllerin hücre içi sentezlenme potansiyelini araştırmışlardır. Mikroalgal

kültür ortamına değişen konsantrasyonlarda (0, 0.25, 0.5, 1, 1.5 mg/l) $Ag^+$ iyonu ilave ederek üretim gerçekleştirmiş ve taramalı elektron mikroskobu (SEM) ile hücre yüzeyinde oluşan nanopartikülleri gözlemlemişlerdir. 0.5 ve 1 mg/l ve 1.5 mg/l gümüş konsantrasyonunun hücre büyümesini dikkate değer oranda azalttığı görülmüştür. SEM ile hücre yüzeyinde 200 nm ve daha küçük boyutlarda nanopartiküller sentezlendiğini gözlemlemişlerdir. Yapılan denemeler sonrası gümüş iyonlarının hücre membranı ve sitoplazmada bulunan enzimler, polisakkaritler, polifosfatlar ve karboksil grupları ile indirgenmiş olabileceğini yorum olarak belirtmişlerdir.

Başka bir çalışmada *Chaetoceros calcitrans* (Paulsen) Takano, 1968, *Isochrysis galbana* Parke, 1949, *Chlorella salina* Butcher, 1952 ve *Tetraselmis gracilis* (Kylin) Butcher, 1959 mikroalgleri kullanılarak farklı yöntemler ile gümüş nanopartiküllerin (AgNP) sentezlenme potansiyeli araştırılmıştır. İlk denemede üretim öncesi besi yerine 1 mM $AgNO_3$ ilave edilmiş ve hücrelerin gümüş varlığında inkübasyonu yapılarak hem hücre büyümesi hem de nanopartikül oluşumu takip edilmiştir. İkinci denemede logaritmik fazda bulunan kültür hasat edilerek süpernatantın ve yaş biyokütlenin gümüş iyonları ile muamelesi sağlanarak AgNPler sentezlenmiştir. Farklı bir deneme olarak ise logaritmik fazdayken hasat edilen hücreler ultrasonikasyon ile parçalanmış ve 1 mM $AgNO_3$ metal çözeltisi ile süspanse edilerek nanopartikül sentezi gerçekleştirilmiştir. Sentezlenen AgNPlerin yüzey plazmon rezonansına bakılarak 420 nm civarında pik verdiği görülmüştür. Ayrıca sentezlenen nanopartiküllerin *Proteus vulgaricus*, *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeroginosa* patojen bakterilerine karşı antimikrobiyal etkisi de test edilmiştir (Merin vd., 2010).

Kırmızı makroalg türü *Lemanea fluviatilis* (Linnaeus) C. Agardh, 1811 ile yapılan çalışmadan kuru biyokütle kullanılarak 530 nm'de maksimum absorbans veren altın nanopartiküller sentezlenmiştir. İndirgenme reaksiyonu sonrası kırmızıya değişen rengin 3 ay boyunca korunduğu belirtilmiştir. Geçirimli elektron mikroskobu (TEM) ile yapılan gözlem sonucu nanopartiküller,n 5-15 nm çapa sahip olduğu görülmüş, antioksidan aktivite testi de yapılarak sentezlenen altın Nanopartiküllerin serbest radikalleri süpürme etkisi olduğu ispatlanmıştır (Sharma vd., 2014).

***Hücre dışı nanopartikül sentezi***

Hücre dışı nanopartiküllerin sentezi, ekstraksiyon sonrası elde edilen ham ekstre, saflaştırılmış biyomüleküller veya kültürün hasat edilmesi ile elde edilen süpernatantın indirgeyici ortam olarak kullanılması ile gerçekleşmektedir. Hücre içi sentez ile karşılaştırıldığında, nanopartikül sentezi sonrası hücre parçalama ve nanopartikülleri hücre kalıntılarından ayırma gibi basamaklar olmadığından işlem kolaylığı ve zamandan kazanç sağlamaktadır. Çalışmaların büyük çoğunluğu hücre içi senteze odaklanmış olsa da metal nanopartiküllerin hücre dışı metabolitler aracılığı ile sentezini araştıran çalışmalar da son yıllarda artış göstermiştir (Dahoumane vd., 2016; Gallon vd., 2019; Caliskan vd., 2022).

Patel ve ark. 2015 yılında yayınladıkları çalışmada 8 mikroalg ve 8 siyanobakteri türünden hücre içi ve hücre dışı AgNP sentezini araştırmışlardır. Üretim sonrası hasat edilen kültürden yaş biyokütle ve süpernatantı indirgeyici ortam olarak kullanmış, nanopartikül sentezi için hem aydınlık hem karanlık koşullarda inkübasyon gerçekleştirmişlerdir. Ek çalışma olarak *Limnothrix* sp. 37-2-1 suşundan saflaştırdıkları C-fikosiyanin ve *Scenesmus* sp. 145-3 suşunun kültür süpernatantından saflaştırdıkları polisakkaritin gümüş iyonlarını indirgeme potansiyelini araştırmışlardır. Karanlık koşulların aydınlık koşula göre daha başarılı bir indirgenme sağladığı yapılan UV-Vis spektroskopik analiziyle tespit edilmiştir. Bunun yanı sıra C-fikosiyaninin gümüş metalini indirgediği fakat zamanla pigmentin metal iyonlarından dolayı denatüre olduğu da belirtilmiştir (Patel vd., 2015). En başarılı sonucu *Spirulina'*dan elde edilen C-fikosiyanin ile etmiş, 13-31 nm aralığında AgNP sentezleyebilmişlerdir.

