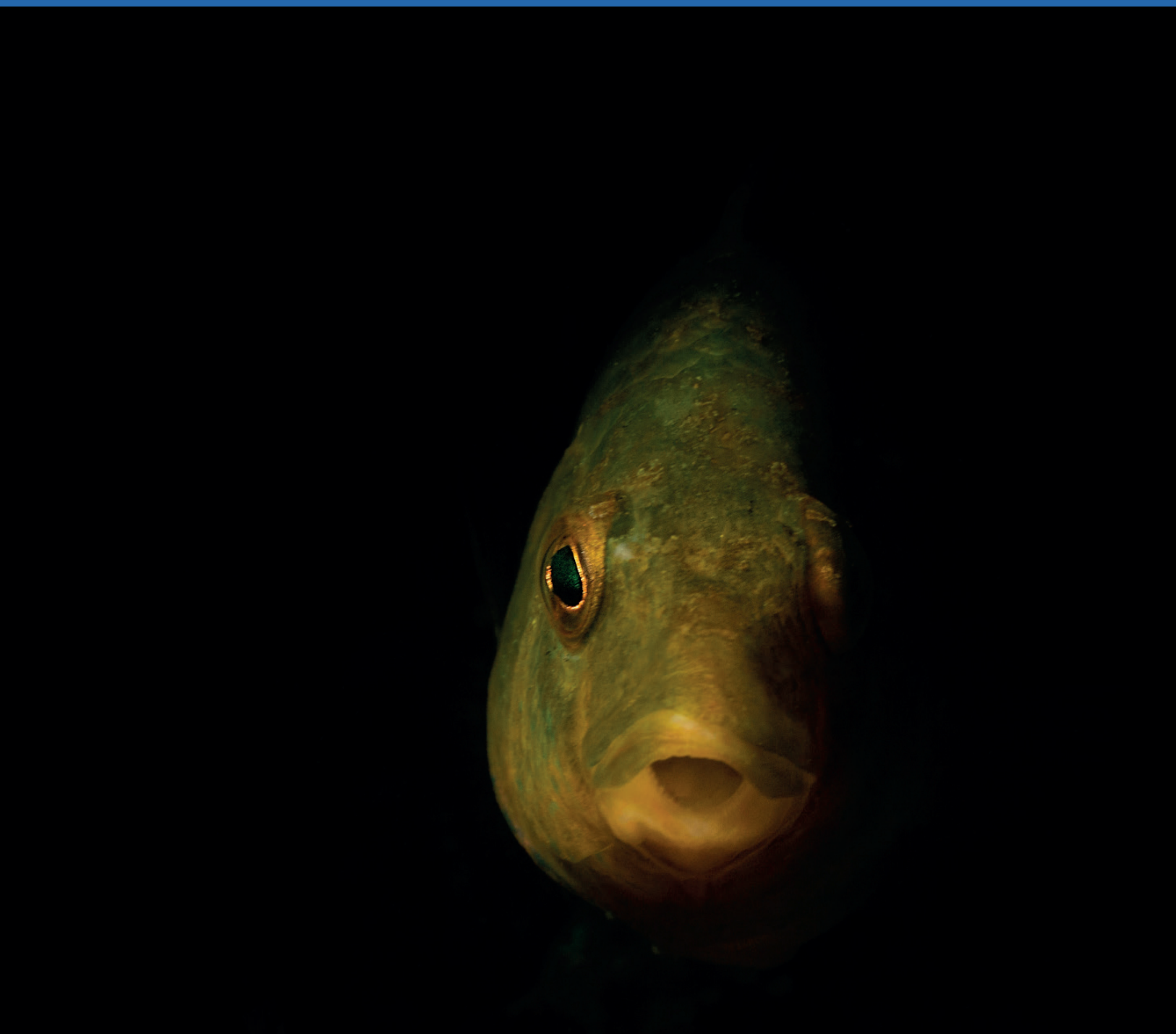
AQUATIC SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

VOLUME: 36 ISSUE: 3 2021 E-ISSN 2602-473X



İSTANBUL UNIVERSITY PRESS

AQUATIC SCIENCES and ENGINEERING

VOLUME: 36 ISSUE: 3 2021 E-ISSN 2602-473X

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Indexing and Abstracting

Web of Science - Emerging Sources Citation Index (ESCI)
SCOPUS
TÜBİTAK-ULAKBİM TR Dizin
Zoological Record
Biological Abstracts
BIOSIS Previews
CAB Abstract
SciLit
DOAJ

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Owner

Prof. Dr. Melek İşinibilir Okyar
Istanbul University, Faculty of Aquatic Sciences, İstanbul, Turkey

Responsible Manager

Prof. Dr. Devrim Memiş
Istanbul University, Faculty of Aquatic Sciences, İstanbul, Turkey

Correspondence Address

Istanbul University Faculty of Aquatic Sciences,
Ordu Caddesi No: 8 34134 Laleli-Fatih / İstanbul, Turkey
E-mail: ase@istanbul.edu.tr
https://iupress.istanbul.edu.tr/tr/journal/ase/home

Publisher

Istanbul University Press
İstanbul University Central Campus, 34452 Beyazıt,
Fatih / İstanbul, Turkey
Phone: +90 (212) 440 00 00

Cover Photo

Ferhan Çoşkun
E-mail: fcoskun@gmail.com
İnstagram: instagram.com/exultsoul

The publication language of the journal is English.

This is a scholarly, international, peer-reviewed and open-access journal published quarterly in January, April, July, and October.

Publication Type: Periodical

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

EDITORIAL MANAGEMENT

EDITORIAL BOARD

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Aims and Scope

Aquatic Sciences and Engineering is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of İstanbul University Faculty of Aquatic Sciences and it is published quarterly on January, April, July, and October. The publication language of the journal is English and continues publication since 1987.

Aquatic Sciences and Engineering aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of aquatic sciences. The journal publishes original research and review articles that are prepared in accordance with the ethical guidelines.

The scope of the journal includes but not limited to; aquaculture science, aquaculture diseases, feeds, and genetics, ecological interactions, sustainable systems, fisheries development, fisheries science, fishery hydrography, aquatic ecosystem, fisheries management, fishery biology, wild fisheries, ocean fisheries, biology, taxonomy, stock identification, functional morphology freshwater, brackish and marine environment, marine biology, water conservation and sustainability, inland waters protection and management, seafood technology and safety.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of aquatic sciences.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Aquatic Sciences and Engineering is covered in Clarivate Analytics Web of Science Emerging Sources Citation Index (ESCI), Clarivate Analytics Zoological Record, Biological Abstracts, BIOSIS Previews, Scopus, DOAJ, Scilit, TUBITAK ULAKBIM TR Index and CAB Abstracts.

Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at https://dergipark.org.tr/ase. The journal guidelines, technical information, and the required forms are available on the journal's web page.

All expenses of the journal are covered by the İstanbul University Faculty of Aquatic Sciences. Potential advertisers should contact the Editorial Office. Advertisement images are published only upon the Editor-in-Chief's approval.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the İstanbul University Faculty of Aquatic Sciences, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

All published content is available online, free of charge at https://dergipark.org.tr/ase. Printed copies of the journal are distributed to the members of the İstanbul University Faculty of Aquatic Sciences, free of charge.

OPEN ACCESS

Editor in Chief: Prof. Devrim MEMİŞ
Address: İstanbul Üniversitesi Su Bilimleri Fakültesi Yetiştiricilik Anabilim Dalı Ordu Cad. No:8 34134 Laleli / İstanbul, Türkiye
Phone: +90 212 4555700/16448
Fax: +90 212 5140379
E-mail: mdevrim@istanbul.edu.tr

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Contents/İçindekiler

Research Article

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2021; 36(3): 101-108 • DOI: https://doi.org/10.26650/ASE2020773014

Original Article

The use of Aloe Vera Gel Functionalized Biogenic Zinc-Oxide Nanoparticles Against Fish Putative Pathogens

Puja Pati[1], Kausik Mondal[1], Madhusudan Mandal[2]

Cite this article as: Pati, P., Mondal, K., & Mandal, M. (2021). The use of Aloe vera gel functionalized biogenic Zinc-Oxide nanoparticles against fish putative pathogens. *Aquatic Sciences and Engineering, 36*(3), 101-108.

ABSTRACT

Indiscriminate uses of antibiotics have resulted in the development of antibiotic-resistance among pathogens which possess a potential risk to the ecosystem, aquaculture and human health. In this study, biogenic zinc oxide nanoparticles (ZnO-NPs) were synthesized using aqueous extract of *Aloe vera* gel (AVGE) and tested against putative pathogenic bacterial strains *in-vitro*. Ultraviolet-Visible (UV-VIS) spectroscopic analysis confirmed the synthesis of AVGE-ZnO-NPs while X-ray diffraction (XRD) and Scanning Electron microscope (SEM) analysis revealed that the average size of synthesized ZnO-NPs is within the nano range. The elemental and chemical compositions of synthesized ZnO-NPs were studied using Energy-dispersive X-ray spectroscopy (EDX) and Fourier-transform infrared (FTIR) spectrometer, respectively. Two widespread bacterial strains, *Aeromonas veronii* strain ONKP1 (MN602971) and *Stenotrophomonas maltophilia* strain ONKP2 (MN602972) that are known as emerging opportunistic pathogens in various marine and freshwater fishes as well as humans and other animals, were used as test organisms. AVGE-ZnO-NPs showed strong antibacterial activity, against the tested Gram-negative multi-drug resistant bacteria in the disc diffusion assay. The results of the present investigation could be useful for the development of new disease management strategies in the fisheries industry.

Keywords: Green nanoparticles, ZnO-NPs, Antibacterial, *Aloe vera*, Fish diseases

ORCID IDs of the author:
P.P. 0000-0001-9366-2422;
K.M. 0000-0003-2677-5966;
M.M. 0000-0003-4277-4541

[1]Department of Zoology, University of Kalyani, Kalyani, Nadia, West Bengal, India

[2]Department of Chemistry, Mahadevananda Mahavidyalaya, Barrackpore, Kolkata, West Bengal, India

Submitted:
24.07.2020

Revision Requested:
19.09.2020

Last Revision Received:
03.10.2020

Accepted:
04.10.2020

Online Published:
08.04.2021

Correspondence:
Puja Pati
E-mail:
pujapati.007@gmail.com

INTRODUCTION

The aquaculture industry highly depends on antibiotics such as erythromycin, tetracycline and streptomycin, to control bacterial infections in the farm. However unregulated and excessive use of antibiotics leads to the emergence of antibiotic resistance in fish pathogenic bacteria. Multi-drug resistant bacteria have been isolated from fish, sediment, and water of farms (Austin & Austin, 2016; Shaalan et al., 2016). Since genetic elements can be shared between aquatic and terrestrial bacteria, human and animal pathogens can acquire such antibiotic-resistance genes from fish pathogens, arising public health issues (Swain et al., 2014; Luis et al., 2019). Thus, there is an urgent need to establish some novel strategies to combat antibiotic-resistance development and disease outbreaks in aquaculture without affecting the aquatic ecosystem. Recently, inorganic metal nanoparticles of silver, gold, titanium and zinc have generated widespread interest among aquaculture scientists as a potential alternative to antibiotics (Swain et al., 2014; Shaalan et al., 2016). They are considered as next-generation nanomedicine due to their unique pharmaceutical characteristics and novel pharmacological functions (De Villiers et al., 2008; Bharti & Singh, 2009; Das et al., 2013).

Interestingly, Zinc oxide nanoparticles (ZnO-NPs) are emerging as a most promising metal based nanodrugs due to their biocompatibility,

selectivity, and high potency (Bisht & Rayamajhi, 2016; Elshama et al., 2018; Jin & Jin, 2019). It is used in the area of drug delivery carrier, wound dressings, biosensors, bioimaging, medical devices, therapeutics, and diagnostics in medicine (Xiong, 2013; Zhu et al., 2016; Martínez-Carmona et al., 2018; Mirzaei & Darroudi, 2017). ZnO-NPs are also valued as a source of the trace element zinc (Pati & Mondal, 2019) which is required for the physiological development of animals, including fish (Watanabe et al., 1997). There are recent reports on the application of ZnO-NPs in aquaculture as an alternative of conventional zinc sources as feed additive to promote growth (Faiz et al., 2015; Wang et al., 2017; Onuegbu et al., 2018) and immunity (Anjugam et al., 2018; Awad et al., 2019). Owing to their nano size and high specific surface area, ZnO-NPs have several advantages over conventional ZnO, such as higher bioavailability, molecular dispersion, and antibacterial properties (Swain et al., 2016; Raje et al., 2018). Currently, Ag-NPs are the most widely used inorganic antimicrobial nanomaterials in water disinfection, wastewater treatment (Dimapilis et al., 2018; Shah & Mraz, 2020) and fish medicine (Shaalan et al., 2016; Khosravi-Katuli et al., 2017), but ZnO-NPs have the potential to be used as multifunctional material in aquaculture in coming years.

Green nanoparticles are mostly synthesized using plant parts like root, flower, leaves, fruit, stem, and seed extracts. Plant products are considered as safe, biocompatible, cheap, and environmentally friendly natural sources (Agarwal et al., 2017; Singh et al., 2018). ZnO-NPs have been successfully synthesized by using extracts of various plants, such as, *Vitex trifolia* (Elumalai et al., 2015), *Catharanthus roseus* (Gupta et al., 2018), *Pongamia pinnata* (Sundrarajan et al., 2015), *Citrullus colocynthis* (Azizi et al., 2017), *Borassus flabellifer* (Vimala et al., 2014) and *Aloe vera* (Sangeetha et al., 2011; Ali et al., 2016). Green ZnO nanoparticles are found to be a more potent antimicrobial agent than chemical ZnO nanoparticles on various human pathogenic strains like *Staphylococcus aureus* (Gunalan et al., 2012), *Escherichia coli* (Elumalai et al., 2015), *Bacillus cereus* (Gupta et al., 2018), *Klebsiella pneumoniae* (Mahendiran et al., 2017), *Pseudomonas aeruginosa* (Ali et al., 2016) and *Bacillus subtilis* (Chandran et al., 2018). However, studies on antibacterial activity of ZnO-NPs as well as green NPs on aquatic pathogens are limited.

Aloe vera (synonym: *Aloe barbadensis* Miller) is a popular and easily available perennial succulent plant of the Liliaceae family. It is well known for its therapeutic properties like antimicrobial, antioxidant, anti-inflammatory, immunomodulatory, and wound healing from ancient times (Sangeetha et al., 2011). *A. vera* gel extract is reported to be rich in phytochemicals like terpenoids, phenols, alkaloids, carbohydrates, flavonoids, saponins, and tannins (Parthasarathy et al., 2017; Mahendiran et al., 2017). These phytochemicals help in nucleation and stabilization of nanoparticles during synthesis (Sangeetha et al., 2011; Ali et al., 2016). In the present study, *A. vera* gel extract has been used as a capping and stabilizing agent for the synthesis of ZnO-NPs. The antibacterial activity of synthesized biogenic ZnO-NPs was evaluated against two Gram-negative fish pathogenic bacteria, *Aeromonas veronii* strain ONKP1 (MN602971) and *Stenotrophomonas maltophilia* strain ONKP2 (MN602972). *A. veronii is* a world-wide distributed fish pathogen, causing hemorrhagic septicemia with high mortality rate (Sun et al., 2016; Tekedar et al., 2019). *S. maltophilia* is emerging as a pathogen in cultured catfishes in India (Abraham et al., 2016) and China (Geng et al., 2010). In China, it has become the most prevalent disease in cultured channel catfish, which spreads rapidly and causes mortality within a few days.

MATERIALS AND METHODS

Synthesis of biogenic zinc oxide nanoparticles

The biogenic ZnO-NPs were synthesized following the standard method of Patel et al., (2017) with slight modifications. Analytical grade zinc chloride ($ZnCl_2$, 99% purity, Himedia, India) salt and sodium hydroxide pellets (NaOH, 99% purity, Merck, India) were used as precursors. A total of 20 g gel was purified from the *Aloe vera* leaves that were collected from the university garden; boiled in double distilled water, and filtered to get the aqueous extract. Later, 50 mL aloe gel extract was added to 50 mL $ZnCl_2$ (quantity = 6.8167 g) solution with constant stirring by a magnetic stirrer. Then the pH was adjusted to 12 by adding 2 M NaOH solution and stirred for another 30 minutes. The suspension was centrifuged and the supernatant was discarded. Then, the precipitate was washed twice with double distilled water and ethanol, respectively. The white product was collected on a watch glass and dried in a hot air oven at 80 ^{0}C for 4 hours. The dried product was then crushed into powder and stored in a vacuum desiccator for future use. The possible chemical reactions were given below (Nath et al., 2018):

$$ZnCl_2 + 2NaOH \rightarrow Zn(OH)_2 + 2NaCl$$

$$Zn(OH)_2 \xrightarrow{80^\circ} ZnO$$

Elemental analysis and chemical characterization of synthesized zinc oxide nanoparticles

The synthesized ZnO-NPs have been characterized following the method of Mahendiran et al., (2017). Optical absorption spectrum for ZnO-NPs was recorded in the UV-Vis (Ultraviolet-Visible) range using a UV-Vis spectrophotometer (UV-3092, LabIndia Analytical Instrument Pvt. Ltd.). The chemical composition of ZnO-NPs was studied by using the Fourier-transform infrared (FTIR) spectrometer (Perkin Elmer L120−000A, spectral range 4000-450 cm^{-1}, KBr pellets). The elemental composition of ZnO-NPs was analyzed using the Energy-dispersive X-ray (EDX) spectroscopy (AMETEK EDAX). The morphology of the products was studied by using Scanning Electron microscope (SEM) (ZEISS Sigma 300). Phase purity and grain size were determined by X-ray diffraction (XRD) analysis (Brucker D8 Advance power-XRD, Cu-Kα-λ= 1.54 Å, range 2θ of 5–80°, scan speed = 0.2 nm, step size = 0.2°).

Determination of antibacterial activity and antibiotics susceptibility

Microorganisms

Two Gram-negative bacteria putative pathogenic strain, viz., *Aeromonas veronii* strain ONKP1 (MN602971), and *Stenotrophomonas maltophilia* strain ONKP2 (MN602972) are used in the present investigation. They were isolated from the gastrointestinal tract of fresh tilapia fish (*Oreochromis niloticus*) which were collected from local wet markets of Kalyani, Nadia, West Bengal. The bacteria

were isolated and characterized (biochemical tests and 16S rRNA sequencing), as in previous studies (Ghosh et al., 2017).

Disc diffusion assay

The antibacterial activity of synthesized biogenic ZnO-NPs and standard antibiotics was tested against the isolates, following the disc diffusion assay (Mahendiran et al., 2017). All discs and materials were autoclaved for sterilization before the experiments. Bacterial inocula were prepared by growing a single colony overnight in 5 ml nutrient broth at 30 °C and streaked onto Mueller-Hinton agar (Himedia, India) plates. Nanoparticle suspension of different concentrations viz. 5, 10, 20, 30, 40 and 50 mg/mL were prepared in double distilled water and sonicated for the uniform suspension of nanoparticles. Then 10 µL suspension was pipetted from each stock and impregnated onto respective 6 mm diameter sterile blank antibiogram discs. Then the dried discs containing different concentrations (50, 100, 200, 300, 400 and 500 µg/disc) were placed onto Mueller-Hinton agar. Discs of streptomycin (100 µg), tetracycline (30 µg) and ciprofloxacin (5 µg) were prepared from their powder form (HiMedia, India) as described above. Commercial discs of erythromycin (15 µg), ampicillin (10 µg), penicillin-G (10 µg), chloramphenicol (30 µg) and amoxicillin (30 µg) (Himedia, India) were also used. After incubation at 37 °C for 24 h, the diameter of the inhibition zones around discs were measured with a ruler. The disc diffusion assay was performed in triplicate for nanoparticle and antibiotics. The results were expressed as means ± standard errors.

RESULTS AND DISCUSSIONS

Elemental analysis and chemical characterization findings of zinc oxide nanoparticles

UV-Vis analysis

The sample exhibits strong UV-absorption spectra with maximum absorption at 340 nm (Fig. 1). Similar observations have been reported on the green synthesis of stable ZnO-NPs (Varghese & George, 2015; Qian et al., 2015). The optical absorbance spectra of noble metal nanoparticles are known to shift to longer wavelengths (red shift) with increasing particle size and to smaller wavelengths (blue shift) with decreasing particle size, due to Surface Plasmon Resonance (SPR). Moreover, only a single SPR band is found in the absorption spectra of spherical nanoparticles, whereas anisotropic and non-spherical shaped particles could give rise to two or more SPR bands depending on symmetry (Sangeetha et al., 2011). Hence, the results of UV absorption spectra of synthesized ZnO-NPs indicate its smaller particle size and spherical shape which is further validated.

XRD analysis

The XRD pattern of AVGE-ZnO-NPs (Fig. 2) shows Bragg reflections at 2θ values of 31.42°(100), 34.01°(002), 36.10°(101), 47.26°(102), 56.22°(110), 62.50°(103), 66.18°(200), 67.38°(112), 68.77°(201), 72.26°(004) and 76.84°(202) that are in good agreement with JCPDS CARD NO: 36-1451. The plane values of XRD patterns confirm the hexagonal wurtzite structure of ZnO-NPs (Sangeetha et al., 2011; Ali et al., 2016). The sample also shows diffraction peaks of the orthorhombic form of $Zn(OH)_2$ (JCPDS CARD NO: 38-0385), which probably arose from surface hydroxylation of ZnO (Deb et al., 2013). The average particle size (D) of

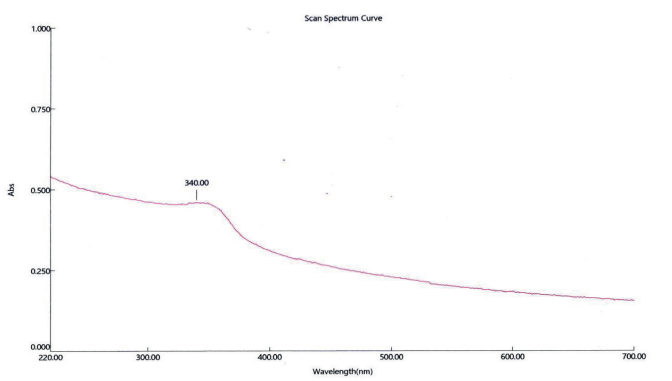


Figure 1. UV-Vis spectra of AVGE-ZnO-NPs.

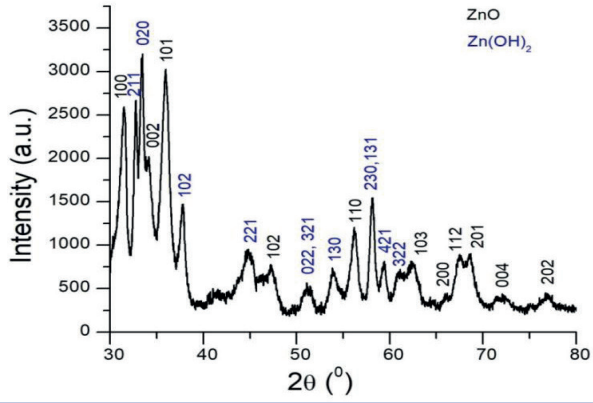


Figure 2. XRD Pattern of AVGE-ZnO-NPs.

synthesized nanoparticles is calculated using the Scherrer's formula, $D = K\lambda/(\beta \cos \theta)$, where D is the crystal size, K is a shape factor (it is a constant; approximately equal to 0.9), λ is the X-ray wavelength, θ is the Bragg's angle in radians and B the full line width at half maximum (FWHM) of the main intensity peak in radians (Mahendiran et al., 2017). The value of D is obtained as 9.72 nm by taking 101 as the main intensity peak for calculation.

SEM and EDX analysis

The SEM images (Fig. 3) show the spherical and rod shaped AVGE-ZnO-NPs. The nanoparticles are found to be agglomerated with a particle size ranging from 37.5-63.75 nm. The agglomeration could be induced by the densification and microstructural changes resulting in the narrow space between particles and also decreased pore size and diameter (Sangeetha et al., 2011). The SEM results of AVGE-ZnO-NPs are similar to previous studies on green ZnO-NPs (Vimala et al., 2014; Qian et al., 2015; Chandran et al., 2018).

The EDX analysis confirms the presence of metallic zinc (Zn) (79.21%) and oxygen (O) (20.79%) as elements in AVGE-ZnO-NPs (Fig. 4). The EDX spectra of AVGE-ZnO-NPs exhibit three characteristic emission peaks of metallic Zn and one small emission peak from O element (Ali et al., 2016). Besides Zn and O, EDX spectra shows a weak signal of Cl element which is probably from precursor $ZnCl_2$ or compounds present in aloe extract.

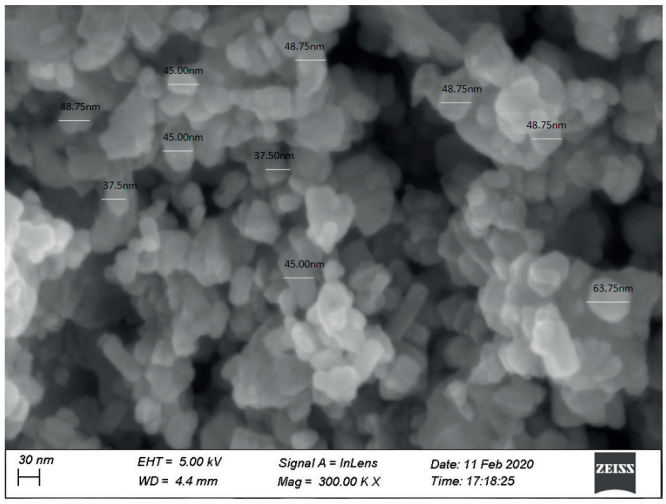


Figure 3. SEM of AVGE-ZnO-NPs (37.3-63.75 nm).

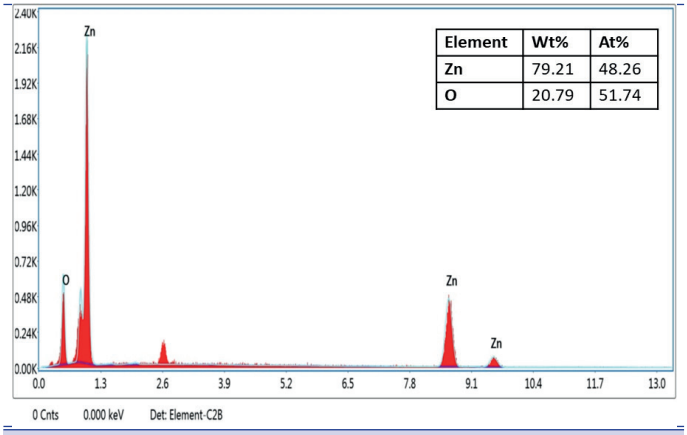


Figure 4. EDX spectrum of AVGE-ZnO-NPs.

FTIR analysis

The infra-red (IR) spectrum of AVGE-ZnO-NPs shows characteristic absorption bands at 470.83 cm^{-1} and 535.57 cm^{-1} owing to the presence of two transverse optical stretching modes of Zn-O (Elumalai et al., 2015; Patel et al., 2017) (Fig. 5). The peak in the region between 600 and 400 cm^{-1} is allotted to Zn-O bond vibrational frequencies (Sangeetha et al., 2011). The absorption band observed at 626.99 cm^{-1} and 708.76 cm^{-1} indicates the deformation of Zn-O bond (Chandran et al., 2018) and C-N stretching of amine group, respectively (Gupta *et al.*, 2018). The peaks at 897.17 cm^{-1}, 1620.40 cm^{-1} and 3460 cm^{-1} are due to C–H bond of alkene group, amide I of proteins/enzymes and stretching vibration of O–H groups in adsorbed moisture, alcohol and phenolic compounds (Mahendiran et al., 2017). 1037.14 cm^{-1} and 2927.15 cm^{-1} are assigned to stretching vibrations of C–H and C–O (Zhou et al., 2017). The peaks in the region of 2900-3700 cm^{-1} also correspond to amide linkages between amino acid residues of the proteins (Sangeetha et al., 2011). The intense band at 1476.92 cm^{-1} can be attributed to alcohols and phenolic groups, C–N groups of aliphatic and aromatic amines and –C–O–C– or –C–O– bonds of alkaloids and flavones (Patel et al., 2017). Thus, the results of FTIR spectrum suggest the role of biological molecules (alkaloid, flavonoid, phenolic compounds, proteins etc.) present in the plant extract as a capping and stabilizing agent for the synthesis of ZnO-NPs.

