Ege Journal of
Fisheries and Aquatic Sciences

www.egejfas.org

E-ISSN 2418-3140

EgeJFAS

Su Ürünleri Dergisi

Volume 42 Number 2

2025

Ege University Faculty of Fisheries

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 Haziran June 15th, 2025

İletişim **Contact**
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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.

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While starting

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

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- Research papers and reviews must not exceed 25 manuscript pages including tables and figures (except checklists).
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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.

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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.

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This should briefly state the major findings of the study.

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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."

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

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

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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."

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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).

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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).

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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.

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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.

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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:

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-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.

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- 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

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Su Ürünleri Dergisi

Ege Journal of Fisheries and Aquatic Sciences

Volume 42 Number 2 ISSN 1300-1590 / E-ISSN 2418-3140

İÇİNDEKİLER *CONTENTS*

ARAŞTIRMA MAKALELERİ RESEARCH ARTICLES

Published by

Ege University Faculty of Fisheries, İzmir, Türkiye

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 42(2), 85-92 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.01

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Length-weight relationship and condition factors of *Puntius chola* (Hamilton, 1822) in Paschim Medinipur (West Bengal, India) with emphasis on seasonal variation

Paschim Medinipur (Batı Bengal, Hindistan)'da *Puntius chola* (Hamilton, 1822)'nın mevsimsel değişime bağlı olarak boy-ağırlık ilişkisi ve kondisyon faktörleri

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Received date: 03.01.2025 *Accepted date: 03.03.2025*

How to cite this paper:
Sit, G., Jana, A., Mukherjee, M.M., & Chanda, A. (2025). Length-weight relationship and condition factors of *Puntius chola* (Hamilton, 1822) in Paschim Medinipur (West Bengal, India) with emphasis on seasonal variation. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 85-92. https://doi.org/10.12714/egejfas.42.2.01

Abstract: This study aims to examine the relationship between *Puntius chola* (Hamilton, 1822) length, weight, condition factor, and relative condition factor, with an emphasis on seasonal variation. The result shows that the species did not strictly follow the anticipated cube law and had negative allometric growth trends in all seasons. The relative condition factor for *P. chola* ranged from 0.93 to 1.64, the length-weight relationship value "b" varied from 2.57 to 2.91, and the condition factor varied from 0.70 to 2.58. While the condition factor (K) peaked during the winter, the average values of "b" peaked during the monsoon season. The Post Hoc test indicates that the seasonal relationship between length, weight, condition factor, and relative condition factor is significant (P<0.05). The present study will assist fishery managers in creating sustainable management plans for *P. chola* in its natural habitats.

Keywords: Length-weight data, fishery, swamp barb, seasonal variation, India

Öz: Bu çalışma, *Puntius chola* (Hamilton, 1822) boyu, ağırlığı, kondisyon faktörü ve bağıl kondisyon faktörü arasındaki ilişkiyi, özellikle mevsimsel değişime vurgu yaparak incelemeyi amaçlamaktadır. Sonuçlar, türün öngörülen küp yasasını tam olarak takip etmediğini ve tüm mevsimlerde negatif allometrik büyüme eğilimlerine sahip olduğunu göstermektedir. *P. chola* için bağıl kondisyon faktörü 0,93 ila 1,64 arasında değişmekte olup, boy-ağırlık ilişki değeri "b" 2,57 ila 2,91 arasında değişmekte olup, kondisyon faktörü 0,70 ila 2,58 arasında değişmektedir. Kondisyon faktörü (K) kışın zirveye ulaşırken, "b"nin ortalama değerleri muson mevsiminde zirveye ulaşmıştır. Post Hoc testi, boy, ağırlık, kondisyon faktörü ve bağıl kondisyon faktörü arasındaki mevsimsel ilişkinin anlamlı olduğunu göstermektedir (P<0,05). Mevcut çalışma, balıkçılık yöneticilerinin *P. chola* için doğal yaşam alanlarında sürdürülebilir yönetim planları oluşturmalarına yardımcı olacaktır.

Anahtar kelimeler: Boy-ağırlık verisi, balıkçılık, bataklık barbusu, mevsimsel değişim, Hindistan

INTRODUCTION

Length and weight, both at the individual and population levels, are two crucial components of species biology. This is especially significant for effectively managing and developing fish populations (Das et al., 2024). The condition factor (CF) is an index that estimates how biotic and abiotic elements interact to affect a fish's physiological state. It portrays the health of the populace at various stages of life. This relationship allows for comparisons of fish life cycles between species and populations as well as an estimate of the fish population's health (Kara and Bayhan, 2008). It also aids in determining the fish species' reproductive seasons without affecting the creatures, which could be a useful tool in developing programmes for monitoring species-specific fisheries and culture (Arellano-Martinez and Ceballos-Vazquez, 2001). The relative condition factor examination is equally important because it reveals a fish's health and resilience. Additionally, length and weight measurements can provide important details on climatic and environmental changes and adjustments to human subsistence practises (Pauly, 1984).

Puntius chola (Hamilton, 1822), also referred to as the Asian swamp barb, has both food and ornamental values. Pollution, habitat destruction, and the selective captive breeding of commercial fish species have all contributed to the slow decline of this species (Sit et al., 2020; Jana et al., 2021a). Saha and Saha (2010) and Muhammad et al. (2016) studied length-weight relationship of *P. chola* in Bangladesh. In India, Kaushik and Bordoloi (2015), studied the 3 species of *Puntius* in Assam including *P. chola* and Gupta and Tripathi (2017) observed 5 species of *Puntius*. In West Bengal, India there are some aspects of various small indigenous fish species had been studied by different researchers such as fourteen fish species in the River Yamuna Tributary (Sani et al., 2010), *Puntius filamentosus* (Palaniswamy et al., 2012), *Puntius binotatus* (Lim et al., 2013), *Puntius sophore* (Pal et al., 2013), five Cyprinidae species from Uttar Pradesh (Gupta and Tripathi, 2017), *Glyptothorax telchitta* (Jana et al., 2021b), *Mystus tengara* (Jana et al., 2022a), *Pachypterus atherinoides* (Jana et al., 2022b), *Puntius sophore* and *Puntius terio* (Sahil et al., 2023), *Puntius terio* (Sit et al., 2022a), *Paracanthocobitis botia* (Sit et al., 2022b), *Puntius chola* (Sit et al., 2023a), *Paracanthocobitis mackenziei* (Sit et al., 2023b), *Pachypterus atherinoides* (Jana et al., 2024a, b), *Puntius chola* (Sit et al., 2024). The current study provided the first baseline data about LWRs and relative condition factor of fish species from the Paschim Medinipur, West Bengal, India. Such data is valuable for establishing a monitoring and management system of these fish species. This information will enhance management and conservation, and allow future comparisons between populations of the same species.

MATERIALS AND METHODS

The specimens (Male-1536 and Female-1536) were collected monthly from 10 aquatic stations (sta.) in the Paschim Medinipur district during the Summer (March to June), Monsoon (July to Oct), and Winter (Nov to Feb) seasons from March 2022 to February 2024 (Table 1 and Figure 1) by the use of cast net.

Table 1. Latitude and longitude of collection sites

Sta. No.	Place	Latitude & Longitude
1	Matkatpur, Kansai River	22°23'55.0"N 87°20'33.9"E
2	Istriganj, Kansai River	22°24'31.3"N 87°17'54.3"E
3	Ghatal, Shilabati River	22°39'54.8"N 87°44'44.8"E
4	Narayanbargh, Kapaleswari River	22°07'08.8"N 87°36'31.8"E
5	Madhavchak, Keleghai River	22°10'23.9"N 87°43'01.5"E
6	Uttarbansbani, Kapaleswari River	22°9'22.34"N 87°36'9.68"E
7	Sabang Pond	22°10'50.3"N 87°35'41.8"E
8	Chak Saora Pond	22°21'24.3"N 87°34'09.5"E
9	Dhoba Pukur, Barageria	22°16'38.6"N 87°35'27.0"E
10	Naoyagan Hazra boro pukur	22°08'53.9"N 87°35'18.6"E

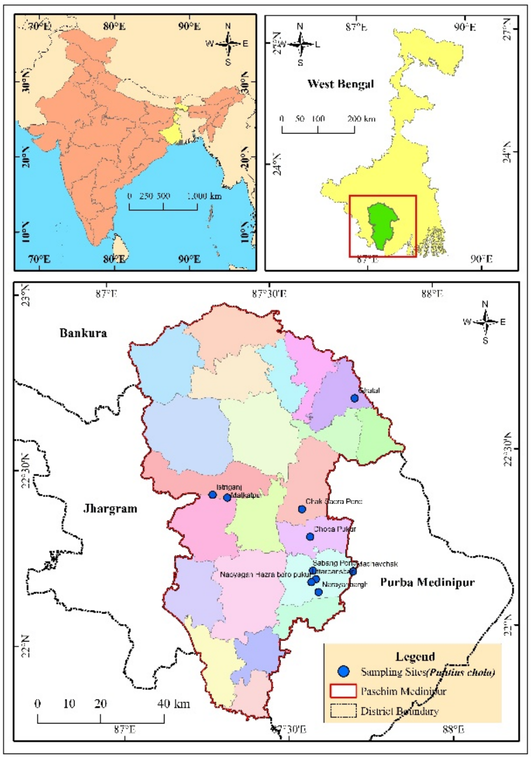

Figure 1. *Puntius chola* specimens' collection sites

Seasonally, total length (*TL*) was measured by a digital slide calliper with ±0.01 mm (length measurements were subsequently used in cm) and total weighted (*TW*) by a digital balance with ±0.01 g accuracy. For length-weight relationships, the adjusted formula of Le Cren (1951) as $W= aL^b$ was used. The logarithmic equation represented as Log W = Log a + b Log L. Condition factor (K) calculated via the following formula of Fulton (1904): K =100×(W/L^3). When calculating Relative Condition Factor (Kn) the following formula of Fulton (1904) was used: $Kn=W/aL^b$.

At last, data have been analysed (Descriptive statistics, Post Hoc test, Pearson's Correlation and Regression) by SPSS (2021), Microsoft Excel (2019), and Origin Pro (2023).

RESULTS

The length and weight of *P. chola* varied from 5.60 to 13.50 cm and 2.98 to 20.30 g, respectively, in the current study (Table 2). The minimum, maximum, and average length and weight for 1536 males, 1536 females and all 3072 specimens of *P. chola* for each season are displayed in Table 2 and Figure 2.

Table 2. Length and weight data of *P. chola* in the West Bengal, India [Male-1536, Female-1536, Combined-3072]

	Length(cm)				Weight (g)			
	Min.	Max.	Mean	SD	Min.	Max.	Mean	SD
SUMMER								
Combined	5.80	13.50	8.08	±1.65	3.11	18.65	7.58	±4.07
Male	5.60	12.20	7.90	±1.84	3.02	14.87	7.22	±3.67
Female	5.90	13.50	8.20	±1.32	3.33	18.65	8.11	±4.22
MONSOON								
Combined	6.70	12.10	9.01	±1.45	3.97	20.30	10.81	±4.91
Male	6.30	11.20	8.88	±1.33	3.21	16.22	9.33	±4.31
Female	7.10	12.10	9.22	±1.56	4.11	20.30	11.23	±5.11
WINTER								
Combined	5.70	9.20	7.30	±1.03	3.01	9.78	4.47	±2.10
Male	5.60	8.80	6.90	±0.99	2.98	10.88	3.76	±2.11
Female	5.80	9.40	7.60	±1.11	3.11	11.66	6.11	±2.61

The mean K' and 'Kn' values were 1.31±0.123 to 1.43±0.133 and 1.22± 0.178 to 1.64±0.166, respectively (Table 3). The largest length and weight were observed during the Monsoon season (Figure 3). The R^2 values show that length and weight have a consistent, positive association throughout the year (Table 4). Pearson's correlation shows that body weight has a very high positive significant association with length; 'K' has a low negative correlation with length; and 'Kn' has a moderately positive, low positive, and very low positive correlation with 'K', body weight, and length, respectively (Figure 4 and Table 4). The 'b' and 'R^2' values varied seasonally from 2.571 to 2.911 and 0.880 to 0.914, respectively (Table 5 and Figure 5). The Monsoon season shows the highest 'b' value, while the Winter season shows the lowest. *P. chola*'s total body relative condition factor (Kn), length and weight in this study area varied significantly throughout the Summer, Winter, and Monsoon seasons in accordance with the results of the post hoc test. Total length and weight substantial difference between the winter and Monsoon seasons but not between the summer and Monsoon seasons. However, 'Kn' does not substantial difference between the winter and Monsoon seasons (Table 6).

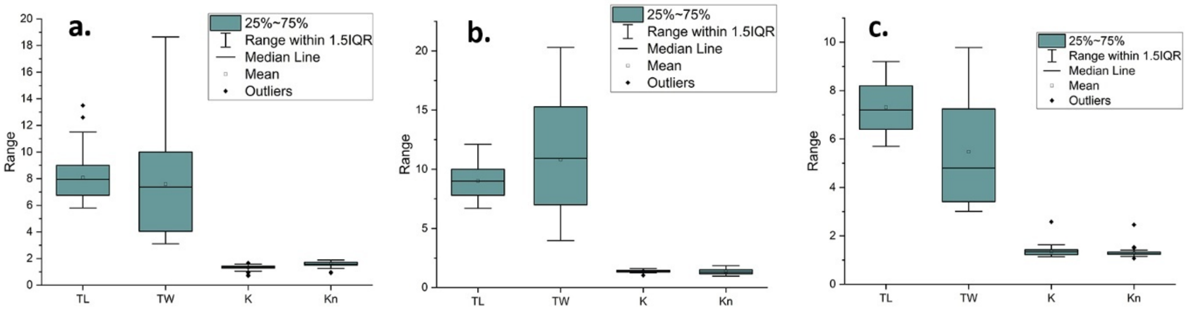

Figure 2. Length, weight, *K*, and K_n of *P. chola* in seasonal variations (a. Summer; b. Monsoon; c. Winter)

Table 3. *K* and *Kn* of *P. chola* in the West Bengal, India [Male-1536, Female-1536, Combined-3072]

	K				Kn			
	Min.	**Max.**	**Mean**	**SD**	**Min.**	**Max.**	**Mean**	**SD**
SUMMER								
Combined	0.70	1.67	1.33	±0.178	0.93	1.89	1.58	±0.188
Male	0.71	1.66	1.31	±0.161	0.93	1.88	1.43	±0.199
Female	0.73	1.68	1.41	±0.181	0.96	1.91	1.64	±0.166
MONSOON								
Combined	1.04	1.60	1.38	±0.122	0.97	1.85	1.35	±0.235
Male	0.92	1.52	1.36	±0.111	0.93	1.76	1.34	±0.213
Female	1.13	1.66	1.43	±0.133	0.98	1.99	1.44	±0.216
WINTER								
Combined	1.14	2.58	1.35	±0.203	1.07	2.46	1.29	±0.179
Male	0.77	1.33	1.31	±0.123	0.98	1.77	1.22	±0.178
Female	1.14	1.93	1.38	±0.176	1.08	1.99	1.31	±0.191

Table 4. Pearson's correlation among Length, Weight, K and K_n of *P. chola*

	Season	TL	TW	K	Kn
Season	1	-0.204**	-0.195**	0.037	-0.496**
TL	-0.204**	1	0.953**	-.305**	0.241**
TW	-0.195**	0.953**	1	-0.059	0.413**
K	0.037	-0.305**	-0.059	1	0.584**
Kn	-0.496**	0.241**	0.413**	0.584**	1

** 0.01 level of significance

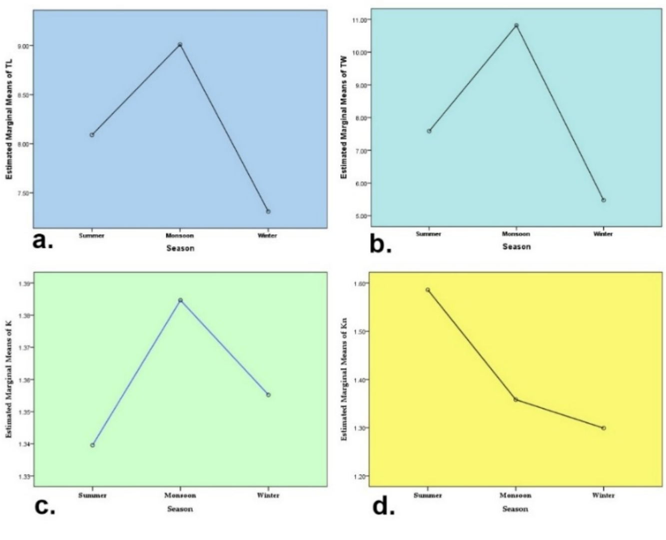

Figure 3. Seasonally changes of length (a), weight (b), *K* (c) and *Kn* (d) of *P. chola*

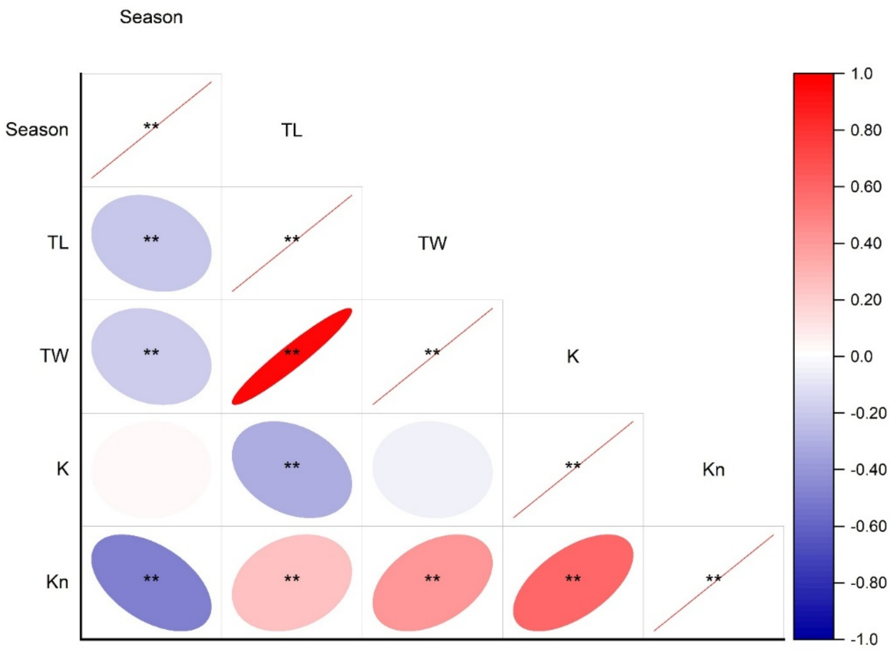

Figure 4. Pearson's correlation among Length, Weight, K and K_n of *P. chola* (* *p≤0.05,* ** *p≤0.01*)

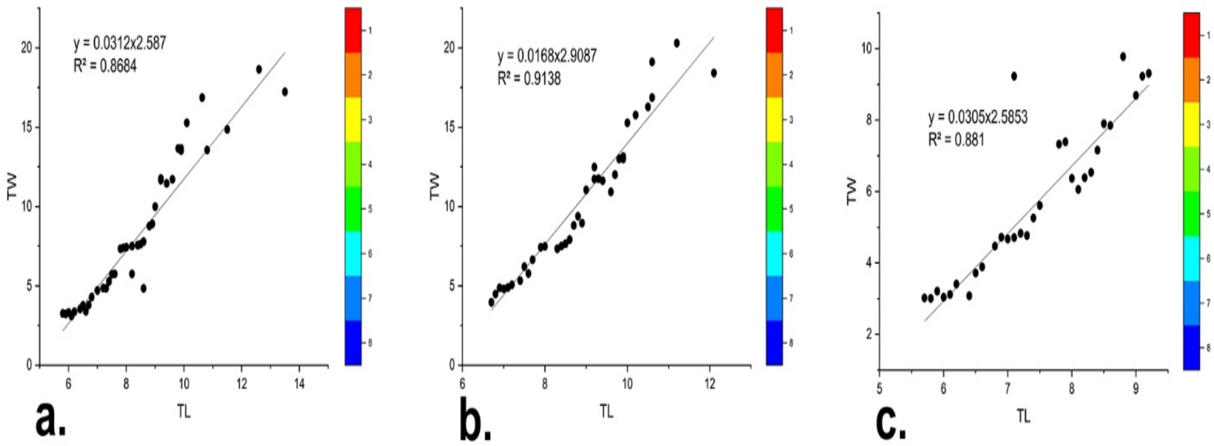

Figure 5. Length-weight relationship of *P. chola* a. Summer; b. Monsoon; c. Winter

Table 5. Seasonally regression parameters of *P. chola* [Male-1536, Female-1536, Combined-3072] (R^2=Regression coefficient, a=intercept; b=slope)

Season	Sex	a	b	R^2	Parabolic	Logarithmic
	Combined	0.0311	2.587	0.868	W=0.03117xTL^2.587	LogW=-1.506+2.587logL
SUMMER	Male	0.0300	2.571	0.860	W=0.03001xTL^2.571	LogW=-1.522+2.571logL
	Female	0.0313	2.596	0.869	W=0.03135xTL^2.596	LogW=-1.503+2.596logL
	Combined	0.0168	2.908	0.913	W=0.0168xTL^2.908	LogW=-1.773+2.908logL
MONSOON	Male	0.0167	2.898	0.914	W=0.01673LxTL^2.898	LogW=-1.776+2.898logL
	Female	0.0171	2.911	0.910	W=0.01716xTL^2.911	LogW=-1.765+2.911logL
	Combined	0.0043	2.585	0.881	W=0.0043xTL^2.585	LogW=-1.515+2.585logL
WINTER	Male	0.0304	2.576	0.880	W=0.03045xTL^2.576	LogW=-1.516+2.576logL
	Female	0.0307	2.591	0.883	W=0.03074xTL^2.591	LogW=-1.512+2.591logL

Table 6. Post Hoc test seasonally among length, weight, K and K_n of *P. chola*

Dependent Variable	(I) Season	(J) Season	Mean Difference (I-J)	Std. Error	P	95% Confidence Interval	
						Lower Bound	Upper Bound
TL	Summer	Monsoon	-0.9214*	0.24869	**0.001**	-1.5089	-0.3339
		Winter	0.7802*	0.24869	0.006	0.1927	1.3676
	Monsoon	Summer	0.9214*	0.24869	**0.001**	0.3339	1.5089
		Winter	1.7016*	0.24869	**0.001**	1.1141	2.2891
	Winter	Summer	-0.7802*	0.24869	0.006	-1.3676	-0.1927
		Monsoon	-1.7016*	0.24869	**0.001**	-2.2891	-1.1141
TW	Summer	Monsoon	-3.2317*	0.68628	**0.001**	-4.8529	-1.6105
		Winter	2.1130*	0.68628	0.007	0.4917	3.7342
	Monsoon	Summer	3.2317*	0.68628	**0.001**	1.6105	4.8529
		Winter	5.3447*	0.68628	**0.001**	3.7235	6.9659
	Winter	Summer	-2.1130*	0.68628	0.007	-3.7342	-0.4917
		Monsoon	-5.3447*	0.68628	**0.001**	-6.9659	-3.7235
K	Summer	Monsoon	-0.0451	0.03035	0.300	-0.1168	0.0266
		Winter	-0.0156	0.03035	0.864	-0.0873	0.0560
	Monsoon	Summer	0.0451	0.03035	0.300	-0.0266	0.1168
		Winter	0.0294	0.03035	0.597	-0.0423	0.1011
	Winter	Summer	0.0156	0.03035	0.864	-0.0560	0.0873
		Monsoon	-0.0294	0.03035	0.597	-0.1011	0.0423
Kn	Summer	Monsoon	0.2276*	0.03582	**0.001**	0.1430	0.3122
		Winter	0.2867*	0.03582	**0.001**	0.2021	0.3713
	Monsoon	Summer	-0.2276*	0.03582	**0.001**	-0.3122	-0.1430
		Winter	0.0591	0.03582	0.227	-0.0255	0.1437
	Winter	Summer	-0.2867*	0.03582	**0.001**	-0.3713	-0.2021
		Monsoon	-0.0591	0.03582	0.227	-0.1437	0.0255

DISCUSSION

Throughout all three seasons, *P. chola* exhibits a negative allometric growth pattern (Table 5). Negative allometric growth may be seen if the fish need to eat more or if their surroundings, including the physicochemical conditions and the breeding season, are not favourable to their growth (Le Cren, 1951; Das et al., 2015). In the current investigation, '*K*' and '*Kn*' values were 1.31±0.123 to 1.43±0.133 and 1.22±0.178 to 1.64±0.166, respectively (Table 3). The average '*K*' value is highest during the Monsoon, and '*Kn*' is highest during the Summer of *P. chola* (Figure 3). When the fish has '*Kn*' values greater than 1, which denotes good nutritional status, the relative condition component, on the other hand, is largely constant from lighter to heavier fish, suggesting the fish's health and general well-being (Jana et al., 2022a). The findings of the current study represent that the species' length-weight ratio is lower after the Monsoon and that the '*Kn*' value is lowest during the Winter, demonstrating that the species' health is poor at this time of season. Different researchers worked on length-weight relationship of different *Puntius* species in the world here observed positive allometric growth pattern, negative allometric growth pattern and also isometric growth pattern (Table 7). The results show similar observations with Bahuguna et al. (2021), Khan et al. (2021), Manorama and Ramanujan (2014), Sarkar et al. (2013), Shafi et al. (2013), and dissimilar to the work of Gupta and Tripathi (2017), Muhammad et al. (2016), Kaushik and Bordoloi (2015), Hossain et al. (2015), Lim et al. (2013), Palaniswamy et al. (2012), Rahman et al. (2012), and Sani et al. (2010). These discrepancies are explained by several factors, including sample structure, reduced feeding ability, gonad maturity, sex, and the high proportion of small specimens (Froese, 2006; Franco et al., 2014). Seasonal variation of condition factors and relative condition factors for this species is supported by the study of Manorama and Ramanujan (2014). Therefore, the fluctuation of growth factors in different seasons is an important concern to the maintenance of this species populations in the study area.

Table 7. Length-weight relationship of *Puntius* species from different parts of the world

Location	Species	Findings	Reference
Isometric Growth			
1. Bangladesh	*P. sophore*	Isometric growth pattern, Positive significant relationships between TL and fecundity	Hossain et al. (2012)
2. Jharkhand	18 species of freshwater fishes including *P. terio*	7 species were positively allometric, seven were negatively allometric, and four were isometric. *P. terio* showed isometric growth, r^2 value 0.974 between TL and TW	Sandhya et al. (2020)
Positive allometric growth			
3. Betwa & Gomti River	14 Indian freshwater fishes with *P. sarana*	'b' value 3.52, r^2 value 0.98 for *P. sarana*	Sani et al. (2010)
4. Kanhirapuzha	*P. filamentosus*	'b' values were 3.239, 3.4243, 3.298 for male, female & combined sex, with mean Kn values 0.931 & 0.877 for males and females, respectively, fluctuating yearly, peaking during December, and high during the breeding season	Palaniswamy et al. (2012)
5. Malayasia	*P. binotatus*	The linear relationship's r^2 value (0.96) was significant at level 0.01, and the 'b' value was 3.356 (> 3)	Lim et al. (2013)
6. Kolkata	*P. sophore*	TL 4.90 to 11.10 cm and TW ranged from 1.37 to 21.11 g. r^2 value 0.871, b value 3.242	Pal et al. (2013)
7. Bangladesh	Nine fish species including *P. conchonius*	'b' value 3.3 as positive allometric growth pattern & r^2 value 0.969	Hossain et al. (2015)
8. Assam, India	*P. sophore*, *P. chola*, *P. ornatus* and *P. ticto*	*P. chola* length 3.90-9.25 cm, b value 3.227 & r^2 value 0.970, except *P. ornatus,* all positive isometric growth	Kaushik and Bordoloi (2015)
9. Indus River, Pakistan	*P. sophore, P. chola, P. conchonius, P. terio, P. ticto*	'b' value 3.18, 3.02, 2.16, 3.20, 4.10; r^2 value 0.84, 0.94, 0.83, 0.81, 0.89 receptively to these species	Muhammad et al. (2016)
10. Uttar Pradesh	*S. sarana*, *P. chola, P. sophore, P. ticto*, and *P. conchonious*	'b' values varied from 2.249 to 3.231 with mean SD 2.801±0.283, with maximum species having negative allometric growth. K values showed all species in good condition. For *P. chola*, length 5.0-11.9 cm, b value 2.959, 2.96, 3.015 & r2 value 0.980, 0.994 & 0.990 for male, female and combined sexes	Gupta and Tripathi (2017)
11. Burhi Gandak River in Bihar	*P. sophore*	'b' value 3.14, positive allometric growth pattern	Sahil et al. (2023)
Negative allometric growth			
12. Ganga, Gomti & Rapti River	*P. sophore, P. ticto*	'b' value 1.92, 1.94, 1.86; 1.93, 1.74, 1. 93 r^2 value 0.98, 0.96, 0.98; 0.95, 0.91, 0.95 against male, female & combined sex	Sarkar et al. (2013)
13. Meghalaya	*P. shalynius*	The 'b' value depicted negative allometric growth in females and males, spatial and temporal variations, and no differences between sexes and seasons	Manorama and Ramanujan (2014)
14. Assan River, Uttarakhand	*P. ticto*	TL 3.9 - 7.5cm and weight 1.15-7.79 g, r^2 values 0.8583 0.8583 and 0.8583 for female, male and combined sex, negative allometric growth pattern	Bahuguna et al. (2021)
15. Panjkora River, Pakistan	8 species including *P. ticto*	'b' value 2.73, r^2 value 0.91	Khan et al. (2021)

CONCLUSION

Study the length-weight relationship and fish condition is essential for assessing the overall health, growth, survival, maturity, and reproduction of fish populations. It is also vital to determine whether the environment is suitable for fish, and it plays a significant role in fish conservation, management, and sustainability. The result demonstrate that the species exhibited allometric development over all seasons and did not adhere precisely to the predicted cube law. The information gathered could be useful in guiding the creation of future biometric research plans for other fish from the study region. Fishery managers will be able to create sustainable management strategies for *P. chola* in their habitats using the current findings.

ACKNOWLEDGEMENTS AND FUNDING

The authors are grateful to the departmental teachers and staff of PG Zoology as well as the Principal of Raja Narendralal Khan Women's College (A) for their unwavering support and encouragement in conducting sustainable research for the advancement of both science and society. The study received funding from a significant research project. A major research project, DST CURIE [DST/CURIE-PG/2023/45], from the Department of Science & Technology, Government of India, provided funding for the work.

AUTHORSHIP CONTRIBUTIONS

Godhuli Sit**:** Collection of specimens, measurements, data analysis, preparation of manuscript**;** Arun Jana**:** Designing, monitoring, analysis of data, communication, preparation of manuscript, reviewing**;** Monalisa Malik Mukherjee: Manuscript preparation**;** Angsuman Chanda: Manuscript preparation.

CONFLICT OF INTEREST

The authors affirm that they have no competing interests.

ETHICAL APPROVAL

Ethical clearance from IAEC, Approval no. 08/1AEC(1)/S/RNLKWC/2023, dated-15/06/2023.

DATA AVAILABILITY

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

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 42(2), 93-104 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.02

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Economic growth and fisheries biocapacity in BRICS+T: An Environmental Kuznets Curve analysis

BRICS+T'de ekonomik büyüme ve balıkçılık biyokapasitesi: Çevresel Kuznets Eğrisi analizi

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Received date: 18.10.2024 *Accepted date: 05.03.2025*

How to cite this paper:
Canbay, Ş., & Şengül, S. (2025). Economic growth and fisheries biocapacity in BRICS+T: An environmental Kuznets Curve analysis. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 93-104. https://doi.org/10.12714/egejfas.42.2.02

Abstract: This study evaluates the robustness of the Environmental Kuznets Curve (EKC) hypothesis by examining the association between economic growth and fisheries biocapacity in BRICS+T countries, namely Brazil, Russia, India, China, South Africa, and Türkiye. Panel bootstrap causality test is utilized to evaluate the causal relationship between the variables using data covering the period 1992-2022. The results show that the EKC hypothesis is held for Russia, South Africa and Türkiye and that economic growth initially causes to a decrease in fisheries biocapacity, but after a certain threshold, biocapacity recovers. In contrast, the EKC hypothesis is not supported for Brazil, China and India. These findings necessitate governments to take policy measures to promote environmental sustainability.

Keywords: Fishing biocapacity, Environmental Kuznets Curve (EKC), economic growth, sustainability, environmental policy

Öz: Bu çalışma, Brezilya, Rusya, Hindistan, Çin, Güney Afrika ve Türkiye'den oluşan BRICS+T ülkelerinde ekonomik büyüme ve balıkçılık biyokapasitesi arasındaki ilişkiyi inceleyerek Çevresel Kuznets Eğrisi (EKC) hipotezinin sağlamlığını değerlendirmektedir. Panel bootstrap nedensellik testi, 1992-2022 dönemini kapsayan veriler kullanılarak değişkenler arasındaki nedensel ilişkiyi değerlendirmek için kullanılmıştır. Sonuçlar, EKC hipotezinin Rusya, Güney Afrika ve Türkiye için geçerli olduğunu ve ekonomik büyümenin başlangıçta balıkçılık biyokapasitesinde bir düşüşe neden olduğunu, ancak belirli bir eşikten sonra biyokapasitenin iyileştiğini göstermektedir. Buna karşılık, EKC hipotezi Brezilya, Çin ve Hindistan için desteklenmemektedir. Bu bulgular, hükümetlerin çevresel sürdürülebilirliği teşvik etmek için politika önlemleri almasını gerektirmektedir.

Anahtar kelimeler: Balıkçılık biyokapasitesi, Çevresel Kuznets Eğrisi (EKC), ekonomik büyüme, sürdürülebilirlik, çevre politikası

INTRODUCTION

The fisheries and aquaculture sector is crucial for maintaining food security and fostering economic growth, especially in developing countries where fish serves as a key source of both protein and income. Approximately 12% of the global population depends on fish for nutrition, with the global trade in fisheries surpassing $406 billion (UN, 2024). The sector supports the livelihoods of 3.3 billion people worldwide, making it a critical resource for both food and economic security (FAO, 2022). However, the rapid pace of industrialization, urbanization, and population growth over the last century has placed immense pressure on marine ecosystems. Economic activities have not only contributed to pollution and ocean warming but also posed severe threats to marine biodiversity and food security (Wei et al., 2023). Oceans, which absorb over 90% of the excess heat generated by greenhouse gas emissions, are undergoing notable temperature increases, particularly in the deeper ocean layers. These rising temperatures lead to oxygen depletion, causing behavioral changes, stunted growth, and increased mortality rates in marine species (Shi et al., 2022). The degradation of marine ecosystems, if left unchecked, could undermine global climate stability and disrupt ecological balance (Pata et al., 2023).

Industrial and commercial activities, particularly those centered around production in developed nations, are major contributors to greenhouse gas emissions and ocean pollution. In many cases, developed nations have shifted pollution-intensive production processes to developing countries to take advantage of lower labor costs, exacerbating global environmental inequality (Damirova and Yayla, 2021). This trend highlights the critical need for a comprehensive understanding of how economic growth interacts with environmental sustainability, a relationship encapsulated by the Environmental Kuznets Curve (EKC) hypothesis. Originally proposed by Grossman and Krueger (1995), the EKC hypothesis posits that environmental degradation intensifies during the early stages of economic growth but eventually decreases after reaching a specific income threshold. This reduction in environmental harm is attributed to greater investments in cleaner technologies, the implementation of stronger regulatory measures, and an increasing awareness of environmental issues.

The Environmental Kuznets Curve (EKC) hypothesis is an important theoretical framework that describes the relationship between economic growth and environmental degradation in an inverted-U shape. It is argued that economic growth increases environmental degradation in the first stage, but after a certain level of income is exceeded, environmental improvements are realized (Grossman and Krueger, 1995; Panayotou, 1993). The main mechanisms of this trend include technological advances, increased environmental awareness and tightening of regulatory policies (Pata, 2018; Farooq et al., 2022). The EKC hypothesis has often been tested on indicators such as carbon emissions and air pollution. However, such indicators mostly address terrestrial ecosystems and provide a limited perspective on the sustainability of marine ecosystems. Therefore, examining indicators for assessing sustainable marine resources in the context of the EKC hypothesis fills an important gap in the literature (Pata and Caglar, 2021).

The main objective of this study is to evaluate the soundness of the EKC hypothesis by investigating the link between economic growth and fisheries biocapacity in BRICS+T countries (Brazil, Russia, India, China, South Africa, and Türkiye). The EKC hypothesis posits that while economic growth initially leads to environmental degradation, a turning point occurs as income levels rise, after which improvements in environmental quality are expected. In this context, fisheries biocapacity, which measures the sustainable use of marine resources, serves as a critical yet underexplored indicator of environmental sustainability. Fisheries biocapacity refers to the biological production capacity that can be sustainably extracted from a region's marine ecosystems. Unlike traditional environmental indicators, this variable allows for a simultaneous assessment of both human pressure on ecosystems and nature's capacity to regenerate itself (Global Footprint Network, 2024c). Marine ecosystems play a critical role in the global carbon cycle and provide livelihoods for millions of people around the world (FAO, 2022). However, marine ecosystems are increasingly threatened by factors such as overfishing, pollution and climate change (Wei et al., 2023). This makes it necessary to consider fisheries biocapacity when analyzing the environmental impacts of economic growth. By focusing on BRICS+T countries—nations characterized by significant natural resources, biodiversity, and rapid economic growth—this study seeks to assess whether the inverted U-shaped relationship proposed by the EKC holds true for marine resource sustainability.

BRICS+T countries have seen their share of global GDP triple over the past 15 years (Wang et al., 2024), and Türkiye, though not a formal BRICS member, is frequently considered in analyses alongside these countries due to its similar economic structure and participation in BRICS meetings (Dogan et al., 2020). These countries also rank high in biocapacity, with Brazil, China, Russia, and India holding leading positions globally (Global Footprint Network, 2024a). China and India, in particular, are global leaders in aquaculture production, while Russia and Brazil also contribute significantly to global fisheries output (UN, 2024).

There is a strong basis for considering Türkiye together with the BRICS countries in terms of both its economic structure and environmental dynamics. Türkiye is among the emerging market economies and exhibits similar development dynamics with BRICS countries in terms of industrialization and global trade (Dogan et al., 2020). Moreover, Türkiye's fisheries sector has significant potential, especially for ecosystems such as the Black Sea and the Mediterranean Sea and is directly related to sustainable resource management policies (Global Footprint Network, 2024b). In addition to having large biocapacity reserves on a global scale, BRICS countries are developing sustainability policies with different environmental governance models (Dogan and Pata, 2022). The inclusion of Türkiye in these countries is important both for understanding the economic growth-environment relationship and for comparing the effects of different development strategies on environmental sustainability.

The BRICS-T countries are becoming increasingly important actors in the global economy and have common dynamics in economic growth processes. Türkiye's inclusion in this group stems from the fact that it exhibits similar structures with BRICS countries in terms of economic, social and political globalization levels (Tekbaş, 2021). BRICS-T countries are among the countries that shape the global economy in terms of factors such as industrialization, trade volume and energy consumption and develop policies in line with sustainable development goals. Considering Türkiye together with BRICS countries provides an important framework for analyzing the effects of globalization on economic growth. In his study, Tekbaş (2021) reveals that globalization indicators are a determining factor in Türkiye's economic growth process and exhibit similar trends when compared to BRICS countries.

In terms of environmental sustainability, BRICS-T countries are among the highest carbon emitters in the world, and energy consumption and industrialization processes play a decisive role in environmental degradation (Samour et al., 2023). However, these countries also have large reserves of ecological capacity and have the potential to implement environmental sustainability policies. Türkiye's marine resource richness and sustainable fisheries policies necessitate comparative analysis with BRICS countries in terms of environmental sustainability. Fisheries biocapacity is not only a measure of human pressure on the ecosystem, but also a critical indicator for sustainable management of natural resources (Erdogan et al., 2020). Therefore, evaluating Türkiye together with the BRICS-T countries allows for a more holistic approach to the relationship between economic growth and environmental sustainability.

Previous research has mainly concentrated on the effects of economic growth on CO_2 emissions and deforestation, often neglecting its impact on marine resources such as fisheries biocapacity. This study addresses a significant gap in the literature by offering a detailed analysis of the relationship between economic growth and the sustainable management of marine resources. Given the increasing pressures global economic activities place on ocean ecosystems, understanding how economic growth affects fisheries biocapacity is crucial for the development of sustainable environmental policies.

To overcome the methodological limitations identified in previous studies, this study applies the panel bootstrap causality test developed by Kónya (2006). Although traditional methods are widely used in panel causality analyses in the literature, it is known that these methods usually ignore the dependence across countries (Dumitrescu and Hurlin, 2012). However, the method of Kónya (2006) takes into account the interconnectedness across countries and leads to a more robust analytical framework by providing a more reliable examination of the causal relationship between economic growth and fisheries biocapacity in BRICS+T countries. Similar methods have also been used in studies analyzing the relationship between environmental sustainability and economic growth and have been shown to yield effective results, especially in studies examining variables such as energy consumption, environmental degradation and biocapacity (Samour et al., 2023; Erdogan et al., 2020). Thus, this study not only tests the EKC hypothesis in a new context, but also provides policy recommendations to increase the sustainability of marine resources.

MATERIALS AND METHODS

Theoretical framework

Kuznets (1955)' foundational work explored the link between economic growth and income distribution, suggesting an inverted U-shaped relationship where inequality initially rises and later declines with development. Grossman and Krueger (1995) extended this concept to environmental degradation, proposing a similar inverted U-curve between economic growth and environmental impact, termed the Environmental Kuznets Curve (EKC) by Panayotou (1993).

The EKC theory posits that in the initial stages of economic growth, there is an increasing trend of environmental degradation and depletion of natural resources. However, once a certain threshold of economic development is surpassed, there tends to be a decrease in environmental degradation and resource depletion. In other words, economic development may have a detrimental short-term effect on the environment; however, it has a tendency to foster long-term environmental improvements. This is particularly feasible in advanced stages of development when governments and citizens allocate their increased incomes to initiatives that mitigate environmental contamination. It is imperative to implement environmental laws and technological advancements to enhance production methods in order to mitigate environmental pollution (Frodyma et al., 2022; Ayad, 2023). As explained, this relationship supports the inverted U-shaped hypothesis (Figure 1), where economic growth first worsens but later improves environmental outcomes (Uche et al., 2023; Wang et al., 2023).

Studies exploring the empirical validity of the EKC hypothesis have produced inconsistent results. While some findings support the EKC hypothesis (Chang, 2009; Kasman and Duman 2015; Pata, 2018; Farooq et al., 2022), others have disputed its validity (Abid, 2017; Allard et al., 2018; Shikwambana et al., 2021; Massagony and Budiono, 2022). These conflicting results stem from variations in country contexts, timeframes, environmental indicators, and methodologies used in the analyses. Most studies measure environmental pollution through CO2 emissions, with some also considering other greenhouse gases like methane and nitrogen oxides (Al-Mulali and Ozturk, 2015). However, these studies have faced criticism for focusing solely on air pollution, while neglecting more comprehensive environmental metrics such as soil and water pollution.

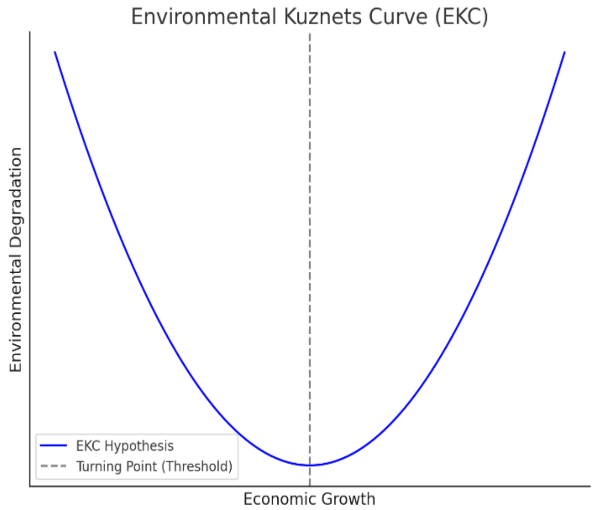

Figure 1. Enviromental Kuznets Curve (EKC)

In response to these limitations, scholars have called for broader indicators of environmental quality, such as the Ecological Footprint (EF). Developed by Wackernagel and Rees (1996), EF encompasses six components: agricultural land, pasture, fishing areas, forest land, built-up area, and carbon footprint (Kitzes et al., 2007). Integrating EF into EKC analyses provides a more holistic view of environmental quality (Yilanci et al., 2022). Nonetheless, studies examining the relationship between EF and the EKC hypothesis have also produced mixed outcomes. Some researchers affirm the EKC hypothesis (Saboori et al., 2016; Udemba, 2021), while others reject it (Hervieux and Darné, 2013; Dogan et al., 2020).

EF has faced criticism for concentrating on the demand side of environmental resources, while overlooking the supply side. To address this gap, Siche et al. (2010) launched the Load Capacity Factor (LCF) as an alternative measure. LCF represents the balance between nature's resource supply and human demand, calculated by dividing biocapacity by EF. This offers a more balanced assessment by considering both supply and demand in environmental quality (Pata and Isik, 2021). Building on this concept, Doğan and Pata (2022) introduced the Load Capacity Curve (LCC) hypothesis, which contrasts with the EKC by proposing a U-shaped relationship. According to the LCC, while economic growth initially depletes LCF, growth beyond a certain point leads to its recovery, reflecting improved environmental outcomes as economies develop (Figure 2).

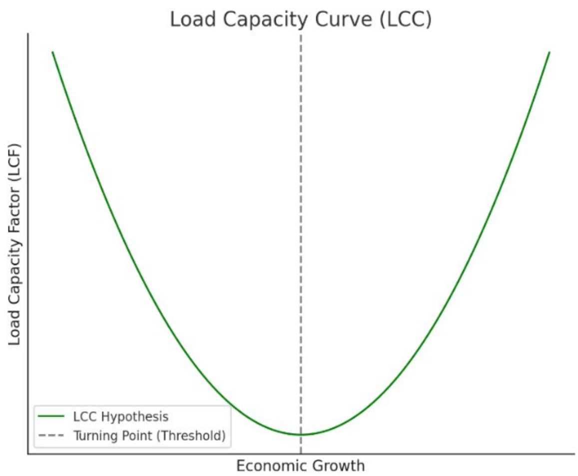

Figure 2. Load Capacity Curve (LCC)

LCF has become a powerful indicator of environmental conditions by providing the opportunity to simultaneously assess air, water, and soil quality. LCF values below 1 indicate that the ecosystem is unsustainable, while values above 1 indicate sustainable environmental conditions (Awosusi et al., 2022). Various studies using LCF, such as Awosusi et al. (2022) and Balsalobre-Lorente et al. (2019), have found that economic growth reduces LCF and thus contributes to environmental degradation. In contrast, studies by Doğan and Pata (2022), and Sun et al. (2024) support the LCC hypothesis. The theoretical framework presented above shows that the EKC hypothesis has been extended to the environmental quality domain, starting from Kuznets' initial findings on income inequality. This framework has been subjected to extensive empirical testing over the years in different geographical, temporal and methodological contexts. Despite strong support for the hypothesis, inconsistencies in the results suggest that the relationship between economic growth and environmental quality is more complex and may vary depending on a variety of factors, including the choice of environmental indicators used, policy interventions and the level of economic development. The ongoing debate around the use of alternative measures, particularly the Ecological Footprint and the Load Capacity Factor, highlights the need for more nuanced approaches to assessing environmental degradation.

In the literature, there are only a few studies that examine the validity of the EKC and LCC hypotheses in the context of marine pollution and biodiversity. One such study is conducted by De Leo et al. (2014), who investigate the EKC/LCC hypothesis alongside various variables related to marine pollution. The authors assert that fishing, storage, and international transportation negatively impact marine biodiversity. In contrast, Sebri (2016) and Paolo Miglietta et al. (2017), using the water footprint variable in different groups of countries, found the EKC hypothesis to be invalid. However, Clark et al. (2018) and Clark and Longo (2019) concluded that economic development is a significant determinant of the FF. Similarly, Kong et al. (2021), in their study on the marine fishery ecological footprint across 11 regions in China, found an inverted U-shaped relationship, supporting the EKC.

Karimi et al. (2022) and Amin et al. (2022), in separate studies conducted on Asia-Pacific countries, confirmed the validity of the EKC using the FF variable. Yıldırım et al. (2022), in their study of 10 Mediterranean countries, concluded that human capital plays a role in reducing FF. Testing the EKC hypothesis within the context of the Chinese economy, Yilanci et al. (2022) found it valid in the long term but not in the short term. In another study, Yilanci et al. (2023), analyzing Indonesian data from 1976 to 2018 using the Autoregressive Distributed Lag (ARDL) Bounds test, confirmed the validity of the EKC. Similarly, Sarkodie and Owusu (2023), conducting a study on over 200 economies using data from 1961 to 2021, also concluded that the EKC holds. Additionally, Pata et al. (2023a), testing both the EKC and Fishing Load Capacity Curve (FLCC) hypotheses with data from the top 20 fishing countries from 2000 to 2018, found both hypotheses to be valid. Lastly, Ayad (2023), focusing on G7 countries from 1970 to 2019, confirmed the validity of the Load Capacity Curve (LCC) hypothesis in the context of fishing grounds.

Data

This study utilizes data from BRICS+T countries to examine the relationship between economic growth and fisheries biocapacity within the Environmental Kuznets Curve (EKC) framework. The key variables used in the analysis are Gross Domestic Product (GDP) and Fisheries Biocapacity (FB), obtained from The World Bank (2024a) and Global Footprint Network (2024b), respectively. The study period is limited to 1992-2022 due to data availability constraints, particularly for Russia.

GDP is included in the model in logarithmic form and adjusted to constant 2015 US dollars to eliminate the effects of inflation and ensure comparability over time. Fisheries Biocapacity (FB) represents the sustainable fish stock that can be harvested from marine ecosystems without depleting future resources. It is calculated based on trophic levels and converted into primary production equivalents, a method widely accepted in ecological accounting (Global Footprint Network, 2024d). Biocapacity is measured in global hectares and serves as an indicator of an ecosystem's ability to regenerate resources relative to human consumption (Wackernagel et al., 2002; Lin et al., 2018). This metric is crucial for understanding whether economic expansion occurs within the limits of environmental sustainability.

The data descriptions of the key variables used in this study are presented in Table 1, while Figure 3 illustrates their trends over the study period.

When analyzing GDP and FB trends across BRICS+T countries, significant variations are observed. As shown in Figure 1, GDP has generally exhibited a continuous increase, particularly in China and India, where rapid economic expansion has been evident since the early 2000s. In contrast, Brazil, Russia, South Africa, and Türkiye have demonstrated a more moderate growth trajectory. This trend is consistent with previous findings in the literature, which highlight the role of industrialization and economic liberalization in fostering economic expansion in emerging economies (Tekbaş, 2021; Samour et al., 2023).

Table 1. Data description

Variable	Description	Source	Unit	Calculation Method
GDP	Gross Domestic Product	The World Bank (2024a)	Constant 2015 USD	Log-transformed
FB	Fisheries Biocapacity	Global Footprint Network (2024b)	Global hectares	Adjusted for trophic levels

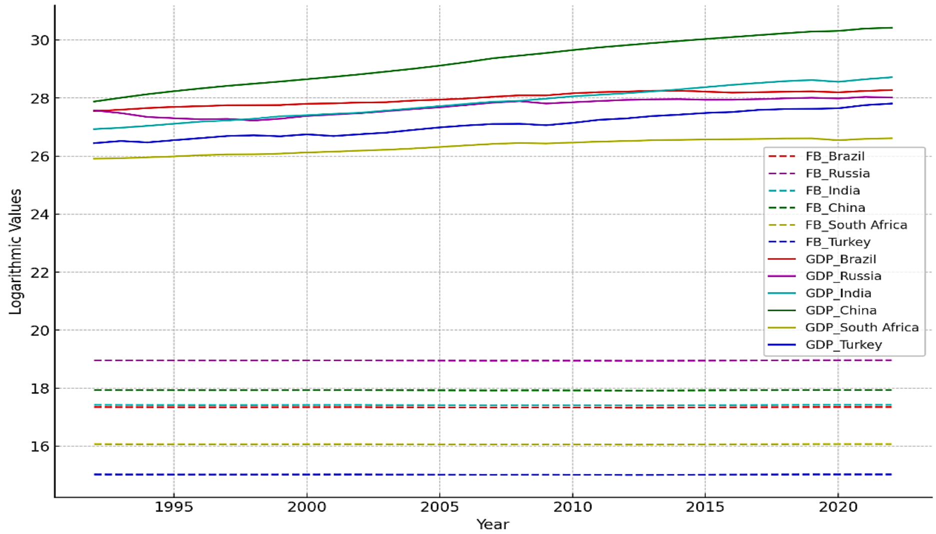

Figure 3. Graphs of FB and GDP variables

However, unlike GDP, fisheries biocapacity has remained relatively stable over time across all countries. This stagnation suggests that while economic output has grown substantially, the regenerative capacity of marine ecosystems has not exhibited a similar upward trend. This finding aligns with earlier studies emphasizing the limitations of biocapacity growth compared to economic expansion (Erdogan et al., 2020). Notably, the declining availability of fish stocks due to overfishing, climate change, and pollution may further constrain fisheries biocapacity in the coming decades (Pauly et al., 2005).

Methodology

In this study, the causal relationships between variables are analyzed using the panel bootstrap causality test developed by Kónya (2006). One of the key advantages of this test is that it does not require the presence of a cointegration relationship between variables and does not necessitate prior examination of their stationarity levels. However, before applying the test, it is essential to determine the existence of cross-sectional dependence in the models and assess whether the model coefficients are homogeneous or heterogeneous. Therefore, in the initial stage of the study, cross-sectional dependence and coefficient heterogeneity are analyzed to ensure the appropriate modeling approach. Only after these critical preliminary tests are completed can the second stage be passed and the Kónya (2006) panel bootstrap causality test be applied.

Disregarding cross-sectional dependence can result in misleading conclusions, as shocks impacting one country may influence others in the same panel (Pesaran, 2004). To account for this, cross-sectional dependence among countries in the sample is examined using specific statistical tests. These tests (Breusch and Pagan (1980)'s BP_{LM} test, Pesaran (2004)'s CD_{LM} test, Pesaran et al. (2008)'s LM_{adj} test and Baltagil et al. (2012)'s LM_{BC} test) ensure that the interdependence between countries is accurately measured, thus enhancing the robustness of the analysis and preventing erroneous interpretations of the relationships within the panel data.

The hypotheses formulated to test cross-sectional dependence are defined as follows:

H_0: The model does not exhibit cross-sectional dependency.

H_1: The model exhibits cross-sectional dependency.

If the probability values of the test statistics obtained from each test are smaller than the 10%, 5%, and 1% statistical significance levels, the H_0 hypothesis is rejected. This result indicates the presence of cross-sectional dependence in the model.

Another prerequisite for the Kónya (2006) panel bootstrap causality test is that the coefficients of the models should exhibit heterogeneity. If the slope coefficients of each country in the sample have the same value, the model is considered homogeneous. However, if the slope coefficients vary across countries, the model is assumed to have a heterogeneous structure.

This study assesses the homogeneity of coefficients using the delta (Δ and Δadj) test statistics developed by Pesaran and Yamagata (2008), which are based on Swamy (1970)'s Random Coefficients Model.

The hypotheses formulated to test the homogeneity of the Model are as follows:

H_0: The model exhibits homogeneity.

H_1: There exists a nation for which the coefficient is not the same. The model is heterogeneous.

If the probability values obtained from these test statistics are smaller than the 10%, 5%, or 1% statistical significance levels, the H_0 hypothesis is rejected. This result indicates that the slope coefficients of the model vary across countries, meaning that the model has a heterogeneous structure.

Kónya (2006) proposed a causality test that extends the Seemingly Unrelated Regressions (SUR) estimator, which was first formulated by Zellner (1962). Based on the Vector Autoregressive (VAR) framework, which Sims (1980) introduced, each equation in the SUR system is derived. The SUR system is used in this research to represent the structural connections among the variables being investigated, as explained by Kónya (2006).

$$\left.\begin{array}{l} FB_{1,t} = \varphi_{1,1} + \sum_{l=1}^{ml\_FB_1} \alpha_{1,1,l} FB_{1,t-1} + \sum_{l=1}^{ml\_GDP_1} \beta_{1,1,l} GDP_{1,t-1} + \sum_{l=1}^{ml\_GDP^2{}_1} \gamma_{1,1,l} GDP^2{}_{1,t-1} + \xi_{1,1,t} \\ FB_{2,t} = \varphi_{1,2} + \sum_{l=1}^{ml\_FB_1} \alpha_{1,2,l} FB_{2,t-1} + \sum_{l=1}^{ml\_GDP_1} \beta_{1,2,l} GDP_{2,t-1} + \sum_{l=1}^{ml\_GDP^2{}_1} \gamma_{1,2,l} GDP^2{}_{2,t-1} + \xi_{1,2,t} \\ \vdots \\ FB_{N,t} = \varphi_{1,N} + \sum_{l=1}^{ml\_FB_1} \alpha_{1,N,l} FB_{N,t-1} + \sum_{l=1}^{ml\_GDP_1} \beta_{1,N,l} GDP + \sum_{l=1}^{ml\_GDP^2{}_1} \gamma_{1,N,l} GDP^2{}_{2,t-1} + \xi_{1,N,t} \end{array}\right\}$$

where N shows the number of countries, and t denotes the time interval (t=1992, 1993, 2022). Additionally, ml denotes the lag length, which is determined by employing configurations that reduce the Akaike and Schwartz Information Criterion values.

RESULTS

The cross-sectional dependence test results for the study are presented in detail in Table 2. This table evaluates the probability values of each test along with their statistical significance levels.

Table 2. Cross-sectional dependence test

Tests	Test-Stat.	Probability
BP_{LM}	369.209*	0.001
CD_{LM}	64.669*	0.001
LM_{BC}	64.569*	0.001
LM_{adj}	19.188*	0.001

*denotes the rejection of null hypothesis at 1%.

The analysis indicates the existence of horizontal cross-sectional dependence within the model. As seen in Table 2, the probability values are below 0.01, allowing us to confidently reject the null hypothesis of no horizontal cross-sectional dependence at the 1% significance level across all models. This confirms the presence of horizontal cross-sectional dependency among the variables. The test results related to homogeneity are presented in Table 3.

Table 3. Homogeneity test results

Test	Test-Stat.	Probability
Δ	-1.918*	0.055
Δadj	-3.322*	0.001

*denotes the rejection of null hypothesis at 1%.

According to the homogeneity test findings presented in Table 3, the test statistic for the Δ test is calculated as -1.918, with a p-value of 0.055. This result indicates that the null hypothesis is rejected at the 10% significance level, suggesting that the model coefficients exhibit a heterogeneous structure.

Similarly, for the Δadj test, the test statistic is -3.232, with a p-value of 0.001, leading to the rejection of the null hypothesis at the 1% significance level, further confirming the heterogeneous nature of the model coefficients. Based on the results of both the Δ and Δadj tests, the model is considered to be heterogeneous.

Since the Kónya (2006) panel bootstrap causality test requires model coefficients to be heterogeneous, these results confirm that the necessary methodological conditions for applying the test have been met.

According to Table 4, the analysis results indicate a causality relationship from GDP and GDP^2 to FB for all countries except India. Specifically, findings reveal a negative causality from GDP to FB in Russia, South Africa, and Türkiye, while a positive causality is observed from GDP^2 to FB in these countries. The findings from Russia, South Africa, and Türkiye are consistent with previous research supporting the EKC hypothesis. Studies by Pata (2018) and Farooq et al. (2022) have similarly demonstrated that in certain countries, economic growth initially leads to environmental degradation, but environmental quality improves once a specific income level is reached. This confirms the presence of an inverted U-shaped relationship between GDP growth and fishing biocapacity in these countries, showing that as GDP grows, fishing biocapacity initially decreases but begins to improve after reaching a certain threshold of economic development. In contrast, a positive causal relationship is observed from GDP and GDP^2 to FB in Brazil and China, indicating that while the EKC hypothesis is not valid in these countries, economic growth still exerts a beneficial effect on fishing biocapacity. Additionally, no significant causal relationship between the variables is detected in India, further underscoring the heterogeneity in environmental outcomes across the BRICS+T.

Table 4. Kónya panel bootstrap causality test results

	GDP	GDP^2	Wald	10%	5%	1%
Brazil	+	+	2.271**	0.710	1.145	2.798
China	+	+	2.994*	0.707	1.174	2.652
India	+	+	0.428	0.750	1.193	2.632
Russia	-	+	6.674*	2.233	3.374	6.626
South Africa	-	+	4.297*	0.762	1.116	2.875
Türkiye	-	+	4.003*	0.657	1.243	2.770

* and **denotes causality at 1%, and 5%, significance level, respectively. H_0: GDP, GDP^2 are not the Granger causality for FB.

The findings for Brazil and China align with the assertion by Balsalobre-Lorente et al. (2019) that GDP growth contributes to environmental quality. Brazil, which boasts the longest coastline in Latin America, harbors coastal ecosystems that host a great diversity of species (Miloslavich et al., 2011). Moreover, Brazil ranks first globally in total biocapacity (Global Footprint Network, 2024a). Despite significant CO^2 emissions growth in BRICS countries since 1990, emissions in Brazil lag behind other countries after India, primarily due to Brazil's industrial structure. Brazil's economic development focuses more on the service sector than industrialization, facilitating a reduction in carbon emissions (Zhang, 2021). This observation aligns with the study of Wu et al. (2015), who demonstrated that in Brazil, CO2 emissions decrease as economic growth progresses, consistent with the results of this study regarding environmental improvements alongside GDP growth among BRICS nations.