Gallón vd. (2019) *Botryococcus braunii* ve *Auxenochlorella pyrenoidosa* (H. Chick) Molinari & Calvo-Perez, 2015 *(=Chlorella pyrenoidosa* H. Chick, 1903*)* türlerine ait kültür süpernatantından hücre dışı polisakkaritleri saflaştırmış ve gümüş nanopartiküllerin sentezi için indirgeyici ve stabilize edici kaynak olarak kullanmışlardır. 5-15 nm boyutlarda AgNP sentezlemiş ve üretilen nanopartiküllerin antimikrobiyal aktivitelerini araştırmışlardır. Gümüş Nanopartiküllerin konsantrasyonuna bağlı olarak patojen bakterilere karşı antimikrobiyal etki gösterdiğini belirtmişlerdir. Aynı zamanda sentezlenen nanopartiküllerin insan dermal fibroblastlarına karşı toksik bir etki göstermediğini de sitotoksisite testleri ile doğrulamışlardır.

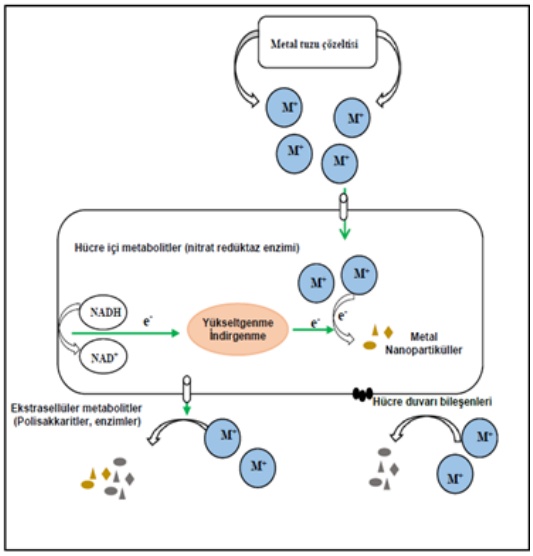
**Mikroalgal nanopartiküllerin sentez mekanizması**

Metal iyonları hücre içi veya hücre dışı metabolitler aracılığı ile metal nanopartikülleri oluşturmak üzere indirgenmektedirler. Biyolojik yollarla indirgenme ve nanopartikülün oluşum mekanizması henüz tam olarak aydınlatılamamış olsa da yayınlanan raporlarda hücre duvarı, hücre membranı bileşenlerinin, hücre içi ve hücre dışı enzimlerin, hücre içi polisakkarit, protein ve enzimlerin indirgenme reaksiyonlarında aktif rol oynadığı belirtilmektedir (Bkz. Şekil 2) (Salunke vd., 2016). Pozitif yüklü metal tuzları ve negatif yüklü hücre çeperi bileşenleri arasında oluşan elektrostatik kuvvet redoks reaksiyonları için sürükleyici gücü oluşturmakta, hücre içi bileşenler ise elektron taşıyıcı sistem olarak görev almaktadır (Skladanowski vd., 2017).

Sentez mekanizmasının aydınlatılmasına yönelik yapılan çalışmalar gümüş metali üzerine yoğunlaşmış, pozitif yüklü gümüş iyonlarının indirgenmesi için elektron taşıma sisteminden elektron alımının NADH bağımlı redüktaz enzimiyle katalizlenerek gerçekleştiği belirtilmiştir. NADH molekülü $NAD^+$ olarak yükseltgenmekte, gümüş iyonu da elektronu alarak elementel gümüşe indirgenmektedir. Elementel hale indirgenmiş gümüş Nanopartiküllerin hücre içi protein ve polisakkaritlerle kaplanması veya bağ yapması ile de stabilizasyonun sağlandığı düşünülmektedir (Nayak vd.,

2016). Bunun yanı sıra gerçekleşen redoks reaksiyonunun başarısı da mikroorganizmanın bulunduğu yaşam evresine göre de değişiklik göstermektedir. Metal iyonlarının metal nanopartiküllere indirgenme başarısı ve üretim verimi erken logaritmik fazda bulunan hücrelerde logaritmik fazın sonlarında olan hücrelere göre daha yüksek olmaktadır (Salunke vd., 2016).