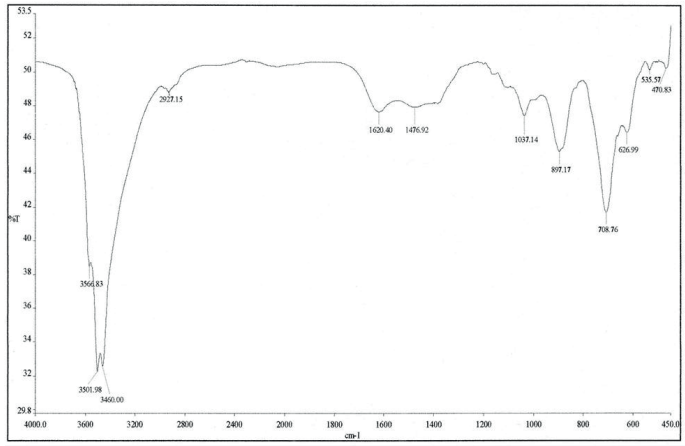


Figure 5. FTIR spectra of AVGE-ZnO-NPs.

Antibacterial activity and Antibiotics susceptibility findings

In the *in-vitro* disc diffusion assay, the presence of clear inhibition zones around the discs indicates the bactericidal activity of AVGE-ZnO-NPs as well as antibiotics (Fig. 6). The antibacterial effect of AVGE-ZnO-NPs and other antibiotics were quantitatively assessed based on the diameter of the inhibition zones which was shown in Table 1. A graphical representation of the zone of inhibition of bacterial pathogens against different antimicrobials was illustrated in Fig 7a. Whereas, In Fig 7b, a graphical representation comparing the zone of inhibition between the highest dose of nanoparticles and the antibiotics to which the bacteria showed susceptibility, was given. The results indicate that the AVGE-ZnO-NPs have good inhibitory activity at all concentrations (50-500 µg/disc) against all the bacterial strains, in comparison to standard commercially available chemotherapeutic agents. In the present study, the inhibitory effect of AVGE-ZnO-NPs increased steadily with the increasing concentration. AVGE-ZnO-NPs showed a maximum zone of inhibition (15.67±1.20 mm) at the highest concentration of 500 µg/disc or 50 mg/ml. As the concentration increases, the diffusion rate of ZnO-NPs increase in the agar medium, causing increased antibacterial activity at higher concentrations (Gunalan et al., 2012).

Both *A. veronii* and *S. maltophilia,* are known to cause serious epidemic disease outbreaks in fish farms, with dominant clinical signs of skin ulcers (Nawaz et al., 2006; Austin & Austin, 2012) and ascites (Geng et al., 2010), respectively. Several studies have isolated *S. maltophilia* from diseased fishes, for example, yellowtail (Furushita et al., 2005), giant gourami (Musa et al., 2008), channel catfish (Geng et al., 2010) and African catfish (Abraham et al., 2016). *A. veronii* is reported to be associated with infections in a number of economically important fish, including cichlid oscar (Sreedharan et al., 2011), gibel carp (Sun et al., 2016), tilapia (Hassan et al., 2017) and channel catfish (Hoai et al., 2019). They are also known as opportunistic pathogens in human and other animals (Gopalakrish-

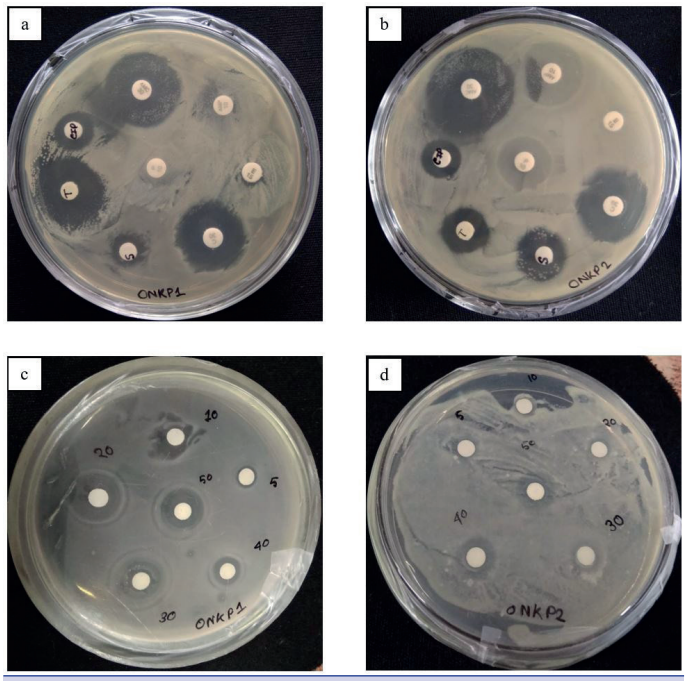


Figure 6. Antimicrobial activity of Standard antibiotics (a,b) and AVGE-ZnO-NPs (c,d) against A. veronii ONKP1 (a,c) and S. maltophilia ONKP2 (b,d). In c and d, nanoparticle concentration was written as per stock value (5-50 mg/ml) which would be 50-500 µg/disc. (E = Erythromycin; C = Chloramphenicol; AMP= Amoxicillin; AMP = Ampicillin; P = Penicillin-G; T= Tetracycline; CIP = Ciprofloxacin; S = Streptomycin).

Table 1. Antimicrobial activity of green synthesized ZnO nanoparticles (AVGE-ZnO-NPs) and standard antibiotics against putative fish pathogenic bacterial strains

Samples (µg/disc)	Bacterial Strain/Zone of inhibition[a] (Mean±SE mm)[b]	
	Aeromonas veronii ONKP1	*Stenotrophomonas maltophilia* ONKP2
AVGE-ZnO-NPs		
50 µg	8.33±0.33	7.67±0.33
100 µg	10.33±0.33	8.67±0.67
200 µg	11.33±0.67	12±1
300 µg	12.33±1.33	14.67±0.33
400 µg	13±1	14.67±0.88
500 µg	15.33±0.33	15.67±1.20
Standard Antibiotics		
Erythromycin (15 µg)	-	-
Chloramphenicol (30 µg)	21.67±0.88	21.33±0.67
Amoxicillin (30 µg)	12.67±0.33	12±0.58
Ampicillin (10 µg)	-	-
Penicillin-G (10 µg)	-	-
Tetracycline (30 µg)	14.67±0.88	15.67±0.88
Ciprofloxacin (5 µg)	12.67±0.33	11.67±0.33
Streptomycin (100 µg)	9.67±0.33	11.33±0.88

SE = standard error, - = No zone; [a] Diameter Zone of inhibition (mm) including disc diameter 6 mm; [b] Results of triplicate analysis.

nan et al., 1999; Nawaz et al., 2006; Janda & Abbott, 2010; Geng et al., 2010). Studies have found that a variety of commercially available antimicrobial agents like, β-lactams, quinolones, aminoglycosides and tetracycline are resisted by *A. veronii* (Sun et al., 2016; Nawaz et al., 2006) and *S. maltophilia* (Looney et al., 2009; Geng et al., 2010) which makes them very difficult to control. In the current study, *A. veronii* and *S. maltophilia* were also found to be resistant against erythromycin, ampicillin and penicillin-G, whereas susceptible to tetracycline, ciprofloxacin, streptomycin, chloramphenicol and amoxicillin. Moreover, *Oreochromis* spp. has been reported to carry antibiotic-resistant pathogenic bacteria such as, *Salmonella* (Budiati et al., 2013), *Aeromonas hydrophila* (Marathe et al., 2016), *Klebsiella pneumoniae* (Marathe et al., 2016; Thongkao & Sudjaroen, 2019) and *Staphylococci* (Thongkao & Sudjaroen, 2019) in their internal organs and is considered as a reservoir of zoonotic diseases. The results of our study also suggest that the marketed Nile tilapias *(O. niloticus) can carry antibiotic-resistant human pathogenic* bacteria like *A. veronii* and *S. maltophilia*, which is a concern of microbiological safety.

Previously, Swain et al., (2014) has studied various metal nanoparticles including Zn, ZnO, CuO, Ag, Al_2O_3, Ag–TiO_2 and Fe_2O_3 (both commercial and laboratory synthesized) as potential antimicrobial agents against bacterial isolates such as *A. hydrophila, Edwardsiella tarda, Pseudomonas aeruginosa, Flavobacterium branchiophilum, Vibrio* sp., *Staphylococcus aureus, Bacillus cereus* and *Citrobacter* sp. which were isolated from various diseased freshwater fish. Chemical ZnO-NPs (105-122 nm), chemical CuO-NPs and green Ag-NPs show better inhibition against all bacteria, than others. However, the authors did not include any antibiotics in the study. Shaalan et al., (2017) also used commercial ZnO-NPs (≈66 nm) and oxytetracycline against six pathogens from infected fish samples. The ZnO-NPs exhibited antibacterial activity against *A. hydrophila* (common bream), *A. salmonicida* subsp. *Salmonicida* (rainbow trout) and *Yersinia ruckeri* (rainbow trout), but fail to inhibit growth of *E. ictaluri* (channel catfish), *E. tarda* (discus) and *Francisella noatunensis* subsp. *orientalis* (Malawi cichlid). In our study, laboratory synthesized green ZnO-NP as well as eight standard antibiotics were tested against the pathogenic strain for direct comparison. Interestingly, AVGE-ZnO-NPs (37.5-63.75 nm) were found to be equally effective, even at lower doses, towards multiple antibiotic-resistant, *A. veronii* and *S. maltophilia*, in the study. There are differences in the results of antibacterial activity of nanoparticles including ZnO, based on size, dose and species (Swain et al., 2014). This is the first *in-vitro* study to demonstrate the inhibitory effects of green

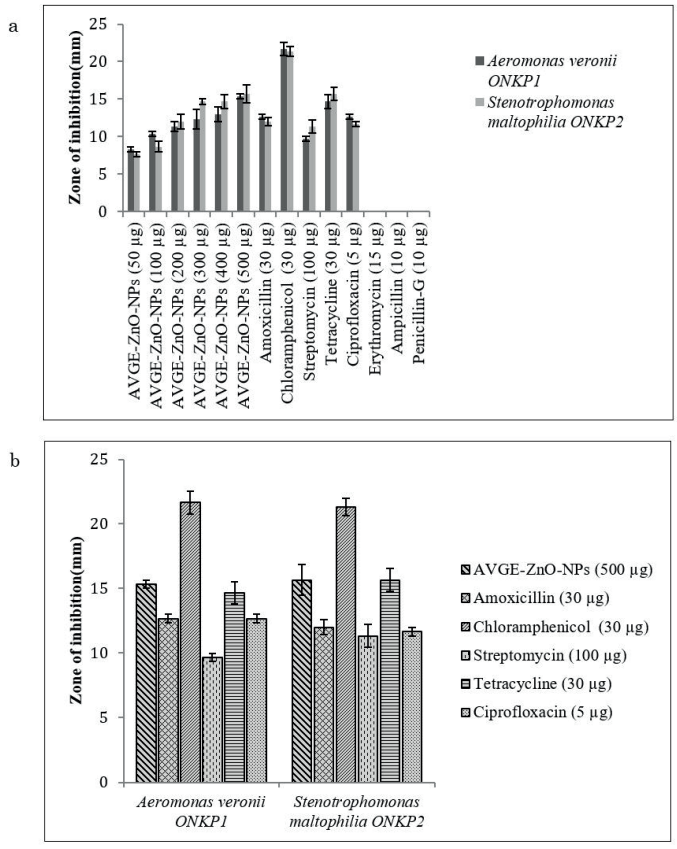


Figure 7. Graphical representation of Zone of inhibition (mm) against bacterial pathogens for antimicrobials; a) Different antimicrobials used in test; b) Comparison between AVGE-ZnO-NPs (highest dose) and the antibiotics to which the bacteria showed susceptibility (error bars represent the mean ± SE of three replicates; concentration of samples as µg/disc).

or *Aloe* functionalized ZnO-NPs against the multi antibiotic-resistant fish pathogen, *A. veronii* and *S. maltophilia*, in addition to their isolation from fresh live marketed, *O. niloticus*. However, synthesized ZnO-NPs may behave differently in live fish and to other pathogenic bacteria. It is a subject of further experimental study which can be carried out in the future.

Nanotechnology is rapidly incorporating into aquaculture by providing new nano-enabled products with novel and unique functions (Luis et al., 2019). But, nanoparticles could be a source of new contamination in the aquatic ecosystem. Studies have shown some toxic effects of ZnO-NPs on aquatic species (Connolly et al., 2016; Kaya et al., 2016; Skjolding et al., 2016; Chupani et al., 2018). The toxicity potential of ZnO-NPs is reported to depend on the exposure route, contact time, concentration, environment and target organism (Swain et al., 2016; Khosravi-Katuli et al., 2017; Elshama et al., 2018; Shah & Mraz, 2020). In freshwater, ZnO-NPs generally tend to dissolve rapidly, increasing the risk of acute toxicity for aquatic organisms (Shaalan et al., 2017).

CONCLUSION

In summary, the results of our study indicates that the green synthesis of ZnO-NPs using *Aloe vera* gel extract is simple, rapid, convenient and cost-effective. *A. vera* has been also found to be equally effective in capping and stabilization of ZnO-NPs. In this study, the synthesized biogenic ZnO-NPs showed strong inhibitory activity towards multi-drug resistant fish pathogenic bacteria, *A. veronii* and *S. maltophilia*, even at a low dose in the disc diffusion assay. Thus the synthesized biogenic ZnO-NPs may be utilized as a potential alternative for disease prevention and treatment in fish. However, detailed *in-vivo* studies accessing the efficacy as well as associated risks and safety of synthesized biogenic ZnO-NPs are needed for application as nano-antibiotics in aquaculture.

Acknowledgements: We express our deep sense of gratitude to the Principal of Mahadevananda Mahavidyalaya for giving us the permission to use UV-Vis spectrophotometer and other laboratory facilities for synthesis of nanoparticles. We also express our gratitude to Prof. Dr. Kumaresh Ghosh, Head of the Department of Chemistry, University of Kalyani, for allowing us to access the FTIR facility. We would like to thank Mr. Prabuddha Sur, Technician of the FTIR instrument, Department of Chemistry, University of Kalyani, for helping us in analysis. We would like to show our gratitude to Dr. Akash Bhattacharya, Technician of the XRD instrument, DST-PURSE-II, Central Instrumentation Laboratory, University of Kalyani. The authors are thankful to Mr. Ayan Mallick, University of Burdwan, for helping in SEM and EDX analysis. We are also thankful to Mr. Pradip Shit and Mr. Saikat Nandi, University of Kalyani, for their help in FTIR and XRD analysis.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics committee approval: Ethics committee approval is not required.

Funding: The research work receives grant from University Research Scholar (URS) Fellowship, University of Kalyani, West Bengal.

REFERENCES

Abraham, T. J., Paul, P., Adikesavalu, H., Patra, A. & Banerjee, S. (2016). *Stenotrophomonas maltophilia* as an opportunistic pathogen in cultured African catfish, *Clarias gariepinus* (Burchell, 1822). *Aquaculture, 450,* 168-172. [CrossRef]

Ali, K., Dwivedi, S., Azam, A., Saquib, Q., Al-Said, M. S., Alkhedhairy, A. A. & Musarrat, J. (2016). *Aloe vera* extract functionalized zinc oxide nanoparticles as nanoantibiotics against multi-drug resistant clinical bacterial isolates. *Journal of Colloid and Interface Science, 472*(2016), 145-156. [CrossRef]

Agarwal, H., Kumar, S. V. & Rajeshkumar, S. (2017). A review on green synthesis of zinc oxide nanoparticles–An eco-friendly approach. *Resource-Efficient Technologies, 3*(4), 406-413. [CrossRef]

Anjugam, M., Vaseeharan, B., Iswarya, A., Gobi, N., Divya, M., Thangaraj, M. P. & Elumalai, P. (2018). Effect of *β*-1, 3 glucan binding protein based zinc oxide nanoparticles supplemented diet on immune response and disease resistance in *Oreochromis mossambicus* against *Aeromonas hydrophila*. *Fish and Shellfish Immunology, 76,* 247-259. [CrossRef]

Austin, B. & Austin, D. A. (2016) Aeromonadaceae Representatives (Motile Aeromonads). In: Bacterial Fish Pathogens. Springer, Cham. [CrossRef]

Awad, A., Zaglool, A. W., Ahmed, S. A. & Khalil, S. R. (2019). Transcriptomic profile change, immunological response and disease resistance of *Oreochromis niloticus* fed with conventional and Nano-Zinc oxide dietary supplements. *Fish and Shellfish Immunology, 93*, 336-343. [CrossRef]

Azizi, S., Mohamad, R. & Mahdavi Shahri, M. (2017). Green microwave-assisted combustion synthesis of zinc oxide nanoparticles with *Citrullus colocynthis* (L.) Schrad: characterization and biomedical applications. *Molecules, 22*(2), 301. [CrossRef]

Bharti, S. K. & Singh, S. K. (2009). Metal based drugs: Current use and future potential. *Der Pharmacia Lettre, 1*(2), 39-51.

Bisht, G. & Rayamajhi, S. (2016). ZnO nanoparticles: a promising anticancer agent. *Nanobiomedicine, 3*(Godište 2016), 3-9. [CrossRef]

Budiati, T., Rusul, G., Wan-Abdullah, W. N., Arip, Y. M., Ahmad, R. & Thong, K. L. (2013). Prevalence, antibiotic resistance and plasmid profiling of *Salmonella* in catfish (*Clarias gariepinus*) and tilapia (*Tilapia mossambica*) obtained from wet markets and ponds in Malaysia. *Aquaculture, 372*, 127-132. [CrossRef]

Chandran, S., Sunny, J. C., Chandran, S. & Bellan, C. (2018). Enhanced Antimicrobial activity of *Aloe vera* blended Zinc Oxide Nanoparticles in PVA matrix. *Materials Today: Proceedings, 5*(8), 16190-16198. [CrossRef]

Chupani, L., Niksirat, H., Velíšek, J., Stará, A., Hradilová, Š., Kolařík, J., Panáček, A. & Zusková, E. (2018). Chronic dietary toxicity of zinc oxide nanoparticles in common carp (*Cyprinus carpio* L.): tissue accumulation and physiological responses. *Ecotoxicology and Environmental Safety*, 147, 110-116. [CrossRef]

Connolly, M., Fernández, M., Conde, E., Torrent, F., Navas, J. M. & Fernández-Cruz, M. L. (2016). Tissue distribution of zinc and subtle oxidative stress effects after dietary administration of ZnO nanoparticles to rainbow trout. *Science of The Total Environment,* 551, 334-343. [CrossRef]

Das, S., Mitra, S., Khurana, S. P. & Debnath, N. (2013). Nanomaterials for biomedical applications. *Frontiers in life science, 7*(3-4), 90-98. [CrossRef]

Deb, S., Kalita, P. K. & Datta, P. (2013). Optical properties of green synthesized ZnO nanocomposites. *Indian Journal of Physics, 87*(12), 1177-1182. [CrossRef]

De Villiers, M. M., Aramwit, P. & Kwon, G. S. (2008). *Nanotechnology in drug delivery*. Springer: Science & Business Media. ISBN 9780387776675 [CrossRef]

Dimapilis, E. A. S., Hsu, C. S., Mendoza, R. M. O. & Lu, M. C. (2018). Zinc oxide nanoparticles for water disinfection. *Sustainable Environment Research, 28*(2), 47-56. [CrossRef]

Elumalai, K., Velmurugan, S., Ravi, S., Kathiravan, V. & Raj, G. A. (2015). Bio-approach: Plant mediated synthesis of ZnO nanoparticles and their catalytic reduction of methylene blue and antimicrobial activity. *Advanced Powder Technology, 26*(6), 1639-1651. [CrossRef]

Elshama, S. S., Abdallah, M. E. & Abdel-Karim, R. I. (2018). Zinc oxide nanoparticles: therapeutic benefits and toxicological hazards. *The Open Nanomedicine Journal, 5*(1), 16-22. [CrossRef]

Faiz, H., Zuberi, A., Nazir, S., Rauf, M. & Younus, N. (2015). Zinc oxide, zinc sulfate and zinc oxide nanoparticles as source of dietary zinc: comparative effects on growth and hematological indices of juvenile grass carp (*Ctenopharyngodon idella*). *International Journal of Agriculture and Biology, 17*(3), 568-574. [CrossRef]

Furushita, M., Okamoto, A., Maeda, T., Ohta, M. & Shiba, T., 2005. Isolation of multidrug-resistant *Stenotrophomonas maltophilia* from cultured yellowtail (*Seriola quinqueradiata*) from a marine fish farm. *Applied and environmental microbiology, 71*(9), 5598-5600. [CrossRef]

Gopalakrishnan, R., Hawley, H. B., Czachor, J. S., Markert, R. J. & Bernstein, J. M. (1999). *Stenotrophomonas maltophilia* infection and colonization in the intensive care units of two community hospitals: a study of 143 patients. *Heart and lung, 28*(2), 134-141. [CrossRef]

Geng, Y., Wang, K., Chen, D., Huang, X., He, M. & Yin, Z. (2010). *Stenotrophomonas maltophilia*, an emerging opportunist pathogen for cultured channel catfish, *Ictalurus punctatus*, in China. *Aquaculture, 308*(3-4), 132-135. [CrossRef]

Ghosh, K., Banerjee, S., Moon, U. M., Khan, H. A. & Dutta, D. (2017). Evaluation of gut associated extracellular enzyme-producing and pathogen inhibitory microbial community as potential probiotics in Nile tilapia, *Oreochromis niloticus*. *International Journal of Aquaculture, 7*(23), 143-158. [CrossRef]

Gunalan, S., Sivaraj, R. & Rajendran, V. (2012). Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. *Progress in Natural Science: Materials International, 22*(6), 693-700. [CrossRef]

Gupta, M., Tomar, R. S., Kaushik, S., Mishra, R. K. & Sharma, D. (2018). Effective antimicrobial activity of green ZnO nano particles of *Catharanthus roseus*. *Frontiers in Microbiology, 9*, 2030. [CrossRef]

Hassan, M. A., Noureldin, E. A., Mahmoud, M. A. & Fita, N. A., 2017. Molecular identification and epizootiology of *Aeromonas veronii* infection among farmed *Oreochromis niloticus* in Eastern Province, KSA. *The Egyptian Journal of Aquatic Research, 43*(2), 161-167. [CrossRef]

Hoai, T. D., Trang, T. T., Van Tuyen, N., Giang, N. T. H. & Van Van, K. (2019). *Aeromonas veronii* caused disease and mortality in channel catfish in Vietnam. *Aquaculture, 513*, 734425. [CrossRef]

Janda, J. M. & Abbott, S. L. (2010). The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clinical microbiology reviews, 23*(1), 35-73. [CrossRef]

Jin, S. E. & Jin, H. E. (2019). Synthesis, Characterization, and Three-Dimensional Structure Generation of Zinc Oxide-Based Nanomedicine for Biomedical Applications. *Pharmaceutics*, *11*(11), 575. [CrossRef]

Kaya, H., Aydın, F., Gürkan, M., Yılmaz, S., Ates, M., Demir, V. and Arslan, Z. (2016). A comparative toxicity study between small and large size zinc oxide nanoparticles in tilapia (*Oreochromis niloticus*): Organ pathologies, osmoregulatory responses and immunological parameters. *Chemosphere, 144*, 571-582. [CrossRef]

Khosravi-Katuli, K., Prato, E., Lofrano, G., Guida, M., Vale, G. & Libralato, G. (2017). Effects of nanoparticles in species of aquaculture interest. *Environmental Science and Pollution Research, 24*(21), 17326-17346. [CrossRef]

Looney, W. J., Narita, M. & Mühlemann, K. (2009). *Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. *The Lancet infectious diseases, 9*(5), 312-323. [CrossRef]

Luis, A. I. S., Campos, E. V. R., de Oliveira, J. L. & Fraceto, L. F. (2019). Trends in aquaculture sciences: from now to use of nanotechnology for disease control. *Reviews in Aquaculture, 11*(1), 119-132. [CrossRef]

Mahendiran, D., Subash, G., Selvan, D. A., Rehana, D., Kumar, R. S. & Rahiman, A. K. (2017). Biosynthesis of zinc oxide nanoparticles using plant extracts of *Aloe vera* and *Hibiscus sabdariffa*: Phytochemical, antibacterial, antioxidant and anti-proliferative studies. *BioNanoScience, 7*(3), 530-545. [CrossRef]

Marathe, N. P., Gaikwad, S. S., Vaishampayan, A. A., Rasane, M. H., Shouche, Y. S. & Gade, W. N. (2016). Mossambicus tilapia (*Oreochromis mossambicus*) collected from water bodies impacted by urban waste carries extended-spectrum beta-lactamases and integron-bearing gut bacteria. *Journal of biosciences, 41*(3), 341-346. [CrossRef]

Martínez-Carmona, M., Gun'ko, Y. & Vallet-Regí, M. (2018). ZnO nanostructures for drug delivery and theranostic applications. *Nanomaterials, 8*(4), 268. [CrossRef]

Mirzaei, H. & Darroudi, M. (2017). Zinc oxide nanoparticles: Biological synthesis and biomedical applications. *Ceramics International, 43*(1), 907-914. [CrossRef]

Musa, N., Wei, L. S., Shaharom, F. & Wee, W. (2008). Surveillance of Bacteria Species in Diseased Freshwater Ornamental Fish from Aquarium Shop. *World Applied Sciences Journal, 3*(6): 903-905.

Nath, M. R., Ahmed, A. N., Gafur, M. A., Miah, M. Y. & Bhattacharjee, S. (2018). ZnO nanoparticles preparation from spent zinc–carbon dry cell batteries: studies on structural, morphological and optical properties. *Journal of Asian Ceramic Societies, 6*(3), 262-270. [CrossRef]

Nawaz, M., Sung, K., Khan, S. A., Khan, A. A. & Steele, R. (2006). Biochemical and molecular characterization of tetracycline-resistant *Aeromonas veronii* isolates from catfish. *Applied and environmental microbiology, 72*(10), 6461-6466. [CrossRef]

Onuegbu, C. U., Aggarwal, A. & Singh, N. B. (2018). ZnO nanoparticles as feed supplement on growth performance of cultured African catfish fingerlings. *Journal of Scientific and Industrial Research, 77*, 213-218.

Parthasarathy, G., Saroja, M. & Venkatachalam, M. (2017). Bio-synthesized nano-formulation of zinc oxide-*Aloe vera* and to study their characterization and antibacterial activities against multiple pathogens. *International Journal of Pharmaceutical Sciences and Research, 8*(2), 900-907.

Patel, V. K., Sundriyal, P. & Bhattacharya, S. (2017). *Aloe vera* vs. poly (ethylene) glycol-based synthesis and relative catalytic activity investigations of ZnO nanorods in thermal decomposition of potassium perchlorate. *Particulate Science and Technology, 35*(3), 361-368. [CrossRef]

Pati, P. & Mondal, K. (2019). A review on the dietary requirements of trace minerals in freshwater fish. *Journal of Environment and Sociobiology, 16*(2), 171-206.

Qian, Y., Yao, J., Russel, M., Chen, K. & Wang, X. (2015). Characterization of green synthesized nano-formulation (ZnO–*A. vera*) and their antibacterial activity against pathogens. *Environmental Toxicology and Pharmacology, 39*(2), 736-746. [CrossRef]

Raje, K., Ojha, S., Mishra, A., Munde, V. K., Rawat, C. & Chaudhary, S. K. (2018). Impact of supplementation of mineral nano particles on growth performance and health status of animals: a review. *Journal of Entomology and Zoology Studies, 6*(3), 1690-1694.