The findings for China indicate that economic growth has a positive impact on fishing biocapacity. Pata and Caglar (2021) support this study's findings by asserting that in China, while the EKC may not be applicable, economic growth reduces CO^2 emissions. Conversely, research by Pal and Mitra (2017) and Hussain et al. (2022) found that the Environmental Kuznets Curve (EKC) hypothesis does not hold true for China. Despite being a developing country, China ranks as the world's second-largest economy, following the U.S., and has shifted its focus towards high-quality economic growth (Jiang and Wang, 2019). Over the past 40 years, China has implemented strategic policies, such as the creation of the Ministry of Natural Resources in 2018, to ensure the stability and resilience of its marine ecosystems. This includes the introduction of Special Marine Protected Areas and consolidating all conservation efforts, both marine and terrestrial, under a unified management system (Hu et al., 2020). The study's findings specific to China can be attributed to its recent shift from rapid growth to higher-quality development, coupled with proactive policies towards marine and aquatic life, which are integral to its economy.

The results of the study suggest that there is no causal relationship between fishing biocapacity and growth in India. This is consistent with the conclusions of Alam and Adil (2019), who hypothesize that India's growth momentum may have been influenced by its acceleration after approximately the year 2000. Furthermore, India's economic growth heavily relies on the service sector, which accounted for 44% of its GDP between 1992 and 2022 (The World Bank, 2024b). The predominant role of the service sector within its growth trajectory may have affected the expected relationship between growth and environmental pollution differently than anticipated. In this regard, India's per capita carbon emissions are relatively low compared to other BRICS countries (Zhang, 2021). On the other hand, India ranks sixth globally in total biocapacity (Global Footprint Network, 2024a).

DISCUSSION

The objective of this study is to evaluate the validity of the EKC hypothesis across BRICS+T countries from 1992 to 2022, with a particular emphasis on the fisheries biocapacity variable. In Russia, South Africa, and Türkiye, evidence has been discovered that supports the validity of the EKC hypothesis through the panel bootstrap causality test. Furthermore, the results of the study imply that the EKC hypothesis may not be valid in Brazil and China. However, they also suggest that economic growth has a beneficial impact on the biocapacity of the fishing industry in these countries. Nevertheless, no causal relationship was identified between fishing biocapacity and growth in India. The results from the other countries, with the exception of India, consistently indicate that sustained economic growth has a positive impact on fisheries biocapacity.

Oceans and seas, which play a central role in stabilizing the world's climate system and providing regulation and livelihood for other dependent species (Pata et al., 2023), are experiencing significant biodiversity loss due to global warming caused by greenhouse gas emissions. This impact is particularly felt in less developed and developing countries, where oceans and seas are crucial sources of income and food. In 2019 alone, air pollution cost the world $8.1 trillion, equivalent to 6.1% of global GDP. This economic burden disproportionately affects less developed and developing countries, where environmental pollution costs range from an estimated 5% to 14% of their GDPs, hindering economic growth and exacerbating poverty and inequality in urban and rural areas (The World Bank, 2024c). Therefore, addressing the warming and pollution observed in oceans and seas, which are crucial for environmental sustainability, should be treated as a global issue. Integrating this critical topic into basic education curricula and raising public awareness through public service announcements can serve as significant initial steps toward addressing these vital concerns.

The research findings indicate that prolonged economic growth can result in a decrease in environmental pollution, supporting the idea that, over time, development can foster environmental improvements through cleaner technologies and stronger regulations. Therefore, especially in sectors driving economic growth in developing countries, it is essential to employ environmentally friendly production techniques while prioritizing conservation and even enhancement of natural habitats. On the other hand, for developed countries aiming at sustainable development goals, shifting pollution-generating production activities to less developed and developing countries, often driven by lower labor costs, poses a significant challenge. To mitigate this issue, multinational corporations engaging in activities that may lead to environmental pollution should be mandated to adopt clean energy sources and environmentally sustainable technologies. Production processes should undergo stricter scrutiny by local and central authorities, ensuring compliance with these requirements.

In order to strengthen the alignment between economic growth and environmental sustainability, several policy recommendations can be developed for BRICS+T countries. First of all, sustainable fisheries management policies need to be supported by more concrete steps. In this context, measures such as determining Total Allowable Catch (TAC), allocating fishing quotas in line with scientific data and increasing Marine Protected Areas (MPAs) should be taken. Long-term sustainability of fish stocks can be ensured by utilizing the practices of countries that are successful in fisheries management, such as the Norwegian model.

In addition, fiscal and legal regulations should be introduced to promote environmentally friendly technologies. Production processes based on renewable energy should be supported through green tax reductions and subsidies, and fossil fuel subsidies should be gradually removed. For example, the special incentive models implemented in China for low carbon technologies can be a guiding light for BRICS+T countries. Making cleaner production techniques mandatory in the industrial sector and expanding environmental taxation mechanisms will also support environmental sustainability.

The activities of multinational companies should be supervised by strict environmental regulations and legislation should be implemented to mandate the use of clean energy. For example, carbon pricing mechanisms such as the European Union Emissions Trading System (EU ETS) could be implemented in BRICS+T countries. In countries like Russia, where there is a lack of environmental data, environmental data collection systems should be strengthened to increase transparency.

Transfer of clean technologies should be encouraged by increasing international cooperation. Especially among BRICS+T countries, joint funds should be established to combat climate change and regional initiatives based on technology sharing should be implemented. Experiences from international cooperation to protect Brazil's Amazon rainforest could be similarly adapted to initiatives to protect marine ecosystems.

Finally, it is crucial to integrate the concept of sustainability into education systems to raise environmental awareness. In particular, more attention should be given to environmental awareness and ecological footprinting in primary and secondary education curricula to encourage individuals to develop attitudes and behaviors that contribute to environmental sustainability in the long term. Governments should also establish regular monitoring and evaluation systems to analyze the effectiveness of implemented environmental policies and make adjustments when necessary. When these policies are considered holistically, it will be possible to harmonize economic growth with the principles of environmental sustainability.

CONCLUSION

This study examined the relationship between economic growth and fisheries biocapacity in BRICS+T countries within the context of the Environmental Kuznets Curve (EKC) hypothesis. The results were mixed: the EKC hypothesis holds true for Russia, South Africa, and Türkiye, where economic growth initially leads to a decline in fisheries biocapacity but eventually improves it. However, the hypothesis was not supported for Brazil, China, and India, where the relationship between economic growth and fisheries biocapacity did not align with the EKC pattern. In Brazil and China, economic growth positively influences fisheries biocapacity, contrary to the EKC hypothesis. The absence of significant relationships in India suggests different dynamics. These variations can be attributed to differences in economic structures, environmental regulations, and fisheries management policies across countries. In Russia, South Africa, and Türkiye, the initial decline in fisheries biocapacity may be linked to rapid industrialization and weak early-stage environmental policies, whereas the eventual improvement could be driven by strengthened regulatory frameworks and technological advancements in marine resource management. Conversely, in Brazil and China, the positive impact of economic growth on fisheries biocapacity might stem from proactive environmental policies, sustainable fisheries initiatives, and investments in marine conservation. The lack of a significant relationship in India suggests that factors such as inconsistent policy enforcement, data limitations, and differing levels of economic dependence on fisheries may play a role.

These findings underscore the importance of tailoring environmental policies to the unique economic structures and growth patterns of each country. For nations experiencing positive biocapacity trends, such as Brazil and China, policies should focus on maintaining and reinforcing sustainable growth patterns. This can be achieved by promoting responsible fisheries management, strengthening conservation incentives, and implementing stricter monitoring mechanisms to ensure continued biocapacity improvement. This trend may be driven by the integration of environmental policies with economic growth strategies, as seen in Brazil's expansion of marine protected areas and China's government-led initiatives to limit overfishing and promote aquaculture sustainability. Strengthening these regulatory frameworks and ensuring long-term policy stability will be crucial for sustaining the positive trajectory of fisheries biocapacity in these countries.

In contrast, for Russia, South Africa, and Türkiye-where the EKC hypothesis holds-policymakers should prioritize the transition to a sustainable phase by integrating stronger environmental regulations, investing in eco-friendly marine technologies, and encouraging public-private partnerships to foster sustainable fisheries management. These countries can also benefit from targeted subsidies for sustainable fishing practices and stricter enforcement of marine protection laws to accelerate the recovery of fisheries biocapacity.

For India, where no significant relationship was found, future policies should focus on improving fisheries data collection and identifying underlying socio-economic and institutional factors affecting biocapacity. Investing in sustainable aquaculture, enhancing marine resource governance, and promoting community-based fisheries management could help establish a clearer link between economic growth and biocapacity changes.

Future research can address in more detail the determinants of the relationship between economic growth and fisheries biocapacity across countries. In particular, by examining the impact of factors such as the level of industrialization, environmental policies, natural resource management strategies and renewable energy use rates on this relationship, sustainable development policies can be developed in line with the unique socio-economic and environmental dynamics of each country. In addition to the panel bootstrap causality test used in this study, the robustness of the findings can be tested by using different methodological approaches and the reasons for cross-country differences can be analyzed in more depth through time series analysis. In addition, issues such as the long-term effects of climate change on fisheries biocapacity and how ecological carrying capacity is shaped by economic growth provide an important basis for future research. Such expanded analyses would make a more comprehensive contribution to efforts to balance economic development and environmental sustainability on a global scale.

ACKNOWLEDGEMENTS AND FUNDING

This research has not received any specific grant, funding or other support from any funding organization in the public, commercial or non-profit sectors.

AUTHOR CONTRIBUTIONS

All authors contributed to the idea and design of the study. Material preparation, research and part of writing was carried out by Şerif Canbay. The rest of writing and editing of the manuscript was done by Serkan Şengül and all authors have read and approved the manuscript.

CONFLICT OF INTEREST STATEMENT

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

ETHICAL APPROVAL

Ethical approval is not required for this study.

DATA AVAILABILITY

All relevant data is inside the article. Additional data sets of the current study will be provided by the corresponding author upon request.

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Ege Journal of Fisheries and Aquatic Sciences, 42(2), 105-121 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.03

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Understanding the status of key fish stocks in the Turkish Black Sea: A graphical approach to sustainable management

Karadeniz'deki önemli balık stoklarının durumunun anlaşılması: Sürdürülebilir yönetim için grafiksel bir yaklaşım

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Received date: 17.12.2024 *Accepted date: 12.03.2025*

How to cite this paper:
Demir, M. (2025). Understanding the status of key fish stocks in the Turkish Black Sea: A graphical approach to sustainable management. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 105-121. https://doi.org/10.12714/egejfas.42.2.03

Abstract: Fish stocks are critical components of aquatic ecosystems, providing essential food sources for humanity and supporting the complex balance of marine food webs. Effective management of these resources is crucial for meeting global food demands and preserving aquatic biodiversity. In this study, we assess fishing pressures—categorized as low, high, and extreme—on six key fish stocks in the Turkish waters of the Black Sea: horse mackerel (*Trachurus mediterraneus*), the Black Sea anchovy (*Engraulis encrasicolus*), sprat (*Sprattus sprattus*), whiting (*Merlangius merlangus euxinus*), bluefish (*Pomatomus saltatrix*), and the Atlantic bonito (*Sarda sarda*) and provide important findings to protect fish stocks. By applying dynamic modeling and stability analysis solely to landing data, we create graphical representations that illustrate the current trends of these stocks, through growth and fishing mortality curves. Our findings reveal that although certain stocks have shown an upward trend over the past 15 years, they remain exposed to high levels of fishing mortality. To support sustainable management, this study establishes the Maximum Sustainable Yields (MSYs) for each stock based on their present conditions and identifies stable equilibrium points where stocks can be sustained over the long term. This method also provides an opportunity to analyze state of fish stocks when only landing data is available.

Keywords: Black Sea fishery, fishing pressures, sustainable fisheries, fishery model, overfishing

Öz: Balık stokları, su ekosistemlerinin temel bileşenleridir; insanlık için çok önemli bir gıda kaynağı sağlarken, besin zincirinin karmaşık dengesine de katkıda bulunurlar. Bu balık stokların etkin bir şekilde yönetimi, gıda taleplerini karşılamak ve denizlerdeki biyolojik çeşitliliği korumak açısından kritik öneme sahiptir. Bu çalışmada, Türkiye'nin Karadeniz kıyısındaki altı ana balık stoğu – istavrit (*Trachurus mediterraneus*), hamsi (*Engraulis encrasicolus*), çaça (*Sprattus sprattus*), mezgit (*Merlangius merlangus euxinus*), lüfer (*Pomatomus saltatrix*) ve palamut (*Sarda sarda*) – düşük/yüksek/aşırı avlanma seviyelerine göre incelenmiştir ve bu stokların korunmasına dair önemli bulgular sunulmuştur. Sadece av istatistikleri kullanılarak dinamiksel bir inceleme ve denge analiziyle, balık stoklarının mevcut dinamiklerini grafiksel temsiller kullanarak analiz ettik. Bu grafikler, balık stoklarının büyüme eğrileri ve balıkçılık kaynaklı avlama (ölüm) eğrilerine dayanarak oluşturulmuştur. Araştırmamız, bazı stoklarda son 15 yılda artış eğilimi gözlense de hala yüksek düzeyde avlanma oranına maruz kaldıklarını ortaya koymaktadır. Bu sorunu ele almak için çalışmamız, her bir balık stoğunun mevcut durumuna bağlı olarak Maksimum Sürdürülebilir Verim (MSV) değerlerini belirlemekte ve bu stokların uzun vadede sürdürülebilir kalabileceği denge noktalarını tespit etmektedir. Bu yöntem, avlanma verileri dışında herhangi bir veri bulunmadığında balık stoklarını analiz etme fırsatı da sunmaktadır.

Anahtar kelimeler: Karadeniz balıkçılığı, av baskıları, sürdürülebilir balıkçılık, balıkçılık modeli, aşırı avlanma

INTRODUCTION

Fish populations are essential to marine ecosystems, maintaining ocean balance and providing a vital food source for humans (Sumaila and Tai, 2020). However, overfishing remains a significant challenge, leading to the depletion of many fish stocks globally (Hilborn, 2012). Such overfishing has also caused the decline of native species and the degradation of entire ecosystems (Nogrady, 2023; Cheikh et al., 2024). Therefore, effective management and conservation of marine ecosystems is fundamental to the sustainability of fisheries and habitats.

One of the well-known habitats for fish stocks facing overfishing is the Black Sea, and it has always been a rich habitat for fish and supported many communities along its shores (Salihoglu et al., 2017; Demirel et al., 2020). However, fish populations are now under significant threat due to increased fishing and environmental problems like pollution and habitat damage (Daskalov, 2002; Nastase et al., 2024; Damir et al., 2024). More fishing, driven by economic needs and new technology, is putting a lot of stress on fish and disrupting the balance of the ecosystems (Llope et al., 2011; Raykov and Duzgunes, 2017). These challenges underscore the urgent need for sustainable management practices to safeguard the future of this invaluable resource and the communities that depend on the habitat.

The specific fish stocks focused on in the present study are horse mackerel, the Black Sea anchovy, sprat, whiting, bluefish, and the Atlantic bonito. Each of these fish stocks has an important role in keeping the Black Sea ecosystem balanced and healthy. However, our primary reason for selecting these species was their high level of landings and their importance in fisheries.

Our main goal of the study was to determine whether these fish populations on the Turkish side of the Black Sea were subject to low, high, or extreme (overfishing) fishing pressure. To evaluate this, we analyzed time-dependent growth and fishing mortality curves derived from the logistic model presented in the material and method section. In assessing the status of these fish populations, we also investigated whether it was possible to maintain the populations at a positive equilibrium for sustainable fishing through a graphical stability analysis. Additionally, we calculated the Maximum Sustainable Yields (MSYs) of these fish populations based on their current status to support maintaining their biomass around a positive equilibrium.

Mathematical modeling serves as a common approach in fishery management, particularly in scenarios with limited or solely landing data (Kot, 2001; Neubert, 2003; Demir, 2019; Demir and Lenhart, 2020). Nonetheless, relying only on mathematical models is inadequate for understanding species dynamics and mitigating the risks of overfishing. Therefore, it is important to combine stability analyses into mathematical models to prevent overfishing and attain a stable equilibrium point, ensuring sustainable fisheries (Kot, 2001; Demir, 2023).

To sum up, this study began by introducing a single-species model (logistic model) (1), followed by the presentation of graphical stability analysis. We then estimated the parameter values of the logistic model. After the parameter estimation, we investigated the time-dependent intrinsic growth and fishing mortality rates over time in graphs. These graphs not only presented fishing status as low, high, or extreme depending on the relation between the intrinsic growth rate (r) and fishing mortality rate (F) but also presented stability conditions for each fish stock. Finally, we present our findings in the results section, followed by a conclusion.

MATERIALS AND METHODS

In our model given in Eq.1, we estimated four parameters: initial biomass (N_0), intrinsic growth rate (*r*), carrying capacity (*K*) and fishing mortality rate (*F*). The number of data points should be at least equal to or larger than the number of parameters being estimated (John, 2015). Our literature review indicated that a minimum of 8 data points is generally considered sufficient to detect a clear pattern in simple linear trends (Jenkins and Quintana, 2020). However, fish stocks typically do not follow simple linear trends due to the complexity of marine ecosystems and various environmental factors. Therefore, in our study, we considered 15 data points to effectively portray the current trends in fish populations. This approach balances the acquisition of sufficient data for trend analysis while avoiding older data that may not reflect the current status of the fish stocks. It is important to state that we are not interested in very old time series data since we only focus on the current status of fish stocks in our study to understand their current status.

We used landing data obtained from the Turkish Statistical Institute for the years 2008 to 2022 (TUIK, 2023). Since this study only considered landing data in stock assessment on the southern part of the Black Sea, we used the landing data collected only from this particular region (specified as West Black Sea (TR8) and East Black Sea (TR9) regions in TUIK data sources). Our study uses graphical visualization to assess fishing pressure levels and determine whether a fish population is experiencing high or extreme exploitation. Fishing pressure is mainly related to fishing mortality rate as indicated in Figure 2 and it refers to the intensity of fishing activity on a particular fish population. We did not include catch per unit effort (CPUE) data, as it is not always available for each fish stock. Instead, we support the landing data with stability analysis and compare our findings in the discussion section with studies that incorporate CPUE and additional data.

We first introduced the logistic model (1) designed to facilitate the analysis of trends and patterns in the size of fish stocks. This model served as a foundational tool, enabling us to investigate the time-dependent intrinsic growth and fishing mortality rates. We visualized these rates over time to discern when the population growth curve surpasses or falls below the fishing mortality curve. When the growth curve exceeds the fishing mortality curve, the population tends to increase, whereas when the growth curve falls behind the fishing mortality curve, the fish stock tends to decrease. Therefore, a comprehensive analysis could provide valuable insights into whether the fish populations in the study area were subjected to low, high, or extreme fishing pressure.

Furthermore, the graphical approach facilitates stability analysis to determine whether a fish population can reach a positive stable equilibrium point for a sustainable fishery. By using this graphical method, we identified key indicators of fish population dynamics and obtained essential information regarding their status, including fishing pressure levels, trends in population biomass, intrinsic growth and fishing mortality rates, and MSYs. We also assessed whether the population could remain at a positive equilibrium point for a sustainable fishery. By leveraging these techniques, we aimed to understand the status of fish stocks when only landing data is available. This is not a new technique and it has been used in stability analysis of the logistic (fishery) models (Kot, 2001).

Model formulation and description

The dynamics of fish stock were described by the following model (Schaefer, 1954), which is a first-order differential equation. This model considered fish stocks growing logistically and being harvested by the term $FN(t)$ where F is a constant fishing mortality and $N(t)$ represents the amount of fish stock in the system at time *t*.

$$\frac{dN(t)}{dt} = rN(t)\left(1 - \frac{N(t)}{K}\right) - FN(t) \qquad (1)$$

Here, initial condition $N(0) = N_0$. The growth term of fish stock was modeled using a logistic equation with an intrinsic growth rate of r and a carrying capacity of K. All the coefficients and initial conditions in the model are positive and have upper bounds. Note that in the equation $F = fq$, f represents fishing effort and q is the catchability rate. In the absence of catch per unit effort (CPUE) data, the catchability rate cannot be determined. Thus, in the study, we assumed $q = 1$, and the fishing mortality rate was directly represented by the fishing effort as $F = f$.

Stability analysis of the model

We examined the stability of the model (1), graphically. The hump-shaped curve corresponds to the density-dependent growth term and the straight line corresponds to the harvest term (or fishing mortality term, see Figure 1). When we investigated the model stability by setting, $\frac{dN(t)}{dt} = 0$ in Eq. 1, we obtained two equilibrium points as $N(t) = N_1^* = 0$ and $N(t) = N_2^* = K\left(1 - \frac{F}{r}\right)$. These equilibrium points were obtained from the equality of the growth term and harvest term as

$$rN(t)\left(1 - \frac{N(t)}{K}\right) = FN(t)$$

The equilibrium point, $N_1^* = 0$ is unstable, but $N_2^* = K\left(1 - \frac{F}{r}\right)$ is stable (see the left plot in Figure 1). When the population biomass is within the range of 0 and N_2^*, the growth term is greater than the harvest term which implies $\frac{dN(t)}{dt} > 0$ in Eq. 1, resulting in an increase in population biomass up to N_2^*. Since population biomass goes from zero to the positive equilibrium point N_2^*, the equilibrium point, $N_1^* = 0$ is called an unstable equilibrium point. On the other hand, $\frac{dN(t)}{dt} < 0$ in Eq.1 when population biomass surpasses N_2^*, leading to a decline in population biomass towards N_2^*. In both cases, when population biomass is below or above the equilibrium point $N_2^* = K\left(1 - \frac{F}{r}\right)$, the biomass of the population approaches towards to the equilibrium point N_2^*. Thus, N_2^* is a stable equilibrium point. Furthermore, in the case of $F = r$, we only have one equilibrium point since $N_1^* = N_2^* = 0$ and this equilibrium point is stable from the right side (see the right plot in Figure 1). This situation indicates severe overfishing, putting fish populations at risk of extinction.

In short, we obtained two equilibrium points: one at zero (N_1^*) and the other at a positive value (N_2^*). Based on the graphical stability analysis, we observed that the first equilibrium point, N_1^* = 0, was unstable, while the second equilibrium point, $N_2^* = K\left(1 - \frac{F}{r}\right)$ was stable. We expected the fish population biomass to remain around this stable point. To help the system stay near this stable point, we provided the current MSYs as MSY$= \frac{rK}{4}$ for each stock, ensuring that the equilibrium point is maintained without shrinking or approaching zero.

The method for investigating the status of fish stocks

By investigating the growth and harvest terms in our model (Eq. 1), we can gather insights into the status of the fish stock and determine whether it is experiencing overfishing. Mainly, we will see three main district statuses for fish stocks: (a) low fishing mortality case, (b) high fishing mortality case, and (c) severe fishing mortality case (Figure 2) (Kot, 2001). Note that although two plots from Figure 1 are also presented in Figure 2, we provide different explanations for the two figures. In Figure 1, we explain the stability analysis using grey arrows to represent the direction of biomass changes, while in Figure 2, we focus on the fishing status of the stocks.

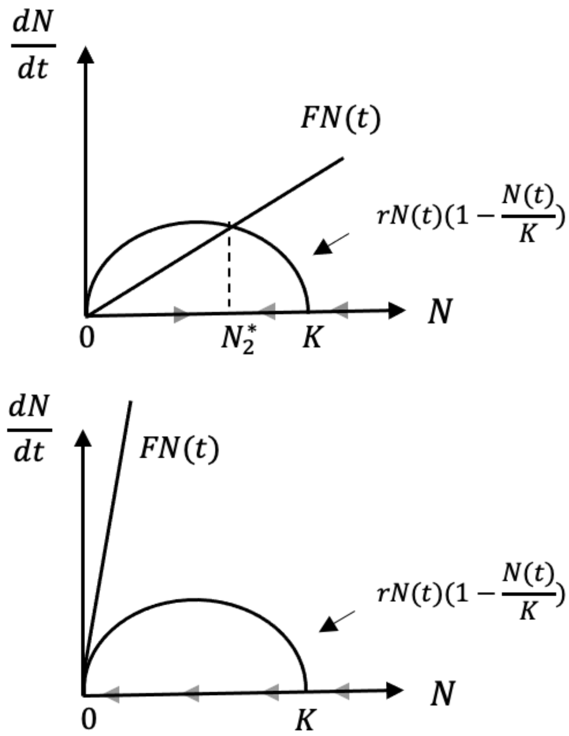

Figure 1. Graphical visualization of the stability analysis. The grey arrows indicate the direction of changes in population biomasses (stocks)

In our model dynamics, we used the estimated fishing mortality rate, F. On the other hand, when examining the 15-year average growth curve corresponds to the term $rN(t)\left(1 - \frac{N(t)}{K}\right)$ and average fishing mortality curve corresponds to the term $FN(t)$ for each month, as shown in the bottom plots of Figures 3-8, we need to extend the fishing mortality rate from a seasonal to a yearly basis to match with the growth term. The growth term is active throughout the year, but the harvest term is seasonal, typically from September to April, with no fishing mortality occurring from May to August.

To create a continuous curve for the entire year, we make a simple assumption. For example, for the horse mackerel stock, we estimated in the parameter estimation section below that the fishing mortality rate was F = 0.431 during the 7-month fishing season (from September to April). During the remaining 5 months (from May to August), there was no fishing mortality. To approximate a continuous annual fishing mortality rate, we assumed that the total harvest effort applied over 7 months is spread evenly across the entire year. This approach yields a constant annual fishing mortality rate. We calculated this by considering how the total harvest effort for 7 months would be distributed over 12 months, resulting in:

$$F^* = \frac{F * 7}{12} = \frac{0.431 * 7}{12} = 0.2514$$

where F was the estimated fishing mortality rate for the 7-month period and F^* for 12 months. This method allowed us to calculate a constant fishing mortality rate that applies throughout the entire year, facilitating comparison with the continuous growth term. Also, note that we used these calculated rates (F^*) to obtain 15-year average growth curves and fishing mortality curves presented in plots *c* and *d* of Figures 3-8 and used F for plots *a* and *b* in Figures 3-8.

Consequently, upon determining the fishing mortality rates outlined in Tables 1-6 (see results section below), we employ the adjusted fishing mortality rates (F^*) rather than F to generate plots *c* and *d* illustrated in Figures 3-8. This method enabled us to capture growth and harvest curves for stability analysis and address one of the scenarios presented in Figure 2. This method is not new; it was originally developed by Schaefer (1954) and is well presented in Kot (2001).

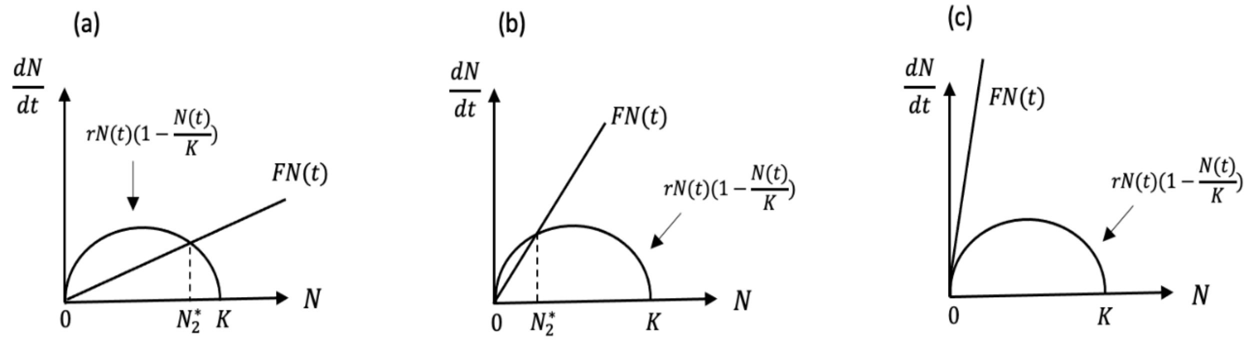

Figure 2. Visualization of the low fishing mortality harvest (case a), high fishing mortality harvest (case b) and severe overfishing harvest (case c)

In Figure 2, the X-axis represents the biomass of fish stock in tonnes, while the Y-axis illustrates the rate of changes in the fish stock's biomass over time. It is noteworthy that when the harvest line surpasses the bell-shaped growth curve, the rate of change of $\frac{dN(t)}{dt}$ in Eq. 1 becomes negative, indicating a decline in the fish stock's biomass. Conversely, when the harvest line falls below the growth curve, the rate of change is positive, signaling an increasing trend in the fish stock's biomass. Based on data and parameter estimations from the model, we projected the status of six important fish stocks on the Turkish side of the Black Sea, including horse mackerel, the Black Sea anchovy, sprat, whiting, bluefish, and the Atlantic bonito.

It is noteworthy that if we only identify one equilibrium point, this point is deemed unstable, leading to the eventual extinction of the fish stock due to severe overfishing mortality (see plot c in Figure 2). Conversely, the presence of two equilibrium points signifies stability (see plots a and b in Figure 2). In such cases, the positive second equilibrium point is stable, indicating that the fish stock will tend towards this equilibrium point over the long term and we aim to determine the values of the second equilibrium point for each fish stock, allowing us to comprehensively grasp the current status of these stocks.

Moreover, the positioning of this equilibrium point concerning the peak of the growth curve holds significance. If it occurs before the peak (plot b in Figure 2), it suggests that the population faces high fishing mortality. Conversely, if it occurs after the peak (plot a in Figure 2), the population is experiencing low fishing mortality. Thus, as the size of area A (plot a in Figure 2) decreases due to the interaction point between the growth term and the harvest term, the equilibrium point shifts from low to high fishing pressure, potentially even reaching extreme fishing pressure. These analyses provide critical insights into the dynamics of fish stocks and help guide efforts toward sustainable management practices.

Thus, this study primarily focuses on equilibrium points to assess the status of fish populations based on their positions. Therefore, we do not analyze dynamics outside these equilibrium points. In Plots d of Figures 3–8, fish biomass fluctuates between the first equilibrium point ($N_1^* = 0$) and the second equilibrium point ($N_2^* > 0$). When fishing pressure decreases, N_2^* increases; otherwise, it declines toward zero.

Parameter estimation

The parameters of the model (1) for each specific fish stock were estimated using the annual landing data between the years 2008 and 2022 (TUIK, 2023), with the Ordinary Least Squares (OLS) method used to minimize the sum of the squares of the differences between the observed annual landing data and the model's predictions. The goodness of fit was assessed by calculating the relative error (e_r) of the fit using the following formula:

$$e_r = \min \left(\frac{\sum_{k=1}^{n}\left(H_k - \widehat{H}_k\right)^2}{\sum_{k=1}^{n}(H_k)^2}\right)$$

where H_k and $\widehat{H}_k$ are the exact and estimated annual landing data, respectively. The term $\widehat{H}_k$ represents the harvest term, $FN(t)$, in our model. To determine the total estimated landing for each fish stock, we sum the estimated harvest over the specified fishery season. For instance, the fishing season for anchovy in the Turkish waters of the Black Sea spans from September to January (Gücü et al., 2017). Accordingly, we calculate the total estimated harvest, ($FN(t)$), over this time period. An ode45 solver with fmincon from the Optimization Toolbox of MATLAB is used in the parameter estimation. See Tables 1-6 for estimated parameters and Figures 3-8 for model fits for each fish stock. Since we fitted model (1) using annual landing data, the parameter units are expressed on a yearly basis. Additionally, to mitigate the risk of converging to a local minimum during parameter estimation, we used 100 different starting points. These starting points were selected based on the initial parameter ranges provided in Tables 1–6, ensuring a more robust minimization of the discrepancy between the observed landing data and the model's estimated values.

In the parameter estimation process, we initially defined a rough range for each parameter based on values reported in the literature to ensure biological plausibility and to accurately capture fish stock trajectories. For anchovy parameters, including growth rate (*K*) and fishing mortality (*F*), we selected values from previous studies that analyzed these factors in similar fish stocks (STECF, 2017; Salihoğlu et al., 2017; Akkuş and Gücü, 2022). Fishing mortality rates for anchovy, sprat, horse mackerel, and whiting were determined based on findings from stock assessments and studies (STECF, 2017; Salihoğlu et al., 2017; Kasapoğlu, 2018). Additionally, the parameter ranges for intrinsic growth rate (*r*), carrying capacity (K), and fishing mortality (*F*) for the Atlantic bonito and bluefish were derived from studies focusing on population dynamics and species-specific modeling (Akkuş and Gücü, 2022; Daskalov et al., 2020). The intrinsic growth rate (*r*) for fish populations generally varies between 0.05 and 1, as reported in global fishery databases and ecological studies (Patrick and Cope, 2014; Daskalov et al., 2020; FishBase, 2025). Thus, we specified the initial ranges of parameters depending on the studies and then made parameter estimations.

After that MATLAB's Optimization Toolbox identified the best value within the specified range, which we used as the estimated parameter value. For example, in the estimate of intrinsic growth rate (*r*) for horse mackerel, we initially set the range to 0.1-0.8. After the estimation process suggested a value of around 0.26, we refined the range to 0.2-0.3. We conducted similar preliminary analyses for all parameters, narrowing down the best intervals. After determining these intervals, we estimated all parameters simultaneously for each fish stock. Once the estimation was complete, we double-checked to ensure that none of the estimated parameters reached the upper or lower bounds of the initial ranges. Additionally, note that we estimated parameters for the entire period, rather than focusing on the initial, middle, or final years. We provided a single estimate that applies to the whole period.

RESULTS

In this section, we looked into the examination and simulation of the current status of fish stocks, based on parameter estimations unique to each fish stock. Our primary focus lay in determining whether fish stocks are subject to low or high fishing mortality, or if they are facing severe overfishing. We initiated our examination with a focus on horse mackerel and subsequently looked into the analysis of other species in the following subsections.

The current status of horse mackerel

We conducted harvesting operations between September and April targeting the horse mackerel fishery on the Turkish side of the Black Sea (STECF, 2017). Based on parameter estimations outlined in Table 1, the Maximum Sustainable Yield (MSY) is estimated at 11405 tonnes from the formula, MSY$=\frac{rK}{4}$. Over the years 2008 to 2020, we observed a decreasing trend in this fishery, followed by signs of an increase in the last two years. While it's challenging to discern two equilibrium points from the bottom left plot in Figure 3, our analysis reveals two equilibrium points: $E_1 = 0$ and $E_2 \cong 12000$ tonnes, as illustrated in the bottom right plot of Figure 3.

Our assessment of fish stocks and stability graphs indicates that the fish stock has been experiencing high fishing mortality since the second positive equilibrium, remaining on the left side of the peak in the bell-shaped growth curve (as shown in the bottom left plot (*c*) of Figure 3). Currently, the annual maximum instantaneous biomass ranges between 5000 and 8000 tonnes in 15 years (see the top right plot (*d*) in Figure 3). With the second equilibrium point at around 12000 tonnes (as depicted in the bottom right plot of Figure 3), the fish stock in this fishery has the potential to boost its instantaneous biomass by up to 12000 tonnes. This is because the growth curve exceeds the fishing mortality curve by the same margin, indicating a promising opportunity for biomass increase. When harvesting horse mackerel at or below the MSY level, there is the potential for the fish stock to increase its instantaneous biomass up to 12000 tonnes. This increase in biomass consequently leads to an elevation in the MSY of this fishery due to the enhanced stock abundance. Thus, to obtain a sustainable fishery for horse mackerel, it is recommended to harvest around or below the MSY estimated in the study.

There may be some confusion when interpreting plot b in Figure 3, as it shows the maximum biomass level ranging from approximately 5000 to 6000 tonnes at the beginning of each year, while our model estimates the maximum sustainable yield (MSY) for horse mackerel stocks to be 11405 tonnes. However, this discrepancy arises because the blue curves in the plot represent the biomass-harvest dynamics rather than the total annual biomass. Since harvesting occurs on a weekly basis between September and April, the cumulative estimated landings are distributed throughout this period rather than being reflected as a single peak in biomass.

Table 1. Parameter descriptions and estimated parameter values for horse mackerel

Parameters	N_0	K	r	F	$\frac{rK}{4}$	e_r
Description	Initial biomass of fish stocks	Carrying capacity	Intrinsic growth rate	Fishing mortality rate	Maximum sustainable yield	Relative errors of fits
Unit	Tonnes	Tonnes	$year^{-1}$	$year^{-1}$	Tonnes	-
Initial range	$5e^4$ - $10e^4$	$1e^5$-$3e^5$	0.2 - 0.3	0.35-0.55	-	-
Horse mackerel	7518	186214	0.254	0.431	11405	0.29

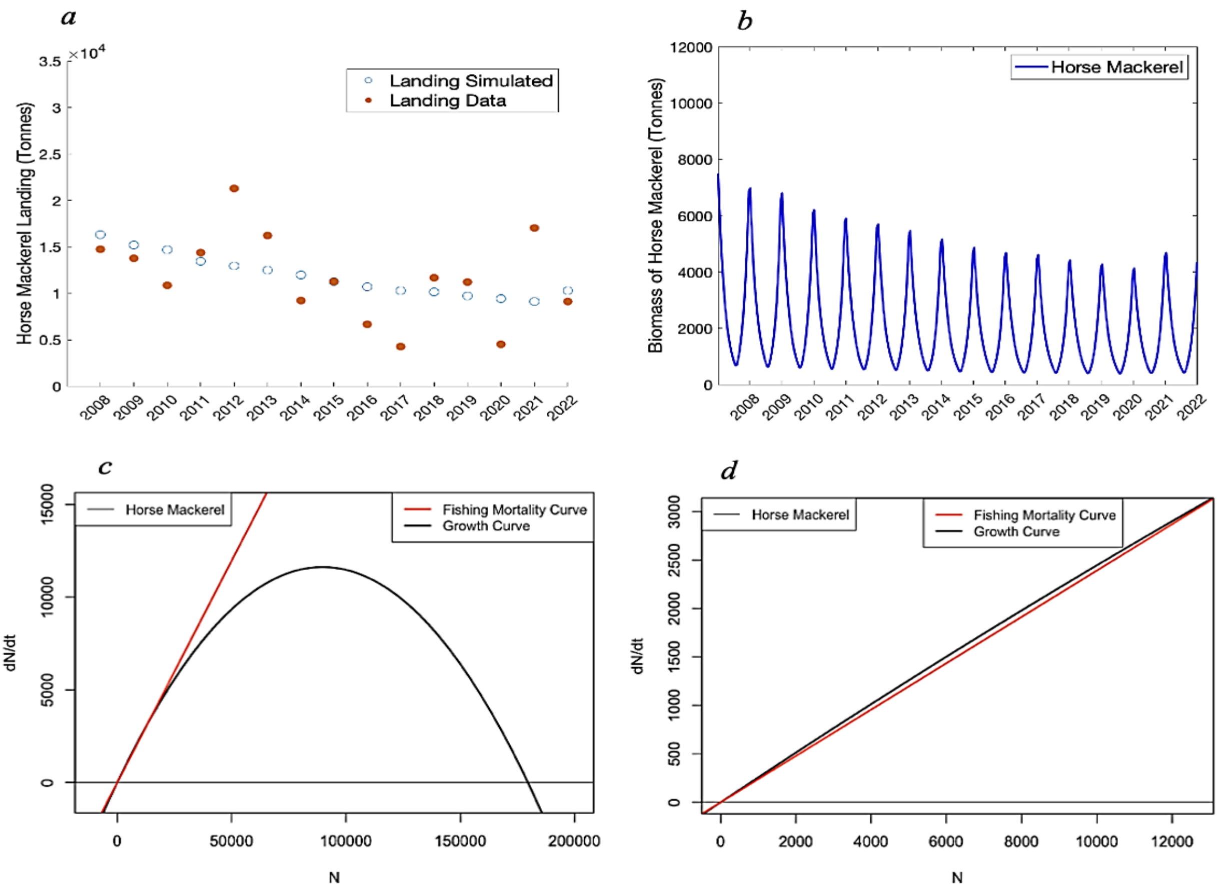

Figure 3. The plot (*a*) denotes the model fit with annual landing data, and the plot (*b*) is the instantaneous change in the fish stock (biomass) with the estimated parameters in Table 1. The plot (*c*) represents the visualization of fish status, the X axis denotes fish stock biomass in terms of tonnes (*N*) and the Y axis denotes the rate of change (dN/dt) in the 15-year average fishing mortality curve (F^*) and growth curve over 15 years. The plot (*d*) is a zoomed version of the plot (*c*) and shows the positive equilibrium point around *N*=12000 tonnes.

The current status of the Black Sea anchovy

We applied the harvest between September and January for the Black Sea anchovy fishery on the Turkish side of the Black Sea (Gücü et al., 2017). The estimated MSY is 169512 tonnes depending on the parameter estimation for this fishery (see Table 2). We have two equilibrium points $E_1 = 0$ (unstable)and $E_2 \cong 230000$ (stable). As indicated in the bottom left plot (*c*) in Figure 4, similar to the horse mackerel, the anchovy population has been also experiencing high fishing mortality in the last 15 years since the second positive equilibrium, remaining on the left side of the peak in the bell-shaped growth curve (as shown in the bottom left plot (*c*) of Figure 4). The annual maximum instantaneous biomass of anchovies fluctuates between 130000 tonnes and 170000 tonnes. However, it has the potential to surge to 230000 tonnes (see the bottom-right plot (*d*) in Figure 4), as indicated by the growth curve consistently surpassing the fishing mortality curve until reaching the 230000-tonnes biomass value.

To achieve an instantaneous biomass increase of up to 230000 tonnes for the anchovy fishery, it's crucial to maintain the annual harvest of anchovy at or below the MSY estimated in this study. Implementing this strategy not only leads to immediate biomass increases but also ensures long-term benefits. As the fish stock reaches stability at 230000 tonnes, the MSY will increase over time, securing the sustainability of anchovy fisheries in the long term.

Table 2. Parameter descriptions and estimated parameter values for the Black Sea anchovy

Parameters	N_0	K	r	F	$\frac{rK}{4}$	e_r
Description	Initial biomass of fish stocks	Carrying capacity	Intrinsic growth rate	Fishing mortality rate	Maximum sustainable yield	Relative errors of fits
Unit	Tonnes	Tonnes	$year^{-1}$	$year^{-1}$	Tonnes	-
Initial range	$1e^5$ - $3e^5$	$1e^6$-$3e^6$	0.3-0.4	0.5-0.9	-	-
Black Sea anchovy	148929	1765750	0.384	0.68	169512	0.32

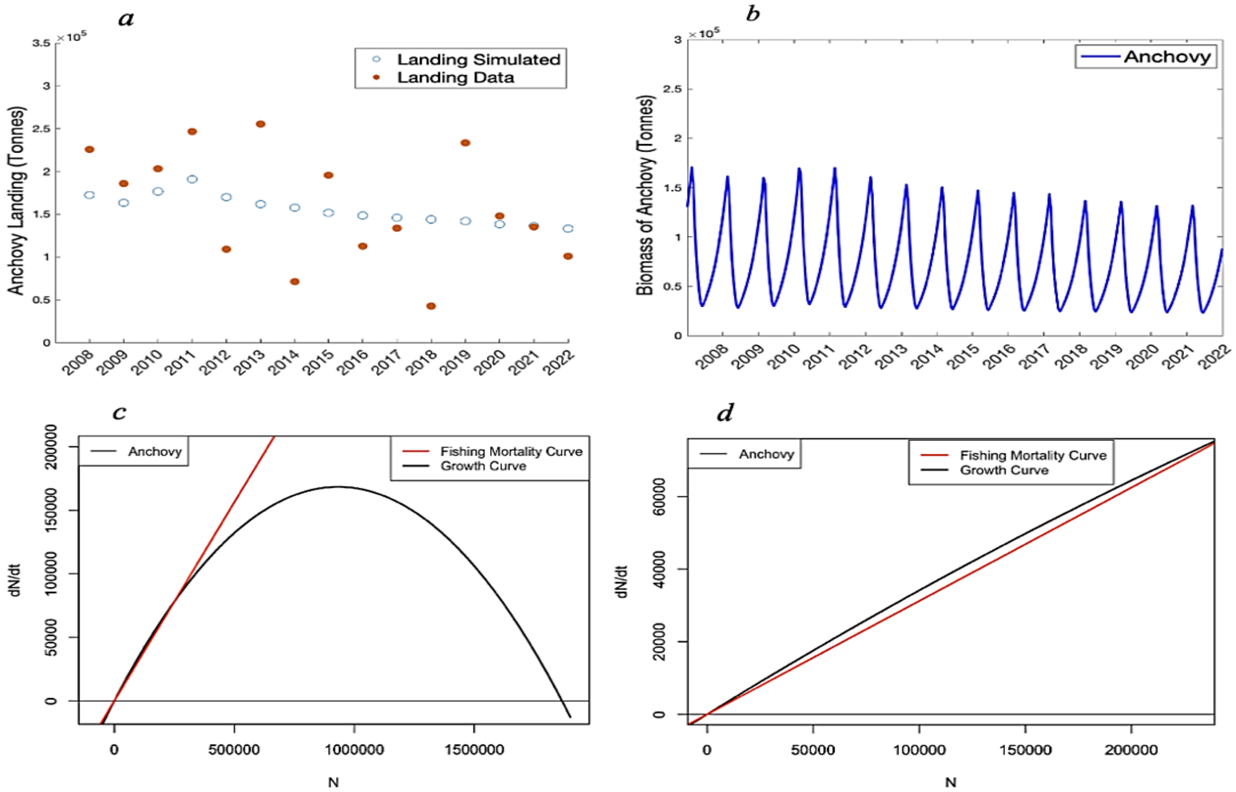

Figure 4. The plot (*a*) denotes the model fit with annual landing data, and the plot (*b*) is the instantaneous change in the fish stock (biomass) with the estimated parameters in Table 2. The plot (*c*) represents the visualization of fish status, the X axis denotes fish stock biomass in terms of tonnes (*N*) and the Y axis denotes the rate of change (dN/dt) in the 15-year average fishing mortality curve (F^*) and growth curve over 15 years. The plot (*d*) is a zoomed version of the plot (*c*) and shows the positive equilibrium point around *N*=230000 tonnes.

The current status of sprat

We conducted harvesting operations between January and April 15th targeting the sprat fishery on the Turkish side of the Black Sea (Zengin and Dincer, 2006; Özsandikçı, 2020). Based on the parameter estimation for this fishery (see Table 3), the MSY is estimated at 36835 tonnes. We captured two equilibrium points for the sprat fishery: $\mathrm{E}_1 = 0$ (unstable) and $\mathrm{E}_2 \cong 55000$ tonnes (stable). Therefore, despite observing a decreasing trend between the years 2008 and 2022 in this fishery, the fish stock does not reach zero due to the existence of two equilibrium points, with the second being stable and being positive at $\mathrm{E}_2 \cong 55000$ tonnes. Consequently, while this fish population does not face severe overfishing, it does experience high fishing mortality.

The annual maximum instantaneous biomass of sprat fluctuates between 20000 tonnes and 50000 tonnes, showing a declining trend. However, there is an opportunity to stop this decreasing trend and enhance this biomass up to 55000 tonnes, as indicated by the growth curve consistently exceeding the fishing mortality curve by this margin. To achieve this improvement, it is advisable to harvest the sprat population around the Maximum Sustainable Yield (MSY) estimated in the study. This approach would allow us to strike a delicate balance between preserving the health of the sprat population and ensuring the continued sustainability of the fishery on the Turkish Side of the Black.

Table 3. Parameter descriptions and estimated parameter values for sprat

Parameters	N_0	K	r	F	$\frac{rK}{4}$	e_r
Description	Initial biomass of fish stocks	Carrying capacity	Intrinsic growth rate	Fishing mortality rate	Maximum sustainable yield	Relative errors of fits
Unit	Tonnes	Tonnes	$year^{-1}$	$year^{-1}$	Tonnes	-
Initial range	$3e^4$ - $7e^4$	$7e^5$-$9e^5$	0.1 - 0.3	0.4 - 0.8	-	-
Sprat	50009	805964	0.183	0.628	36835	0.41

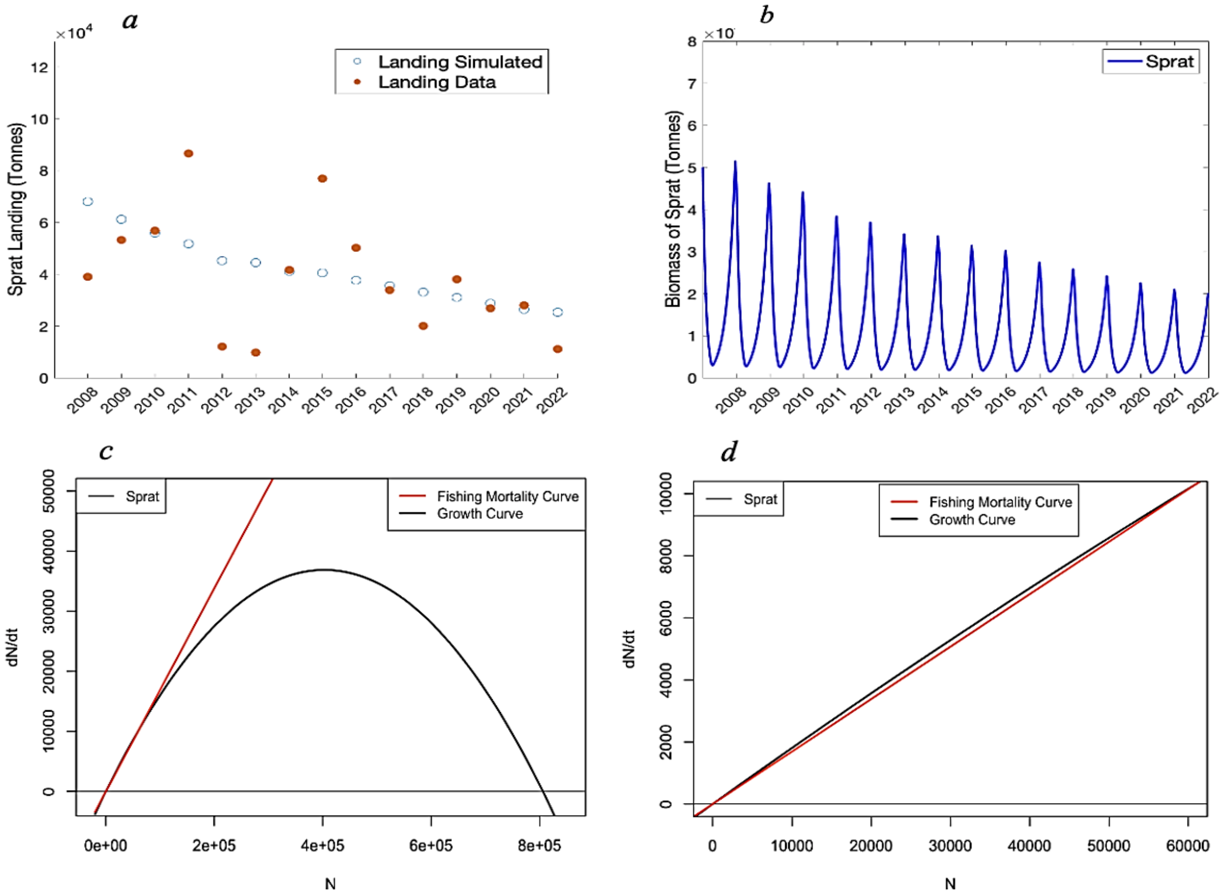

Figure 5. The plot (*a*) denotes the model fit with annual landing data, and the plot (*b*) is the instantaneous change in the fish stock (biomass) with the estimated parameters in Table 3. The plot (*c*) represents the visualization of fish status, the X axis denotes fish stock biomass in terms of tonnes (*N*) and the Y axis denotes the rate of change (dN/dt) in the 15-year average fishing mortality curve (F^*) and growth curve over 15 years. The plot (*d*) is a zoomed version of the plot (*c*) and shows the positive equilibrium point around *N*=55000 tonnes.

The current status of whiting

We applied our harvest operations between September and April 15th targeting the whiting fishery on the Turkish side of the Black Sea (STECF, 2017). The estimated MSY stands at 8158 tonnes, dependent on the parameter estimation for this fishery, as outlined in Table 4. Our analysis reveals two equilibrium points: $E_1 = 0$ and $E_2 \cong 8000$ tonnes, as illustrated in the bottom plot of Figure 6. Despite observing a high fishing mortality in this fishery since the second positive equilibrium, remaining on the left side of the peak in the bell-shaped growth curve (as shown in the bottom left plot (*c*) of Figure 5), there hasn't been a sharp decline in the whiting stock. The underlying reasons remain unclear; however, we have noticed consistent oscillations in this fishery every five years. Further investigation is needed to understand the dynamics driving these fluctuations.

The annual maximum instantaneous biomass of whiting fluctuates between about 5000 tonnes and 7000 tonnes. However, it has the potential to surge to 8000 tonnes (see the bottom-right plot (*d*) in Figure 5), as indicated by the growth curve consistently surpassing the fishing mortality curve until reaching the 8000-tonnes biomass value which is stable. Thus, to obtain a sustainable fishery for whiting, it is recommended to harvest around or below the MSY estimated in the study.

Table 4. Parameter descriptions and estimated parameter values for whiting

Parameters	N_0	K	r	F	$\frac{rK}{4}$	e_r
Description	Initial biomass of fish stocks	Carrying capacity	Intrinsic growth rate	Fishing mortality rate	Maximum sustainable yield	Relative errors of fits
Unit	Tonnes	Tonnes	$year^{-1}$	$year^{-1}$	Tonnes	-
Initial range	$5e^3$ - $8e^3$	$6e^4$-$8e^4$	0.3 - 0.6	0.6 - 0.8	-	-
Whiting	6523	68174	0.463	0.723	7891	0.17

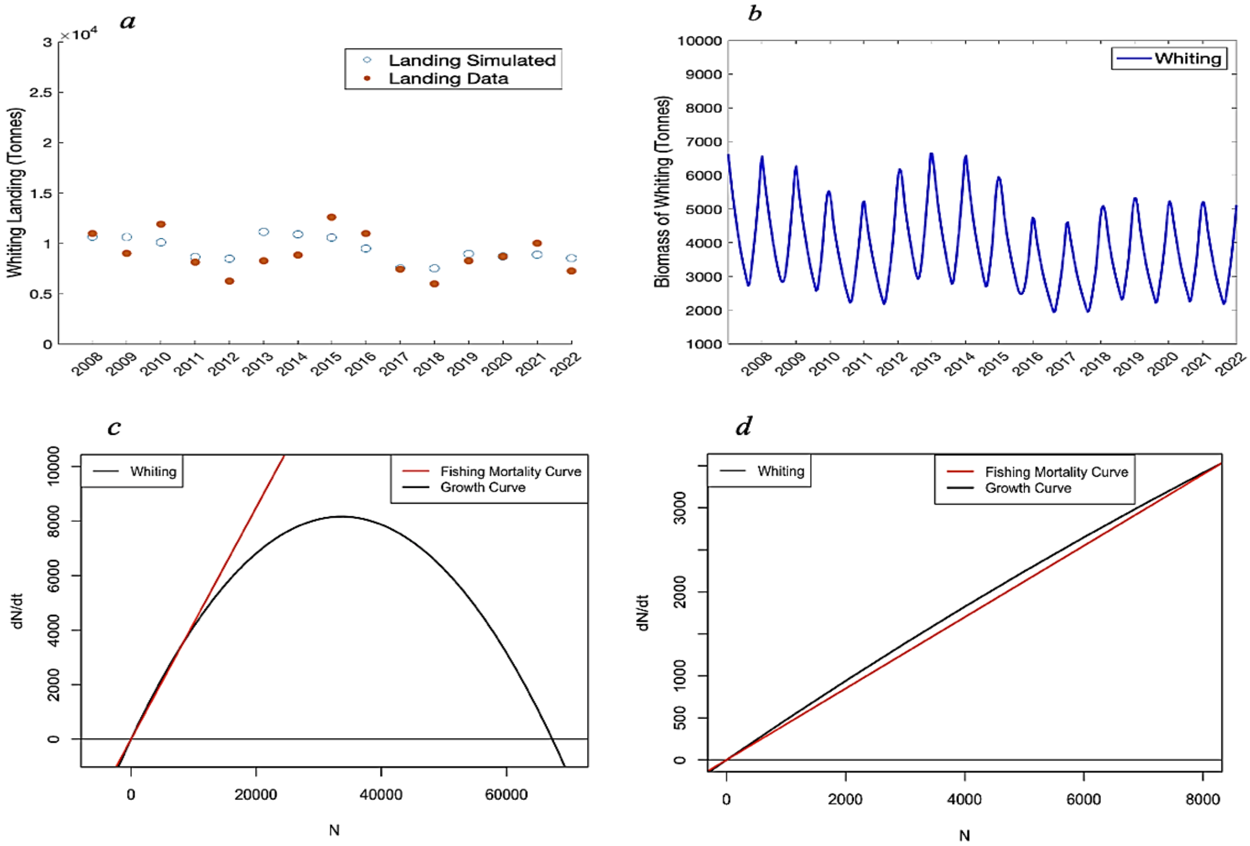

Figure 6. The plot (*a*) denotes the model fit with annual landing data, and the plot (*b*) is the instantaneous change in the fish stock (biomass) with the estimated parameters in Table 4. The plot (*c*) represents the visualization of fish status, the X axis denotes fish stock biomass in terms of tonnes (*N*) and the Y axis denotes the rate of change (dN/dt) in the 15-year average fishing mortality curve (F^*) and growth curve over 15 years. The plot (*d*) is a zoomed version of the plot (*c*) and shows the positive equilibrium point around *N*=8000 tonnes.

The current status of bluefish

We applied harvesting operations between September and January targeting the bluefish fishery on the Turkish side of the Black Sea (Gücü et al., 2017). The estimated MSY stands at 4087 tonnes, dependent on the parameter estimation for this fishery (see Table 5). Despite observing fluctuations in the fishery landing, there is a clear increasing trend over time (see the top-left plot in Figure 7). Our analysis reveals two equilibrium points: $E_1 = 0$ and $E_2 \cong 6000$ tonnes, as illustrated in the bottom right plot (*c*) of Figure 7. This indicates that while the annual maximum instantaneous fish biomass has fluctuated between 1500 tonnes to 3500 tonnes, as shown in the top right plot (*d*) of Figure 7, it has the potential to increase up to 6000 tonnes since the equilibrium point, $E_2 \cong 6000$ is stable.

Hence, it is crucial to harvest this fish stock around or below the MSY estimated in the study. This approach serves the dual purpose of maintaining a sustainable fishery and aiming to attain its maximum potential biomass of 6000 tonnes for current status. By following this recommendation, we endeavor to achieve a harmonious balance between harvesting bluefish and safeguarding the long-term health and productivity of the fishery on the Turkish Side of the Black Sea.

Table 5. Parameter descriptions and estimated parameter values for bluefish

Parameters	N_0	K	r	F	$\frac{rK}{4}$	e_r
Description	Initial biomass of fish stocks	Carrying capacity	Intrinsic growth rate	Fishing mortality rate	Maximum sustainable yield	Relative errors of fits
Unit	Tonnes	Tonnes	$year^{-1}$	$year^{-1}$	Tonnes	-
Initial range	$1e^4$ - $3e^4$	$8e^4$-$12e^4$	0.1 - 0.3	0.4 - 0.6	-	-
Bluefish	1459	95623	0.171	0.471	4087	0.43

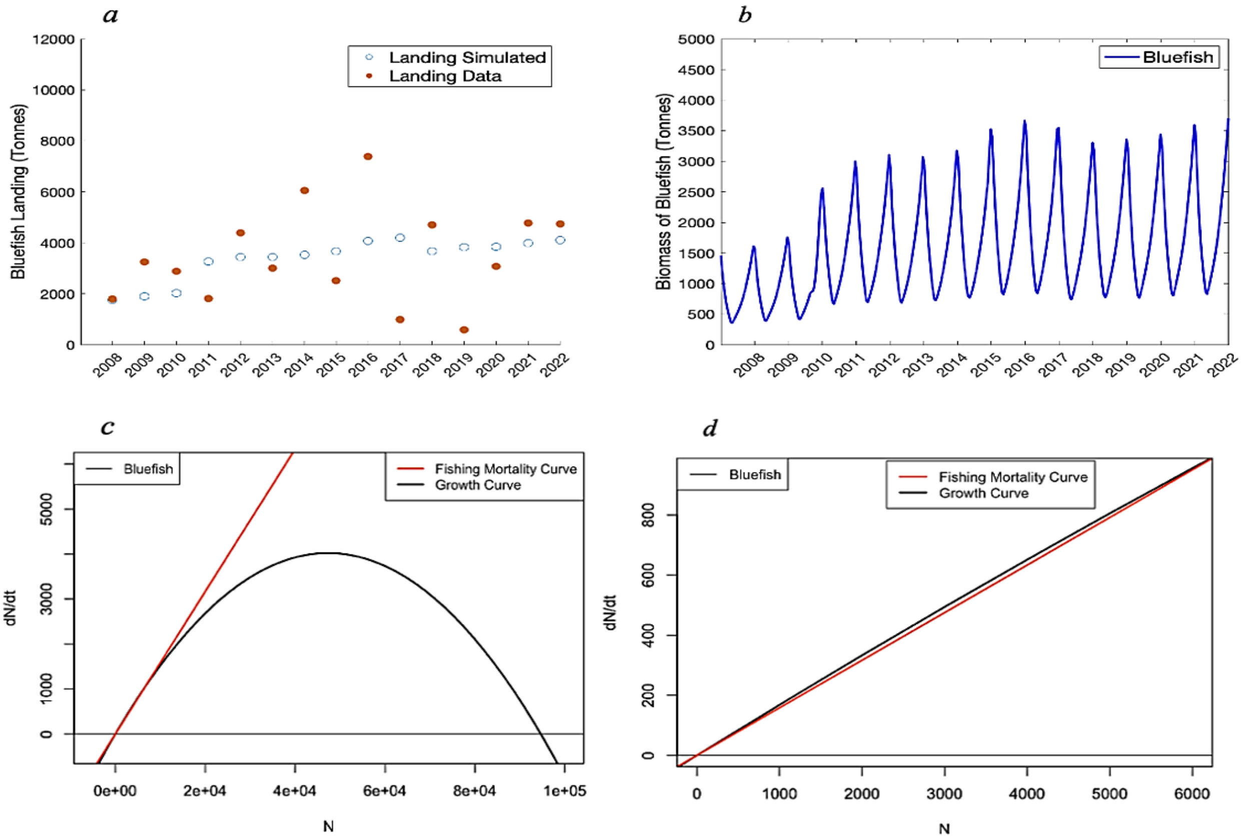

Figure 7. The plot (*a*) denotes the model fit with annual landing data, and the plot (*b*) is the instantaneous change in the fish stock (biomass) with the estimated parameters in Table 5. The plot (*c*) represents the visualization of fish status, the X axis denotes fish stock biomass in terms of tonnes (*N*) and the Y axis denotes the rate of change (dN/dt) in the 15-year average fishing mortality curve (F^*) and growth curve over 15 years. The plot (*d*) is a zoomed version of the plot (*c*) and shows the positive equilibrium point around *N*=6000 tonnes.