Hücre dışı sentez reaksiyonlarında ise indirgenme basamaklarında ekzopolisakkaritlerin etkin rol oynadığı düşünülmektedir. Mikroalgler tarafından sentezlenen ekzopolisakkaritler çoğunlukla negatif yüklü sülfat ve glukuronik asit gruplarından oluştuğundan pozitif yüklü metal iyonları için indirgeyici ajan olarak görev yağmaktadırlar. Bunun yanı sıra polisakkaritler sentezlenen nanopartikülün etrafını saran bir kaplayıcı ajan olarak da stabiliteyi artırıcı etki göstermektedir (Lutzu vd., 2017).




**Şekil 2.** Biyojenik nanopartiküllerin sentez mekanizması (Salunke vd., 2016'den değiştirilerek hazırlanmıştır.)

**Figure 2.** Synthesis mechanism of biogenic nanoparticles (Modified from Salunke et al., 2016)

### Mikroalgler aracılığı ile sentezlenen nanopartiküllerin karakterizasyon teknikleri

Sentezlenmiş olan Nanopartiküllerin kullanım alanına uygunluğunun tespiti için farklı teknikler ile karakterize edilmesi ve boyut dağılımı, şekil, yüzey yükü gibi morfolojik özellikleri ile kimyasal özelliklerinin tespit edilmesi gerekmektedir. Her bir nanopartikül sahip olduğu morfolojik özelliklere göre farklı fizikokimyasal özellikler ve fonksiyona sahip olmaktadır, bu durum da nanopartikülün potansiyel kullanım alanını etkilemektedir. Örneğin ilaç taşıma sistemlerinde veya kozmetik formülasyonlarda kullanılmak üzere sentezlenmiş nanopartiküllerin boyut aralığı ve nanopartiküllerle biyoaktif bileşenler arasındaki elektrostatik kuvvetleri etkileyen yüzey yükü işlevsellik açısından önemli parametrelerdir (Pal vd., 2011).

Nanopartiküllerin morfolojik özelliklerinin karakterizasyonu için çoğunlukla geçirimli elektron mikroskobu (TEM), taramalı elektron mikroskobu (SEM), atomik kuvvet mikroskobu (AFM) gibi mikroskobik görüntüleme metotları kullanılmaktadır. Partiküllerin yüzey yükünün belirlenmesi ve süspansiyon içindeki ortalama boyut dağılımının ölçümü için zeta potansiyeli ve dinamik ışık saçılımı (DLS) yöntemleri kullanılmaktadır. Bunun yanı sıra metal iyonlarının elementel hale indirgenmesi ile sentezlenen nanopartikülün yüzey plazmon rezonansı her metal için karakteristik olduğundan, sentezlenme sonrası süspansiyonun maksimum absorbans verdiği dalga boyu indirgenme reaksiyonunun gerçekleştiği hakkında ön bilgi vermektedir. Bu nedenle nanopartikül sentezinin başarısına dair hızlı bir sonuç verdiğinden UV-Vis spektroskopik analizi de metal nanopartiküllerin karakterizasyonu için kullanılan bir tekniktir (Djurišić vd., 2014; Pal vd., 2011). Kendine özgü yüzey plazmon rezonansından dolayı her bir metal nanopartikülün kolloidal süspansiyonu da karakteristik bir renge sahiptir. Örneğin gümüş iyonları gümüş nanopartiküllere indirgendiğinde süspansiyonun şeffaf rengi sarıdan kızıl kahverengiye doğru değişmektedir (Ahn vd., 2019). Benzer şekilde titanyum nanopartikül oluşumunda ise şeffaf metal çözeltisinin rengi nanopartikül konsantrasyonuna bağlı olarak kırık beyaz ile gri arasında bir renk almaktadır (Caliskan vd., 2022). Çinko ve demir nanopartikül sentezinde ise metal çözeltisinin şeffaf rengi nanopartikül oluşumunun başladığı andan itibaren kahverengiye dönmektedir (Agarwal vd., 2019; Dhandapani vd., 2020). Renk değişimi her bir metal iyonu için karakteristik olduğundan, metal iyonlarının indirgenerek metal nanopartiküllerin oluşumu ve deneyin başarısı hakkında kısa sürede fikir vermesi açısından bir indikatör görevi görmektedir.

Sentezlenen nanopartiküllerin karakterizasyonu için yaygın olarak kullanılan bir diğer teknik ise Fourier dönüşümlü kızılötesi spektroskopisi (FTIR)'dir. Sentezlenen nanopartikülleri ve nanopartiküller ile biyomoleküller arasındaki kimyasal bağları tanımlayabilmek için FTIR analizi gerekmektedir (Agarwal vd., 2019; Dhandapani vd., 2020). Örneğin $TiO_2$, ZnO gibi metal oksit nanopartiküllerde O-Ti-O bağlarını veya Zn-O bağını temsil eden bağ titreşimlerinin varlığı ile sentezlenen nanopartiküllerin moleküler yapısı hakkında bilgi sahibi olunmaktadır. Bunun yanı sıra sentezlenen nanopartiküller ile biyomoleküller arasındaki bağlar da tanımlanarak hangi moleküllerin nanopartikül sentezinde ve stabilizasyonun sağlanmasında görev aldığı belirlenebilmektedir (Agarwal vd., 2019; Dhandapani vd., 2020).