Sangeetha, G., Rajeshwari, S. & Venckatesh, R. (2011). Green synthesis of zinc oxide nanoparticles by *Aloe barbadensis* miller leaf extract: Structure and optical properties. *Materials Research Bulletin, 46*(12), 2560-2566. [CrossRef]

Shaalan, M., Saleh, M., El-Mahdy, M. & El-Matbouli, M. (2016). Recent progress in applications of nanoparticles in fish medicine: a review. *Nanomedicine: Nanotechnology, Biology and Medicine, 12*(3), 701-710. [CrossRef]

Shaalan, M. I., El-Mahdy, M. M., Theiner, S., El-Matbouli, M. & Saleh, M. (2017). *In-vitro* assessment of the antimicrobial activity of silver and zinc oxide nanoparticles against fish pathogens. *Acta Veterinaria Scandinavica, 59*(1), 49. [CrossRef]

Shah, B. R. & Mraz, J. (2020). Advances in nanotechnology for sustainable aquaculture and fisheries. *Reviews in Aquaculture, 12*(2), 925-942. [CrossRef]

Singh, A., Singh, N. B., Afzal, S., Singh, T. & Hussain, I. (2018). Zinc oxide nanoparticles: a review of their biological synthesis, antimicrobial activity, uptake, translocation and biotransformation in plants. *Journal of Materials Science, 53*(1), 185-201. [CrossRef]

Skjolding, L. M., Sørensen, S. N., Hartmann, N. B., Hjorth, R., Hansen, S. F., & Baun, A. (2016) A critical review of aquatic ecotoxicity testing of nanoparticles - the quest for disclosing nanoparticle effects. *Angewandte Chemie, 55*, 15224–15239. [CrossRef]

Sreedharan, K., Philip, R. & Singh, I. B. (2011). Isolation and characterization of virulent *Aeromonas veronii* from ascitic fluid of oscar *Astronotus ocellatus* showing signs of infectious dropsy. *Diseases of aquatic organisms, 94*(1), 29-39. [CrossRef]

Sundrarajan, M., Ambika, S. & Bharathi, K. (2015). Plant-extract mediated synthesis of ZnO nanoparticles using *Pongamia pinnata* and their activity against pathogenic bacteria. *Advanced Powder Technology, 26*(5), 1294-1299. [CrossRef]

Sun, J., Zhang, X., Gao, X., Jiang, Q., Wen, Y. & Lin, L. (2016). Characterization of virulence properties of *Aeromonas veronii* isolated from diseased Gibel Carp (*Carassius gibelio*). *International journal of molecular sciences, 17*(4), 496. [CrossRef]

Swain, P., Nayak, S. K., Sasmal, A., Behera, T., Barik, S. K., Swain, S. K., Mishra, S. S., Sen, A. K., Das, J. K. & Jayasankar, P. (2014). Antimicrobial activity of metal based nanoparticles against microbes associated with diseases in aquaculture. *World Journal of Microbiology and Biotechnology, 30*(9), 2491-2502. [CrossRef]

Swain, P. S., Rao, S. B., Rajendran, D., Dominic, G. & Selvaraju, S. (2016). Nano zinc, an alternative to conventional zinc as animal feed supplement: A review. *Animal Nutrition, 2*(3), 134-141. [CrossRef]

Tekedar, H. C., Kumru, S., Blom, J., Perkins, A. D., Griffin, M. J., Abdelhamed, H., Karsi, A. & Lawrence, M. L. (2019). Comparative genomics of *Aeromonas veronii:* Identification of a pathotype impacting aquaculture globally. *PloS one, 14*(8), e0221018. [CrossRef]

Thongkao, K. & Sudjaroen, Y. (2019). Beta-lactamase and integron-associated antibiotic resistance genes of *Klebsiella pneumoniae* isolated from Tilapia fishes (*Oreochromis niloticus*). *Journal of Applied Pharmaceutical Science, 9*(1), 125-30.

Varghese, E. & George, M. (2015). Green synthesis of zinc oxide nanoparticles. *International Journal of Advance Research in Science and Engineering, 4*(1), 307-314.

Vimala, K., Sundarraj, S., Paulpandi, M., Vengatesan, S. & Kannan, S. (2014). Green synthesized doxorubicin loaded zinc oxide nanoparticles regulates the Bax and Bcl-2 expression in breast and colon carcinoma. *Process biochemistry, 49*(1), 160-172. [CrossRef]

Xiong, H. M. (2013). ZnO nanoparticles applied to bio-imaging and drug delivery. *Advanced Materials, 25*(37), 5329-5335. [CrossRef]

Zhou, Q., Lv, J., Cai, L., Ren, Y., Chen, J., Gao, D., Lu, Z. & Wang, C. (2017). Preparation and characterization of ZnO/AGE MNPs with aloe gel extract and its application on linen fabric. *The Journal of The Textile Institute, 108*(8), 1371-1378. [CrossRef]

Zhu, P., Weng, Z., Li, X., Liu, X., Wu, S., Yeung, K. W. K., Wang, X., Cui, Z., Yang, X. & Chu, P. K. (2016). Biomedical applications of functionalized ZnO nanomaterials: from biosensors to bioimaging. *Advanced Materials Interfaces, 3*(1), 1500494. [CrossRef]

Wang, J., Wang, A. & Wang, W. X. (2017). Evaluation of nano-ZnOs as a novel Zn source for marine fish: importance of digestive physiology. *Nanotoxicology, 11*(8), 1026-1039. [CrossRef]

Watanabe, T., Kiron, V. & Satoh, S. (1997). Trace minerals in fish nutrition. *Aquaculture, 151*(1-4), 185-207. [CrossRef]

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2021; 36(3): 109-115 • DOI: https://doi.org/10.26650/ASE2021744926

Original Article

Host Selection of *Potomida semirugata* (Unionidae: Bivalvia) in Reproduction Strategy

Hülya Şereflişan[1]

Cite this article as: Sereflisan, H. (2021). Host selection of *Potomida semirugata* (Unionidae: Bivalvia) in reproduction strategy. *Aquatic Sciences and Engineering, 36*(3), 109-115.

ABSTRACT

Potomida semirugata, which belongs to the Unionidae family, require a host fish to complete their life cycle. In this study, the host fish preference of this species was investigated. Besides, mussel species and hence the attachment state of larvae on the host fish were also investigated. Five different fish species namely *Cyprinus carpio, Oreochromis niloticus, Clarias gariepinus, Anguilla anguilla*, and *Carasobarbus luteus* were used as host candidates. In this study, five glass aquaria each containing 140 liters of water without any substrate were used and 3 mussels and 10 fish were placed in each aquarium. The aquaria were monitored for 120 days and the glochidia release on the fish was determined by examining gill tissue, dorsal and caudal fins by using a stereomicroscope. Results indicated that *C. carpio* and *O. niloticus* were more preferable in terms of mussel-host fish relationship. The best host fish was determined as *C. carpio* in terms of gill-fullness and juvenile mussel transformation at the ground of the aquarium. These observations provide significant insight and information regarding the effective culture of freshwater mussels.

Keywords: Freshwater bivalve, host fish, *P. semirugata*, glochidia, Unionidae

ORCID IDs of the author:
H.Ş. 0000-0002-2510-3714

[1]Department of Aquaculture, Iskenderun Technical University, Marine Sciences and Technology Faculty, Hatay, Turkey

Submitted:
29.05.2020

Revision Requested:
04.11.2020

Last Revision Received:
10.01.2021

Accepted:
18.01.2021

Online Published:
08.04.2021

Correspondence:
Hülya Şereflişan
E-mail:
hulya.sereflisan@iste.edu.tr

INTRODUCTION

Freshwater mussels (Unionidae), which are important components of the aquatic ecosystems, are among the most threatened animal groups on the planet (Lopes-Lima et al., 2017; 2018). The life cycle of unionids is characterized by a unique obligatory period of larval parasitism on a fish host (Haag, 2012). The glochidium process is defined as a parasitic stage in the life cycle of freshwater bivalve (Unionoidae) molluscs (Kat, 1984; O'Connell & Neves, 1999; Wachtler et al., 2001; Treasurer & Turnbull, 2000; Treasurer et al., 2006). Therefore, the fate of glochidia is highly dependent on the presence of a suitable host fish (Barnhart et al., 2008). *Lasmigona subviridis*, which belongs to the family of Unionidae, can complete its life cycle without the existance of a host (Lellis & King, 1998). Glochidia complete the embryonic development process in the parts of the female mussel gills called marsupial and then are released from the parent and connect to a host fish (Schwartz & Dimock, 2001). Potamida live a parasitic stage at the skin, gill and fin of host fish (Meyers & Millemann, 1977; Haag & Warren, 2003). Morphologically, glochidia can be rectangular, circular and axe head shaped and are divided into two as with or without a hook (Graf & Cummings, 2006; Şereflişan et al., 2009; Wen et al., 2018). The glochidia are encapsulated in a cyst, which they form on the epithelial and connective tissue of the host fish after attachment. When they connect to a non-suitable type of fish species, the cyst they produce is deformed and releases the glochidia from the epithelial and/or connective tissue of the fish within a few days (Kat, 1984; Nezlin et al., 1994; Kirk & Layzer, 1997; Wachtler et al., 2001; Rogers-Lowery & Dimock, 2003). The host fish is an obligate nutrient source for glochidia (Araujo & Ramos, 1998; Araujo et al., 2002). The attach-

ment of glochidia also affects the respiratory performance of the host fish (Thomas et al., 2010). The time spent on the host fish varies from a couple of days to a couple of weeks depending on the host species. Glochidia undergoing metamorphosis leave the host fish and start a new life at the sediment (Fisher & Dimock, 2002; Jones & Neves, 2002). As a result of several biological and anthropogenic factors, extinction of freshwater mussels was predicted (Lyons et al., 2007; Zieritz et al., 2012). Causes, such as a long-life time, the long time required to reach sexual maturity, sedentary lifestyles and high glochidia mortality rates, make the mussels vulnerable and difficult to spread (Gatenby et al., 2000). In addition to all these problems, the search for host fish suitable for the glochidia of the mussel species also complicates the continuity of the life cycle (O' Connell & Neves, 1999). Due to the drying out of wetlands and mass agricultural activities, *P. semirugata* distribution has decreased in most of Turkey's southern rivers and lakes (Seddon et al., 2014). *P. semirugata* has a spawning activity in June in Gölbaşı Lake or the southern parts of Turkey (Şereflişan et al., 2013). Possible host fish populations for *P. semirugata* are in danger of decreasing due to intense fishing activities in Gölbaşı Lake. This situation threatens mussel life (Şereflişan, 2014). Since there are decreases in *Potomida* populations, *P. littoralis* has recently been listed as Endangered in the IUCN Red List. Therefore, priority protection areas should be created for *Potomida* species (Seddon et al., 2014).

The aim of this study is to determine the suitable host fish species for *P. semirugata i*n Gölbaşı Lake in the province of Hatay. We believe that the results of this study will provide valuable information for the determination of the status of this freshwater ecosystem and the culture of this potentially threatened mussel species.

MATERIALS AND METHODS

Mussel collection

Mussels were collected from Gölbaşı Lake (36° 30′ 16″ N; 36° 29′ 42″ E) with a surface area of 12,000 acres, which is located in the Kırıkhan district of Hatay, Turkey (Fig 1). Twenty taxa belonging to 9 families, namely Anguillidae, Bagridae, Chiclidae, Cobitidae, Cyprinidae, Cyrinodontidae, Mugilidae, and Poecilidae, have been identified in Lake Gölbaşı (Şereflişan and Şereflişan, 2001). Among these, *C. carpio, O. niloticus, C. gariepinus, A. anguilla*, and *C. luteus* are the most abundant species in the lake. These fish species were preferred in this study, since the relationship of them with mussels has previously been investigated in a preliminary study. Mussels were randomly collected from the shallow muddy or sandy areas of the lake with scoops and hand rakes of various sizes and by SCUBA diving from the deeper (1-6 m) parts of the lake in early March 2014.

Reproduction monitoring stage

Collected mussels were transferred to a laboratory in water containers. Before starting the study, mussel samples were kept in an aquarium supplied with tap water for two days so that they can adapt to laboratory conditions. The gender of the mussels was determined by controling its gill glochidia fullness and only female ones were selected. The length, width, height and live weight of female mussels were measured as 6.18 ± 0.37 cm, 4.13 ± 0.23 cm, 2.81 ± 0.20 cm and 35.08 ± 7.10 g, respectively. During this resting period, it was observed that the valves of the mussels were opened upto 8 mm, since the adductor muscles were loosend because of the lack of stress factors. Five (glass) aquaria of 140 liters (35cm x 20cm x 20cm) capacity supplied with 22 – 26 °C freshwater and continious aeration were prepared without any substrates in the bottom. Three mussels (*P. semirugata*) and 10 fish samples were placed in each aquarium [A: (*C. carpio - P. semirugata*), B: (*O. niloticus - P. semirugata*) C: (*A. anguilla - P. semirugata*), D: (*C. gariepinus - P. semirugata*) E: (*C. luteus - P. semirugata*)]. While the fishes were fed with standard pellet feed, the mussels were fed with a suspended fito-zooplankton mixture containing (*Compylodiscus clypeus, Pediastrum bonyanum, Keratella cochlearis, Filinia erminali, Brachionus angularis, Lepadella ovalis, Keratella cochlearis*). Glochidia release from the gill and fin of fish was controlled by eye at 2 days intervals. Water discharged from the aquaria was filtered by a 120 µm sieve and the waste product was examined under a light microscope (Olympus CX 41). Detected glochidias were visualized by scanning electron microscope (SEM). The samples were then dehydrated in an ethanol series of 60%, 70%, and 80% for 30 min in each. The samples were mounted on stubs with conductive double-sided carbon tape and coated with gold/palladium in a sputter coater (Polaron SC7620, UK) for 90 sec at 9 mA. The samples were examined and photographed using a JEOL JSM 5500 scanning electron microscope (SEM) at an accelerating voltage of 5 kV. (Şereflişan et al., 2009).

Statistical analysis

The t-test, which is used to compare the means of two independent groups, was used.

Figure 1. Map of the study area (Gölbaşı Lake).

RESULTS

Reproduction strategy of *P. semirugata*

Aquarium A (Relationship between *C. carpio* and *P. semirugata*)

In the first week of May, mussel shell opening and glochidia release were frequently observed. When the gills of *C. carpio* were examined, denser glochidia were examined in the gill lamellae. Aquarium water has a higher turbidity with a more suspended view. There were glochidia detected in the dorsal and caudal fin

of the fish. The glochidia released occured twice and 25 days after the last release, juvenile mussels were detected in the bottom of the aquarium. A third release occured in the first week of June (with a water temperature of 26 °C) Glochidium detected in the fish gill was determined to be on average 140-170 µm in size. At the bottom of the aquarium, juvenile mussels were detected in the size of 180-200 µm (Table 1). As a result, *C.carpio* was found as a suitable host for *P. semirugata* (Fig 2. A).

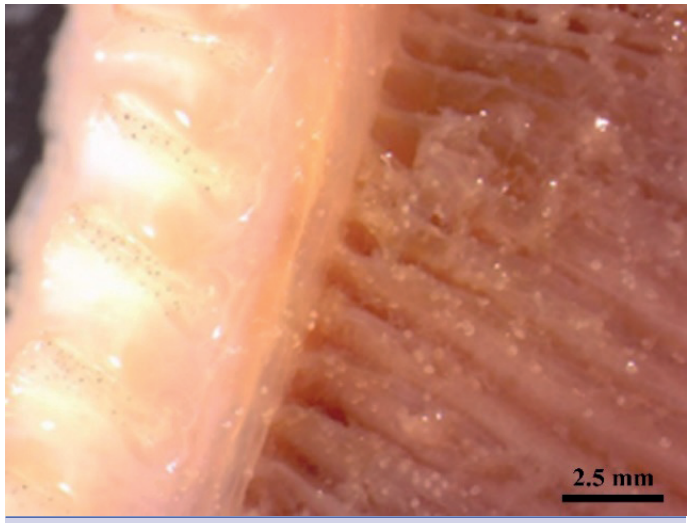


Figure 2A. *P. semirugata* glochidia attached to the gill filaments of *C. carpio*.

Aquarium B (Relationship between *O. niloticus* and *P. semirugata*) Glochidia release was first observed in the second week of April. Even though less dense glochia were observed in the gill of *O. niloticus* than the gill of *C. carpio*, a denser glochidia population was found in the aquarium bottom. The aquarium water was slightly suspended and turbid. Shells of *P. semirugata* were usually closed; they were rarely opened. Release occured twice at intervals (Fig 2.B). The third release was observed in the second week of May and juveniles of *P. semirugata* were observed at the aquarium bottom. Glochidium detected in the fish gill was determined to be on average 120-160 µm in size. At the bottom of the aquarium, juvenile mussels were detected in the size of 150-190 µm. (Table 1). These results confirm the preferance of *O.niloticus* as host.

Aquarium C (Relationship between *A. anguilla* and *P. semirugata*) Starting from the first week of April, mussels were monitored continuoulsy and no changes were observed in the openings of shells until the first of May. When the waste obtained from the bottom siphon of the aquarium was examined, it was found that most of the glochidia packs were in a non-dispersed state. *P. semirugata* did not shape the mantle tissue to attract *A. anguilla*. When the fish's back and tail fin were examined with a stereomicroscope, there were no glochidia detected (Fig 2.C). Even though, glochidia was released four times in P. semirugata, A. anguilla was found to be an unsuitable host.

Table 1. Length measurements (µm) of glochidium detected in the gill and the bottom of the aquarium of fish chosen as host by *P. semirugata* (Mean Length±SD)

Glochidium	*C. Carpio*		*O. niloticus*	
	Mean Length ±SD (µm)	min-max. (µm)	Mean Length ±SD (µm)	min-max. (µm)
Glochidium size in the fish gill	162 ± 0.45[a]	140-170	145 ±0.38[b]	120-160
Sizes of glochidium detected in the aquarium	190±0.22[a]	180-200	70±0.31[b]	150-190

* Mean values with different superscript on the same row are significantly different (p <0.05).

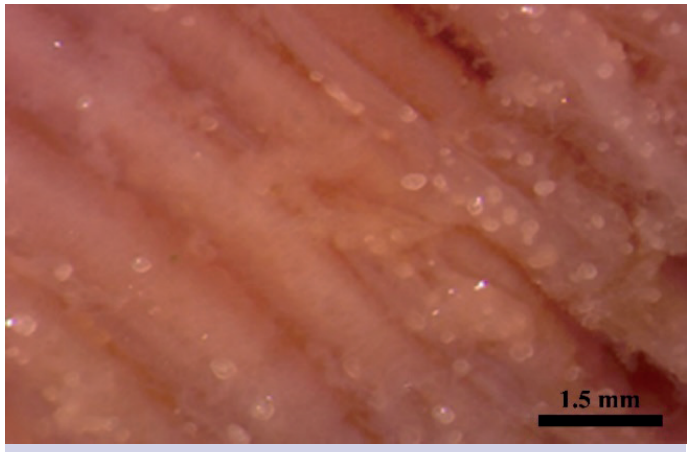


Figure 2B. *P. semirugata* glochidia attached to the gill filaments of *O. niloticus*.

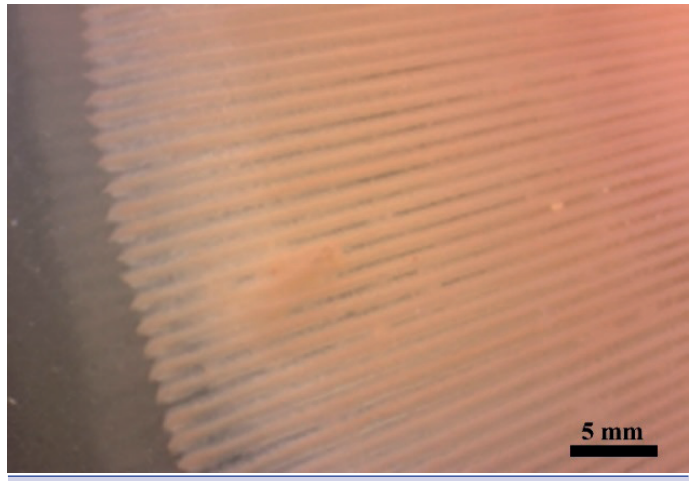


Figure 2C. Gill filaments of *A. anguilla* free from glochidia.

Aquarium D (Relationship between *C. gariepinus* and *P. semirugata*)
When the bottom of the aquarium was examined in the second week of May, glochidia release was observed. However, glochidia were not on the gills and dorsal and caudal fins of C. gariepinus (Fig 2.D). It was observed that the aquarium water was clear and the mussel shells were closed. Glochidia release occurred three times by the end of May, however, it was understood that *C. gariepinus* was not a suitable host for *P. semirugata*.

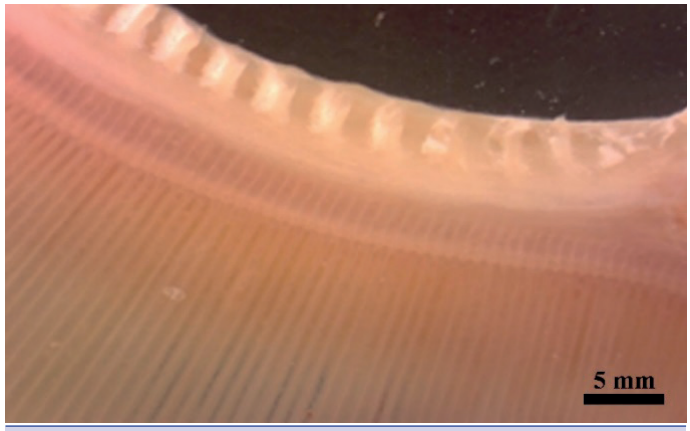


Figure 2D. Gill filaments of *C. gariepinus* free from glochidia.

Aquarium E (Relationship between *C. luteus* and *P. semirugata*)
Glochidia release was observed twice at the third and fourth week of May. Results showed that *C. Luteus* were not preferred as a host by *P. semirugata* since there were no glochidia observed at the gills as well as on the dorsal and caudal fins (Fig 2.E). It is worth mentioning that glochidia packets were found scattered rather than whole at the aquarium bottom.

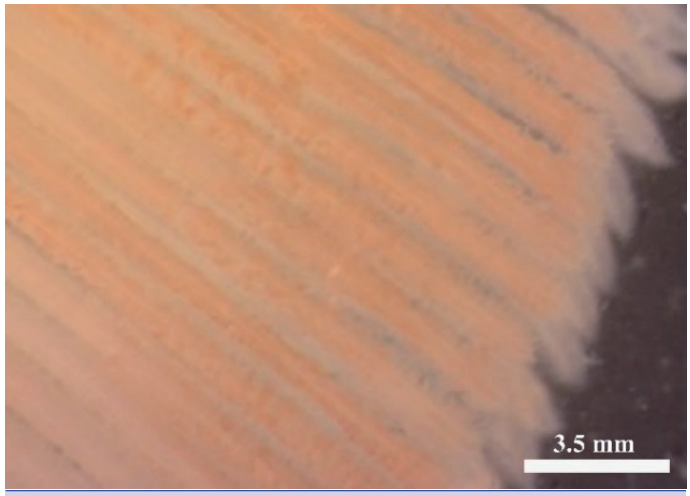


Figure 2E. Gill filaments of *C. luteus* free from glochidia.

Unlike many other members of Unionidae, no hooks were found at the front end of the dorsal and ventral shell of *P. semirugata* larvae (Fig 3.A; 3.B; 3.C; 3.D) and 3-4 rows of protruding structures were observed in this region (Fig 4.A; 4.B; Fig 5).

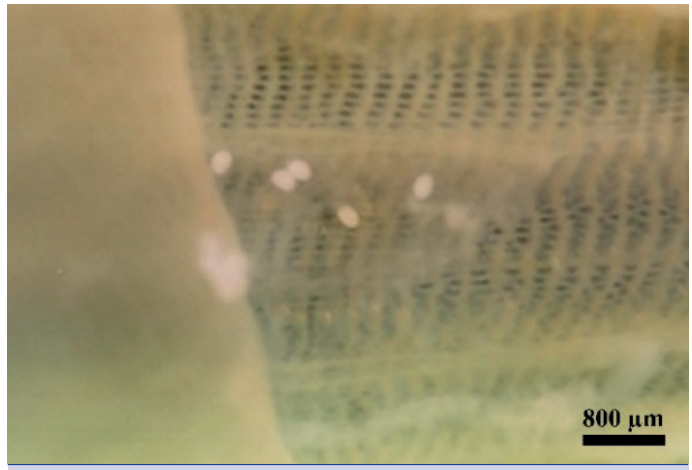


Figure 3A. The preglochidia in the marsupial region of *P. semirugata* gill.

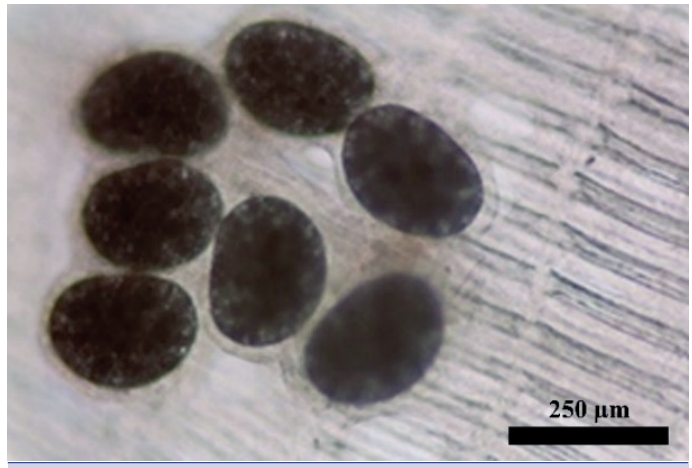


Figure 3B. Preglochidia group in the marsupial region of the gill of *P. semirugata*.

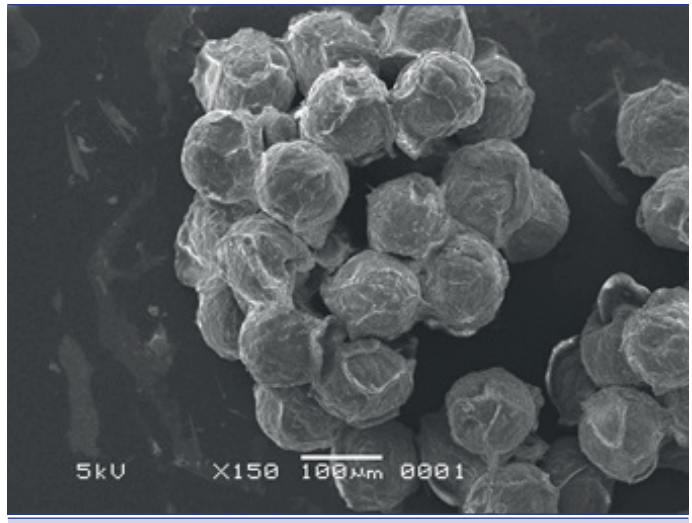


Figure 3C.Preglochidia, which were fertilized in the gonad of *P. semirugata*.

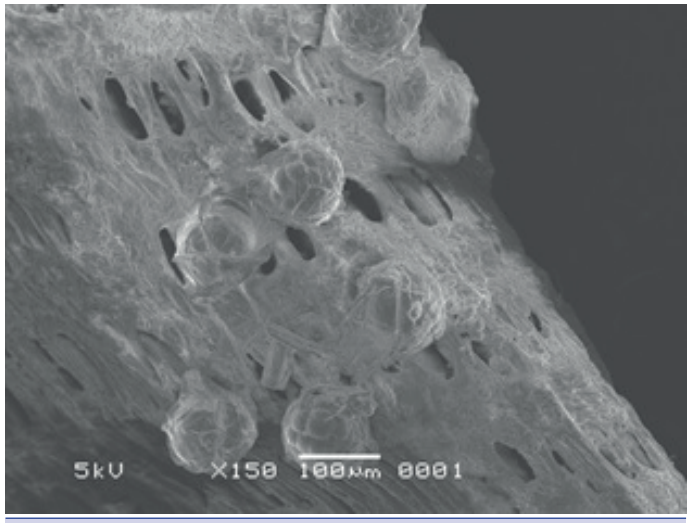


Figure 3D. SEM image of preglochidia in the gill of P. semirugata.