The current status of Atlantic bonito

We conducted harvesting operations from September to December for the Atlantic bonito fishery on the Turkish side of the Black Sea (Gücü et al., 2017). The estimated Maximum Sustainable Yield (MSY) is 16032 tonnes based on parameter estimations specific to this fishery (see Table 6). Despite fluctuations in fishery landing, a noticeable upward trend persists over time.

Our analysis reveals two equilibrium points: $E_1 = 0$ and $E_2 \cong 40000$ tonnes. This indicates that although the annual maximum instantaneous fish biomass has varied between 6000 tonnes to 19000 tonnes, as illustrated in the top-right plot (*c*) of Figure 8, it has the potential to rise up to 40000 tonnes (see the bottom-right plot (*d*) in Figure 8), as indicated by the growth curve consistently surpassing the fishing mortality curve until reaching the 40000 tonnes. To achieve an instantaneous biomass increase of up to 40000 tonnes for the Atlantic bonito, it's crucial to maintain the annual harvest of the Atlantic bonito at or below the MSY estimated in this study.

Table 6. Parameter descriptions and estimated parameter values for the Atlantic bonito

Parameters	N_0	K	r	F	$\frac{rK}{4}$	e_r
Description	Initial biomass of fish stocks	Carrying capacity	Intrinsic growth rate	Fishing mortality rate	Maximum sustainable yield	Relative errors of fits
Unit	Tonnes	Tonnes	$year^{-1}$	$year^{-1}$	Tonnes	-
Initial range	$4e^4$ - $8e^4$	$5e^5$-$8e^5$	0.05 - 0.2	0.2 - 0.4	-	-
Atlantic bonito	5864	640318	0.100	0.281	16032	0.49

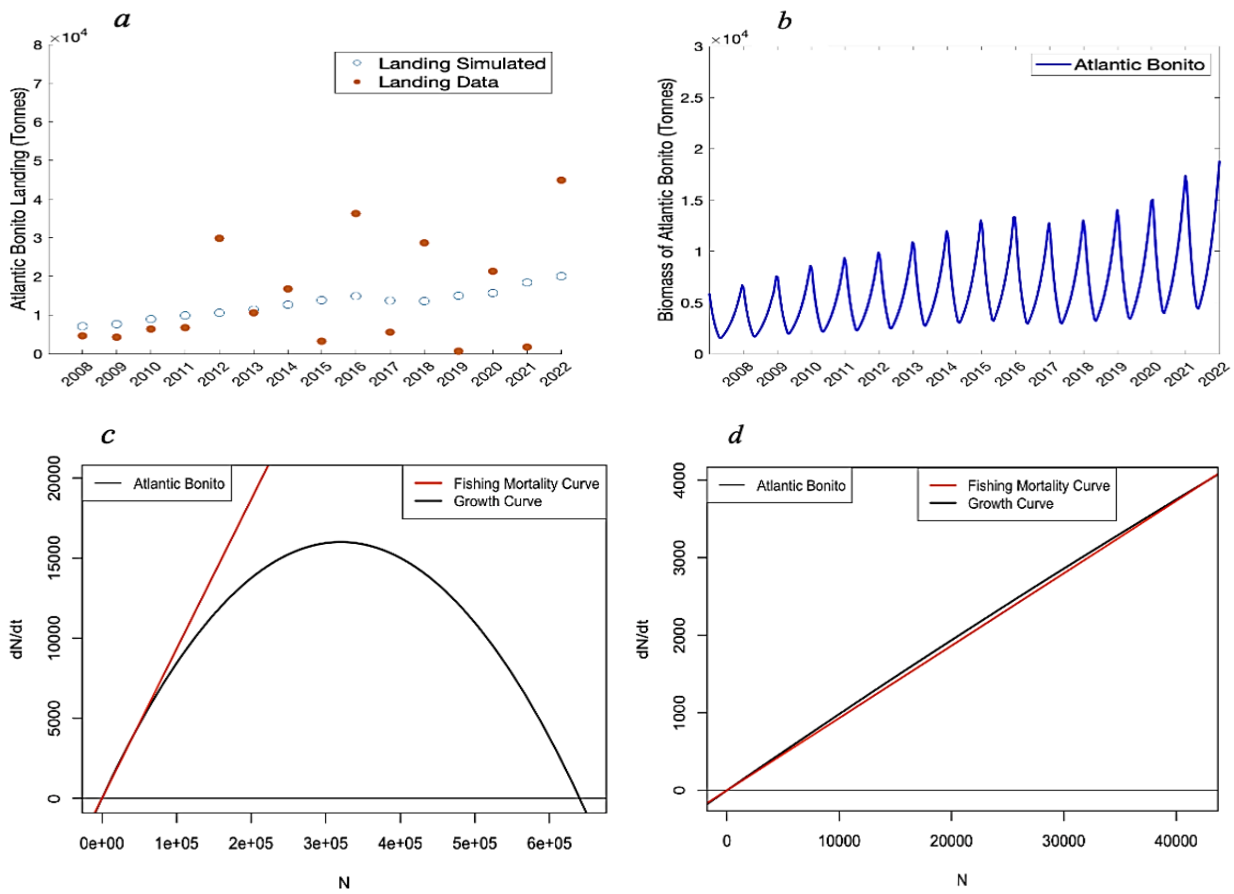

Figure 8. The plot (*a*) denotes the model fit with annual landing data, and the plot (*b*) is the instantaneous change in the fish stock (biomass) with the estimated parameters in Table 6. The plot (*c*) represents the visualization of fish status, the X axis denotes fish stock biomass in terms of tonnes (*N*) and the Y axis denotes the rate of change (dN/dt) in the 15-year average fishing mortality curve (F^*) and growth curve over 15 years. The plot (*d*) is a zoomed version of the plot (*c*) and shows the positive equilibrium point around *N*=40000 tonnes.

Sensitivity analysis of the parameters

To determine the key parameters influencing fish stock dynamics and assess how small variations impact model outcomes, we performed a sensitivity analysis. This analysis employed Latin Hypercube Sampling (LHS) alongside the Partial Rank Correlation Coefficients (PRCC) method, following the approach described by Marino et al. (2008). Using the parameter ranges specified in Table 7, we generated samples from a uniform distribution and incorporated them as inputs to simulate system (1) over 15 years. The total fish stock yield or biomass served as the output variables. Table 7 presents the PRCC values and parameter ranges used in the sensitivity analysis of parameters for each fish stocks. We selected these ranges based on their biological significance, as outlined in the parameter estimation section, while ensuring that the estimated parameter values in Tables 1–6 remain within their respective lower and upper bounds.

The sensitivity analysis indicates that the parameters r, K and F are statistically significant, as evidenced by their high PRCC values. Among these, the intrinsic growth rate (r) has the greatest influence on model outcomes, followed by the carrying capacity (K) and then the fishing mortality rate (F). Our sensitivity analysis for each fish stock indicates that the initial condition is not statistically significant, meaning the model outcomes are not sensitive to initial conditions. Figure 9 provides a representative plot from the sensitivity analysis of the anchovy population.

It is also worth noting that the positive correlation between fishing mortality rate (F) and annual yield weakens as the fishing mortality rate increases (Figure 9). This outcome is expected, as the sensitivity analysis spans 15 years rather than a single year. Higher fishing mortality rates impact future yields, preventing a perfectly positive correlation between increased fishing mortality and annual yield.

Table 7. Results of sensitivity analysis with partial rank correlation coefficient (PRCC) with respect to the output of total yields at final time. We used ranges (0.01,1) for the parameters *r* and *F* for all the species in the sensitivity analysis.

PRCC values					Parameter ranges used in sensitivity analysis	
Parameters	$N(0) = N_0$	K	r	F	$N(0) = N_0$	K
Description	Initial biomass of fish stocks	Carrying capacity	Intrinsic growth rate	Fishing mortality rate	Initial biomass of fish stocks	Carrying capacity
Horse mackerel	0.05	0.61	0.76	0.51	5000-20000	100000-300000
Black Sea anchovy	0.04	0.53	0.76	0.44	100000-300000	1000000-3000000
Sprat	0.01	0.59	0.74	0.43	20000-200000	500000-2000000
Whiting	0.08	0.58	0.69	0.37	2000-20000	30000-200000
Bluefish	0.05	0.57	0.69	0.41	1000-10000	50000-200000
Atlantic bonito	0.04	0.63	0.72	0.40	2000-20000	300000-1000000

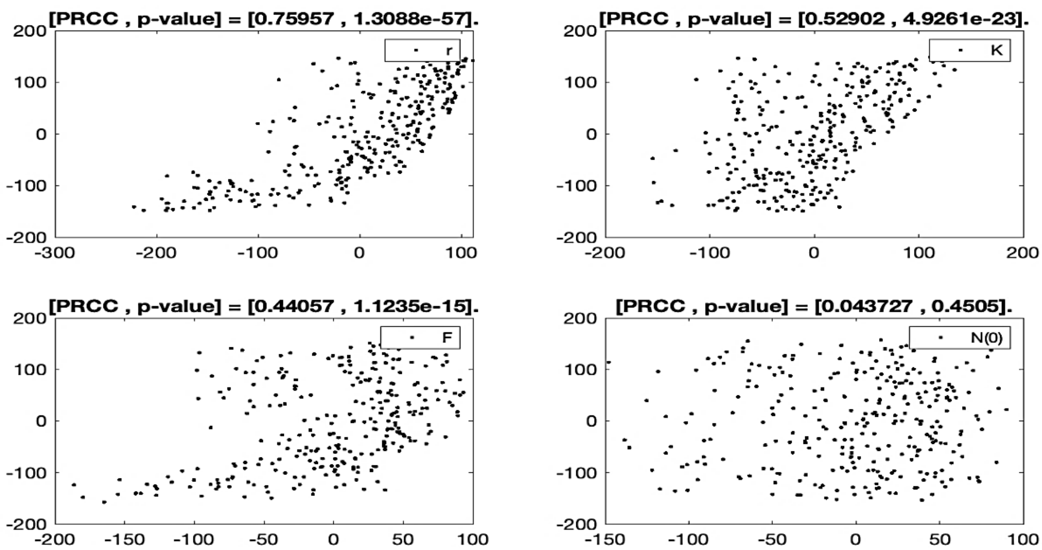

Figure 9. Visualization of sensitivity analysis for anchovy population: X axis denotes the variation of parameters with the ranked initial range of parameters given in Table 2 and Y axis denotes the change in the annual anchovy yields as we vary parameters in X axis.

Our sensitivity analysis was initially based on the total yield at the final time. However, when conducted using the biomass of the fish stock at the final time, the results differ slightly. As shown in Table 8 and Figure 10, the significance and sensitivity levels of the parameters increase, with the exception of the initial conditions. Thus, regardless of the scenario at the final time, the initial condition has no significant impact on the model outputs.

Table 8. Results of sensitivity analysis with partial rank correlation coefficient (PRCC) with respect to the biomass of fish stock at final time. We used ranges (0.01,1) for the parameters *r* and *F* for all the species in the sensitivity analysis.

PRCC Values					Parameter ranges used in sensitivity analysis	
Parameters	$N(0) = N_0$	K	r	F	$N(0) = N_0$	K
Description	Initial biomass of fish stocks	Carrying capacity	Intrinsic growth rate	Fishing mortality rate	Initial biomass of fish stocks	Carrying capacity
Horse Mackerel	0.09	0.80	0.81	-0.73	5000-20000	100000-300000
Black Sea anchovy	0.04	0.78	0.82	-0.71	100000-300000	1000000-3000000
Sprat	0.01	0.77	0.80	-0.66	20000-200000	500000-2000000
Whiting	0.03	0.80	0.76	-0.61	2000-20000	30000-200000
Bluefish	0.08	0.77	0.81	-0.60	1000-10000	50000-200000
Atlantic Bonito	0.03	0.77	0.80	-0.60	2000-20000	300000-1000000

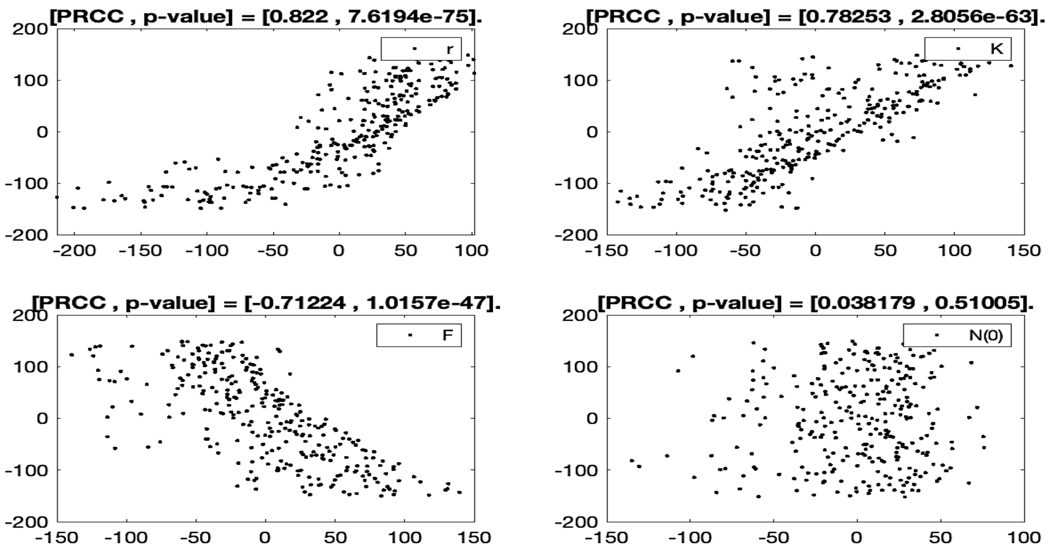

Figure 10. Visualization of sensitivity analysis for anchovy population: X axis denotes the variation of parameters with the ranked initial range of parameters given in Table 2 and Y axis denotes the change in the biomass of anchovy stock as we vary parameters in X axis.

DISCUSSION

Overall, the study's findings shed light on the dynamics and fishing pressure levels of the six fish stocks on the Turkish side of the Black Sea. Our analysis of different fish stocks, including horse mackerel, the Black Sea anchovy, sprat, whiting, bluefish, and the Atlantic bonito, revealed valuable insights into their fishing status and potential for sustainable management by implementing MSYs.

Our sensitivity analysis revealed that the initial conditions of fish stocks have no significant impact on the model results, as the correlation between initial conditions and biomass at the final time is close to zero. In contrast, the parameters r, K and F are statistically significant, meaning any variation in these parameters strongly influences the model outputs. Therefore, we compared our parameter estimates to the rates reported in the literature.

The annual landing data and our model fits indicated that the fish stocks: horse mackerel, anchovy, whiting, and sprat were in a decreasing trend. Atlantic bonito and bluefish stocks were on an increasing trend even if we saw sharp ups and downs in these fish stocks over the years (see Figures 7 and 8). Depending on the method for investigation of fish stocks given in the material and method section, all the fish stocks covered in the study were experiencing high fishing mortality on the Turkish side of the Black Sea. To address the high fishing pressure on these fish stocks, this study offered the Maximum Sustainable Yields (MSYs) for their management. Implementing the estimated MSYs for these fisheries could mitigate the high fishing pressure and alleviate its adverse impacts on the Turkish side of the Black Sea. Despite these fish stocks experiencing high fishing mortality, there is optimism that they can attain the second equilibrium point and prevent severe overfishing if the estimated MSYs are applied for each fishery.

Upon examining the horse mackerel fishery, our investigation reveals promising potential. With an estimated MSY of 11405 tonnes, the fishery showcases a positive trajectory despite historical fluctuations. The presence of two equilibrium points suggests stability, emphasizing the opportunity for sustainable harvesting practices. Harvesting the horse mackerel around the MSY estimated in this study could be pivotal in maintaining the fishery's health and productivity while ensuring its long-term sustainability. The estimated fishing mortality rate was about 0.44 in this study, but this rate was 0.65 between the years 2008 and 2011 in the study presented by Kasapoğlu (2018). This might relate to the reduction in harvesting efforts for horse mackerel fishery in recent years.

In a similar fashion to the horse mackerel fishery, the anchovy fishery also exhibits a declining trend, coupled with a high fishing mortality rate of approximately 0.69. Despite these challenges, the anchovy fishery boasts an estimated MSY of 169512 tonnes. Despite fluctuations in landings attributed to this high fishing mortality rate, the fishery maintains stability, evident from the capture of two equilibrium points based on landing data between 2008 and 2022. Harvesting anchovy around the MSY not only promises to sustain the fishery but also maximizes its potential biomass, underlining the importance of adhering to sustainable harvesting practices.

The MSY was estimated as 244000 between the years 1963 and 2014 (Akkuş and Gücü, 2022) and estimated as 222250 tonnes between the years 2002 and 2017 (Demir and Lenhart, 2020). However, in our study, the MSY is estimated as 169512 tonnes between the years 2008 and 2022. This discrepancy suggests that the status of anchovy fishing may have worsened in recent years, as indicated by the decline in the estimated MSYs from 1963 to 2022. A lower MSY indicates that the fishery may be experiencing decreased productivity or facing overexploitation, which could be a cause for concern regarding the sustainability of anchovy stocks. Moreover, the constant fishing mortality rate was estimated as 0.48 between the years 2002 and 2017 (Demir and Lenhart, 2020) and 0.5 between the years 2005 and 2014 (Akkuş and Gücü, 2022) but in our estimate, the fishing mortality rate is estimated as 0.69 between the years 2008 and 2022. This may be related to the choice of model selection. We used a single equation but the study (Demir and Lenhart, 2020) used a food chain model consisting of 4 tropic levels and considered the effect of predators on anchovy. Or, it may be related to the increased fishing mortality rate of anchovy fishery in recent years.

Similarly, the sprat fishery has displayed a concerning declining trend over the years, raising significant questions about its long-term sustainability. Despite the presence of two equilibrium points, the fishery suffers from high fishing mortality, as evidenced by the fishing mortality rate of approximately 0.63 as given in Table 3 and depicted in Figure 5. This estimate coincides with the estimate of 0.64 obtained for the single year 2014 in the study presented by Özsandikçı (2020). Our findings necessitate the implementation of careful management strategies to avoid further decline. As highlighted in the results section, it is imperative to apply the estimated MSY to ensure the sustainability of the sprat fishery.

We estimated the MSY as 7891 tonnes for the whiting fishery. Despite encountering high fishing mortality, the fishery maintains stability with two equilibrium points. However, the consistent oscillations observed every five years warrant further investigation into the underlying factors influencing the fishery's dynamics. The estimated fishing mortality rate is about 0.72 above the 0.69 estimated in STECF (2017) and below 0.76 estimated in Kasapoğlu (2018).

In contrast, the bluefish fishery, with an estimated MSY of 4087 tonnes, shows a clear increasing trend over time. The presence of two equilibrium points indicates potential for sustainable harvesting practices, underscoring the importance of adhering to MSY guidelines to ensure the fishery's long-term viability. It also has the potential to increase its biomass up to 6000 tonnes when the estimated MSY is applied in the long term (see the bottom right plot in Figure 7).

Finally, the Atlantic bonito fishery presents an intriguing scenario, with an MSY estimated at 16032 tonnes. Despite fluctuations in landing rates, the fishery displays a consistent increasing trend, with two equilibrium points suggesting stability. Harvesting the Atlantic bonito around the estimated MSY offers promising prospects for sustaining the fishery while maximizing its potential biomass. The MSY for Atlantic bonito was estimated at approximately 17000 tonnes, with a range of 14700 to 19800 tonnes, according to the study by Daskalov et al., (2020). It's worth noting that our estimated MSY for Atlantic bonito, which stands at 16032 tonnes, closely aligns with this result. In Daskalov et al., (2020), the study period ranged from the 1950s to 2016. However, in our study, we focused on the more recent period from 2008 to 2022. According to these studies, while the carrying capacity of Atlantic bonito in the Black Sea has increased, there has been a decline in its intrinsic growth rate.

Limitations and benefits of model selection

There have been many data-limited stock assessment methods primarily requiring landing data to assess the current status and abundance of fish populations, including CMSY, OCOM, JABBA, SPICT, and DBSPR (Dick and MacCall, 2011; Froese, 2017; Zhou, 2017; Winker et al., 2018; Bouch et al., 2021; Froese, 2023). Similar to these methods, we only used landing data in our analysis. Our analysis mainly depended on time-dependent graphs of intrinsic growth and fishing mortality rates. These graphs not only provided these rates but also provided equilibrium points and the status of fishing pressure such as low, high, or extreme fishing. Thus, the main benefit of the method we used was obtaining such outputs in a single graph. Additionally, we could obtain the biomass dynamics of fish populations and MSYs from the logistic model. This method was not new but served as an alternative to the approaches mentioned above (Kot, 2001). Our main reason for choosing this method was its graphical simplicity when analyzing data-limited fish stocks. Additionally, it was important to include stochasticity in a fishery assessment method, as it allowed in the surplus production models (Schaefer, 1954; Fox, 1970). In the model used in this study, stochastic elements and optimal control tools could be incorporated into the mathematical model for further analysis (Demir and Lenhart, 2021; Demir, 2024), or conducting a sensitivity analysis if required (Aslan et al., 2022; Marino et al., 2008). Therefore, using this mathematical model has provided flexibility for conducting additional analyses.

Single-species models tend to oversimplify the ecosystem by disregarding interspecies interactions, such as competition, predation, and mutualism, which can significantly influence the target species' population dynamics (Demir, 2019). This lack of complexity can make the model less accurate in predicting changes driven by ecological or environmental shifts, including those caused by climate change or invasive species introductions (Bellard et al., 2013; Mainali et al., 2015; Demir and Lenhart, 2020). Furthermore, a narrow focus on a single species may lead to unintended ecological consequences, as management efforts aimed at benefiting one species could inadvertently harm others and disrupt overall ecosystem stability (Botsford et al., 1997).

On the other hand, in the absence of detailed information and data, this approach can still be valuable if applied cautiously (Beverton and Holt, 1957). In this study, rather than relying directly on model outputs from the single-species model such as $E_{0.1}$, $E_{0.5}$, E_{max}, E_{cur}, $F_{0.1}$, $F_{0.5}$, F_{max}, F_{cur}, SPR, B, and B_{max}, we focused on examining the direction of change in the size of fish stocks to understand when the population stock size increases or decreases. Given that changes in fish stock size are directly influenced by the intrinsic growth rate and fishing mortality rate, we not only conducted a stability analysis but also captured the current levels of fishing pressure. Thus, instead of using the estimated rates directly to assess fish stock status, we used them to indicate periods when fish stock sizes were trending upward or downward in our graphs (Figures 3-8). We first examined fishing pressure levels based on these trends and then analyzed the potential for achieving positive equilibrium points to prevent overfishing of these stocks.

Limitations of data

Since we used a deterministic model that does not account for noise and measurement errors, our parameter estimates may be affected by errors in the data. For instance, landing data often excludes discards, which can represent a significant portion of the total catch. Additionally, underreporting or misreporting in landing data is common, potentially introducing biases into the analysis. To address these limitations, a stochastic state-space model can be employed, allowing for the incorporation of both measurement and process errors. It is also worth noting that incorporating additional data, such as CPUE or independent survey data, can improve parameter estimates and enhance model accuracy. However, our approach remains still applicable even in the absence of such data.

CONCLUSION

In conclusion, despite the inherent limitations of single-species models, our approach demonstrates their potential utility when detailed ecological data is lacking. By focusing on the direction of stock changes rather than absolute biological reference points, we identified patterns of increasing and decreasing stock trends, enabling us to assess fishing pressure levels effectively. This method provides a practical framework for fishery management, offering valuable insights into sustainable harvest levels and equilibrium points to mitigate overfishing risks while promoting stock stability if the estimated MSY for each fish stock is applied.

We implemented this method to assess which fish stocks are experiencing high fishing pressure and to identify those at risk of overfishing. By categorizing these stocks according to their levels of fishing pressure—low, high, or extreme—we gained a clearer understanding of their status and potential vulnerabilities (Figures 3-8). To further validate our findings, we conducted a graphical stability analysis to ensure that the stock sizes could reach a positive, stable state, supporting a sustainable fishery over time. This approach offers an effective strategy for managing fish populations in data-limited stock assessments, ultimately contributing to long-term resource stability and sustainability. Thus, our investigation reveals that, despite an increasing trend in some stocks over the past 15 years, they remain subject to high fishing mortality. This study also identifies stable equilibrium points, indicating conditions under which these stocks can be sustained long-term.

Overall, this study presents a graphical investigation not only to analyze the stability of stocks (Figure 1) but also to understand and capture important features of the current status of fish stocks such as MSYs, stable equilibrium points, and fishing mortality levels of fish stocks such as low fishing mortality case, high fishing mortality case, extreme fishing mortality case (Figure 2). Such investigations can help sustainable management practices in maintaining the health and productivity of fisheries on the Turkish side of the Black Sea.

ACKNOWLEDGEMENTS AND FUNDING

There are no funding sources to declare in this study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

ETHICAL STATEMENT

No ethical approval is required for this study.

DATA AVAILABILITY

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

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Ege Journal of Fisheries and Aquatic Sciences, 42(2), 122-130 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.04

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Methylene blue dye removal utilizing biomass of the macroalgae (*Padina* sp.): Adsorption, kinetic studies and mechanism

Makroalg (*Padina* sp.) biyokütlesi kullanılarak metilen mavisi boyasının uzaklaştırılması: Adsorpsiyon, kinetik çalışmalar ve mekanizma

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Received date: 20.01.2025 *Accepted date: 24.03.2025*

How to cite this paper:
Türe, H., & Akdoğan Şirin, P. (2025). Methylene blue dye removal utilizing biomass of the macroalgae (*Padina* sp.): Adsorption, kinetic studies and mechanism. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 122-130. https://doi.org/10.12714/egejfas.42.2.04

Abstract: Macroalgae, which are abundant in marine ecosystems, are promising natural adsorbents for water purification because of their high surface area, rich functional groups, and natural adsorption capabilities. In this study, the adsorption capacity of *Padina* sp., a brown macroalgae in dried powdered form, was evaluated for the removal of toxic methylene blue (MB) dye from water. A series of batch adsorption tests were conducted to investigate the effects of several operational parameters, as initial MB concentrations (2, 5, 10, 20, and 50 mg L^{-1}), pH levels (2, 4, 7, and 11), and contact time (0, 0.08, 0.5, 1, 2, 4, 6, and 24 h) on MB removal. Furthermore, powdered *Padina* sp. was analyzed before and after MB adsorption using Scanning Electron Microscopy and Fourier Transform Infrared Spectroscopy. The results showed optimal removal efficiency under neutral and alkaline conditions, as opposed to acidic environments. The Freundlich model accurately represents experimental adsorption data (R^2= 0.9902). Kinetic analysis revealed that MB adsorption onto *Padina* sp. primarily followed a pseudo-second order mechanism (R^2= 0.9982). The characterization and experimental findings suggest that the proposed mechanisms for MB adsorption by *Padina* sp. involve electrostatic interactions, pore filling, and hydrogen bonding. The experimental results suggest that powdered *Padina* sp. is a promising adsorbent for removing MB from water.

Keywords: Water pollution, adsorption, dye removal, bio-adsorbent, *Padina* sp.

Öz: Deniz ekosistemlerinde yaygın olarak bulunan makroalgler, yüksek yüzey alanları, zengin fonksiyonel grupları ve doğal adsorpsiyon kabiliyetleri nedeniyle su arıtımı için umut vadeden doğal adsorbanlardır. Bu çalışmada, kurutulmuş toz formunda kahverengi bir makroalg olan *Padina* sp.'nin sudan toksik metilen mavisi (MB) boyasının uzaklaştırılması için adsorpsiyon kapasitesi değerlendirilmiştir. Başlangıç MB konsantrasyonları (2, 5, 10, 20 ve 50 mg L^{-1}), pH seviyeleri (2, 4, 7 ve 11) ve temas süresi (0, 0.08, 0.5, 1, 2, 4, 6, and 24 saat) dahil olmak üzere çeşitli operasyonel faktörlerin MB eliminasyonu üzerindeki etkilerini incelemek için bir dizi adsorpsiyon testi yürütülmüştür. Ayrıca, toz haline getirilmiş *Padina* sp., MB adsorpsiyonundan önce ve sonra Fourier dönüşümlü kızılötesi spektroskopisi ve taramalı elektron mikroskobu kullanılarak analiz edilmiştir. Sonuçlar, asidik ortamların aksine, nötr ve alkali koşullar altında optimum bir giderme verimliliği olduğunu göstermiştir. Freundlich modeli, deneysel adsorpsiyon verilerini doğru bir şekilde temsil etmektedir (R^2 = 0.9902). Kinetik analiz, MB adsorpsiyonunun *Padina* sp. üzerine öncelikle psödo-ikinci dereceden bir mekanizmayı izlediğini ortaya koymuştur (R^2 = 0.9982). Karakterizasyon ve deneysel bulgulara dayanarak, *Padina* sp.'nin önerilen MB adsorpsiyon mekanizmaları arasında hidrojen bağı, elektrostatik etkileşimler ve gözenek doldurma yer almaktadır. Deneysel sonuçlar, toz halindeki *Padina* sp.'nin sudan MB'yi uzaklaştırmak için umut verici bir adsorban olduğunu göstermektedir.

Anahtar kelimeler: Su kirliliği, adsorpsiyon, boya giderimi, biyo-adsorban, *Padina* sp.

INTRODUCTION

Even at low levels, synthetic organic dyes are important industrial contaminants that seriously endanger human health, aquatic ecosystems, and environment (Benhouria et al., 2023; Saheed et al., 2021). Methylene blue (MB; 3,7-bis(dimethylamino) phenothiazine chloride tetra methylthionine chloride) is used for fabric dyeing in the textile industry and is also preferred for dyeing paper and leathers (Oladoye et al., 2022; Khodaie et al., 2013). MB is a cationic synthetic dye known for its toxic effects, including teratogenicity, mental confusion, nausea, hypertension, mutagenicity, neurotoxicity, and damage to nucleic acid (Martínez Cadena et al., 2025; Ucuncu et al., 2022; Ghosh et al., 2021). Due to the monoamine oxidase inhibitor properties of MB dye, doses exceeding 5 mg kg^{-1} can pose a threat to the fauna in aquatic ecosystems and may also cause fatal serotonin toxicity in humans (Oladoye et al., 2022). To prevent the harmful effects of this toxic feature of dyes on humans and all living organisms, wastewater must be properly treated.

Several methods for dye removal have been employed, including coagulation (Mcyotto et al., 2021), flocculation (Ihaddaden et al., 2022), oxidation (Bravo-Yumi et al., 2022), photodegradation (Gomes et al., 2023), ion exchange (Joseph et al., 2020), and membrane separation (Zhou et al., 2023). Nevertheless, these methods are difficult to carry out, and highly expensive (Benhouria et al., 2023). Adsorption with inexpensive biological materials is widely considered to be a successful method for treating water that contains dyes (Şenol et al., 2024; Şen and Şenol, 2023; Aragaw and Bogale, 2021). Biologically derived adsorbents, such as bacterial, fungal, and agricultural wastes, have been utilized extensively to treat diluted dye solutions (Tsoutsa et al., 2024; Hamad and Idrus, 2022; Eyupoglu et al., 2025). Among these, algae have emerged as particularly promising biosorbents, attributed to their rich functional groups, high binding capacity, and porous cell walls that allow for effective pollutant uptake (Sheng et al., 2004; Srinivasan and Viraraghavan, 2010). Additionally, algae are widely available and exhibit straightforward growth requirements, making them an attractive and sustainable option for water treatment applications (Aragaw and Bogale, 2021).

Macroalgae species (e.g., *Fucus spiralis, Ulva intestinalis, Corallina officinalis, Codium* sp.*, Padina sanctae-crucis*) are non-toxic, easily available, and provide effective results in the treatment of dye-contaminated waters (Boukarma et al., 2024; Şahin et al., 2024; Boukarma et al., 2023; Mahini et al., 2018). Algae of the class Phaeophyceae contain extracellular biopolymers, mainly alginic acid or alginate, which include small amounts of fucoidan that facilitate the transition of small ions (Vieira and Volesky, 2000). *Padina* sp., a macroalgae species from the Phaeophyceae class, is commonly found in intertidal to subtidal zones and is often referred to as sea fan ribbon weed (Ansari et al., 2019).

Previous studies have investigated both micro- and macroalgae as biosorbents for dye removal from aqueous solutions (Kousha et al., 2012; Omar et al., 2018; da Fontoura et al., 2017; Ucuncu et al., 2022). However, the direct application of *Padina* sp. as a biosorbent for MB dye remains relatively underexplored. While some studies have utilized *Padina* sp. in the removal of MB via the green synthesis of zinc oxide nanoparticles (Alprol et al., 2024; Pandimurugan and Thambidurai, 2016), its direct biosorption potential has not been comprehensively examined.

The primary aim of this work was to evaluate the ability of *Padina* sp*.,* biomass to adsorb MB dye from aqueous solution. Dye removal capacity of *Padina* sp. was assessed under varying conditions, including different dye concentrations (2, 5, 10, 20, and 50 mg L^{-1}), pH values (2, 4, 7, and 11), and contact time time (0, 0.08, 0.5, 1, 2, 4, 6, and 24 h). The intrinsic mechanisms involved in the adsorption were explained by means of Fourier Transform Infrared Spectroscopy (FT-IR), equilibrium isotherm models, Scanning Electron Microscopy (SEM), and kinetic models.

MATERIAL AND METHODS

Macroalgae

Padina sp. (family Dictyotaceae, class Phaeophyceae) was collected from Black Sea coast, (Ordu Province, Türkiye) (41°07'34.5"N 37°41'32.7"E). The collected *Padina* sp. has a light brown thallus with a slightly calcareous structure, shaped like a semicircle or circle.

After being taken from their natural habitat, the samples were cleaned in the laboratory using distilled water to get rid of any sand or other organisms. Following this, the samples were dried in an oven at 60 °C until their weight stayed constant (Ucuncu et al., 2022). Following grinding and sieving utilizing a 941 µm mesh sieve, dried macroalgae samples were used in tests.

Experimental design

MB, a cationic dye obtained from Pancreac®, was used in this study. Initial pH values of trials were adjusted to 2, 4, 7, and 11 utilizing 0.1 M NaOH and HCl. Using a stock dye solution, various dye concentrations (2, 5, 10, 20, and 50 mg L^{-1}) were prepared. Adsorbent dosage was adjusted at 0.1 g L^{-1} for all experimental groups. Erlenmeyer flasks containing 100 ml of dye solution were used for the experiments. The samples were shaken continuously at 80 rpm and 30 °C using a shaking culture incubator for different time (0, 0.08, 0.5, 1, 2, 4, 6, and 24 h). UV-vis spectroscopy was utilized to evaluate absorbance value of MB at 664 nm (Ucuncu et al., 2022; Türe, 2023). All the experiments were conducted in fresh distilled water to evaluate the adsorption performance of *Padina* sp.

Isotherm models

The adsorption capacity of *Padina* sp*.* was assessed utilizing the following equation (Ucuncu et al., 2022; Türe, 2023).

$$q_e = \frac{C_o - C_e}{m} x\, V \qquad (1)$$

While C_0 and C_e (mg L^{-1}) denote the initial and equilibrium concentrations of MB, respectively, q_e (mg g^{-1}) stands for the equilibrium adsorption capacity in this context. The dry weight of the adsorbent is indicated by *m* (g), and the volume of the test solution is *V* (L).

The Langmuir and Freundlich models were employed for identifying mechanisms involved in adsorption. Langmuir adsorption isotherm model was assessed using the following equation: (Ucuncu et al., 2022; Türe, 2023)

$$\frac{Ce}{qe} = \frac{1}{qmaxb} + \frac{1}{qmax} \qquad (2)$$

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

The following equation shows the Freundlich model, which may be applied to heterogeneous surfaces (Ucuncu et al., 2022; Türe, 2023):

$$ln\, q\, e = ln\, K\, f + \frac{1}{n} ln\, C\, e \qquad (3)$$

In this context, *n* and K_f (L mg^{-1}) are constants, which represent intensity and adsorption capacity, respectively.

Adsorption kinetics

Kinetics of adsorption were evaluated using 0.1 g algal powder with an initial MB concentration of 10 mg L^{-1}, contact time of 0 to 24 h, and pH of 8.0. Intra-particle diffusion and pseudo-first and pseudo-second order models were used to fit experimental data for explaining adsorption kinetics of MB on biomass (Sahu et al., 2021; Türe, 2023).

$$\ln(q_e - q_t) = lnq_e - k_1 t \qquad (4)$$

Here, k_1 represents the equilibrium rate constant of the pseudo-first order model (min^{-1}), while q_e and q_t indicate the amounts of MB adsorbed on the adsorbent (mg g^{-1}) at equilibrium and at time *t*, respectively.

The linear equation for the pseudo-second order kinetic model is shown in the following equation:

$$\frac{t}{q_t} = \frac{1}{k_2 {q_e}^2} + \frac{t}{q_e} \qquad (5)$$

where k_2 is the equilibrium rate constant (g mg^{-1} min^{-1}) for the pseudo-second order model.

The following is an expression for intra-particle diffusion:

$$q_t = k_{dif}\, t^{1/2} \qquad (6)$$

where k_{dif} is intra particle constant.

Characterization of the adsorbent

Using an FT-IR spectrometer (Bruker Tensor 27), the functional groups found on the surface of *Padina* sp. were investigated. Additionally, the surface morphology was investigated with a SEM (Hitachi SU 1510).

Statistical analysis

The analyses were performed twice. Following data analysis using ANOVA, Tukey's test was utilized to determine group average differences at a significance level of 0.05. OriginPro2024-Learning Version (Software, USA) was used for graphing and analysis.

RESULTS

Adsorption experiments results

Effect of pH

Figure 1 illustrates the effect of pH on MB uptake by *Padina* sp. at 30 °C. The most efficient removal was observed in neutral and basic media, in contrast to acidic pH. Furthermore, no significant differences were observed between pH values of 7 and 11, with a calculated q_e of approximately 9.88.

Effect of MB initial concentration

To investigate the effect of dye concentration, 0.1 g of *Padina* sp. underwent exposure to 0.1 L solutions of MB dye at concentrations of 2, 5, 10, 20, and 50 mg L^{-1}. The treatment was conducted at 30 °C and pH of 8 for 4 h (Figure 2). According to the findings, *Padina* sp. was able to remove more dye when the concentration of dye increased. Figures 3a and 3b show the linear fitting of the Langmuir and Freundlich models, respectively, to the equilibrium data. Table 1 lists the experimental constants for both models. The Freundlich model, as indicated by its high correlation coefficient (R^2=0.9902), offers a more accurate representation of the isotherm data when *Padina* sp. was utilized as an adsorbent (Figure 3b). Additionally, the values of K_F and *n* were greater than 1, which supports the suitability of the model for adsorption applications (Table 1) (Batool et al., 2018). The Langmuir constant q_{max} represents the maximum adsorption capacity and indicates that *Padina* sp. can adsorb up to 107.5 mg of MB per gram of adsorbent. The Langmuir constant, *b*, is related to the affinity of the adsorbent for the adsorbate, indicating good adsorption interactions (Table 1) (Ucuncu et al., 2022).

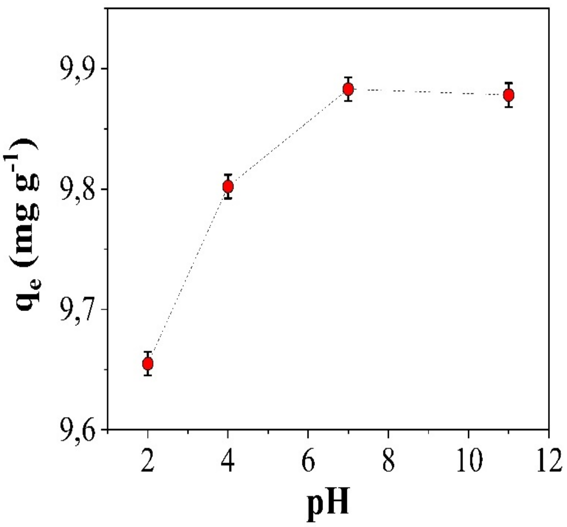

Figure 1. Effect of pH on MB adsorption capacity (q_e) of *Padina* sp. (Weight = 0.1 g, Volume = 0.10 L, initial MB dye level = 10 mg L^{-1}, temperature = 30 ˚C, and stirring rate = 80 rpm, contact time= 4 h)

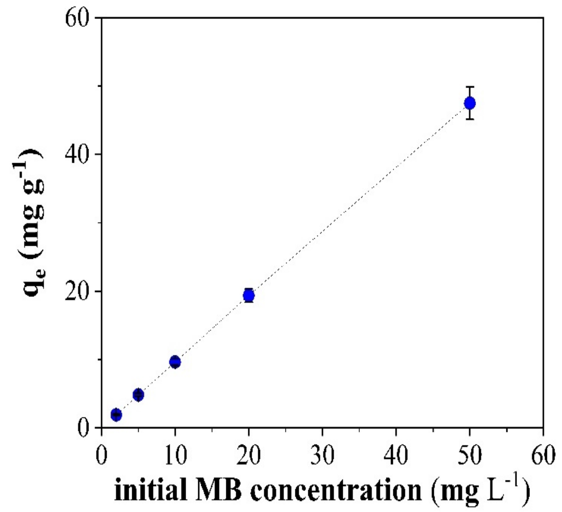

Figure 2. Effect of MB dye concentrations on adsorption capacity (q_e) of *Padina* sp. (Weight = 0.1 g, Volume = 0.10 L, temperature = 30 ˚C, and stirring rate = 80 rpm, contact time= 4 h, pH=8)

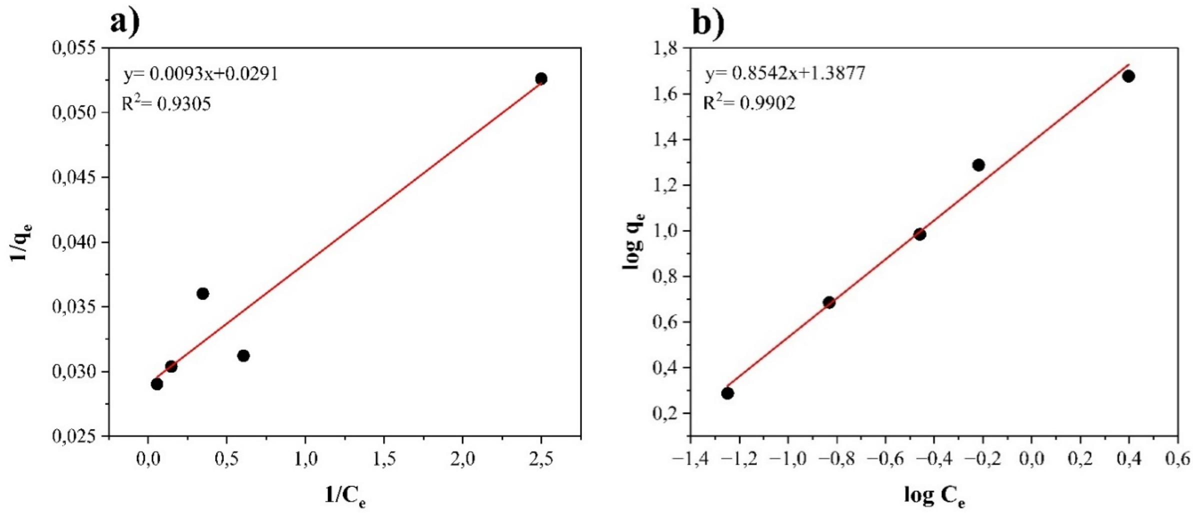

Figure 3. Linear fitting graphs of (a) Langmuir and (b) Freundlich, isotherm models

Table 1. Values of isotherm for MB adsorption on *Padina* sp.

Models	Parameters	Values
Langmuir	q_{max} (mg g^{-1})	107.5
	b (L mg^{-1})	0.32
Freundlich	K_F	24.42
	n	1.17

Effect of contact time

The effect of various contact times (0, 0.08, 0.5, 1, 2, 4, 6, and 24 h) on MB sorption yield was investigated at a MB concentration of 10 mg L^{-1}, pH 8, and 0.1 g L^{-1} of *Padina* sp. The findings, which are displayed in Figure 4, reveal that the removal efficiency increases with contact time up to 6 hours, at which point the sorption of MB onto *Padina* sp. has almost achieved equilibrium.

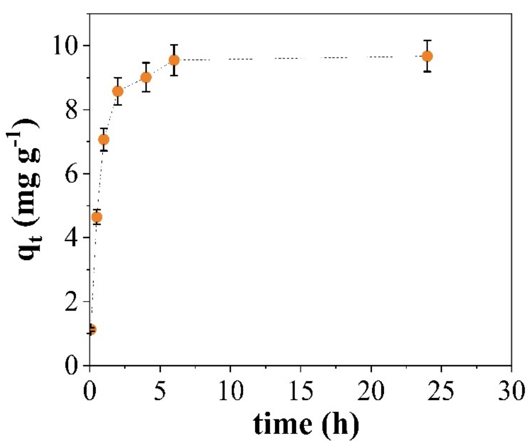

Figure 4. Effect of time on the biosorption of MB dye by *Padina* sp. (Weight = 0.1 g, Volume = 0.10 L, initial MB dye level = 10 mg L^{-1}, temperature = 30 °C, stirring rate = 80 rpm, pH=8)

Additionally, the adsorption kinetics were examined to obtain a deeper understanding of the kinematics of MB adsorption on *Padina* sp. The kinetic data was calculated using pseudo-first order (PFO) and pseudo-second order (PSO) models, which were derived from Eqns. (4) and (5). Using the linear plots in Figures 5a and 5b, the adsorption kinetic parameters for the PFO and PSO models were determined, and results are listed in Table 2. The PSO kinetics were found to offer the best correct explanation for the kinetic data, with an R^2 value of 0.9982. Furthermore, the measured adsorption capacity of 9.65 mg g^{-1} (Figure 4) and predicted q_e (10.5) obtained by plotting time vs. t/q_t on the PSO graph are quite comparable. Additionally, process design requires an understanding of an adsorption system's dynamic behavior. Removal of MB dye by adsorption on biomass was initially rapid but subsequently decelerated as the contact time increased.

Table 2. Values for kinetic model of MB adsorption on *Padina* sp.

Models	Parameters	Values
Pseudo-first order kinetic	$q_{e,\ calc}$ (mg g^{-1})	6.43
	k_1 (min^{-1})	9.2x10^{-5}
Pseudo-second order kinetic	$q_{e,\ calc}$ (mg g^{-1})	10.5
	k_2 (g mg^{-1} min^{-1})	1.2x10^{-3}

Based on intra-particle diffusion (IPD) process, which explains the sorption of MB from its aqueous phase, dye molecules move from the bulk solution to the surface of the sorbent, traversing the boundary layer to reach the surface where adsorption and IPD take place (Long et al., 2024). The intercept was C (1.94), and the slope directly predicted the rate constant K_{dif} (0.46), as shown in Figure 5c.

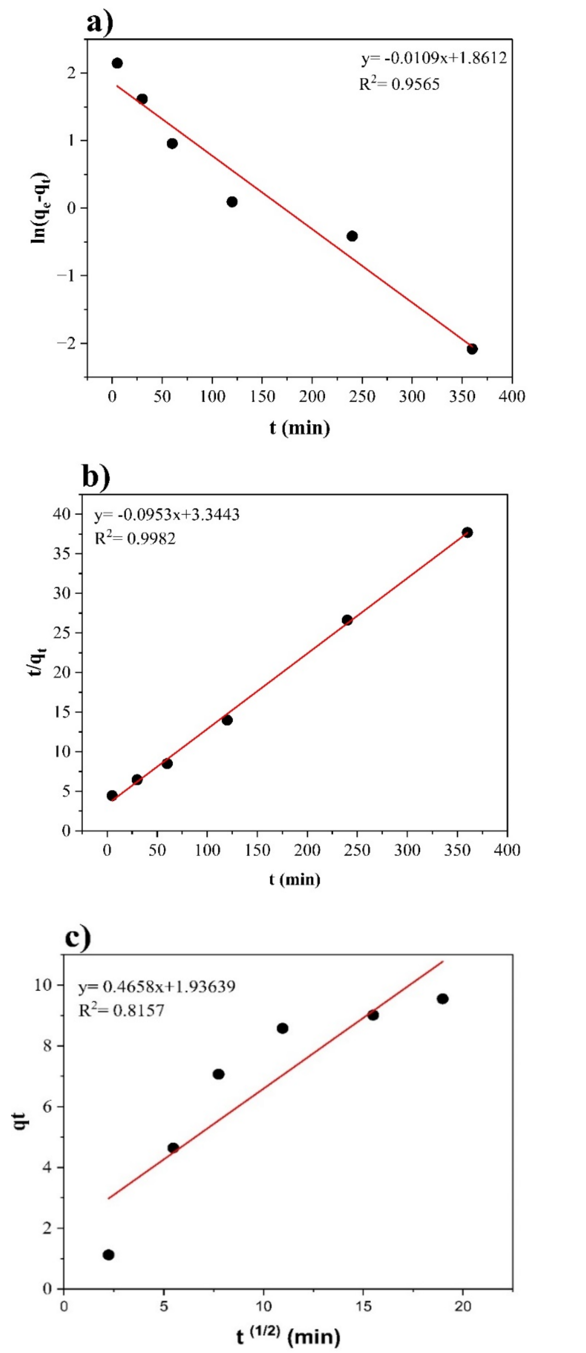

Figure 5. Adsorption kinetics of MB dye on *Padina* sp. (a) Pseudo-first order kinetic; (b) Pseudo-second order kinetic and (c) intra-particle diffusion models.

FT-IR analysis

The FT-IR spectra of *Padina* sp. biomass before and after MB removal are displayed in Figure 6. Broad peaks at 3291 and 3295 cm^{-1} show the existence of -OH groups, whereas C-H bond stretching is indicated by the peaks at 2920 and 2928 cm^{-1}. The Amide I band was attributed to the region between 1600 and 1690 cm^{-1}. The peaks at 1453 and 1458 cm^{-1} are associated with the symmetrical stretching of the C=O group and the bending vibration of the CH_2 and CH_3 groups in the production of carboxyl (-COOH). The peaks that show C-H stretching are at around 850 and 700 cm^{-1} (Seoane et al., 2022; Samar et al., 2022; Abdel-Raouf et al., 2019). Figure 6 shows that the biomass's FT-IR spectra either showed minor changes in the peaks corresponding to certain functional groups or were comparable before and after adsorption. This finding implies that the compound's main biosorption mechanism could be electrostatic attraction.

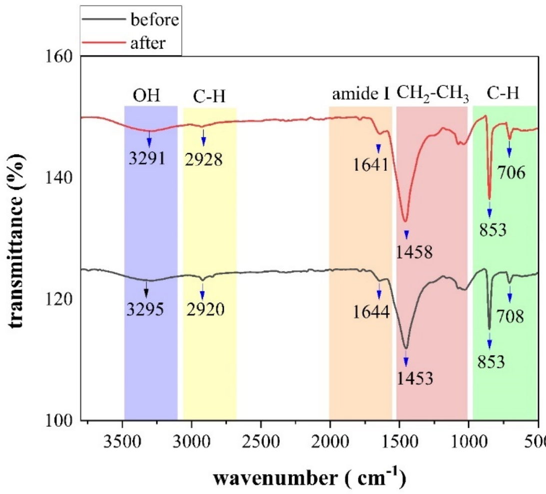

Figure 6. FT-IR spectra of *Padina* sp. before and after the removal process of MB (Weight = 0.1 g, Volume = 0.10 L, initial MB dye concentration = 10 mg L^{-1}, temperature = 30 °C, stirring rate = 80 rpm, contact time= 4 h, pH=8)

Surface morphology

Images of *Padina* sp. taken with SEM before and after dye absorption are depicted in Figure 7. As can be seen in Figure 7a, adsorbent's porosity structure is consistent. The adsorbent surface's pores may aid in the efficient absorption of MB dye. Following MB adsorption, the surface morphology underwent notable changes, and discrete aggregates formed on their surfaces (Figure 7b). Furthermore, the porous texture filled and disappeared when coming into contact with MB dye.

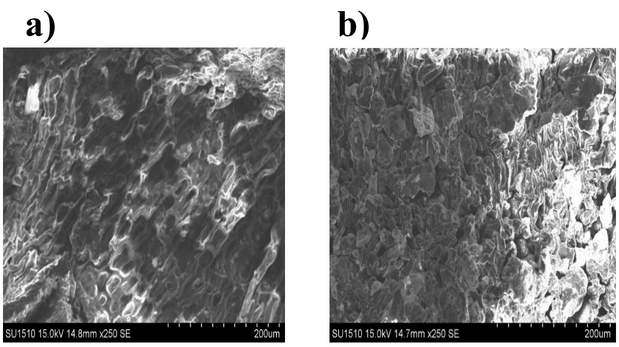

Figure 7. SEM image of *Padina* sp. (a) before and (b) after adsorption of MB dye. (Weight = 0.1 g, Volume = 0.10 L, initial MB dye concentration = 10 mg L^{-1}, temperature = 30 °C, stirring rate = 80 rpm, contact time= 4 h, pH=8)

DISCUSSION

Effect of pH

Because pH influences adsorbate's surface characteristics and degree of ionization, it is a crucial component in determining a material's adsorption capability (Benhouria et al., 2023). Both the functional groups of the dye and the macroalgal surface may be influenced by the pH of the dye solution (Chin et al., 2020). Due to the increased negative surface charge of biomass at higher pH value, which electrostatically attracts cationic dyes, efficient MB adsorption occurs on the surface of the adsorbent (Ali, 2010). Furthermore, the adsorption of MB may be explained by the strong interactions between adsorbate molecules (MB) and the several functional groups found in the phenols and flavonoids within the non-cellulosic cells of *Padina* sp. (Mansour et al., 2022). Similar patterns on how pH affects MB remediation in the aqueous phase have been documented in earlier research (Chin et al., 2020; Mansour et al., 2022).

Isotherm and kinetic models

The Langmuir and Freundlich models, two of the most widely used adsorption models, were chosen to fit the experimental findings (Zhu et al., 2018). The Langmuir isotherm model depicts an adsorbate-adsorbent system where the adsorption process is not distributed, the surface is homogenous, and each adsorbate molecule occupies a single site (Al-Trawneh et al., 2021). The Freundlich model, which validates solid phase's heterogeneity and rough surface properties, was also used to assess the experimental MB adsorption values. This isotherm highlights how surface-bonded and unbound molecules interact in the liquid state to produce sorbate molecules in many layers (Alobaidi and Alwared, 2023). The isotherm analysis indicated that the removal of MB by *Padina* sp. showed a stronger correlation with the Freundlich model. This illustrates how MB was adsorbed onto *Padina* sp. via a multilayer process on a surface that was heterogeneous. The MB biosorption value of n = 1.17 (>1) shows strong MB/biosorbent interactions (Alobaidi and Alwared, 2023). This finding supports the conclusion that the Freundlich model more effectively describes the use of marine algae adsorbents for MB removal. However, the Langmuir model's constant q_{max} can be used to compare the adsorption capacity of *Padina* sp. with other adsorbents because its R^2 (0.9305) value is comparable. The data shown in Table 3 indicate that the q_{max} values of MB dyes on *Padina* sp. are comparable to or higher than those of the other adsorbents.

Figure 4 demonstrates that the adsorbent's MB adsorption rate is quick in the first phase of the experiment. This result can be attributed to the active sites on its surface and a greater concentration of MB molecules in the solution that can easily reach the adsorbent's surface. Over time, however, the rate of MB biosorption slowed because there were fewer active sites available on the *Padina* sp. surface. This typical tendency of decreasing biosorption effectiveness as the adsorbent and adsorbate's contact duration increases has been revealed by numerous studies (Long et al., 2024; Sahu et al., 2021).

The removal rate at the solid-liquid interface that establishes the adsorbate's retention time is known as adsorption kinetics (Pandey et al., 2010). The structure of Figure 5c, which illustrates that straight lines do not pass through origin, indicates that IPD is not the only rate-controlling step; other processes, such as boundary layer mass transfer and contact, also influence the adsorption process (Long et al., 2024).

Table 3. Comparison of various adsorbents utilized for the removal of MB

Adsorbent	q_{max} (mg g^{-1})	Reference
Chlorella vulgaris	10.142	Chin et al. (2020)
Chlamydomonas moewusii	212.41	Seoane et al. (2022)
Treptacantha barbata	73.53	Ucuncu et al. (2022)
Sargassum latifolium	0.819	Mansour et al. (2022)
Sargassum ilicifolium	99.7	Tabaraki and Sadeghinejad (2017)
Ulva lactuca	10.99	El Sikaily et al. (2006)
Rice husk	8.07	Shih (2012)
Glass fibres	2.24	Chakrabarti and Dutta (2005)
Padina sp.	107.5	This study

Mechanism of MB on *Padina* sp.

The way that a pollutant is adsorbed onto a particular material depends on nature of adsorbate, properties of adsorbent, and possible interactions between them (Türe, 2023). Figure 8 shows a model of MB adsorption. According to pseudo-second order kinetic model, adsorption between *Padina* sp. and MB was hypothesized to be chemical. However, the absence of significant differences observed in the FT-IR analysis between *Padina* sp. before and after dye adsorption suggests that classical chemical bonds (such as covalent or ionic bonds) may not be present. In this case, adsorption may occur through weak chemical interactions or strong physical interactions. Several possibilities may explain this phenomenon: MB is a positively charged dye, and its binding is facilitated by negative charges, which are provided by a variety of components primarily found on the cell surface of macroalgal biomass (Pandey et al., 2010). This characteristic may be one of the factors contributing to the suitability of macroalgal biomass for biosorption of cationic sorbates. These electrostatic attractions can conform to pseudo-second order kinetics but might not demonstrate distinct chemical changes in FT-IR. Groups such as -OH in *Padina* sp. can form weak hydrogen bonds with the functional groups in MB. These hydrogen bonds are considered to be more physical interactions than strong chemical bonds. Such interactions may not be detectable by FT-IR or produce very weak signals. Additionally, a comparison of the SEM images taken before and after adsorption showed that the adsorbed *Padina* sp. had a rougher surface, which could be a sign that

MB molecules had partially filled the pores. When the algal cell surface interacted with MB, its porous texture changed to an uneven shape. Mansour et al. (2022) also noted similar results when *Sargassum latifolium* was utilized as an adsorbent to remove MB from water.

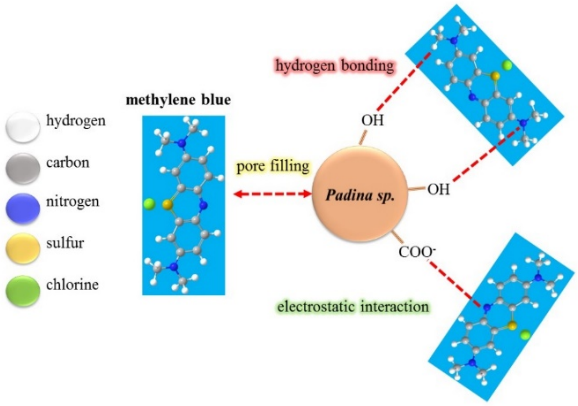

Figure 8. The suggested MB adsorption process on *Padina* sp.

CONCLUSION

In this work, MB was removed from aqueous solutions using the macroalgae *Padina* sp. as an adsorbent. The results demonstrate that *Padina* sp. is effective as a natural sorbent for eliminating cationic dyes. The isotherm analysis revealed that MB removal by *Padina* sp. aligned more closely with Freundlich model, suggesting multilayer adsorption during experiments. The pseudo-second order model provided the best explanation for the kinetic data for MB adsorption. Additionally, an investigation of adsorption mechanism identified hydrogen bonding, electrostatic interactions, and pore filling as the primary factors contributing to the significantly enhanced adsorption capacity. These findings indicate that the dried powder of this macroalga serves as an efficient biosorbent, making it highly suitable for elimination of MB dye from water. This study demonstrates the potential of natural adsorbents to address water contamination issues and contribute to the development of green technologies for environmental remediation. The regeneration and reusability of *Padina* sp. as an adsorbent can be further studied to increase its applicability in large-scale wastewater treatment applications.

ACKNOWLEDGMENTS AND FUNDING

No grant, money, or other assistance from a governmental, private, or nonprofit organization was obtained for this study.

AUTORSHIP CONTRIBUTIONS

Hasan Türe contributed through conceptualization, drafting, writing, reviewing, editing, and analyzing data and supervision. Pınar Akdoğan Şirin was responsible for the collecting of the samples and conducting laboratory experiments. The final version of the work has been reviewed and approved by all authors.