Yayınlanan raporlar incelendiğinde SEM ve TEM mikroskopi teknikleri de nanopartiküllerin boyut ve şeklini belirlemek için en çok tercih edilen karakterizasyon teknikleridir. Fakat mikroskobik yöntemlerde alınan sonuçlar taranan bölgeye bağlı olduğundan genel bir sonuçtan ziyade belirli bir bölgeye özgü sonuçlar alınabilmektedir. Bu nedenle mikroskopi görüntülerinin yanı sıra ortalama partikül boyut dağılımı hakkında bilgi almak adına DLS ile de karakterizasyon yapılarak elde edilen sonuçlar doğrulanmalıdır (Hassellöv vd., 2008; Pal vd., 2011).

**Mikroalgler aracılığı ile sentezlenen nanopartiküllerin antimikrobiyal aktiviteleri**

Antibiyotikler yıllardır bakteriyel enfeksiyonları tedavi etmek amacıyla kullanılmasına rağmen son zamanlarda antibiyotiklere dirençli bakteri sayısında artış olması, yeni nesil antibakteriyel ajanların gündeme gelmesini ve araştırılmasını sağlamıştır. Gümüş, bakır, altın, titanyum ve çinko gibi metallerin uzun zamandır bilinen antimikrobiyal özelliklerinden dolayı metal nanopartiküllerin de antimikrobiyal aktivitesi olabileceği düşünüldüğünden nanopartikül sentezine odaklanan çalışmaların büyük çoğunluğu antibakteriyel etkiyi de araştırmıştır. Gümüş, titanyum dioksit, silikon, bakır oksit, çinko oksit, magnezyum oksit ve kalsiyum oksit nanopartiküllerin antimikrobiyal etkisi *in vitro* çalışmalarla ispatlanmıştır (Bhattacharyaa vd., 2019; Caliskan vd., 2022; Silva Ferreira vd., 2017; Sathishkumar vd., 2019).

Nanopartiküllerin antibakteriyel etkisi henüz tam olarak aydınlatılamamış olsa da iki temel yaklaşım geçerliliğini korumaktadır:

**1.** Metallerin bakteri ile teması sonrası nanopartikül yüzeyinden çözünerek metal iyonlarına dönüşmesi ve bakteri için ağır metal toksisitesi yaratması.

**2.** Nanopartikül yüzeyinde reaktif oksijen türlerinin (ROS) oluşmasına bağlı oksidatif stresin meydana gelmesi (Besinis vd., 2014).

Antibakteriyel aktivitesi test edilen metal nanopartiküller çoğunlukla AgNP üzerine yoğunlaşmış olsa da altın, çinko, titanyum metal nanopartiküllerinin de farklı bakteri türlerine olan antibakteriyel etkileri bulunmaktadır. Antimikrobiyal etki için gram pozitif ve gram negatif bakteriler, patojen bakteriler (gıda patojeni vb.), antibiyotik dirençli bakteriler ve funguslar üzerine çalışmalar yoğunlaşmıştır. Antimikrobiyal aktivite çalışmalarında en sık rastlanan mikroorganizma türleri, *Streptococcus mutans, Escherichia coli, Salmonella typhimurium, Vibrio parahaemolyticus, Staphylococcus epidermidis, Bacillus megaterium, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans, Aspergillus fumigates, Aspergillus niger* vb. türleridir (Dizaj vd., 2014).