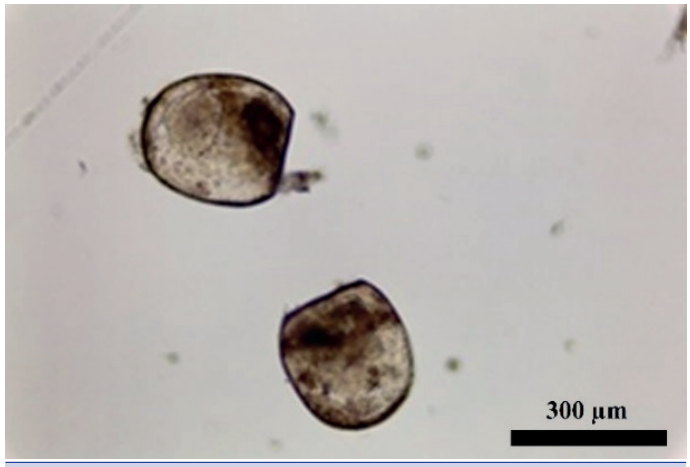


Figure 4A. Image of *P. semirugata* juveniles separated from the host fish (under a stereo microscope).

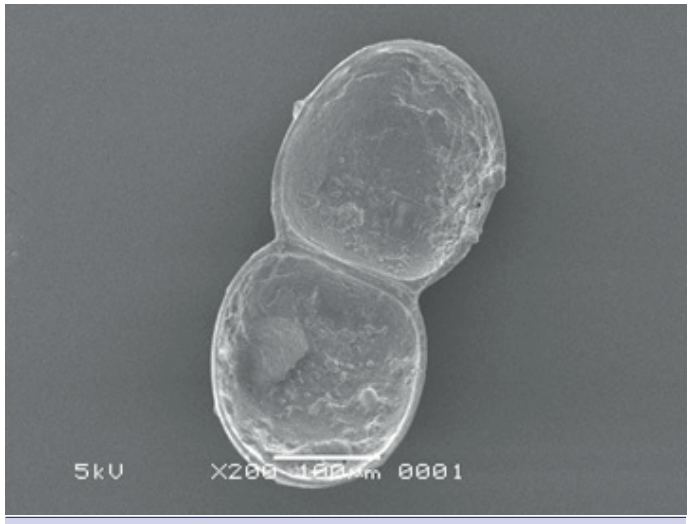


Figure 4B. SEM image of *P. semirugata* juveniles separated from the host fish.

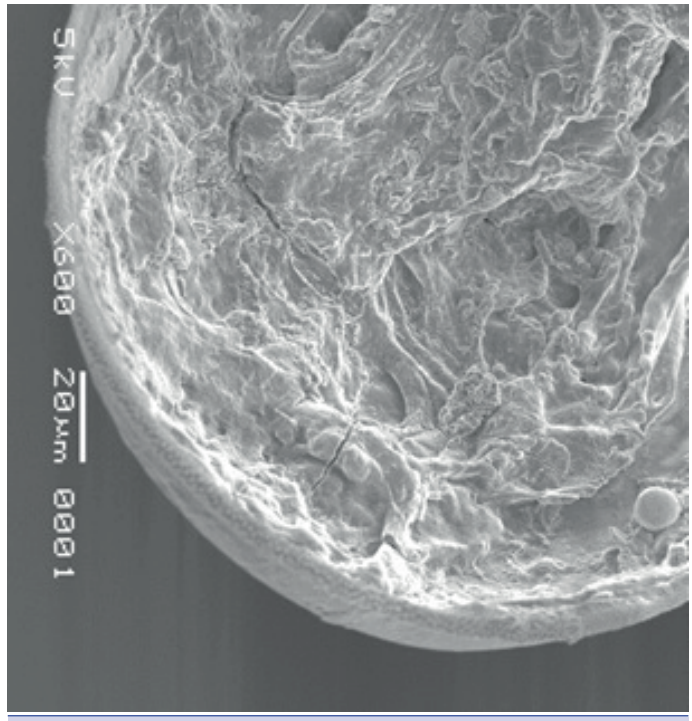


Figure 5. SEM image of ventral valve of *P. semirugata* without hooks.

DISCUSSION

Host fish, and the members of various fish families such as Centrarchidae, Cyprinidae, Percidae, Ictaluridae, Cottidae and Sciaenidae etc. were prefered as a host by various mussel species (Haag et al., 1999; O'Dee & Watters, 2000; Haag & Warren, 2003; Şereflişan et al., 2009; Haag, 2010; Şereflişan, 2018; Modesto et al., 2018; Lopes-Lima et al., 2020); whereas, Unionid mussels prefer fishes which belong to the Cyprinidae family (Haag et al., 1999; Haag & Warren, 2003; O'Dee & Watters, 2000; Larsson, 2015). There are some studies that report *Margaritifera margaritifera, Unio crassus* and *Unio terminalis* prefer *Salmo trutta, Alburnus alburnus* and *Cyprinus carpio* as a host for glachidia release, respectively (Poulin, 2000; Thomas et al., 2010; Şereflişan, 2018). Similarly, *P. semirugata* samples examined in this study preferred the members of Cyprinidae and Cichlidae families as host.

The importance of glochidia release to find suitable host fish in freshwater mussels has been reported (Riusech & Barnhart, 2000). These toothed or toothless hook structures are the most important structure of mussel larvae since they help them to connect to the gill or fins of host fish. Glochidia with a hook are attached to the skin and gills of the host fish while the ones without a hook are usually found encapsulated among the gill filaments of the host (Barnhart et al., 2008). On the other hand, the ones which do not have hook structure are usually encapsulated inside the gills of host fish (Wachtler et al., 2001). Similarly, in this study, hookless glochidia of *P. semirugata* were only detected at the gills of host fish.

Hookless glochidia have been observed in the Unionoidae including Potomida (Graf & Cummings, 2006). Glochidia of *P.*

semirugata have a large number of protrusions with 4 to 5 rows without hooks inside the anterior part of the dorsal and ventral of the two shells (Giusti, 1973; Şereflişan et al., 2009). Approximately 6 hours after release of hookless glochidials by the mussel to the appropriate host, the glochidia are encapsulated by the epithelial tissue of the fish and a cyst is formed (Rogers-Lowery & Dimock, 2006). When glochidia produced a cyst on an unsuitable host fish, it was rejected by the immune system and the glochidia died (Wachtler et al., 2001; Rogers-Lowery & Dimock, 2003). In this study, 5-9 hours after glochidia release, cyct formation as a result of encapsulation of glochidia in the gill tissue was detected. Previous studies reported that anti-glochidial factors are found in the serum of fish infected with glochidia and dead glochidials are poured from the fish gills (Meyers et al., 1980; Waller & Mitchell, 1989; Kirk & Layzer, 1997; O'Connell & Neves, 1999). Despite no studies having been conducted on the fish serum, the attachment of *P. semirugata* glochidia on *C. carpio* and *O. niloticus* may be due to lack of these anti-glochidia factors in these hosts. In this study, Glochidia were found only in *C. carpio* and *O. niloticus* gills on the 2nd, 4th, 6th, and 8th days after *P. semirugata* glochidia release. Others were rejected by the host on the 2nd and 4th day. Especially, in the *A. anguilla* aquarium, glochidia packets were detected undisturbed which indicates that glochidia did not prefer *A. anguilla* as the host. Some mussels use mantle tissue to lure fish in the glochidia release (Barnhart & Roberts, 1995; Haag & Varren, 2003). In this study, *P.semirugata* did not alter its mantle tissue; it only kept its shells 3-4 mm open during glochidia release.

Fertilized eggs in female mussels produce larvae called glochidium and are kept in the marsupial pockets of the gills ready to be released to the host fish (Lang, 1998; von Proschwitz, 1999; Şereflişan, 2009).

CONCLUSIONS

In this study *P. semirugata* collected from Gölbaşı Lake in the province of Hatay was investigated in terms of host preferences using five different fish species. Also, hookless glochidia of *P.semirugata* were examined by SEM to understand their binding strategy to the fish gill. Results provided in this study provide significant insight and information regarding host selection in freshwater mussel production when their presence in the ecological chain gets under a threat. With this study, determining the fish preferred by *P. semirugata* as a host, the prey pressure will be reduced and the mussel-host fish relationship will be preserved. In this study, preferable fish host species were determined but the host selection criteria of *P. semirugata* is still worth exploring (Ziuganov & Nezlin, 1988; Bauer & Wachtler, 2001; Geist, 2010; Şereflişan, 2018).

Ethics committee approval: Ethics committee approval was not required.

Conflict of Interests: There are no conflicts of interest to declare.

Acknowledgement: The author would like to thank Menderes Şereflişan for his assistance with the collection of the mussels and Ece Kılıç for her English language corrections.

Author contribution: H. Şereflişan designed the study, collected the mussels, planned and performed all the procedures and drafted the manuscript. The author interpreted the results, edited the manuscript and accepted its final version.

Disclosure: -

REFERENCES

Araujo, R., Ramos, M.A. (1998). Description of the glochidium of *Margaritifera auricularia* (Spengler 1793)(Bivalvia, Unionoidea). Philosophical Transactions of the Royal Society of London. Series B: *Biological Sciences*, 353: 1553-1559. [CrossRef]

Araujo, R., Cámara, N., Ramos, M.A. (2002). Glochidium metamorphosis in the endangered freshwater mussel *Margaritifera auricularia* (Spengler, 1793): a histological and scanning electron microscopy study. *Journal of Morphology*, 254: 259-265. [CrossRef]

Barnhart, M. C., Roberts, A.D. (1995). Reproduction and fish hosts of unionids from the Ozark Uplifts. In Conservation and management of freshwater mussels II. Proceedings of a UMRCC Symposium 16: 18.

Barnhart, M.C., Haag, W.R., Roston, W.N. (2008). Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society,* 27: 370-394. [CrossRef]

Bauer, G., Wächtler, K. (2001). Environmental relationships of naiads: threats, impact on the ecosystem, indicator function. In Ecology and evolution of the freshwater mussels Unionoida Springer, Berlin, Heidelberg pp. 311-315. [CrossRef]

Fisher, G. R. Dimock, J.R.V. (2002). Ultrastructure of the mushroom body: digestion during metamorphosis of *Utterbackia imbecillis* (Bivalvia: Unionidae). *Invertebrate Biology*, 121: 126-135. [CrossRef]

Gatenby, C.M., Morrison, P. A. Neves, R.J., Parker, B.C. (2000). A protocol for the salvage and quarantine of unionid mussels from zebra mussel-infested waters. In: Freshwater Mollusk Symposia Proceedings, (R. A. Tankersley, D. I. Warmolts, G. T. Watters, B. J. Armitage, P. D. Johnson, and R. S. Butler, eds), 9-18. Ohio Biological Survey, Columbus, Ohio.

Geist, J. (2010). Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of conservation genetics and ecology. *Hydrobiologia*, 644: 69-88. [CrossRef]

Giusti, F. (1973). The minute shell structure of the glochidium of some species of the genera *Unio*, *Potomida* and *Anodonta* (Bivalvia, Unionacea). *Malacologia*, 14: 1-30.

Graf, D.L. Cummings, K.S. (2006). Palaeoheterodont diversity (Mollusca: Trigonioida+ Unionoida): what we know and what we wish we knew about freshwater mussel evolution. *Zoological Journal of the Linnean Society,* 148: 343-394. [CrossRef]

Haag, W.R., Warren, M.L., Shillingsford, M. (1999). Host fishes and host-attracting behavior of *Lampsilis altilis* and *Villosa vibex* (Bivalvia: Unionidae). *The American Midland Naturalist*, 141: 149-158. [CrossRef]

Haag, W.R., Warren, J.M.L. (2003). Host fishes and infection strategies of freshwater mussels in large Mobile Basin streams, USA. *Journal of the North American Benthological Society*, 22: 78-91. [CrossRef]

Haag, W.R. (2010). A hierarchical classification of freshwater mussel diversity in North America. *Journal of Biogeography*, 37: 12-26. [CrossRef]

Haag, W.R. (2012). North American Freshwater Mussels: Natural History, Ecology, and Conservation. Cambridge University Press, Cambridge. [CrossRef]

Jones, J.W., Neves, R.J. (2002). Life history and propagation of the endangered fanshell pearl mussel, *Cyprogenia stegaria* Rafinesque (Bivalvia: Unionidae). *Journal of the North American Benthological Society*, 21: 76-88. [CrossRef]

Kat, P.W. (1984). Parasitism and the Unionacea (bivalvia). *Biological Reviews*, 59: 189-207. [CrossRef]

Kirk, S. G., Layzer, J.B. (1997). Induced metamorphosis of freshwater mussel glochidia on nonhost fish. Nautilus-Paper Edition 110: 102.

Lang, K. (1998). Die Bachmuschel *Unio crassus* (Philipsson, 1788) im Niederbach: Bestandssituation, Fortpflanzungsbiologie und Wanderverhalten. Dipl.-Arb. Univ. Freiburg.

Larsson, M. (2015). Interaction between fish and freshwater mussels. Degree project for Master of Science in Biology Animal Ecology, 45 hec, ht 2015 Department of Biological and Environmental Sciences University of Gothenburg.

Lellis, W.A., King, T.L. (1998). Release of metamorphosed juveniles by the green floater, *Lasmigona subviridis*. *US Fish and Wildlife Service*, 16: 23-23.

Lopes-Lima, M., Sousa, R., Geist, J., Aldridge, D.C., Araujo, R., Bergengren, J., Bespalaya, Y., Bódis, E., Burlakova, L., van Damme, D., Douda, K., Froufe, E., Georgiev, D., Gumpinger, C., Karatayev, A., Kebapçı, Ü., Killeen, I., Lajtner, J., Larsen, B.M., Lauceri, R., Legakis, A., Lois, S., Lundberg, S., Moorkens, E., Motte, G., Nagel, K.-O., Ondina, P., Outeiro, A., Paunovic, M., Prié, V., von Proschwitz, T., Riccardi, N., RudzĪte, M., Rudzītis, M., Scheder, C., Seddon, M., Şereflişan, H., Simić, V., Sokolova, S., Stoeckl, K., Taskinen, J., Teixeira, A., Thielen, F., Trichkova, T., Varandas, S., Vicentini, H., Zajac, K., Zajac, T., Zogaris, S. (2017). Conservation status of freshwater mussels in Europe: state of the art and future challenges. *Biological Reviews*, 92: 572–607. [CrossRef]

Lopes-Lima, M., Burlakova, L.E., Karatayev, A.Y., Mehler, K. Seddon, M., Sousa, R. (2018). Conservation of freshwater bivalves at the global scale: diversity, threats and research needs. *Hydrobiologia*, 810: 1–14. [CrossRef]

Lopes-Lima, M., Hinzmann, M., Varandas, S., Froufe, E., Reise, J., Moreira, C., Araújo, S., Miranda, F., Gonçalves, D.V., Beja, P., Sousa, R., Teixeira, A. (2020). Setting the stage for new ecological indicator species: A holistic case study on the Iberian dolphin freshwater mussel *Unio delphinus* Spengler, 1793. *Ecological Indicators*, 111:105987 [CrossRef]

Lyons, M.S., Krebs, R.A., Holt, J.P., Rundo, L.J., Zawiski, W. (2007). Assessing causes of change in the freshwater mussels (Bivalvia: Unionidae) in the Black River, Ohio. *The American Midland Naturalist*, 158: 1-16. [CrossRef]

Meyers, T.R., Millemann, R.E. (1977). Glochidiosis of salmonid fishes. I. Comparative susceptibility to experimental infection with *Margaritifera margaritifera* (L.)(Pelecypoda: Margaritanidae). *The Journal of Parasitology*, 2:728-733. [CrossRef]

Meyers, T.R., Millemann, R.E., Fustish, C.A. (1980). Glochidiosis of salmonid fishes. IV. Humoral and tissue responses of Coho and Chinook salmon to experimental infection with *Margaritifera margaritifera* (L.) (Pelecypoda: Margaritiferidae). *The Journal of Parasitology*, 66: 274–281. [CrossRef]

Modesto, V., Ilarri, M., Souza, A.T., Lopes-Lima, M., Douda, K., Clavero, M., Sousa, R. (2018). Fish and mussels: importance of fish for freshwater mussel conservation. *Fish Fish*. 19, 244–259. [CrossRef]

Nezlin, L.P., Cunjak, R.A., Zotin, A.A., Ziuganov, V.V. (1994). Glochidium morphology of the freshwater pearl mussel *(Margaritifera margaritifera*) and glochidiosis of Atlantic salmon (*Salmo salar*): a study by scanning electron microscopy. *Canadian Journal of Zoology*, 72: 15-21. [CrossRef]

O'Connell, M.T., Neves, R.J. (1999). Evidence of immunological responses by a host fish (*Ambloplites rupestris*) and two non-host fishes (*Cyprinus carpio* and *Carassius auratus*) to glochidia of a freshwater mussel (*Villosa iris*). *Journal of Freshwater Ecology*, 14: 71-78. [CrossRef]

O'Dee, S.H., Watters, G.T. (2000). New or confirmed host identifications for ten freshwater mussels. In Freshwater mollusk symposia proceedings. part I. Proceedings of the conservation, captive care and propagation of freshwater mussels symposium. Ohio Biological Survey Special Publication (pp. 77-82).

Poulin, R. (2000). Variation in the intraspecific relationship between fish length and intensity of parasitic infection: biological and statistical causes. *Journal of Fish Biology*, 56: 123-137. [CrossRef]

Riusech, F.A., Barnhart, M.C. (2000). Host suitability and utilization in *Venustaconcha ellipsiformis* and *Venustaconcha pleasii* (Bivalvia: Unionidae) from the Ozark Plateaus. In Freshwater mollusk symposia proceedings. *Ohio Biological Survey Special Publication* (pp. 83-91). Columbus, Ohio.

Rogers-Lowery, C.L., Dimock, J.R.V. (2003). Acquired resistance of bluegill sunfish *Lepomis macrochirus* to glochidia larvae of the freshwater mussel *Utterbackia imbecillis* (Bivalvia: Unionidae) after multiple infections. *Journal of Parasitology*, 89: 51-57. [CrossRef]

Rogers-Lowery, C.L., Dimock J.R.V. (2006). Encapsulation of attached ectoparasitic glochidia larvae of freshwater mussels by epithelial tissue on fins of naive and resistant host fish. *The Biological Bulletin,* 210: 51-63. [CrossRef]

Seddon, M.B., Kebapçı, Ü., Lopes-Lima, U.M., van Damme, D., Smith, K.G. (2014). Freshwater mollusks. In: Smith, K.G., Barrios, V., Darwall, W.R.T., Numa, C. (Eds.) *The Status and Distribution of Freshwater Biodiversity in the Eastern Mediterranean (*pp. 43–56). Cambridge, UK, Malaga, Spain and Gland, Switzerland.

Schwartz, M.L., Dimock, J.R.V. (2001). Ultrastructural evidence for nutritional exchange between brooding unionid mussels and their glochidia larvae. *Invertebrate Biology*, 120: 227-236. [CrossRef]

Şereflişan, H. (2014). Gölbaşı Gölü'nde (Hatay) bulunan tatlı su midyelerinin (Unionidae) çevresel koşullarının belirlenmesi. *Yunus Araştırma Bülteni*, (4): 29-36. [CrossRef]

Şereflişan, H. (2018). Determination of host Fish Suitability for *Unio terminalis delicatus* (Bivalvia: Unionidae) from Gölbaşı Lake in Turkey. *Journal of Advances in VetBio Science and Techniques*, 3: 15-22. [CrossRef]

Şereflişan, H., Çek, Ş., Şereflişan, M. (2013). The reproductive Cycle of *Potomida littoralis* (Cuvier, 1798) (Bivalvia: Unionidae) in Lake Gölbaşı, Turkey. *Pakistan Journal of Zoology*, 45(5):1311-1319.

Şereflişan, H., Şereflişan, M., Soylu, S. (2009). Description of glochidia of three species of freshwater mussels (Unionidae) from southeastern Turkey. *Malacologia*, 51: 165-173. [CrossRef]

Sereflisan, M., Şereflişan Hülya (2001). Kırıkhan Gölbası Gölü Hatay Balık Faunası. XI. Ulusal Su Ürünleri Sempozyumu 04-06 Eylül 2001 Hatay.

Thomas, G.R., Taylor, J., Leaniz, C.G. (2010). Captive breeding of the endangered freshwater pearl mussel *Margaritifera margaritifera*. *Endangered Species Research*, 12: 1-9. [CrossRef]

Treasure, J.W., Turnbull, T. (2000). The pathology and seawater performance of farmed Atlantic salmon infected with glochidia of *Margaritifera margaritifera. Journal of Fish Biology*, 57: 858-866. [CrossRef]

Treasurer, J.W., Hastie, L.C., Hunter, D., Duncan F., Treasurer, C.M. (2006). Effects of (*Margaritifera margaritifera*) glochidial infection on performance of tank-reared Atlantic salmon (*Salmo salar*). *Aquaculture*, 256: 74-79. [CrossRef]

von Proschwitz, T. (1999). Art Databanken 2015-12-02. Faktablad: *Unio crassus* – tjockskalig målarmussla. – ArtDatabanken, SLU 2015.

Wachtler, K., Dreher-Mansur M.C., Richter, T. (2001). Larval types and early postlarval biology in naiads (Unionoida). *In Ecology and evolution of the freshwater mussels Unionoida* (pp. 93-125). Springer, Berlin, Heidelberg. [CrossRef]

Waller, D.L., Mitchell, L.G. (1989). Gill tissue reactions in walleye Stizostedion vitreum vitreum and common carp Cyprinus carpio to glochidia of the freshwater mussel Lampsilis radiata siliquoidea. Diseases of Aquatic Organisms, 6: 81-87. [CrossRef]

Wen, H.B., Jin, W., Ma, X.Y., Zheng, B.Q., Xu, P., Xu, L., Gu, R.B. (2018). Vitro culture of axe-head glochidia in pink heelsplitter *Potamilus alatus* and mechanism of its high host specialists. *Plos One,* 13: 1-19. [CrossRef]

Zieritz, A., Gum, B., Kuehn, R., Geist, J. (2012). Identifying freshwater mussels (Unionoida) and parasitic glochidia larvae from host fish gills: a molecular key to the North and Central European species. *Ecology and Evolution*, 2: 740-750. [CrossRef]

Ziuganov, V.V., Nezlin, L.P. (1988). Evolutionary aspects of the symbiosis of pearl mussels and salmonid fishes. *The problems of macroevolution* (pp. 110–111), Nauka, Moscow.

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2021; 36(3): 116-125 • DOI: https://doi.org/10.26650/ASE2020821658

Original Article

Determination of Benthic Macroinvertebrate Fauna and Some Physicochemical Properties of Balaban Lake (Menderes- Izmir)

Alperen Ertaş[1], Tugba Boz[1], İnci Tuney Kızılkaya[1]

Cite this article as: Ertas, A., Boz, T., & Tuney Kizilkaya, I. (2021). Determination of benthic macroinvertebrate fauna and some physicochemical properties of Balaban lake (Menderes- Izmir). *Aquatic Sciences and Engineering, 36*(3), 116-125.

ABSTRACT

In this study, we aimed to identify the benthic macroinvertebrate fauna and some physicochemical characteristics of Balaban Lake located in Menderes, Izmir. For this, benthic macroinvertebrate and water samples were taken from 5 stations of the lake from October 2019 to August 2020. As a result, a total of 25 taxa were identified and were classified as Annelida (Oligochaeta and Hirudinea), Mollusca (Bivalvia and Gastropoda), Crustacea and other Insecta (Trichoptera, Coleoptera and Diptera). The sampling stations were clustered by using the Bray-Curtis UPGMA analysis in terms of the distribution of the benthic macroinvertebrates. As a result of the UPGMA analysis, the 2nd and 3rd stations (88%) were the most similar to each other. The second most similar stations to each other were determined as the 1st and 4th stations (75%). This situation can be explained by the bottom structure (rich vegetation) of these stations. According to Pearson Correlation, there is a strong positive correlation *($p<0.01$)* of TU, EC, TDS and TP with *Nais communis*, while there is strong positive correlation *($p<0.05$)* of TU, EC and TDS with *Nais elinguis*. On the other hand, there is a strong negative correlation *($p<0.01$)* of TU, pH, EC, TDS and TP with *Gammarus* sp. This study is the first study for determining Balaban Lake benthic fauna. That's why all the taxa diagnosed in the stream has been recorded for the first time.

Keywords: Balaban Lake, Benthic Macroinvertebrates, Physicochemical Parameters

ORCID IDs of the author:
A.E. 0000-0001-8510-6100;
T.B. 0000-0001-5491-4367;
İ.T.K. 0000-0003-0293-6964

[1]Ege University, Faculty of Science, Department of Biology, Izmir, Turkey

Submitted:
05.11.2020

Revision Requested:
15.12.2020

Last Revision Received:
17.12.2021

Accepted:
20.12.2021

Online Published:
08.04.2021

Correspondence:
Alperen Ertaş
E-mail:
alperenertas@hotmail.com

INTRODUCTION

The general purpose of limnological studies is to reveal the natural richness of aquatic ecosystems. In this context, the natural wealth of inland waters undoubtedly depends on the flora and fauna of the environment. It is necessary to determine the relationships of living groups living in aquatic ecosystems among themselves and with the physical and chemical characteristics of the ecosystem in which they are located, both in determining the limits of benefiting from the ecosystem in terms of fisheries and aquaculture, and in determining the basic biological efficiency of the aquatic ecosystem (Kırgız, 1984).

Studies on benthic fauna in inland waters constitute one of the important parts of limnological studies. The groups that make up the benthic fauna, the distribution of these groups and the investigation of various factors affecting their distribution are the subjects of study in this context. Benthic macroinvertebrates are defined as organisms that live on the bottom of water bodies, larger than 0.5 mm, visible and have no vertebrae. They are called "benthos" for short. Benthic macroinvertebrates are organisms that inhabit sediment and live on or in the bottom substrates of freshwater and marine ecosystems. In the water, they live on rocks, sediments, debris and aquatic plants, they can make sheaths attached to the substrate they live on or in during their lifetime or for some time in their lives. They have limited mobility. They live in seas, lakes, rivers, pools, swamps and ponds. While some groups feed on organ-

isms such as algae and bacteria at the bottom of the food chain, some groups feed by breaking down plant and wood pieces and debris in the water (Wetzel, 2001; Tanyolaç, 2004). Benthic macroinvertebrates living in lake ecosystems constitute the third link of the food chain in the lake after phytoplanktonic and zooplanktonic organisms and gain importance as the active organisms in the biological productivity of the lake.

It is an undeniable fact that the distribution and taxonomic and ecological characteristics of benthic invertebrates, which constitute one of the basic building blocks of aquatic ecosystems, should be taken in the form of an inventory. In this regard, although there have been some studies done in Turkey (Balık et al., 2004; Balık et al., 2005; Balık et al., 2006; Toksöz and Ustaoğlu, 2005; Yardım et al., 2008; Yıldız et al., 2008; Taşdemir et al., 2010; Akkan Kökçü, 2016; Özbek et al., 2016), faunistic data on aquatic ecosystems is still quite inadequate. On the other hand, there are some studies showing the relationships between physicochemical characters and benthic invertebrate species distribution in our country (Kalyoncu et al., 2008; Kalyoncu and Zeybek, 2009; Ünlü et al., 2008; Zeybek et al., 2014; Yorulmaz et al., 2015; Zeybek 2016). For this reason, both taxonomic and ecological studies on benthic invertebrates, which are distributed in the inland waters of our country, should accelerate and new interpretations should be made in the light of the information to be obtained.

Anatolia is a region that has always preserved its importance in terms of zoogeography throughout various geographical periods. Due to its location between Asia, Europe and Africa, it constitutes one of the important passages and refuge areas for both terrestrial and aquatic species. For this reason, Anatolia is in a very rich situation in terms of species diversity. Izmir with its historical importance is localized on the Mediterranean coast in the West of Anatolia of Turkey. Izmir is the third-largest city in Turkey. Izmir is the fastest-growing city with a population of 4,320 million and an annual population growth rate of 9.5% in Western Anatolia. Balaban Lake was determined as a case area because it is an important drinking water source for Izmir. Balaban Lake is one of the important water sources of the Tahtalı Dam Lake basin in the Menderes district of Izmir. The Lake basin supplies 40% of city water needs. There are many industrial establishments, agricultural lands, and animal farms around Balaban Lake. There has been no study on the benthic macroinvertebrate fauna and physicochemical parameters of Balaban Lake thus far. The aim of this study is to identify the benthic macroinvertebrate fauna and physicochemical characteristics of Balaban Lake. Additionally, it was aimed to demonstrate the effective physicochemical parameters on benthic macroinvertebrates by using Pearson correlation and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis. This study further aimed to contribute to the taxonomical and environmental studies performed in Turkish lakes.