CONFLICT OF INTEREST

The authors claim that there are no conflicts of interest.

ETHICS APPROVAL

For this investigation, no ethical approval was needed.

DATA AVAILABILITY

The relevant author should be contacted with any queries about the datasets.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 42(2), 131-137 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.05

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Assessment of bathing water quality in the Sea of Marmara (2022-2024) and recommendations

Marmara Denizi'nde yüzme suyu kalitesinin değerlendirilmesi (2022-2024) ve öneriler

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Received date: 02.03.2025 *Accepted date: 07.04.2025*

How to cite this paper:
Zeki, S. (2025). Assessment of bathing water quality in the Sea of Marmara (2022-2024) and recommendations. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 131-137. https://doi.org/10.12714/egejfas.42.2.05

Abstract: The Sea of Marmara, a semi-enclosed inland sea in Türkiye, has a naturally sensitive ecosystem increasingly impacted by anthropogenic pressures. This study evaluated the bathing water quality along the Sea of Marmara, including the Straits of İstanbul and Çanakkale, and provided recommendations for enhancing the dissemination of monitoring results. Bathing water quality data from 176 sites, monitored by the Turkish Ministry of Health, were analyzed over three consecutive bathing seasons (2022-2024). The results indicate that in 2024, 66% of the monitored beaches were classified as excellent, a proportion lower than the European Union average. Furthermore, only 24% of those beaches received the Blue Flag certification. Spatial analysis revealed distinct provincial disparities, with Balıkesir and Çanakkale exhibiting the highest bathing water quality, whereas Yalova and Bursa recorded the poorest conditions, with roughly a quarter of their bathing waters classified as poor quality. These findings underscore the need for targeted interventions to improve bathing water quality, particularly in provinces with lower classifications, and to align Türkiye's bathing water standards more closely with EU benchmarks. In addition, the study emphasizes the importance of improving public access to monitoring results. Developing comprehensive national and regional reports, including data on beach closures, is recommended to enhance public awareness and policymaking. Such implementations are crucial for not only protecting public health but also facilitating targeted interventions, promoting sustainable beach management, and fostering tourism development.

Keywords: Bathing water quality, Blue Flag, fecal indicator bacteria, Sea of Marmara

Öz: Türkiye'de yarı kapalı bir iç deniz olan Marmara Denizi, antropojenik baskılardan giderek daha fazla etkilenen doğal olarak hassas bir ekosisteme sahiptir. Bu çalışma, İstanbul ve Çanakkale Boğazlarını da kapsayacak şekilde Marmara Denizi'nde yüzme suyu kalitesini değerlendirmiş ve izleme sonuçlarının yaygınlaştırılması için öneriler sunmuştur. Türkiye Sağlık Bakanlığı tarafından izlenen 176 yüzme alanının verileri, birbirini takip eden üç yüzme sezonu (2022-2024) boyunca analiz edilmiştir. Sonuçlarımız, izlenen plajların %66'sının 2024 yılında yüzme suyu kalitesi açısından mükemmel olarak sınıflandırıldığını ve bu oranın Avrupa Birliği ortalamasından daha düşük olduğunu göstermektedir. Ayrıca, bu plajların sadece %24'ü Mavi Bayrak sertifikası almıştır. Mekânsal analiz, iller arasında belirgin farklılıklar olduğunu ortaya koymuştur. Balıkesir ve Çanakkale en yüksek yüzme suyu kalitesini sergilerken, Yalova ve Bursa yüzme sularının yaklaşık dörtte birinin kötü kalite olarak sınıflandırılmasıyla en kötü koşulları kaydetmiştir. Bu bulgular, özellikle düşük sınıflandırmaya sahip illerde yüzme suyu kalitesini iyileştirmek ve Türkiye'nin yüzme suyu kalitesini AB ile daha uyumlu hale getirmek için hedefe yönelik müdahalelere duyulan ihtiyacı göstermektedir. Buna ek olarak, çalışmamız izleme sonuçlarının kamuoyuna daha iyi duyurulmasının önemini vurgulamaktadır. Politika yapıcıları ve kamuoyunu yüzme suyu kalitesi hakkında daha iyi bilgilendirmek için, plajların kapatılmasına ilişkin veriler de dahil olmak üzere, kapsamlı ulusal ve bölgesel raporların hazırlanması önerilmektedir. Bu tür uygulamalar sadece halk sağlığını korumak için değil, aynı zamanda hedefe yönelik müdahaleleri kolaylaştırmak, sürdürülebilir plaj yönetimini teşvik etmek ve turizm gelişimini desteklemek için de çok önemlidir.

Anahtar kelimeler: Yüzme suyu kalitesi, Mavi Bayrak, fekal indikatör bakteriler, Marmara Denizi

INTRODUCTION

Water sports such as swimming, diving, rowing, and fishing are popular recreational activities, especially during the summer months, in both marine and freshwater ecosystems. Ensuring the safe conduct of these activities requires maintaining high water quality standards. However, these aquatic environments are often contaminated with microorganisms that pose risks to human health (World Health Organization, 2021). Exposure to microbiologically contaminated waters is linked to an increased risk of gastrointestinal diseases, skin infections, and other pathogen-induced infections (Fewtrell and Kay, 2015; Leonard et al., 2018; Wade et al., 2022). Studies estimate that exposure to contaminated recreational waters causes 90 million illnesses annually in the United States, with an economic burden of approximately $2.9 billion (DeFlorio-Barker et al., 2017). Therefore, regular monitoring of recreational waters and maintaining safe microbial water quality are essential for protecting public health. Additionally, these measures support ecosystem sustainability and provide economic benefits for tourism and local communities (Devane et al., 2020; Holcomb and Stewart, 2020).

Microbial water quality assessment primarily relies on fecal indicator organisms (FIOs), including bacteria, viruses, or other microorganisms that signal potential fecal contamination. Elevated FIO levels indicate a higher risk of pathogenic microorganisms. Currently, *Escherichia coli* (*E. coli*) and intestinal enterococci are the most widely used indicators for microbial contamination in both freshwater and marine environments (Tiwari et al., 2021; World Health Organization, 2021). The classification of microbial water quality based on these indicators varies by national standards and regulations. In the European Union (EU), bathing waters are monitored and classified under the Bathing Water Directive (European Commission, 2006). Similarly, in the United States, the Environmental Protection Agency (EPA) enforces microbial water quality standards through the Recreational Water Quality Criteria (USEPA, 2012) to safeguard public health. In Türkiye, microbial water quality is regulated by the Regulation on the Management of Bathing Water Quality (RMBWQ) (2019), which aligns with the EU Bathing Water Directive. This regulation replaced total coliform and fecal coliform analysis with *E. coli* analysis, improving the precision of bathing water quality assessments. The Ministry of Health of Türkiye (MoH) conducts water quality monitoring and publishes results online via the Bathing Water Monitoring System (https://yuzme.saglik.gov.tr). Meanwhile, the Ministry of Environment, Urbanization and Climate Change of Türkiye (MoEUCC) is responsible for establishing bathing water profiles, which are available through the Bathing Area Information System (https://plaj.csb.gov.tr).

Another national regulation governing the microbial water quality classification is the Regulation on Surface Water Quality (2012), which defines the microbial standards for coastal and transitional waters used for recreational purposes. Additionally, the Blue Flag Program complements national regulations by promoting high bathing water quality standards in Türkiye. The Blue Flag is an international environmental award granted to beaches, marinas, and tourism boats that meet strict environmental criteria. Managed globally by the Foundation for Environmental Education (FEE) and represented in Türkiye by the Turkish Environmental Education Foundation (TÜRÇEV). The program has been implemented in Türkiye since 1993, leading to a steady increase in the number of qualifying beaches. The program consists of 33 criteria, covering environmental education, bathing water quality, environmental management, and safety. Among these, Criterion 10 mandates that microbiological parameters influencing bathing water quality remain within specified limits (TÜRÇEV, 2019). Together, these regulations and programs ensure that bathing waters in Türkiye remain safe for public health and environmentally sustainable.

Türkiye has a total coastline of 8,592 km along the Mediterranean, Aegean, Marmara, and Black Seas (excluding islands), providing numerous bathing water areas essential for tourism and recreation. The Sea of Marmara accounts for approximately 17% of this coastline, extending 1,474 km (Uzun and Celik, 2014). Over the past decade, the ecological condition of the Sea of Marmara has deteriorated significantly due to increasing human activities, pollution, and climate change. Inputs from domestic and industrial wastewater, riverine pollution, and agricultural runoff, have negatively impacted microbial water quality (Akoglu et al., 2024; Demirel et al., 2023; İşinibilir et al., 2024). Given the fragile nature of this ecosystem, monitoring of bathing waters is crucial for both public health and environmental sustainability. However, academic studies on the microbial water quality of the Sea of Marmara remain limited, focusing mainly on the Golden Horn (Çelik and Zeki, 2024; Karabas et al., 2018; Zeki et al., 2021), the southwest coast of İstanbul (Altuğ and Hulyar, 2020; Sönmez and Sivri, 2022), and the Prince's Islands (Karaman Baş and Altuğ, 2022). While nationwide bathing water quality monitoring is conducted annually during the bathing season, the collected data are not comprehensively evaluated, and country- and region-specific reports are either not prepared or not effectively communicated to the public. In this context, the objectives of this study were (1) to evaluate the bathing water quality in the Sea of Marmara from 2022 to 2024 and (2) to provide recommendations for enhancing the transparency and effectiveness of bathing water quality monitoring in Türkiye.

MATERIALS AND METHODS

Study area

This study focuses on the Sea of Marmara, the Strait of İstanbul, and the Strait of Çanakkale, collectively forming the Turkish Straits System. The annual microbial water quality status for the years 2022, 2023, and 2024 was assessed for a total of 176 bathing areas along the Sea of Marmara coast, monitored by the Turkish MoH during the bathing season (Figure 1). Lakes in the Marmara Basin were not included in the study.

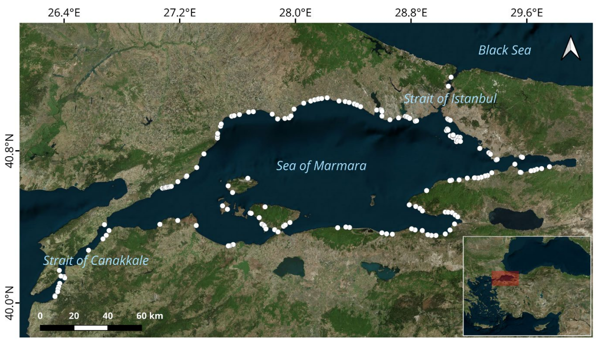

Figure 1. Study area and monitored bathing areas along the Turkish Strait System coast

Data compilation and analysis (2022-2024)

A list of monitored bathing areas along the Sea of Marmara, including Tekirdağ, İstanbul, Kocaeli, Yalova, Bursa, Balıkesir, and Çanakkale, was compiled from the Bathing Water Monitoring System of the MoH (https://yuzme.saglik.gov.tr). From the same system, annual water quality classification results were obtained for 176 beaches for the years 2022, 2023, and 2024, categorized as excellent, good, sufficient, or poor (Table S1). These classifications are determined by the MoH according to the RMBWQ (2019) as outlined in Table 1. This assessment is carried out at the end of the bathing season, using the data collected for that bathing season and the previous three. Waters exceeding the threshold for sufficient quality (Table 1) are classified as poor. Bathing areas that are added to the monitoring program later, without a continuous data set for four consecutive years, are not classified and are defined as new.

Table 1. Quality criteria and classifications for coastal and transitional waters under RMBWQ (2019)

	Water Quality Classification		
Indicator (CFU or MPN/100 ml)	Excellent	Good	Sufficient
E. coli	250*	500*	500**
Intestinal enterococci	100*	200*	185**

*Based on a 95^{th}-percentile evaluation. Upper 95-percentile = antilog (μ+1,65 σ)
**Based on a 90^{th}-percentile evaluation. Upper 90-percentile = antilog (μ+ 1,282 σ)
- μ is arithmetic mean of the log10 values and σ is standard deviation of the log10 values

In order to analyze temporal and spatial variations in bathing water quality, water quality data were first grouped by year and then by province, with the frequency of each quality category (excellent, good, sufficient, poor, and new) recorded accordingly. To examine associations between categorical variables, Pearson's Chi-Square Test was used to assess whether water quality classifications were independent of year and province. All statistical tests were performed at a significant level of $p<0.05$.

To evaluate changes in bathing water quality, we compared the classifications between 2022 and 2024 to determine whether individual sites showed improvement or degradation. If water quality remained consistent between 2022 and 2024, it was classified as unchanged, regardless of fluctuation in 2023. If a change in water quality was observed between 2022 and 2024, it was classified as either improved or degraded (Table S1). In addition, Blue Flag awarded beaches were identified through the Bathing Water Monitoring System and verified on the Blue Flag Türkiye website (Blue Flag Türkiye, n.d.). These beaches were then compared with the relevant water quality classifications to verify compliance with the Blue Flag criteria, which require excellent water quality status, and to identify any discrepancies.

RESULTS

Quality of bathing water in the Sea of Marmara: 2022-2024

Of the 1501 bathing waters monitored by the MoH Provincial Directorates of Health, 176 are located along the Sea of Marmara, including the Strait of İstanbul and the Strait of Çanakkale. These waters are assessed annually for microbial water quality during the bathing season. While the number of monitored beaches in the Sea of Marmara remained 176 in 2022 and 2023, it decreased to 174 in 2024. A review of the dataset revealed that "Yenice Marmara Sahil Sitesi" and "Eriklice Köyü Halk Plajı" in Tekirdağ, previously included in the monitoring, were excluded from the 2024 dataset.

The analysis of bathing water quality (2022–2024) showed a significant association between water quality classification and year (χ^2=15.94 and $p<0.05$), with an increase in the number of bathing areas classified as excellent. However, while the number of poor quality bathing areas remained stable in 2022 and 2023, it increased to 15 in 2024 (Figure 2). The proportion of bathing areas with excellent water quality ranged from 53% to 66%, which is relatively low compared to the European Union (EU) average.

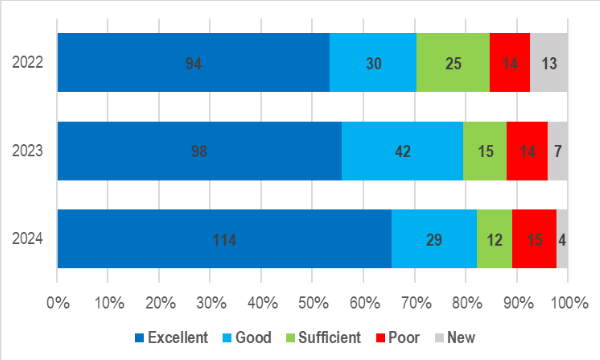

Figure 2. Bathing water quality status of the Sea of Marmara between 2022 and 2024. The numbers inside the boxes represent the absolute counts (n), n=176 for 2022 and 2023, and n=174 for 2024

In 2024, İstanbul had the highest number of monitored bathing waters (53), followed by Tekirdağ (30), Balıkesir (22), Bursa (20), and Yalova and Çanakkale (18 each). Kocaeli had the lowest number, with 13 monitored bathing waters (Figure 3). These variations likely reflect differences in coastline length and tourism potential. A significant association was found between water quality classification and province (χ^2 = 49.14 and $p < 0.05$). Balıkesir had the highest proportion of beaches with excellent water quality (95%), followed by Çanakkale (83%) and Kocaeli (69%). İstanbul (68%) and Tekirdağ (67%) represented the average level of water quality in the Sea of Marmara. In contrast, Yalova (28%) and Bursa (25%) exhibited the lowest bathing water quality, with 10 beaches classified as poor in 2024 (Figure 3).

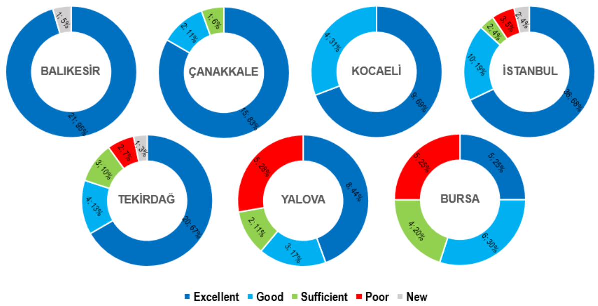

Figure 3. Bathing water quality status of the Sea of Marmara provinces in 2024. The first numbers represent the absolute counts (n); the second values indicate the relative proportion of each category as a percentage (%)

For the 2024 season, the number of samples collected per province ranged from 7 to 10, in accordance with regulatory criteria. The province with the highest number of samples during the season was Kocaeli with 10 samples, followed by Balıkesir with 9, and Yalova with 8. İstanbul and Bursa exhibited the lowest number of samples, with 7 each. In Tekirdağ and Çanakkale, the number of samples varies between 7 and 8. Among provinces, Kocaeli demonstrated the most consistent monitoring with two samples collected per month from May to September, ensuring a comprehensive assessment of bathing water quality.

An evaluation of bathing water quality changes over a three-year period revealed that water quality improved at 30 sites (18 in İstanbul, 4 in Bursa, 3 in Tekirdağ, 3 in Kocaeli, and 2 in Yalova), deteriorated at 14 sites (5 in Bursa, 3 in Tekirdağ, 3 in Yalova, 2 in İstanbul, and 1 in Çanakkale), and remained unchanged at 129 bathing areas. Among the provinces, İstanbul demonstrated the most significant improvement, with 18 beaches showing enhanced water quality. In contrast, Bursa experienced the highest level of degradation, with five beaches exhibiting a decline in microbial water quality (Table S1).

Blue Flag status in the Sea of Marmara: 2024

In 2024, a total of 567 beaches, 27 marinas, 9 individual yachts, and 18 tourism boats in Türkiye were awarded the Blue Flag. In this context, Türkiye ranks third in terms of the number of Blue Flag awarded beaches among the 51 countries participating in the Blue Flag Program, following Spain (639 beaches) and Greece (583 beaches) (Blue Flag, n.d.). Accordingly, 38% (567/1501) of the beaches that have their bathing water quality monitored in Türkiye have received the Blue Flag, with 27 of these located along the coast of the Sea of Marmara.

An analysis of Blue Flag beaches by province showed that Balıkesir and Tekirdağ led with eight Blue Flag beaches each. However, when considering the ratio of Blue Flag beaches to the total monitored beaches, Balıkesir and Çanakkale demonstrate the highest percentages of 36% and 28%, respectively (Table 2).

Table 2. The number and ratio of Blue Flag awarded beaches by province

	BF awarded beach numbers	Total beach numbers	BF awarded beach ratio
Balıkesir	8	22	36%
Tekirdağ	8	30	27%
Çanakkale	5	18	28%
Kocaeli	2	13	15%
Bursa	2	20	10%
İstanbul	1	53	2%
Yalova	1	18	6%
Sea of Marmara	27	174	16%

Of the 27 Blue Flag beaches in the Sea of Marmara, 26 demonstrated excellent water quality. However, Çanakkale Municipality Güzelyalı Public Beach was classified as good rather than excellent for the period 2022–2024.

DISCUSSION

This study evaluated the bathing water quality of the Sea of Marmara from 2022 to 2024, highlighting spatial and temporal trends, possible sources of contamination, and monitoring practices. The results showed that the proportion of bathing areas classified as excellent remained relatively low compared to the EU average, with values ranging between 53% and 66%. In contrast, the percentage of bathing areas with poor water quality has averaged 8% over the past three years, significantly exceeding the EU's 2023 average of 1.5% (European Environment Agency, 2024). The highest rates of poor water quality were recorded in Bursa and Yalova, where 10 beaches failed to meet the sufficient quality standard in 2024. Additionally, monitoring frequency varied between provinces, suggesting a lack of standardization in sampling practices.

The omission of two bathing areas from the 2024 dataset, may be due to a technical error in data entry. However, if no such error exists, the historical data for these beaches should remain accessible in the Bathing Water Monitoring System, along with an explanation for their removal to ensure transparency in the monitoring program.

In 2023, 85% of EU beaches met the excellent water quality standard (European Environment Agency, 2024). Notably, the bathing water quality of the Sea of Marmara (Figure 2) is more comparable to that of Poland (54.9%), Hungary (62.5%), and Estonia (66.2%) which recorded some of the lowest values among EU countries. However, it is important to note that assessments in these EU countries include both marine and inland waters, whereas the present study focuses solely on marine bathing areas. For the 2024 bathing season, 15 sites were classified as having poor water quality, 10 of which are located in Bursa and Yalova (Figure 2 and 3). Of these sites, 80% (12/15) are located near river mouths, while 20% (3/15) are close to piers or fishing ports. This suggests that microbial pollution originates primarily from riverine inputs or port activities. Previous studies have identified rivers (Basili et al., 2021; Bruschi et al., 2021; Ferrarin et al., 2021; Kataržytė et al., 2019) and port activities (Kraus et al., 2022) as potential contamination sources for bathing waters. Based on these findings, efforts to improve bathing water quality in the Sea of Marmara should prioritize Bursa and Yalova, followed by Tekirdağ and İstanbul (Figure 3).

The RMBWQ (2019) sets the number of samplings to be taken from bathing areas during the season at a minimum of five, including one sampling 15 days before the start of the monitoring calendar. Although the number of samples taken is sufficient for all provinces in the SoM according to the RMBWQ, the comparability of the results and the consistency of the assessment will be increased if the total number of samples taken for bathing areas in the same basin and under similar climatic conditions is as high and as equal as possible.

This study identified both improvements and deterioration in water quality across different locations. The measures implemented in İstanbul bathing areas, where the highest number of improvements in microbial water quality were observed, can serve as examples of best practices. In contrast, for bathing areas where degradation occurred, the relevant authorities from the MoH and the MoEUCC should ensure that an environmental assessment is conducted for these bathing areas within their operational areas where water quality has deteriorated, identify the factors contributing to this deterioration, and implement the necessary measures to minimize it.

Türkiye effectively implements regular monitoring of bathing waters. Despite this, there is a need for further efforts in disseminating monitoring results and related activities to ensure public awareness and engagement. As stated in the RMBWQ (2019), one of the objectives of the regulation is to inform the public about bathing water quality. For instance, a study from the UK reported that most beachgoers were unfamiliar with available water quality information (Quilliam et al., 2019). Similarly, other studies on beachgoers in Georgia, USA, found that 36.1% of visitors were unaware of regular water quality monitoring, and 64.7% considered the existing signage insufficient (Aslan et al., 2023; Jones et al., 2024). These examples highlight the common challenge of public awareness regarding water quality, which is also relevant to the Turkish context. A survey conducted in Türkiye as part of the Updating Swimming Water Profiles project reported that 73.2% of bathing sites have sufficient warning and informational signage, according to institutional representatives from relevant institutions (Yüksek et al., 2021). While this rate appears relatively high compared to findings from other studies, it is important to note that the survey responses came from institutional representatives rather than the general public. Another critical gap in public communication concerns short-term pollution events and beach closures. Currently, information regarding short-term pollution and beach closures is not available in the Bathing Water Monitoring System nor it reported. This highlights a gap in accessibility to real-time updates for beachgoers.

The limit values set for microbiological parameters in the Blue Flag Criteria Guide for Türkiye (TÜRÇEV, 2019) align with the excellent water quality criteria of the RMBWQ (Table 1). Similarly, in EU countries that implement the Blue Flag Program, applicant beaches must be classified as having excellent water quality to be awarded the Blue Flag (Foundation for Environmental Education, 2024). In light of these standards, the Blue Flag status of Güzelyalı Public Beach in Çanakkale should be re-evaluated, as it has maintained only good water quality over the past three years rather than excellent.

In a study conducted in the European Western Mediterranean Basin, Blue Flag beaches have significantly cleaner waters than non-Blue Flag beaches (Merino and Prats, 2022). However, in contrast to these findings, in the Sea of Marmara, 59 beaches, in addition to the 26 already awarded the Blue Flag, have maintained excellent water quality over the past three years. These beaches should be encouraged to meet the additional criteria for Blue Flag certification and apply for the designation. Expanding the number of Blue Flag beaches in the Sea of Marmara would further contribute to sustainable beach management, as previous studies have demonstrated that Blue Flag certification promotes tourism (Castillo-Manzano et al., 2021) and environmentally responsible coastal practices (Albaladejo-García and Zabala, 2023; Merino and Prats, 2020).

CONCLUSION

This study evaluated the bathing water quality of the Sea of Marmara for the period 2022–2024 and provided a summary of the current status of bathing waters and their monitoring practices. The results revealed that the proportion of bathing areas with excellent water quality in the Sea of Marmara lagged behind the European Union average of 85%. In the regional assessment, Balıkesir and Çanakkale provinces have a high percentage of beaches with excellent water quality, while Bursa and Yalova have significantly higher rates of poor water quality. Additionally, discrepancies in the number of samples collected per province suggest a lack of standardization in monitoring practices. In the context of the Blue Flag Program, 16% of the monitored beaches in the Sea of Marmara were found to have received Blue Flag certification. However, the criteria compliance should be reviewed at Güzelyalı Public Beach in Çanakkale.

In line with the findings of this study, the following recommendations are proposed to enhance the transparency and effectiveness of bathing water quality monitoring in the Sea of Marmara and Türkiye. Firstly, it is necessary to improve public access to water quality information by ensuring clear dissemination of monitoring results and potential health risks. In this way, public engagement must be encouraged by enabling citizens to verify monitoring activities and requesting additional water quality analyses when needed. In addition, it is important to prepare annual regional and national reports summarizing the monitoring results, including beach closure information during the relevant bathing season. These recommendations aim to ensure the transparent management of bathing water quality and improve public access to information. Finally, it is also important to increase training and awareness programs for bathing area managers, health officials, and relevant institutions to improve consistency and quality in monitoring.

Implementation of all these steps will significantly contribute to better bathing water management, protecting public health and contributing positively to tourism and the local economy in the Sea of Marmara.

ACKNOWLEDGEMENTS AND FUNDING

The author would like to thank Zeynep Akdoğan for her contribution to data acquisition, as well as Prof. Dr. Nazli Demirel and Assoc. Prof. Dr. Fuat Dursun for their valuable feedback on this paper. This study did not receive any financial support.

AUTHORSHIP CONTRIBUTIONS

Sibel Zeki: Conceptualization, Methodology, Investigation, Visualization, Methodology, Data Curation, Writing-Original Draft Preparation, Writing-Reviewing and Editing.

CONFLICT OF INTEREST

The author declares no competing interests.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY

The data sets generated for the present study are available from the corresponding author upon reasonable request.

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Table S1. Annual water quality classification of 176 beaches for the years 2022, 2023, and 2024 in the Sea of Marmara

Name of Bathing Area	District/Province	Coordinates	Blue Flag	Bathing Water Quality			Water quality change from 2022 to 2024	Sample number in 2024
				2022	2023	2024		
Kovalık Mevki Halk Plajı	Şarköy/Tekirdağ	40.605164, 27.080686		Excellent	Excellent	Excellent	unchanged	8
Marmara Evleri Önü 19 Halk Plajı	Şarköy/Tekirdağ	40.606666, 27.094078		Excellent	Excellent	Excellent	unchanged	8
Süleyman Altınok Halk Palјı	Şarköy/Tekirdağ	40.606806, 27.094926	yes	Excellent	Excellent	Excellent	unchanged	8
Belediye Sosyal Tesisleri Önü	Şarköy/Tekirdağ	40.607799, 27.099890	yes	Excellent	Excellent	Excellent	unchanged	8
Tekirdağ B.B Atatürk Parkı Halk Plajı	Şarköy/Tekirdağ	40.608693, 27.104240	yes	Excellent	Excellent	Excellent	unchanged	8
Marmara Evleri Önü	Şarköy/Tekirdağ	40.609714, 27.107537	yes	Excellent	Excellent	Excellent	unchanged	8
Tekirağ B.B Şarköy 1 Nolu Halk Plajı	Şarköy/Tekirdağ	40.610966, 27.121842	yes	Excellent	Excellent	Excellent	unchanged	8
SSK Evleri (Halı Saha) Önü Plajı	Şarköy/Tekirdağ	40.612464, 27.130165		Excellent	Excellent	Excellent	unchanged	8
SSK Evleri Önü Halk Plajı	Şarköy/Tekirdağ	40.613162, 27.133179		Excellent	Excellent	Excellent	unchanged	8
Tekirdağ B.B. Şarköy 2 Nolu Halk Plajı	Şarköy/Tekirdağ	40.614420, 27.141701	yes	Excellent	Excellent	Excellent	unchanged	8
Eriklice Köyü Halk Plajı	Şarköy/Tekirdağ	40.639086, 27.186991		Excellent	Excellent		unchanged	
23 Nisan Kafe Önü Plajı	Şarköy/Tekirdağ	40.665530, 27.245124	yes	Excellent	Excellent	Excellent	unchanged	8
Mürefte Halk Plajı	Şarköy/Tekirdağ	40.669447, 27.249107	yes	Excellent	Excellent	Excellent	unchanged	8
Hoşköy Sağlık Ocağı Önü	Şarköy/Tekirdağ	40.712965, 27.317436		Excellent	Excellent	Excellent	unchanged	8
Uçmakdere Halk Plajı	Şarköy/Tekirdağ	40.784101, 27.367610		Excellent	Excellent	Excellent	unchanged	8
Kumbağ Balıkçıbarınağı Yanı Halk Plajı	Süleymanpaşa/Tekirdağ	40.867145, 27.459877		Excellent	Good	Good	degraded	8
Kumbağ Belediye Plajı	Süleymanpaşa/Tekirdağ	40.875168, 27.460346		Excellent	Good	Excellent	unchanged	8
Kumbağ Askeri Kampı	Süleymanpaşa/Tekirdağ	40.887931, 27.461809		Sufficient	Excellent	Excellent	improved	8
Barbaros Gündal Halk Plajı	Süleymanpaşa/Tekirdağ	40.890607, 27.462111		Excellent	Excellent	Excellent	unchanged	8
Barboros Topağaç Halk Plajı	Süleymanpaşa/Tekirdağ	40.928889, 27.482161		Good	Excellent	Excellent	improved	8
Altınova Halk Plajı	Süleymanpaşa/Tekirdağ	40.940399, 27.486915		Good	Good	Excellent	improved	8
Dereağzı Halk Plajı	Süleymanpaşa/Tekirdağ	40.980992, 27.559614		Poor	Poor	Poor	unchanged	7
Değirmenaltı Halk Plajı	Süleymanpaşa/Tekirdağ	40.984311, 27.576404		Sufficient	Poor	Poor	degraded	7
Beyazköy Şekerkamp Halk Plajı	Süleymanpaşa/Tekirdağ	40.989594, 27.612953		Excellent	Good	Good	degraded	7
Tekirdağ Salat Yağ Fabrikası Çamlık Plajı	Süleymanpaşa/Tekirdağ	41.002700, 27.676993		Sufficient	Sufficient	Sufficient	unchanged	7
Yenice Marmara Sahil Sitesi Önü	Çorlu/Tekirdağ	41.004795, 27.708841		Good	Good		unchanged	
Yeniçiftlik Halk Plajı	Marmaraereğlisi/Tekirdağ	40.991948, 27.839896		Sufficient	Good	Sufficient	unchanged	7
Marmara Ereğlisi Kaptan 2 Önü Halk Plajı	Marmaraereğlisi/Tekirdağ	40.966875, 27.876812		Good	Excellent	Good	unchanged	8
Dallas Kampı Halk Plajı	Marmaraereğlisi/Tekirdağ	40.969443, 27.927013		Good	Good	Good	unchanged	8
Marmara Ereğlisi Halk Plajı	Marmaraereğlisi/Tekirdağ	40.972310, 27.956284		Sufficient	Sufficient	Sufficient	unchanged	7
Kamaradere Halk Plajı	Marmaraereğlisi/Tekirdağ	40.982728, 27.969820		New	New	New	unchanged	7
Sultanköy Halk Plajı	Marmaraereğlisi/Tekirdağ	41.018287, 27.990545		Excellent	Excellent	Excellent	unchanged	7
Gümüsyaka Belediye Çadır Yeri Mevkii	Silivri/İstanbul	41.049080, 28.057666		Sufficient	Good	Good	improved	7
Çanta Albayraklar-Kınalı Mevki Önü	Silivri/İstanbul	41.060031, 28.105983		Excellent	Excellent	Excellent	unchanged	7
Uyumkent Sitesi Önü	Silivri/İstanbul	41.066498, 28.132422		Excellent	Excellent	Excellent	unchanged	7

Table S1. Continued.

Name of Bathing Area	District/Province	Coordinates	Blue Flag	Bathing Water Quality 2022	2023	2024	Water quality change from 2022 to 2024	Sample number in 2024
Semizkum Basınkent 4 Site Önü	Silivri/İstanbul	41.069910, 28.153613		Excellent	Excellent	Excellent	unchanged	7
Semizkum Çadır ve Kamp Yeri	Silivri/İstanbul	41.071680, 28.161011		Excellent	Excellent	Excellent	unchanged	7
Altınorak Sitesi Önü	Silivri/İstanbul	41.073319, 28.181972		Excellent	Excellent	Excellent	unchanged	7
Silivri Kumluk Mevkii	Silivri/İstanbul	41.077044, 28.219787	yes	Excellent	Excellent	Excellent	unchanged	7
Bizimköy-Parkköy Sitesi Önü	Silivri/İstanbul	41.060435, 28.301813		Good	Good	Excellent	improved	7
Selimpasa Baskent Sitesi Önü	Silivri/İstanbul	41.057522, 28.344377		Good	Good	Excellent	improved	7
Selimpasa Duruman Mevkii	Silivri/İstanbul	41.052939, 28.371773		Good	Good	Excellent	improved	7
Celaliye Beldesi Belediye Tesisleri Önü	Büyükçekmece/İstanbul	41.045236, 28.407311		Poor	Good	Good	improved	7
Kamiloba Beldesi Ağar Kamping Önü	Büyükçekmece/İstanbul	41.039468, 28.426275		Sufficient	Good	Excellent	improved	7
Kumburgaz Plajı	Büyükçekmece/İstanbul	41.030679, 28.450827		Good	Good	Good	unchanged	7
Mimarsinan Sahili	Büyükçekmece/İstanbul	41.013153, 28.561752		Sufficient	Good	Excellent	improved	7
Büyükçekmece Halk Plajı	Büyükçekmece/İstanbul	41.015874, 28.590863		Good	Good	Good	unchanged	7
Büyükçekmece Çocuk Sahili	Büyükçekmece/İstanbul	41.014015, 28.595287		Sufficient	Good	Good	unchanged	7
Albatros Sahili	Büyükçekmece/İstanbul	41.012666, 28.597040		Good	Good	Excellent	improved	7
Gürpinar Sahili	Büyükçekmece/İstanbul	41.006721, 28.598745		Excellent	Excellent	Excellent	unchanged	7
Beylikdüzü Gürpinar Sahili Halk Plaj	Beylikdüzü/İstanbul	40.981508, 28.598711		Good	Good	Excellent	improved	7
West İstanbul Marina Plajı	Beylikdüzü/İstanbul	40.962808, 28.653902		Sufficient	Poor	Poor	degraded	8
Denizköşkler	Avcılar/İstanbul	40.972319, 28.713483		New	Good	Good	unchanged	7
Menekşe Plajı Sahil Parkı Önü	Küçükçekmece/İstanbul	40.980518, 28.761041		Poor	Good	Sufficient	improved	7
Menekse Plajı Cankurtaran Kulesı Önü	Küçükçekmece/İstanbul	40.979062, 28.770776		Poor	Sufficient	Good	improved	7
Menekse Plajı Iskele Önü	Küçükçekmece/İstanbul	40.977433, 28.774018		Poor	Sufficient	Good	improved	7
Florya Güneş Plajı	Bakırköy/İstanbul	40.969993, 28.789924		New	New	New	unchanged	7
Yesilköy Polis Merkezi Önü	Bakırköy/İstanbul	40.955219, 28.829444		Sufficient	Sufficient	Poor	degraded	7
Yeşilköy international Hospital Önü	Bakırköy/İstanbul	40.957325, 28.837974		Poor	Poor	Poor	unchanged	7
Tarabya Plajı	Sarıyer/İstanbul	41.137100, 29.057890		Sufficient	Good	Good	improved	7
Rumeli Kavagı Plajı	Sarıyer/İstanbul	41.184322, 29.076590		Sufficient	Sufficient	Sufficient	unchanged	7
Caddebostan Plajı Büyük Kulüp Arkası	Kadıköy/İstanbul	40.967511, 29.052877		Good	Good	Excellent	improved	7
Caddebostan Plajı Irmak Okulları Arkası	Kadıköy/İstanbul	40.964281, 29.057729		Good	Good	Excellent	improved	7
Suadiye Plajı	Kadıköy/İstanbul	40.959965, 29.070769		Good	Good	Excellent	improved	7
Kınalıada Ülker Restaurant Önü	Adalar/İstanbul	40.906427, 29.044207		Good	Excellent	Excellent	improved	7
Kınalıada Su Sporları Kulübü Önü	Adalar/İstanbul	40.914504, 29.050315		Excellent	Excellent	Excellent	unchanged	7
Kınalıada Vapur Iskelesı Sag Taraf Önü	Adalar/İstanbul	40.911514, 29.054934		Excellent	Excellent	Excellent	unchanged	7
Kınalıada Vapur Iskelesı Sol Taraf Önü	Adalar/İstanbul	40.908268, 29.056040		Excellent	Excellent	Excellent	unchanged	7
Burgazada Su Sporları Kulübü Önü	Adalar/İstanbul	40.878801, 29.071783		Excellent	Excellent	Excellent	unchanged	7
Burgazada Deniz Kulübü	Adalar/İstanbul	40.878697, 29.070994		New	Excellent	Excellent	unchanged	7

Table S1. Continued.

Name of Bathing Area	District/Province	Coordinates	Blue Flag	Bathing Water Quality			Water quality change from 2022 to 2024	Sample number in 2024
				2022	2023	2024		
Heybeliada Ada Beach Club Önü	Adalar/İstanbul	40.870617, 29.088226		Excellent	Excellent	Excellent	unchanged	7
Heybeliada Asaf Beach	Adalar/İstanbul	40.879485, 29.088573		New	Excellent	Excellent	unchanged	7
Heybeliada Sadıkbey Plajı Önü	Adalar/İstanbul	40.880482, 29.090188		Excellent	Excellent	Excellent	unchanged	7
Heybeliada Su Sporları Kulübü Önü	Adalar/İstanbul	40.882312, 29.088921		Excellent	Excellent	Excellent	unchanged	7
Değirmenburnu Tabiat Parkı Plajı	Adalar/İstanbul	40.883335, 29.090919		New	New	New		7
Halik Koyu Eskibag Mesire Alanı Halk Plajı	Adalar/İstanbul	40.848355, 29.113735		Good	Good	Excellent	improved	7
Büyükada Prenses Koyu	Adalar/İstanbul	40.858878, 29.112632		New	Excellent	Excellent	unchanged	7
Büyükada Yörükalı Plajı Önü	Adalar/İstanbul	40.859599, 29.112769		Excellent	Excellent	Excellent	unchanged	7
Büyükada Kayıkhane Blue Beach	Adalar/İstanbul	40.872431, 29.122753		Excellent	Excellent	Excellent	unchanged	7
Büyükada Su Sporları Kulübü Önü	Adalar/İstanbul	40.870556, 29.139156		Excellent	Excellent	Excellent	unchanged	7
Büyükada Nakıbey Plajı	Adalar/İstanbul	40.863668, 29.133911		Excellent	Excellent	Excellent	unchanged	7
Büyükada Aya Nikola Halk Plajı	Adalar/İstanbul	40.854298, 29.125331		Excellent	Excellent	Excellent	unchanged	7
Büyükada Tabiat Parkı Plajı	Adalar/İstanbul	40.852641, 29.125507		Excellent	Excellent	Excellent	unchanged	7
Sedef Adası Halk Plajı	Adalar/İstanbul	40.849850, 29.141486		Excellent	Excellent	Excellent	unchanged	7
Tuzla Beledıyesi Halk Plajı	Tuzla/İstanbul	40.813139, 29.275073		Good	Excellent	Good	unchanged	7
Bayramoğlu Ada	Darıca/Kocaeli	40.790361, 29.337584		Sufficient	Good	Good	improved	10
Kadınlar Plajı	Darıca/Kocaeli	40.783901, 29.348597		Sufficient	Good	Good	improved	10
Bayramoğlu Halk	Darıca/Kocaeli	40.782567, 29.349454		Good	Good	Good	unchanged	10
Balyanoz Koyu Plajı	Darıca/Kocaeli	40.753105, 29.388674		New	New	Good	unchanged	10
Darıca Ş.Er Gökhan Hüseyinoğlu Sahil Parkı Plajı	Darıca/Kocaeli	40.755639, 29.395530		Good	Excellent	Excellent	improved	10
Tavşancıl	Dilovası/Kocaeli	40.769301, 29.562482		Excellent	Excellent	Excellent	unchanged	10
Tavşancıl Sahili 2	Dilovası/Kocaeli	40.766560, 29.570316		New	Excellent	Excellent	unchanged	10
Halıdere	Gölcük/Kocaeli	40.717179, 29.757211		Excellent	Excellent	Excellent	unchanged	9
Ulaşlı Haık Plajı	Gölcük/Kocaeli	40.707384, 29.702787		Excellent	Excellent	Excellent	unchanged	10
Güzelkıyı Sahili	Karamürsel/Kocaeli	40.703854, 29.669009		Excellent	Excellent	Excellent	unchanged	10
Ereğli Kumyalı Defne	Karamürsel/Kocaeli	40.698482, 29.650082	yes	Excellent	Excellent	Excellent	unchanged	10
Altınkemer	Karamürsel/Kocaeli	40.695678, 29.629647	yes	Excellent	Excellent	Excellent	unchanged	10
Dereköy Sahili	Karamürsel/Kocaeli	40.687053, 29.560087		Excellent	Excellent	Excellent	unchanged	10
Saralkent Sahili	Altınova/Yalova	40.688025, 29.555197	yes	Excellent	Excellent	Excellent	unchanged	8
Hersek Sahili	Altınova/Yalova	40.730719, 29.517622		Excellent	Good	Excellent	unchanged	8
Aydınkent-Ceylankent Sahili	Çiftlikköy/Yalova	40.685507, 29.358296		Excellent	Excellent	Excellent	unchanged	8
Baskent 3 Sıtesı Plajı	Çiftlikköy/Yalova	40.671495, 29.329224		Poor	Poor	Poor	unchanged	8
Çiftlikköy Halk Plajı	Çiftlikköy/Yalova	40.669153, 29.312164		Poor	Poor	Sufficient	improved	8
Arastırma Sahili Plajı	Merkez/Yalova	40.662408, 29.286469		Sufficient	Sufficient	Poor	degraded	8
Akasya Park Plajı	Merkez/Yalova	40.658153, 29.264047		Sufficient	Poor	Poor	degraded	8

Table S1. Continued.

Name of Bathing Area	District/Province	Coordinates	Blue Flag	Bathing Water Quality 2022	2023	2024	Water quality change from 2022 to 2024	Sample number in 2024
Su Ürünleri Plajı	Merkez/Yalova	40.659448, 29.242248		Poor	Poor	Poor	unchanged	8
Kamplar Bölgesi Plajı	Merkez/Yalova	40.658871, 29.238005		Good	Good	Good	unchanged	8
Koru Halk Plajı	Çınarcık/Yalova	40.657429, 29.188486		Sufficient	Sufficient	Sufficient	unchanged	8
Taslıman Mevkii Plajı	Çınarcık/Yalova	40.648267, 29.131986		Excellent	Excellent	Excellent	unchanged	8
Üçreisler Özenler Mevkii Plajı	Çınarcık/Yalova	40.646639, 29.079767		Sufficient	Sufficient	Poor	degraded	8
Kumluk Mavıs Plajı	Çınarcık/Yalova	40.644513, 29.068776		Excellent	Excellent	Excellent	unchanged	8
Esenköy Çamlıbel Mevkii	Çınarcık/Yalova	40.592205, 28.907283		Excellent	Good	Excellent	unchanged	8
İhlas Armutlu Tatil Koyu Plajı	Armutlu/Yalova	40.517341, 28.787403		Excellent	Excellent	Excellent	unchanged	8
İskele Mevkii	Armutlu/Yalova	40.508903, 28.832107		Sufficient	Sufficient	Excellent	improved	8
Yılandar Mevkii Plajı	Armutlu/Yalova	40.498841, 28.861102		Good	Good	Good	unchanged	8
Fıstıklı Köy Sahili	Armutlu/Yalova	40.485600, 28.878375		Good	Sufficient	Good	unchanged	8
Narlı Halk Plajı	Gemlik/Bursa	40.479217, 29.036347		Good	Good	Good	unchanged	7
Karacaali Gençlik Kampı	Gemlik/Bursa	40.478509, 29.056135		Good	Good	Good	unchanged	7
Büyükkumla Halk Plajı	Gemlik/Bursa	40.476489, 29.084309		Good	Sufficient	Sufficient	degraded	7
B.B.B. Küçükkumla Halk Plajı	Gemlik/Bursa	40.464170, 29.104912		Poor	Poor	Poor	unchanged	7
Hasanağa Kadınlar Plajı	Gemlik/Bursa	40.454352, 29.122133		Poor	Poor	Poor	unchanged	7
Gemsaz Halk Plajı	Gemlik/Bursa	40.416962, 29.100608		Good	Good	Good	unchanged	7
B.B.B. Kumsaz Halk Plajı	Gemlik/Bursa	40.384925, 29.069430		Poor	Poor	Sufficient	improved	7
B.B.B.Kurşunlu Kadınlar Plajı	Gemlik/Bursa	40.364283, 29.036363		Poor	Poor	Sufficient	improved	7
Altıntaş Halk Plajı	Mudanya/Bursa	40.356554, 28.971953		New	New	Excellent	unchanged	7
Burgaz Altınkum Halk Plajı	Mudanya/Bursa	40.356801, 28.940741		Sufficient	Poor	Poor	degraded	7
Burgaz Halk Plajı	Mudanya/Bursa	40.360564, 28.915482		Sufficient	Sufficient	Poor	degraded	7
Coşkunöz Halk Plajı	Mudanya/Bursa	40.382264, 28.877543		Sufficient	Sufficient	Sufficient	unchanged	7
Kumyaka Halk Plajı	Mudanya/Bursa	40.385797, 28.827732		Poor	Poor	Poor	unchanged	7
Zeytinbağı Halk Plajı	Mudanya/Bursa	40.391404, 28.803451		Good	Good	Excellent	improved	7
B.B.B. Eskel Halk Plaji	Mudanya/Bursa	40.362955, 28.670669		Sufficient	Good	Excellent	improved	7
B.B.B. Egerce Halk Plaji	Mudanya/Bursa	40.364926, 28.630684		Good	Good	Good	unchanged	7
B.B.B. Mesudiye Halk Plajı	Mudanya/Bursa	40.370194, 28.598117		Excellent	Good	Good	degraded	7
Yeniköy Halk Plaji	Karacabey/Bursa	40.398482, 28.374206		Excellent	Good	Good	degraded	7
Malkara Halk Plaji	Karacabey/Bursa	40.400727, 28.348805	yes	Excellent	Excellent	Excellent	unchanged	7
Kursunlu Halk Plaji / Karacabey	Karacabey/Bursa	40.397140, 28.292495	yes	Excellent	Excellent	Excellent	unchanged	7
Tatlısu Mah. Halk Plajı Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.405014, 27.918214		Excellent	Excellent	Excellent	unchanged	9
Bakraç Plajı-Tatlısu Mevkii Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.411760, 27.930055	yes	Excellent	Excellent	Excellent	unchanged	9
Dalyan Plajı-Tatlısu Mevkii Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.424894, 27.962442	yes	Excellent	Excellent	Excellent	unchanged	9

Table S1. Continued.

Name of Bathing Area	District/Province	Coordinates	Blue Flag	Bathing Water Quality 2022	2023	2024	Water quality change from 2022 to 2024	Sample number in 2024
Turan Mah. Halk Plajı Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.506795, 27.790100		Excellent	Excellent	Excellent	unchanged	9
Moda Plajı Narlı Mevkii Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.474492, 27.687026	yes	Excellent	Excellent	Excellent	unchanged	9
Laka Plajı Narlı Mevkii Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.472833, 27.694301		Excellent	Excellent	Excellent	unchanged	9
Ocaklar Belediye Plajı Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.450070, 27.749723	yes	Excellent	Excellent	Excellent	unchanged	9
Toma Deresi Mev. Çugra Plajları Yüz. Su. İz. Nok.	Erdek/Balıkesir	40.416345, 27.768357		Excellent	Excellent	Excellent	unchanged	9
Kafkas Otel Çugra Mevkii Yüzme Suyu İz. Noktası	Erdek/Balıkesir	40.410060, 27.782051		Excellent	Excellent	Excellent	unchanged	9
Orman Kampı Mevkii Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.405515, 27.786261		Excellent	Excellent	Excellent	unchanged	9
Kurbağalıdere Mevkii Yüzme Suyu İzleme Noktası	Erdek/Balıkesir	40.391191, 27.796135	yes	Excellent	Excellent	Excellent	unchanged	9
Pınar Otel Plajı-Gedeve Mevkii Yüzme Suyu İz. Nok.	Erdek/Balıkesir	40.386816, 27.856198	yes	Excellent	Excellent	Excellent	unchanged	9
Edincik Altı Halk Plajı Yüzme Suyu İzleme Noktası	Bandırma/Balıkesir	40.375308, 27.879595		Excellent	Excellent	Excellent	unchanged	9
Saraylar Abroz Plajı Yüzme Suyu İzleme Noktası	Marmara/Balıkesir	40.656692, 27.671027	yes	Excellent	Excellent	Excellent	unchanged	9
Çınarlı Sahili Yüzme Suyu İzleme Noktası	Marmara/Balıkesir	40.616850, 27.535073		New	New	New		9
Aba Plajı Yüzme Suyu İzleme Noktası	Marmara/Balıkesir	40.582190, 27.565425		Excellent	Excellent	Excellent	unchanged	9
Avşa Halk Plajı Yüzme Suyu İzleme Noktası	Marmara/Balıkesir	40.512200, 27.496083	yes	Excellent	Excellent	Excellent	unchanged	9
Yiğitler Halk Plajı Yüzme Suyu İzleme Noktası	Marmara/Balıkesir	40.494083, 27.530241		Excellent	Excellent	Excellent	unchanged	9
Sendika Önü Yüzme Suyu İzleme Noktas	Gönen/Balıkesir	40.308702, 27.569719		Excellent	Excellent	Excellent	unchanged	9
Pınarkent Yüzme Suyu İzleme Noktası	Gönen/Balıkesir	40.302305, 27.546329		Excellent	Excellent	Excellent	unchanged	9
66 Evler Plajı Yüzme Suyu İzleme Noktası	Gönen/Balıkesir	40.301795, 27.540503		Excellent	Excellent	Excellent	unchanged	9
Akınkent Yüzme Suyu İzleme Noktası	Gönen/Balıkesir	40.301757, 27.535793		Excellent	Excellent	Excellent	unchanged	9
Karabıga Halk Plajı	Biga/Çanakkale	40.408972, 27.314188	yes	Excellent	Excellent	Excellent	unchanged	8
Aksaz Halk Plajı	Biga/Çanakkale	40.435111, 27.186333		Excellent	Excellent	Excellent	unchanged	8
Kemer Halk Plajı	Biga/Çanakkale	40.414757, 27.062819		Excellent	Excellent	Excellent	unchanged	8
Çardak Halk Plajı	Lapseki/Çanakkale	40.382360, 26.711374		Excellent	Excellent	Excellent	unchanged	8
Beledıye Plajı (Dalyan)	Lapseki/Çanakkale	40.357195, 26.691874		Excellent	Excellent	Excellent	unchanged	8
Kökez Mevkıı Plajı	Lapseki/Çanakkale	40.338421, 26.669528		Excellent	Excellent	Excellent	unchanged	8
Umurbey Sahil	Lapseki/Çanakkale	40.278638, 26.569475		New	New	Excellent		8
Mega Beach Önü Belediye Halk Plajı	Merkez/Çanakkale	40.139309, 26.399981		Excellent	Excellent	Excellent	unchanged	8
Yenıkordon Barış Plajı	Merkez/Çanakkale	40.132766, 26.405264	yes	Excellent	Excellent	Excellent	unchanged	7
Kepez Halk Plajı	Merkez/Çanakkale	40.103829, 26.370265	yes	Excellent	Excellent	Excellent	unchanged	8
Dardanos Halk Plajı	Merkez/Çanakkale	40.087163, 26.363751	yes	Excellent	Excellent	Excellent	unchanged	7
Dardanos Orman Kampı Yanı Plajı	Merkezçanakkale	40.074441, 26.358132		Excellent	Excellent	Excellent	unchanged	8
Özel Eğitim Merkezi Komutanlığı Plajı	Merkezçanakkale	40.060142, 26.356016		New	Excellent	Excellent	unchanged	8
Güzelyalı Belediye Halk Plajı	Merkez/Çanakkale	40.037943, 26.337731	yes	Good	Good	Good	unchanged	7
Intepe Gençlık Kampı	Merkez/Çanakkale	40.034371, 26.337274		Excellent	Excellent	Good	degraded	8

Table S1. Continued.

Name of Bathing Area	District/Province	Coordinates	Blue Flag	Bathing Water Quality 2022	2023	2024	Water quality change from 2022 to 2024	Sample number in 2024
Kılıtbahır Zargana Plajı	Eceabat/Çanakkale	40.142417, 26.378484		Excellent	Excellent	Excellent	unchanged	8
Eceabat Plajı	Eceabat/Çanakkale	40.172302, 26.367459		Sufficient	Sufficient	Sufficient	unchanged	8
Hamzakoy Plajı	Gelibolu/Çanakkale	40.413573, 26.679640		Excellent	Excellent	Excellent	unchanged	8

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 42(2), 138-147 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.06

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

Population genetics of *Pomatomus saltatrix* (Linnaeus, 1766) in the seas of Türkiye based on microsatellite DNA

Türkiye denizlerindeki *Pomatomus saltatrix* (Linnaeus, 1766)'in mikrosatelit DNA tabanlı popülasyon genetiği

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Received date: 25.12.2024 *Accepted date: 07.04.2025*

How to cite this paper:
Kutlu, İ., Düzgüneş, Z.D., Firidin, Ş., Alemdağ, M., Cebeci, A., & Turan, İ. (2025). Population genetics of *Pomatomus saltatrix* (Linnaeus, 1766) in the seas of Türkiye based on microsatellite DNA. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 138-147. https://doi.org/10.12714/egejfas.42.2.06

Abstract: Bluefish, (*Pomatomus saltatrix* (Linnaeus, 1766)) a commercially important and highly migratory predatory fish species, is found along the coasts of Türkiye. Despite it's widespread presence along these coasts, there has been no detailed study on the genetic structure of bluefish populations in Türkiye. In order to protect the biological diversity of countries, the genetic diversity present in natural resources must be identified. In this study, we examined the population structure of bluefish in the coastal regions of Türkiye; We aimed to identify bluefish samples collected from 14 regions along Türkiye coast by analyzing microsatellit DNA. For the microsatellite analysis, eight loci (ELF 17, ELF 37, ELF 49, ELF 19, ELF 39, ELF 46, ELF 44, ELF 50) were analyzed. A total of 433 samples from 14 populations were studied. In total 207 alleles and 61 specific alleles were identified across all populations and all loci. The highest observed (Ho) heterozygous values are ELF 50 (Ho: 0,991) while the lowest value is ELF 19 (Ho: 0.716). The highest expected (He) heterozygous values are ELF 39 (He: 0.952) while the lowest value is ELF 50 (He: 0.518). According to the Hardy-Weinberg analysis results, it was determined that there was a significant deviation in all populations. When bluefish populations are clustered according to their phylogenetic lineages by applying principal coordinate analysis (PCoA), the first three axes show 94% of the total genetic variation. The highest variation values and eigenvalues were found on the 3rd axis. When the analysis results are examined, it is clearly seen that the Mersin bluefish population is clustered differently from other populations. According to the Mantel test, a low correlation (R^2 = 0.3061, P = 0.01) was detected between genetic and geographical distance. Admixed individuals and low genetic differentiation were observed in all populations.

Keywords: Bluefish, loci, microsatellite, allele, Fst

Öz: Lüfer (*Pomatomus saltatrix* (Linnaeus, 1766)), ticari açıdan oldukça önemli ve göç eden bir yırtıcı balık türü olup, Türkiye kıyılarında bulunmaktadır. Buna rağmen, Türkiye'deki Lüfer popülasyonlarının genetik yapısı hakkında detaylı bir çalışma yapılmamıştır. Ülkelerin biyolojik çeşitliliğini korumak için doğal kaynaklarda bulunan genetik çeşitliliğin belirlenmesi gerekmektedir. Bu çalışmada, Türkiye'nin kıyı bölgelerindeki Lüfer popülasyon yapısını inceledik; Türkiye kıyılarındaki 14 bölgeden toplanan Lüfer örneklerini mikrosatelit DNA analizi yaparak tanımlamayı amaçladık. Mikrosatelit analizi için sekiz lokus (ELF 17, ELF 37, ELF 49, ELF 19, ELF 39, ELF 46, ELF 44, ELF 50) analiz edildi. Toplam 14 popülasyondan 433 örnek incelendi. Toplamda tüm popülasyonlarda ve tüm lokuslarda 207 alel ve 61 spesifik alel tanımlanmıştır. Gözlemlenen en yüksek (Ho) heterozigot değerler ELF 50 (Ho: 0,991) iken en düşük değer ELF 19'dur (Ho: 0,716). Beklenen en yüksek (He) heterozigot değerler ELF 39 (He: 0,952) iken en düşük değer ELF 50'dir (He: 0,518). Hardy-Weinberg analiz sonuçlarına göre tüm popülasyonlarda önemli bir sapma olduğu belirlenmiştir. Lüfer popülasyonları filogenetik soylarına göre temel koordinat analizi (PCoA) uygulanarak kümelendirildiğinde ilk üç eksen toplam genetik varyasyonun %94'ünü göstermektedir. En yüksek varyasyon değerleri ve öz değerler 3. eksende bulunmuştur. Analiz sonuçları incelendiğinde Mersin lüfer popülasyonunun diğer popülasyonlardan farklı olarak kümelendiği açıkça görülmektedir. Mantel testine göre genetik ve coğrafi mesafe arasında düşük bir korelasyon (R^2 = 0,3061, P = 0,01) tespit edilmiştir. Sonuç olarak Tüm popülasyonlarda karışık bireylerin olduğu ve düşük genetik farklılaşma gözlenmiştir.

Anahtar kelimeler: Lüfer, lokus, mikrosatelit, allel, Fst

INTRODUCTION

Bluefish (*Pomatomus saltatrix* (L., 1766)) are predatory fish that spread in temperate and warm waters around the world, generally on the continental margin, and migrate to warm waters between seas (Briggs, 1960; Wilk, 1977; Juanes et al., 1996). Adult bluefish are fast-swimming fish that migrate in response to seasonal changes (Wilk, 1977). Although bluefish prefer sandy substrates, they are also found in clayey and muddy ground (Bal, 2015).

Bluefish are fish that migrate between seas to warm waters. Bluefish are capable of long-distance movement, and in geographically isolated populations bluefish are known to undertake extensive seasonal migrations (Van der Elst, 1976).

Since Türkiye is located in the temperate climate zone of the world, bluefish can be found in all four of our regional seas (Bal et al., 2018). In Türkiye waters, it is known that bluefish migrates from the Sea of Marmara and The Aegean Sea after September (Türgan, 1959; Akşiray, 1987).

When we look at genetically bluefish population structure, there are very few studies on bluefish in literature. There is no detailed genetic study on Türkiye seas. According to the results of Pardinas et al. (2010) study; They reported that the populations in the Eastern Atlantic Ocean (including the Eastern Mediterranean) and the populations in the Western Atlantic Ocean did not share any haplotypes. They also revealed complete genetic isolation between the two sides of the North Atlantic Ocean. In addition to; Miralles et al. (2014b) reported that genetically, there are two barriers, one in the middle of the Atlantic Ocean and the other in the Mediterranean, but regional permeability and migration occur in both. Miralles et al. (2016) also revealed a mixture of Eastern and Western Mediterranean strains in the farm located in Guardamar in the Western Mediterranean. They also reported that although most of the individuals caught around the facility genetically belonged to the local population, 7.14% to 11.9% of the individuals belonged to the genetic population living in Turkish waters.

Despite their complicated migration patterns, there are no compherensive molecular studies on bluefish in Türkiye, although some limited research has been conducted globally.

In the study aims to determine the genetic structure of the bluefish, an economically valuable species found in Türkiye seas, through microsatellite analysis. Thus, the study was conducted contribute to the studies aimed at revealing the biological diversity of Türkiye.

MATERIALS AND METHODS

Sampling

Samples were collected from 14 stations along the Türkiye coast: Hopa, Trabzon, Giresun, Samsun, Sinop, Ereğli, İğneada, Rumeli Feneri (İstanbul), Çanakkale, Erdek (Marmara sea), Bodrum, İzmir, Mersin and Adana (Figure 1). A 2-3 cm^2 of caudal fin tissue was sampled from a sufficient number of fish at each station and stored in 96% ethanol at room temperature.