Fiziksel karakterizasyon tekniklerine ilave olarak yeşil sentez yaklaşımıyla sentezlenen nanopartiküllerde araştırılan en yaygın biyo-fonksiyonel özellik sahip oldukları antimikrobiyal aktivitedir. Özellikle gıda ve biyomedikal uygulamalar için sentezlenen nanopartiküllerin hedef mikroorganizmalar üzerinde antimikrobiyal aktivite göstermesi beklenmektedir. Bu nedenle güncel raporlarda hedeflenen alana yönelik mikroorganizmalar belirlenerek sentezlenen nanopartiküllerin antimikrobiyal aktiviteleri test edilmektedir. Algotiml ve diğerleri, 2022 yılında yayınladıkları çalışmada *Ulva rigida, Cystoseira myrica*, ve *Gracilaria foliifera* algal ekstrelerinin gümüş iyonlarını indirgeme potansiyelini incelemiş ve 12-24 nm arasında değişen gümüş nanopartiküller (AgNP) sentezlemişlerdir. Farklı algal kaynaklarla sentezledikleri AgNP'lerin farklı antimikrobiyal aktiviteler gösterdiğini, *Ulva rigida* ile sentezlenen AgNP'lerin hem funguslar hem de bakteriler üzerinde en yüksek antimikrobiyal aktiviteye sahip olduğunu belirtmişlerdir (Algotiml vd., 2022). Başka bir çalışmada ise Patel ve ekibi gümüş nanopartiküllerin antibakteriyel aktivitesinin partikül boyutuna bağlı olduğunu belirtmiştir. Yaptıkları çalışmada nanopartikül boyutu arttıkça antimikrobiyal aktivitenin azaldığını görmüş, 10 nm'den daha büyük partiküllerin mikroorganizmalar ile yüzey temasında azalmalar meydana geldiği ve antimikrobiyal etki mekanizmasını gerçekleştiremediği şeklinde bir yorum yapmışlardır (Patel vd., 2015). Farklı kaynaklar aracılığı ile üretilen gümüş nanopartiküllerde farklı antimikrobiyal aktivitelerin görülmesinin temel sebebi, nanopartiküllere bağlanan metabolitlerin farklı olmasıdır. İndirgenme reaksiyonu boyunca nanopartiküller üzerine stabilize edici ve kaplayıcı ajan olarak bağlanan biyomoleküller her bir mikroalg için farklılık gösterdiğinden, elde edilen antimikrobiyal aktivitelerde de farklılıklar meydana gelmektedir. Metal nanopartiküllerin antimikrobiyal aktivitesini araştıran çalışmaların büyük çoğunluğu gümüş metaline odaklanmış olsa da çinko, bakır, titanyum nanopartiküllerin antimikrobiyal aktivitesi olduğunu doğrulayan çalışmalar da bulunmaktadır (Ali vd., 2020; Caliskan vd., 2022; Bhattacharyaa vd., 2019; Zayadi ve Bakar, 2020). Caliskan ve ark. *Phaeodactylum tricornutum* mikroalginin kültür süpernatantı aracılığı ile titanyum nanopartikülleri sentezlemiş, ardından sentezledikleri nanopartikülleri kitosanla kaplayarak kitosanın antimikrobiyal aktivite üzerindeki etkisini incelemişlerdir. Kitosan kaplanmış nanopartiküllerin antibakteriyel aktivitesinde de artış olduğunu belirtmişlerdir (Caliskan vd., 2022).

**Sonuç**

Özetlenecek olursa; bilişim sistemleri, uzay ve malzeme teknolojisi, kozmetik, gıda, tekstil endüstrileri, biyomedikal uygulamalar gibi hayatın her alanında karşımıza çıkan nanopartiküllerin yeşil sentezi, daha temiz bir dünya için çevre dostu ve sürdürülebilir üretim yaklaşımında önemli bir paya sahiptir. Bu amaç doğrultusunda, fotosentetik canlılar olan mikroalgler de sentezledikleri hücre içi ve hücre dışı metabolitler sayesinde yeşil nanopartikül sentezi için yüksek potansiyel göstermektedirler. Açık havuz sistemleri veya kapalı fotobiyoreaktör sistemleriyle laboratuvar ölçeğinden ticari ölçeklere kadar kontrollü koşullarda ve yüksek verimlilikte üretim potansiyeline sahip olmaları, ağır metalleri hücre içine alarak elementel hale indirgeyebilmeleri ve yüksek metal konsantrasyonlarını tolere edebilmeleri sayesinde nanopartikül sentezinde başarılı sonuçlar vermektedirler. Mikroalglerin bu alandaki potansiyeli son yıllarda fark edilmiş ve yayınlanan araştırma sayısı artış göstermiş olsa da literatürde hala tam olarak aydınlatılamamış noktalar bulunmaktadır. Özellikle iyonların indirgenme mekanizması için farklı yorumlar ve varsayımlar olmasına rağmen, her metal için hangi elektron taşıyıcı sistemin görev aldığı, hangi metabolitlerin hangi metal iyonlarını indirgeyebildiği ve indirgenme yolakları tam olarak

aydınlatılabilmiş değildir. Bu nedenle hücre içi ve hücre dışı sentezin yanı sıra her bir molekülün saflaştırılarak metal iyonlarına olan etkilerinin incelenmesi ve her bir metal iyonuna özgü indirgenme yolaklarının araştırılması gelecek çalışmalar için önerilmektedir. Aydınlatılması gereken bir diğer konu ise pH, sıcaklık, karıştırma hızı, metal konsantrasyonu, inkübasyon süresi gibi farklı üretim parametrelerinin nanopartiküllerin morfolojik yapısı ve fonksiyonel özelliklerine olan etkisinin araştırılmasıdır. Yayınlanmış çalışmalar genellikle tek bir parametre üzerine odaklanmış olsa da birden fazla parametrenin bir arada incelenmesi ve nanopartikül sentezine olan etkileri araştırılarak optimizasyon çalışmalarının yapılması gerekmektedir. Böylelikle sentezlenen nanopartiküllerin şekil, boyut gibi morfolojik yapıları, dolayısıyla fizikokimyasal özellikleri ve fonksiyonları daha iyi kontrol edilebilecek, istenen özellikleri sağlayan, hedeflenen uygulama alanına spesifik nanopartiküller daha kontrollü olarak üretilebilecektir.

**Teşekkür ve Maddi Destek**

Bu çalışma, Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (TUBİTAK) tarafından 117M052 numaralı 1001 projesi kapsamında finansal olarak desteklenmiştir.