MATERIALS AND METHODS

The study area is located 40 km south of the city center of Izmir, within the area surrounded by the villages Yeniköy and Efemçukuru. The rock structure of Balaban Lake consists of highly metamorphic rocks, recrystallized limestones, metaquartzite, phyllite, and schist rocks (Hetzel et al., 1995). The deepest part of Balaban Lake is 10 meters. The lake's long axis is 4 km and the widest part of the lake is 250m. Kozluoluk Stream is one of the main sources bringing water to the lake. The lake water flows through underground water channels and Balaban Stream to Tahtalı Dam. The study was carried out seasonally at five sampling points in Balaban Lake. Spring sampling was carried out in April 2020, summer sampling in August 2020, autumn sampling in October 2019 and winter sampling in January 2020. Sampling points and coordinates are also given in Table 1 and Figure 1.

The 1st and 2nd stations are located just near Balaban Stream. Both stations have rich vegetation. The 3rd and 4th stations are located in the middle of the lake. The 5th station is located where the Kozluoluk Stream meets the lake and has rich vegetation, being the shallowest part of the lake. The water samples were preserved in the study area by using 2.0 L polyethylene plastic bottles. In this study, 8 physicochemical parameters were monitored over a period of a year. The pH, Total Dissolved Solids (TDS), Electrical Conductivity (EC) and Turbidity (TU) were measured by using CyberScan Series 600 Waterproof- Portable Meter while the Temperature (T), Dissolved oxygen (DO) and Oxygen saturation (Sat. O_2) of each water sample were measured at the sampling sites by using Oxi 315i/ SET WTW Oxygenmeter. Total Phosphate (TP) was analyzed by using appropriate Merck kits according to manufacturer's instructions (Merck Phosphate Test Kits).

Benthic macroinvertebrates were collected from each station by using a classic 50x30 sized 250 µm mesh hand net and Ekman grab (approximately 250 cm^2) according to the literature (AQEM Consortium 2002). The AQEM sorting protocol was done completely in the laboratory and required that a defined subsample was taken prior to sorting. The subsample corresponded to 1/6 of the sample and at least 700 individuals were sorted. If 1/6 of the sample contained < 700 individuals, the sub sample was increased until ≥ 700 organisms are sorted. All individuals of the subsample were picked and counted without magnification and then determined. The result was a taxa list giving the number of individuals extrapolated to the whole sample. The taken samples were kept in 70% alcohol and 4% formaldehyde throughout the field study, and then brought to the Ege University Hydrobiology Research Laboratory. The benthic macroinvertebrate samples were categorized and diagnosed to the level of genus or species under a stereomicroscope. Brauer (1909), Almeida & Mise (2009) were used to diagnose the Coleoptera samples. Edington and Hildrew (1981), Morse (1983), Wallace et al. (1887) were used to diagnose the Trichoptera samples. Only Gledhill et al. (1993) was used in the diagnosis of the Crustacea sub-class. Korneyev & Evstigneev (2013) was used to diagnose the Diptera samples. Brinkhurst and Jamieson (1971) and Timm (1999) were used to diagnose the Oligochaeta and Hirudinea samples. Norman (1998) and Siddiqui et al. (2007) were used to diagnose the Mollusca samples based on morphological characteristics of the shell.

All the mathematical and statistical analyses between the physicochemical data sets and biotic parameters were made using Excel 2019 (Microsoft Office^R) and PAST 3 software. In this study, the dominance of the species was calculated by dividing the number of individuals of a species determined in the station by the total number of individuals of all species. In this study, the faunal sim-

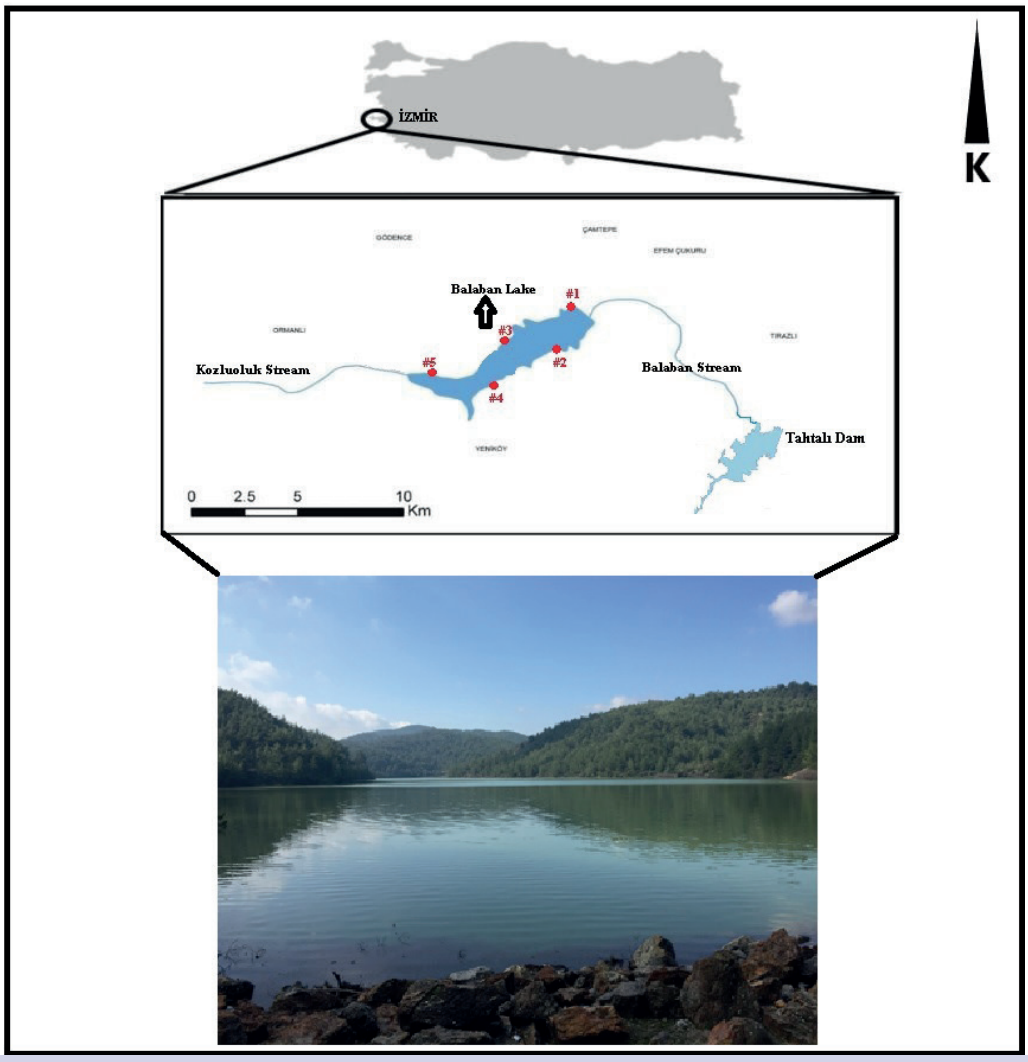


Figure 1. Study Area.

Table 1. Balaban Lake sampling points, coordinates and average depths.

Stations	Coordinates
Station 1	38°23'19.17"N-27°02'68.31"E
Station 2	38°22'98.69"N-27°02'78.50"E
Station 3	38°22'94.14"N-27°02'25.07"E
Station 4	38°22'68.85"N-27°02'23.68"E
Station 5	38°22'69.70"N-27°01'93.42"E

ilarities based on benthic macroinvertebrates between the sampling stations were assessed by using Bray-Curtis similarity index. UPGMA was used to illustrate existent clustering relationships based on Bray-Curtis similarity index. Bray-Curtis is the most frequently preferred method for clustering faunistic data (Somerfield, 2008). In this study, the relationship between physicochemical parameters and macroinvertebrates were determined by using Pearson correlation analysis.

RESULTS AND DISCUSSION

Physical and chemical parameters

The results of the analyzed physicochemical variables of water in five sampling stations along the stream are presented in Table 2.

Temperature (T) is a very important parameter for aquatic life, as it changes the viscosity and density of water, affects the rate of biochemical reactions occurring in the aquatic environment and the solubility of gases. It is known that the metabolism of organisms, especially fish and benthic macroinvertebrates, varies with temperature. Macroinvertebrates are generally eurothermal and tolerant up to 30°C, except for stenothermal organisms such as Plecoptera found in regions close to the source. However, if there is organic contamination in the environment besides the increasing water temperature, the decrease in oxygen concentration creates a problem for macroinvertebrates (Wetzel, 2001; Tanyolaç, 2004). For example, carp is euriterm but begins feeding (8-10°C) and breeding (15°C) only after certain temperatures (Nikolsky, 1963). The most suitable water temperature for trout is 8-16°C (Tanyolaç, 2004). The T level of the Balaban Lake fluctuat-

Table 2. Statistics of physicochemical parameters in sampling stations.

Parameters		Station 1	Station 2	Station 3	Station 4	Station 5
T	R	13.6-14.8	13.9-15.2	14.9-16.9	14.4-15.7	15.0-17.1
(°C)	M±Sd.	14.2±0.51	14.6±0.57	15.9±0.95	15.1±0.57	16.2±1.01
pH	R	6.95-7.15	6.70-6.95	6.70-6.95	6.90-7.05	6.80-6.90
	M±Sd.	7.02±0.09	6.84±0.11	6.84±0.11	6.99±0.06	6.88±0.05
EC	R	78.0-94.0	222-267	241-303	82.0-91.0	249-276
(µS/cm)	M±Sd.	89.3±7.63	248.5±20.5	282.8±28.2	87.8±4.03	262.8±12.8
TDS	R	83.0-100	229-274	248-311	87.0-96.0	254-280
(ppt)	M±Sd	94.5±7.85	257.5±20.3	289±28.1	92.5±4.04	268.8±12.4
TU	R	2.05-2.91	41.3-50.1	52.1-60.1	2.22-3.12	47.4-56.8
(ppt)	M±Sd.	2.46±0.36	46.1±3.73	56.3±3.74	2.62±0.45	51.1±4.07
DO	R	13.4-14.1	12.4-14.0	11.1-13.5	12.6-13.5	10.8-12.4
(mg/l)	M±Sd.	13.8±0.29	13.4±0.71	12.1±1.07	13.0±0.40	11.5±0.73
Sat. O_2	R	90.0-93.0	87.0-90.0	80.0-84.0	92.0-95.0	78.0-82.0
(%)	M±Sd.	91.5±1.29	88.5±1.29	82.0±1.83	93.8±1.26	80.3±1.71
TP	R	4.02-4.97	8.23-10.14	11.8-13.6	3.11-4.23	10.2-12.5
(mg N/L)	M±Sd.	4.49±0.38	9.42±0.84	12.6±0.76	3.82±0.49	11.6±0.98

R: Range; M: Mean; Sd: Standard deviation

ed between 14.2°C and 16.2°C during the study period. This fluctuation was caused by seasonal temperature changes in the weather. Similar physicochemical results were found in various studies conducted in Kılıçkaya Dam Lake (Dirican, 2008); Karacalar D am Lake (Mutlu et al., 2014); Çamlıgöze Dam Lake (Dirican, 2015); Sivas 4 Eylül Dam Lake (Yıldız & Karakuş, 2018).

?? Dissolved oxygen(DO) and saturated oxygen level (Sat. O_2), which are vital for aquatic organisms, varies depending on the temperature as well as the photosynthesis rate of plants and the trophic level of the lakes. In addition, the oxygen holding capacity of water is affected by temperature, pressure and salts dissolved in water. Ephemeroptera, Plecoptera and Trichoptera are abundant in waters with high DO content. These organisms are intolerant of low oxygen conditions. Diptera and Oligochaeta species can survive at the bottom of lentic ecosystems with low DO content. They are tolerant of high water temperatures and low DO concentrations (Wetzel, 2001; Tanyolaç, 2004). It is desired that waters in which fish are grown be saturated with oxygen. Bremond & Vuichard (1973) stated that the minimum amount of DO required for the cyprinid species to survive should be 5.0 mg/L. For trout, the oxygen of the water should be at least 7.0 mg/L (Özdemir, 1994). In Balaban Lake, the DO level was observed to fluctuate between 11.5 mg/L to 13.8 mg/L during the study period. The Sat. O_2 level was observed to fluctuate between 80.3% to 93.8% during the study period The DO and Sat. O_2 levels did not vary much among the stations. Further, in Karacalar Dam Lake, it was reported that DO was 11.12 mg/L on average (Mutlu et al., 2014). In a study conducted by Yıldız & Karakuş (2018), it was reported that the DO amount in surface waters was found to be 7.88 mg/L on average.

pH, which is an indicator of the acidity of water, is one of the important factors affecting aquatic life. In lake waters that are not contaminated in any way, the pH value varies between 6.0 and 9.0. Most industrial wastes are highly alkaline or acidic, but other factors make it difficult to determine the direct effect of pH on freshwater communities. While gastropods are seen above pH 7, bivalves are found in the range of pH 5.6-8.3. Among insects, Coleoptera tolerate a wide tolerance range, Helminths tolerate pH 4.5-8.5 range. While Chironomidae (Diptera) is dominant at pH> 8.5 and at a pH <4.5, Orthocladinae family is not encountered. Some members of Plecoptera, Trichoptera and Hemiptera are tolerant to high pH and some to low pH (Wetzel, 2001; Tanyolaç, 2004). While many fish species show good growth in waters with a pH of 6.5-8.5 (Arrignon, 1976; Dauba, 1981), waters with a pH higher than 10.8 and less than 5.0 have a lethal effect for the Cyprinidae (especially carp) (Svobodá et al., 1993). Generally, alkaline waters are more suitable for trout production. Although trout can live in waters with a pH between 4.5-10, the best are waters with a pH of 7.5-8.0 (Özdemir, 1994). The waters of the stations detected on Balaban Lake have a slightly alkaline character in terms of pH values. For cyprinid health, the mandatory pH range in waters is expected to be 6.00 – 9.00 (EC, 2006). According to the pH data determined in the region, there is no risk for Cyprinid species. In the study performed in Kılıçkaya Dam Lake (Sivas) and Çamlıgöze Dam Lake (Sivas), it were reported that both lakes were suitable for aquatic life (Dirican 2008; Dirican 2015). In another other study which was performed by Mutlu et al., (2014) in Karacalar Dam Lake (Ulaş-Sivas), it was reported that the lake water had a pH level of 8.33 on average. Further, in the 4 Eylül Dam Lake (Sivas) by Yıldız & Karakuş (2018) the pH level of the surface water was reported to be 7.73 on average. According to Kesici et al., (2012) the average pH values were changing between the 6.89-9.12 in Bafa Lake.

The ?? Electrical conductivity (EC) of waters is a measure of the amount of salts or soluble substances in the water and depends on both geological and external factors (Höll, 1979). As a result of the wastes, the increase in the concentration of the salts found naturally in rivers and the deterioration of the balance cause toxic conditions. Macroinvertebrates vary in their tolerance to salinity.

Of the leeches, Piscicola geometra is less tolerant than the Erpobdellidae and Glossiphonidae families. However, salinity tolerance is higher in cold waters than in warm waters (Wetzel, 2001; Tanyolaç, 2004). Tolerant groups to high chloride concentrations (> 1000 mg/L) include only the Baetidae family from Odonata, Diptera (especially Chironomidae) and Ephemeroptera. Plecoptera is not seen in waters with increased salinity (Kalyoncu et al., 2008). The EC level of the Balaban Lake water was observed to have values ranging from 87.8 µS/cm to 282.8 µS/cm. The highest values of EC were recorded at the 3rd and 5th station. These stations are the points where the Kozluoluk Stream meets the lake. The pollution from the stream may have increased when combined with the dam. The EC value may be high due to pollution. In a study conducted at 4 Eylül Dam Lake, Yıldız & Karakuş (2018) reported that the surface water had an EC level of 181.5 µS/cm.

?? Total dissolved solids (TDS) originate from natural resources, domestic and industrial wastewater and agricultural areas. The main ions that contribute to the total amount of dissolved solids are carbonate, bicarbonate, chloride, sulfate, nitrate, sodium, potassium, calcium, magnesium, etc. In addition, silt, clay, small particles of organic structures, inorganic substances, organic compounds that can be dissolved, plankton and other microscopic organisms compose TDS (Tanyolaç, 2004). The amount of TDS affects the properties of drinking water such as taste, hardness and corrosion. The TDS level of the Balaban Lake water was observed to have values ranging from 92.5 ppt to 289 ppt.

?? Turbidity (TU) is seen in waters containing suspended solids, which prevent the passage of light. TU can be caused by many organic or inorganic substances (Wetzel, 2001). TU is important for 3 reasons in terms of the environment. First, the aesthetic use is avoided, the second is the low filterability and finally the disinfectability is not effective (Wetzel, 2001). It is recommended not to exceed 1 NTU (turbidity unit) by the EPA (1979) and World Health Organization (WHO, 2011). TU leads to an increase in the amount of prey by reducing the predator's influence in the prey-predator relationship. It has been reported that the amount of leeches (Hirudinae), which are the food of the fish, is increased in waters with high turbidity (Wetzel, 2001; Tanyolaç, 2004). The TU level of the Balaban Lake water was observed to have values ranging from 2.46 ppt to 56.3 ppt.

Phosphorus is a necessary element for aquatic life. Phosphorus is the most basic element of eutrophication occurring in water (Harper, 1992). It is found in very small amounts in uncontaminated waters and determines the richness of lakes (Tepe & Boyd, 2003). Phosphorus values above 0.3mg/L are entirely due to the pollution produced by humans in surface waters. It can be thought that algae and aquatic plants are overfed as a result of a phosphorus concentration above 0.5 mg/L and the presence of sufficient nitrogen compounds at the same time. As a result, biological equality is disturbed in stagnant waters. In this case, eutrophication is accelerated. Oxygen-free conditions caused by eutrophication adversely affect the population of Ephemeroptera, Plecoptera and Trichoptera groups (Wetzel, 2001; Tanyolaç, 2004). In Balaban Lake, the TP level was reported to fluctuate between 3.82 mg/L and 12.6 mg/L during the study period. In a study performed by Mutlu et al., (2014), the phosphorus level was reported to be between 0.001 mg/L and 0.017 mg/L in Karacalar Dam Lake. Uslu & Türkman (1987) reported that detergents used in cleaning had reached the receiving water environment through wastewater and is a factor affecting phosphorus concentration. It has been calculated that 91% of phosphate comes from domestic and industrial wastewater and 9% from agricultural areas to receiving waters (Egemen & Sunlu 1999).

Benthic macroinvertebrate data

In this study, a total of 2,008 benthic macroinvertebrate samples were collected; all the specimens collected belong to four groups: Mollusca, Annelida, Crustacea, and Insecta. The maximum numbers of individual were collected at station #3 (613 individuals), while the minimum numbers of individual were collected at station #5 (247 individuals). The lowest number of individuals was determined in winter while the highest number of individuals was determined in autumn with the collection of all benthic invertebrate samples (Figure 2).

As a result of diagnosis, the most dominant group in all benthic macroinvertebrate groups was Insecta in the stream (Figure 3). In this study, Crustacea was represented by a total of 219 individuals, Annelida with a total of 258 individuals, Mollusca with a total of 678 individuals and Insecta with a total of 853 individuals. In Kanak Lake the Insecta group was found as the dominant group. This may be due to either the high DO observed in the lake that can maintain the life cycle of insects, or it may be related to the age of the lake. In a study conducted at Sarıkum Lake (Sinop) by Akbulut et al., (2002) the Insecta group was also found to be the dominant group.

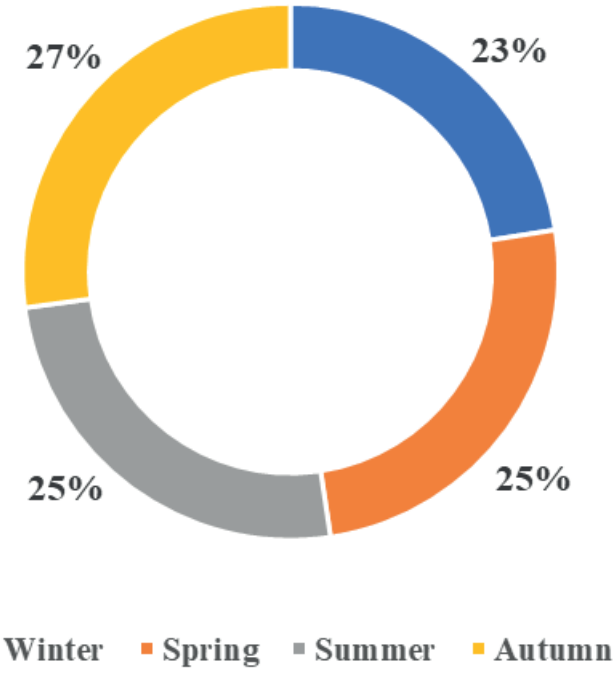


Figure 2. The total percent of benthic macroinvertebrates according to season.

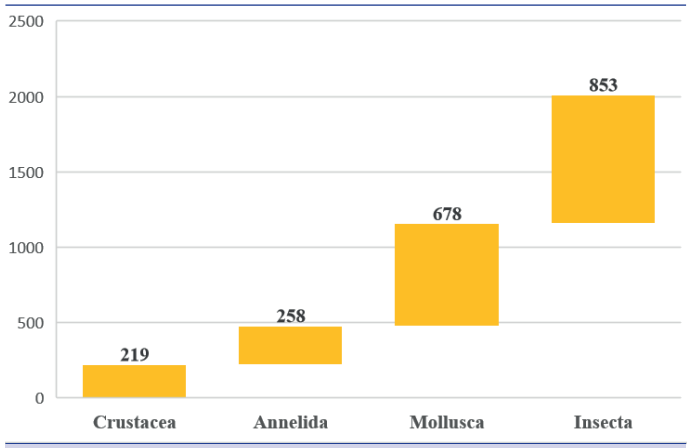


Figure 3. Benthic invertebrate groups in Balaban Lake.

Distributions of the recorded macrozoo-benthic invertebrates in Balaban Lake are shown in Table 3.

Considering all taxonomic groups in Balaban Lake, Bivalvia were the most dominant group in summer (25%) and winter (28%). The second most dominant group was Diptera in summer (21%) and winter (20%). Diptera was the most dominant group in autumn (27%) and spring (24%). The second most dominant group was Bivalvia in autumn (25%) and spring (19%) (Figure 4).

Out of 12 families identified, Insects were the richest group represented with 5 families: Trichoptera (3), Coleoptera (1) and Diptera (1) making up 41.7% of the macroinvertebrates of the Balaban Lake. Crustacea were represented with 2 families: Gammaridae, and Geryonidae which consisted 16.7% of the macroinvertebrates. Annelida were represented with 2 families: Naididae and Erpobdellidae, which consisted 16.7% of the macroinvertebrates. Mollusca were represented with 3 families: Physidae, Pla-

Table 3. Distributions and relative occurrence (%) of benthic macroinvertebrates in the stations.

	Station 1	Station 2	Station 3	Station 4	Station 5
MOLLUSCA					
BIVALVIA					
Dreissenidae					
Dreissena sp.	-	44.7	41.9	-	-
GASTROPODA					
Physidae					
Physa acuta Draparnaud, 1805	7.21	2.77	5.05	-	6.88
Planorbidae					
Gyraulus albus O. F. Müller, 1774	-	4.35	6.03	-	5.26
Planorbis planorbis Linnaeus, 1758	-	1.78	1.95	2.77	3.23
ANNELIDA					
HIRUDINEA					
Erpobdellidae					
Erpobdella octoculata Linnaeus, 1758	-	-	-	11.7	-
OLIGOCHAETA					
Naididae					
Nais communis Piguet, 1906	-	1.58	2.28	-	3.64
Nais elinguis Müller, 1774	-	2.57	2.44	-	2.83
Tubifex costatus Claparède, 1863	4.07	1.18	2.61	2.77	1.61
Tubifex tubifex O. F. Müller, 1774	9.40	1.78	1.95	13.2	4.85
CRUSTACEA					
Gammaridae					
Gammarus sp.	37.3	-	-	29.9	-
Geryonidae					
Potamon potamios Olivier. 1804	-	-	0.48	-	-
INSECTA					
Trichoptera					
Ecnomidae					
Ecnomus tenellus Rambur, 1842	4.07	-	1.46	4.32	-
Hydropsychidae					
Hydropsyche sp.	5.32	2.57	1.79	7.40	4.04
Hydropsyche fulvipes Curtis, 1834	4.07	2.97	2.12	6.17	5.26
Hydropsyche bulbifera McLachlan, 1878	2.82	-	-	3.08	-
Leptoceridae					
Oecetis ochracea Curtis, 1825	-	1.78	1.14	-	3.23
Oecetis furva Rambur, 1842	-	1.58	1.14	-	2.83

Table 3. Continue

	Station 1	Station 2	Station 3	Station 4	Station 5
Coleoptera					
Dytiscidae					
Cybister sp.	2.19	2.17	1.63	-	4.85
Hydaticus sp.	2.82	3.36	1.95	3.08	4.85
Methles sp.	2.19	1.98	1.63	-	4.04
Diptera					
Chironomidae					
Orthocladius sp.	3.76	6.73	6.19	3.70	10.9
Polypedilum sp.	3.13	4.15	4.24	4.93	9.31
Procladius sp.	4.07	3.96	3.58	2.77	7.28
Sergentia sp.	2.19	3.16	3.75	-	6.07
Tanytarsus sp.	5.32	4.75	4.56	4.01	8.90

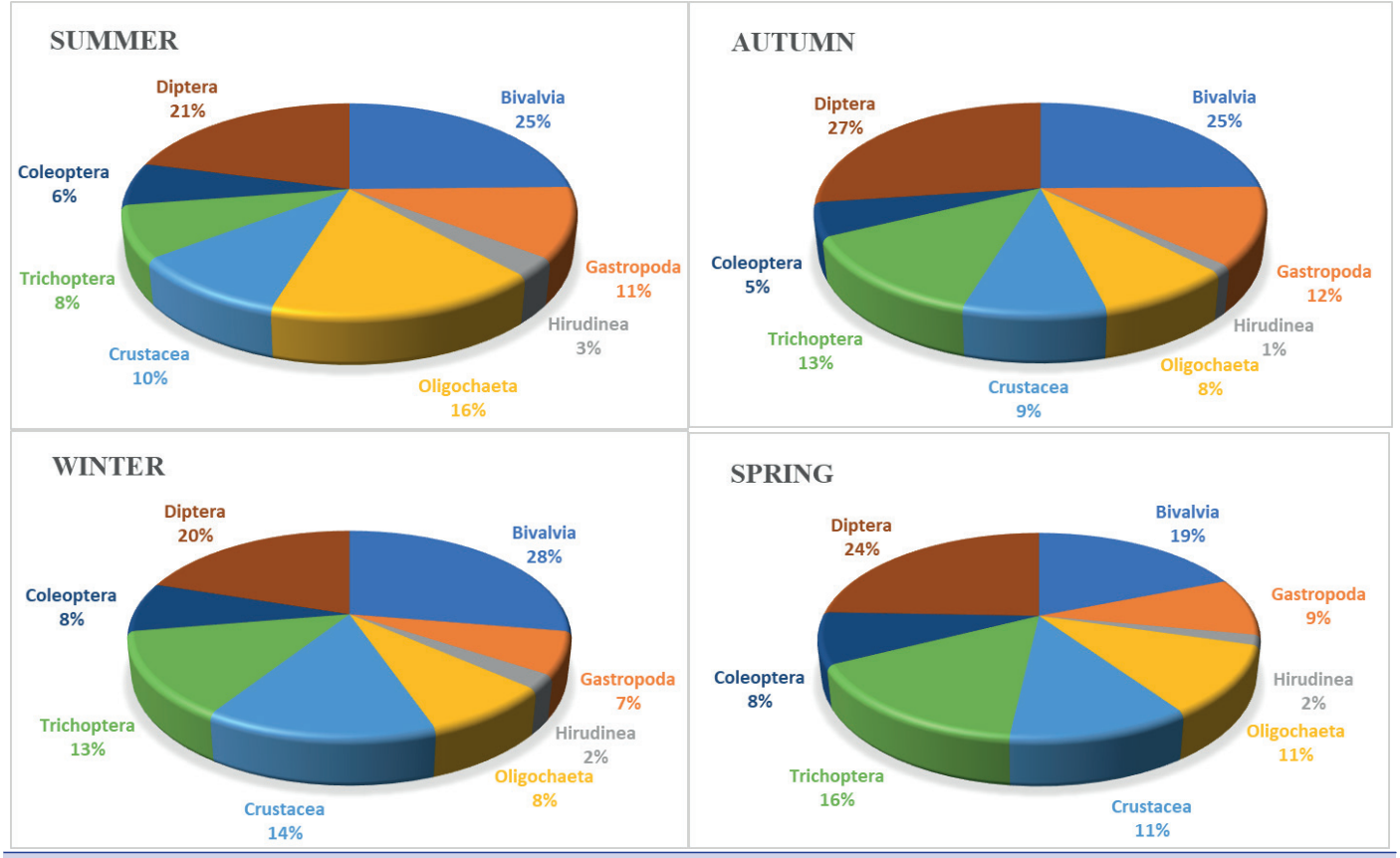


Figure 4. Distribution of taxonomic groups in Balaban Lake.

norbidae and Dreissenidae, which consisted 24.9% of the macroinvertebrates.