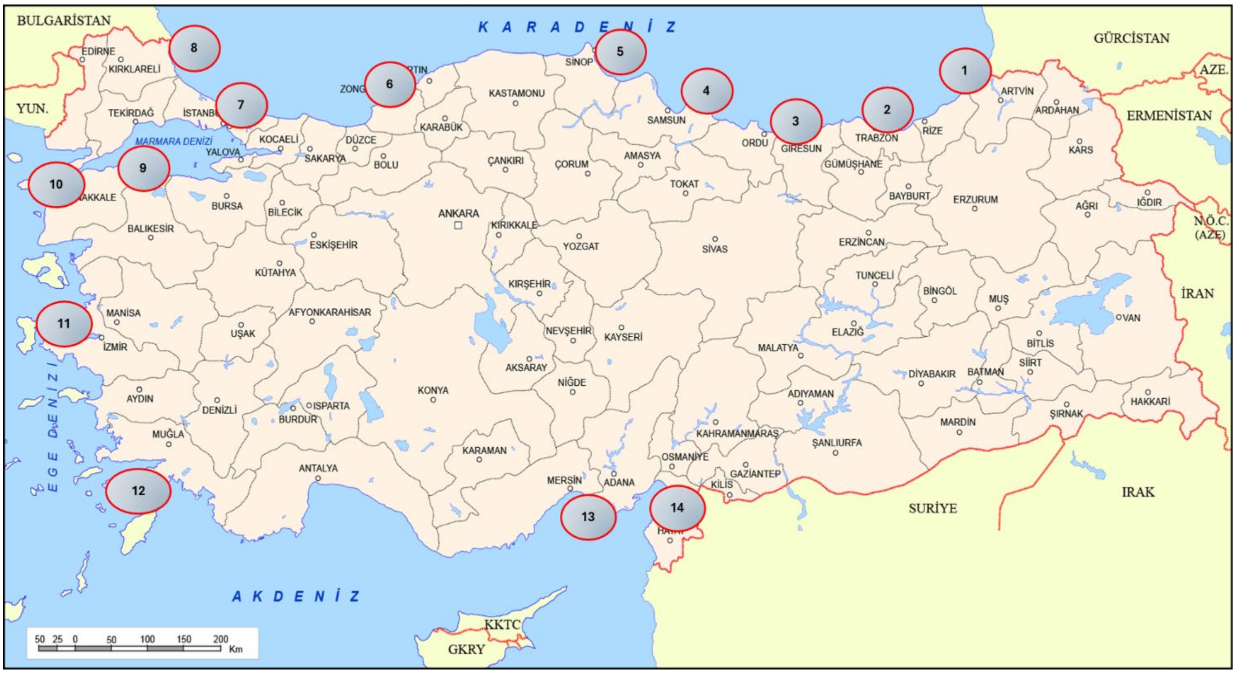

Figure 1. Map of sampling locations for *P. saltatrix* in coastal of Türkiye (1-Hopa, 2-Trabzon, 3-Giresun, 4-Samsun, 5-Sinop, 6-Ereğli, 7-Rumeli Feneri, 8-İğneada, 9-Erdek, 10-Çanakkale, 11-İzmir, 12-Bodrum, 13-Mersin, 14-Adana)

DNA extraction, amplification and microsatellite analysis

Genomic DNA was extracted using the kit (QIAamp DNA HT, Qiagen®, Germany) following the protocol suggested by the manufacturer. The samples were visualized through electrophoresis on 1,5 % agarose gel, dyed with SafeViewTM (NBS Biologicals, UK) and visualized under ultraviolet light.

Multiplex PCR was performed with 2x Master Mix (Type-it Microsatellite PCR Kit; 2000, Qiagen®, Germany), 10µl Forward Primer (10µM), 1µl Reverse Primer (10µM), 1µl, DNA (~50 ng/ml), 1µl Nuclease-free water and up to 20µl) with using primers in literature (Table 1) and the products were checked on agarose gel. Then, optimized the annealing temperatures that were created in three groups designated as 55-56-58°C.

In the next step, microsatellite locus-specific primers labelled with different fluorescently labelled dyes to determine allele sizes for multiplex PCR were synthesized (Table 1).

The resulting products of the PCR reaction and the displayed samples were diluted 1/50 in preparation for fragment analysis. After 0.5 µl of the diluted product was taken and 9.5 µl of formamide and 0.05 µl standard (LIZ-600 Genescan Size Standard, Applied Biosystems™, Lithuanian) were added.

It was placed on ice after being denatured at 95°C for 5 minutes. Afterwards, fluorescently labelled PCR products were electrophoresed along on the ABI 3500 Sequencer (Applied Biosystems) and allele lengths obtained for the microsatellite locus were determined on each samples.

Table 1. Microsatellite loci and primers (Dos Santos et al., 2008) used in the multiplex PCR study

Group	Lokus	Primer sequence (5'–3')	GenBank Accession Number	Fluorescent dye	Repeat Motif	Allele References Range (bp)
Group 1 Annealing 55 °C	ELF 17	F:TTCCACTTCCTCCTACTTTC R:GCAGGCTAATAATCGTTGAC	EU289407	FAM-Blue	$(TATC)_{21}$	136-216
	ELF 19	F:GCGACGGCTCTGTCTATGTG R:GAGGCTGAGACGGGTTCTTG	EU289408	PET-Red	$(TATC)_{22}$	234-394
	ELF 37	F:TGCTCGGCTACAATAACG R:GACCTGTCAGTGGAGATTC	EU289409	VIC-Green	$(TATC)_{28}$	216-324
	ELF 49	F:TACACCATGAGTGAACAAAG R:ATGAGAAGAAGGAAGCTAAG	EU289413	NED-Yellow	$(TATC)_{14}$	158-234
Group 2 Annealing 58 °C	ELF 39	F:TAGTGGTTCTGGGCAACAGG R:TATCCGGGCTGTACTGTTGG	EU289410	FAM- Blue	$(TATC)_{30}$	157-285
	ELF 44	F:ACTTGGGGTTGGGCAATATG R:ATTTCACAGCACAGGAAGAC	EU289411	VIC- Green	$(TATC)_{34}$	216-320
	ELF 46	F:TCAGATTACCCTCCCTGTTC R:TGTAGATGTGCTGGTGATCC	EU289412	NED- Yellow	$(TATC)_{25}$	268-376
Group 3 Annealing 56 °C	ELF 50	F: CTGCACAGGAACACGTCAGT R: ATCTGCCCCAAAACAGACAC	EU289414	FAM- Blue	(TATC)09	130-218

Microsatellite data analysis

The raw data obtained from fragment analysis were processed using the Convert program (Glaubitz, 2004) and analyzed with the Genemarker (Soft Genetics LLC) to determine allele sizes and frequencies.

Null-allele, allele overlap (stuttering) and allele loss (large allele dropout) were determined using Microchecker v2.2.3 (Van Oosterhout et al., 2004), and null allele frequencies were determined using the maximum likelihood method ML-NULLFREQ (Kalinowski and Taper, 2006) program.

The compliance of genotypic ratios with the Hardy-Weinberg (HW) equilibrium was determined by the “exact test” method of the GENEPOP v.4.2 (Rousset, 2008) program. To control the false discovery rate (FDR), new probability threshold values (Threshold P) were calculated and adjusted with the Bonferroni method (Benjamini and Hochberg, 1995). Probability values (P value) were determined based on 10,000 demomorizations, 500 batches, and 5,000 repetitions for each batch.

Total number of alleles (NA), expected heterozygosity (He), observed heterozygosity (Ho) and Polymorphic information content (PIC) were calculated with the Cervus 3.0.7 (Kalinowski et al., 2007) program.

Pedigree coefficient (FIS) and Allelic Richness (AR) values were calculated with the FSTAT v.2.9.3 (Goudet, 1995) program. The presence of unique alleles (private alleles) in the populations and the allele frequency were determined and calculated with the GenAlEX 6.5 program. Due to the possible presence of null allele, FST and null allele frequency were recalculated with 25 000 replicates in the Freena (Chapuis and Estoup, 2007) program.

To identify genetic differences between populations, interpopulation fixation indices (FST) based on allele frequency variation of loci (Weir and Cockerham, 1984) GENEPOP 4.2. It was calculated in the program (Raymond and Rousset, 1995).

The presence of genetically different populations in the data set was investigated using the Bayesian multi-locus clustering method in the STRUCTURE v.2.3.4 (Falush et al., 2003) program. Geographical and genetic distance relationships were examined with the Mantel test in the GenAlEx 6.5 (Mantel, 1967). To determine the most appropriate number of clusters, default clusters between 2-7 were tested in three independent repetitions. MCMC searches were created with a total of 108 steps, 107 of which were burning. The probable K value was determined with the delta K (ΔK) statistics using the online software Structure Harvester.

And principal coordinate analysis (PCoA) was performed with the same program to obtain more information about the relationship between populations (Liu and Muse, 2005)

The Analysis Molecular Variance (AMOVA) was performed on Arlequin (Excoffier et al., 1992) software to detect differences between populations.

RESULTS

Polymorphism of microsatellite loci

Eight microsatellite loci (ELF 17, ELF 37, ELF 49, ELF 19, ELF 39, ELF 46, ELF 44, ELF 50) were analyzed, Of the 433 samples from 14 populations seven loci were polymorphic, while one locus (ELF 44) was monomorphic. The polymorphic loci exhibited moderate to high polymorphism, and with PIC values ranging from 0.400 to 0.949 (average 0.730, Table 2). A total of 207 alleles were identified across all loci. The most common allele was stated in Table 3. The highest observed heterozygosity (Ho: 0.991) occurred at ELF 50 while the lowest (Ho: 0.716) was at ELF 19. Expected heterozygosity ranges from 0.518 (ELF 50) to 0.952 (Table 2).

According to the results of the Hardy-Weinberg analysis, it was determined that there was a significant deviation in bluefish populations (Table 3' blue colors).

At the population level, the average NA (number of alleles) number varies from 9.5 for the Mersin population to 14.25 for Çanakkale (average 12.4). The highest average number of alleles was observed in Çanakkale and İzmir populations (14.3-14.1). The average He (expected heterozygosity) and Ho (observed heterozygosity) for each population range were calculated between 0.730 and 0.716, respectively (Table 4).

In all populations, the highest observed heterozygosity (0.781) was determined in the R. Feneri population, while the lowest observed heterozygosity (0.647) was determined in the Sinop population (Table 4).

Genetic differentiation and structure among populations

61 specific alleles were identified in all populations. The populations and numbers where special alleles were seen were as follows. 9 alleles in Adana, 8 alleles each in Çanakkale and İzmir, 7 alleles in R. Feneri, 5 alleles in Mersin and Sinop, 4 alleles in Bodrum, 3 alleles each in İğneada and Erdek, 2 alleles in Ereğli, Samsun, Trabzon and Hopa and 1 allele in Giresun (Table 5). When allele frequencies were compared between populations, 3 loci (ELF 49, ELF 19 and ELF 50) were found to deviate significantly from HWE in all populations ($p < 0.01$) in Table 5.

Table 2. Parametric properties of microsatellite loci (NA: number of alleles; HObs: observed heterozygosity; HExp: expected heterozygosity; PIC: Polymorphic Information Content; HW; deviate significantly from Hardy-Weinberg Equilibrium, F(null) null allele frequency, Fis: Coefficient of nobility, Fit: Fixation index of Individual relative to gametes of the Total Population, Fst: fixation indices, NS: Non Significant Value, ND: Not Detected)

No	Locus	Na	HObs	HExp	PIC	HW	F (Null)	Fis	Fit	Fst
1	ELF 17	22	0.755	0.831	0.814	NS	0.0448	0.066	0.098	0.034
2	ELF 37	47	0.804	0.946	0.942	ND	0.0801	0.127	0.148	0.024
3	ELF 49	27	0.767	0.912	0.904	***	0.0867	0.132	0.152	0.023
4	ELF 19	29	0.716	0.923	0.916	***	0.1262	0.194	0.220	0.033
5	ELF 39	52	0.857	0.952	0.949	NS	0.0514	0.068	0.098	0.032
6	ELF 46	24	0.845	0.924	0.918	NS	0.0442	0.054	0.085	0.032
7	ELF 44	1	0.000	0.000	0.000	ND	ND	0.000	1.000	1.000
8	ELF 50	5	0.991	0.518	0.400	***	-0.3195	-0.888	-0.852	0.019

*** P<0.001 Summary of Chi-Square Tests for Hardy-Weinberg Equilibrium

Table 3. Stations and loci with significant deviations according to the HW analysis results

	ELF 17	ELF 19	ELF 37	ELF 39	ELF 44	ELF 46	ELF 49	ELF 50
Adana		■	■	■		■		■
Mersin	■	■		■				■
Bodrum	■	■	■	■		■		■
Çanakkale			■					■
İzmir		■	■				■	■
Erdek			■	■				■
Rumeli Feneri			■					■
İğneada	■	■	■			■	■	■
Ereğli	■		■	■		■		■
Sinop	■	■	■	■		■	■	■
Samsun		■						■
Giresun			■				■	■
Trabzon			■	■		■		■
Hopa		■	■				■	■

Table 4. Heterozygosity and polymorphism in population (N: Analyzed number of the Samples, Na:Average alllels number, Ne: Number of effective alleles, F: Fixation Index, I: Information Index, UHe: Unbiased Expected Heterozygosity, HO: observed heterozygosity, HE: expected heterozygosity)

Population	N	Na	Ne	I	Ho	He	uHe	F
Çanakkale	32	14.3	8.957	2.047	0.758	0.744	0.756	-0.070
Erdek	32	13.4	8.717	2.011	0.773	0.740	0.752	-0.093
R. Feneri	32	12.5	8.367	1.955	0.781	0.733	0.745	-0.125
Giresun	32	12.0	8.513	1.957	0.758	0.736	0.748	-0.094
Samsun	32	13.1	8.135	1.928	0.711	0.719	0.730	-0.059
Trabzon	32	12.4	7.474	1.918	0.766	0.729	0.740	-0.113
Hopa	32	13.0	8.117	1.957	0.711	0.733	0.744	-0.036
İğneada	32	11.0	7.188	1.818	0.680	0.713	0.725	-0.011
İzmir	32	14.1	9.079	2.040	0.719	0.741	0.752	-0.038
Adana	32	13.1	8.952	2.029	0.688	0.745	0.757	0.007
Sinop	34	12.9	7.607	1.903	0.647	0.718	0.729	0.031
Ereğli	32	10.8	6.698	1.776	0.668	0.703	0.714	-0.003
Bodrum	32	11.3	7.982	1.920	0.672	0.732	0.743	0.014
Mersin	15	9.5	6.759	1.830	0.700	0.738	0.764	0.053

Table 5. Specific allele frequencies seen in populations (Note: The same allele is specific because it is at different loci and was observed only at that station)

Population	Locus	Allele	Frequency	Population	Locus	Allele	Frequency
Çanakkale	ELF 17	132	0.016	Mersin	ELF 39	459	0.033
Çanakkale	ELF 17	183	0.016	Mersin	ELF 50	158	0.233
Çanakkale	ELF 37	210	0.016	İzmir	ELF 37	242	0.031
Çanakkale	ELF 49	379	0.016	İzmir	ELF 37	248	0.016
Çanakkale	ELF 39	466	0.016	İzmir	ELF 37	283	0.016
Çanakkale	ELF 46	240	0.047	İzmir	ELF 37	287	0.016
Çanakkale	ELF 46	257	0.016	İzmir	ELF 37	324	0.016
Çanakkale	ELF 46	269	0.031	İzmir	ELF 39	401	0.016
Erdek	ELF 37	292	0.016	İzmir	ELF 39	414	0.016
Erdek	ELF 49	214	0.016	İzmir	ELF 39	418	0.016
Erdek	ELF 39	411	0.016	Adana	ELF 17	114	0.031
R. Feneri	ELF 37	221	0.016	Adana	ELF 17	139	0.016
R. Feneri	ELF 37	281	0.016	Adana	ELF 37	229	0.047
R. Feneri	ELF 49	168	0.016	Adana	ELF 37	279	0.016
R. Feneri	ELF 49	198	0.016	Adana	ELF 49	204	0.031
R. Feneri	ELF 49	210	0.016	Adana	ELF 49	286	0.016
R. Feneri	ELF 39	446	0.031	Adana	ELF 39	387	0.031
R. Feneri	ELF 39	456	0.031	Adana	ELF 39	393	0.031
Giresun	ELF 37	215	0.016	Adana	ELF 46	303	0.031
Samsun	ELF 37	202	0.016	Sinop	ELF 37	264	0.015
Samsun	ELF 37	291	0.016	Sinop	ELF 37	366	0.015
Trabzon	ELF 39	455	0.016	Sinop	ELF 49	148	0.015
Trabzon	ELF 46	304	0.016	Sinop	ELF 49	297	0.015
Hopa	ELF 49	259	0.016	Sinop	ELF 49	365	0.015
Hopa	ELF 49	318	0.016	Ereğli	ELF 37	234	0.016
İğneada	ELF 37	256	0.016	Ereğli	ELF 37	318	0.016
İğneada	ELF 46	258	0.063	Bodrum	ELF 37	299	0.016
İğneada	ELF 46	278	0.063	Bodrum	ELF 49	244	0.016
Mersin	ELF 17	147	0.067	Bodrum	ELF 39	403	0.047
Mersin	ELF 17	162	0.033	Bodrum	ELF 46	261	0.031
Mersin	ELF 37	185	0.033				

AMOVA analysis determined that genetic diversity was 1% between populations and 95% within populations. (Table 6, $p < 0.01$).

Bluefish populations were clustered according to their phylogenetic lineages by applying principal coordinate analysis (PCoA) with Genalex 6 software. The first three axes show 94% of the total genetic variation. The highest variation values and Eigen values were found on the 3rd axis. The analysis results clearly show that the Mersin population is clustered differently from other populations (Figure 2).

Null allele frequencies were estimated, and the effect of null alleles on the fixation index FST was calculated with and without excluding null alleles. Patterns of genetic differentiation between populations at all locations are shown using pairwise Fst analyses. Pairwise FST comparisons showed low levels of genetic differentiation (Fst < 0.05) in Table 7.

Table 6. Analysis of molecular variance between populations

Source of variation	df	SS	MS	Est. Var.	%
Between Populations	13	69.071	5.313	0.036	1
Between Individuals	419	1286.958	3.071	0.102	4
Within Individuals	433	1241.500	2.867	2.867	95
Total	865	2597.529		3.006	100

The results of the pairwise population matrix of genetic similarity between populations in this study are presented in Table 8. The pairwise population matrix value between Trabzon and Mersin is 0.208, which is the highest of the pairwise genetic similarity indices between populations.

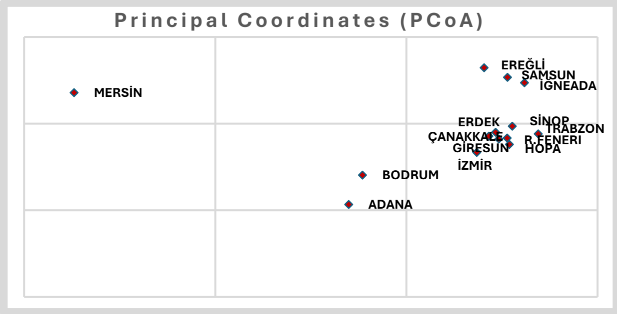

Figure 2. PCoA graph created using FST for bluefish populations

Table 7. FST (Weir and Cockerham, 1984) estimates using correction and without ENA correction for pairwise comparisons of allele distributions

FST	Çanakkale	Erdek	R. Feneri	Giresun	Samsun	Trabzon	Hopa	İğneada	İzmir	Adana	Sinop	Ereğli	Bodrum	Mersin
Çanakkale	0.0000													
Erdek	-0.001414	0.0000												
R. Feneri	-0.001755	-0.000658	0.0000											
Giresun	0.001223	0.005226	0.004029	0.0000										
Samsun	0.009449	0.012652	0.012223	0.002972	0.0000									
Trabzon	0.005123	0.009768	0.006411	-0.00217	0.004514	0.0000								
Hopa	0.006620	0.009483	0.008797	0.000440	0.011084	0.001306	0.0000							
İğneada	0.007795	0.007690	0.008048	0.010640	0.009168	0.011146	0.012595	0.0000						
İzmir	0.006098	0.010181	0.010064	0.002823	0.009323	0.010264	0.007655	0.013887	0.0000					
Adana	0.013216	0.014386	0.014646	0.012057	0.032007	0.017460	0.013414	0.027085	0.008713	0.0000				
Sinop	0.004559	0.004318	0.001901	0.004145	0.013752	0.003923	0.003326	0.007444	0.009646	0.020337	0.0000			
Ereğli	0.012014	0.008945	0.012176	0.010770	0.012389	0.012959	0.012495	0.001500	0.016499	0.032589	0.001704	0.0000		
Bodrum	0.013955	0.016440	0.015401	0.013428	0.025682	0.019623	0.014360	0.028764	0.007778	0.008690	0.014042	0.022788	0.0000	
Mersin	0.032540	0.034522	0.036222	0.033867	0.035409	0.040540	0.037184	0.038030	0.033751	0.025459	0.036032	0.032222	0.024779	0.00
FST+ENA	Çanakkale	Erdek	R. Feneri	Giresun	Samsun	Trabzon	Hopa	İğneada	İzmir	Adana	Sinop	Ereğli	Bodrum	Mersin
Çanakkale	0.0000													
Erdek	-0.000763	0.0000												
R. Feneri	-0.001842	-0.000420	0.0000											
Giresun	0.001941	0.005292	0.004086	0.0000										
Samsun	0.010623	0.012924	0.012471	0.002584	0.0000									
Trabzon	0.005927	0.010405	0.007225	-0.00097	0.005912	0.0000								
Hopa	0.006927	0.009754	0.009272	0.001297	0.011338	0.002531	0.0000							
İğneada	0.007683	0.007449	0.008017	0.009134	0.009432	0.010822	0.011950	0.0000						
İzmir	0.006235	0.009545	0.009186	0.002938	0.009143	0.010323	0.007740	0.012709	0.0000					
Adana	0.013820	0.014192	0.015301	0.013028	0.032532	0.019085	0.015384	0.025337	0.009647	0.0000				
Sinop	0.004709	0.004026	0.001609	0.003725	0.012646	0.005107	0.003125	0.008754	0.007752	0.020739	0.0000			
Ereğli	0.011008	0.006689	0.010201	0.009979	0.012256	0.012500	0.011500	0.002122	0.014976	0.030881	0.003514	0.0000		
Bodrum	0.012791	0.015716	0.014958	0.013348	0.025576	0.019527	0.013527	0.025484	0.006502	0.011025	0.012861	0.020120	0.0000	
Mersin	0.033667	0.032988	0.034723	0.034321	0.037267	0.042072	0.036800	0.036669	0.033012	0.027898	0.037110	0.033573	0.026624	0.0000

Table 8. Pairwise population matrix of the proximities of populations to each other

	Çanakkale	Erdek	R. Feneri	Giresun	Samsun	Trabzon	Hopa	İğneada	İzmir	Adana	Sinop	Ereğli	Bodrum	Mersin
Çanakkale	0.000													
Erdek	0.042	0.000												
R. Feneri	0.039	0.042	0.000											
Giresun	0.049	0.061	0.055	0.000										
Samsun	0.072	0.080	0.077	0.052	0.000									
Trabzon	0.059	0.073	0.061	0.037	0.055	0.000								
Hopa	0.067	0.075	0.071	0.047	0.076	0.048	0.000							
İğneada	0.066	0.065	0.065	0.073	0.068	0.074	0.080	0.000						
İzmir	0.067	0.079	0.076	0.055	0.072	0.076	0.071	0.085	0.000					
Adana	0.092	0.094	0.093	0.086	0.147	0.101	0.091	0.129	0.078	0.000				
Sinop	0.057	0.056	0.048	0.055	0.081	0.054	0.054	0.063	0.073	0.108	0.000			
Ereğli	0.075	0.066	0.074	0.071	0.075	0.076	0.077	0.046	0.090	0.142	0.046	0.000		
Bodrum	0.091	0.098	0.092	0.088	0.122	0.105	0.091	0.131	0.072	0.077	0.086	0.108	0.000	
Mersin	0.189	0.193	0.194	0.189	0.186	0.208	0.202	0.194	0.194	0.169	0.191	0.167	0.160	0.000

According to the Mantel test, a low correlation ($R^2 = 0.3666$, $P = 0.01$) was detected between genetic and geographical distance (Figure 3). The K values for the mixed model analysis were chosen between 2-5 and the calculations were repeated 20 times. The graph depicting the assignment of individuals to ancestral populations, based on the barcode method, is shown in Figure 4. For K = 2 and K = 3, individuals from all 14 regions could not be assigned to a different cluster (Figure 4).

The accuracy of the analysis for each K in structure is indicated by the selection of K based on LnP(D) (Taki et al., 2021). The value of LnP(D) is almost maximized when K=3 (Figure 5).

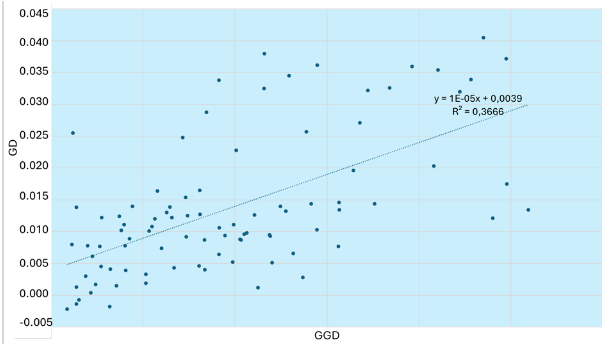

Figure 3. Mantel test between genetic and geographic distance of bluefish populations

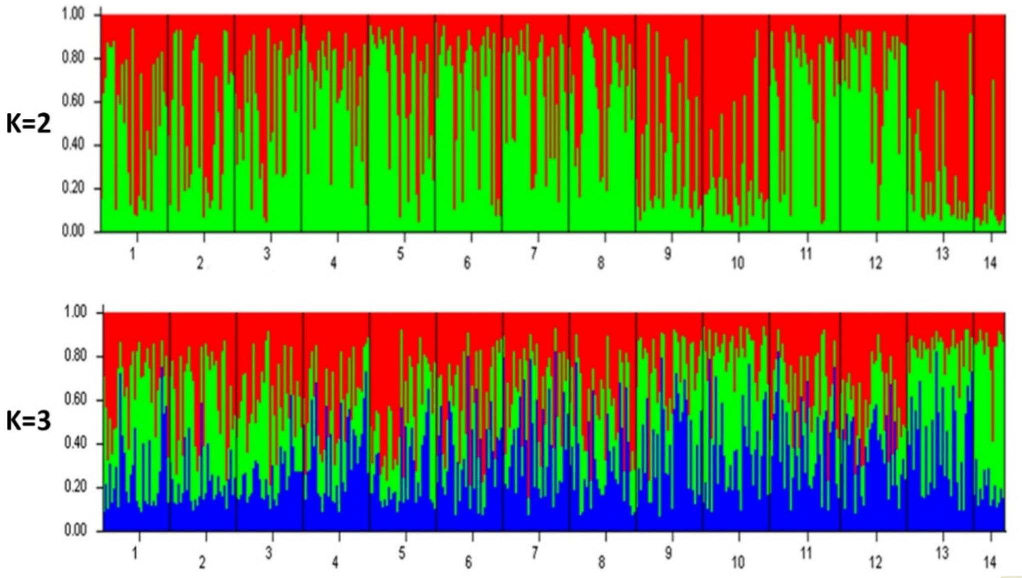

Figure 4. Population structure of bluefish for K = 2 and K = 3 (A thin vertical line represents each individual, and each color represents the probability of belonging to one of the genetic clusters. Black lines separate individuals from different sampling areas) (1-Çanakkale, 2-Erdek, 3-R. Feneri, 4-Giresun, 5-Samsun, 6-Trabzon, 7-Hopa, 8-İğneada, 9-İzmir, 10-Adana, 11-Sinop, 12-Ereğli, 13-Bodrum, 14-Mersin)

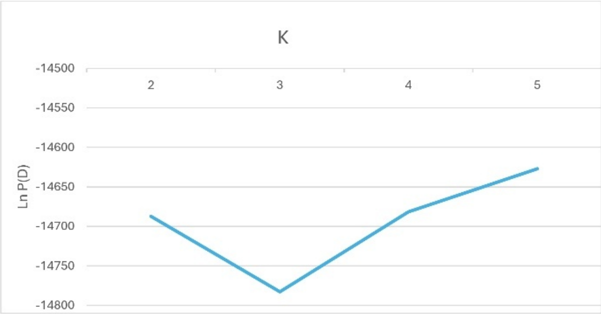

Figure 5. In STRUCTURE, LnP (D) was almost maximized at K=3

DISCUSSION

Genetic studies on bluefish are very limited worldwide, with only a few studies focusing samples taken from İstanbul and Çanakkale in Türkiye.

Miralles et al. (2014a) analyzed eight loci from a total of 120 *Pomatomus saltatrix* samples collected from 2004 and 2009, they calculated microsatellite allele sizes, null alleles, conformity to Hardy-Weinberg equilibrium, microsatellite variation (such as the number of alleles per locus, allelic richness, and observed and expected heterozygosity). According to Bayesian analysis (K=3), bluefish belong to three different genetic units with moderate admixture between them as indicated by genetic distances (K = 3, STRUCTURE test), mitochondrial and nuclear FST results and AMOVA analysis, which revealed some permeability between these genetic units). Their findings confirmed that bluefish populations belongs to the distinct genetic unit. However our results are inconsistent with this conclusion as we observed mixed individuals and low genetic differentiation across all clusters.

Miralles et al. (2014b) reported values close to our AMOVA test results, with (among population variation at 2,96 and within population variation at 96,3) Table 6. Furthermore their study suggests that the Strait of Gibraltar does not act as a barrier to gene flow for *Pomatomus saltatrix* as Spanish samples from both sides of the strait (Cadiz and Barcelona) showed no significant genetic differences.

Miralles et al. (2016) compared the genetic diversity of adult bluefish caught around an aquaculture farm in the Spanish waters of the Western Mediterranean with reference individuals from offshore stocks in the Eastern and Western Mediterranean. According to the study results, bluefish collected from around the fish farm exhibited very high genetic diversity in terms of both microsatellite and mitochondrial DNA, showing that the high genetic diversity of the bluefish caught in the farm is due to the mixing of populations (the Eastern and Western Mediterranean)although most of the individuals caught around the facility belonged genetically to the local population, 7.14% to 11.9% of the individuals belonged to the genetic population living in Türkiye waters. It was revealed that there was some degree of hybridization between the Eastern and Western Mediterranean bluefish stocks in the farm located in Guardamar in the Western Mediterranean. It confirms that individuals from a single population are mixed in all the stations we found.

Dos Santos et al. (2008) also investigated the genetic structure of bluefish populations that are widely distributed from Mozambique to Namiba on the coast of South Africa. They reported high polymorphic differences loci in eight polymorphic regions in their applied studies.

It is seen that eight loci are sufficient to distinguish polymorphic differences between bluefish populations. It is seen that the same loci and the study we examined revealed monomorphism in only one locus and polymorphic differences in the other seven.

When the principal coordinate analysis results are – examined, it is clearly seen that the Mersin population is clustered differently from other populations. However, according to the Mantel test, a low correlation (R^2 = 0.3666, P= 0.01) was detected between genetic and geographical distance. According to the structure analysis results, individuals in 14 regions could not be assigned to a different cluster for K = 2 and K = 3. It was observed that there were mixed individuals in all clusters and there was low genetic differentiation as a structure analysis result.

Reid et al. (2016) examined bluefish samples collected from the Africa coast to the Indian Ocean, analyzing variation in 15 polymorphic microsatellite loci. Contrary to our results, their results showed that; both sequence and microsatellite data showed population partitioning between southern Africa and other locations (South Africa, East Atlantic and another location), which could be explained by the Benguela upwelling as a barrier to gene flow. The absence of subgroups among bluefish populations in Türkiye seas indicates no barrier to gene flow between locations reported in the literature (Miralles et al., 2014a; Reid et al., 2016).

CONCLUSION

Our results showed that, contrary to the literature that;

- Loci showed highly significant deviations from the Hardy-Weinberg equilibrium.

- Pairwise FST comparisons revealed low levels of genetic differentiation (Fst < 0,05).

- For K= 3 structure analysis, individuals in 14 regions could not be assigned to dictinct cluster. (Figure 4).

- Mersin population was different from others according to principal coordinate analysis (Figure 2) but According to the Mantel test, a low correlation (R^2 = 0,3666, P = 0,01) was detected between genetic and geographical distance (Figure 3).

- AMOVA analysis showed that variation within populations was greater than variation between populations (Table 6).

In conclusion, our study reveals a high degree of genetic mixing among bluefish populations, contrary to previous findings that suggested distinct genetic units. The presence of mixed individuals across all clusters and low genetic differentiation indicates that gene flow is extensive and barriers to genetic exchange are minimal. The results emphasize the need for further studies incorporating broader geographic sampling and advanced genomic techniques to fully understand the population structure and genetic connectivity of bluefish. These findings have significant implications for conservation and fisheries management, underscoring the importance of considering genetic diversity and connectivity when developing sustainable management strategies.

ACKNOWLEDGEMENTS AND FUNDING

The authors would like to thank Assoc. Prof. Dr. Rafet Çağrı Öztürk for his valuable contributions in the article. This study was supported by the Republic of Türkiye Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies Agricultural (Funding No: TAGEM/ HAYSÜD /Ü/20/A6/P3/1814).

AUTHORSHIP CONTRIBUTIONS

İlyas Kutlu: Conceptualization, investigation, methodology, writing -original draft; Zehra Duygu Düzgüneş: Analysing statistic, writing -review and editing; Şirin Firidin: Writing -review and editing; Melike Alemdağ: Writing -review and editing; Ayşe Cebeci: Visualization, writing -review and editing; İbrahim Turan: Supervision, writing- review and editing.

CONFLICT OF INTEREST

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

ETHICS APPROVAL

All experiments were carried out considering the ethical rules of the authorities, with the approval coded as 325.04.02-12 by the Ethical Committee of Animal Experiments of Central Fisheries Research Institute.

DATA AVAILABILITY

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

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 42(2), 148-157 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.07

RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

The health management and medication use in trout farms located in Erzurum province and its districts

Erzurum il ve ilçelerinde bulunan alabalık çiftliklerinde sağlık yönetimi ve ilaç kullanımı

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Received date: 23.01.2025 *Accepted date: 12.05.2025*

How to cite this paper:
Karadaş, N.F., Duman, H., & Karadaş, K. (2025). The health management and medication use in trout farms located in Erzurum province and its districts. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 148-157. https://doi.org/10.12714/egejfas.42.2.07

Abstract: The aquaculture sector is very important both in terms of employment and the contribution of the products obtained to healthy nutrition. In order for this sector to grow at a sufficient speed and at a sustainable level, it is important to determine the diseases and control methods seen in the sector and to take more effective measures in this context. In this study, the level of use of drugs and vaccines currently used in land and dam fish facilities in Erzurum province and districts was determined, information and effective approaches were determined about the infectious diseases of fish in aquaculture environment and the drugs and vaccines used for these diseases. In this context, facility management practices were examined through questionnaires conducted with 41 farm managers in the region. At the end of the study, it was determined that the enterprises used an average of 51.58 kg of medicines, produced with a capacity of 50-100 tonnes and the most preferred water source (59%) was lakes. The enterprises mostly (54%) use open circuit system and 84% use pallet feed and 75% of the production is rainbow trout. The most common diseases are bacterial and fungal infections, parasitic and viral diseases. The preferred medicines are disinfectants, antibiotics, antiparasitics, supplements and fungicides. Although the producers work with 68% capacity, they stated insufficient demand, high costs and diseases as the reasons for this situation. Statistical analyses revealed important findings specific to the region through clustering analysis which identified two different groups on the basis of 22 important criteria. The results, in which the 'Diana' method was determined as the most appropriate clustering approach, provide applicable recommendations for improving facility management strategies. The findings provided detailed information on the drug utilisation patterns of the farms and made it possible to better understand the needs of the enterprises in the region. As a result, this study will provide important contributions not only to the strategic management of enterprises, but also to policy development processes for regional development.

Keywords: Fish disease, medication, control, protection, management

Öz: Su ürünleri sektörü gerek istihdam ve gerekse elde edilen ürünlerin sağlıklı beslenmeye katkısı nedeniyle oldukça önemlidir. Bu sektörün yeterli hızda ve sürdürülebilir düzeyde büyüyebilmesi için sektörde görülen hastalıklar ve mücadele yöntemlerinin belirlenmesi ve bu bağlamda daha etkin önlemlerin alınması önem arzetmektedir. Bu çalışmada Erzurum il ve ilçelerinde bulunan kara ve baraj balık tesislerinde hali hazırda kullanılan ilaç ve aşıların kullanım düzeyleri belirlenmiş, aquakültür ortamında balıkların enfeksiyon hastalıkları ve bu hastalıklar için kullanılan ilaçlar ve aşılar hakkında bilgiler ve etkili yaklaşımlar belirlenmiştir. Bu kapsamda bölgedeki 41 çiftlik yöneticisiyle yapılan anketler aracılığıyla tesis yönetimi uygulamalarını incelenmiştir. Çalışma sonunda işletmelerin ortalama 51,58 kg ilaç kullandıkları, 50-100 ton kapasite ile üretimde bulundukları ve en çok tercih edilen su kaynağının (%59) göller olduğu belirlenmiştir. İşletmeler çoğunlukla (%54) açık devre sistem ve %84 oranında palet yemi kullanmakta olup üretimin %75'ini gökkuşağı alabalığı oluşturmaktadır. En sık görülen hastalıklar bakteri ve mantar enfeksiyonları ile paraziter ve viral hastalıklardır. Tercih edilen ilaçların dezenfektan, antibiyotik, antiparaziter, takviye ve fungusitler olduğu tespit edilmiştir. Üreticiler %68 kapasite ile çalışmakla birlikte bu duruma yetersiz talep, yüksek maliyetler ve hastalıkları sebep olarak ifade etmişlerdir. Yapılan istatistiksel analizler, 22 önemli kriter temelinde iki farklı grup belirleyen kümeleme analiziyle bölgeye özgü önemli bulgular ortaya koymuştur. 'Diana' yönteminin en uygun kümeleme yaklaşımı olarak belirlendiği sonuçlar, tesis yönetimi stratejilerinin iyileştirilmesi için uygulanabilir öneriler sunmaktadır. Elde edilen bulgular, çiftliklerin ilaç kullanım alışkanlıklarına dair detaylı bilgiler sağlamış ve bölgedeki işletmelerin ihtiyaçlarını daha iyi anlamayı mümkün kılmıştır. Sonuç olarak, bu çalışma, yalnızca işletmelerin stratejik yönetimine değil, aynı zamanda bölgesel kalkınmaya yönelik politika geliştirme süreçlerine de önemli katkılar sağlayacaktır.

Anahtar kelimeler: Balık hastalığı, ilaç, kontrol, koruma, yönetim

INTRODUCTION

Aquatic product production is becoming an increasingly significant sector not only due to its importance in nutrition but also because of its contribution to global food security. This sector, which has seen rapid growth in production in recent years, is making a significant impact on the global economy through its growing share in international trade. The export of fish and fish products plays a key role in the economic structure of many countries (Çöteli, 2024).

Technological innovations and the rising demand for fish as an animal protein source are among the primary factors supporting the growth of the sector. With the expansion of the industry, farming methods have become more intensive in order to achieve higher yields (Rico et al., 2012). Today, aquaculture has become the fastest-growing agricultural food production sector in the world and makes significant contributions to the socio-economic development and food security of many countries. Global aquaculture production practices have transitioned from traditional, extensive methods to intensive and semi-intensive systems, where industrial feeds are used to farm key fish species at higher stocking densities (Assefa and Abunna, 2018; Plant and LaPatra, 2011). In recent years, Türkiye has shown significant progress in aquaculture. As of 2023, aquaculture accounted for 55% of Türkiye's total aquatic product production. Of this aquaculture production, 72% is in marine environments, and 28% is in inland waters. Among marine fish, sea bass and gilt-head bream are the most preferred species, while trout is the most preferred species among freshwater fish. Türkiye is progressing towards becoming one of the leading countries in Europe for aquaculture. Aquaculture in Türkiye has become diversified with the production of various species. Of the species raised in both marine and inland waters, 40% are trout, 29% are sea bass, and 28% are gilt-head bream. Additionally, species like salmon and tuna stand out as high-value products, while local species such as carp, catfish, and crayfish are widely consumed in the Turkish market. These species are in high demand, particularly in European and Asian markets (Çöteli, 2024).

The sustainable growth of the aquaculture sector faces several challenges, with diseases being one of the primary obstacles. Particularly in intensive and semi-intensive farming systems, diseases can lead to significant economic losses. The healthy and disease-free cultivation of fish is a fundamental condition for sustainable aquaculture production. Preventing and controlling fish diseases requires the implementation of measures that minimize losses in this field. This is particularly important in commercial fish farms, where treatment and intervention options are limited (Chong et al., 2011). A large portion of the fish produced in aquaculture is intended for human consumption. However, various reasons have caused significant production losses in the aquaculture sector. The primary cause of these losses is diseases, which lead to job losses and income reductions, negatively affecting farmers' livelihoods. According to studies, nearly half of production losses are caused by diseases, and these losses are more pronounced in developing countries. This is because the majority of aquaculture businesses are located in developing countries. Annual income losses due to diseases are reported to reach as high as 6 billion dollars (Assefa and Abunna, 2018).

The improvement of genetic material (germplasm) and the support of effective management practices, along with the careful use of chemicals, biological products, and veterinary medicinal products (VMPs), play a critical role in ensuring the economic sustainability of the sector. Pharmaceutical products and chemicals have made significant advancements in global human and animal health and livestock production. Similarly, in aquaculture, the use of chemicals, biological agents, and VMPs that improve environmental and health conditions is applied to achieve higher production efficiency. In aquaculture businesses, chemicals, nutritional supplements, probiotics, disinfectants, antimicrobials, and antiparasitic drugs are commonly used to maintain environmental quality, improve health status, promote growth and production, and combat microbial and parasitic diseases (Patil et al., 2022). To reduce infection-related losses in aquaculture, it is important to intervene in each health constraint based on scientifically supported and locally applicable methods (Peeler and Taylor, 2011). Climate change, limited water resources, and growth pressures increase the need for epidemiological approaches to protect aquatic animal health. Based on the principle "prevention is better than cure," focusing on preventing the emergence of diseases is more logical (Romero et al., 2012). In the control of infectious diseases, advanced management practices, movement restrictions, genetically resistant stocks, diet supplements, general immune stimulants, vaccines, probiotics, prebiotics, medicinal plant products, water disinfection, biological control, antimicrobial agents, and movement control are the most effective methods (Kumar et al., 2016).

The main objective of this study is to identify the diseases occurring in fish farming facilities operating in Erzurum province and its districts and to determine the usage rates of vaccines, medications, and disinfectants used for the treatment of these diseases. It is believed that this study will benefit the aquaculture and fishing industry in terms of disease management, treatment methods, cost analysis, sustainability, and awareness.

MATERIALS AND METHODS

Erzurum province has an area of 25,066 km², located between 40° 14' 15" and 42° 33' 35" east longitudes, and 40° 54' 57" and 39° 06' 10" north latitudes, with an approximate surface area of 25,000 km². Erzurum province consists of 19 districts. This study focuses on the medications, antimicrobials, dietary supplements used by fish farms operating in Erzurum province and its districts, their quantities, and the annual fish production capacities. Through interviews and surveys conducted with fish farms, detailed data was collected on the health management practices and production performance of the businesses. The findings will provide significant contributions to evaluating the fish farming practices in the region and the sustainability of these practices. According to official records, there are currently 54 active fish farms in Erzurum province, of which 19 are land-based facilities and 35 are dam-based facilities. The fish farms are located in the districts of Aziziye, Yakutiye, İspir, Oltu, Olur, Pasinler, Pazaryolu, Şenkaya, Tortum, and Uzundere. Rainbow trout, brown trout, and brook trout are cultivated in these facilities.

The study was conducted to cover fish producers operating in Erzurum province (Figure 1). The data were collected through surveys conducted via telephone. The study adopted a census method, and a total of 53 fish producers were contacted. However, due to varying levels of participation from the businesses, 41 facility managers agreed to respond to the survey questions. This was taken into account during the process of evaluating the study's findings, and the data were processed using relevant analytical methods. The research used a 27-question survey form consisting of both open-ended and closed-ended questions, which were developed by the researchers. Prior to analysis, responses to the open-ended questions were standardized to reduce interpretive variability and ensure a consistent data structure. Synonymous expressions were merged into unified categories, spelling errors were corrected for consistency, and longer responses were examined to extract and classify significant patterns. Additionally, some responses were assigned to multiple codes or thematic groups, facilitating a multidimensional evaluation of the data. This approach aligns with established qualitative coding practices, where similar meanings are grouped under the same code to enhance clarity and analytical depth (Brailas et al., 2023; Hensley et al., 2024).

Figure 1. Map of the fish farms reached in the study

The Shapiro-Wilk test was used to test the normal distribution assumption in the analyses. The homogeneity of the data was examined using the Levene test. It was observed that the continuous variables in the data set did not show normal distribution. Therefore, the Mann-Whitney U test, which does not require parametric assumptions, was preferred when comparing mean differences between groups.

While preparing the graphs, cumulative percentage totals were incorporated in accordance with the principles of Pareto analysis. Pareto Analysis, also known as the Pareto Principle or the 80/20 Rule, is an analytical method used as a rough approach to understand and prioritize the root causes of a problem or situation (Parmenter, 2007). A Pareto diagram typically consists of two elements: vertical bars and a cumulative percentage curve. The bars are vertical columns that represent the factors ranked according to their impact. The cumulative percentage curve is represented by a line illustrating the effects of these bars and the percentage of total impacts. This analysis is commonly applied to identify critical factors essential for productivity improvements or the efficient use of resources (Souza, 2022).

In the preprocessing phase, the dataset was first examined for any missing data. It was found that there was a missing observation in the "medicine" variable, which was addressed using the bagging tree algorithm. This imputation method was chosen due to its ability to handle missing data effectively by generating multiple decision trees and combining them to make predictions (Kuhn and Johnson, 2013).

Subsequently, the dataset was linearized using the Yeo-Johnson method (Yeo and Johnson, 2000). This transformation was applied to stabilize variance and make the data more normally distributed, which is essential for ensuring the reliability of subsequent analyses.

After linearization, all values were normalized to z-scores, standardizing the dataset with a mean of zero and a standard deviation of one to prevent bias in scale-sensitive methods (Brailas et al., 2023).

Clustering is one of the fundamental data mining techniques used to uncover patterns and latent structures in multidimensional datasets. The primary objective of this method is to create a meaningful structure by grouping items within a dataset that share similar characteristics (Kassambara, 2017). Clustering analysis is performed using various algorithms, which are based on features such as distances between data points, density values, or statistical distributions (Toomey, 2014).

In this study, clustering analysis was conducted due to its suitability for identifying underlying patterns and grouping similar items within the dataset. Before proceeding with clustering analysis, the suitability of the dataset for clustering was assessed using the Hopkins test (Hopkins and Skellam, 1954). The Hopkins test is a method employed to determine whether a dataset has a random structure or one conducive to clustering (Banerjee and Dave, 2004). This statistical test evaluates whether a given dataset was generated by a uniform distribution and analyzes the spatial randomness of the data (Kassambara, 2017).

Standard clustering methods are broadly classified into hierarchical and non-hierarchical techniques. Non-hierarchical approaches aim to find the optimal partition for items in clusters by minimizing a cost function, while hierarchical methods iteratively merge (agglomerative) or split (divisive) clusters, producing a sequence of partitions. Traditional clustering methods are termed "hard" clustering, where each item is assigned to a single cluster. In contrast, fuzzy and model-based clustering allow more flexibility, termed "soft" clustering (Giordani et al., 2020). Agglomerative clustering (e.g., Agnes) builds clusters bottom-up by merging the most similar ones, while divisive clustering (e.g., Dıana) works top-down, splitting the most heterogeneous clusters (Kassambara, 2017).

Center-based algorithms, such as k-means, k-medoids Pam and Clara are effective for large datasets. K-means minimizes the total within-cluster sum of squares but is sensitive to outliers. Pam, uses medoids instead of means, offering robustness against noise. Clara extends Pam by sampling subsets of data to improve scalability (Gan et al., 2020). Model-based clustering assumes data are generated by probabilistic processes, modeling clusters as components of a mixture model. Parameters are estimated using the Expectation-Maximization algorithm, and cluster validity is assessed using Bıc (Kassambara, 2017).

For the clustering analysis, the Ward.D2 method was applied in conjunction with the Euclidean distance metric. The Ward.D2 method was chosen for its effectiveness in minimizing the variance within clusters while maximizing the variance between them. Euclidean distance was used as the similarity measure to calculate the distance between data points, enabling the identification of natural groupings within the data (Charrad et al., 2014; Kassambara, 2017).

In this study, the “NbClust” package in R was used to determine the optimal number of clusters by considering various indices. Clustering analyses were conducted using seven different consistency criteria, including internal consistency and stability. Hierarchical clustering, k-means, Dıana, Pam, Clara, Agnes, and model-based clustering methods were applied. These procedures were performed using the “clValid” package in the R statistical programming environment. Analyses were conducted in R (v4.2.2) using packages such as dplyr, ggplot2, NbClust, mclust, and clValid. Code implementation was adapted from Duman (2024).

RESULTS

Both the quantitative and qualitative aspects of the survey data were descriptively analyzed. The duration of activity of the participating businesses ranges from 1 to 22 years (Figure 2). The average number of years in operation is 7.29 years, with a standard deviation of 5.58 years. According to the results of the Shapiro-Wilk test, no statistical evidence was found to suggest that the data follows a normal distribution (W=0.820; p<0.001). It was found that the annual effective capacity amounts of the businesses showed a wide range of distribution. Among the participating businesses, there are fish farms of different scales, producing between 10 tons and 1500 tons of fish annually. The average effective capacity is 199.49 tons, with a standard deviation of 308.19 tons. However, it was determined that these data do not conform to a normal distribution (W=0.610, p<0.001).

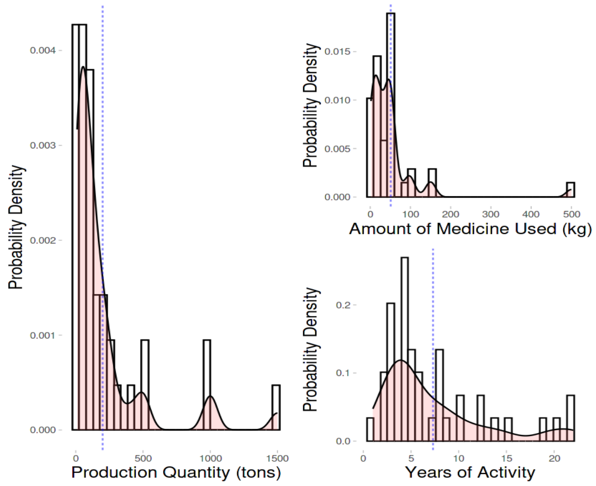

Figure 2. Histogram charts of quantitative variables

In terms of the amount of medication used, a high variation and a wide range [2-500 tons] were observed, similar to the variation in business sizes. The average amount of medication used was calculated to be 51.58 kg, with a standard deviation of 81.10 kg. Additionally, according to the results of the Shapiro-Wilk test, it was determined that the data does not follow a normal distribution (*W=0.493, p<0.001*). It was observed that the data for all three variables (the establishment year of the business, annual production quantity, and the amount of medication used) did not follow a normal distribution. When examining the districts where the businesses are located, it was determined that 34% of the participants operate in Oltu, 20% in İspir, 16% in Aziziye, 11% in Olur, 7% in Tortum, 5% in Pasinler and Yakutiye, and 2% in Pazaryolu. Furthermore, it was found that three businesses operate in multiple districts.

Survey participants were asked about the theoretical capacity of their businesses. It was determined that 7% of the businesses have a production capacity of 0-10 tons (n=3), 15% have a capacity of 10-50 tons (n=6), 61% have a capacity of 50-100 tons (n=25), and 17% have a capacity of over 100 tons (n=7). These data show that a large portion of the businesses are concentrated in the 50-100 ton capacity range. According to the research results, the most common source for meeting the water needs of businesses is lakes. 59% of the participating businesses indicated that they mainly meet their water needs from lakes. Rivers follow with 22%, and groundwater accounts for 12%. Other sources make up 7% in total.

54% of the participating businesses reported using the open circuit system. This system is followed by other systems with 44%, and the closed circuit system ranks third with the lowest percentage of 2%. Looking at the cumulative values, almost all businesses (98%) use either the open circuit or other systems. The findings revealed that the dominant production type is rainbow trout, which constitutes approximately 75% of total production. Rainbow trout is followed by brown trout, other trout species, and brook trout. These results indicate that rainbow trout is the dominant species in trout production in Erzurum. This situation may have been influenced by various factors, including the physical and chemical properties of the ecosystem, as well as production strategies applied in the past.

68% of the participants stated that they are unable to fully utilize their production capacities, while 32% reported that they are utilizing it fully. When examining the reasons for not reaching theoretical capacity, 41% of the participants (n=24) cited insufficient demand, 32% (n=19) pointed to high costs, and 20% (n=12) mentioned that diseases prevent them from reaching theoretical capacity. The remaining 7% (n=4) highlighted other various reasons.

When evaluating the annual production changes over a five-year period, 35% of the participants (n=14) reported a 35% decrease in production, while 32% (n=13) expressed a 32% increase in production. The remaining 32% of participants (n=13) reported that the production level remained constant. When examining fish feed preferences, it was found that 84% of the participants (n=36) prefer pellet feed. The proportion of those who prefer other types of feed was found to be 12% (n=5). Only 5% of participants (n=2) reported using organic feed.

In terms of preferred types of medication, 27.62% of participants (n=29) most frequently preferred disinfectants. Disinfectants were followed by antibiotics at 24.76% (n=26), antiparasitics at 20.95% (n=22), supplements at 15.24% (n=16), fungicides at 10.48% (n=11), and vaccines at 0.95% (n=1). The results of the Pareto analysis show that disinfectants, antibiotics, and antiparasitics are the most frequently preferred types of medication (Figure 3).

When examining the factors affecting the frequency of medication use, 51% of participants (n=32) identified disease frequency, and 46% (n=29) identified preventive measures as the most important determinants. Other factors and legal obligations were mentioned by only 4% of participants (n=2). These findings indicate that disease frequency and preventive measures are the factors that most influence medication use decisions. When asked about the methods of medication application, the most frequently preferred method was adding medication to feed (52%, n=33). This was followed by the method of mixing medication into water (46%, n=29). Injection application was preferred by only 2% of participants (n=1).

When examining the most common fish diseases in the farms operating in Erzurum province over the past year (Figure 4), it was found that *Aeromonas* bacteria and fungal infections were the most prevalent diseases, each accounting for 26.67% (n=16) of cases. These were followed by *Yersinia Ruckeri* infection (18.33%, n=11), parasitic diseases (10%, n=6), and viral diseases (6.67%, n=4). Other diseases made up only 2% of the total cases. When the frequency of these diseases was examined, it was observed that most cases occurred "occasionally" (49%, n=20), but some diseases were reported as occurring "frequently" (39%, n=16). Particularly, *Aeromonas* and fungal infections were defined as "very frequent" in 7% of the cases (n=3). When participants were asked about the methods used to combat diseases, it was found that the most commonly preferred method was medication (40%, n=23). This was followed by preventive measures (18%), vitamin supplementation (14%), vaccination and feed adjustment (9%), disinfection (7%), and isolation with probiotic use (2%). These results indicate that fish farmers prioritize medication when dealing with diseases. However, it was also observed that methods such as preventive measures, vitamin supplementation, and vaccination were significantly used. Specifically, feed adjustment and disinfection can be considered critical methods in disease prevention. Additionally, the use of alternative methods for disease control was examined. While 72% of participants (n=29) stated that no alternative methods other than medication were used, 28% (n=11) indicated that such methods were employed.

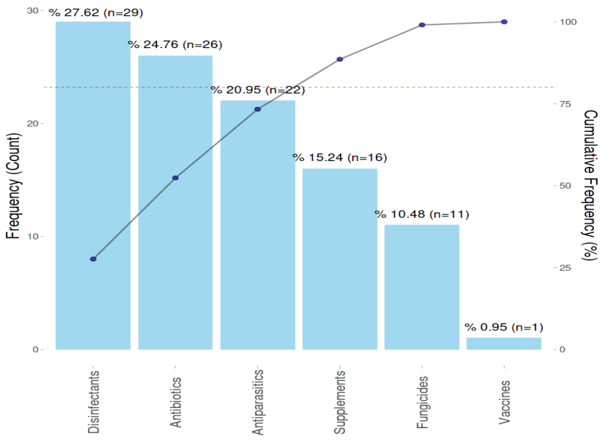

Figure 3. Types of medications most frequently used in fish farms

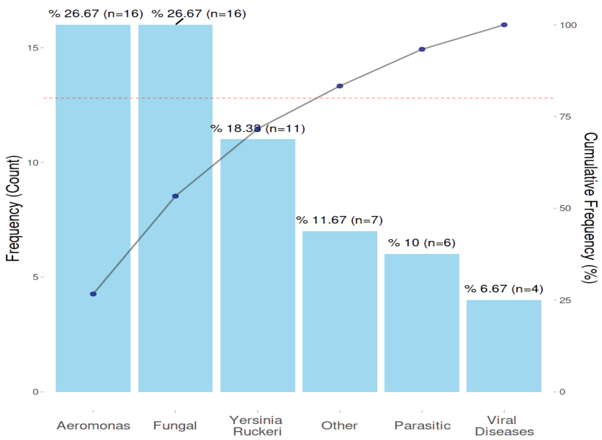

Figure 4. Common fish diseases

When the preventive measures taken by participants for fish health and production efficiency were examined, appropriate feeding (32.14%, n=18) emerged as the most common practice, followed by hygiene and water quality measurement (16.07%, n=9). Other preventive measures included capacity-appropriate production (7.14%, n=4), disinfection (5.36%, n=3), medication (5.36%, n=3), isolation (5.36%, n=3), vitamin supplementation (5.36%, n=3), egg quality (3.57%, n=2), and others (3.57%, n=1). Based on the evaluation of the findings, feeding emerged as a key factor; a large portion of participants indicated that appropriate feeding is essential for fish health. This reflects the direct impact of nutritional balance on the growth, development, and disease resistance of the fish. Hygiene and water quality measurement were also commonly used as important preventive measures, indicating that farmers are aware of the impact of water quality on fish health (Figure 5).

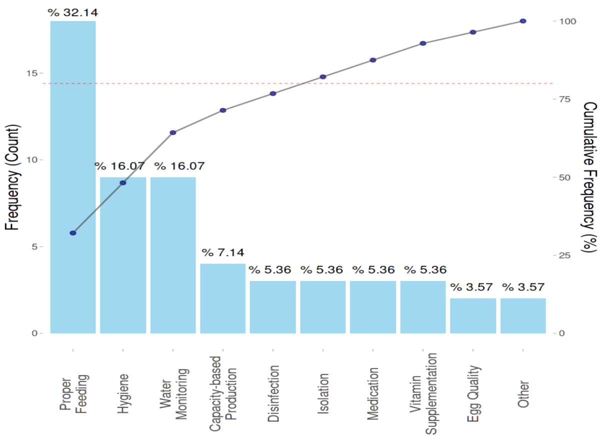

Figure 5. Preventive measures implemented for fish disease management

The current study did not include data on the frequency and parameters of water quality measurements or specific details about the disinfection process, including items subjected to disinfection and substances used. These factors are crucial in understanding the influence of environmental conditions on disease occurrence and spread in trout farming. Future research should focus on these aspects to provide a more comprehensive understanding of the interplay between water quality, disinfection practices, and disease management.

The widespread practice of capacity-appropriate production highlights that fish density is critical for healthy production, preventing fish from experiencing stress and the increase of diseases. Disinfection and medication are commonly used methods for controlling diseases, but it should be noted that excessive use of medication could lead to issues such as antibiotic resistance.

The responses to the open-ended question "What are your suggestions for improving fish health and production in your farm?" have been categorized and standardized under six main thematic groups: Feeding and Nutrition, Health and Hygiene, Environmental and Production Conditions, Marketing and Economic Factors, Production Strategies, and Support and Policies. Each response contains suggestions associated with these groups, and one response may cover multiple groups (Figure 6). For example, suggestions such as using quality feed (Feeding and Nutrition) and ensuring hygienic conditions (Health and Hygiene) to improve fish health have been evaluated simultaneously. This standardized approach allows for a systematic analysis of the responses and ensures that the suggestions are more effectively assessed in practice, contributing to the development of more effective strategies for increasing production efficiency and ensuring sustainability in fish farms.

According to the Pareto analysis, Health and Hygiene (29%), Feeding and Nutrition (27%), and Marketing and Economic Factors (24%) account for a total of 80% of the impact. The remaining three factors Production Strategies (11%), Environmental and Production Conditions (8%), and Support and Policies (2%) represent only 20% of the total impact. This analysis indicates that when prioritizing areas for improvement in fish farms, special attention should be given to Health and Hygiene, Feeding and Nutrition, and Marketing and Economic Factors. These three areas have been perceived by farm managers as the most critical factors affecting performance and production on the farms.

When participants ranked the biggest challenges in the sector, the main issues identified were costs (37%), diseases (34%), market competition (22%), and other factors (7%). These findings indicate that the main challenges in the sector stem from cost pressure, health issues, and competitive conditions. Rising feed, energy, and labor costs have been predicted to increase production costs and reduce profit margins, while fish diseases can lead to both production losses and cost increases. Increased market competition may lead producers to sell at lower prices, thus reducing their profit margins. Furthermore, seasonal fluctuations, raw material supply, environmental regulations, and changes in consumer preferences should also be considered as factors that could affect the challenges in the sector.