**Yazarlık Katkısı**

Tüm yazarlar çalışma fikrine ve tasarımına katkıda bulunmuştur. Makalenin yazılması ve düzenlenmesi aşağıda belirtilen isimler tarafından yapılmış olup, tüm yazarlar makaleyi okuyup onaylamıştır.

Tuğçe Mutaf: İnceleme, yazma- özgün taslak hazırlama, literatür taraması

Gülizar Çalışkan: araştırma, yazma-gözden geçirme ve düzenleme

Suphi S. Öncel ve Murat Elibol: Gözden geçirme, düzenleme, denetleme

**Çıkar/Rekabet Çatışması Beyanı**

Yazarlar herhangi bir rekabet veya çıkar çatışması olmadığını beyan eder.

**Etik Onay**

Bu çalışma için özel bir etik onay gerekli değildir.

**Veri Kullanılabilirliği**

Bu makalenin sonuçlarını destekleyen veriler makalede mevcuttur.

## KAYNAKÇA

Abdel-Raouf, N., Alharbi, R.M., El-Anazi, N.M., Alkhulaifi, M.M., & Ibraheem, I.B.M. (2018). Rapid biosynthesis of silver nanoparticles using the marine red alga *Laurencia catarinensis* and their characterization. *Beni-Suef University Journal of Basic and Applied Sciences, 7*, 150–157. https://doi.org/10.1016/j.bjbas.2017.10.003

Ahn, E.Y., Hang, J., & Youmie, P. (2019). Assessing the antioxidant, cytotoxic, apoptotic and wound healing properties of silver nanoparticles green-synthesized by plant extracts. *Materials Science & Engineering, 101*, 204–216. https://doi.org/10.1016/j.msec.2019.03.095

Agarwal, H., Amatullah, N., Soumya, M., & VenkatKumar, S. (2019). Eco-friendly synthesis of zinc oxide nanoparticles using *Cinnamomum Tamala* leaf extract and its promising effect towards the antibacterial activity. *Journal of Drug Delivery Science and Technology*, *53*, 101212. https://doi.org/10.1016/j.jddst.2019.101212

Algotiml, R., Ali, G.A., Roshdi, S., Hussein, H.A., Mahmoud, Z.E., & Khaled, E. (2022). Anticancer and antimicrobial activity of biosynthesized Red Sea marine algal silver nanoparticles. *Scientific Reports*, *12*, 2421. https://doi.org/10.1038/s41598-022-06412-3

Ali, M.A., Temoor, A., Wenge, W., Afsana, H., Rahila, H., Mahidul, I.M., Yanli, W., Qianli, A., Guochang, S., & Bin, L. (2020). Advancements in Plant and Microbe-Based Synthesis of Metallic Nanoparticles and Their Antimicrobial Activity against Plant Pathogens. *Nanomaterials*, *10*, 1146. https://doi.org/10.3390/nano10061146

Besinis, A., De Peralta, T., & Handy, R.D. (2014). The antibacterial effects of silver, titaniumdioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on *Streptococcus mutans* using a suite of bioassays. *Nanotoxicology, 8*, 1–16. https://doi.org/10.3109/17435390.2012.742935

Bhattacharyaa, P., Swarnakar, S., Ghosh, S., Majumdar, S., & Banerjee, S. (2019). Disinfection of drinking water via algae mediated green synthesized copper oxide nanoparticles and its toxicity evaluation. *Journal of Environmental Chemical Engineering*, *7*, 102867. https://doi.org/10.1016/j.jece.2018.102867

Borowitzka, M.A. (2013). High-Value products from microalgae—their development and commercialisation. *Journal of Applied Phycology*, *25*, 743–756. https://doi.org/10.1007/s10811-013-9983-9

Caliskan, G., Mutaf, T., Agba, H.C., & Elibol, M. (2022). green synthesis and characterization of titanium nanoparticles using microalga, *Phaeodactylum tricornutum. Geomicrobıology Journal, 39* (1), 83–96. https://doi.org/10.1080/01490451.2021.2008549

Dahoumane, S.A., Mechouet, M., Alvarez, F.J., Agathos, S.N., & Jeffryes, C. (2016). Microalgae: an outstanding tool in nanotechnology. *Bionatura*, *1*(4), 196-201. https://doi.org/10.21931/RB/2016.01.04.7

Dhandapani, K.V., Devipriya, A., Arumugam Dhanesh, G., Purandaradas, A., Bala Sundaram, M., Purushothaman, K., & Babujanarthanam, R. (2020). Green route for the synthesis of zinc oxide nanoparticles from *Melia azedarach* leaf extract and evaluation of their antioxidant and antibacterial activities. *Biocatalysis and Agricultural Biotechnology*, *24*, 101517. https://doi.org/10.1016/j.bcab.2020.101517

Dizaj, S.M., Lotfipour, F., Barzegar-Jalali, M., Zarrintan, M.H., & Adibkia, K. (2014). Antimicrobial activity of the metals and metal oxide nanoparticles. *Materials Science and Engineering*, *44*, 278–284. https://doi.org/10.1016/j.msec.2014.08.031