The dominance of benthic macroinvertebrate species according to the stations is shown in Figure 5. As a result of the observations, *Gammarus* sp. was dominant in the 1st and 4th stations. *Gammarus* sp., which belongs to the group of Amphipoda, was found in low polluted river sections (Meyer, 1987). *Orthocladius* sp. and *Tanytarsus* sp. were dominant species in the 2nd and 5th stations. These species are an indicator for oliosaprobic (clean) aquatic systems (Tanyolaç, 2004). According to Tanyolaç (2004) these organisms tolerance range is low. They can be found in high DO (mg/l) concentration, Sat O_2 (%) and T (°C). Existent abundance of the organic matter is favorable for benthic macroinvertebrates such as Diptera and Oligochaeta (Rashid and Pandit, 2014).

Oligochaeta and Diptera species are one of the most important freshwater species and are important food resources for some benthic macroinvertebrates and fishes (Brinkhurst & Jamieson, 1971). Numerous studies have shown a correlation between the population of Diptera and the number of diverse species of Oli-

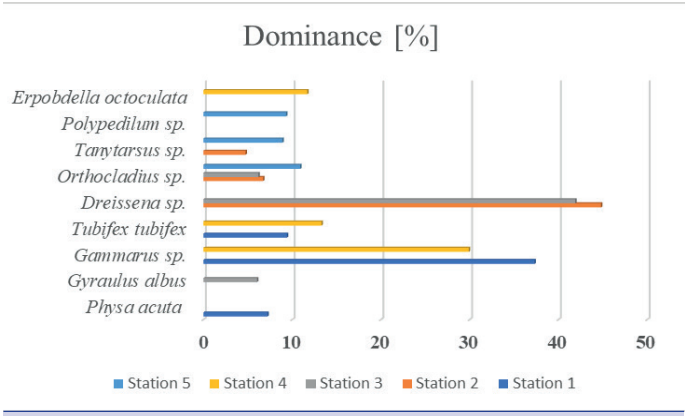


Figure 5. Dominance (%) of taxon of benthic macroinvertebrates at the stations.

gochaeta, and this correlation was observed to be negative (Darby, 1962; Ponyı, 1983). In these studies, Diptera was found to be the dominant group when larval Oligochaeta were found at a low density. In a study conducted by Kırgız (1988), it was reported that Oligochaeta had an abundance level of 18.16% while Chironomidae had 77.27% in Seyhan Lake of the Adana Province of Turkey. In another study conducted at Terkos Lake in the province of Istanbul, a contrasting result was reported, that Oligochaeta was the dominant group in the lake (82% Oligochaeta, 10% and 8% in other groups) Çamur-Elipek (2003). Further, in a study by Balık et al., (2004), Diptera was found as the dominant group with a 86.50% abundance, while Oligochaeta was found to have a 8.72% abundance in Buldan Reservoir in the province of Denizli. In Kemer Lake in the province of Aydın, Oligochaeta was found to be the dominant group with 10 taxa (Yıldız et al., 2008).

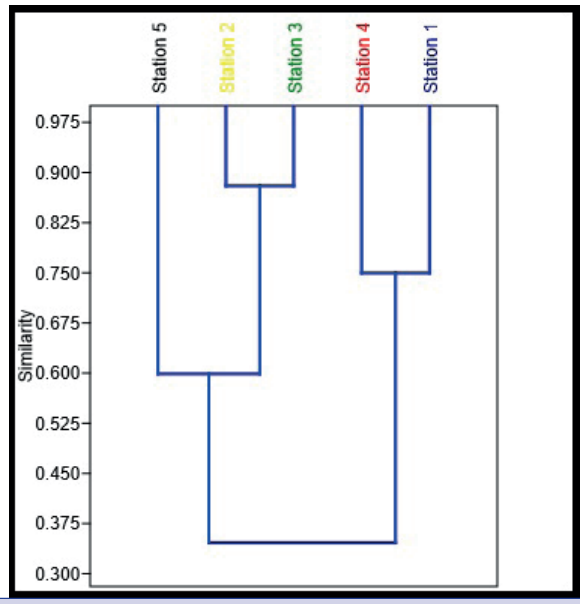


Figure 6. Classification of stations based on similarities of in Balaban Lake.

Table 4. Pearson's based correlation assessment between physicochemical parameters and macroinvertebrate species of the lake.

	T	DO	Sat. Oxy.	TU	pH	EC	TDS	TP
Dreissena sp.	0.099	-0.072	-0.337	0.669	-0.781	0.678	0.683	0.618
Physa acuta	0.188	-0.214	-0.602	0.501	-0.346	0.504	0.501	0.596
Gyraulus albus	0.484	-0.469	-0.69	0.875	-0.891*	0.878	0.878	0.873
Planorbis planorbis	0.663	-0.611	-0.446	0.655	-0.764	0.651	0.65	0.614
Erpobdella octoculata	-0.066	0.135	0.625	-0.605	0.467	-0.612	-0.614	-0.631
Nais communis	0.679	-0.682	-0.872	0.966**	-0.925*	0,966**	0,964**	0,978**
Nais elinguis	0.408	-0.405	-0.665	0.914*	-0.956*	0.918*	0.921*	0.874
Tubifex costatus	-0.138	0.187	0.098	-0.161	0.19	-0.159	-0.163	-0.058
Tubifex tubifex	-0.387	0.433	0.798	-0.928*	0.876	-0.932*	-0.935*	-0.899*
Gammarus sp.	-0.638	0.656	0.818	-0.983**	0.982**	-0.983**	-0,.984**	-0.939*
Potamon potamios	0.463	-0.417	-0.496	0.511	-0.48	0.509	0.504	0.591
Ecnomus tenellus	-0.35	0.41	0.627	-0.771	0.758	-0.774	-0.779	-0.688
Hydropsyche sp.	-0.493	0.548	0.900*	-0.888*	0.758	-0.890*	-0.889*	-0.910*
Hydropsyche fulvipes	-0.185	0.251	0.681	-0.537	0.341	-0.54	-0.538	-0.61
Hydropsyche bulbifera	-0.567	0.598	0.85	-0.989**	0.957*	-0,990**	-0.992**	-0.957*
Oecetis ochracea	0.5	-0.532	-0.77	0.957*	-0.957*	0.960**	0.963**	0.897*
Oecetis furva	0.52	-0.547	-0.787	0.974**	-0.974**	0.977**	0.979**	0.922*
Cybister sp.	0.329	-0.396	-0.8	0.829	-0.717	0.833	0.835	0.825
Hydaticus sp.	0.019	-0.036	-0.27	0.657	-0.797	0.666	0.676	0.519
Methles sp.	0.273	-0.332	-0.771	0.812	-0.705	0.818	0.82	0.817
Orthocladius sp.	0.486	-0.49	-0.739	0,953*	-0.973**	0.957*	0.959*	0.920*
Polypedilum sp.	0.798	-0.786	-0.809	0,928*	-0.931*	0.925*	0.922*	0.917*
Procladius sp.	0.417	-0.441	-0.79	0,936*	-0.899*	0.940*	0.942*	0.926*
Sergentia sp.	0.483	-0.503	-0.831	0,924*	-0.862	0.927*	0.927*	0.940*
Tanytarsus sp.	0.447	-0.462	-0.789	0,922*	-0.884*	0.926*	0.927*	0.926*

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

According to Tanyolaç (2004) the most dominant group was Diptera (*Chironomus thummi*) followed by Oligochaeta (*Limnodrilus* sp. and *Limnodrilus hoffmeisteri*) in eutrophic (polluted) lakes. In this study, *Nais communis* and *Nais elinguis* diagnosed in addition to Limnodrilus species in Balaban Lake. In a study conducted in the Lakes Region, Yıldız & Balık (2006) reported that *Dero digitata* was the most dominant organism in the region and the second most dominant organism in Topçam Dam Lake. Trichoptera are a good indicator of pollution-free water as they dwell in clean water and are very sensitive to polluted water. They can be found anywhere from warm streams to cool streams including lakes, ponds and marshes (Haldar et al., 2016). In this study the DO value of the lake was found to be abundant.

The classification of the stations based on benthic macroinvertebrates composition was illustrated by using Bray-Curtis UPGMA analysis (Figure 6). As a result of the UPGMA analysis, the 2nd and 3rd stations (88%) were the most similar to each other. The second most similar stations to each other were determined in the 1st and 4th stations (75%). This situation can be explained by the bottom structure (rich vegetation) of these stations.

In this study, the random sample cases (*10% select case*) were made on the biotic indices and physicochemical parameters to verify data sets and to determine that the data was transferred without errors in the PAST3. Table 4 indicates the correlations of physicochemical parameters and benthic macroinverterates species.

There is a strong positive correlation ($p<0.01$) of TU, EC, TDS and TP with *Nais communis*, while there is strong positive correlation ($p<0.05$) of TU, EC and TDS with *Nais elinguis*. There is a strong negative correlation ($p<0.01$) of TU, pH, EC, TDS and TP with *Gammarus* sp. There is a strong positive correlation ($p<0.01$) of TU, EC, TDS and TP with Chironomidae species.

CONCLUSIONS

With this study, we aimed to determine some physicochemical properties and benthic macroinvertebrate fauna of Balaban Lake. As a result of the research, 25 taxa were identified. It was observed that the benthic macroinvertebrates were presented as Insecta group > Annelida group > Mollusca group > Crustacea group. The identified taxa were the first recorded for the lake. Relationships between certain benthic macroinvertebrate species and physicochemical parameters were revealed in this study. We believe that this study will provide data for future monitoring studies. However, the change of populations in freshwater, which is under threat due to drought caused by climate change, must be determined over time.

Conflict of interests: The authors declare that they have no conflict of interest.

Ethics committee approval: Ethics committee approval was not required.

Funding: This research was supported by Unit of Scientific Research Projects (BAP) at Ege University (Project no: 21348).

Acknowledgments: The author wishes to thank Prof. Dr. Hasan Kalyoncu who contributed to the study with her vast knowledge and experience.

Disclosure: -

REFERENCES

Akbulut, M., Öztürk, M. & Öztürk, M. (2002). The Benthic Macroinvertebrate Fauna of Sarıkum Lake and Spring waters (Sinop). *Turkish Journal of Marine Sciences, 8*, 102-119.

Akkan Kökçü, C. (2016). Sapanca Gölü Ekolojik Kalitesinin Makroomurgasızlara Dayalı Olarak Su Çerçeve Direktifi (SÇD) Doğrultusunda Değerlendirilmesi. Doktora Tezi. Eskişehir Osmangazi Üniversitesi Fen Bilimleri Enstitüsü Biyoloji Anabilim Dalı Hidrobiyoloji Bilim Dalı.

Almeida, L. M., & Mise, K. M. (2009). Diagnosis and key of the main families and species of South American Coleoptera of forensic importance. *Revista Brasileira de Entomologia, 53*(2), 227- 244. [CrossRef]

Arrignon, J. (1976). Aménagement Ecologique et Piscicole des Eaux Douces, Bordas, Paris, 322 p.

AQEM Consortium (2002). The AQEM sampling method to be applied in STAR. Chapters 7-8.

Balık, S., Ustaoğlu, M. R., Özbek, M., Taşdemir, A., & Yıldız, S. (2004). Buldan Dam Lake (Denizli, Turkey) Benthic Fauna. *Ege Journal of Fisheries and Aquatic Sciences, 21*(1-2), 139-141.

Balık, S., Ustaoğlu, M. R., Taşdemir, A., Yıldız, S., & Özbek, M. (2005). Kuş Gölü (Bandırma) Makrobentik Omurgasız Faunası Hakkında Bir Ön Araştırma. *E.Ü. Su Ürünleri Dergisi, 22*(3-4), 347–349.

Balık, S., Ustaoğlu, M. R., Sarı, H. M., Özdemir Mis, D., Aygen, C., Taşdemir, A., Yıldız, S., Topkara, E. T., Sömek, H., Özbek, M., & İlhan, A. (2006). Bozalan Gölü'nün (Menemen-İzmir) Biyolojik Çeşitliliği Hakkında Bir Ön Araştırma. *E.Ü. Su Ürünleri Dergisi, 23*(3-4), 291–294.

Brauer, A. (1909). Die Süsswasserfauna Deutschland (Coleoptera). Verlang von Gustav Fischer Jena, Berlin, 235p.

Bremond, R., & Vuichard, R. (1973). Parameters de la qualite des eaux: Ministere de la Protection de la Nature et de Environnement. Documentation, Française, Paris, 179 p.

Brinkhurst, R. O., & Jamieson, B. G. M. (1971). Aquatic Oligochaeta of the World. University of Toronto Press, Toronto. 860 pp.

Çamur-Elipek, B. (2003). The dynamics of Benthic Macroinvertabrates in a Mesotrophic Lake: Terkos, Turkey, *Acta Biologica Iugoslavica - Serija D: Ekologija, 38*(1-2), 31-40.

Dauba, F. (1981). Etude comperative de la fauna des poissons dans les ecosystemes de deux reservoirs: Luzech (Lut) et Chastang (Dordogone): These de troisieme cycle L'Institut National Polytechnique de Toulouse. 179 p.

Darby, R. E. (1962). Midges associated with California rice field, with special reference to their ecology (Diptera: Chironomidae). *Hilgardia, 32,* 1-206. [CrossRef]

Dirican, S. (2008). Kılıçkaya BarajGölü (Sivas-Türkiye)'nin fzikokimyasal özelliklerinin gökkuşağı alabalığı yetiştiriciliği için değerlendirilmesi. *Kafkas Üniversitesi Fen Bilimleri Dergisi, 1*(2), 11-21.

Dirican, S. (2015). Assessment of water quality using physicochemical parameters of Çamlıgöze dam lake in Sivas. Turkey, *Ecologia, 5*(1), 1-7. [CrossRef]

EC (European Communities) (2006). EC of the European Parliament and of the council of 6 September 2006 on the quality of fresh waters needing protection or improvement in order to support fish life. Directive 2006/44.

Edington, J. M., & Hildrew, A. G. (1981). Caseless Caddis Larvae of the British Isles, London, 92p.

Egemen, O., & Sunlu, U. (1999). Water quality. Ege University Fisheries Faculty Issue number: 14, İzmir. ISBN: 9754831416.

EPA (United States Environmental Protection Agency) (1979). A review of the EPA red book quality criteria for water. Environmental Protection Agency, USA. 311s.

Gledhill, T., Sutcliffe, D. W., & Williams, W. D. (1993). British Freshwater Crustacea Malacostraca. Freshwater Biological Association, No. 52, 173 p.

Haldar, R., Kosankar, S., & Sangolkar, L. N. (2016). Ecological significance of macro ınvertebrates as an ındicator of environmental pollution. *International Journal of Engineering Science and Computing, 6*(11), 3302-3307.

Harper, D. (1992). Eutrophication of fresh waters: Principles, problems and restoration. Chapman and Hall, London, UK.

Hetzel, R., Ring, U., Akal, C., & Troesch, M. (1995). Miocene NNE-directed extensional unroofing in the Menderes Massif, Southwestern Turkey. *Journal of Geological Society, 152*, 639-654p. [CrossRef]

Höll, K. (1979). Wasser (untersuchung, beurteilung, aufbereitung, chemie, bakteriologie, virologie, biologie). Berlin: Auflage, de Gruyter 586p. [CrossRef]

Kalyoncu, H., Yorulmaz, B., Barlas, M., Yıldırım, M.Z., & Zeybek, M. (2008). Aksu Çayı'nın su kalitesi ve fizikokimyasal parametrelerin makroomurgasız çeşitliliği üzerine etkisi. *Fırat Üni. Fen ve Müh. Bil. Dergisi, 20*(1), 23-33.

Kalyoncu, H., & Zeybek, M. (2009). Benthic fauna of Ağlasun and Isparta streams and determination of water quality according to physicochemıcal parameters and Belgium Biotic Index. *Biyoloji Bilimleri Araştırma Dergisi, 2* (1), 41-48.

Kesici, K., Tüney, Ġ., Kesici, E., & Sukatar, A. (2012). Bafa Gölü'nde Ani Alg Çoğalmasına Neden Olan Nodularia spumigena Türünün Morfolojik Tayini. 21. Ulusal Biyoloji Kongresi. 1122-1145, İzmir.

Kırgız, T. (1984). Seyhan Baraj Gölü Bentik Hayvansal Organizmaları ve Bunların Nitel ve Nicel Dağılımları. Çukurova Üniversitesi Fen Bilimleri Enstitüsü Biyoloji ABD Doktora Tezi, Adana. 86s.

Kırgız, T. (1988). A morphological and Ecological study on the larvae of Chironomidae (Diptera) of Seyhan Dam Lake (in Turkish with English abstract). *Doğa Turkish Journal of Zoology, 12*(3), 231-245.

Korneyev, V. A., & Evstigneev, D. A. (2013). Key to European Species of the Genus Oxyna Robineau-Desvoidy, 1830 (Diptera: Tephritidae), with New Records from Russia. *Ukrainska Entomofaunistyka, 4*(1), 1– 23.

Meyer, D. (1987). Makroskopisch- Biologische Feldmethoden zur Wassergütebeurteilung von Fliegewässern, 3. Auflage, A.L.G., 6, 3000, Hannover, 140p.

Morse, J. C. (1983). Proceedings of the Fourth International Symposium on Trichoptera, South Carolina, Junk Publishers. 486p.

Mutlu, E., Kutlu, B., Yanık, T. & Demir, T. (2014). Evaluation of water quality of Karacalar Dam Lake (Ulaş-Sivas) by using Physicochemical methods. Journal of Selçuk University Natural and Applied Science, Online ISSN:2147-3781, 30-40.

Nikolsky, G. V. (1963). The Ecology of Fishes (Translated by L. Birkett), Academic Press, London and New York, 352 p.

Norman, M. D. (1998). Octopodidae (Benthic octopuses). In: Carpenter, K.E. and Niem, V.H. (eds.), FAO species Identification Guide for Fishery Purposes. The Living Marine Resources of the western Central Pacific. Rome, FAO. 2, 800-826.

Özdemir, N. (1994). Tatlı ve Tuzlu Sularda Alabalık Üretimi. Fırat Üniversitesi, Yayın No: 35, Elazığ.

Özbek, M., Taşdemir, A., & Yıldız, S. (2016). Benthic macroinvertebrate of Adıgüzel Reservoir (Denizli, Turkey). *Ege Journal of Fisheries and Aquatic Sciences, 33*(3), 259-263. [CrossRef]

Ponyı, J. E. (1983). Quantitative Studies on Chironomidae and Oligochaeta in the Benthos of Lake Balaton. Arch Hydrobiol, 97, 196-207.

Rashid, R., & Pandit, A. K. (2014). Macroinvertebrates (oligochaetes) as indicators of pollution: A review. *Journal of Ecology and the Natural Environment, 6*(4), 140-144. [CrossRef]

Siddiqui, K. U., Islam, M. A., Kabir, S. M. H., Ahmad, A. T. A., Rahman, A. K. A., Haque, E. U., Ahmad, Z. U., Begum, Z. N. T., Hassan, M. A., Khondker, M., & Rahman, M. M. (2007). Encyclopedia of flora and fauna of Bangladesh, Mollusks. Asiatic Society of Bangladesh, Dhaka, Vol. 17, 415p.

Somerfield, P.J. (2008). Identification of the Bray-Curtis similarity index: Comment on Yoshioka (2008). *Mar Ecol Prog Ser., 372*, 303-306. [CrossRef]

Svobodá, Z., Lloyd, R., Máchová, J., & Vykusová, B. (1993). Water quality and fish health, FAO, EIFAC technical paper, No:54.

Tanyolac, J. (2004). Limnology (3rd edition) (in Turkish), Hatipoğlu Press, Ankara.

Taşdemir, A., Yıldız, S., Özbek, M., Ustaoğlu, M. R., & Balık, S. (2010). Tahtalı Baraj Gölü'nün (İZMİR) makrobentik (Oligochaeta, Chironomidae, Amphipoda) faunası. *Journal of FisheriesSciences. com, 4*(4), 376-383. [CrossRef]

Tepe, Y., & Boyd, C. E. (2003). A reassessment of nitrogen fertilization for sunfish ponds. *Journal of World Aquaculture Society, 34*(4), 505-511. [CrossRef]

Timm, T. (1999). A Guide to the Estonian Annelida. Naturnalist's Handbooks 1, Tart- Tallinn, 208 pp.

Toksöz, A., & Ustaoğlu, M. R. (2005). Gölcük Gölü'nün (Bozdağ, Ödemiş) Profundal Makrobentik Faunası Üzerine Araştırmalar. E.Ü. *Su Ürünleri Dergisi, 22*(1-2), 173–175.

Uslu, O., & Türkman, A. (1987). Su kirliliği ve kontrolü. Ankara: T.C. Başbakanlık Çevre Genel Müdürlüğü Yayınları 398s.

Ünlü, A., Çoban, F., & Tunç, S. (2008). Investigation of Lake Hazar water quality according to physical and inorganic chemical parameters (in Turkish with English abstract). *Journal of The Faculty of Engineering and Architecture of Gazi University, 23*(1), 119-127.

Wallace, I. D., Wallace, B., & Philipson, G. N. (1887). A Key to the Case-Bearing Caddish Larvae of Britain and Ireland. *Freshwater Biological Association*, No. 51, 237p.

Wetzel, R.G. (2001). Limnology: Lake and river ecosystems. Elsevier Academic Press 1006 p.

WHO (World Heath Organization) (2011). Guidelines for drinking-water quality. World Heath Organization Library Cataloguing-in-Publication Data, NLM classification: WA 675.

Yardım, Ö., Şendoğan, E., Bat, L., Sezgin, M., & Çulha, M. (2008). Sarıkum Gölü (Sinop) Makrobentik Mollusca ve Crustacea Faunası. E.Ü. *Su Ürünleri Dergisi, 25*(4), 301–309.

Yıldız, S., & Balık, S. (2006). The Oligochaeta (Annelida) Fauna of Topçam Dam-Lake (Aydın, Turkey). *Turkish Journal of Zoology, 30*(83-89).

Yıldız, S., Tasdemir, A., Balık, S., & Ustaoğlu, M. R. (2008). Macrobentic (Oligochaeta, Chironomidae) Fauna of Kemer Dam Lake (Aydın). *Journal of Fisheries Sciences.com, 2*(3), 457-465. [CrossRef]

Yıldız, S., & Karakuş, C. B. (2018). Mapping of water quality-level relation of Sivas 4 Eylül Dam with Geographic Information System (GIS). *APJES, 6-1*, 64-75.

Yorulmaz, B., Sukatar, A., & Barlas, M. (2015). Comparative analysis of biotic indices for evaluation of water quality of Esen River in South-West Anatolia, Turkey. *Fresenius Environmental Bulletin*, Vol. 24, No.1a.

Zeybek, M., Kalyoncu, H., Karakuş, B., & Özgül, S. (2014). The use of BMWP and ASPT indices for evaluation of water quality according to macroinvertebrates in Değirmendere Stream (Isparta, Turkey). Turkish Journal of Zoology, 38, 603-613. [CrossRef]

Zeybek, M. (2017). Macroinvertebrate-based biotic indices for evaluating the water quality of Kargı Stream (Antalya, Turkey). *Turkish Journal of Zoology, 41*, 476-48. [CrossRef]

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2021; 36(3): 126-132 • DOI: https://doi.org/10.26650/ASE2020838973

Original Article

Digenean Parasites of Labrid Fishes (Labridae: *Symphodus*) from Turkish Coasts of the Black Sea: New Records

Türkay Öztürk[1], Arzu Güven[1,2]

Cite this article as: Öztürk, T., & Güven, A. (2021). Digenean Parasites of Labrid Fishes (Labridae: *Symphodus*) from Turkish Coasts of the Black Sea. *Aquatic Sciences and Engineering, 36*(3), 126-132.

ABSTRACT

In the present study digenean parasite faunas of four labrid fishes, *Symphodus tinca, S. rossali, S. cinereus* and *S. ocellatus* were investigated and compared. A total of 52 fish specimens were caught on the Black Sea coast near Sinop, Turkey, over two years. Eight digenean species were found in the four labrid fishes. These are *Helicometra fasciata, Gaevskajatrema perezi, Proctoeces maculatus, Phyllodistomum crenilabri, Galactosomum lacteum, Condylocotyla pilodora, Metadena pauli* and *Opecoelidae* gen. sp. Each digenean species on their respective hosts were counted, their prevalence, mean intensity and abundance values were determined. While the maximum parasite diversity was found in *S. tinca, S. ocellatus* was infected with the fewest parasites. Total parasite abundance was significantly high in S. roissali, which was infected with four species. A close resemblance was observed in the digenean parasite faunas of *S. tinca* and *S. roissali*. The core, secondary, satellite, and rare species in the digenean parasite community of each host were determined. This study contains the first data on the digenean parasites of labrid fish on Turkish Black Sea coast. *Phyllodistomum crenilabri* and *Metadena pauli* are new parasite records in Turkish fish parasite fauna, while *Condylocotyla pilodora* is a new parasite record for the labrid fishes.

Keywords: Trematode, digenea, parasites, *Symphodus* spp., Black Sea

ORCID IDs of the author:
T.Ö. 0000-0001-5568-3214;
A.G. 0000-0002-8220-5282

[1]Sinop University, Faculty of Fisheries and Aquatic Sciences, Sinop, Turkey

[2]Malatya Turgut Özal University, Vahap Küçük Vocational High School, Malatya, Turkey

Submitted:
11.12.2020

Revision Requested:
28.01.2021

Last Revision Received:
30.01.2021

Accepted:
31.01.2021

Online Published:
08.04.2021

Correspondence:
Türkay Öztürk
E-mail:
turkay.ozturk@gmail.com

INTRODUCTION

The family Labridae is one of the large taxons of fish, comprising approximately 580 species in 82 genera and they are widely dispersed in tropical and temperate marine waters around the world (Hanel, Westneat, & Sturmbauer, 2002). A total of 20 species of this family have been recorded from the Turkish Sea to date (Bilecenoğlu, Kaya, Cihangir, & Çiçek, 2014). A total of 5 out of 8 species of this family, which inhabit the Black Sea, are the species of the *Symphodus* genus. The native habitats of the genus *Symphodus* are the coastal zones of the East Atlantic Ocean, the Mediterranean and the Black Sea. They are found to a depth of 1–50 m near rocks and eel-grass beds. Labrid fish are not target species commercially. However, labrid fish are often caught by fishermen that focus on fishing for other species (Choat & Bellwood, 1998; Nelson, 2006).

Digenean parasites provide important information about the aquatic ecosystem since they have complex life cycles, during which several groups of marine animals are used as intermediate hosts. There are a lot of published data on digenean parasites of labrid fishes from the Mediterranean and Black Sea (Sey, 1970; Nikolaeva & Solonchenko, 1970; Parukhin, Naidenova, & Nikolaeva, 1971; Gaevskaya & Solonchenko, 1989; Campos, Carbonell, & Pellicer, 1990; Campos & Carbonell, 1994; Sasal, Niquil, & Bartoli, 1999; Korniychuk, 2001; Bartoli, Gibson, & Bray, 2005; Gargouri, Elbohli, & Maamouri, 2010; Radujkovic & Sundic, 2014; Munoz & Diaz, 2015).