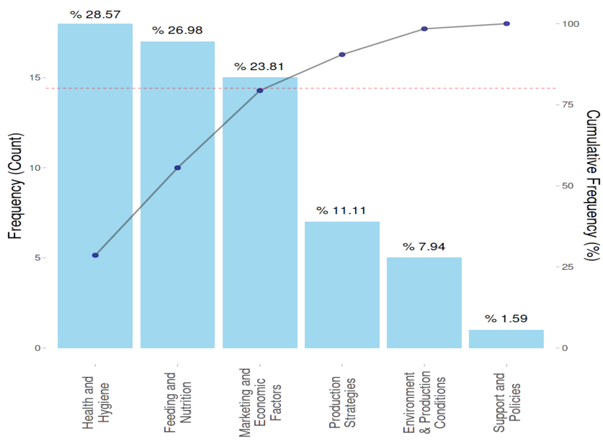

Figure 6. Suggestions for improving fish health and production on the farm

When the open-ended question "Which diseases do you use vaccines for?" was subjected to content analysis, it was found that 66% (n=21) of the participants did not use any vaccines. Among the participants who used vaccines, the most commonly reported vaccines were for *Yersinia Ruckeri* (45%, n=5), vaccines for all diseases (27%, n=3), *Aeromonas* (18%, n=2), and parasites (9%, n=1). When evaluating all participants, the vaccine usage rates for *Yersinia Ruckeri* (16%, n=5), vaccines for all diseases (9%, n=3), *Aeromonas* (6%, n=2), and parasites (3%, n=1) were identified. This study relies on subjective responses from business owners, which may lead to misclassifications or misunderstandings, such as claims of using vaccines against parasites an option not currently available in routine aquaculture.

When the responses of the participants in the study were examined regarding the challenges they faced in obtaining medications and vaccines, it was found that cost (64%, n=21) emerged as the most significant barrier. Other factors such as the procurement process, lack of qualified personnel, insufficient knowledge, indifference, and usage preferences were also frequently mentioned challenges by the participants (24%, n=8). Additionally, 12% (n=4) of the participants stated that they did not encounter any difficulties in this regard.

In the evaluation of the use of natural or herbal medicines in the farms, the vast majority of participants (%76) reported that they do not use such medicines. However, a significant proportion (%24) prefers natural or herbal medicines. Among those who use natural or herbal medicines, the most commonly preferred product is salt, with a rate of %47 (n=9). This is followed by clove oil at %26 (n=5) and other products (such as mint, lemon, garlic, etc.) at %26 (n=5). Before starting the clustering process, the data underwent certain preprocessing steps. First, the dataset was checked for any missing data, and it was found that there was a missing observation in the "medicine" variable. This missing observation was completed using the bagging tree algorithm. The data was then linearized using the Yeo-Johnson method, and all values were normalized by converting them into z-scores.

As a result of the clusters analysis, based on the suggestion of 7 different indices, the optimal number of clusters was determined to be 2. Additionally, in the evaluations made to select the best clustering method, the "diana" method, indicated by 3 measures according to the majority rule, was preferred. The silhouette plot for the clusters showed that the clusters are consistent and distinct (Figure 7).

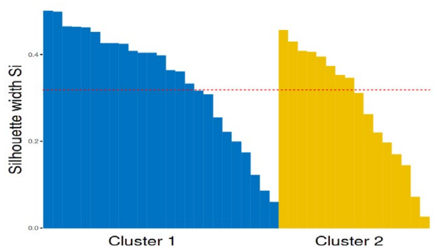

Figure 7. Silhouette chart

The mean differences of the continuous variables between the clusters in the dataset were examined using the Mann-Whitney U test, as it was determined that these variables do not exhibit a normal distribution. As a result of the examination conducted for the obtained clusters, it was found that the mean differences of these variables between the clusters are statistically significant. Descriptive statistics for these variables are presented in the table below for Cluster 1 and Cluster 2 (Table 1).

Table 1. Examination of continuous variables between clusters

Variable	Cluster 1	Cluster 2
Farm service duration (years)	9.48^{a}±6.06	3.88 ±1.96
Actual production capacity (tons/year)	305.00^{a}±357.98	34.62^{b}±26.58
Drug consumption (kg/year)	75.40^{a}±95.20	11.87^{b}±9.23

No statistically significant difference was found between the means of the clusters with the same letter group (α=0.05).

These statistics clearly show the magnitude of the differences between the groups. The means of the farm service duration, actual production capacity, and drug consumption variables are higher in Cluster 1 compared to Cluster 2. This indicates that the farms represented by Cluster 1 have a longer service duration, higher production capacities, and greater drug consumption amounts. Therefore, Cluster 1 can be named "Large and Established Companies." Based on the findings, it is expected that these farms are generally well-established, large-scale, and corporate entities. Cluster 2, on the other hand, can be named "Small and Newly Established Companies." This cluster includes farms with shorter service durations and lower production capacities.

When examining the geographical distribution of the clusters (Figure 8), it was observed that the farms in the "Large and Established Companies" cluster are concentrated in the Aziziye and Yakutiye regions. Similarly, farms in the "Small and Newly Established Companies" cluster were predominantly located in the Pasinler, Pazaryolu, Olur, and Tortum districts. A more heterogeneous distribution was observed in the İspir and Oltu districts, with a notable concentration of "Large and Established Companies" in İspir.

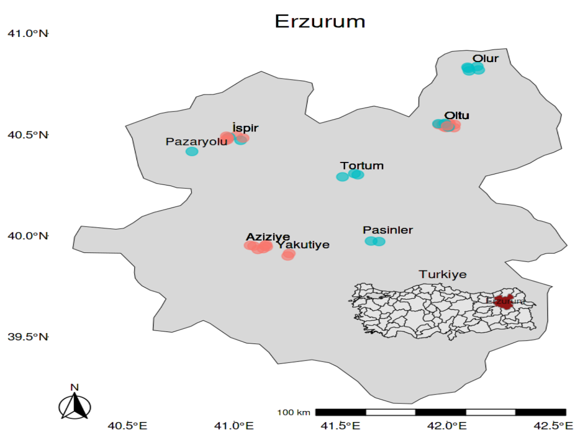

Figure 8. Geographical distribution of the clusters

DISCUSSION

At the and of the study, it was observed that chemical drugs and antibiotics are commonly used in the farms for disease prevention. This raises concerns regarding environmental and consumer health. Similar findings have been reported in other studies; Samanidou and Evaggelopoulou (2007), unconsciously excessive they reported that the use of drugs will lead to accumulation of these drugs in the bodies of fish, destruction of internal organs, resistance of bacteria to these drugs in long-term use and economic losses due to excessive use of drugs. Türk and Oğuz (2013) stated that Türkiye has an important aquaculture potential and when the drug consumption rates are analysed, antibiotics are mostly used in fish farming. Yonar and Sağlam (2013) stated that aquaculture in Türkiye is a rapidly developing sector due to its favourable geographical location and climatic characteristics, but the production potential in aquaculture cannot be fully utilised, sufficient yield level cannot be reached due to feeding errors and diseases, sudden changes in environmental conditions, stress factors such as stock density and the presence of pathogenic microorganisms increase the mortality rate and can cause significant economic losses. Topal (2017) analysed the concentrations of tetracycline and its degradation products in a trout farm and surrounding waters and stated that farm activities may cause antibiotic residues in the environment. Dandi et al. (2024) examined the knowledge, attitudes and practices of fish farmers in Ghana on antibiotic use and found that the level of education had a significant effect on the correct use of antibiotics. Furthermore, improper and indiscriminate drug use can trigger health problems such as bacterial resistance. In the trout farms of Erzurum, production rates were generally found to be satisfactory, although in some farms, production capacity was found to be below potential, and productivity could be improved. This indicates the need for improvements in the management processes of the farms. Similarly, studies conducted in different regions of Türkiye emphasize the need for adopting practices that enhance the productivity of trout farming. Environmental impacts of trout farms, particularly waste management and water use, are discussed. In this context, it is crucial for farm owners to adopt environmentally friendly practices and develop new strategies to protect ecosystems. To increase the efficiency of trout farms and ensure sustainable production, local governments and the Ministry of Agriculture should develop policies and regulations to manage health and drug use. Additionally, promoting biological control methods and organic farming techniques in the farms could reduce the use of drugs. In this regard, increasing educational and awareness programs is also important (Koca et al., 2011). The findings regarding health management, drug use and production rates in the trout farms of Erzurum suggest that existing practices need to be improved. By adopting environmentally friendly and sustainable farming practices, farm owners can enhance productivity and reduce environmental impacts. In the future, regulations and training programs in this field will contribute to moving Türkiye's trout farming sector toward a more sustainable future.

The results of the study indicate that fish farm owners report using vaccines against parasites as part of their disease management practices. However, this finding contrasts with the current state of vaccine availability and usage in aquaculture. At present, vaccines for parasitic diseases are extremely limited. Existing commercial fish vaccines primarily address bacterial and viral pathogens, and no widely available or routinely applied vaccines for parasites exist. The discrepancy observed in the responses may stem from a lack of technical knowledge or misinterpretation of the term "vaccine" by the farm owners. This highlights a critical need for education and outreach efforts to improve understanding of disease prevention tools and their appropriate applications within the aquaculture sector.

CONCLUSION

The phrase "Prevention is more effective than treatment" holds significant importance in the context of fish diseases and health preservation. Disease prevention is not limited to treatment with modern vaccines but begins with the proper management of the organisms. The concept, defined as "good care" in mammals, is also applicable to fish management, where it is critical to raise fish in the most optimal conditions. This means maintaining high water quality and planning the necessary steps to achieve this in advance. Additionally, reducing the pathogen load in the water environment is also important. To achieve this, stocking density should be carefully adjusted, fish should be sourced from "clean" sources, ponds should be regularly emptied and cleaned, and "clean" feed should be used. In marine aquaculture, the cyclical use of farming areas and the proper disposal of dead fish play a key role in maintaining a clean environment. These measures are fundamental strategies to protect fish health and prevent the spread of diseases (Chong et al., 2011).

The results of this study provide detailed descriptive information about the drug usage habits of rainbow trout production farms operating in Erzurum province. Cluster analysis identified two main clusters, referred to as "Large and Established Companies" and "Small and Newly Established Companies." It was observed that the farms in the "Large and Established Companies" cluster are concentrated in the Aziziye and Yakutiye regions, while farms in the "Small and Newly Established Companies" cluster are primarily located in the Pasinler, Pazaryolu, Olur, and Tortum districts. Cluster analysis helps better understand the needs and characteristics of businesses in the region, offering significant advantages from a strategic, economic, and operational perspective. Public institutions and sectoral support organizations can develop broader incentives for large enterprises and support programs focused on training, guidance, and modernization for small businesses based on these analysis results. Additionally, identifying the resource needs of businesses allows for more efficient resource allocation. Cluster analysis can also contribute to monitoring the overall health of regional economic activities and the development of growth strategies, while fostering cooperation between businesses and enabling them to gain competitive advantages. In terms of risk and crisis management, this analysis enables businesses to understand their risk profiles and provides valuable information, such as larger firms being more resilient to crises and smaller firms being more vulnerable.

This study also underscores the importance of addressing knowledge gaps among aquaculture practitioners, as misconceptions about available disease control measures can lead to ineffective practices and hinder advancements in disease management strategies. Future studies should aim to validate self-reported data and investigate the factors contributing to these misinterpretations to enhance disease control practices and support sustainable aquaculture development.

In conclusion, it can be said that the clustering analysis conducted in this study provides valuable contributions not only to the strategic management of businesses but also to the policy development processes for regional development. The findings allow for targeted planning for the growth and development of businesses.

ACKNOWLEDGEMENTS AND FUNDING

We would like to thank Edip Bingöl and Canip Koçak, fisheries engineers at the Erzurum Provincial Directorate of Agriculture, for facilitating our connection with the fish farm authorities for the survey work.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no financial interests or personal relationships that could influence this work.

AUTHOR CONTRIBUTIONS

Study design, literature review, methodology, data collection: Naime Filiz Karadaş, Hakan Duman. Data analysis: Hakan Duman. Writing the article: Naime Filiz Karadaş, Köksal Karadaş. Supervision: All authors have approved the final draft.

ETHICAL APPROVAL STATEMENT

As this study involved a survey, the necessary Ethical Committee Approval (no. 2024/28) was obtained from Iğdır University.

DATA AVAILABILITY STATEMENT

The data used in this study can be obtained from the corresponding author upon reasonable request.

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Ege Journal of Fisheries and Aquatic Sciences, 42(2), 158-172 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.08

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Depth-gradient biometrics of the invasive seaweed *Caulerpa taxifolia* spreading northward along the Turkish Aegean coast

Türkiye Ege kıyılarında kuzeye doğru yayılan istilacı deniz yosunu *Caulerpa taxifolia*'nın derinlik-meyilli biyometrisi

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Received date: 15.05.2025 *Accepted date: 02.06.2025*

How to cite this paper:
Mutlu, E., Akçalı, B., Özvarol, Y., Zabun, Z., Narlı, Z., & Aslan, B.E. (2025). Depth-gradient biometrics of the invasive seaweed *Caulerpa taxifolia* spreading northward along the Turkish Aegean coast. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 158-172. https://doi.org/10.12714/egejfas.42.2.08

Abstract: The presence of the invasive green alga *Caulerpa taxifolia* has been documented in the Turkish Aegean Sea, prompting a comprehensive investigation into its depth biometry and ecological interactions. This study sought to assess the distribution, density, and morphological characteristics of *C. taxifolia* at four distinct depths (10, 15, 20, and 30 m) in Dikili Bay, İzmir, during the summer of 2024. A total of 321 sampling stations were surveyed, and *C. taxifolia* specimens were identified at four locations. Sampling was performed by SCUBA divers using a 0.4 x 0.4 m quadrat. Biometric measurements included shoot density, stolon length, frond length, and number of pinnae per frond. Environmental parameters such as temperature, salinity, pH, and light intensity were simultaneously recorded to evaluate their influence on algal growth. The internodal distance (frond) on stolon exhibited a range from 18.4 mm at the 15-m depth to 38.4 mm at the 30-m depth. The maximum shoot density (1397 shoots/m^2) was recorded at the deepest sampling depth. The minimum density of 343 shoots/m^2 was observed at 15 m. The number of fronds (shoots) per stolon showed a gradual decrease with depth up to 20 m. The length of the stolons ranged from 178 mm to 355 mm, with the shortest stolon occurring in the shallowest water, followed by a gradual increase with increasing bottom depth. Frond length exhibited a range from 49.9 mm at 10 m to 87 mm at 20 m, followed by 71 mm at 30 m. Frond width remained within a narrow range of 0.855 cm to 1.1139 cm across the bottom depth. The widest frond was observed at 30 m, while the narrowest frond was recorded at 10 m. A total of 35% of the specimens examined at 10 m were found to be budded, 12% at 15 m, and 7% at 20 m, and 20% at 30 m. The number of branchlets ranged from 6-18 to 156-210, with an average of 76 to 94 and 10-12 per 1 cm frond length.

Keywords: *Caulerpa taxifolia*, invasive species, biometric, depth distribution, Turkish Aegean Sea

Öz: İstilacı yeşil alg *Caulerpa taxifolia*'nın varlığı Türkiye Ege Denizi'nde belgelenmiş olup, bu durum derinlik biyometrisi ve ekolojik etkileşimleri hakkında kapsamlı bir araştırma yapılmasını sağlamıştır. Bu çalışma, 2024 yazında İzmir, Dikili Körfezi'nde dört farklı derinlikte (10, 15, 20 ve 30 m) *C. taxifolia*'nın dağılımını, yoğunluğunu ve morfolojik özelliklerini değerlendirmeyi amaçlamıştır. Toplam 321 örnekleme istasyonu araştırılmış ve *C. taxifolia* örnekleri dört lokasyonda tanımlanmıştır. Örnekleme, 0,4 x 0,4 m'lik bir kuadrat kullanılarak SCUBA dalgıçları tarafından gerçekleştirilmiştir. Biyometrik ölçümler sürgün yoğunluğu, stolon uzunluğu, yaprak uzunluğu ve yaprak başına pinna sayısını içermiştir. Sıcaklık, tuzluluk, pH ve ışık yoğunluğu gibi çevresel parametreler, alg büyümesi üzerindeki etkilerini değerlendirmek için eş zamanlı olarak kaydedilmiştir. Stolon üzerindeki sürgün arası mesafe (frond) 15 m derinlikte 18,4 mm'den 30 m derinlikte 38,4 mm'ye kadar bir aralık gösterdi. Maksimum sürgün yoğunluğu (1397 sürgün/m^2) en derin örnekleme derinliğinde kaydedildi. Minimum yoğunluk 343 sürgün/m^2 olarak 15 m'de gözlendi. Stolon başına düşen frond (sürgün) sayısı, 20 m'ye kadar derinlikle kademeli bir azalma gösterdi. Stolonların uzunluğu 178 mm ile 355 mm arasında değişti; en kısa stolon en sığ suda meydana geldi, ardından artan dip derinliğiyle kademeli bir artış izledi. Frond uzunluğu 10 m'de 49,9 mm'den 20 m'de 87 mm'ye kadar bir aralık gösterdi, ardından 30 m'de 71 mm oldu. Frond genişliği dip derinliği boyunca 0,855 cm ile 1,1139 cm arasında dar bir aralıkta kalmıştır. En geniş frond 30 m'de gözlenirken, en dar frond 10 m'de kaydedilmiştir. 10 m'de incelenen örneklerin toplam %35'inin tomurcuklu, %12'sinin 15 m'de, %7'sinin 20 m'de ve %20'sinin 30 m'de olduğu bulunmuştur. Pinna sayısı 6-18 ile 156-210 arasında değişmiş olup, ortalama 76 ila 94 ve 1 cm'lik frond uzunluğu başına 10-12 pinna bulunmaktadır.

Anahtar kelimeler: *Caulerpa taxifolia*, istilacı türler, biyometrik, derinlik dağılımı, Türk Ege Denizi

INTRODUCTION

The Mediterranean basin, particularly the eastern basin and seas such as the Levant and Aegean, has become a notable location for the introduction and invasion of exotic species. Biological invasions have the potential to pose a significant threat to the conservation of endangered species in natural communities of plants and animals. Researchers have found that these invasions can facilitate a deeper understanding of the biometric interactions and effects of seagrasses on submerged algal communities (Ceccherelli and Cinelli, 1998). Fish, benthic fauna, and macrophytes comprise the majority of intentionally and accidentally introduced species in the eastern Mediterranean (Ceccherelli and Cinelli, 1998). The invaders are predominantly Indo-Pacific and temperate and tropical species, and they affect ecosystems already established in the Mediterranean, causing an extension of their succession and spatial changes in the ecosystem. The abundance and population growth rate of seaweeds serve as indicators of theoretical and applied processes, as they facilitate the understanding of how variations in their life history interact in isolated and mixed populations (Schemske et al., 1994). Clonal vegetative growth is a prevalent phenomenon in areas with high-density aggregations, where seaweeds forage and move to suitable adjacent spaces, undergo rapid expansion, and face a reduced risk of mortality (Wright, 2005).

A total of 143 species of marine algae have been introduced into the Mediterranean, including nine species previously (*Caulerpa taxifolia* (M. Vahl) C. Agardh; *Caulerpa racemosa* (Forsskal) J. Agardh; *Sargassum muticum* (Yendo) Fensholt; *Saccharina japonica* (Areschoug) C.E.Lane, C.Mayes, Druehl & G.W.Saunders; *Asparagopsis armata* Harvey; and *Undaria pinnatifida* (Harvey) Suringar; and *Womersleyella setacea* (Hollenberg) R. E. Norris; *Acrothamnion preissii* (Sonder) E.M. Wollaston; *Lophocladia trichoclados* (C.Agardh) F.Schmitz) and additionally *Caulerpa cylindracea* Sonder; and *Rugulopteryx okamurae* (E.Y.Dawson) I.K.Hwang, W.J.Lee & H.S.Kim and *Caulerpa taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini have been found to have invasive and economic impacts (Siguan and Ribera, 2002; Galanidi et al., 2023). The density of fifty species has increased in the last two decades. Nevertheless, the western Mediterranean basin contains approximately 90 nonindigenous species (of Japanese or Pacific origin) (Galanidi et al., 2023). The eastern basin, on the other hand, contains increased 72 nonindigenous species, predominantly of Red Sea or Indian Ocean origin, as updated following the inclusion of nonindigenous macrophyte species in the Mediterranean Sea (Zenetos and Galanidi, 2020; Galanidi et al., 2023) and the Turkish waters of the eastern Mediterranean (Cinar et al., 2021; Galanidi et al., 2023).

Caulerpa taxifolia (Vahl) C. Agardh (Ulvophyceae, Caulerpales) is a coenocytic (siphonous) green alga. Members of the genus *Caulerpa* are typically found in warm tropical and subtropical waters. Recently, *C. taxifolia* was reported from the northern Mediterranean Sea (Meinesz and Boudouresque, 1996). The first documented instance of *C. taxifolia* in the Mediterranean occurred in 1984, and it is believed that this species may have escaped from the tropical display aquaria of the Oceanographic Museum in Monaco (Meinesz and Boudouresque, 1996; Meinesz and Hesse, 1991). The alga has become established and is found in abundance in the northern Mediterranean, with no previous reports of its presence in tropical native regions. The invasive nature of the alga was demonstrated by its remarkable spread: by the end of 1996, an area of 3096 ha had been colonized by *C. taxifolia*, most of which was recorded off the coasts of France and Italy (between Toulon, France and Alassio, Italy) (Meinesz et al., 1997). Numerous isolated occurrences have been documented in Italy (Liguria, Elba, Sicily), Croatia (Isle of Hvar, Isle of Krk), Spain (Balearic Islands) (Meinesz and Boudouresque, 1996), and Tunisia (Meinesz et al., 2001), and Türkiye (Gulf of İskenderun) (Cevik et al., 2007) (Gulf of İzmir) (Turan et al., 2011). *C. taxifolia* in the Mediterranean is regarded as a significant threat to native flora (and fauna) due to its rapid growth rate and production of secondary metabolites, many of which are toxic (Lemée et al., 1993; Meinesz et al., 1993).

The species *C. taxifolia* is grazed by certain species of grazers, including *Oxynoe olivacea* (Gianguzza et al., 2007) and *Elysia subornata* (Thibaut et al., 2001) and the sea urchin *Paracentrotus lividus* and the fish *Sarpa salpa*, as previously documented by Lemée et al. (1996) and Boudouresque (1997). It is a poor substrate for epiphytes for most of the year. The alga has been observed to cover up to 100% of the substrate across a range of depths, primarily between 0 and 50 meters (although it has been recorded at 100 meters), and on diverse substrate types (Meinesz et al., 1993; Belsher and Meinesz, 1995; Meinesz et al., 1995). Komatsu et al. (1997) demonstrated that *C. taxifolia* from the northern Mediterranean Sea exhibited survival capacity across a temperature range of 10 to 31.5 °C, with optimal irradiance levels ranging from 88 to 332 µmol $m^{-2}.s^{-1}$. The authors of the study posited that the Mediterranean strain of *C. taxifolia* possesses considerable potential for expansion throughout the Mediterranean region and for invasion into adjacent tropical and temperate seas (Gillespie et al., 1997). However, following its initial proliferation, *C. taxifolia* has undergone a shift in its kinetic phase, resulting in a substantial decrease or stabilization of its distribution across most regions of the Mediterranean Sea. Consequently, it is no longer regarded as a threat to the same extent as previously observed (Montefalcone et al., 2015).

In addition to aquaria, ships and fishing gear have been identified as significant vectors for the dissemination of *C. taxifolia* through fragments of the species destroyed by anchoring within and between localities (West et al., 2007; Relini et al., 2000). A notable distinction between the life cycle of *C. taxifolia* and that of other Mediterranean macrophytes pertains to the timing of its peak biomass, which occurs in the autumn, a season coinciding with the decline in productivity of autochthonous algae (Ballesteros, 1989). This phenomenon, characterized by the reduction in photophilic algal communities, has been shown to facilitate competition (Verlaque and Fritayre, 1994). During the winter months, the minimum biomass consists of a dense network of thick stolons with short, highly branched primary axes (Meinesz et al., 1995), which still completely cover the substrate and ensure algal regrowth in spring (Komatsu et al., 1997).These characteristics lead to a modification of habitat structure with various direct or indirect consequences for flora and fauna (Boudouresque et al., 1995).

In the ecosystem, the species has a role, effect, and impact; *C. taxifolia* changes the shoot density and orthotropic/plagiotropic rhizomes ratio of *P. oceanica* (Molenaar et al., 2009). When *P. oceanica* coexists with *C. taxifolia*, it reduces leaf length and biomass, while *C. taxifolia* increases frond length (Pergent et al., 2008). However, the presence of *C. taxifolia* did not significantly affect *Cymodocea nodosa* shoot density, and an increase in nutrient availability in the sediment did not alter this pattern (Ceccherelli and Sechi, 2002). A slightly insignificant effect on fish fauna biomass occurred only in a colonized *C. taxifolia* sample (Francour et al., 1995). The invasion of *C. taxifolia* did not cause significant changes in the density of the main taxa forming the macrobenthic community (Caronni, 2011). However, *C. racemosa* affects macroalgal communities more than does *C. taxifolia* when cooccurring with *C. racemosa* (Piazzi et al., 2003; Balata et al., 2004). Furthermore, *C. taxifolia* has been observed to impact the invertebrate fauna inhabiting *Posidonia* beds following its invasion (Francour et al., 2009) and within soft-bottomed environments (McKinnon et al., 2009).

A variety species of *C. taxifolia*, *Caulerpa taxifolia* var. *distichophylla* has been documented along the Turkish coastline, with occurrences reported in İskenderun Bay (Cevik et al., 2007) and Antalya Bay (Mutlu et al., 2022), Alanya Bay, Kaş Bay, Sea of Marmara (Taşkın et al., 2023), and the Aegean coast (Mutlu et al., 2025a). Cevik et al. (2007) reported the occurrence of *C. taxifolia* from İskenderun Gulf, located in the eastern Mediterranean waters of Türkiye. However, this species was subsequently corrected by Jongma et al. (2013) to be *C. taxifolia* var. *distichophylla*. The initial documentation of *C. taxifolia* in Turkish Aegean waters was reported from Uzunada Island with a special permission to study at one bottom depth from Turkish Republic Navy Forces Command, in İzmir Gulf (Turan et al., 2011). Subsequent to the initial documentation of *C. taxifolia* in the Aegean Sea by Turan et al. (2011), the present study, which examines a subsequent occurrence of the species extending northward in the Turkish Aegean Sea, has yielded substantial insights into the morphological and biometric characteristics of this invasive species. The species occurred second times in the Aegean Sea as well as the eastern Mediterranean basin. Of the 321 SCUBA sampling stations deployed during the present study, however, the species occurred only at four different bottom depths of one site, Dikili Bay. Therefore, the present study was designed to provide an exhaustive biometric analysis of *C. taxifolia* across the depth gradient and to compare distinguished biometrics with congeneric species, *C. taxifolia* var. *distichophylla*.

MATERIALS AND METHODS

Specimen and environment sampling

A research cruise was conducted to ascertain the distribution and species composition of seagrasses and seaweeds as submerged vegetation along the Turkish coast of the Aegean Sea from May to August of 2024 (Figure 1). During the survey, which was conducted during daylight hours, 321 stations were sampled. In August of 2024, four stations recorded the presence of *Caulerpa taxifolia*. The stations were located in Dikili Bay, İzmir, Aegean Sea, Türkiye (Figure 1).

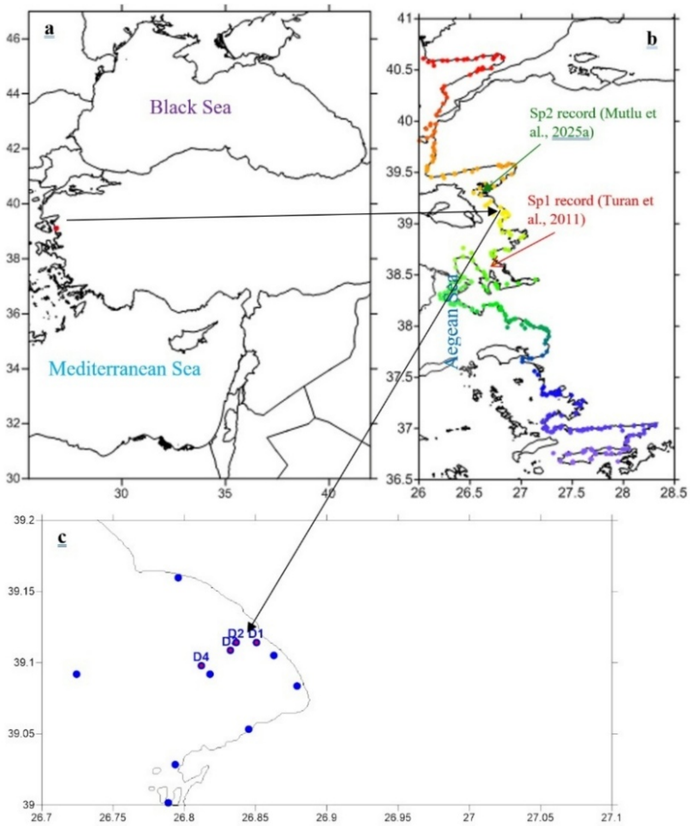

Figure 1. Study area (a, b), sampling stations (b) colored basing the geographic latitude and stations in Dikili Bay where *Caulerpa taxifolia* occurred (D1-D4 in red circle) (c). Red arrow denotes the first occurrence of Sp1: *C. taxifolia* in the Aegean Sea (Turan et al., 2011), and dark green Sp2: *C. taxifolia* var. *distichophylla* (Mutlu et al., 2025a) during the present survey (Appendix Figure A1)

A transect was meticulously established from the shore to the seaward side at four different bottom depths (10, 15, 20, and 30 m at stations D1, D2, D3, and D4, respectively). At these stations, runner stolons of specimens were triply replicated and collected by SCUBA divers in a 0.4 m × 0.4 m quadrat. On board the *R/V Akdeniz Su,* the tangled fronds, stolons, and rhizoids were untangled and prepared for biometric measurements (Figure 2). Measurements were taken from fresh, unpreserved specimens.

Figure 2. *Caulerpa taxifolia* appearance on entire specimens (a and b) and close-up view of fronds (c and d). F: frond, St: stolon, Rh: rhizoid, FL: frond length, FW: frond width, P: pinnae, R1: rachis 1, and R2: rachis 2. Specimen in Figure 2c could be scaled from same specimen indicated by a arrow in Figure 2e

During the shipboard sampling, physicochemical parameters (temperature, salinity, pH, oxygen, and total suspended solids) and optical parameters (Secchi disk depth and photosynthetically active radiation, PAR) were measured from surface and near-bottom waters. Water samples were collected on board using a 5-liter Niskin bottle, and the physicochemical parameters were measured using multiparameter probes (AZ Combo, model 84051). The PAR was measured using an ampoule (Spherical SPQA-4671 model, LI-COR, Inc.) and a multiparameter recorder (LI-1400 model). The D1 sample was collected at 12:40, the D2 sample at 12:13, the D3 sample at 11:40, and the D4 sample at 09:07. The ampoule was casted with the cable from the surface to the near-bottom depth, and the profiled PAR values were subsequently converted to percent values with respect to the sea surface value as 100% throughout the water depth.

Biometrical measurements

The material examined included unpreserved specimens, with eight stolons and 88 fronds at D1, six stolons and 75 fronds at D2, five stolons and 76 fronds at D3, and five stolons and 95 fronds at D4. The total frond count at D1, D2, D3, and D4 was 63, 67, and 87, respectively. The fronds were collected at locations specified by geographical coordinates: 39.1142° N and 26.85061° E, 39.11427° N and 26.83638° E, and 39.10874° N and 26. 83232 E, and 39.09793 N and 26.81195 E from depths of 10, 15, 20, and 30 m by SCUBA diving, respectively, on August 8, 2024, by Yaşar Özvarol and Barış Akçalı.

The biometry was characterized by the following parameters: density (number of shoots/m^2 and per quadrat; TS, buds: leaf or frond growing from main frond; BNo, internodal distance: the distance between two fronds along a stolon; IND, number of fronds per stolon; FNo, number of stolons; #S, number of paired rachises or pinnae (ramuli) per rachis and per 1 cm rachis; #F, leaf area: frond width x length; LA) and morphometry (stolon length; SL, frond length: FL, and frond width: FW) of the samples (Figure 2). The morphometric parameters were measured with an accuracy of µm using a caliper. Additionally, the buds on the fronds were enumerated, and the biometric parameters were measured.

Statistical interpretation of biometrics

In order to ascertain the biometric characteristics of the species, the relationships between frond length-width and frond length-number were established using Pearson correlation and regression models. Subsequently, differences in the frond length-number of pinnae relationships among the bottom depths were tested using analysis of co-variance (ANOCOVA). Following the testing of normality (dispersion index, randomness test, FAO, 1991), all the biometric variables were subjected to an analysis of variance (ANOVA) to determine differences among the four sampling depths and a post-hoc test (Least Significant Difference, LSD) was then applied. Spearman's correlation coefficient was employed to assess the relationship between biometric data and environmental variables. The confidence level established for the statistical significance of the tests was $p < 0.05$. All analyses were conducted using the statistical software MATLAB (version 20221a, MathWorks, Inc.).

RESULTS

Study environment

A total of 321 sampling stations were surveyed, and four of these stations (D1 at 10 m, D2 at 15 m, D3 at 20 m, and D4 at 30 m; Table 1) were located in coastal waters around the town of Dikili in the city of İzmir. The study area was frequently visited by recreational boats, vacationers and tourists.

Table 1. Minimum, maximum, mean and standard deviation values of the morphometrical variables of *C. taxifolia* at the depths three times replicated (Rx) for the sampling. S#: number of stolons per quadrat, SL: stolon length in mm, FNo: number of fronds per stolon, FL: frond length in mm, FW: frond width in mm and IND: internodal distance in mm. nd is not detected in the specimens

		SL	FNo	FL	FW	IND	SL	F#	FL	FW	IND	SL	F#	FL	FW	IND
		R1					R2					R3				
S#		33														
Min	D1	35	1	5	2.45	4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Max		570	19	181	18.5	74	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S#		5					3									
Min	D2	130	3	8	2.4	1	130	8	14	3.44	3	nd	nd	nd	nd	nd
Max		557	28	170	17.6	54	350	17	142	15.13	67	nd	nd	nd	nd	nd
S#		28					2									
Min	D3	178	1	7	2	1	493	30	12	2.8	3	nd	nd	nd	nd	nd
Max		533	21	166	13.3	64	493	39	250	18.1	53	nd	nd	nd	nd	nd
S#		95					13					36				
Min	D4	150	1	7	2.9	3	45.3	1	4.2	3	3	45.3	1	5.9	2.9	7
Max		908	46	171	17.8	134	740	13	174	107.4	122	740	14	164	16	103

Mutlu et al. (2025a) provided a comprehensive overview of the physical properties of the waters throughout the study area. These properties included sea surface temperature variations ranging from 20.5 to 28.5°C and near-bottom water temperatures fluctuating between 18 and 28°C. The presence of cold water in the Black Sea and the Sea of Marmara was observed in the Dardanelles Strait exit in the Aegean Sea, while warmer water was identified in the northern part of the Aegean Sea. The impact of the river Meriç was evident in this region, where less saline water was recorded (Figure 3).

The sea surface temperature of Dikili Bay was approximately 25.5 °C. However, a decrease in sea surface salinity was observed from the coast (34 PSU) to the open waters (32 PSU), with the exception of the Black Sea water in the northernmost Aegean Sea (Figure 3). The pH of the sea surface ranged from 8.35 to 8.4, while the pH of the near-bottom water varied from 8.4 to 8.45. Within Dikili Bay, the dissolved oxygen levels in surface waters exhibited moderate values ranging from 7.4 to 11.2 mg/L, and the total suspended solids levels varied from 23 to 27 mg/L (Figure 4). These measurements were obtained across the entire study area.

The salinity exhibited a gradient of decreasing values from south to north within the study area, a trend that was more pronounced in the near-bottom waters. Conversely, the oxygen content and pH levels exhibited a slight increase from south to north, contrasting with the variation in total suspended solids (Figure 4).

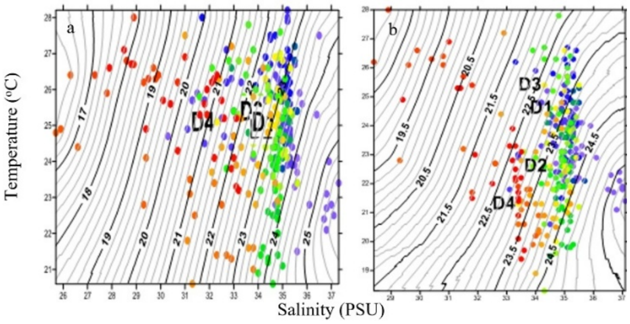

Figure 3. Sea surface (a) and near-bottom water (b) T-S diagram with isoclines of density, σ_t, at the stations colored according to geographical latitude (see Figure 1b for the station locations)

With respect to the sea surface water, the TS diagram revealed that the temperatures at stations in Dikili Bay were comparable, yet D4 (σt=21.5) exhibited a divergence from D1, D2, and D3 with respect to water density (σt=23). In contrast to the surface waters, the near-bottom waters at the four stations exhibited distinct salinity and temperature values, yet shared the same temperature value of σt=23.2 (Figure 3).

Along the transect with D1-D2, which exhibited a vertical distribution of water temperature, the warmest water body was located within the first 10 meters, followed by the seasonal thermocline in the second layer from 10-15 meters to 20 meters, and the coldest water body started after a water depth of 20 meters (Figure 4).

The open station (D4) exhibited lower levels of saline surface water, with a gradual increase in salinity toward the coastal waters. In the initial five meters of the bottom, the salinity exhibited a decrease from the surface, followed by a slight increase up to fifteen meters and a subsequent gradual decrease up to thirty meters (Figure 4). The total suspended solids (24-25.6 mg/l) and pH (8.3-8.5) exhibited analogous vertical profiles to those of the salinity, while the oxygen profile exhibited contrast with that of temperature (Figure 4). The coastal water had a lower irradiance value (PAR) than did the open waters of Dikili Bay. The Secchi disk depth exhibited a strong correlation with an irradiance value of 4.6 mol photons/cm^2/s, indicating the presence of an euphotic zone extending from 5 to 2.5 m from D1 to D4. The lowest percentage value (99.64%) was observed at a water depth of 20 m, as depicted in Figure 4.

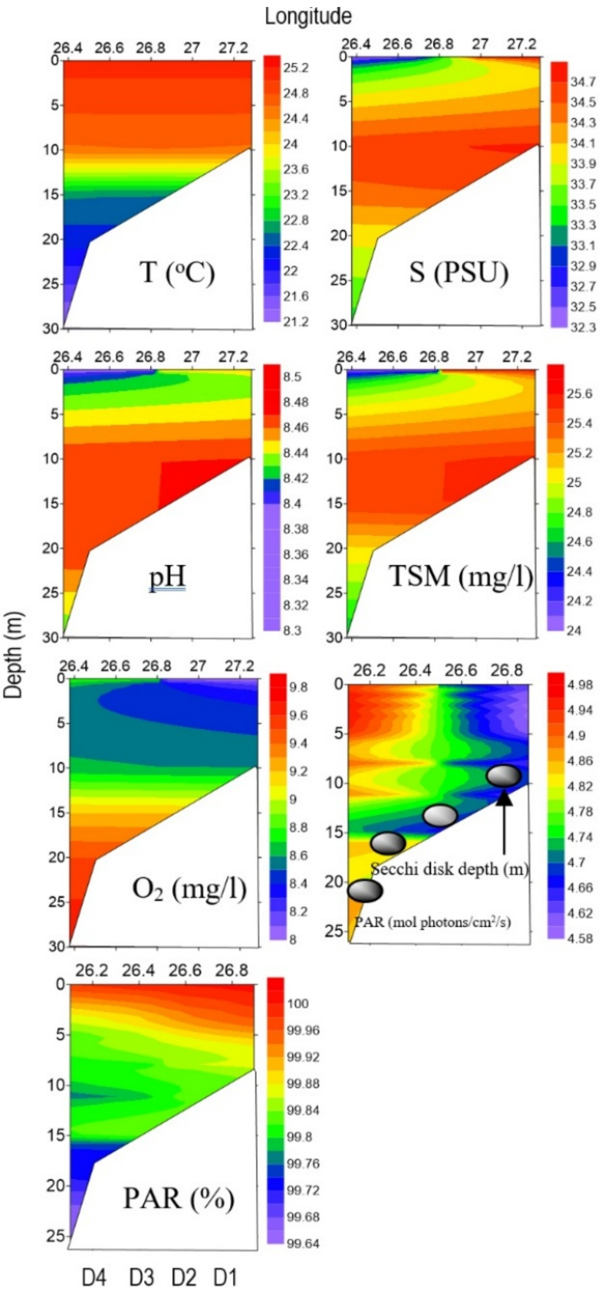

Figure 4. Profiles of water temperature in °C, salinity in PSU, pH, dissolved oxygen in mg/l, total suspended matter in mg/l, PAR in mol photons/cm^2/s and percent across D1 to D4 across geographical longitudes

Plan traits

Caulerpa taxifolia (M.Vahl) C.Agardh is a green macroalga belonging to the class Ulvophyceae (subkingdom Viridiplantae, kingdom Plantae).

Description

The plants were characterized by a green, prostrate axis (stolon) with downward-growing colorless rhizophores, which were attached to a hard substrate or anchored in the unconsolidated substrate (Figure 2).The stolons were smooth (glabrous) and produced erect photosynthetic fronds (assimilators). The assimilators constituted a central axis (rachis) bearing lateral branches (ramuli or pinnae) in a distichous configuration and a sickle shape (falcate) on a pedicel (Figure 2). The rachises exhibited regular constrictions (annulations). The basal part of the ramuli attached to the rachis was constricted and contracted (Figure 2). The maximum frond length ranged from 16.6 to 18.1 cm, and the frond width ranged from 13.3 to 18.5 mm. The number of branchlets varied between 6-18 and 156-210, with an average of 76 to 94 and 10-12 per 1 cm frond length, respectively. The relationship between frond length and the number of branchlets was found to be best fit by a slope of less than 1 (Figure A2).

Remarks

It has a similar structure to *Caulerpa mexicana,* and both *C. mexicana* and *C. taxifolia* have distichous fronds, but *C. mexicana* has a type between clavate and club-shaped (wider than tip parts of ramuli) falcate ramuli, and *C. taxifolia* has a variant of falcate, sickle-shaped ramuli. The basal part of the ramuli of both species contracts, but it is not contracted in *C. taxifolia* var. *distichophylla* (Verlaque et al., 2015). For *C. taxifolia*, the maximum frond length ranged from 16.6 to 18.1 cm, and the width ranged from 13.3 to 18.5 mm. The number of branchlets varied from 6-18 to 156-210, with an average of 76 to 94 and 10-12 per 1 cm frond length. The slope of the relationship between frond length and the number of branchlets was estimated to be 2.15 greater than 2 for *C. taxifolia* var. *distichophylla* (Mutlu et al., 2025a). The slope of the relationship between frond length and number of branchlets was estimated to be less (0.81-0.97) than 1 for *C. taxifolia* (Table A1, Figure A2).

Distribution

In addition to the previous record in the Gulf of İzmir, Aegean Sea (Figure 1), *Caulerpa taxifolia* was collected from four different locations in the shallow and coastal waters of the Aegean Sea (Dikili Bay, Türkiye). The distribution ranged from 10 m to 30 m.

Biometrics

The biometric parameters of the species were recognized as density and morphometric variables to characterize the recent measurements made from the live specimens that occurred for the second time in the Aegean Sea. Basic descriptive statistics of the biometric variables of *C. taxifolia* are presented in Table 1. The number of stolons recorded at each depth interval is listed in Table 1. At 10 m (D1), the number of stolons was 33; at 15 m (D2), it was 3–5; at 20 m (D3), it was 2–28; and at 30 m (D4), it was 13–95 stolons per quadrat.

Density

The internodal distance (IND) is indicative of the rarefaction and densification of shoot density. The IND exhibited a range of values between 18.4 mm, measured at the 15-m depth, and 38.4 mm, measured at the 30-m depth (Table 2). A positive correlation was observed between the IND and depth, with an increase in IND with decreasing depth after 10 m (Figure 5). The first two depths exhibited slightly greater variations in the IND measurements compared to the deeper depths. The IND exhibited a significant and negative correlation with the sea surface total suspended solids and near-bottom pH (Table 3).

The LSD test revealed that the internodal (shoot) distance (IND) was minimal at bottom depths of 15 and 20 m, where a significant difference in IND was observed compared to bottom depths of 10 m and 30 m (Figure 5). The IND was found to be significantly differentiated by bottom depth (Figure 5).

Table 2. Depthwise distribution of density variables for *C. taxifolia* (mean±SD). IND: internodal distance (mm), LA: leaf area per quadrat (cm^2), TS: shoot density (fronds/m^2), LAI: leaf area index (m^2/m^2, and FNo; number of fronds per stolon

Depth	IND	LA	TS	LAI	FNo
10	23.1±1.1	$5.56*10^{-4}$±$3.09*10^{-5}$	560±330	3.43±4.82	11.0±0.6
15	18.4±1.2	$8.17*10^{-4}$±$3.91*10^{-5}$	343±330	3.89±4.82	10.5±1.1
20	19.9±0.9	$9.78*10^{-4}$±$3.39*10^{-5}$	431±572	25.38±8.36	10.2±1.0
30	38.4±0.9	$9.01*10^{-4}$±$3.33*10^{-5}$	1397±330	16.83±4.82	7.4±0.6

Leaf area (LA): In contrast to the actual leaf area of seagrass leaves, *Caulerpa* species, except for *C. prolifera*, had fronds and ramuli that did not reflect the total leaf area. LA exhibited an increase with depth, ranging from an estimated $5.56*10^{-4}$ m^2 at 10 m to $9.78*10^{-4}$ m2 at 20 m, followed by $9.01*10^{-4}$ m^2 at 30 m (Table 2, Figure 5). However, LA and leaf area index (LAI) were not correlated with any of the other environmental parameters (Table 3). LA per quadrat is a function of frond length and width and was a minimum of 5 m. LA was significantly different among the bottom depths (Figure 5). The maximum LA was found at 20 m, followed by a depth of 30 m (Figure 5).

Shoot density (TS): In contrast to the IND treatment, the maximum shoot density (1397 shoots/m^2) was found at the deepest sampling depth (Table 2), while the minimum density of 343 shoots/m^2 occurred at 15 m. The TS exhibited a tendency to increase seaward by 15 m (Figure 5). Furthermore, a negative correlation was observed between TS and sea surface salinity, total suspended solids, and near-bottom pH at a significance level of $p < 0.05$ (Table 3). However, no significant differences in TS were detected among the different bottom depths. Notably, TS at 30 m showed a significant increase compared to 15 m (Figure 5).

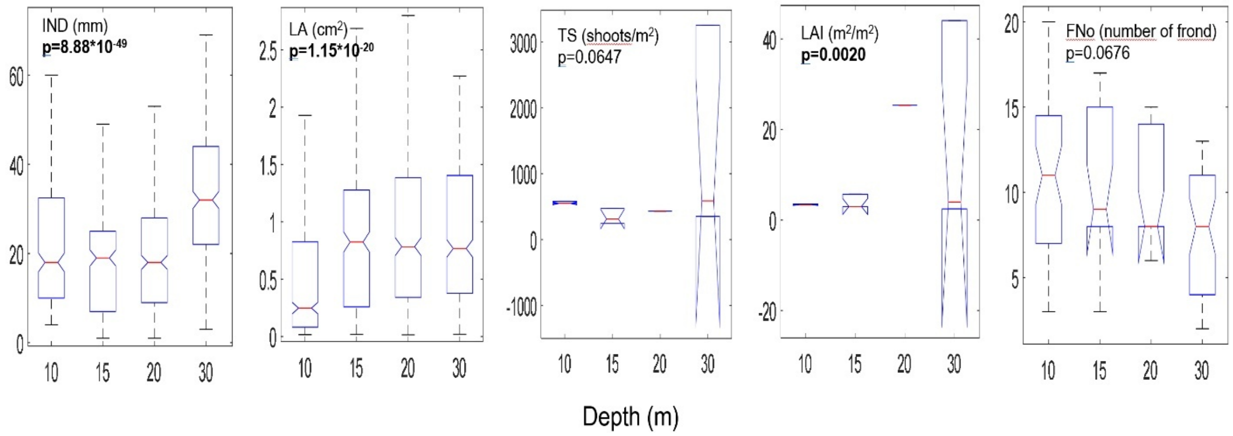

Figure 5. Notch plots of density variables of *C. taxifolia* at the sampling depths. TS: number of shoots per sampler, LAI: single-sided leaf area index (cm^2/m^2), and FNo: number of fronds per stolon. The bold p value denotes a significant difference in the variable among the bottom depths, in other words, from station D1 to D4

The leaf area index (LAI) exhibited a significant increase in the deeper layers of the water column, reaching a maximum at 20 m and then decreasing at 30 m (Table 2, Figure 5). This increase in LAI at 20 m was notably higher compared to the values at 10 and 15 m (Figure 5).

The number of fronds per runner (FNo) also showed a distinct pattern. The number of fronds (shoots) per stolon exhibited a gradual decrease with depth up to 20 m, with the lowest recorded number of fronds per stolon observed at 30 m within the study area. FNo demonstrated a positive correlation with salinity, pH, total suspended solids in surface waters, and pH in near-bottom waters, and a negative correlation with bottom depth (Table 3). However, FNo exhibited no significant change with bottom depth, although a notable difference in FNo was observed between 5 and 30 m (Figure 5).

Number of stolons (#S): The number of stolons per quadrat exhibited a tendency to increase with depth greater than 10 m, reaching a maximum of 95 stolons per quadrat at 30 m.

Table 3. Spearman correlation coefficients between the biometrical variables and sea surface and near-bottom water environmental variables (T: temperature, S: salinity: S, TSM: total suspended matter, DOx: Dissolved oxygen, Depth: bottom depth). Bold coefficients denote significant correlations between the variables at $p < 0.05$ (sample size, n = 4)

	Sea surface water					Near-bottom water					
	T	S	Ph	TSM	DOx	Depth	T	S	Ph	TSM	DOx
SL	0.3913	-0.6662	-0.7685	-0.6518	0.3617	0.871	**-0.9552**	-0.8644	-0.5335	-0.7824	**0.9908**
FL	0.6047	-0.2917	-0.421	-0.2788	-0.0877	0.5788	-0.7449	-0.5949	-0.1187	-0.4927	0.8301
FW	-0.2631	-0.7383	-0.8061	-0.7142	0.7658	0.8243	-0.9267	-0.7496	-0.7121	-0.6732	0.8475
IND	-0.0361	-0.9449	-0.8845	**-0.9519**	0.8792	0.79	-0.5164	-0.7911	**-0.9769**	-0.8573	0.4786
LA	0.4291	-0.4702	-0.5953	-0.4529	0.1606	0.7296	-0.8902	-0.7222	-0.3197	-0.6174	0.9381
TS	-0.0253	**-0.956**	-0.9006	**-0.9622**	0.882	0.8113	-0.5464	-0.8116	**-0.9829**	-0.8732	0.5097
LAI	0.849	-0.4481	-0.5253	-0.4513	-0.0536	0.6461	-0.6235	-0.7103	-0.2781	-0.6735	0.7494
FNo	-0.0175	**0.9956**	**0.9933**	**0.9917**	-0.8777	**-0.9564**	0.8061	0.9395	**0.979**	0.9492	-0.7714

Morphometry

The morphometry of *C. taxifolia* included stolon length (SL), frond length (FL), and frond width (FW).

Stolon length (SL): The length of the stolons ranged between 178 mm and 355 mm, with the shortest stolon occurring in the shallowest water. A gradual increase with increasing bottom depth was observed (Table 4, Figure 6). However, a negative correlation between SL and the near-bottom water temperature was observed at a significance level of $p < 0.05$ (Table 3). The stolon length exhibited significant variation across different bottom depths. The shortest length was recorded at 10 m, and at greater depths, the stolon length did not differ significantly. The length of the stolons gradually increased with increasing bottom depth (Figure 6).

Frond length (FL): A similar trend was observed in frond length along the bottom gradient (Table 4, Figure 6), ranging from 49.9 mm at 10 m to 87 mm at 20 m, followed by 71 mm at 30 m. However, none of the environmental parameters exhibited a significant correlation with variations in FL (Table 3). A significant relationship was identified between frond length and bottom depth (Figure 6). FL exhibited an increasing trend up to a depth of 20 m, followed by a decrease at 30 m. The minimum length was recorded at 10 meters. Notably, the FL exhibited no significant variation between 15 m and 30 m (Figure 6).

Table 4. Depthwise distribution of morphometrical variables of *C. taxifolia* (mean±SD). SL: stolon length in mm, FL: frond length in mm and FW: frond width in mm

Depth	SL	FL	FW
10	178.5±26.3	49.9±2.0	0.855±0.021
15	277.2±37.2	67.5±2.5	1.063±0.025
20	347.4±34.4	87.1±2.2	0.979±0.022
30	355.1±25.7	71.8±2.1	1.139±0.021

Frond width (FW): In a manner analogous to FL, frond width exhibited no significant correlation with any of the other environmental parameters (Table 3). Furthermore, it remained within a narrow range of 0.855 cm to 1.1139 cm (Table 4). The frond with the greatest width was observed at a depth of 30 m, while the narrowest frond was measured at 10 m (Table 4, Figure 6). A significant decrease in FW was observed at 5 m compared to the other depths (Figure 6). FW exhibited an increase from 10 m to 15 m, followed by a decrease at 20 m and an increase at 30 m. The variation in FW among the different depths was found to be statistically significant (Figure 6).

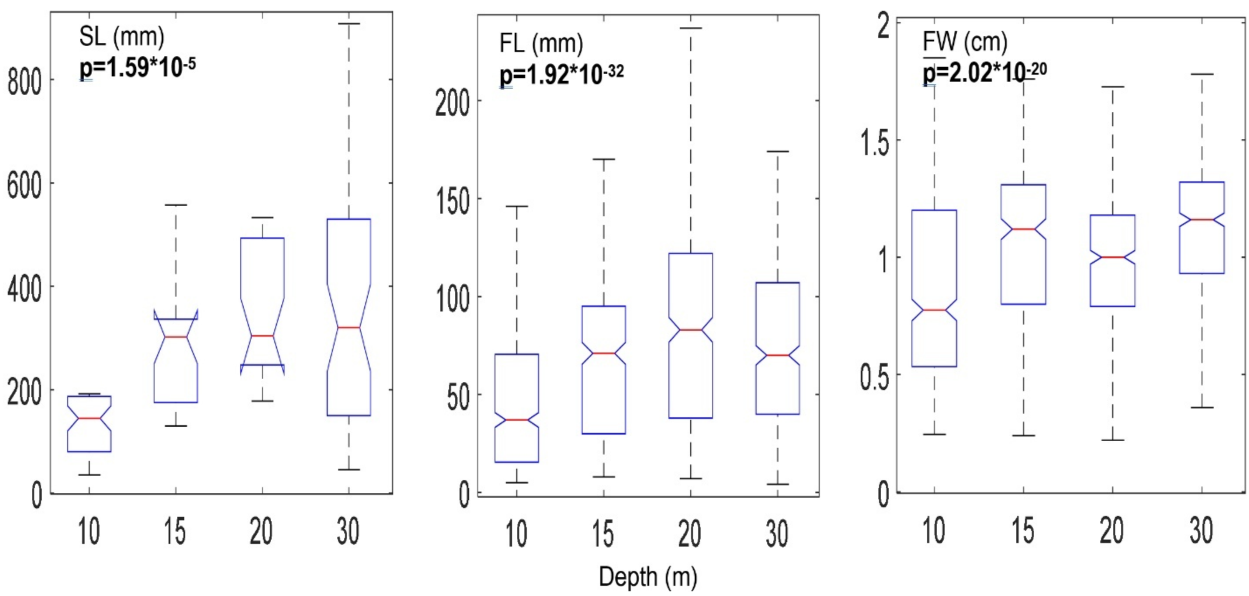

Figure 6. Notch plots of morphometrical variables of *C. taxifolia* at the sampling depths. SL: stolon length in mm, FL: frond length in mm, and FW: frond width in mm. The bold p value denotes a significant difference in the variable among the bottom depths, in other words, from station D1 to D4

Budding

The stolons of the species manifested as a continuous line formed by the buds (Figure 2). A total of 1 stolon, 31 shoots, 11 buds at 10 m, 6 stolons, 63 shoots, 8 buds at 15 m, 6 stolons, 67 shoots, 5 buds at 20 m, 8 stolons, 84 shoots, and 17 buds at 30 m were identified among the total specimens examined in the present study area (Figure 7). The frequency of budding was as follows: 35% of the total numbers of specimens examined at 10 m were found to be budded, 12% at 15 m, 7% at 20 m, and 20% at 30 m. Multiple buds rarely occurred on one main shoot of the specimens (Figure 7).

Interbiometric relationship

The interbiometric relationship was characterized by the number of pinnae vs. frond length and frond length vs. frond width (Figures 8, 9). This relationship could be useful for the identification of congeneric species of *Caulerpa*.

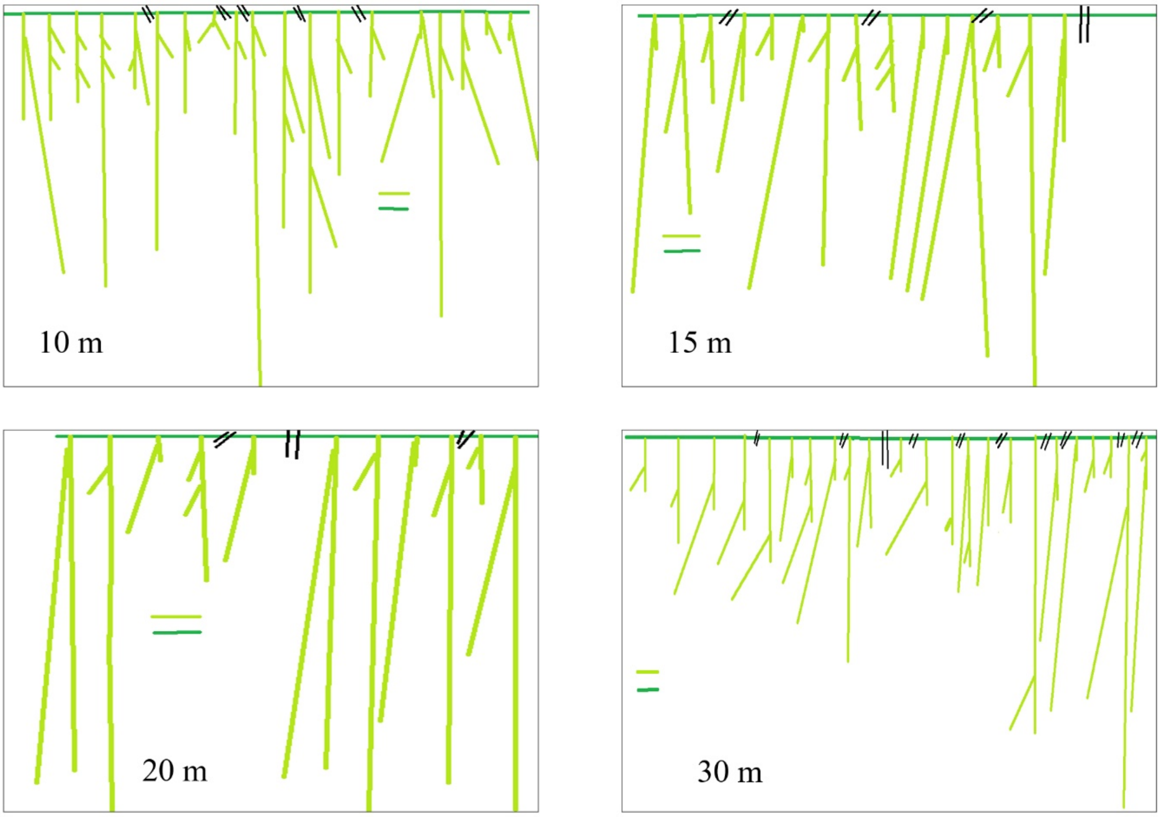

Figure 7. Schematized frond buds (see Figure 2 for the real appearance of budding reticulated with branches) of *C. taxifolia* were found at 10-30 m, in other words, from station D1 to D4. The light green represents fronds (scale: 2 cm), and the dark green represents unscaled stolons. The diagonal line denotes fronds at each stolon, and the perpendicular line represents the separation of replicate samples

The number of ramuli (pinnae) was significantly correlated with frond length at $p < 0.05$ (Figure 8). The estimated slope of this relationship was approximately 1, indicating that a 1-unit increase in frond length corresponds to a 1-unit increase in pinnae number. The minimum and maximum number of pinnae per 1 cm frond length are documented in Table 5, and the average number of fronds ranged from 9.6 to 11.8 pinnae per 1 cm frond length (Table 5). The relationship at each bottom depth was found to be significant at $p < 0.05$ (t-test). However, a significant difference in the relationships between the bottom depths was observed (ANOCOVA, $p = 3.16 \times 10^{-6}$) (Figure 8).