Djurišić, A.B., Leung, Y.H., Alan, M.C., Xu, X.Y., Lee, P.K.H., Degger, N., & Wu, R.S.S. (2014). Toxicity of metal oxide nanoparticles: mechanisms, characterization, and avoiding experimental artefacts. *Small, 11*(1), 26-44. https://doi.org/10.1002/smll.201303947

Gallón, S.M.N., Alpaslan, E., Wang, M., Larese-Casanova,P., Londono, M.E., Atehortua, L., Pavon, J.J., & Webster, T.J. (2019). Characterization and study of the antibacterial mechanisms of silver nanoparticles prepared with microalgal exopolysaccharides. *Materials Science & Engineering*, *99*, 685-695. https://doi.org/10.1016/j.msec.2019.01.134

Hassellöv, M., Readman, J.W., Ranville, J.F., & Tiede, K. (2008). Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. *Ecotoxicology*, *17*, 344-361. https://doi.org/10.1007/s10646-008-0225-x

Hulla, J., Sahu, S.C., & Hayes, A.W. (2015). Nanotechnology: history and future. *Human and Experimental Toxicology*, *34*(12), 1318-1321. https://doi.org/10.1177/0960327115603588

Khezri, S., Kia, E.M., Seyedsaleh, M.M., Abedinzadeh, S., & Dastras, M. (2016). Application of nanotechnology in food ındustry and related health concern challenges. *International Journal of Advanced Biotechnology and Research*, *7*(2), 1370-1382.

Lutzu, G.A., Zhang, L., Zhang, Z., & Liu, T. (2017). Feasibility of attached cultivation for polysaccharides production by *Porphyridium cruentum*. *Bioprocess Biosystems Engineering*, *40*, 73-83. https://doi.org/10.1007/s00449-016-1676-8

Merin, D.D., Prakash, S., & Bhimba, B.V. (2010). Antibacterial screening of silver nanoparticles synthesized by marine microalgae. *Asian Pacific Journal of Tropical Medicine*, *3*(10), 797-799. https://doi.org/10.1016/S1995-7645(10)60191-5

Mora-Godinez, S., Abril-Martinez, F., & Pacheco, A. (2022). Green synthesis of silver nanoparticles using microalgae acclimated to high $CO_2$. *Materials Today: Proceedings*, *48*, 5-9. https://doi.org/10.1016/j.matpr.2020.04.761

Mu, L., & Sprando, L. (2010). Application of nanotechnology in cosmetics. *Pharmaceutical Research*, *27*, 1746-1749. https://doi.org/10.1007/s11095-010-0139-1

Narayanan, K.B., & Sakthivel, N. (2010). Biological synthesis of metal nanoparticles by microbes. *Advances in Colloid and Interface Science*, *156*, 1-13. https://doi.org/10.1016/j.cis.2010.02.001

Nayak, P.S., Arakha, M., Kumar, A., Asthana, S., Mallick, B.C., & Jha, S. (2016). An approach towards continuous production of silver nanoparticles using *Bacillus thuringiensis*. *The Royal Society of Chemistry, 6,* 8232-8242.

Önem, B. (2016). Çinko, civa ve kalay toksisitesinin *Arthrospıra platensıs* gomont alginin gelişimi ve antioksidan enzimlerinin üzerine etkisi. (Yüksek lisans tezi). Sakarya Üniversitesi, Sakarya.

Pal, S.L., Jana, U., Manna, P.K., Mohanta, G.P., & Manavalan, R. (2011). Nanoparticle: An overview of preparation and characterization. *Journal of Applied Pharmaceutical Science*, *01*(06), 228-234.

Pantidos, N., & Horsfall, L.E. (2014). Biological synthesis of metallic nanoparticles by bacteria, fungi and plants. *Nanomedicine & Nanotechnology*, *5*, 5. https://doi.org/10.4172/2157-7439.1000233

Patel, V., Berthold, D., Puranik, P., & Gantar, M. (2015). Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity. *Biotechnology Reports*, *5*, 112-119. https://doi.org/10.1016/j.btre.2014.12.001

Raab, C., Simko, M., Fiedeler, U., Nentwitch, M., & Gazso, A. (2011). Production of nanoparticles and nanomaterials. *Nano Trust Dossiers*, 006.

Rai, M., & Posten, C. (2013). *Green biosynthesis of nanoparticles: Mechanism and applications.* UK: Berforts Information Press Ltd.