To date, there have been many survey studies on digenean parasites in Turkey (Oğuz & Bray, 2006; Akmırza, 2013; Tepe, Oğuz, & Heckmann, 2014; Çınar, 2014; Öztürk & Özer, 2016; Öztürk & Güven,

2020). On the other hand, there are no previous studies on the digenean parasite fauna of labrid fish on the Black Sea coast of Turkey.

The aim of this research study is to investigate digenean parasites of four labrid fishes collected from the Sinop coast of the Black Sea. This study is the first survey on digenean parasites of the labrid fishes on the Turkish Black Sea coast.

MATERIALS AND METHODS

The present study was carried out between May 2015 and April 2017. The fish specimens were sampled with gill nets by fishermen from the Sinop coast of the Black Sea (42°01′ 55′ N, 35°16′ 36′ E). The caught fish were transported to the parasitology laboratory of the Faculty of Fisheries and Aquatic Sciences at Sinop University. A total of 52 specimens of the four labrid species, *Symphodus tinca* (27), *S. cinereus* (16), *S. roissali* (7) and *S. ocellatus* (2) were investigated for the digenean parasites. The fish were examined within 24 h of capture. At necropsy, the fishes were measured and weighed. Skin, fins, gills, eyes, brain, liver, stomach, intestine, kidney, urinary and gall bladder were examined under the dissecting microscope. The number of parasites was counted individually and the site of infection was recorded. Parasite specimens were studied in both alive and permanent preparations. For identification, the parasites were fixed in Bouin's fluid between slide and coverglass without pressure, stained with acetic carmine and mounted in Canada balsam. Permanent preparations were examined using a light microscope (Olympus microscope BX53) at magnification X10 and X100. The prevalence (P, %), mean intensity (MI), and abundance (A) values of each digenean parasites were calculated following the definitions of Bush, Lafferty, Lotz, & Shostak, (1997). The standard deviation (SD) of the mean intensity was calculated. The Kruskal-Wallis test (Nonparametric ANOVA) was performed to compare the mean intensity values of digenean parasites in each fish host. The analyses were carried out using the computer programmes GraphPad Instat 3.0. P-values less than 0.05 were considered to be significant. The significance of the digenean parasite fauna was determined by using an abundance (A) index according to the scale presented in Zander, Reimer, Barz, Dietel, & Strohbach, (2000), as follows; A>2: core species, A=0.6-2: secondary species, A=0.2-0.6: satellite species, A<0.2: rare species. The Czekanowski–Sørensen Index (ICS, %) was used to compare the digenean faunas of four *Symphodus* spp. (Sørensen, 1948).

RESULTS AND DISCUSSION

In the present study, a total of eight digenean parasite species including adults of *Helicometra fasciata, Gaevskajatrema perezi, Proctoeces maculatus, Phyllodistomum crenilabri* and metacercariae of *Galactosomum lacteum, Condylocotyle pilodora, Metadena pauli* and Opecoelidae gen. sp. were determined in four labrid fish (Table 1 and Figure 1).

Table 2 summarises the digenean parasite list with indications of prevalence (%), mean intensity (MI), and abundance (A) values of identified digenean parasites in their respective fish hosts. Our results showed that *S. tinca* has the richest digenean fauna with 7 digenea species, but *S. ocellatus* was infected with only 2 digenea species (Table 2). In other words, the most diverse digenean fauna was found in *S. tinca*; *S. ocellatus* showed a low diversity with 2 species (Table 2). In this study, the parasite species richness observed in labrid fish may be related to sampling effort. Many more specimens of *S. tinca* (27 individuals) were examined, compared to *S. ocellatus* (2 individuals).

The overall mean intensity values of digenea parasite species varied significantly among the four labrid fish ($P<0.05$). Overall, the highest prevalence and mean intensity values (100% and 73.86) were found in *S. roissali*, which was infected with 4 digenea species. *Galactosomum lacteum* occurred only in *S. tinca* and *Condylocotyle pilodora* occurred only in *S. cinereus (*Table 2).

Metadena pauli was observed as a core species, but *G. lacteum* and *C. pilodora* were found as rare species (Table 2). *Helicometra fasciata* and *G. perezi* played the role of core in *S.tinca* and *S. roissali. Proctoeces maculatus* played the role of core, satellite or

Table 1. List of digenean parasite species identified in four labrid fish in the present study and their infection site (microhabitat)

Family	Digenean parasite species	hosts	Infection site (microhabitat)
Opecoelidae	*Helicometra fasciata* Rudolphi, 1819	*S. tinca, S. roissali*	intestine
	Opecoelidae gen. sp. (met.)	*S. tinca, S. roissali*	gills
	Gaevskajatrema perezi (Mathias, 1926)	*S. tinca, S. roissali, S. cinereus*	intestine
Fellodistomidae	*Proctoeces maculatus* (Looss, 1901), Odhner, 1911	*S. tinca, S. roissali, S. cinereus*	intestine
Gorgoderidae	*Phyllodistomum crenilabri* Dolgikh & Naidenova, 1968	*S. tinca, S. ocellatus*	urinary bladder
Heterophyidae	*Galactosomum lacteum* Jägerskiöld, 1896, (met.)	*S. tinca*	gills
	Condylocotyle pilodora Pearson & Prevot, 1805 (met.)	*S. cinereus*	brain
Cryptogonimidae	*Metadena pauli* (Vlasenko, 1931) Yamaguti, 1958 (met.)	*S. tinca, S. roissali, S. ocellatus*	gills, eyes

(met.: metacercariae)

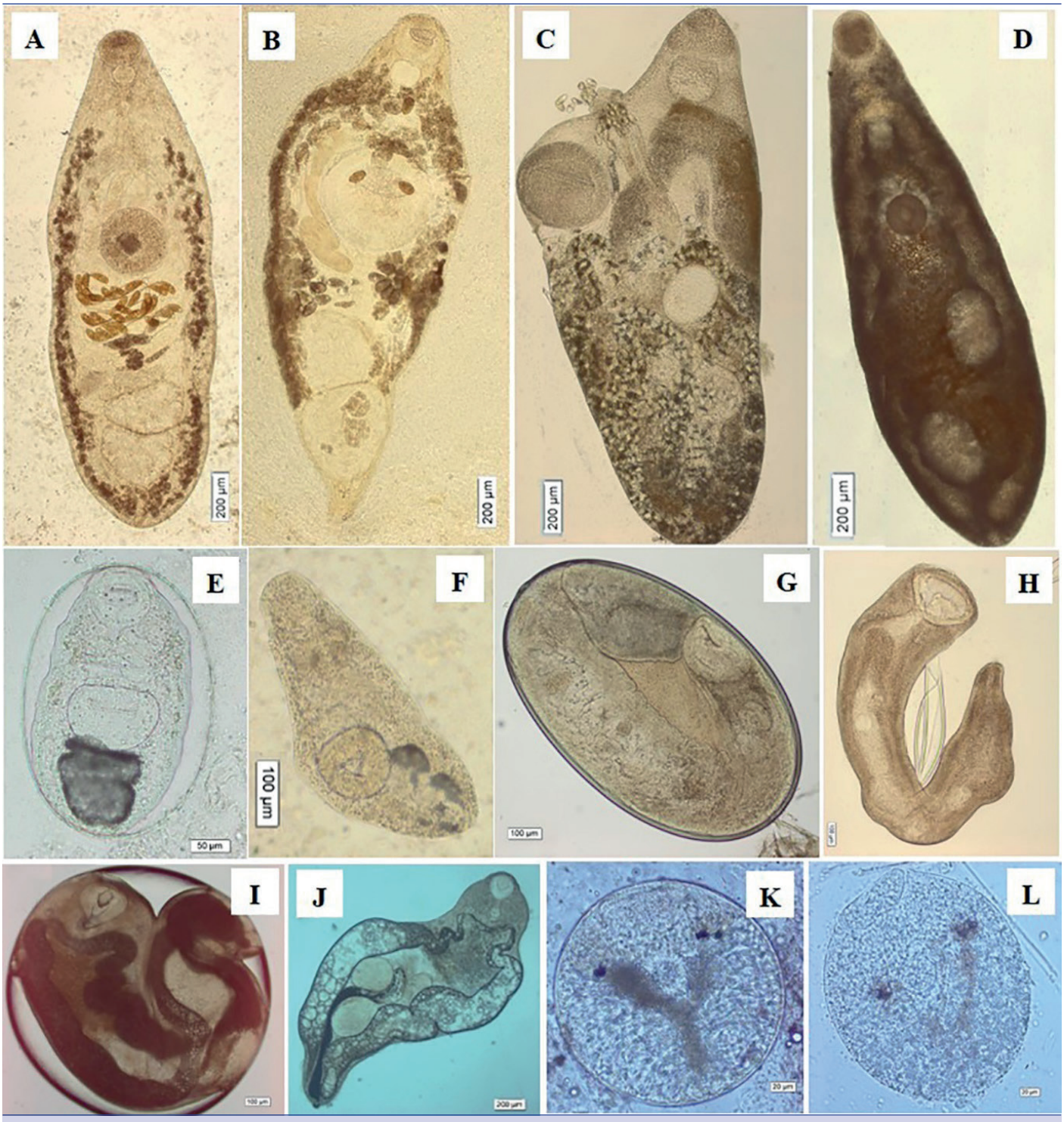


Figure 1. Digenean parasites identified in four labrid fish in this study (original), **A)** *Helicometra fasciata*, **B)** *Gaevskajatrema perezi*, **C)** *Proctoeces maculatus*, **D)** *Phyllodistomum crenilabri*, **E)** encysted metacercariae of Opecoelidae gen. sp., **F)** excysted metacercariae of Opecoelidae gen. sp. **G**) encysted metacercariae of *Condocotyla pilodora*, **H)** excysted metacercariae of *C. pilodora*, **I)** encysted metacercariae of *Galactosomum lacteum*, **J)** excysted metacercariae of *G. lacteum*, **K)** encysted metacercariae of *Metadena pauli*, **L)** excysted metacercariae of *M. pauli*.

Table 2. Overall infection prevalence (%), mean intensity (MI), and abundance (A) values of digenean parasite species from four labrid fishes in the present study

	Symphodus tinca (n=27)			*Symphodus roissali* (n=7)			*Symphodus cinereus* (n=16)			*Symphodus ocellatus* (n=2)*		
Digenean species	**P**	**MI±SD**	**A**	**P**	**MI±SD**	**A**	**P**	**MI±SD**	**A**	**P**	**MI±SD**	**A**
Helicometra fasciata	37.5	9.22±6.78	**2.07**	85.7	5.60±7.55	**4.86**						
Opecoelidae gen. sp. met.	37.5	2.30±1.00	1.02	85.7	3.00±0.00	0.43						
Gaevskajatrema perezi	22.2	34.00±15.2	**7.55**	14.3	105.00±0.00	**15.0**	18.8	11.5±9.85	1.43			
Proctoeces maculatus	11.1	3.67±2.51	0.41	42.8	90.67±15.36	**38.9**	6.25	1.00±0.00	0.06			
Phyllodistomum crenilabri	44.4	10.75±14.5	**4.78**							50.0	2.00±0.00	1.00
Metadena pauli met.	33.3	8.67±6.34	**2.89**	14.3	115.00±9.89	**32.9**				100	12.5±2.12	**12.5**
Galactosomum lacteum met.	3.7	1.00±0.00	0.04									
Condylocotyle pilodora met.							6.25	1.00±0.00	0.06			
Total	81.5	21.27±17.6[a]	17.33	100	73.86±59.82[b]	73.9	18.8	8.33±7.95[a]	1.56	100	13.5±0.71	13.5

Means followed by the same superscript letter are not significantly different ($P < 0.05$). * too few values for statistical analysis; (n: number of examined fish, S.D.: standard deviation, met.: metacercariae)

rare parasite depending on the host species. *Phyllodistomum crenilabri* was core in *S. tinca*, but secondary in *S. ocellatus* (Table 2). Moreover, all digenean species found in *S. roissali* were determined as core species (Table 2).

The digenean species composition and the digenean species richness of the four labrid fishes were found to be different in the present study. A close similarity was observed in the digenea fauna of *S. tinca* and *S. roissali* (ICS = 80.0%) (Table 3). In this study, it was observed that the similarity among the digenean parasite fauna of the *Symphodus* species was low in general. Moreover, a similarity was not detected in digenea parasite fauna of two of the labrid fish, *S. cinereus* and *S. ocellatus* (Table 3).

Table 3. Czekanowski-Sorensen Index (%) in digenean fauna of four labrid fish in the study area

	S. tinca	***S. roissali***	***S. cinereus***	***S. ocellatus***
S. tinca	100			
S. roissali	80.0	100		
S. cinereus	44.4	57.1	100	
S. ocellatus	40.0	33.3	0.0	100

A high Czekanowski–Sorensen index, indicating a close similarity, was observed in the digenean parasite fauna of the *S. tinca* and *S. roissali* (80%). But the digenea fauna of *S. cinereus* and *S. ocellatus* were not similar in the present study (Table 3). This may be related to the diet of the labrid hosts. The composition of the parasite fauna depends on many factors. The type of diet is one of them. This may be related with the diet of the labrid hosts. *S. cinereus* is carnivorous with a slight tendency toward omnivory, mostly consuming polychaetes, foraminiferans and decopod non-crustaceans (Fernandez, Freire, & Gonzalez-Gurriaran, 1995). Whereas *S. ocellatus* is omnivorous, tending toward herbivory, mostly consume algae, and to a lesser extent bryozoans and hydroids (Kabasakal, 2001).

According to previous studies, it should be considered that the majority of the listed digenea species have been reported from *S. tinca* (Table 4).

Labridae is one of the most important families of marine fish with its biological diversity and ecological importance, and labrid fishes have distribution worldwide (Nelson, 2006). To date, a total of 134 records of digenean parasites have been enlisted from 127 labrid fish (Munoz & Diaz, 2015). So far, 35 nominal digenean species have been reported in the four labrid fishes in the Mediterranean basin and Black Sea according to data of various authors (Table 4). Four species, *H. fasciata*, *P.maculatus*, *P. acceptum* and *G. perezi*, are typical digenean parasites of the labrid fishes and have also been reported in both basins (Table 4). Considering the number of digenea species reported in the four labrid fishes, except for the digenea species reported in both basins, it is noteworthy that the species diversity in the Mediterranean basin (19 species) is higher than from the Black Sea (9 species) (Table 4). The difference in di-

Table 4. List of the digenean parasites reported by various authors in four labrid fish. [Reports in the Black Sea are shown with square brackets]

Digenean Species	*Symphodus tinca*	*Symphodus roissali*	*Symphodus cinereus*	*Symphodus ocellatus*
Skrjabiniella aculeata	6, 12			
Prosorhynchus crucibulum	12			
Metadena depressa	12			
Metadena pauli	[18], **[PS]**	[18], **[PS]**	[18]	[18], **[PS]**
Proctoeces maculatus	10, 12, 16, [18], 19, 21, 22, **[PS]**	[5], [18], **[PS]**	[18], 22, **[PS]**	[5], 14, [18]
Steringotrema pagelli	22		22	
Tergestia laticollis	22		[3], 22	
Theledera skrjabini	[1]		[18]	
Phyllodistomum acceptum	[4], 12, [18]	[18]	[18], 22	[18]
Phyllodistomum crenilabri	[2], [18], **[PS]**		[18]	[18], **[PS]**
Lecithochirium musculus	22		19, 22	
Lecithochirium rufoviride	12	6		
Synaptobothrium caudiporum	6	6		
Lecithaster gibbosus			3, 22	
Lecithaster stellatus				14, 16, 17, 19
Holorchis micracanthum		16		
Holorchis pycnoporus		13, 19		14
Lepidauchen stenostoma	8, 10, 12			
Centroderma spinosissimum	3			
Monorchis monorchis	[4]			
Genitocotyle mediterranea				14, 16, 17, 19
Gaevskajatrema perezi	[9], [18], 12, **[PS]**	16, [18], 19, **[PS]**	16, [18], 19 **[PS]**	19
Helicometra fasciata	10, 12, [15], 16, [18], 21, **[PS]**	3, 16, [18], **[PS]**	3, 11, [18]	14, 16, [18]
Helicometra pulchella	21		11, [18]	[18]
Macvicaria alacris	16, 19, 20, 21		16, 19, 22	14, 16, 17, 19
Peracreadium idoneum	22		3, 22	
Peracreadium sp.	[4]	[4]		
Gaevskajatrema pontica	[4]	[4]		
Caudotestis skrjabini	[4]			
Caudotestis trachuri	[4]	[4]		
Deretrema scorpaenicola				19
Diphtherostomum brusinae	8, 10, 12, 22	7	7	
Zoogonus rubellus	10, 12, 19, 21, 22			
Galactosomum lacteum	[18], **[PS]**	[18]	[18]	[18]
Cardiocephaloides longicollis				[18]
Condocotyla pilodora			**[PS]**	
Number of species	**28**	**14**	**16**	**14**

[1]. Koval & Tsarychkova (1964), [2]. Dolgikh & Naidenova (1968), 3. Sey (1970), [4]. Nikolaeva & Solonchenko (1970), [5]. Parukhin, Naidenova, & Nikolaeva, (1971), 6. Papoutsoglou (1976), 7. Bray & Gibson (1986), 8. Orecchia, Paggi, & Radujkovic, (1988), [9]. Gaevskaya & Solonchenko (1989), 10. Campos, Carbonell, & Pellicer, (1990), 11. Reversat & Silan (1993), 12. Campos & Carbonell (1994), 13. Bartoli & Bray (1996), 14. Bartoli & Boudouresque (1997), [15]. Korniychuk & Gaevskaya (1999), 6. Sasal, Niquil, & Bartoli, (1999), 17. Bartoli, Morand. Riutort, & Combes, (2000), [18]. Korniychuck (2001), 19. Bartoli, Gibson, & Bray, (2005), 20. Oğuz & Bray (2006), 21. Gargouri, Elbohli, & Maamouri, (2010), 22. Radujković & Šundic (2014), **[PS]**. Present Study.

versity of digenean parasites reported from the Mediterranean basin and Black Sea may be related to the number of the intermediate hosts and the variation in physical and chemical parameters of the environment that can influence the host. So far, *Condylocotyle pilodora* has not been previously recorded in labrid fishes. It is remarkable that this digenean parasite is reported for the first time in labrid fish in the present investigation (Table 4). Thus, the number of digenean parasite species in the four labrid fishes reported in both basins has increased to 36 with the addition of *C. pilodora*.

CONCLUSIONS

This study revealed the first data on the digenean fauna of four fish species belonging to *Sypmhodus* genera in Turkish waters, and the data recovered in this paper contribute to the digenean parasites list of labrid fish inhabiting the Black Sea. In addition, these results contain some valuable knowledge, which can be used in future digenean parasite research.

Conflict of interest: The authors declare that they have no conflicts of interest.

Ethics Committee Approval: All applicable international, national and institutional guidelines for the care and use of animals were followed. The study protocol no. 19 of 13/07/2015 was approved by the Republic of Turkey, Sinop University Experimental Animals Local Ethics committee.

Acknowledgements: Authors are grateful to the Turkish Scientific and Technological Council (TUBİTAK) for financial support. Some parts of this study were previously orally presented in an international symposium.

Financial disclosure: This study contains a part of the project that was supported financially by the Turkish Scientific and Technological Council (TUBİTAK) with number of 215O224.

REFERENCES

Akmirza, A. (2013). Digenean trematodes of fish in the waters off Gökceada, the Aegean Sea, Turkey. *Journal of the Black Sea / Mediterranean Environment,* 19(3), 283–298. [CrossRef]

Bartoli, P. & Bray, R.A. (1996). Description of three species of *Holorchis* Stossich, 1901 (Digenea: Lepocreadiidae) from marine fishes off Corsica. *Systematic Parasitology, 35*(2), 133-143. [CrossRef]

Bartoli, P. & Boudouresque, C.F. (1997). Transmission failure of parasites (Digenea) in sites colonized by the recently introduced invasive alga *Caulerpa taxifolia*. *Marine Ecology Progress Series,* 154, 253-260. [CrossRef]

Bartoli, P., Morand. S., Riutort, J.J. & Combes, C. (2000). Acquisition of parasites correlated with social rank and behavioural changes in fish species. *Journal of Helminthology, 74*(4), 289-293. [CrossRef]

Bartoli, P., Gibson, D.I. & Bray, R.A. (2005). Digenean species diversity in teleost fish from a nature reserve of Corsica, France (Western Mediterranean), and a comparison with other Mediterranean regions. *Journal of Natural History, 39*(1), 47-70. [CrossRef]

Bilecenoğlu, M., Kaya, M., Cihangir, B. & Çiçek, E. (2015). An updated checklist of the marine fishes of Turkey. *Turkish Journal of Zoology, 38*, 901-929. [CrossRef]

Bray, R.A. & Gibson, D.I. (1986). The Zoogonidae (Digenea) of fishes from the north-east Atlantic. *Bulletin of the British Museum (Natural History) Zoology, 51*, 127-206. [CrossRef]

Bush, A.O., Lafferty, K.D., Lotz, J.M. & Shostak, A.W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Parasitology, 83*, 575-583. [CrossRef]

Campos, A., Carbonell, E. & Pellicer, M. (1990). Helmintofauna de *Symphodus tinca* (L.) y *Labrus merula* (L.) (Pisces: Labridae) del litoral Valenciano. 1. Trematoda. *Revista Iberica de Parasitologia, 50*(1-2), 37-42.

Campos, A. & Carbonell, E. (1994). Parasite community diversity in two Mediterranean labrid fishes *Symphodus tinca* and *Labrus merula*. *Journal of Fish Biology, 44*(3), 409-413. [CrossRef]

Choat, J.H. & Bellwood, D.R. (1998). Wrasses and parrotfishes. In: Paxton, J.R. and Eschmeyer, W.N. (Eds), *Encyclopedia of fishes*, (pp. 211-115). San Diego, Calif: Academic Press. ISBN: 0125476655 9780125476652

Çınar, M.E. (2014). Checklist of the phyla Platyhelminthes, Xenacoelomorpha, Nematoda, Acanthocephala, Myxozoa, Tardigrada, Cephalorhyncha, Nemertea, Echiura, Brachiopoda, Phoronida, Chaetognatha, and Chordata (Tunicata, Cephalochordata, and Hemichordata) from the coasts of Turkey. *Turkish Journal of Zoology, 38*(6), 698-722. [CrossRef]

Dolgikh, A.V. & Naidenova, N.N. (1968). Some comments to trematodes of the family Gorgoderidae with description of a new species. *Zoologicheskii Zhurnal, 47*, 1717-1719 [In Russian].

Fernandez, L., Freire, J. & Gonzalez-Gurriaran, E. (1995). Diel feding activity of demersal fishes in the Ria de Arousa (Galicia, NW Spain): an area of intense mussel raft culture. *Cahiers de Biologie Marine, 36*(2), 141-151.

Gaevskaya, A.V. & Solonchenko, A.I. (1989). New data on the trematodes from the Black Sea fishes. *Biologicheskie Nauki, 5*, 43-47 [In Russian].

Gargouri, L.B.A., Elbohli, S. & Maamouri, F. (2010). Digenean diversity in labrid fish from the Bay of Bizerte in Tunisia. *Journal of Helminthology, 84*(1), 27–33. [CrossRef]

Hanel, R., Westneat, M.W. & Sturmbauer, C. (2002). Phylogenetic relationships, evolution of broodcare behavior, and geographic speciation in the wrasse tribe Labrini. *Journal of Molecular Evolution, 55*(6), 776–789. [CrossRef]

Kabasakal, H. (2001). Description of the feeding morphology and the food habits of four sympatric labrids (Perciformes, Labridae) from south-eastern Aegean Sea, Turkey. *Netherlands Journal of Zoology, 51*(4), 439-455. [CrossRef]

Korniychuk, J.M. & Gaevskaya, A.V. (1999). Reproductive strategy of trematode, *Helicometra fasciata* (Trematoda: Opecoelidae) as an index of favourability of its environment. *Ekologiya Morya, 48,* 42-47.

Korniychuck, J.M. (2001). Structure of the trematode fauna of the Black Sea labrid fishes (Pisces: Labridae). *Ekologiya Morya, 59,* 21-22.

Koval, V.P. & Tsarychkova, D.B. (1964). A study of the trematodes of fishes from the Black Sea. *Visnyk Kyyivs Koho Universytetu Seriya Biolohiyi, 6*, 141-146.

Munoz, G. & Diaz, P.E. (2015). Checklist of parasites of labrid fishes (Pisces: Labridae). Viña del Mar, Chile.

Nelson, J.S. (2006). *Fishes of the World* (4th Ed). John Wiley and Sons, USA. 601 p. ISBN: 0471250317

Nikolaeva, V.M. & Solonchenko, A.I. (1970). Helminth fauna of some benthopelagic fish in the Black Sea. *Biologiya. Morya, 20*, 129-166 [In Russian].

Oğuz, M.C. & Bray, R.A. (2006). Digenetic trematodes of some teleost fish off the Mudanya Coast (Sea of Marmara, Turkey). *Helminthologia, 43*(3), 161-167. [CrossRef]

Orecchia, P., Paggi, L., & Radujkovic, B.M. (1988). Digeneans of fishes from the Adriatic Sea with a description of *Lecithaster atherinae* n. sp. from *Atherina (Hepsetia) boyeri*. *Parassitologia, 30*, 225-229.

Öztürk, T. & Özer, A. (2016). Digenean parasites of Atlantic horse mackerel (*Trachurus trachurus*) in the Turkish Black Sea coast. *Ege Journal of Fisheries and Aquatic Sciences, 33*(1), 35-40. [CrossRef]

Öztürk, T. & Güven, A. (2020). New Data on Digenean Parasites of Rusty Blenny, *Parablennius sanguinolentus* (Pallas, 1814) in the Black Sea. *Sinop University Journal of Natural Sciences, 5*(1), 26-37. [CrossRef]

Papoutsoglou, S.E. (1976). Metazoan parasites of fishes from Saronicos Gulf Athens-Greece. *Thalassographica,* 1, 69-102.

Parukhin, A.M., Naidenova, N.N. & Nikolaeva, V.M. (1971). The parasite fauna of fishes caught in the Mediterranean Sea. In: Vodjanichky VA (ed). Expeditionary investigations in the Mediterranean Sea in May-July 1970 (A. Kovalevsky), pp. 64-87. Naukova Dumka, Russia.

Radujković, B.M. & Śundic, D. (2014). Parasitic flatworms (Platyhelminthes: Monogenea, Digenea, Cestoda) of fish from Adriatic Sea. *Natura Montenegrina, 13*(1), 7-280.

Reversat, J. & Silan, P. (1993). Comparative population biology of fish digenea: the case of three *Helicometra* (Trematoda: Opecoelidae) mesoparasites of marine teleosts in a Mediterranean lagoon. *Annales de Parasitologie Humaine et Comparee, 68*(3), 128-135. [CrossRef]

Sasal, P., Niquil, N. & Bartoli, P. (1999). Community structure of digenean parasites of sparid and labrid fishes of the Mediterranean Sea: a new approach. *Parasitology, 119*(6), 635-648. [CrossRef]

Sey, O. (1970). Parasitic helminths occurring in Adriatic fishes, part II (Flukes and Tapeworms). *ACTA Adriatica. 13*, 3-15.

Sorensen, T.A. (1948). A new method of establishing groups of equal amplitude in plant sociology based on similarity of species content and its application to analysis of vegetation on danish commons. *Biologiske Skrifter, 5*, 1-34.

Tepe, Y., Oğuz, M.C. & Heckmann, R.A. (2014). Digenean and cestode parasites of teleost fish from the Eastern Black Sea Region. *Turkish Journal of Zoology, 38*(2), 209-215. [CrossRef]

Zander, C.D., Reimer, L.W., Barz, K., Dietel, G. & Strohbach, U. (2000). Parasite communities of the Salzhaff (northwest Mecklenberg, Baltic Sea). II. Guild communities, with special regard to snails, benthic crustaceans, and small-sized fish. *Parasitology Research, 86*(5), 359–372. [CrossRef]

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2021; 36(3): 133-139 • DOI: https://doi.org/10.26650/ASE2020823908

Original Article

Catch Performance of Deep Water Cast Nets Used for Whiting along the Turkish Coast of the Black Sea (Turkey)

Uğur Karadurmuş[1], Ertuğ Düzgüneş[2], Mehmet Aydın[3]

Cite this article as: Karadurmus, U., Duzgunes, E., & Aydin, M. (2021). Catch performance of deep water cast nets used for whiting along the Turkish coast of the Black Sea (Turkey). *Aquatic Sciences and Engineering, 36*(3), 133-139.