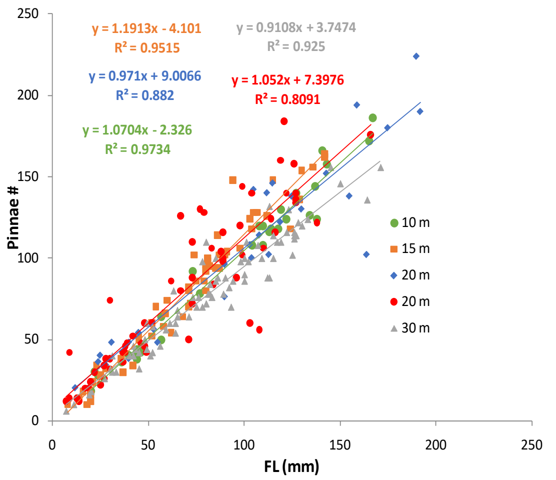

Figure 8. Relationships between frond length and the number of pinnae (#P) at 10-30 m, in other words, from station D1 to D4

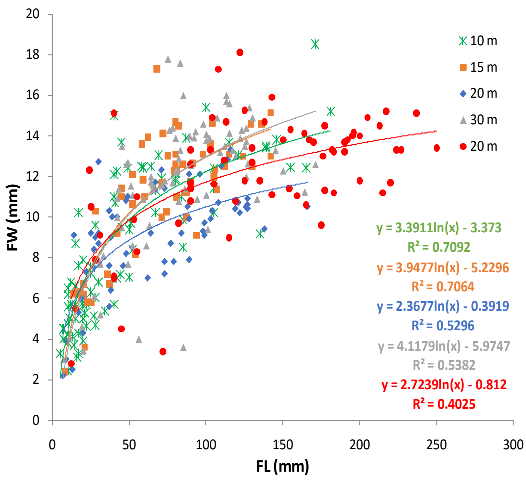

Figure 9. Relationships between frond length (FL) and frond width (FW) at 10-30 m depth, in other words, from station D1 to D4

Table 5. Minimum, maximum, mean and standard deviation values of the number of pinnae per 1 cm long frond

Pno/FL	D1	D2	D3	D4
Min	8.64	5.6	5.2	7.1
Max	13.64	15.7	24.7	13.8
Mean	10.40	11.1	11.8	9.6
SD	1.10	1.7	3.0	1.2

However, the frond length-width relationship was significantly estimated with a curved linear (logarithmic) model as opposed to a linear model (Figure 9). The relationship exhibited considerable variability in the scatter plot (Figure 9). Nevertheless, there were significant differences in the relationships between bottom depths (ANOCOVA, $p = 0.0046$) (Figure 9).

DISCUSSION

Caulerpa species were identified as target species of interest due to their distinctive characteristics (e.g., unique siphonous thallus composed of a single multinucleate cell, utilization as food, and invasive characteristics) (Paul and Fenical, 1987; Ceccherelli et al., 2002). The invasive nature of these organisms is attributable to their remarkable flexibility and capacity to thrive under a broad spectrum of temperatures and irradiances (Uchimura et al., 2000; Ljiljana et al., 2006). Consequently, these plants have developed vegetative reproduction, fragmentation, and morphological plasticity in a variety of marine environments (Ceccherelli and Cinelli, 1999a, b; Ceccherelli and Piazzi, 2001; Wright, 2005). It has been documented that certain species within this group exhibit remarkably high levels of morphological plasticity, a phenomenon that has contributed to significant taxonomic confusion and the description of nearly 400 species, varieties, forms, and ecads (i.e., environmentally modified morphologies) (Zubia et al., 2020). Consequently, the collection of morphometrics from diverse locations and seas has become imperative for effective species identification.

It shares a structural resemblance to *Caulerpa mexicana*, and both *C. mexicana* and *C. taxifolia* possess distichous fronds. However, a distinguishing characteristic of *C. mexicana* is the presence of a type of frond that exhibits an intermediate form between clavate and club-shaped (wider than the tip parts of ramuli) falcate ramuli, while *C. taxifolia* manifests a variant of falcate, sickle-shaped ramuli (Verlaque et al., 2015). The basal part of the ramuli of both species was contracted, but this was not observed in *C. taxifolia* var. *distichophylla* (Verlaque et al., 2015) (Figure A1).

In the Mediterranean basin, *Caulerpa taxifolia* is an invasive alga that was introduced into the ecosystem from an aquarium in France (Meinesz and Hesse, 1991). In Turkish waters, the species was observed in İskenderun Bay (Mediterranean Sea), followed by the Gulf of İzmir and Dikili Bay (Aegean Sea). However, its biometry was not extensively detailed because of its previous occurrence along the bottom depth gradient. Following the sporadic introduction of specimens to the Mediterranean Sea in 1991 (Meinesz and Hesse, 1991), the species proliferated at depths ranging from 45 to 100 m. Along the French coast, the species was found to consist of a stolon measuring approximately 20 cm, bearing a cluster of approximately ten living fronds, each ranging from 5 to 15 cm in length (Belsher and Meinesz, 1995). In areas where *Posidonia oceanica* was present, the positive impact of *P. oceanica* on *C. taxifolia* was more pronounced at the deepest edge of the seagrass stand, where shoot density was lower. This phenomenon was partially explained by the observation that the algal leaves were larger where they occurred at the edge of the mimic transparent plants. This suggests that protection comes at a cost. Therefore, dense *P. oceanica* meadows are likely to be less vulnerable to algal invasion, while sparse meadows represent an optimal compromise between protection and shading (Villele and Verlaque, 1995; Ceccherelli and Cinelli, 1999b).

Biometric parameters are critical variants that are identical to species or congeneric species, as evidenced by the analysis of seaweeds and seagrasses. In addition, the environment can modify biometric measurements, thereby creating measurements specific to the location and broad-scale space. Furthermore, depth-dependent environments are very effective for the flora and fauna of bottom ecosystems. At a depth of 20 m, where *C. taxifolia* colonized the lake, Molenaar et al. (2006) observed that the density remained within normal parameters, measuring 311 to 515 ± 32 shoots/m^2 (Cap Martin, France). This finding aligns closely with our own measurements at a similar depth (431 ± 572 shoots/m^2). The photosynthetic capacity of *C. taxifolia* exhibited a positive or null trend within the temperature range of 5-35 °C, yet demonstrated a negative response at 40 °C (Gacia et al., 1996). The maximum photosynthetic capacity was observed in November, followed by a slight but significant decline in July, and an approximate twofold reduction in April, September, and January (Gacia et al., 1996). Consequently, *C. taxifolia* demonstrated pronounced seasonal patterns in terms of light-saturated photosynthetic rates, which exhibited no correlation with the environmental parameters that predominantly influence the productivity of Mediterranean seaweeds, namely light availability, water temperature, and nutrient availability (Ballesteros, 1989).

Extreme salt loading, defined as a concentration of more than 50 kg per square meter, has been observed to significantly reduce the abundance of *C. taxifolia*. A study conducted by Glasby et al. (2005) reported a 75-90% decrease in salt loading at the *C. taxifolia* site compared to the control site. The study also noted that shoot density and the number of fronds per stolon remained constant for *C. taxifolia*, irrespective of bottom depth. However, the analysis revealed significant variations in other biometric variables with changes in bottom depth. The findings suggest that *C. taxifolia* may be nutrient limited, as evidenced by the positive correlation between leaf density and nutrient addition (Ceccherelli and Cinelli, 1997). Moreover, the removal of *C. taxifolia* has been shown to result in the sustained presence of high nutrient concentrations in fertilized sediments during competition with *Cymodocea nodosa* (Ceccherelli and Cinelli, 1997). At a depth of 9 m (Cap Martin, Alpes-Maritimes, France), the length of *C. taxifolia* stolons exhibited a range from 150 to 352 mm throughout the year. The rhizoid columns were regularly spaced and demonstrated no seasonal variation, with an average of one column every 1.63 ± 4.3 cm along the stolon. The density of primary fronds exhibited a range from 5100 to 13920 m^{-2}, with the maximum number of branches recorded in spring, at 25000 m^{-2} (Meinesz et al., 1995).

The variables that demonstrated a tendency to increase or decrease with depth included stolon length, frond length and width, leaf area, internodal distance, and the number of fronds per stolon. The leaf size of *C. taxifolia* was found to be greater at 10 m than at 2 m depth, while controlling for the presence of *P. oceanica* (Ceccherelli and Cinelli, 1999b). The greatest blade length of *C. taxifolia* was found in individuals at the edge of *P. oceanica*, while the blade length was intermediate within *C. nodosa* beds (Ceccherelli and Cinelli, 1998). A reduction in blade density was observed in spring in sandy and *C. nodosa* habitats but not in *P. oceanica* habitats (Ceccherelli and Cinelli 1998). However, the mean biomass of *C. taxifolia* at 5 m depth was greater than that at 20 m depth on the French Mediterranean coast (Thibaut et al., 2004), which contrasts with our estimates of the density variables. The biomass changes in exposed areas are abrupt due to frequent thermal fluctuations, while biomass changes progress in sheltered areas (Thibaut et al., 2004). The optimal temperature for maximum stolon and assimilator elongation was determined to be 25 °C, with growth observed up to 30 °C and slight growth at 15 °C. The growth patterns of *C. taxifolia* remained consistent across the range of irradiances studied. The maximum mean stolon and assimilator elongation was recorded at an irradiance of 75 µmol photons m^{-2} s^{-1} (Gillespie et al., 1997). The number of fronds was 10-12 per 10 cm of stolon, and the fronds were 2.0-10.0 cm high and 1.9-3.5 mm wide. A comparison of the Turkish Levantine coast (Cevik et al., 2007) with the Turkish Aegean coast revealed that the former had a higher number of fronds, though these were longer and narrower than those measured in the present study. The Turkish Aegean coast had an average photosynthetic photon flux (PAR) of approximately 4.8 mol photons/cm^2/s, which is indicative of higher levels of photosynthesis (Burfeind and Udy, 2009). It has been established that light and nutrients play a pivotal role in the growth of *C. taxifolia* (Burfeind and Udy, 2009).

Interbiometric relationships can be used to characterize species, and in the present study, these relationships were differentiated by bottom depth for *C. taxifolia* as well as *C. mexicana* and *C. taxifolia* var. *distichophylla*. Two congeneric species were identified, and they exhibited significant differences in their relationships with each other (Figure A2). The relationship between frond length and the number of lateral branches exhibited a slope greater than 2 for *Caulerpa taxifolia* var. *distichophylla* and a slope less than 1 for *C. taxifolia* (Figure A2) (Mutlu et al., 2025a). The length-width relationship of the frond of *C. taxifolia* var. *distichophylla* specimens collected from Antalya Gulf of the Turkish Mediterranean coast (Mutlu et al., 2022) exhibited a comparable relationship with specimens of the Aegean Sea (Mutlu et al., 2025a) (Figure A2). The relationships between the seas were not significantly different at p <0.05 (ANOCOVA, p: 0.457). Consequently, the plasticity of the specimens due to the different marine environment could not be valid for *C. taxifolia* var. *distichophylla* in terms of the biometrical relationship (Figure A2). This is further supported by the observation that the sampling dates of both seas were highly different (Mutlu et al., 2022, 2025a).

However, the basal part of ramuli of the specimens published by Erduğan et al. (2009) exhibited no contraction, and the frond blade was sickle-shaped (Verlaque et al., 2015). The species could be identified as *C. taxifolia* var. *distichophyla*. In contrast, the specimen reported from the Turkish coast of the Aegean Sea by Turan et al. (2011) is *C. taxifolia*, as the basal part of the ramuli was contracted (Verlaque et al., 2015). Turan et al. (2011) characterized the biometry of the pinnules of *C. taxifolia* collected from the İzmir Gulf, Aegean Sea, as follows: The mean stolon diameter, frond width, maximal length of pinnules, and width of pinnules of a total of 50 *C. taxifolia* samples were 1.6 ± 0.5 mm, 9.9 ± 2.3 mm, 5.4 ± 1.3 mm, and 1.1 ± 0.1 mm, respectively. Cevik et al. (2007) described the fronds of the putative species *C. taxifolia* isolated from İskenderun Bay as green, simple to 1-2 times laterally branched, 2.0-10.0 cm long, and 1.9-3.5 mm wide. In a similar study along the Turkish coast of the Aegean Sea, Mutlu et al. (2025a) reported that the frond length of *C. taxifolia* var. *distichophylla* ranged from 2.10 mm to 105 mm, with a mean of 46.51 ± 1.4 mm. Similarly, the frond width varied between 1.30 and 4.10 mm, with a mean of 2.68 ± 0.003 mm. The specimens isolated from the aquarium were characterized by green fronds ranging in length from 1.5 to 15.0 cm and in width from 10.0 to 13.0 mm (Cevik et al., 2007). Based on these observations, it was estimated that the FL of *C. taxifolia* ranged from 2 mm. to 250 mm, with 49.9 mm at 10 m to 87 mm at 20 m, followed by 71 mm at 30 m on average, and frond width remained within a narrow range of 8.55 mm to 11.139 mm. However, Erduğan et al. (2009) measured frond length overall in a range of 1-15 cm (0.4-23 cm) and frond width in a range of 6-9 mm (3-13 mm) of a putative species *C. mexicana* (most probably, *Caulerpa taxifolia* var. *distichophylla* according to our description in Table A1 and Mutlu et al. 2025a, b) collected from İskenderun Bay. However, three studies (Cevik et al., 2007; Erduğan et al., 2009; Turan et al., 2011) did not establish the biometric relationship. Molecular data has corroborated these morphological similarities and suggests that *C. distichophylla* is a brittle form of *C. taxifolia* (Famà et al., 2002; Stam et al., 2006).

Cevik et al. (2007) conducted a molecular analysis to identify specimens found in İskenderun Bay (Türkiye) by comparing them to specimens from other world seas and an aquarium in İzmir. They recognized the İskenderun specimens as *C. taxifolia*. Notably, the İskenderun specimens exhibited an absence of contracted structure at the basal part of the ramuli attached to the rachis (Verlaque et al., 2015), which suggests that this species differs from *C. taxifolia* collected from the aquarium (Turan et al., 2011). Indeed, both locus specimens (İskenderun and İzmir) exhibited biometric differences at the time of description performed by Cevik et al. (2007). The specimens inhabiting İskenderun Bay were subsequently corrected for genetically misidentification by Jongma et al. (2013). The following abstract was prepared to emphasize the significance and challenges encountered in both genetic and morphological studies: "On the basis of morphological and molecular studies, we identified the Australian endemic green alga known as *Caulerpa distichophylla* along the coasts of Sicily (Italy, Mediterranean Sea). The slender *Caulerpa* previously reported as *C. taxifolia* from southeastern Türkiye (Gulf of İskenderun) also belongs to *C. distichophylla*. Morphologically, *C. distichophylla* clearly differs from *C. taxifolia* in its slender thallus and the lack of large rhizoidal pillars. However, genetic data do not provide undisputed evidence that the species are distinct. Sequences of the tufA cpDNA gene and of the cp16S rDNA intron-2 sequences separated the two taxa by only one single nucleotide mutation, whereas ITS rDNA sequences did not clearly distinguish them". Specimens of *C. taxifolia* var. *distichophylla* from Rhodes and Cyprus Islands do not exhibit the usual morphologies of *C. mexicana* or *C. taxifolia*, or the typically robust morphology of the invasive *C. taxifolia* in the aquarium strain, but was found in range of plant traits of specimens from the eastern Levant coasts, İskenderun and Antalya bay, Türkiye (Aplikioti et al., 2016; Cevik et al., 2007; Mutlu et al., 2022). We listed their biometry in detail (Table A1) including the Levant specimens (Mutlu et al., 2022) since we observed these two species of *Caulerpa* during the present study (Mutlu et al., 2025a) in addition to *C. mexicana* (Mutlu et al., 2025a, b). Verlaque et al (2015) described the species for their featured traits.

The bud and stolon structure of *C. taxifolia* (Figure 2) was also found to be comparatively simple when compared to those of *C. taxifolia* var. *distichophylla* (Mutlu et al., 2025a).

In conclusion, this study provides valuable insights into the depth biometry of the invasive species *Caulerpa taxifolia* in the Turkish Aegean Sea. The biometry and biometrical relationship could be specific to similar species of the *Caulerpa* genus. These results demonstrated the ability of *C. taxifolia* to adapt to and thrive under a range of environmental conditions in the Aegean Sea, and the biometric plasticity of the species likely contributes to its invasive success by allowing it to exploit different niches. This study establishes a foundation for the region's invasive alga monitoring and ecological impact assessment. Subsequent studies will prioritize the analysis of seasonal depth-wise population and biometric dynamics to inform ecosystem management.

ACKNOWLEDGEMENTS AND FUNDING

The authors would like to express their sincere gratitude to the Scientific and Technological Research Council of Turkey (TÜBİTAK) for providing financial support through project number 124Y031. The authors also wish to acknowledge the dedicated efforts and support provided by the crew of the R/V Akdeniz Su during this project.

CONFLICT OF INTEREST

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

AUTHOR CONTRIBUTIONS

All the authors contributed to the study conception and design. Material preparation and measurements, data collection, scripting, and analysis were performed by Erhan Mutlu, Barış Akçalı, Yaşar Özvarol, Zeynep Zabun, Zeynep Narlı and Berivan Elif Alsan. The first draft of the manuscript was written by Erhan Mutlu and reviewed by Barış Akçalı, and all the authors commented on previous versions of the manuscript. All the authors read and approved the final manuscript.

ETHICAL APPROVAL STATEMENT

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

DATA AVAILABILITY STATEMENT

Not applicable.

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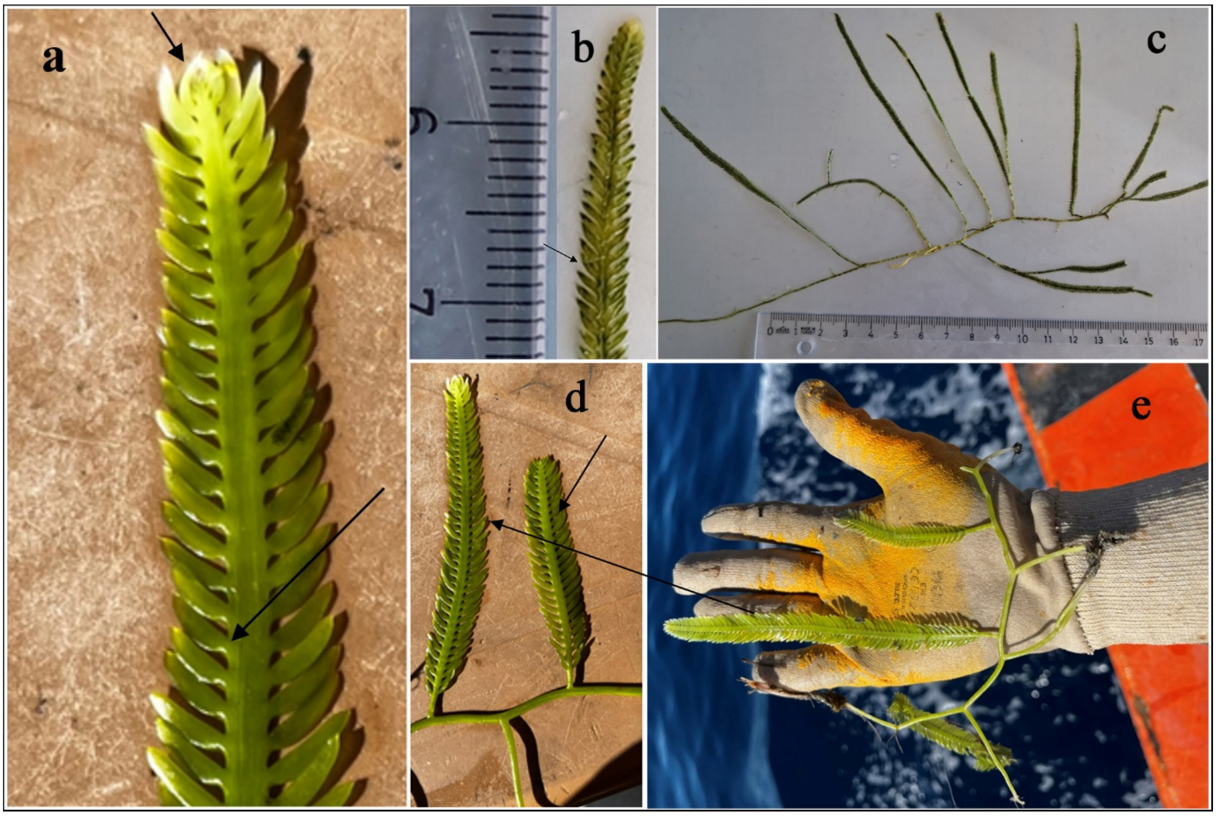

Figure A1. Fronds, rachises and pinnae of *C. taxifolia* (a, d, e) and *C. taxifolia* var. *distichophylla* (b, c, Mutlu et al., 2025a) from the Turkish Aegean waters. Specimen in Fig. A1a could be scaled regarding to same specimen in Fig. A1d, e (a hand of SCUBA diver, Barış Akçalı)

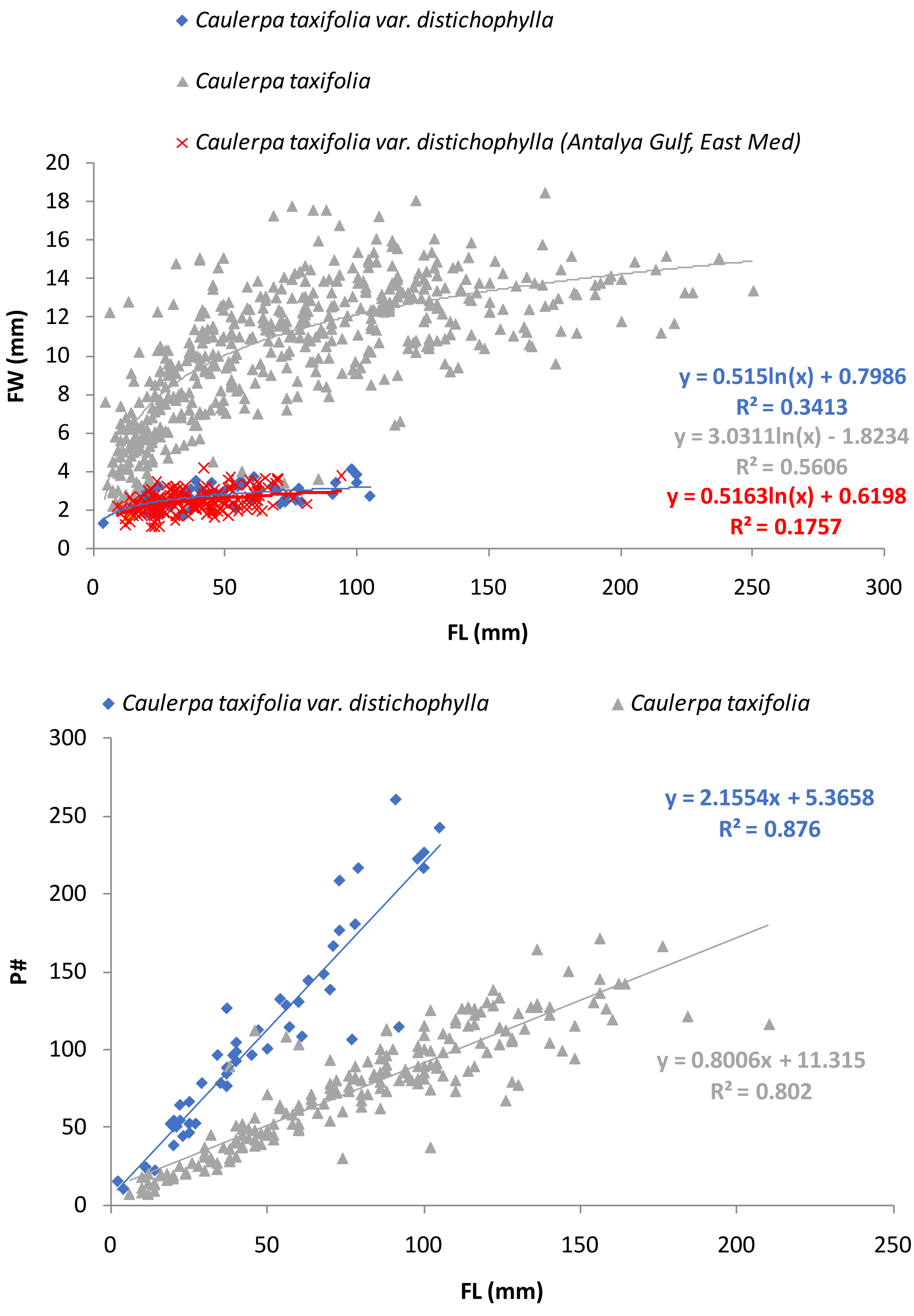

Figure A2. Frond length (FL)-width (FW) and frond length (FL)-number of pinnae (P#) of *Caulerpa taxifolia* and *C. taxifolia* var. *distichophylla* specimens collected during the present study conducted in the Turkish coast of the Aegean Sea (Mutlu et al. 2025a) and *C. taxifolia* var. *distichophylla* from Antalya Gulf, Eastern Mediterranean Sea for FL-FW, regardless of bottom depth and season (data from Mutlu et al. 2022)

Figure A3. *Caulerpa taxifolia* var. *distichophylla* at sea and air from the Antalya bay of the Mediterranean Sea (Mutlu et al. 2022)

Table A1. Plant trait comparison between *Caulerpa taxifolia* in the Aegean Sea (the present study) and *Caulerpa taxifolia* var. *distichophylla* (*C. t*. var. *distichophylla*) in the Mediterranean Sea (Mutlu et al. 2022) (see Fig. A3) and Aegean Sea (Mutlu et al. 2025a). * Additional data from Antalya Bay. +: identical characters for difference between two species. Variable coded below with [1] denoted excluding both ends of the frond. Variable coded below with [2] was given for the number per 1 cm frond length with the related table in a publication by Mutlu et al. (2025a), but the number was indeed per 1 mm frond length

Variable	*Caulerpa taxifolia*	*C. t.* var. *distichophylla*
+Frond shape [1]	ellipsoid	rectangular
Frond length (mm)	49.9-87, 69.1	2.1-105, 46.5 up to 100, 27-48*
+Frond width (mm)	8.55-11.14, 10.1	1.3-4.1, 2.6 up-to 4.8, 2.6-4.8*
+Widest frond	in mid-frond	all through frond
Ramuli shape	sickle	sickle
+Ramuli base	contracted	not contracted
Ramuli tip	knife-shape	knife-shape
+Ramuli tip color	green	blackish
+frond color	green	green in sea, dark green in air
+Rhizoid color	green	cream
+Rhizoid hairs (thallus)	available	almost rudimental
+Thallus aggregation shape	ball-shape	almost rudimental
Stolon shape	slender	slender
+Stolon color	green	dark green-creamy
Budding structure	simple	little complex
Frond apex	similar	similar
Ramuli per 1 cm frond		
+Min-max	3.6-24.3	7.1-34.1 [2]
+Average	9.8	22.7 [2]
+Slope for FL-FW	greater than 2 (>2.4)	less than 1* (~0.5)
+for FL-P#	less than 1 (<1.1)	greater than 2
Rachis shape	similar	similar
+Rachis color	green	dark green
+Rachis midrib	apparent	not-apparent
+Thallus	much hairy	few hairy

http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 42(9), 173-182 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.09

RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Determination of fatty acids and evaluation of nutritional quality of *Barbus lacerta* from Murat River, Türkiye

Murat Nehri, Türkiye'den *Barbus lacerta*'nın yağ asitlerinin belirlenmesi ve besin kalitesinin değerlendirilmesi

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Received date: 08.04.2025 *Accepted date: 13.06.2025*

How to cite this paper:
Özdemir, N. Ş., & Koyun M. (2025). Determination of fatty acids and evaluation of nutritional quality of *Barbus lacerta* from Murat River, Türkiye. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 173-182. https://doi.org/10.12714/egejfas.42.2.09

Abstract: The ultimate goal, to investigate for differences in seasonally fatty acid composition of liver, muscle and gonad from *Barbus lacerta* females' fish, bought from fishermen who hunt from the Murat River, but also to reveal the food quality in edible muscle tissue with nutritional quality index fatty acids. Fatty acid composition of *B. lacerta* varied different among the three tissues/organs and the most important difference was seen between muscle and liver. *B. lacerta* had found higher proportions in 14:0, 16:0, EPA and DHA in muscle and liver than the gonads. In contrast, the gonads had higher proportions of ARA because of the important role of ARA in the ovulation processes (late winter-early spring). In parallel, there were changes in the food quality indexes (AI, TI, h/H, DFA, OFA, ω6/ω3, DHA+EPA, HPI, PUFA/SFA) used based on fatty acids in determining the food quality in edible muscle tissue. The highest change was in AI (Atherogenicity index), although there were no significant seasonal differences. The lowest AI values as good quality index was determined in spring (0.42) and. winter (0.41). The food quality index that stood out in each season was different. Therefore, it can be suggested that *B. lacerta* can be consumed as a high quality and healthy food, partially except for summer.

Keywords: Atherogenicity index, arachidonic acid, *Barbus lacerta*, food quality

Öz: Murat Nehri'nde avlanan balıkçılardan satın alınan dişi B. lacerta balıklarının karaciğer, kas ve gonadlarındaki mevsimsel yağ asidi bileşimlerindeki farklılıkları araştırmak ve ayrıca besin kalitesi indeksi yağ asitleri ile yenilebilir kas dokusundaki besin kalitesinin ortaya konulması amaçlanmıştır. *B. lacerta*'nın yağ asiyi bileşimi üç doku/organ arasında farklılık göstermiştir ve en önemli fark kas ve karaciğer arasında görülmüştür. *B. lacerta*, kas ve karaciğerlerinde gonadlarından daha yüksek oranlarda 14:0, 16:0, EPA ve DHA bulmuştur. Buna karşılık, ARA'nın yumurtlama süreçlerindeki (kış sonu-ilkbahar başı) önemli rolü nedeniyle gonadlar daha yüksek ARA oranlarına sahipti. Buna paralel olarak, yenilebilir kas dokusundaki besin kalitesini belirlemede yağ asitlerine dayalı olarak kullanılan besin kalitesi indekslerinde (AI, TI, h/H, DFA, OFA, ω6/ωω3, DHA+EPA, HPI, PUFA/SFA) değişiklikler olmuştur. En yüksek değişim AI (Aterojenite indeksi) da olmakla birlikte önemli mevsimsel farklılıklar yoktu. Besin kalite indeksi olarak en düşük AI değerleri ilkbaharda (0,42) ve kışın (0,41) belirlendi. Her mevsimde öne çıkan gıda kalite indeksi farklıydı. Bu nedenle, *B. lacerta*'nın yüksek kaliteli ve sağlıklı bir gıda olarak tüketimi kısmen yaz hariç önerilebilir.

Anahtar kelimeler: Aterojenite indeksi, araşidonik asit, *Barbus lacerta*, gıda kalitesi

INTRODUCTION

Fatty acids as dietary nutrients are important compounds as energy-rich biochemical for consumers and can be used as tracers of organic matter pathways in aquatic food webs. Aquatic ecosystems are the primary source of omega-3 fatty acids (ω3 FAs), because of supporting both aquatic and terrestrial heterotrophs by the trophic transfer of these key essential fatty acids (EFAs) via food webs (Gladyshev et al., 2013). It is known that fish meat is a valuable food in human nutrition (Ahmed et al., 2022). A large body of research has detailed how the growth and reproduction of many aquatic consumers can be restricted by the limitation of EFA, suggesting that fatty acids are a promising biochemical metric for use as a proxy for ecosystem-scale food quality (Galloway and Winder 2015). In nature, fatty acids are found in the form of mixtures of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). Especially, PUFA are vital dietary elements that play an important role in human nutrition. In addition to providing the body with an important source of energy such as SFA, PUFA obtained from fish play a role in cell membrane structure, blood pressure regulation, and coagulation; participate in the proper functioning of the immune system and the assimilation of fat-soluble vitamins; affect the synthesis of pro- and anti-inflammatory substances; and protect the cardiovascular system (Mititelu et al., 2024).

Therefore, their nutritional and medicinal values should be determined. In recent years, classical indices such as SFA, MUFA, PUFA, ω6, ω3 and ω6/ω3 have not been taken into account much. Instead, indices such as PUFA/SFA, atherogenicity index (AI), thrombogenicity index (TI), hypocholesterolemic/hypercholesterolemic ratio (h/H), health-promoting index (HPI) and sum of eicosapentaenoic acid and docosahexaenoic acid (EPA + DHA) are taken into account for fish species (Chen and Liu, 2020).

FA composition of different tissues and organs may vary according to their specific physiological roles (Parzanini et al., 2021). Muscle and liver are the main tissues that mainly consist of triacylglycerols (TAG) and storage fats (Pierron et et al., 2009). The liver is the main site of lipogenesis (Tocher, 2003) and energy conversion (Hansen and Abraham, 1983) in fish. Gonads are more resistant to changes in fatty acid composition when compared to other tissues, since its composition is genetically determined to allow adequate embryo development (Sargent et al., 2002). For example, ARA (Arachidonic acid, 20:4ω6) is one of the major nutrients to ensure reproductive success in many fish species (Tocher, 2010), as is the main precursor for the 2-series prostaglandins (PG-2), eicosanoids that stimulate steroid synthesis in the ovary, trigger oocyte maturation and affect the sexual behavior of females (Tocher, 2003).

Although there are many studies on seasonal changes in many different freshwater fish species and some Barbus species, no reports have yet been published on the effects of seasonal changes on the fatty acid composition and nutritional quality with food quality index fatty acids of the freshwater female Kura Barbell (*Barbus lacerta,* Heckel 1843*)*. *Barbus lacerta* is an Asian origin and distributed in the Euphrates-Tigris rivers in the Mesopotamian basin. *B. lacerta* is a member of Cyprinidae family from ray-finned fishes. It is an endemic fish species for inland waters of Eastern Anatolia, Türkiye (Geldiay and Balık, 2007). Based on all these, it was aimed to evaluate the fatty acid composition of *B. lacerta* both as an ecological and nutritional source. We put forward the hypothesis that these changes will show significant differences in different tissues seasonally and this may have important effects on the nutritional quality of edible muscle tissue. We think that this will shed light on the hunting and consumption of this fish species during the period when the nutritional quality is best. Tissues (muscle, liver and gonad) of female *B. lacerta* was used in the study because females had higher length and weight than males. (Dopeikar et al., 2015; Şen Özdemir et al., 2023). All these increase the economic value of female *B. lacerta*. For these reasons, we preferred to examine the differences in fatty acid composition among the three tissues of females.

MATERIALS AND METHODS

Preliminary preparations

Barbus lacerta samples were obtained from contracted commercial fishermen. Samplings were done randomly in different months to represent the seasons from different points in Göynük Stream, Murat River (Türkiye). Nets with different eye apertures were used in catching the fishes. The catching fish were brought to the laboratory on the ice (September 2018-August 2019). Sexually mature fish were used in the analysis. Fish were cut according to the butterfly fillet technique (Liu et al., 2022). The long-based fins, head and skin were cut off. The meat along the "top" of the fish was cut and separated from the bones. The backbone was separated from the tail and lift the spine from the tail and pull out the meat, removing a butterfly fillet from the fish. The muscle tissues of fish were separated from the inedible parts of the deceased fish. The internal organs (liver and gonad) were removed by pliers. The gender determination was made macroscopically from the gonads of the fish catching fish. The study was carried out on the adult female fish (mean total weight=32 kg; mean total length=15 cm) (32-53 individuals). Then, every tissue/organ sample was sealed in plastic bags. All the fish muscle were stored at -80 ˚C for further analysis.

Lipid extraction and fatty acid derivatization

Lipid was extracted from muscle, liver and gonad tissues separated from caught fish. Hara and Radin (1978) method were used in hexane: isopropanol (3:2) solution for lipid extraction. These tissues are separately taken from each sampling period and then centrifuged at 4000 rpm for 10 min, leading to the formation of two separate layers. The supernatant layer was removed from the organic layer and transferred into new tubes. 5 ml 2% methanolic sulfuric acid solution was added to the supernatant layer and mixed completely with the vortex. The mixture was left to be methylated in an oven at 55 ˚C for 15 hours. Then, 5 ml of 5% NaCl was added and mixed. 5 ml of hexane was added to the tubes and waited about 3 hours at room temperature. The hexane phase formed was taken from the top, 5 ml 2% $KHCO_3$ solution was added to the tubes. The final extracts were evaporated using a gentle stream of nitrogen evaporator (Allsheng WD-12). Then, 1 ml hexane was added to the dry lipid layer and the mixture was vortexed. Every sample was taken into 2 ml labelled autosampler vials and waited -20 ˚C until mass spectrometer gas chromatograph (GC/MS) analysis.

GC/MS analysis

GC/MS (American, Agilent 5975 C) was used for the FAME analysis. Machery-Nagel (Germany) capillary column (30 m x 0.25 mm, 0.25 µm) was used. The column temperature was 120-220 °C, the injection temperature was 240 °C and the detector temperature was 280 °C. Helium (He) (0.5 ml/min) was used as a carrier gas (David et al., 2005). FAME of the catching fish were made from the retention times of each fatty acid using standard (Supelco: 37 component FAME mix (Product number 47885-U). After analysis, wsearch32 software was used in the identification of the peaks of each fatty acid. Individual FA data were reported as percentage weights (%) of total identified FA.

Determination of nutritional quality of *B. lacerta*

There is certain fatty acid groups used as nutritional quality index in fish. In this study, the following 9 indices were used to determine the nutritional quality index of *B. lacerta*.

1. Atherogenicity index (AI)

AI= [12:0 + (4×14:0) + 16:0]/(ω3PUFA + ω6PUFA + MUFA)] **(**Ulbricht and Southgate 1991; Garaffo et al., 2011; Luczynska and Paszczyk 2019**)**

2. Thrombogenicity Index (TI)

TI= [14:0 + 16:0 + 18:0]/[(0.5×18:1) + (0.5×ΣMUFA) + (0.5×ΣPUFAω6) + (3×ΣPUFAω3) + ΣPUFAω3/ΣPUFAω6)] (Ulbricht and Southgate 1991; Santos-Silva et al., 2002; Garaffo et al., 2011; Łuczyńska and Paszczyk 2019)

3. Hypocholesterolemic/hypercholesterolemic ratio (h/H)

h/H=[(18:1 + 18:2 + 18:3 + 20:3 + 20:4 + 20:5 + 22:4 + 22:5 + 22:6) / (14:0 + 16:0)] (Santos-Silva et al., 2002)

4. Hypercholesterolaemic fatty acids (OFAs)

OFA= 12:0 + 14:0 + 16:0

High OFA content reduces the lipid quality of food. (Łuczyńska and Paszczyk 2019)

5. Polyunsaturated fatty acids/saturated fatty acids ratio (PUFA/SFA)

6. Total of eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA)

7. Polyunsaturated Omega 6 Fatty Acids/ Polyunsaturated Omega 3 Fatty Acids (ω6/ω3)

8. Desirable fatty acids (DFAs)

DFA=18:0 + ΣMUFA+ΣPUFA (Costa et al., 2008; Silva et al., 2019; Łuczyńska and Paszczyk 2019; Tibaoui et al., 2020).

9. The health-promoting index (HPI)

HPI =∑MUFA+∑PUFA/[12:0+(4 × 14:0) + 16:0] (Chen and Liu 2020).

Statistical analysis

Only individual FAs present with mean proportions ≥0.5% across the different tissues/organs (muscle, liver and gonad) were analyzed statistically. Multivariate statistics were used to analyze differences in fatty acid composition in PRIMER-e 2017. The Bray Curtis similarity coefficient was used for PERMONAVA, principal coordinates (PCO) and CLUSTER analysis for similarity ranges. In analyses of the entire fatty acid data's (total fatty acids, food quality index fatty acids) of *B. lacerta* factored by season, the tissues/organs (muscle, liver, gonad). PERMANOVA tests and SIMPER (Cut off for low contributions: 70%) were conducted on Bray–Curtis similarities with the unrestricted permutation of raw data method (number of permutations 9999; type III sums of squares). The tests were used to identify the fatty acids that contributed the most to the similarities between/within the factor groups.

To further investigate the seasonal effects on changes in nutritional quality in edible muscle tissue and differences in fatty acid composition among tissues/organs, one-way analysis of variance (ANOVA) (Post-Hoc, Homogeneous Groups, Significant Differences) was conducted. ANOVA tested the significance ($p<0.05$) of the differences in the effect of seasons and tissues/organs (liver, muscle, gonad) on fatty acids using STATISTICA software.

RESULTS

Fatty acid composition of *B. lacerta* tissues/organs (muscle, liver, gonad)

The fatty acid composition in all *B. lacerta* tissues/organs taken during the sampling period was investigated regardless of the sampling season. FA composition varied differently among the three tissues/organs (PERMANOVA, Pseudo-F=3.48, P(*perm*)=0.0002) (Figure 1). The most important difference was between the muscle and liver, muscle and gonad with the same P(*perm*) value=0.002. However, the difference between muscle and liver was higher (t=1.99) than the other (t=1.97). The average similarity within tissues/organs changed between 71-76 % (liver-muscle) according to SIMPER results. The fatty acids that contribute the most to the similarity were 16:0 (20%; 22%, respectively) within the liver and gonads. EPA was the first contributor within muscle with 21%. However, EPA was in the second contributor within liver and gonad with the same value 17%. The highest difference between liver and gonad was in ARA from PUFA, between liver and muscle in ι16:0 from SFA (Figure 2, Table 1). ARA was the lowest in the liver, the highest in the gonad, while the EPA and DHA were lower in the gonad and the highest EPA was in the liver, the lowest DHA was in the muscle tissue. However, the percentage of LC-PUFA in the muscle and liver of *B. lacerta* was higher than gonad (Table 1). The highest difference between liver and gonad was in ARA from PUFA, between liver and muscle in ω16:0 from SFA. There was no difference between the tissues/organs in ∑SFA, while there was a difference between the tissues/organs in ∑MUFA, ∑PUFA and ω3 fatty acids, (Table 1, $p<0.05$).

Table 1. Mean fatty acid composition for muscle, liver and gonad in catching fish of female *B. lacerta* during the sampling period (% of total FA)

FAs	LIVER (n=39)	MUSCLE (n=53)	GONAD (Ovary) (n=32)
12:0	1.07±0.99	1.10±0.98	1.15±1.11
14:0	2.91±2.07	2.78±1.80	3.30±2.21
15:0	0.54±0.40	0.45±0.27	0.54±0.48
ι16:0	0.58±0.57[b]	1.36±1.00[a]	1.13±0.92[a]
16:0	16.71±4.18	17.70±5.32	18.37±4.31
17:0	1.30±1.08	0.81±0.53	0.70±0.58
18:0	3.88±2.18	3.22±2.01	4.04±2.45
20:0	0.76±0.56	0.54±0.41	0.73±0.52
∑SFA	**28.30±6.01**	**28.57±4.94**	**30.43±5.90**
MSFA*	0.55±0.49[a]	0.61±0.78[a]	0.47±0.22[b]
14:1	0.73±0.68	0.67±0.50	0.84±0.51
16:1ω7	13.87±5.52[a]	11.52±3.37[b]	13.85±4.42[a]
17:1	0.62±0.47	-	-
18:1ω9	10.86±6.78	8.90±4.81	10.39±6.31
20:1ω9	2.28±2.03[a]	1.37±1.07[b]	1.10±0.79[b]
22:1ω11	-	-	0.62±0.55
22:1ω9	0.59±0.45	-	-
22:1ω7	-	-	0.62±0.42
∑MUFA	**29.25±10.12[a]**	**24.03±6.55[b]**	**28.33±10.36[ab]**
MMUFA*	0.30±0.26[c]	1.57±0.86[a]	0.91±0.62[b]
16:2ω4	0.52±0.47	1.26±0.74	1.09±0.58
18:2ω6	2.64±2.32	2.76±2.01	2. 08±1.49
18:3ω3	3.39±2.03	4.47±2.31	3.79±2.87
20:4ω6 (ARA)	4.55±2.78[b]	5.70±2.22[b]	7.46±3.37a
20:5ω3 (EPA)	15.11±5.78[b]	18.76±4.66[a]	14.81±5.14[b]
22:2	1.28±0.77	1.36±0.59	1.13±0.38
22:6ω3 (DHA)	14.05±6.26[a]	12.56±4.94ab	10.00±5.40[b]
∑PUFA	**42.45±9.07[b]**	**47.40±7.08[a]**	**41.32±8.79[b]**
MPUFA*	0.91±0.82[a]	0.53±0.42[b]	0.96±0.69[a]

*Minor FA<0.5 (19:0, 21:0, 23:0 from SFA; 15:1, 20:1ω7, 20:1ω11 from MUFA, 18:3ω4, 18:3ω6, 20:2ω6, 22:5ω3 from PUFA. Means followed by different letters (a, b, c), letter groups in the same row are significantly different ($p<0.05$), while means do not differ if there are no letters in the same row ($p<0.05$). , ± SD (Standard Deviation), '-' non defined.

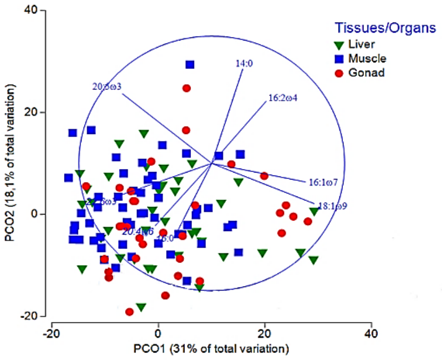

Figure 1. Differences in the fatty acid composition of *B. lacerta* tissues/organs plotted using PCO. The lower triangular matrix was created using Bray–Curtis similarity coefficients. Pearson correlation (p>0.60).

Seasonally FA composition of *B. lacerta* tissues/organs (muscle, liver, gonad)

Liver

The FA composition of *B. lacerta* liver varied across seasons (PERMANOVA, Pseudo-F=2.79, *P (perm)*=0.0003, *Pearson Correlation* p>0.55). The most seasonally differences were between summer and autumn (t=1.97 *P (perm)*=0.004). The most average similarity was within winter (77%). The fatty acids that contributed the most to this similarity were 16:0 (palmitic acid) (21%), EPA (19%), 16:1ω7 (15%), DHA (14%) and 18:1ω9 (8%) (Bray-Curtis Similarity, Cut off for low contributions: 70 %). Seasonally fatty acid composition of liver was important for 14:0, 18:0, 18:ω9, EPA, ω3 fatty acids. 14:0, ARA, EPA and DHA were characteristic fatty acids for all seasons in liver. However, 16:1ω7, 18:1ω9 and 18:3ω4 were more characteristic fatty acids for autumn than other seasons with similarity 66.7% (Figure 2). The most important difference was only ∑MUFA in total FA groups (Table 2), especially between autumn and winter (p<0.05). The most significant difference was between summer and winter for 14:0 autumn and winter for 16:1ω7 and following ω3 fatty acids between spring and autumn. The difference between autumn and winter, where 18:1ω9 differed, was partially significant

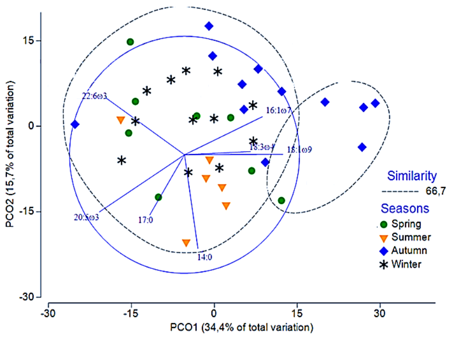

Figure 2. Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in liver of *B. lacerta*. The lower triangular matrix was created using Bray–Curtis similarity coefficients. Pearson Correlation (p>0.55)

Table 2. Seasonally fatty acid composition for liver of *B. lacerta* (% of total FA)

FA	LIVER			
	Spring (n=9)	**Summer (n=6)**	**Autumn (n=12)**	**Winter (n=12)**
12:0	1.19±0.99	1.29±0.62	0.55±0.38	1.43±0.34
14:0	3.16±1.51[ab]	5.50±3.13[a]	2.26±0.66[b]	2.15±0.86[b]
ι16:0	-	-	0.62±0.23[b]	0.94±0.45[a]
15:0	0.61±0.32	0.51±0.25	0.53±0.31	0.50±0.28
16:0	15.46±4.24	14.72±4.83	17.15±6.79	18.20±4.04
17:0	2.67±1.56[a]	1.52±0.12[ab]	-	1.01±0.23[b]
18:0	3.02±1.02[b]	6.53±3.18[a]	3.69±1.58b	3.63±1.99[b]
20:0	1.17±0.34	1.27±0.56	-	0.60±0.45
23:0	-	0.67±0.48	-	-
***MSFA**	0.84±0.76[a]	0.56±0.45[b]	0.60±0.48[ab]	0.46±0.65[b]
ΣSFA	**28.12±4.12**	**32.57±4.78**	**25.40±3.12**	**28.92±2.78**
14:1	0.50±0.30	1.35±1.03	0.51±0.25	0.80±0.42
16:1ω7	13.72±3.12	9.06±3.25	16.19±7.10	14.06±4.44
17:1	0.94±0.22	0.86±0.31	-	-
18:1ω9	9.35±5.22[ab]	9.54±2.70[ab]	15.66±8.44[a]	8.38±3.84[b]
20:1ω9	1.39±0.98	2.84±2.12	2.55±2.10	2.02±1.40
22:1ω9	-	-	1.18±0.56	0.58±0.36
22:1ω11	-	-	-	-
***MMUFA**	0.57±0.46[b]	1.43±0.84[a]	1.63±1.34[a]	0.91±0.76[b]
ΣMUFA	**26.47±7.14[b]**	**25.08±6.23[b]**	**37.72±8.56[a]**	**26.75±5.67[b]**
16:2ω4	-	-	0.83±0.34	0.70±0.42
18:2ω6	1.41±0.85	2.70±1.53	4.56±3.22	1.77±1.02
18:3ω3	2.91±1.70	4.86±3.22	3.65±3.12	2.94±2.05
ARA	3.51±2.20	3.40±1.80	4.52±3.45	5.78±2.19
EPA	17.60±3.54[a]	17.57±3.96[a]	10.64±5.74[b]	16.97±3.72[a]
22:2	1.81±0.97[a]	1.39±0.93[ab]	0.88±0.35[b]	1.22±0.62[ab]
DHA	18.29±9.37	11.76±6.66	11.55±5.23	14.24±5.89
***MPUFA**	0.78±0.72	0.67±0.34	0.23±0.18	0.71±0.48
ΣPUFA	**46.31±8.54**	**42.35±6.96**	**36.86±4.12**	**44.33±6.23**
Σω3	**38.79±5.12[a]**	**34.19±4.87[ab]**	**25.84±3.18[b]**	**34.15±3.89[ab]**
DHA/EPA	**1.16±0.34**	**0.68±0.26**	**1.18±0.45**	**0.86±0.42**

*Minor FA<0.5 (19:0, 21:0, 23:0 from SFA; 15:1, 20:1ω7, 20:1ω11 from MUFA, 18:3ω4, 18:3ω6, 20:2ω6, 22:5ω3 from PUFA). Means followed by different letters (a,b), letter group in the same row are significantly different (p<0.05), while means do not differ if there are no letters in the same row (p<0.05). ± SD (Standard Deviation), '-' non defined.

Muscle

The FA composition of *B. lacerta* muscle varied across the seasons (PERMANOVA, pseudo-F=2.10, *P(perm)*=0.008, *Pearson correlation* p>0.60). The most seasonally differences were between spring and autumn (t=1.71, *P (perm)*=0.02, p>0.60). The most average similarity was within autumn (79 %). The FA that contributed the most to this similarity were 16:0 (21%), EPA (17%), DHA (14%), 16:1ω9 (13%) and 18:1ω9 (10%) (Bray-Curtis Similarity, Cut off for low contributions: 70%). 14:0, 12:0, 16:0, 16:1ω7, EPA and DHA were characteristic FA for all seasons. However, ι16:0 and ARA were more characteristic FA for winter and 18:1ω9 for autumn than other seasons (Figure 3). No difference was in total FA groups (Table 3) (p<0.05). The most significant difference was between spring and autumn for EPA and following 22:2 between spring and summer, ι16:0 between summer and winter (Table 2, p<0.05).

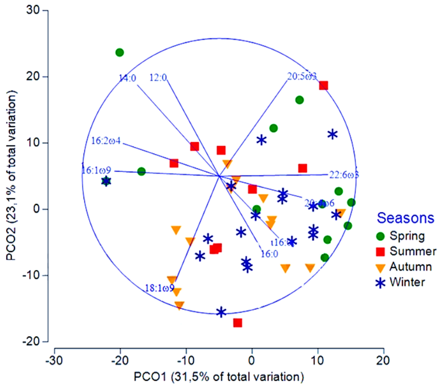

Figure 3. Differences in seasonally fatty acid composition of *B. lacerta* muscle plotted using PCO. The lower triangular matrix was created using Bray–Curtis similarity coefficients. Pearson correlation (p>0.60).

Table 3. Seasonally fatty acid composition for muscle of *B. lacerta* (% of total FA)

FA	MUSCLE			
	Spring (n=12)	**Summer (n=9)**	**Autumn (n=14)**	**Winter (n=18)**
12:0	1.56±1.37	1.12±0.41	0.83±0.77	0.99±0.70
14:0	3.51±1.15^{ab}	3.90±1.48^{a}	2.44±1.06^{ab}	2.00±1.15^{b}
ι16:0	1.32±0.36^{ab}	0.71±0.41^{b}	1.24±0.47^{ab}	1.81±0.51^{a}
15:0	-	0.63±0.45	-	-
16:0	14.61±3.90	19.38±4.74	18.42±5.95	18.38±2.58
17:0	1.26±0.56	0.93±0.45	-	0.75±0.38
18:0	2.74±2.01	3.18±1.71	3.97±2.06	2.74±1.44
20:0	0.80±0.58	0.67±0.35	-	-
23:0	-	-	-	0.77±0.46
***MSFA**	3.00±2.30^{a}	0.56±0.41^{b}	1.79±0.88^{b}	0.97±2.26^{ab}
ΣSFA	**28.80±3.17**	**31.08±0.41**	**28.69±5.85**	**28.41±3.88**
14:1	0.51±0.32	0.98±0.67	0.55±0.34	0.72±0.41
16:1ω7	10.42±4.34	12.78±1.32	12.05±2.77	11.21±3.15
17:1	0.60±0.52	-	-	-
18:1ω9	8.42±3.44	6.72±4.82	10.77±6.04	8.85±4.84
20:1ω9	1.91±1.43	0.96±0.77	1.19±1.12	1.37±0.79
22:1ω9	-	-	-	-
22:1ω11	-	-	-	0.78±0.36
***MMUFA**	0.73±0.59	1.08±0.52	1.78±1.52	1.02±0.96
ΣMUFA	**22.59±5.92**	**22.52±4.32**	**26.34±8.14**	**23.95±7.19**
16:2ω4	1.72±1.05	0.89±2.19	1.26±1.23	1.15±1.21
18:2ω6	1.98±0.95	3.11±1.87	3.27±2.75	2.71±1.36
18:3ω3	3.15±2.25^{b}	5.86±2.00^{a}	5.08±1.96^{ab}	4.16±2.84^{ab}
ARA	5.40±2.46	5.07±0.59	4.95±1.56	6.81±2.25
EPA	21.12±4.54^{a}	18.57±3.75^{ab}	16.01±5.61b	19.41±3.65^{ab}
22:2	1.67±0.77a	0.95±0.25^{b}	1.21±0.43^{ab}	1.48±0.39^{ab}
DHA	15.12±7.21	11.46±1.70	12.66±3.68	11.34±3.04
***MPUFA**	0.45±0.28	0.49±0.18	0.53±0.21	0.58±0.32
ΣPUFA	**50.61±4.93**	**46.40±5.19**	44.97±9.07	47.64±5.08
Σω3	**39.39±6.47**	**35.88±3.06**	**33.76±9.17**	**34.91±5.77**
DHA/EPA	**0.75±0.43**	**0.64±0.24**	**0.84±0.32**	**0.59±0.18**

*Minor FA<0.5 (19:0, 21:0, 23:0 from SFA; 15:1, 20:1ω7, 20:1ω11 from MUFA, 18:3ω4, 18:3ω6, 20:2ω6, 22:5ω3 from PUFA). Means followed by different letters, letter groups in the same row are significantly different (p<0.05), while means do not differ if there are no letters in the same row (p<0.05). ± SD (Standard Deviation), '-' non defined.

Gonad (Ovary)

The FA composition of *B. lacerta* gonads did not vary significantly across the season (PERMANOVA, Pseudo-F=1.30, *P (perm)*=0.21, *Pearson correlation* p>0.60). The most seasonal differences were between summer and winter (t=1.38, *P(perm)*=0.09). The most average similarity was within winter (77%).

The fatty acids that contributed the most to this similarity were 16:0 (21%), 16:1ω9 (17%), EPA (17 %), 18:1ω9 (11%) and DHA (9%), and (Bray-Curtis Similarity, Cut off for low contributions: 70%). ι16:0, 16:0, ARA and DHA were characteristic fatty acids for all seasons in gonads. However, 18:1ω9 and 16:1ω7 were more characteristic fatty acids for autumn and winter. Additionally, EPA, 14:0, 16:2ω4 were more characteristic fatty acids for summer and spring than other seasons with 70% similarity (Figure 4). No difference was in total fatty acid groups (Table 4) (p<0.05). The most significant difference was between spring and winter for ι16:0 and the following 14:0 between summer and winter, summer and autumn (Table 2, p<0.05).

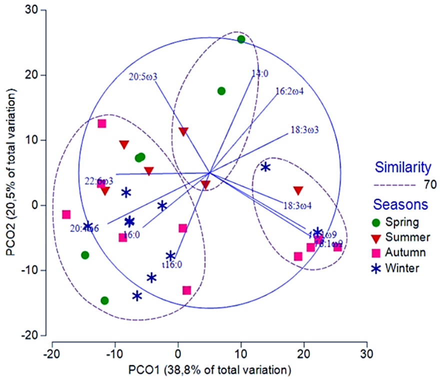

Figure 4. Two-dimensional configuration plot of a PCO analysis of a resemblance matrix of fatty acids in gonad of *B. lacerta*. The lower triangular matrix was created using Bray–Curtis similarity coefficients. Pearson Correlation (p>0.60).

Seasonally FA composition of *B. lacerta* edible muscles

The nutritional quality of *B. lacerta* edible muscle tissue did not vary significantly across the season to SIMPER results; the most average similarity was within winter (94%) for nutritional quality index fatty acids of *B. lacerta* muscle tissue. The FA that contributed the most to this similarity were DFA (56%) and EPA+DHA (19%) (Bray-Curtis Similarity, Cut off for low contributions: 70%). DFA and EPA+ DHA were the most characteristic nutritional indexes for all the seasons. The lowest dissimilarity was between spring and winter (7%), while the highest dissimilarity was between summer and. autumn, spring and summer with 10%. No difference was in nutritional indices of *B. lacerta* except for AI. The most significant difference was between winter and summer for AI (Table 5, p<0.05).

Table 4. Seasonally fatty acid composition for gonads of *B. lacerta* (% of total FA)

FA	GONAD (Ovary)			
	Spring (n=6)	Summer (n=6)	Autumn (n=10)	Winter (n=10)
12:0	1.50±1.20	1.90±1.60	0.77±0.73	0.88±0.53
14:0	3.84±2.68^{ab}	5.67±2.16^{a}	2.49±0.82^{b}	2.37±1.78^{b}
ι16:0	0.86±0.97^{b}	-	1.04±1.06^{b}	1.99±1.02^{a}
15:0	-	-	0.52±0.30	0.81±0.46
16:0	16.82±4.36	20.28±4.75	17.53±4.16	19.01±4.28
17:0	-	1.39±0.71	0.51±0.32	0.62±0.36
18:0	4.86±2.64	3.87±2.22	4.98±3.72	2.71±3.75
20:0	1.31±0.73	-	0.64±0.48	0.61±0.28
23:0	-	-	-	-
***MSFA**	1.17±0.87^{a}	1.25±0.79^{a}	0.44±0.28^{b}	0.62±0.41^{b}
ΣSFA	**30.36±1.03**	**34.36±2.15**	**28.92±3.12**	**29.62±0.87**
14:1	0.62±0.43	1.09±0.76	0.59±0.32	0.86±0.68
16:1ω7	12.23±1.42	11.82±3.36	14.11±5.67	15.58±4.87
17:1	-	-	-	-
18:1ω9	6.95±3.32	8.41±3.38	13.60±±8.25	10.41±3.75
20:1ω9	1.46±0.96	1.23±0.85	1.22±0.71	0.72±0.42
22:1ω9	1.47±1.18	1.43±1.15	2.59±3.12	2.27±3.67
22:1ω11	0.72±0.45	0.56±0.24	-	-
***MMUFA**	0.52±0.35	-	-	-
ΣMUFA	**23.35±5.06**	**24.54±6.28**	**32.11±6.12**	**29.82±5.76**
16:2ω4	2.44±1.34	0.96±0.19	0.93±0.54	0.51±0.45
18:2ω6	1.94±0.89	1.73±0.68	2.74±2.24	1.73±1.05
18:3ω3	3.50±1.49	4.85±2.20	4.77±2.58	2.37±1.03
ARA	7.72±4.28	5.06±3.56	7.05±4.92	9.16±3.90
EPA	16.93±4.06^{ab}	17.44±5.25^{b}	11.56±4.91^{a}	15.20±3.50^{ab}
22:2	1.17±0.36	1.17±0.41	1.13±0.39	1.10±0.66
DHA	11.80±6.71	8.86±4.75	9.79±5.60	9.80±4.34
***MPUFA**	**1.40±0.81**	1.03±0.42	1.00±0.57	0.69±0.36
ΣPUFA	**46.29±4.56**	**41.10±5.83**	**38.97±3.85**	**40.56±3.45**
Σω3	**32.23±7.27**	**31.15±5.12**	**26.12±1.39**	**27.37±2.45**
DHA/EPA	**0.81±0.22**	**0.53±0.19**	**0.85±0.24**	**0.65±0.27**

*Minor FA<0.5 (19:0, 21:0, 23:0 from SFA; 15:1, 20:1ω7, 20:1ω11 from MUFA, 18:3ω4, 18:3ω6, 20:2ω6, 22:5ω3 from PUFA) , Means followed by different letters, letter groups in the same row are significantly different (p<0.05), while means do not differ if there are no letters in the same row (p<0.05). ± SD (Standard Deviation), '-' non defined.