Rajkumar, R., Ezhumalai, G., &Gnanadesigan, M. (2021). A green approach for the synthesis of silver nanoparticles by *Chlorella vulgaris* and its application in photocatalytic dye degradation activity. *Environmental Technology & Innovation*, *21*, 101282. https://doi.org/10.1016/j.eti.2020.101282

Saifuddin, N., Wong, C.W., & Nur Yasumira, A.A. (2009). Rapid biosynthesis of silver nanoparticles using culture supernatant of bacteria with microwave ırradiation, *E-Journal of Chemistry*, *6*(1), 61-70. https://doi.org/10.1155/2009/734264

Salunke, B.K., Sawant, S.S., Lee, S., & Kim, B.S. (2016). Microorganisms as efficient biosystem for the synthesis of metal nanoparticles: current scenario and future possibilities. *World Journal of Microbiololgy and Biotechnology*, *32* (5), 88. https://doi.org/10.1007/s11274-016-2044-1

Sathishkumar, R.S., Sundaramanickam, A., Srinath, R., Ramesh, T., Saranya, K., Meena, M., & Surya, P. (2019). Green synthesis of silver nanoparticles by bloom forming marine microalgae *Trichodesmium erythraeum* and its applications in antioxidant, drug-resistant bacteria, and cytotoxicity activity. *Journal of Saudi Chemical Society, 23*, 1180–1191. https://doi.org/10.1016/j.jscs.2019.07.008

Shah, M.S., Fawcett, D., Sharma, S., Tripathy, S.K., & Poinem, G.E.J. (2015). Green synthesis of metallic nanoparticles via biological entities. *Materials*, *8*, 7278-7308. https://doi.org/10.3390/ma8115377

Sharma, B., Purkayastha, D.D., Hazra, S., Thajamanbi, M., Bhattacharjee, C.R., Ghosh, N.N., & Rout, J. (2014). Biosynthesis of fluorescent gold nanoparticles using an edible freshwater red alga, *Lemanea fluviatilis* (L.) C.Ag. and antioxidant activity of biomatrix loaded nanoparticles. *Bioprocess Biosystrm Engineering*, *37*(12), 2559-65. https://doi.org/10.1007/s00449-014-1233-2

Sharma, A., Sharma, S., Sharma, K., Chetri, S.P.K., Vashishtha, A., Singh, P., Kumar, R., Rathi, B., & Agrawal, V. (2016). Algae as crucial organisms in advancing nanotechnology: a systematic review. *Journal of Applied Phycology*, *28*, 1759-1774. https://doi.org/10.1007/s10811-015-0715-1

Silva Ferreira, V., ConzFerreira, M.E., Lima, L.M.T.R., Frases, S., de Souza, W., & Sant'Anna, C. (2017). Green production of microalgae-based silver chloride nanoparticles with antimicrobial activity against pathogenic bacteria. *Enzyme and Microbial Technology*, *97*, 114–121. https://doi.org/10.1016/j.enzmictec.2016.10.018

Singh, P., Kim, Y.J., Zhang, D., & Yang, D.C. (2016). Biological synthesis of nanoparticles from plants and microorganisms, *Trends in Biotechnology*, *34*(7), 588-599. https://doi.org/10.1016/j.tibtech.2016.02.006

Singh, N.A. (2017). Nanotechnology ınnovations, Industrial Applications and Patents. *Environmentel Chemistry Letters*, *15*:185-191. https://doi.org/10.1007/s10311-017-0612-8

Skladanowski, M., Wypij, M., Laskowski, D., Golinska, P., Dahm, H., & Rai, M. (2017). Silver and gold nanoparticles synthesized from streptomyces sp. ısolated from acid forest soil with special reference to its antibacterial activity against pathogens. *Journal of Cluster Science, 28*, 58-79. https://doi.org/10.1007/s10876-016-1043-6

Thakkar, K.N., Mhatre, S.S., & Parikh, R.Y. (2010). Biological synthesis of metallic nanoparticles. *Nanomedicine: Nanotechnology, Biology and Medicine*, *6*, 257-262. https://doi.org/10.1016/j.nano.2009.07.002

Tüylek, Z. (2017). Drug Delivery Systems and Nanotechnological Interaction, *Bozok Tıp Dergisi*, 7(3), 89-98. (in Turkish with English abstract)

Wishkerman, A., Arad, M. S. (2017). Production of silver nanoparticles by the diatom *Phaeodactylum tricornutum*. *Nanotechnology VIII*, 1048. https://doi.org/10.1117/12.2264706

Wong, Y.W.H., Yuen, C.W.M., Leung, M.Y.S., Ku, S.K.A., & Lam, H.L.I. (2006). Selected applications of nanotechnology ın textiles. *AUTEX Research Journal*, *6*(1).

Yildirim, O., Tunay, D., Ozkaya, B., & Demir, A. (2021). Effect of green synthesized silver oxide nanoparticle on biological hydrogen production. *International Journal of Hydrogen Energy*, In Press. https://doi.org/10.1016/j.ijhydene.2021.11.176

Zayadi, R.A., & Bakar, F.A. (2020). Comparative study on stability, antioxidant and catalytic activities of biostabilized colloidal gold nanoparticles using microalgae and cyanobacteria. *Journal of Environmental Chemical Engineering, 8*, 103843. https://doi.org/10.1016/j.jece.2020.103843

Zhang, Y., Gao, G., Qirong, Q., & Cui, D. (2012). Chloroplasts-mediated biosynthesis of nanoscale au-ag alloy for 2-butanone assay based on electrochemical sensor. *Nanoscale Research Letters*, 7(475). http://www.nanoscalereslett.com/content/7/1/475