Table 5. Seasonally nutritional quality index fatty acids in muscle of *B. lacerta* (% of total FA)

FAs	Spring (n=12)	Summer (n=9)	Autumn (n=14)	Winter (n=18)
AI	0.47±0.18^{ab}	0.57±0.15^{b}	0.42±0.12 ^{a}	0.41±0.09^{a}
TI	0.54±0.08	0.55±0.11	0.58±0.07	0.56±0.08
h/H	3.10±0.77	2.40±1.07	4.47±2.61	2.73±0.51
OFAs	20.34±3.45	24.34±5.23	19.77±5.59	21.53±2.79
DFA	75.51±14.78	72.10±24.00	77.18±13.91	74.05±17.91
HPI	2.59±0.82	2.04±0.70	3.00±1.56	2.76±0.72
∑PUFA/∑SFA	2.05±0.50	1.68±0.68	2.08±1.41	1.90±0.44
EPA+DHA	36.24±4.31	30.03±4.79	28.67±6.04	30.75±6.36
ω6/ω3	0.18±0.11	0.16±0.06	0.19±0.09	0.23±0.09

*Means followed by different letters (a, b), letter groups (ab) in the same row are significantly different (p<0.05), while means do not differ if there are no letters in the same row (p<0.05). ± SD (Standard Deviation).

DISCUSSION

There were differences among the FA composition of the tissues/organs of wild female *B. lac*erta. The most important difference was between muscle and gonad. However, these differences were not valid for all fatty acids. 14:0, 16:0, 16: 1ω7, 18:1ω9, DHA, EPA, ARA were in the foreground in the three tissues in PCO analysis in *B. lacerta*. 14:0, 16:1ω7, 18:3ω3, 18:2ω6 (Linoleic acid, LA) and ARA have been reported to be typical freshwater fatty acids (Parzanini et al., 2021). Ugoala et al. (2009) reported that the predominant fatty acids in freshwater fish are 14:0 and 16:0 from SFA and 18:1 from MUFA. The predominant PUFA is from the ω6 series and is mainly 18:2 FA. In addition, EFA compounds 18:3ω3 (Alpha-linolenic Acid, ALA) and 18:2ω6 are importance in PUFA. However, freshwater fish have an irregular FA pattern, which are good sources of ω6 EFA (Ugoala et al., 2009). We showed that *B. lacerta* had higher percentage of ω3 FA than ω6 FA. There were only two major fatty acids, ARA and LA from ω6 FA. A similar result was found by Parzanini et al. (2021) in tissues/organs (muscle, liver and eyes) of the European eel. They showed that ω3 FA were higher than ω6 FA in the tissues/organs in all circumstances. On the other hand, it was found that the balanced ratio of ω3 and ω6 FA in freshwater fish increased the reproductive performance and hatchability of zebrafish, *Danio rerio* females by Jaya-Ram et al. (2008). In this study, the most important differences among the tissues/organs were seen in ∑PUFA and ∑ω3 FA and ARA, EPA and DHA from PUFA. Also, the change of ι16:0 from SFA between the tissues/organs was important. ARA was the lowest in the liver, the highest in the gonad, while the EPA and DHA were lower in the gonad, and the highest EPA was in the liver, the lowest DHA was in the muscle tissue. However, the percentage of LC-PUFA in the muscle and liver of *B. lacerta* was higher than gonad. The possible reason for the high percentage of this situation in the muscle of fish is that the LC-PUFA serves as the main energy provider in fish muscle (Hong et al., 2014) and liver (Tocher, 2003). Some recent studies highlighted the key role of LC-PUFA, particularly ARA, in regulating physiological functions of reproduction (Tocher et al., 2010; Majdoubi et al., 2020). Gonad fatty acid composition varies within each mature stage (Anido et al., 2015). The spawning period of *B. lacerta* generally occurs from late March to August with a peak in April (Dopeikar et al., 2015). Highest ARA in gonad of *B. lacerta* was in winter (9%) and spring (8%). ARA is the precursor of several eicosanoids which are produced by the ovarian tissues and play an important role in the ovulation process (Suloma and Ogata, 2011). Thus, ARA was higher in spawning seasons than the other seasons (Majdoubi et al., 2020). Stream invertebrates have a limited innate ability to transform ALA to EPA (trophic upgrading). Thus, their PUFA composition mostly resembles dietary PUFA (Masclaux et al., 2012). Probably, the EPA content of *B. lacerta* was related to terrestrial transport as well as the rich benthic fauna of the Murat River, because EPA percentages of three tissues/organs were higher than the other seasons in spring and summer. In particular, primary production increases in the Murat River due to the excess of terrestrial origin transport in the spring season, which ensures the enrichment of benthic fauna and the fatty acid composition of *B. lacerta*, a benthopelagic freshwater fish, is also affected. 16:1ω7 was the first high abundance MUFA (12-14%) in *B. lacerta the* tissues/organs. The second highest abundance MUFA was 18:1ω9 (9-11%). In contrast, some studies reported that 18:1ω9 was the high abundance MUFA in total lipids in freshwater fish species including the genus *Barbus* (Olgunoğlu et al., 2011; Gokce et al., 2011).

ARA, EPA and DHA proved to be the three most abundant members for *Barbus barbus* (Mancini et al., 2011). Similarly, it was found that EPA, DHA, and ARA were the most abundant fatty acids. Also, 18:1ω9, 16:1ω7 and 16:0 were the most abundant fatty acids in all the three tissues/organs of *B. lacerta* in all seasons. In a study conducted by Gokce et al. *(*2011) in the lake located at the Euphrates River in South Eastern Türkiye, was reported that MUFA were the highest in the *Barbus crypus* muscle tissue, followed by SFA and PUFA. Similarly, Bayır et al. (2011) found that PUFA were at the lowest percentage in muscle tissue of *Barbus capito capito* in different lipid fractions (polar and neutral lipids) for all seasons in Aras River, Türkiye. In our study, the mean highest PUFA were found in muscle tissue. However, the period in which the study was May and June, which is the breeding season of Barbus species (Bayır et al., 2011). If we make a comparison based on the spring period, the most abundant FA group in the spring in the muscle tissue was ∑PUFA (51%), ∑SFA (29%) and ∑MUFA (23%). Although *B. lacerta*, *B. capito capito*, *B. crypus* are members of the same genus (Barbus), it is possible that there are some differences due to the location difference and different Barbus species.

Seasonally nutritional indices in muscle of *B. lacerta*

We examined the FA used as food quality indexes such as AI, TI, h/H, OFAs, DFA, HP, ∑PUFA/∑SFA, EPA+DHA, ω6/ω3 to examine the seasonal changes in nutritional quality of *B. lacerta*. Studies evaluating the nutritional quality of fish based on the food quality index fatty acids are very limited, and no information on this subject has been found for *B. lacerta*. Thus, the evaluation was made by comparing with a limited number of studies conducted with other fish species.

This study determined that there were seasonal changes in the food quality index FA, but the most significant changes were in AI. No difference was found in terms of other indices. The low AI value indicates that the tissues of the examined fish are beneficial for health (Łuczyńska and Paszczyk, 2019**)**. It was determined the lowest AI values in spring (0.42) and winter (0.41) while highest AI value was in summer (0.57). Therefore, we can say that the highest quality *B. lacerta* in terms of AI was found in winter and spring periods. It was reported as 0.37-0.42 in *Abramis brama,* 0.36 in *Cyrinus caprio,* 0.33 in *Oncorhynchus mykiss,* 0.37 in *Perca fluviatilis* (Łuczy´nska et al., 2017), 0.64-0.72 in *Salmo trutta* (Dal Bosco et al., 2013*)*, 0.37-0.67 in *Cyrinion macrostomus* (Şen Özdemir et al., 2023) and 0.29-0.68 in *Micropterus salmoides* (Subhadra et al., 2006) when we looked at the studies conducted with some other freshwater fish. In other indices, seasonal differences within season were not significant and high similarity rates were found in SIMPER analyses and the food quality index that contributed the most to this was fatty acids DFA (55%) and EPA+DHA (22%). The DFA (neutral and hypocholesterolemic fatty acids) index reports the hypocholesterolemic (total cholesterol-lowering) properties of the analyzed lipids (Batkowska et al., 2021). Therefore, the periods when DFA is high represent the most suitable periods for food consumption. Although we did not detect a significant difference among the seasons, we can say that the most suitable consumption periods of *B. lacerta* are autumn (77%) and spring (75%). EPA + DHA is a globally recognized index. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommended an intake of 0.25-2 g EPA + DHA per day (FAO, WHO, 2010). This index is mostly used to evaluate the nutritional value of seafood, especially fish, due to the low content of EPA and DHA in terrestrial plants and animals. Rincón-Cervera et al. (2020) studied the FA composition of fish and shellfish caught in the South Pacific. Their results showed that EPA + DHA varied between 115.15 and 1370.67 mg/100 g in all the studied fish species (Rincón-Cervera et al., 2020). However, there are limited studies on DHA+EPA as a nutritional quality index in inland fish. The EPA+DHA value given as food quality index in fresh water fish *Megalobrama amblycephala* was 5.52-7.36 (Xu et al., 2017). This value was quite low compared to the EPA+DHA of *B. lacerta*. This may indicate that *B. lacerta* has a moderate quality in terms of EPA+DHA as a freshwater fish. According to SIMPER results; EPA+DHA that contributed as the third contributor the difference among the seasons were OFA except for spring and winter. We know that high OFA content reduces the lipid quality of food. In this study, OFA content of *B. lacerta* was relatively high (20-24 %), and spring and autumn were the periods when it was relatively low. TI from the other food quality index FA characterizes the thrombogenic potential of FA, indicating the tendency to form clots in blood vessels and provides the contribution of different FA indicating the relationship between pro-thrombogenic FA (12:0, 14:0 and 16:0) and anti-thrombogenic FA (MUFA and ω3, ω6 families) (Ulbricht et al., 1991). Therefore, consumption of foods or products with lower IT is beneficial for cardiovascular diseases (CVD). In studies on freshwater fish, TI was reported to be 0.16 in *Oncorhynchus mykiss*, 0.20 in *Perca fluviatilis,* 0.18 in *Esox lucius* (Łuczynska et al., 2017), 0.21-0.30 in *Salmo trutta* (Dal Bosco et al., 2013), 0.82-0.87 in *Oreochromis niloticus* (Tonial et al., 2014) and 0.26-0.39 in *Cyprinion macrostomus* (Şen Özdemir et al., 2023). We determined the TI value in *B. lacerta* to be between 0.53-0.58 (winter-autumn). When we compare these values with other reported freshwater fish, it is seen that they are at medium level and low TI increases the nutritional quality of *B. lacerta*. H/h is based on research on the regulation of dietary FA and plasma low density cholesterol (LDL-C) (Dietschy,1998). It characterizes the relationship between hyp ocholesterolemic (18:1 and PUFA) and hypercholesterolemic FA. It is an important nutritional quality index reflecting the effect of FA composition on CVD (Chen and Liu, 2020). h/H changed between 2.40 (summer) and 4.47 (autumn) in *B. lacerta* edible muscle tissue. The h/H value was reported as 1.56-1.63 in freshwater fish *Oreochromis niloticus* (Tonial et al., 2014), 1.88–2.16 in *Salmo trutta* (Dal Bosco et al., 2013) and 1.34-2.20 in *Cyprinion macrostomus* (Şen Özdemir et al., 2023). We saw that *B. lacerta* is a very high-quality food in terms of h/H. Especially, autumn was seen as the best period for consuming *B. lacerta* in terms of h/H.

ω6/ω3 is a useful indicator of the nutritional value of fish lipids and a lower ratio is more effective in preventing CVD associated with plasma lipid levels (Rhee et al., 2017). ω6/ω3 should not exceed 5.0 in the human diet. Therefore, it is suggested that increasing ω3 and decreasing ω6 consumptions to decrease the ω6/ω3 ratio is beneficial to human health (Fernandes et al., 2014). Here, it is not the excess of ω6 in the diet but rather the deficiency of ω3 that increases this risk (FAO, 2014). Matos et al. (2019) reported that the ω6/ω3 ratio was 8.16 for Nile tilapia (cage), 5.40 for Common carp (5.40), 5.27 for Grass carp. ω6/ω3 was reported in 0.22-0.29 for *Cyprinion macrostomus* by Şen Özdemir et al. (2023). In the study, the highest ω6/ω3 for *B. lacerta* was determined in winter (0.23) while the lowest ω6/ω3 of *B. lacerta* was in summer (0.16). The values did not exceed 5.0 and Σω3 fatty acids were higher than Σω6 FA, since high ω3 and low ω6 increase the nutritional quality of the diet, *B. lacerta* appears to be a quality food source. Additionally, the higher the PUFA/SFA ratio will be the higher positive effect (Liu and Chen, 2020). Foods with a PUFA/SFA ratio below 0.45 are considered undesirable foods for the human diet due to their potential to trigger an increase in cholesterol in the blood (Kromhout 2010). In this study, PUFA/SFA ratio was found to be above 0.45 (1.68-2.08; spring-autumn) in *B. lacerta* for every period.

CONCLUSIONS

In the study, muscle, gonad and liver tissues were used as factors to determine seasonal changes of *B. lacerta*. Additionally, by determining the food quality index fatty acids (AI, TI, h/H, OFA, DFA, HPI, ∑PUFA/∑SFA, EPA+DHA, ω6/ω3) in edible muscle tissue, the healthiest consumption period was tried to be determined.

The study indicated that although FA of all the tissue/organs for *B. lacerta* were differed, the most important difference was between muscle and gonads. The seasonal FA composition of each tissue/organ of *B. lacerta* changes in *B. lacerta*. It was observed that ARA was higher in the gonads than the other tissues during the periods when spawning was active, such as the end of winter and the beginning of spring. Because, muscle and liver lipids are mostly used for energy, while gonadal lipids are used for reproduction. ω3 FA were more abundant than ω6 fatty acids. The most abundant ω6 fatty acids were ARA and LA. The most abundant ω3 fatty acids were DHA and EPA.

It was seen that the highest seasonal change was in AI, when the seasonal changes of the food quality index fatty acids were evaluated in edible muscle tissue. We determined that different indexes came to the fore in different seasons except summer, when the food quality index FA were evaluated as a whole. Therefore, it can be suggested that *B. lacerta* can be consumed as a high quality and healthy food partially except summer. It can be seen that *B. lacerta* has a rich PUFA content in all three tissues in all seasons. And this makes it a healthy food that protects against cardiovascular, immune system diseases and high cholesterol.

ACKNOWLEDGEMENTS AND FUNDING

We would like to thank Bingöl University BAP (Grant/Award Number: BAP-FEF.2018.00.004) unit for financial contribution to the project, Dr. Zeynep Ürüşan from the Central Laboratory for helping with GC/MS analyses, and all the fishermen who contributed to providing fish samples.

AUTHORSHIP CONTRIBUTIONS

Nurgül Şen Özdemir and Mustafa Koyun contributed to the design and implementation of the study. Also, Mustafa Koyun bought fish samples from fisherman from the sampling area and prepared the catching fish for analysis. Nurgül Şen Özdemir was responsible for data curation and analysis, and writing the original draft of the manuscript. All the authors reviewed and edited the draft.

CONFLICT OF INTEREST

The authors declare no competing interests.

ETHICS APPROVAL

No ethical approval was required, since fish samples were obtained from commercial.

DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

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http://www.egejfas.org
Ege Journal of Fisheries and Aquatic Sciences, 42(2), 183-190 (2025)

Su Ürünleri Dergisi (2025)
DOI: 10.12714/egejfas.42.2.10

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Atatürk Barajı Gölü'ndeki sazanların (*Cyprinus carpio* L.) iyon homeostazisi ve ozmoregülasyonunda rol oynayan enzimler üzerine kirliliğin etkisi

Effect of pollution on enzymes playing role in ion homeostasis and osmoregulation of carps (*Cyprinus carpio* L.) in Atatürk Dam Lake

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Received date: 30.12.2024 *Accepted date: 03.03.2025*

How to cite this paper:
Fırat, Ö., Temiz, Ö., & Fırat Ö. (2025). Effect of pollution on enzymes playing role in ion homeostasis and osmoregulation of carps (*Cyprinus carpio* L.) in Atatürk Dam Lake. *Ege Journal of Fisheries and Aquatic Sciences, 42*(2), 183-190. https://doi.org/10.12714/egejfas.42.2.10

Öz: Atatürk Barajı Gölü Türkiye'deki en büyük baraj olması (i); çevresindeki yerleşim bölgelerindeki yoğun faaliyetlerden kaynaklı kirleticilerden etkilenmesi (ii); ekonomik, ticari ve besin değeri yüksek su ürünleri içermesi (iii); su kalitesinde ve/veya balıklarda istenmeyen değişimlerin insanları da etkileme potansiyeli (iv) nedeniyle son yıllarda toksikolojik araştırmaların odağı halindedir. Sunulan çalışmada baraj gölünün Adıyaman il sınırlarındaki göreceli olarak temiz olan Samsat ve kentsel aktivitelerden etkilenen Sitilce bölgeleri ile Şanlıurfa il sınırlarındaki tarımsal aktivitelerden etkilenen Bozova bölgesinde 2021 yılının Eylül ayında yakalanan sazanların (*Cyprinus carpio*) dokularındaki Na^+/K^+-ATPaz (EC 3.6.3.9), Ca^{+2}-ATPaz (EC 3.6.3.8) ve Mg^{+2}-ATPaz (EC 3.6.3.2) aktivitelerindeki değişimler araştırılmıştır. Samsat bölgesi ile karşılaştırıldığında Sitilce ve Bozova bölgesi balıklarının solungaç, karaciğer, bağırsak ve böbrek dokularında incelenen tüm ATPaz enzim aktivitelerinde anlamlı azalışlar gözlemlenirken, kas dokusu enzim aktivitelerinde bir değişim gözlenmemiştir. Çalışmamız Atatürk Baraj Gölü'ndeki kirleticilerin balıklarda iyon dengesi, iyon homeostazisi ve ozmoregülasyon mekanizmaları üzerinde toksik etkilere sahip olduğunu ve ATPazların sucul ekosistemlerde kirliliğin belirlenmesinde yararlı biyobelirteçler olarak kullanılabileceğini açıkça ortaya koymuştur.

Anahtar kelimeler: Atatürk Barajı Gölü, *Cyprinus carpio*, balık, ATPaz, su kirliliği

Abstract: The Atatürk Dam Lake has recently become the focus of toxicological research due to the fact that it is the largest dam in Türkiye (i); that it is affected by pollutants originating from the intensive activities in the residential areas around the dam (ii); that it contains aquatic products with high economic, commercial and nutritional value (iii); and that undesirable changes in water quality and/or fish have the potential to affect humans (iv). In the current work, changes in the Na^+/K^+-ATPase (EC 3.6.3.9), Ca^{2+}-ATPase (EC 3.6.3.8) and Mg^{2+}-ATPase (EC 3.6.3.2) activities in the tissues of carps (*Cyprinus carpio*) caught in Samsat (relatively clean area) and Sitilce (area affected by urban activities) in the Adıyaman province borders and Bozova (area affected by agricultural activities) in the Şanlıurfa province borders of the dam lake in september 2021 were determined. When the Samsat region was compared, significant decreases were observed in all ATPase enzyme activities examined in gill, liver, intestine and kidney tissues of fish from Sitilce and Bozova regions, while enzyme activities in muscle tissue did not show any significant change. Our study clearly demonstrated that the pollutants in the Atatürk Dam Lake have toxic effects on ion balance, ion homeostasis and osmoregulation mechanisms in fish and that ATPases can be used as useful biomarkers in determining pollution in aquatic ecosystems.

Keywords: Atatürk Dam Lake, *Cyprinus carpio*, fish, ATPaz, water pollution

GİRİŞ

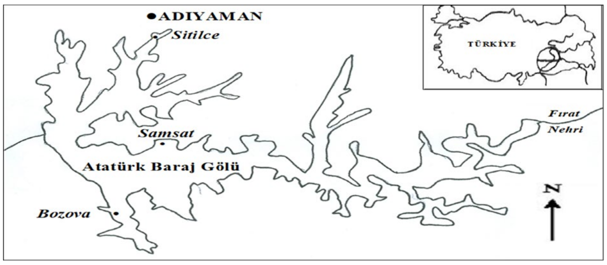
Akuatik ortamların günümüzde artan antropojenik faaliyetler nedeniyle kirlenmesi sucul ekosistemin bileşenleri üzerinde toksikolojik etkileri nedeni ile başta balıklar olmak üzere sucul yaşam için büyük bir risk oluşturmaktadır (Apiamu vd., 2022). Kirleticilerin biyokimyasal ve fizyolojik etkilerinin belirlenmesinde çoğunlukla biyokimyasal belirteçlerin tepkilerine dayalı araştırmalar yapılmaktadır (Temiz ve Kargın, 2024). Çünkü bu toksikantlar canlıların iç dinamiklerinde moleküler ve hücresel düzeylerde değişikliklere neden olmaktadır (Karadag vd., 2014).

Ozmoregülasyon, çevredeki ortamın ozmolaritesine (tuzluluğuna) rağmen, ekstraselüler sıvılarda ozmotik konsantrasyonları aktif olarak koruma yeteneği olup (de la Torre vd., 2007), adenozin trifosfatazlar (ATPazlar) bu regülasyonda önemli rol oynayan ve toksisitenin hassas belirteçleri olarak kabul edilen birkaç izoenzimden oluşan bir gruptur (Lopez-Lopez vd., 2011). Bu enzimler, Na, K ve Ca gibi iyonların biyolojik zarlar boyunca aktif taşınmasından sorumlu olan zara bağlı enzimlerdir (Sancho vd., 2003). Na^+/K^+-ATPaz, Ca^{+2}-ATPaz ve Mg^{+2}-ATPaz biyolojik sistemler için önemli ATPazlar olup iyon konsantrasyonu, enerji metabolizması stabilitesi, membran bütünlüğü ve ozmotik basıncı korumada kritik fonksiyonları vardır (Wang vd., 2020). Hayvan hücrelerindeki mitokondriler tarafından üretilen enerjinin yaklaşık üçte birinin sadece Na^+/K^+-ATPaz'ın işlevi için kullanılması ATPazların canlılar için ne kadar hayati olduğunu göstermesi bakımından önemlidir (Lopez-Lopez vd., 2011). Na^+/K^+-ATPaz, Ca^{+2}-ATPaz ve Mg^{+2}-ATPaz aktivitelerindeki değişimler, tatlı su ekosistemlerindeki kirleticilerin izlenmesi için popüler belirteçlerdir (Veedu vd., 2022). Bu iyon bağımlı ATPazların aktivitesindeki inhibisyonların balıkların iyon dengesi, hücre sinyalizasyonu / metabolizması, hücre zarı bütünlüğü/geçirgenliği ve zar akışkanlığında bir dizi değişikliğe yol açabileceği ve bunun da hücrenin yaşamsal döngüsünde önemli bir bozulmaya neden olabileceği ifade edilmektedir (Temiz vd., 2018).

Tatlı su kaynakları üzerine kirleticilerin etkilerinin değerlendirilmesi bu ortamların geleceği açısından elzemdir. Atatürk Baraj Gölü Fırat Nehri üzerinde 169 m yüksekliğinde ve 1820 m uzunluğunda bir setle kurulan, 817 km^2 yüzey alanı ve 48,7 milyar m^3 su rezervi ile Türkiye için çok önemli bir elektrik enerjisi üretme ve geniş tarımsal alanları sulama potansiyeline sahip (Fırat ve Alıcı, 2012), içerdiği 8 familyaya ait yaklaşık 25 balık türü (Bayhan, 2021) ile su ürünleri bakımından da zengin olan bir rezervuardır. Atatürk Barajı Gölü Türkiye'nin en büyük barajı olması, Adıyaman, Şanlıurfa ve Diyarbakır illerinin arasında yer alması nedeniyle antropojenik aktivitelerinden kaynaklı kirleticilerden olumsuz etkilenmesi, sadece çok büyük bir su hacmini değil ekonomik ve yöre halkı tarafından tüketilen besin değeri yüksek balıkları da barındırması, su kalitesi ve canlı içeriğinde arzu edilmeyen değişimlerin insanları da etkileme potansiyeli nedeni ile son yıllarda toksikolojik araştırmaların sıklıkla yapıldığı bir alan haline gelmiştir. Önceki çalışmalarda barajın birçok bölgesinin insan aktivitelerinden kaynaklı kirleticilerden etkilendiği gösterilmiş ve barajın özellikle Sitilce ve Bozova bölgeleri bu su kütlesinin etrafındaki yoğun nüfus, kentsel faaliyetler, endüstriyel ve tarımsal aktivitelerden etkilendiği, Samsat bölgesinin ise bu aktivitelerden uzak göreceli olarak temiz bölge olarak ifade edilmiştir (Karadag vd., 2014; Firat, 2016; Uçkun, 2017). Sitilce, 2016/2017 yıllarında faaliyete geçen Adıyaman evsel/endüstriyel atık su arıtma tesislerine kadar bu şehirden gelen arıtılmamış atık suların etkisinde uzun yıllar kalmış bir bölgedir (Fırat ve Kılınç, 2022). Bozova bölgesi ise özellikle yoğun tarımsal aktivitenin bir sonucu olarak kullanılan pestisitlerin etkisi altında kalmıştır (Uçkun, 2017). Sunulan çalışmanın temel amacı baraj gölünün farklı bölgelerinden (Samsat, Sitilce ve Bozova) yakalanan sazanların (*Cyprinus carpio*) solungaç, karaciğer, bağırsak, böbrek ve kas dokularındaki Na^+/K^+-ATPaz, Ca^{+2}-ATPaz ve Mg^{+2}-ATPaz aktivitelerindeki değişimlerin belirlenerek kirliliğin balıkların iyon dengesi ve ozmoregülasyon mekanizmaları üzerine etkilerini değerlendirmektir.

MATERYAL VE METOT

Sunulan alan çalışmasında araştırma materyali olarak Dünyada en fazla kültürü yapılan balık türlerinden biri olan, Atatürk Baraj Gölü'nde yaygın olarak bulunan ve yöre halkının da önemli bir besin kaynağı olan *C. carpio* kullanılmıştır. Bu balık üzerinde yapılacak prosedürler Adıyaman Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan alınan etik onaya uygun olarak yürütülmüştür (Protokol No: 2020/067). Ayrıca Tarım ve Orman Bakanlığı Doğa Koruma ve Milli Parklar Genel Müdürlüğü'nden de baraj gölünde yapılacak çalışma için gerekli araştırma izni alınmıştır (Tarih: 16.06.2021, Sayı No: E-21264211-288.04-1734404). Barajın Adıyaman il sınırlarındaki Samsat ve Sitilce bölgeleri ile Şanlıurfa il sınırlarındaki Bozova bölgesi çalışma alanları olarak seçilmiştir (Şekil 1). Sitilce ve Bozova bölgeleri sırasıyla kentsel ve tarımsal aktivitelerden etkilenen kirli bölgeler, Samsat bölgesi ise insan aktivitelerinden etkilenmeyen göreceli temiz bölge olarak dikkate alınmıştır.



Şekil 1. Atatürk Baraj Gölü ve Samsat (37º34'03"N 38º29'47"E), Sitilce (37º43'20"N 38º20'10"E) ve Bozova (37º24'27"N 38º33'03"E) çalışma bölgeleri

Figure 1. Atatürk Dam Lake and study areas of Samsat (37º34'03"N 38º29'47"E), Sitilce (37º43'20"N 38º20'10"E) and Bozova (37º24'27"N 38º33'03"E)

Araştırmamızda yaklaşık olarak ortalama 40 cm total uzunlukta ve 1000 g ağırlıkta sazanlar kullanılmış, her bir bölgeden on adet balık alınmış ve çalışmamız totalde 30 balıkla yürütülmüştür. Samsat ve Sitilce bölgelerinden balık örnekleri aynı gün, Bozova bölgesinde ise takip eden gün alınmıştır. Üç bölgede ağ atmak suretiyle profesyonel balıkçılar tarafından yakalanan sazanlar MS222 (3 amino benzoik asit etil ester, 75 mg/L) ile bayıltılmış ve diseksiyon işlemlerin yapılacağı laboratuvara soğuk zincirle götürülmüştür (Karadag vd., 2014). Balıkların yakalandığı bölgelerdeki baraj suyunun pH, sıcaklık ve çözünmüş oksijen düzeyleri alanda yapılacak ölçümler için uygun olan taşınabilir ölçüm cihazı (YSI 556 MPS) ile belirlenmiştir.

Steril aletler kullanılarak buz üzerinde disekte edilen sazanların solungaç, karaciğer, bağırsak, böbrek ve kas dokuları % 0,59 NaCl ile yıkanmış ve ağırlıkları alındıktan sonra analizlere kadar -80 °C'de muhafaza edilmiştir. Derin dondurucudan alınan dokular çözündükten sonra 1/10 ağırlık/hacim (w/v) olacak şekilde 0,25 M sükroz içeren 0,05 M soğutulmuş Na-P tamponu (pH: 7,4) ile buz içerisinde ultra-turrax homojenizatörde 3 dakika süreyle 10.000 rpm'de homojenize edilmiştir. Homojenatlar +4 °C'de 10.000 rpm'de 30 dakika süreyle santrifüj edildikten sonra eppendorf tüplere alınan doku süpernatantları enzim aktiviteleri ve protein düzeylerinin belirlenmesi için kullanılmıştır. ATPaz aktiviteleri Atkinson vd. (1973), protein düzeyleri ise Lowry vd. (1951)'nin önerdiği yöntemlere göre belirlenmiştir. Protein hesaplamalarında sığır serum albümin standart olarak kullanılmıştır. ATPaz aktivite tayininde, ATP'den açığa çıkan inorganik fosfatın (Pi) polioksietilen 10 lauril eter ile fosfomolibdatın sarı renk oluşturması ve bunun 290 nm'de ölçülmesi ve 100-1000 µM KH_2PO_4 çözeltisi ile oluşturulan standart eğriden elde edilen Pi regresyon eşitliği (y=0,0013x, R^2=0,992) kullanılarak enzim aktiviteleri µmol/mgprotein/saat olarak hesaplanmıştır. Na^+/K^+-ATPaz; oubain içeren Mg^{+2}-ATPaz aktivitesinin oubain içermeyen toplam ATPaz aktivitesinden çıkarılarak hesaplanırken; Mg^{+2}-ATPaz ve Ca^{+2}-ATPaz aktiviteleri ise sırasıyla $MgCl_2$ ve $CaCl_2$'nin varlığı ve yokluğunda ölçülen aktiviteler arasındaki farka bağlı olarak hesaplanmıştır. ATPaz ölçümleri sırasında Atlı ve Canlı (2008)'nın önerdiği yöntemde enzim aktivitesinin en yüksek olduğu inkübasyon ortamları kullanılmıştır.

Üç bölgeden elde edilen verilerin istatistik analizleri SPSS 21.0 bilgisayar paket programı kullanılarak One Way Anova ve takiben çoklu karşılaştırma testlerinden Duncan testi uygulanarak yapılmıştır. Her bir bölgedeki her bir parametre için veriler 10 tekrarlı olarak analiz edilmiş (n=10) ve sonuçlar aritmetik ortalama±standart hata şeklinde verilmiştir. Sonuçlar % 95 güven aralığı ile anlamlı kabul edilmiştir.

BULGULAR

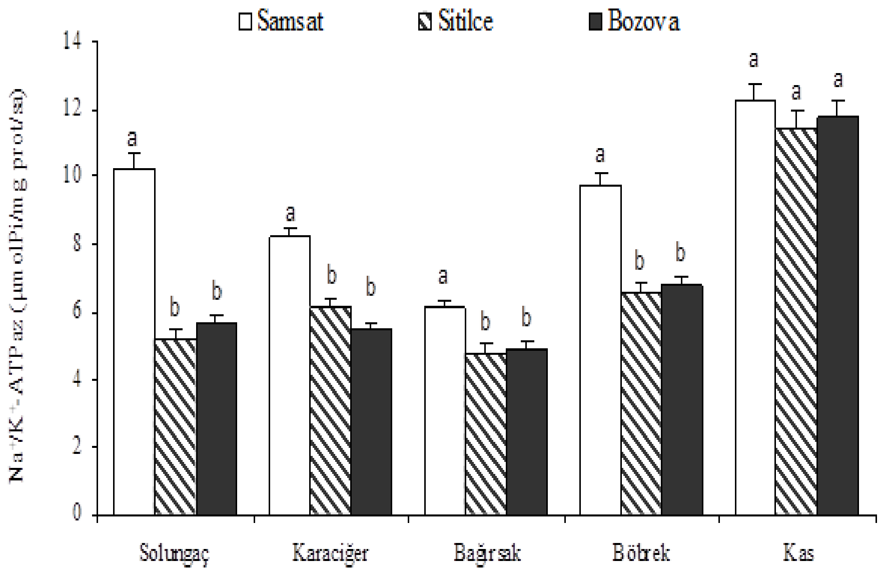
Atatürk Barajında balık örneklerinin alındığı Samsat, Sitilce ve Bozova bölgelerindeki suyun bazı fizikokimyasal parametreleri Tablo 1'de verilmiştir. Çalışma bölgelerindeki suların sıcaklık ve pH değerleri birbirine yakın iken Sitilce bölgesindeki suyun çözünmüş oksijen düzeyi diğer iki bölgeye göre daha düşük olduğu belirlenmiştir.

Tablo 1. Çalışma bölgelerindeki suların bazı fiziko-kimyasal özellikleri

Table 1. Some physical-chemical properties of water in the study areas

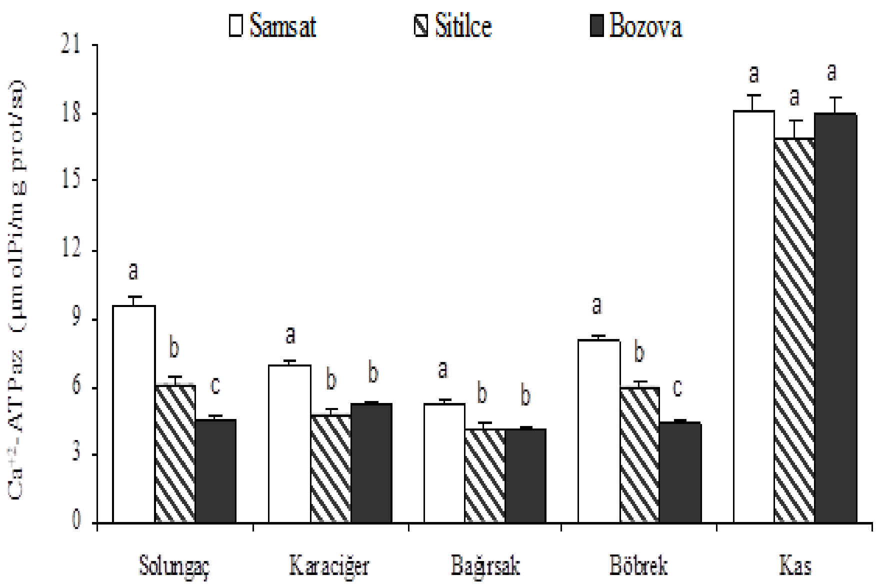
Parametre	Samsat	Sitilce	Bozova
pH	8,14	8,23	8,09
Sıcaklık (°C)	27,4	27,6	28,1
Çözünmüş Oksijen(mg/L)	7,81	5,49	7,18

Atatürk Barajı Gölü'nün Samsat, Sitilce ve Bozova bölgesinde yakalanan *C. carpio*'nun solungaç, karaciğer, bağırsak, böbrek ve kas dokularındaki ATPaz aktivitelerindeki değişimler belirlenmiş ve Na^+/K^+-ATPaz, Ca^{+2}-ATPaz ve Mg^{+2}-ATPaz için elde edilen bulgular sırasıyla Şekil 2, 3 ve 4'te verilmiştir. Çalışılan bölgelere ve incelenen dokulara bağlı olarak ATPaz aktivitelerinde anlamlı değişimler gözlemlenmiştir. Samsat bölgesi ile karşılaştırıldığında Sitilce ve Bozova bölgesi balıklarının solungaç, karaciğer, bağırsak ve böbrek dokularında incelenen tüm ATPaz enzim aktivitelerinde anlamlı azalışlar gözlemlenirken (P<0,05), kas dokusu enzim aktiviteleri anlamlı bir değişim göstermemiştir (P>0,05). Her üç ATPaz aktivitesinde de en fazla azalışlar solungaç dokusunda bulunmuş ve yüzdelik olarak bu azalışların Sitilce ve Bozova bölgesi balıklarında Na^+/K^+-ATPaz için sırasıyla %49 ve %44, Ca^{+2}-ATPaz için sırasıyla %29 ve %48 ve Mg^{+2}-ATPaz için sırasıyla %33 ve %28 düzeylerinde olduğu hesaplanmıştır. Sitilce ve Bozova bölgelerinde enzim aktivitelerindeki azalış sıralaması dokularda genel olarak solungaç>böbrek>karaciğer >bağırsak>kas şeklindedir.



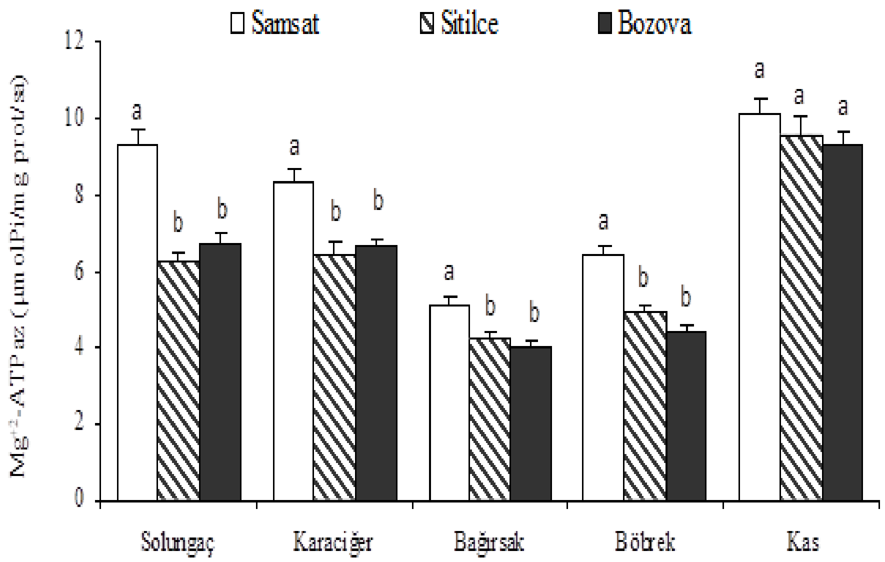
Şekil 2. Atatürk Baraj Gölü sazanlarının (*C. carpio*) doku Na^+/K^+-ATPaz aktivitesi. "a ve b" harfleri çalışma bölgeleri arasındaki P<0,05 düzeyindeki istatistiksel farkı ifade etmektedir.

Figure 2. Tissue Na^+/K^+-ATPase activity of carps (*C. carpio*) in Atatürk Dam Lake. Letters "a and b" indicate statistical differences at P<0.05 level between the study regions.



Şekil 3. Atatürk Baraj Gölü sazanlarının (*C. carpio*) doku Ca^{+2}-ATPaz aktivitesi. "a, b ve c" harfleri çalışma bölgeleri arasındaki P<0,05 düzeyindeki istatistiksel farkı ifade etmektedir.

Figure 3. Tissue Ca^{+2}-ATPase activity of carps (*C. carpio*) in Atatürk Dam Lake. Letters "a, b and c" indicate statistical differences at P<0.05 level between the study regions.



Şekil 4. Atatürk Baraj Gölü sazanlarının (*C. carpio*) doku Mg^{+2}-ATPaz aktivitesi. "a ve b" harfleri çalışma bölgeleri arasındaki P<0,05 düzeyindeki istatistiksel farkı ifade etmektedir.

Figure 4. Tissue Mg^{+2}-ATPase activity of carps (*C. carpio*) in Atatürk Dam Lake. Letters "a and b" indicate statistical differences at P<0.05 level between the study regions.

TARTIŞMA

Su kalite parametreleri kirlilikten etkilenebilmektedir. Çalışmamızda da Sitilce bölgesindeki baraj suyunun düşük çözünmüş oksijen düzeyinin bu bölgede baskın olan evsel kökenli kirleticilerle ilişkili olabileceği düşünülmektedir. Karadag vd. (2014) barajın Sitilce ve Samsat bölgelerinde çeşitli su kalite parametrelerini ölçtükleri çalışmalarında Samsat bölgesi ile karşılaştırıldığında Sitilce bölgesinde örneklenen sularda çözünmüş oksijen düzeylerini daha düşük; amonyak, nitrit, nitrat, sülfat ve fosfat düzeylerini ise daha yüksek bulmuşlardır. Araştırıcılar Sitilce bölgesinde baraja giren Adıyaman şehir atık sularına bağlı olarak su kalite parametrelerinde değişimlerin meydana geldiğini belirtmişlerdir.

Tatlısu (nehirler ve göller) ve tuzlu (denizler ve okyanuslar) sular tuz derişimi, su hacmi ve canlı içeriği yönünden birbirinden önemli farklılıklar göstermesine rağmen günümüzün en önemli çevre sorunlarından biri olan kirlilikten benzer şekilde etkilenmektedir (Fırat ve Kaya, 2019). Artan insan nüfusunun gereksinmeleri için artan endüstriyel gelişme, tarımsal uygulamalar ve kentleşme, sulardaki kirletici yükünü artırmakta ve bazen tolere edilebilir düzeylerin bile üzerine çıkmasına neden olmaktadır. Geçmişten günümüze ağır metaller ve pestisitler gibi daha iyi bilinen geleneksel kirleticiler ile birlikte her geçen gün çeşidi ve miktarı artan nanopartiküller ve mikroplastikler de su ekosistemlerini açıkça tehdit etmektedir. Öncelikli olarak içme suyu olmak üzere tarımsal alanların sulanması ve elektrik enerjisi üretimi için nehirler üzerine kurulan barajlar da yerleşim bölgelerine yakınlıkları nedeni ile insan aktivitelerinden kaynaklı kirleticilerin açık hedefi haline gelmektedir. Önceki çalışmalar (Firat, 2016; Uçkun, 2017; Gündoğdu, 2023) Atatürk Baraj Gölü'nün de kirlilikten etkilendiğini, çeşitli kirleticilerin (ağır metaller, pestisitler ve mikroplastikler gibi) su, sediment ve balıklarda biriktiğini, su kalitesinde önemli değişikliklere neden olduğunu ve balıklarda fizyolojik ve biyokimyasal yanıtları oluşturduğunu göstermiştir. Sunulan çalışmada da göreceli olarak temiz bölge ile karşılaştırıldığında barajın insan aktivitelerinden etkilenen bölgelerindeki balıklarda Na, K, Ca ve Mg gibi iyonların taşınmasından ve regülasyonundan sorumlu enzimlerde toksik stres yanıtlarının oluştuğu belirlenmiştir.

Balıklar su ekosisteminin önemli öğelerinden biri olup su kirliliğinin değerlendirilmesinde biyoindikatör türler olarak sıklıkla kullanılmaktadır (Temiz ve Kargın, 2024). Cyprinidae familyasından olan *C. carpio* önceleri Avrupa ve Asya'ya özgü tatlı su balığı türü iken günümüzde dünyanın çok çeşitli bölgelerinde yaygın olarak yetiştirildiği, davranışları açısından oldukça uyumlu ve dirençli oldukları, çok çeşitli su koşullarına dayanabildikleri ve genellikle yavaş akan nehirlerde, göletlerde ve göllerde yaşadıkları belirtilmektedir (Khoshnood, 2024). Sazanlar stenohalin bir türdür (Metz vd., 2003). Düşük gereksinimler, yüksek adaptasyon ve yaygın dağılımları nedeniyle ticari ve ekonomik öneminin yanı sıra sazan balığı, çevresel kirleticilerin etkilerini değerlendirmede de deneysel bir tür olarak yaygın olarak kullanılan bir balıktır (Ji vd., 2012). Sunulan çalışmada da Atatürk Baraj Gölü'nde oldukça bol bulunan ve yöre halkının da besin kaynağı olan *C. carpio* kirliliğin toksik etkilerinin belirlenmesi için biyoindikatör bir tür olarak seçilmiştir.

Balıkların ortamlarında bulunan kirleticileri su ve besin yoluyla ile aldığı ve dokularında biriktirebildiği iyi bilinmektedir. Balıkların metabolik yönden aktif dokuları toksikantların etkisine daha açıktır. Balık solungaçları gaz değişimi, asit-baz düzenlemesi, iyonların taşınması ve azotlu artıkların atılımında görevlidir (Atlı ve Canlı, 2008; Khoshnood, 2024). Karaciğer dokusu toksikantların detoksifikasyonu ve depolanmasında rol oynar (Firidin, 2018). Kas dokularının biyoanalizi, insan tüketimi ve sağlığı amacıyla balık kalitesini izlemek için önemlidir (Fırat ve Kılınç, 2022). Böbrekler, bir organizmanın iç ortamının korunması, hücre dışı sıvı hacmi ve bileşiminin yanı sıra asit-baz dengesinin düzenlenmesinde hayati rolleri olan ama aynı zamanda da işlevlerini bozabilen ve homeostazın geçici veya kalıcı olarak bozulmasına neden olabilen toksik kimyasalların hedefi olabilen bir dokudur (Temiz ve Kargın, 2024). Bağırsak dokusu sindirim ve emilim olaylarının merkezidir ve besin yoluyla alınan kirleticilerin kan dolaşımına da alındığı vücut bölümü olması nedeniyle toksik etkilere açık bir dokudur (Dane ve Sisman, 2020). Sunulan araştırmada da sudaki toksikantların alınım, atılım ve depolanmasında önemli rolleri olan solungaç, karaciğer, bağırsak, böbrek ve kas dokuları kirleticilerin hedef dokuları olarak seçilmiştir.

Endüstriyel/teknolojik alanlardaki ve tarımsal uygulamalardaki hızlı gelişmelerin etkisindeki dünyada ekosistemin ve insan sağlığının korunması için sucul sistemin kirliliğinin biyokimyasal parametrelerle izlenmesine her zamankinden daha fazla ihtiyaç duyulmaktadır (Temiz vd., 2021). Su ekosistemleri üzerine kirleticilerin ve çevresel stres faktörlerinin etkilerini değerlendirmek için balıklardaki biyokimyasal belirteçleri kullanmak bu stres yapıcıların biyolojik organizasyonun farklı seviyelerini nasıl etkilediğine dair bilgilerimizi geliştirmeye yardımcı olmaktadır (Abdallah vd., 2024). Sunulan çalışmada Atatürk Baraj Gölü'ndeki sazanlar üzerine kirliliğin etkisi biyokimyasal indikatörler olarak atfedilen ATPaz enzim aktiviteleri ile değerlendirilmiştir. İyon düzenleyici enzimler olan ve hücre zarlarında bulunan ATPazlar hücre içi iyon derişimlerini ayarlayan, hücre zarı geçirgenliğini ve iyonların (Na^+, K^+, Ca^{+2} ve Mg^{+2} gibi) taşınmasını fizyolojik süreçlerde düzenleyen enzimlerdir (Langeswaran vd., 2012). Tatlı su balıklarında major iyonların düzeyleri ve bu iyonların fizyolojik regülasyonlarında rol oynayan enzim aktiviteleri çevresel stres faktörlerine karşı çok hassastır ve kirleticilere yanıt olarak düzeyleri/aktiviteleri değişim göstermektedir. Araştırmamızda da baraj gölündeki çalışma bölgelerine ve incelenen dokulara bağlı olarak ATPaz enzim aktivitelerinde anlamlı değişimler belirlenmiştir.

Na^+/K^+-ATPaz, hücresel iyon dengesini, hücre hacmini ve membran dinlenme potansiyelini korumada kritik bir rol oynayan, sodyum iyonlarını hücrelerden dışarı ve potasyum iyonlarını ise hücrelere aktif olarak pompalayarak hücre membranı boyunca bir elektrokimyasal gradienti oluşturan zara bağlı önemli bir enzimdir (Khoshnood, 2024). Hücresel ve organizma düzeyindeki iyon homeostazı için gerekli olan bu enzim ksenobiyotiklerin enerji üreten yolları etkileyerek ve/veya doğrudan enzimle etkileşime girerek aktivitelerinde azalışlara neden olabilmektedir (Gupta vd., 2023). Ca^{+2}-ATPaz önemli bir ATPazdır. Ca^{+2}'nin yüksek hücre içi derişimleri (>10^{-7} M) sitotoksik etkilere neden olduğundan zarlarda bulunan ve kalsiyum pompası olarak görev yapan bu enzim aktif taşıma ile bu iyonları hücreden uzaklaştırılarak hücresel Ca^{+2} düzeylerini korumada hayati bir rol oynamaktadır (Wong ve Wong, 2000). Mg^{+2}-ATPaz balıklarda Na^+/K^+-ATPaz ile birlikte bulunur ve oksidatif fosforilasyon, hücre zarının bütünlüğü ve iyonik taşımada önemli bir rol oynar ayrıca bu enzim Mg^{+2}'nin transepitelyal taşınımıyla da ilişkilidir (Suvetha vd., 2010).

Araştırmamızda Sitilce ve Bozova bölgesi balıklarının solungaç, karaciğer, bağırsak ve böbrek dokularında Samsat bölgesine oranla azalan Na^{+}/K^{+}-ATPaz, Ca^{+2}-ATPaz ve Mg^{+2}-ATPaz enzim aktivitelerin kirleticilerin toksik etkilerinin bir sonucu olarak düşünülmektedir. Bu ATPaz'ların aktivitelerindeki inhibisyonun iyon dengesi, taşınımı ve homeostazisini bozarak ve ozmoregülasyona zarar vererek balıklarda ozmotik ve iyon düzenlemesinde başarısızlığa yol açacak toksikolojik sonuçları tetiklemiş olabilir. Bu enzimlerin yapısında yer alan sülfidril gruplarına karşı ağır metaller gibi çeşitli kirleticilerin yüksek ilgisine bağlı olarak doğrudan ya da kirleticilerin etkisinde oluşan oksidatif strese bağlı olarak membran bütünlüğünün bozulması sonucu aynı zamanda birer membran enzimi olan bu ATPaz'lar üzerine olan dolaylı bir etkinin sonucunda bu enzimlerin aktiviteleri düşüş göstermiş olabilir. Sunulan çalışmada Na^{+}/K^{+}-ATPaz aktivitesindeki azalışlara bağlı olarak sodyum ve potasyum iyonlarının hücre zarlarındaki aktif taşınmasının engellenebileceği ve bunun da hücreler için gerekli olan iyonik ve elektriksel gradiente zarar verebileceği tahmin edilmektedir. Ca^{+2}-ATPaz aktivitesindeki azalışların hücre içi kalsiyum düzeylerinin artışına neden olarak sitotoksik etkilere neden olabileceği düşünülmektedir. Mg^{+2}-ATPaz enzim inhibisyonunun ise magnezyum iyon dengesine olumsuz etkileri olduğu ve bu iyonun taşınmasını bloke edebileceği öngörülmektedir. Balıkların dinlenme durumunda bile Na^{+}, Cl^{-} ve ozmotik regülasyon mekanizmalarında rol oynayan enzimlerin fonksiyonlarını yaparken önemli miktarda enerji harcadığı bilindiğinden çalışmamızda ATPaz enzim aktivitelerindeki inhibisyonlara bağlı olarak iyon-regülatör kapasitedeki azalmaların, iyon ve su düzenleme ile ilişkili metabolik maliyetleri önemli ölçüde arttırmış olabileceği tahmin edilmektedir. Araştırmamızda analiz edilen tüm ATPaz aktivitelerinde en fazla azalışın solungaç dokusunda belirlenmesinin bu dokunun sudaki toksikantların ilk alınım yolu olması nedeniyle kirleticilerin doğrudan hedef dokusu olması ve özellikle de hidromineral homeostazis ve ozmoregülasyondaki merkezi rolü ile ilişkili olabileceği öngörülmektedir.

Atatürk Baraj Gölü'nde yapılan bir çalışmada barajın iki kirli bölgesi olan Kahta ve Bozova bölgelerinde yakalanan *C. carpio*'nun solungaç dokusu ATPaz aktivitelerini değerlendiren Uçkun ve Uçkun (2021a) Bozova ile karşılaştırıldığında Kahta Na^{+}/K^{+}-ATPaz aktivitesinde anlamlı azalışlar belirlerken Ca^{+2}-ATPaz ve Mg^{+2}-ATPaz enzim aktivitelerinde anlamlı değişimler gözlemlememişlerdir. Araştırıcılar Na^{+}/K^{+}-ATPaz aktivitesindeki bu azalmaya bağlı olarak balıkların solungaç dokularındaki hücresel iyon düzenlemesinin zarar görebileceğini ve aktif Na^{+} ve K^{+} iyonlarının taşınmasının bloke edilebileceğini ifade etmişlerdir. Sentetik piretriot bir insektisit olan sipermetrin etkileşimini takiben *C. carpio*'nun solungaç, karaciğer ve böbrek dokularında Na^{+}/K^{+}-ATPaz, Ca^{+2}-ATPaz ve Mg^{+2}-ATPaz enzim aktivitelerinde önemli azalışların olduğu ve bu enzim inhibisyonlarının dokulardaki iyon dengesi ve homeostazisini bozduğu rapor edilmiştir (Balaji vd., 2015). Tatlı su midyeleri (*Unio mancus*) üzerine yapılan bir laboratuvar çalışmasında toksikant etkisinde solungaç dokusundaki Na^{+}/K^{+}-ATPaz, Mg^{+2}-ATPaz ve Ca^{+2}-ATPaz aktivitelerindeki azalışların sırasıyla bu dokunun zar geçirgenliğini bozduğu, mitokondri zarlarına zarar verdiği ve hücrelerde Ca^{+2} düzeylerini arttırarak sitotoksik etkiye neden olduğu öne sürülmüştür (Uçkun ve Uçkun, 2021b). *Danio rerio* embriyolarında çinko oksit nanopartikülleri ozmoregülasyonda rol oynayan Na^{+}/K^{+}-ATPaz aktivitesinde anlamlı azalışlara neden olmuştur (Deenathayalan vd., 2024). Sun vd. (2024) akut amonyak stresi etkisinde *Thunnus albacares* türü balıkların karaciğerinde ATPaz aktivitesindeki azalışların doku hasarına, hücre zarlarının geçirgenliğinin bozulmasına ve iyon taşıma kapasitelerinin azalmasına yol açtığını vurgulamışlardır. Dogan vd. (2014), *Oreochromis niloticus*'un dokularında ATPaz aktivitesinin metallerin etkisinde azaldığını ve ATPazların azalma eğiliminin, enzim sentezini engelleyen enzim moleküllerine metallerin bağlanması, hücre membranların hasar görmesi, ozmoregülasyon işlev bozuklukları ve iyon homeostazının bozulmasıyla ilişkili olabileceğini bildirmişlerdir. Firidin (2018) kurşun etkileşimini takiben *O. niloticus*'un karaciğer ve böbrek dokularında total ATPaz aktivitelerinde anlamlı azalışların olduğunu rapor etmiştir. Gupta vd. (2023) pestisit etkisinde balıklarda azalan Na^{+}/K^{+}-ATPaz aktivitesinin bu kimyasalın enzime bağlanarak aktivitesini engellenmesi ile ilişkili olduğunu bildirmiştir. Temiz ve Kargın (2024) *O. niloticus*'un böbrek dokusu ATPaz aktivitesini thiametokzam etkisinde değişimini inceledikleri araştırmalarında en yüksek ortam derişiminde Na^{+}/K^{+}-ATPaz enzim aktivitesinin %73 oranında Ca^{+2}-ATPaz enzim aktivitesinin %62 oranında azaldığını hesap etmişlerdir. Kurşun ve çinkonun etkisinde Afrika yılanbalığında (*Clarias gariepinus*) Na^{+}/K^{+}-ATPaz aktivitesinin azaldığı ve bu inhibisyonun enzimin oynadığı biyolojik rolleri üzerine engelleyici bir sonuç yaratacağını ve bu azalışların metallerin enzimin yapısında bulunan sulfidril gruplarına olan yüksek afinite ile ilişkili olabileceği belirtilmiştir (Apiamu vd., 2022). Hegazi vd. (2015) yaptıkları alan çalışmasında kirli bölgelerden toplanan *Siganus rivulatus* balığının solungaç dokusunda Na^{+}/K^{+}-ATPaz, Mg^{+2}-ATPaz ve Ca^{+2}-ATPaz aktivitelerin inhibe olduğunu ve bu azalışların, sitozolde aşırı Ca^{+2} birikmesi, metabolik işlev bozukluğu ve hücresel anormalliklere neden olabileceğini belirtmişlerdir. Lopez-Lopez vd. (2011) Yuriria Gölü'nün (Meksika) evsel ve endüstriyel atık sulardan etkilenen bölgelerinden *alınan Goodea atripinnis* türü balıkların karaciğer Na^{+}/K^{+}-ATPaz aktivitesinde anlamlı azalışlar belirlemiş ve enzim aktivitesinin membranın yapısal bütünlüğüne bağlı olduğunu ve kirleticilerin oksidatif stresin bir sonucu olarak oluşan lipid peroksidasyonu gibi membran hasarlarına bağlı olarak enzim aktivitesinin azalmış olabileceğini bildirmişlerdir.

Atatürk Baraj Gölü'nün faklı bölgelerinde su, sediment ve omurgalı/omurgasız su canlılarının dokularında kirletici düzeylerini araştıran çalışmalarda ağırlıklı olarak barajın Sitilce bölgesinde ağır metal kirliliği Bozova bölgesinde ise pestisit kirliliği belirlenmiştir (Firat, 2016; Uçkun, 2017; Uçkun vd., 2017). Bu çalışmalarda Firat (2016) Samsat bölgesi ile karşılaştırıldığında Sitilce bölgesindeki *C. carpio*'nun solungaç, karaciğer ve kas dokularında anlamlı kadmiyum, kurşun, krom, bakır, çinko ve demir birikimi; Uçkun (2017) Bozova bölgesinde yine Samsat'a oranla sediment ve sazan karaciğerinde anlamlı organoklorlu pestisit (Dieldrin, p,p'-DDE; p-p'-DDD; o,p'- DDD; o,p'-DDT ve p,p'-DDT) kalıntıları; Uçkun vd. (2017) Sitilce bölgesi su, sediment ve *U. mancus* türü tatlı su midyelerinde Samsat'a göre genel olarak daha yüksek ağır metal düzeyleri belirlenmiş ve bu üç çalışmadaki araştırmacılar tarafından Sitilce ve Bozova bölgeleri kentsel, endüstriyel ve tarımsal aktivitelerden etkilenen kirli bölgeler olarak tanımlanmıştır. Bu araştırmaları dikkate aldığımızda çalışmamızda Sitilce ve Bozova bölgesi ATPaz aktivitelerindeki azalışların bu kirleticilerle ilişkili olabileceği tahmin edilmektedir. Sitilce bölgesi 2016 yılında yapılan atık su arıtma tesisine kadar uzun yıllar Adıyaman şehrinden gelen arıtılmamış kanalizasyon sularının etkisi altında kalmıştır. Araştırmamızda atık su arıtma tesisine rağmen Sitilce bölgesi balıklarında toksikolojik sonuçlar elde edilmiş olması şaşırtıcıdır. Bu nedenle tesisin arıtma kapasitesi veya diğer özelliklerinin gözden geçirilmesinin önemli olabileceği düşünülmektedir. Sonuçlarımız barajın Sitilce ve Bozova bölgesindeki balıkların insan aktivitesinden kaynaklı kirleticilerin açık hedefi halinde olduğunu göstermektedir.

SONUÇ

Tatlı sular sınırlı kaynaklardır ama insanların geleceği için hayati bir öneme sahiptir. Günümüzde özellikle küresel ısınma gibi farklı çevre sorunlarının da bu sular üzerindeki ekolojik stres etkileri göz önüne alındığında bu kaynaklardaki kirliliğin izlenmesi ve önlenmesi için gerekli tedbirlerin alınması bu su kütlelerinin kalitesi, içerdiği canlıların sağlığı, gelecek nesillerin temiz su ihtiyacı ve sağlıklı tarımsal sulama için oldukça elzemdir. Araştırmamızdaki toksikolojik sonuçlar Türkiye'nin en önemli tatlı su kaynaklarından biri olan Fırat Nehri'nin üzerinde bulunan Atatürk Baraj Gölü'nün ne yazık ki kirleticilerin etkisi altında olduğunu kanıtlamaktadır. Çalışmamız baraj gölündeki kirleticilerin balıklarda iyon dengesi, iyon homeostazı ve ozmoregülasyon mekanizmaları üzerinde toksik etkilere sahip olduğunu ve ATPazların sucul ekosistemlerde kirliliğin belirlenmesinde yararlı biyobelirteçler olarak kullanılabileceğini açıkça ortaya koymuştur.

TEŞEKKÜR VE MADDİ DESTEK

Bu çalışma Adıyaman Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından desteklenmiştir. Proje Numarası: KMYOMAP/2021-0001.

YAZARLIK KATKISI

Özge Fırat: Kavramsallaştırma, Metodoloji, Yazılım, Veri küratörlüğü, Yazma-Orijinal taslak hazırlama, Gözetim ve denetim, Yazılım, doğrulama, Proje yönetimi, kaynaklar, finansman edinme, Yazma-gözden geçirme ve Düzenleme; Özge Temiz: Kavramsallaştırma, Metodoloji, Yazılım, Görselleştirme, araştırma, Gözetim ve denetim, Yazma-gözden geçirme ve Düzenleme; Özgür Fırat: Kavramsallaştırma, Metodoloji, Yazılım, Veri küratörlüğü, Yazma-Orijinal taslak hazırlama, Görselleştirme, araştırma, Gözetim ve denetim, Yazılım, doğrulama, Yazma-gözden geçirme ve Düzenleme.

ÇIKAR/REKABET ÇATIŞMASI BEYANI

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

ETİK ONAY

Adıyaman Üniversitesi Hayvan Deneyleri Yerel Etik Kurulu'ndan onay alındı (Tarih: 24/12/2020, Karar No:4, Protokol No: 2020/067).

VERİ KULLANILABİLİRLİĞİ

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

KAYNAKÇA

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