ABSTRACT

Deep water cast net fishing is regarded as a traditional method that has been used since antiquity in the Eastern part of the Black Sea. Unlike ordinary cast nets, it is used in deep waters from shallow up to 140 meters for catching whiting (*Merlangius merlangus* euxinus). In this study, important findings were obtained about the operational success and duration, catch efficiency and composition of traditional deep water cast nets. The highest operation success rate was obtained from 60 to 120 meter depth with 84.2%. The highest mean CPUE was established at 0.42 kg/operation in August. The catch rate of whiting, the main target species of the study, of the total catch was calculated as 98.8%. In this study, the effect of deep water cast nets on the ecosystem was also investigated. According to the findings, fishing season and operation depth significantly affect the catch per unit effort. In terms of ecosystem-based fisheries management due to high selectivity, the low impact of ghost fishing and high survival rate of the individuals, the deep water cast net fishing was found to be beneficial. It is recommended that the performance of deep water cast nets on different species in different seas should be investigated.

Keywords: Cast net, fisheries, Black Sea, Whiting, *Merlangius merlangus* euxinus

ORCID IDs of the author:
U.K. 0000-0002-5827-0404;
E.D. 0000-0001-5456-0925;
M.A. 0000-0003-1163-6461

[1]Bandırma Onyedi Eylul University, Maritime Vocational School, Bandırma, Balıkesir, Turkey

[2]Black Sea Technical University, Faculty of Marine Sciences, Trabzon, Turkey

[3]Ordu University, Faculty of Marine Sciences, Ordu, Turkey

Submitted:
10.11.2020

Revision Requested:
28.11.2020

Last Revision Received:
14.12.2020

Accepted:
14.12.2020

Online Published:
08.04.2021

Correspondence:
Uğur Karadurmuş
E-mail:
ukaradurmus@bandirma.edu.tr

INTRODUCTION

Cast nets have been used to collect fish in shallow water canals surrounding marshes and to collect nearshore lake species (Meador & Kelso, 1990). The cast net covers a large area per deployment and large areas can be sampled quickly (Emmanuel, Chukwu, & Azeez, 2008). The usage and purpose of cast nets vary according to the region and species (Türkmen, & Akyurt, 2000; Smith, Tun, Chid, Winb, & Moeb, 2009; Zappes, Andriolo, Simoes Lopes, & Beneditto, 2011; Stein, Smith, & Smith, 2014). The Eastern Black Sea has been the most important region for whiting fisheries in Turkey where more than 46% of the total whiting (*Merlangius merlangus*) production was obtained from this region (Turkish Statistical Institute, 2019). Gillnets, hand lines and deep water cast nets are used for whiting fisheries in the Eastern Black Sea which is closed to trawling.

Deep-water cast net fishing is regarded as a traditional method of catching whiting that has been used since antiquity in the Black Sea. Deep water cast nets are released from the boat into the sea and down to the bottom with spread out like a parachute (Karadurmuş, Düzgüneş, & Aydın, 2020). There are a few studies on deep water cast nets in the literature. Emanet & Ayaz (2018) carried out surveys about catch efficiency with deep cast nets used for whiting on the coast of Sürmene.

Some fish stocks around the world, including Turkey have been damaged, some have been over-exploited and others have been completely destroyed (Vasilakopoulos, Maravelias, & Tserpes, 2014; Tsikliras, Dinouli, Tsiros, & Tsalkou, 2015; Demirel, Zengin, & Ulman, 2020). Despite the implementation of policies and limitations, fishermen`s desire to obtain more prey by using existing

technology makes the measures inadequate. With damage to pelagic stocks, fishermen have turned to demersal stocks in recent years (Avşar, 2005). Demersal stocks, which are already limited, have also begun to be over-exploited. Therefore, discovery or development of alternative fishing gear and fishing methods is important in terms of sustainable fishing. In this study, it was aimed to determine the catch performance of traditional deep-water cast nets used for whiting fishing in the Black Sea of the Turkish coast.

MATERIALS AND METHODS

This study was carried out in the Eastern Black Sea region (Figure 1) during the July 2016-June 2017 period by monthly samplings. The sampling operations were made from shallow to deep waters by the traditional deep-water cast nets. The operations were carried out by a small fishing boat, which is named 'Gürşen' with a length of 8 m and width of 3 m. Standard traditional nets were used in the operations with a 14 mm mesh size and 190 meshes along the depth. The circumference of the net mouth was 18.6 m and the area of the net mouth was 27.5 m^2. An area approximately 20-30 m^2 was covered in each operation.

The stations selected were from 40-60 m (D_1), 60-80 m (D_2), 80-100 m (D_3), 100-120 m (D_4), 120-140 m (D_5) depth and these stations were defined as *internal depth contours*. The stations with shallow depths of less than 40 m (D_-) and deeper depths of more than 140 m (D_+) were defined as *external depth contours*. A total of 994 operations were carried out in 52 days throughout the year. The number of operations performed according to depth contours and months is given in Table 1. Operation details (date, operation duration, depth etc.) and meteorological conditions (intensity of wind, wave and flow) were noted on data forms daily.

During the study, a total of 4,860 specimens were examined, which included 4,804 whiting. Length frequencies of individuals were measured on the boat to the nearest 1 mm. All species were defined at the lowest possible taxonomic level and the catching quantities of each species were noted on the forms (Slastenenko, 1956; Mater, Kaya, & Bilecenoğlu, 1989; Bilecenoğlu, Taşkavak, Mater, & Kaya, 2002). Concerning the success rate of operations, when the fishing gear worked without any problems (no curling and tangling), the operation was defined as successful. The success rate of operations (OS) was calculated as follows,

OS (%) = [The number of successful operation / The number of total operation] x 100 (1)

Classification of total catch, targeted catch and bycatch were made according to the definitions given below;

- Total catch: All living and dead materials caught with the fishing gear

- Targeted Catch: Amount of a species over minimum landing size with certain fishing gear for a given fishery

- Bycatch: Amount of untargeted catch (discards due to any reason and undersized individuals of the targeted species).

In this study, whiting and the other commercial species were evaluated as target species excluding smaller individuals according to the legal length limit. The non-commercial species, undersized individuals of the targeted species and forbidden species were determined as non-target species. In terms of a fisheries management-based approach, protected species and smaller individuals than the legal length limit should be returned to the sea (Kasapoğlu & Düzgüneş, 2017).

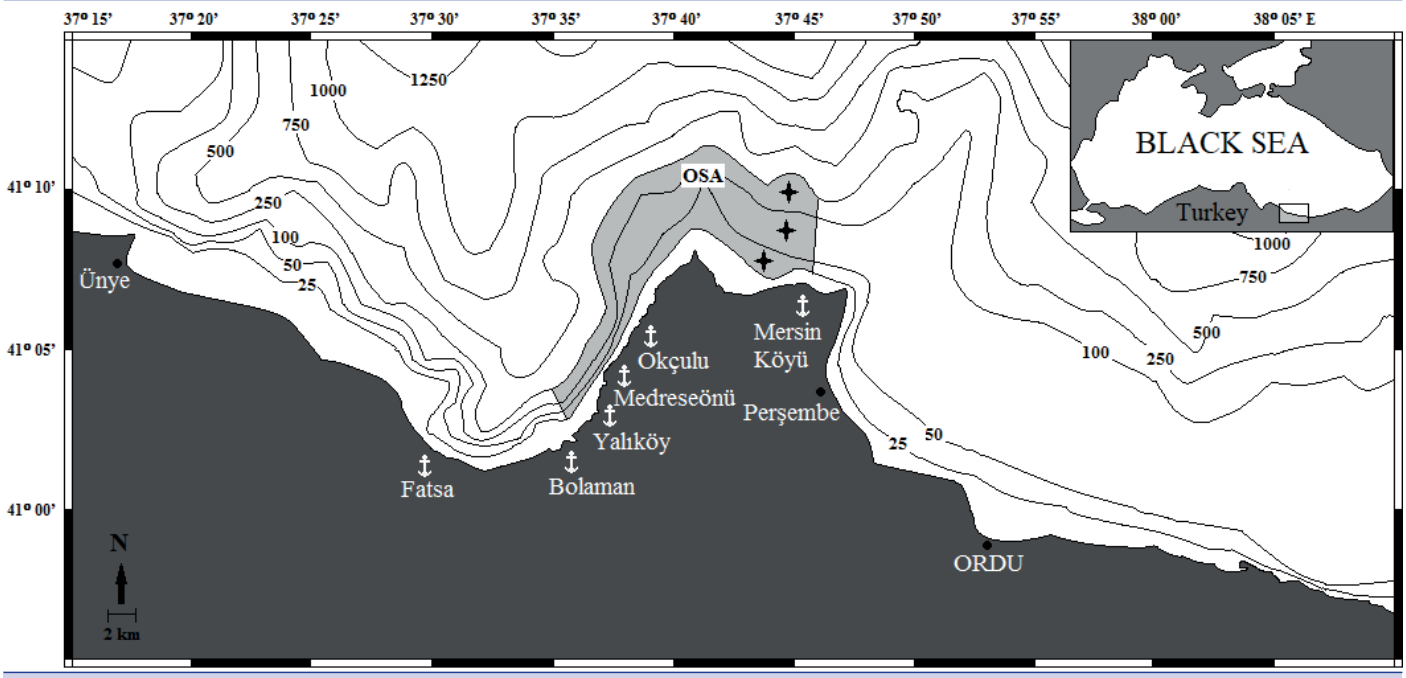


Figure 1. Map of the study area (Ordu Shelf Area).

Table 1. The number of operations performed according to depth contours and months

Months	Internal Depth Contours* (40 - 120 m)					External Depths Contours* (<40 m and >140 m)		Total
	D_1	D_2	D_3	D_4	D_5	D_-	D_+	
Jul.16	16	17	17	17	18	11	9	105
Aug.16	19	16	15	15	18	-	-	83
Sept.16	19	15	19	18	18	-	-	89
Oct.16	19	17	16	17	18	12	7	106
Nov.16	17	16	17	17	18	-	-	85
Dec.16	10	13	16	14	12	-	-	65
Jan.17	14	11	13	11	9	10	10	78
Feb.17	11	14	17	11	13	-	-	66
Mar.17	11	16	14	16	17	-	-	74
Apr.17	14	15	15	17	17	9	11	98
May.17	14	15	13	17	14	-	-	73
June.17	14	13	16	15	14	-	-	72
Total	178	178	188	185	186	42	37	994

*(D_-: <40 m, D_1: 40-60 m, D_2: 60-80 m, D_3: 80-100 m, D_4: 100-120 m, D_5: 120-140 m, D_+: >140)

Catch per Unit Effort (CPUE) for each species was calculated and standardized in kg per operation (Sparre & Venema, 1992) as follows;

$$CPUE = \sum C / n \qquad (2)$$

In this equation: ∑C: Quantity of catch in each operation (kg), n: Number of operations. The statistical analyses of the data were made using the SPSS package program 22.0 (Sokal & Rohlf, 1969; Düzgüneş, Kesici, & Gürbüz, 1983).

RESULTS AND DISCUSSION

Operation success

A total of 915 operations at the internal depth contour (40-120 m) and 79 operations at external depth contours (<40 m and >140 m) were performed throughout the year. The average operation success rate was determined as 78.3% (716 operations) at internal depth contours and as 40.5% (32 operations) at external depth contours. In 25.1% of successful operations (180 operations) where carried out at internal depth contours and 53% of successful operations (17 operations) where carried out at external depth contours where no catch was obtained (Figure 2).

The operation success rates of depth contours are given in Figure 3. The highest operation rate was obtained at D_2 depth contour (60-80 m) with 84.2%. The operation success rate was found to be low at external depth contours (<40 m and >140 m). It was determined that at depths deeper than 80 m, the operation success rate was decreased. The average operation success rate was calculated as 67%. Depth contours that obtain above the average success rate were defined as efficient. It was observed that sometimes the net mouth did not open when hauling the nets at shallow water operations (<40 m). That's way it is understood that shallow water did not have enough depth to open the gear. It was observed that the brail lines and sinker line were hanging out with each other due to increased operation duration in deeper water (>140 m). It was found that the relationship between operation depth and operation success rate was statistically significant ($P<0.05$).

The lowest operation success rate was obtained as 64.7% in January whereas the highest operation success rate was obtained as 88.9% in October. The average operation success rate performed at internal deep contour operations was calculated as 77.3%. Months that obtain above the average success rate were defined as efficient. The monthly variations in operation success rate are given in Figure 4. It was observed that the meteorological conditions (wind, wave and deep flow) affected the operation success rate and operation efficiency.

Operation duration

The duration of operation was defined as the time that the deep water cast net remained in the water. The duration of operation is given in Table 2. The operation duration changed between 4 and 29 minute in internal depth contours throughout the study. As the operation depth increased, the duration of operation increased. A high linear correlation was found between operation duration and operation depth (R: 0.915; $P<0.05$). Deep-water cast nets can be used with twin gear if more than one person is present on board. In this way, from 24 to 31 percent more operations can be performed per unit of time.

Meteorological conditions like wind and deep flow affected the operation duration. The length of pulling cord was changed according to the different meteorological conditions at same depth. The number of fishing days was determined by considering the operation success rate and observed meteorological conditions. It was determined that fishing activities with deep water cast nets could be conducted 195 days/year, in particular, fishing activities reached a peak in summer. Malkoç, Durukanoğlu, & Özer (1995) reported that the average wind speed was low in summer in the Eastern Black Sea. Their findings are support our findings.

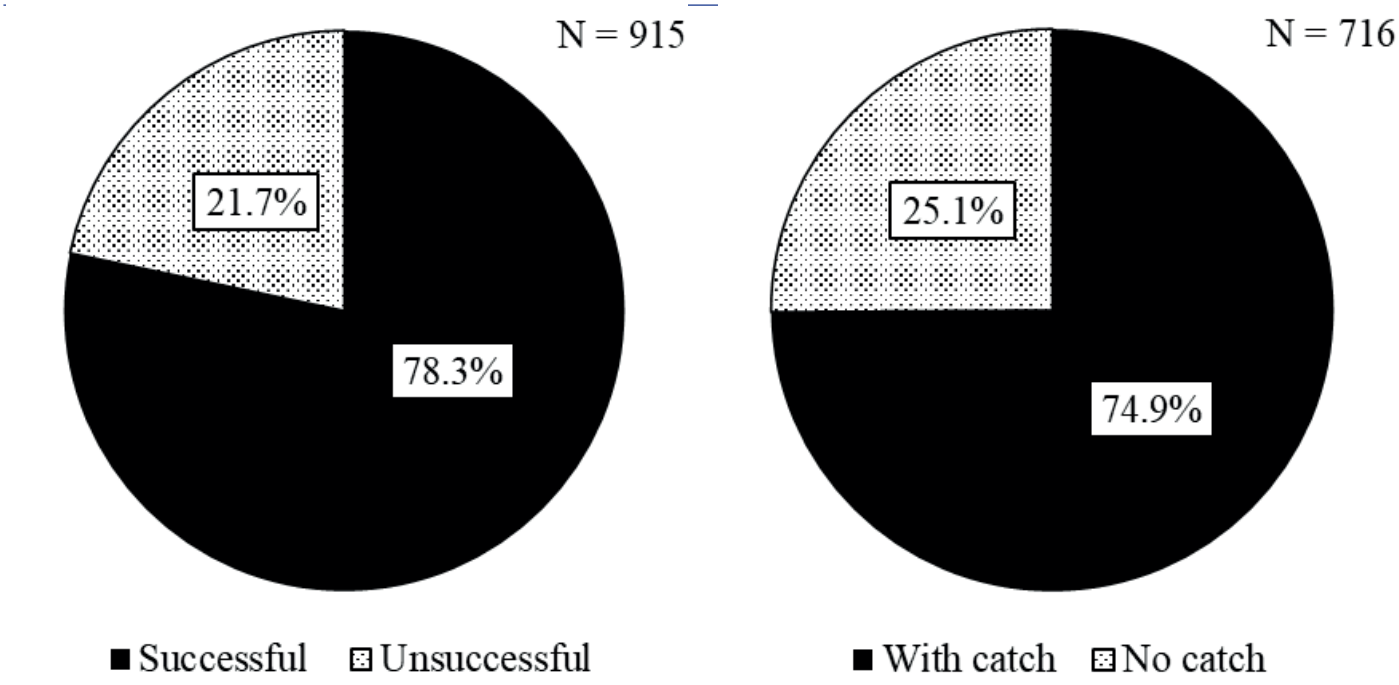


Figure 2. The operation success rate and capture rate in internal depth contours.

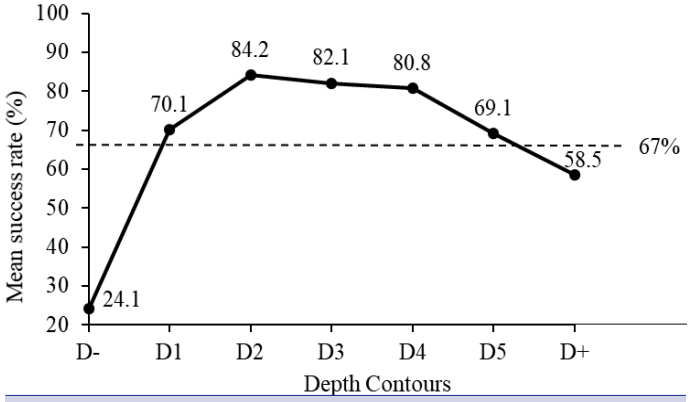


Figure 3. Change of mean operation success rate according to depth classes (D_-: <40 m, D_1: 40-60 m, D_2: 60-80 m, D_3: 80-100 m, D_4: 100-120 m, D_5: 120-140 m, D_+: >140).

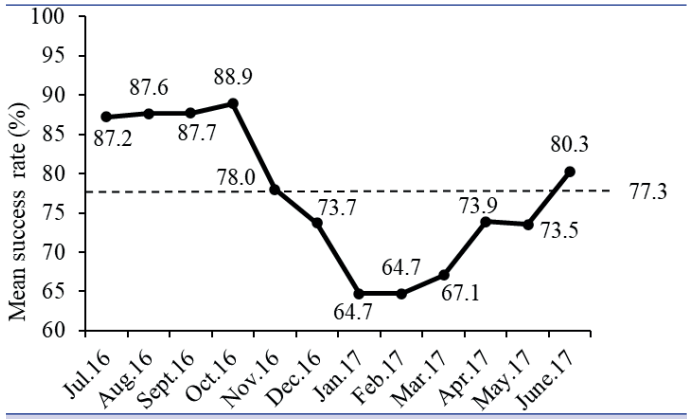


Figure 4. The monthly variations in mean operation success rate.

Table 2. Change of operation duration according to depth contour

	Depth Contours*				
	D_1	D_2	D_3	D_4	D_5
Mean ± S.E.	6.4 ± 0.1	10.1 ± 0.2	15.3 ± 0.1	18.7 ± 0.2	20.9 ± 0.2
Min.	4	7	11	10	16
Max.	9	16	20	27	29

*(D_-: <40 m, D_1: 40-60 m, D_2: 60-80 m, D_3: 80-100 m, D_4: 100-120 m, D_5: 120-140 m, D_+: >140)

Catch efficiency

During the study, a total of 137.9 kg of whiting was caught at internal depth contours throughout the year. The mean CPUE for all operations was calculated as 140 g/operation. Emanet & Ayaz (2018) reported the mean CPUE for deep water cast net fishing as 1.1 kg/operation on the Sürmene coast. The majority (86.7%) of individuals were captured between a depth of 60 and 120 meters. Most of the individuals, 47.5 kg (34.5%), were captured at D_2 depth contour (60-80 m). The mean CPUE values in different depth contours are given in Figure 5. The lowest and highest mean CPUE were established to be 0.04 kg/operation and 0.27 kg/operation at D5 and D2 depth contours, respectively. It was determined that differences among CPUE and operation depth were statistically significant ($P<0.05$). Approximately 0.5 kg of

whiting were caught in 32 successful operations performed at external depth contours (<40 m and >140 m). Although the amount of catch per operation seems to be low, it is possible to catch at a satisfactory level with repetitive operations in a day. The maximum catch in an operation was obtained in August with 15.2 kg. The lowest CPUE was established to be 0.0007 kg/operation in

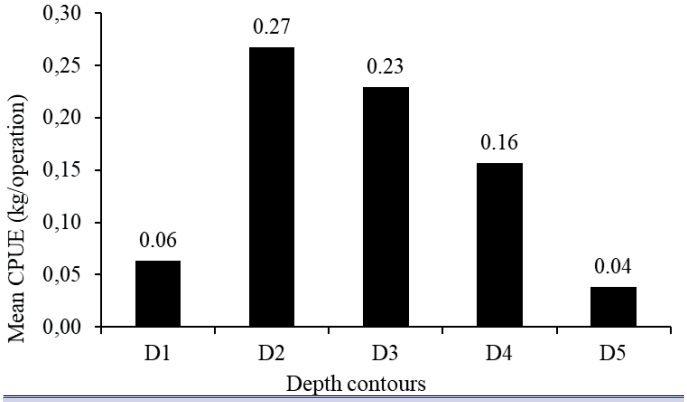


Figure 5. Change of mean CPUE values according to depth (D-: <40 m, D_1: 40-60 m, D_2: 60-80 m, D_3: 80-100 m, D_4: 100-120 m, D_5: 120-140 m, D_+: >140).

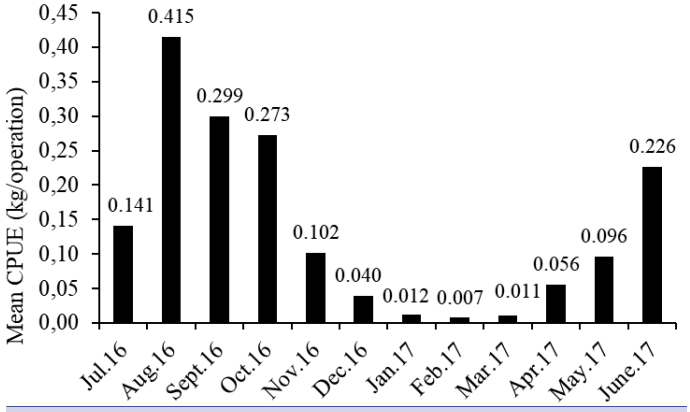


Figure 6. The monthly variations of mean CPUE values in internal depth contours.

February whereas the highest CPUE was 0.42 kg/operation in August. The majority of individuals, 74.4% (101 kg), were captured from June to October. The monthly variations in CPUE values were significantly different (*P<0.05*) (Figure 6).

Akşıray (1954) reported that whiting can exist up to a depth of 200 m in winter and in shallow waters up to 20 meters. In spite of the presence of whiting in external depth contours, the catch efficiency was found to be very low. Based on these findings, it is thought the fishing gear is not working in external depth contours. The success rate of operations would affect catching efficiency indirectly. It is thought that the high deep flows affect the mouth opening of the net and narrow the area of influence of the gear. In another study, it is stated that hydrographic factors like temperature, salinity and dissolved oxygen affected the distribution of the marine species (Uçal et al., 1986). It was determined that there was a strong relation between catch efficiency and the level of population to depth, operation success rate and hydrographic factors.

Length frequency disribution

In total of 2,950 whiting specimens were measured in the study period. The length of whiting changed between 5 and 24 cm. The majority of the population (70.6%) was composed of individuals in the 12-17 cm length group. The cumulative rate of individuals with a smaller than legal length limit ($L.L.L._{whiting}$ < 13 cm) was calculated as 22.4%. The length frequency distribution of the individuals is given in Figure 7. The abundance of whiting with a smaller than legal length limit were determined at D_1, D_2, D_3, D_4 and D_5 depth contours as 27.25%, 20.15%, 20.12%, 22.12% and 27.24%, respectively (Table 3.). Erdem (2018) reported that size at first maturity was esti-

Table 3. Rate of whiting with smaller than legal length limit according to depth contour

Depth Contours*	Length Groups	
	< 13 cm (%)	≥ 13 cm (%)
D_1	27.25	72.75
D_2	20.15	79.85
D_3	20.12	79.88
D_4	22.12	77.88
D_5	27.24	72.76

*(D_1: 40-60 m, D_2: 60-80 m, D_3: 80-100 m, D_4: 100-120 m, D_5: 120-140 m)

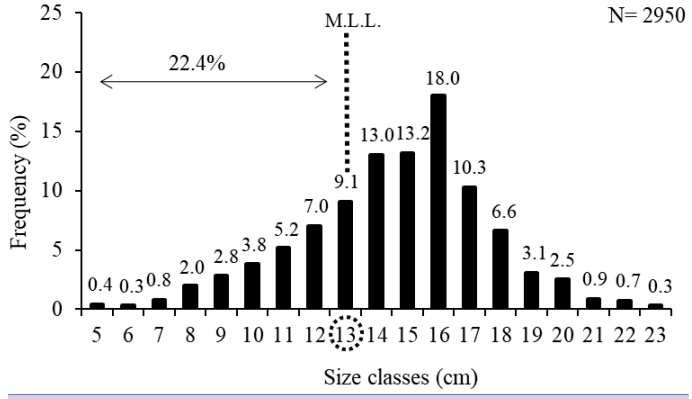


Figure 7. The distribution of length frequencies of the samples according to the size classes (L.L.L.: Legal length limit).

mated as 10.73 cm for females, 10.95 cm for males and 10.88 cm for all whiting specimens. Larger individuals were caught at D2 and D3 depth contours. From this point of view, it is seen that catching with deep-water cast nets is more beneficial at depths between 60 and 100 meter in terms of fisheries management.

Catch composition

During the study period, a total of 12 species belonging to 3 classes, 7 orders, and 12 families were determined in the deep

Table 4. Catch composition of the deep water cast net

Class order	Family	Species	N	N%	Status*
Gadiformes	Gadidae	*Merlangius merlangus euxinus*	4,804	98.85	MT
	Lotidae	*Gaidropsarus mediterraneus*	3	0.06	NT
Perciformes	Carangidae	*Trachurus mediterraneus*	21	0.43	T
	Trachinidae	*Trachinus draco*	7	0.14	NT
	Mullidae	*Mullus barbatus*	6	0.12	T
Neogastropoda	Muricidae	*Rapana venosa*	5	0.10	NT
Mytilida	Mytilidae	*Mytilus galloprovincialis*	3	0.06	NT
Syngnathiformes	Syngnathidae	*Hippocampus hippocampus*	3	0.06	NT
Pleuronectiformes	Soleidae	*Solea solea*	2	0.04	T
	Scophthalmidae	*Psetta maxima*	1	0.02	T
Scorpaeniformes	Scorpaenidae	*Scorpaena porcus*	3	0.06	T
	Triglidae	*Chelidonichthys lucerna*	2	0.04	T

*(N: number of caught individuals; N%: frequency; MT: Main target; T: Target; NT: Non-target)

water cast net. The catch rate of whiting, as the main target species of the study, in the total catch was calculated as 98.8% (N: 4884 individuals). The proportion of non-target species was 23.35% of the total catch and 22.4% of this rate consisted of small individuals (< 13 cm) of whiting. The catch composition of the deep-water cast net is given in Table 4.

The amount of non-target species caught was found to be very low. It is thought that the residence time in water and the narrow impact area of gear are determinative for low catch composition. Also, it is thought that other species, excluding whiting, had an opportunity to escape by seeing the net during the landing. It was observed that the captured individuals were not damaged and they were turned back to the water and survived. It was observed that the net or accessories were not lost or ruptured for any reason. The amount of catch, the species diversity and low impact of ghost fishing shows the benefit of the deep water cast nets in terms of ecosystem-based fisheries management.

CONCLUSIONS

Our study indicated that the mean CPUE of whiting showed a difference depending on the month of the fishing operation and operation depth contour class. When the operation success is low, the fishing gear does not work efficiently so this situation leads to loss of time, labor and fishing income. Meteorological conditions like wind and deep flow affected operation success and catch efficiency. The results showed that the deep-water cast net did not work effectively in shallow water less than 40 m and deeper water over 140 m but it worked better in depths from 60 to 120 meters. Also, the catch efficiency level was determined to be sufficient from April to November. In this period, fishermen can catch about 0.25 kg of whiting per operation. It was observed that the larger individuals can be caught at depths from 60 to 120 meters. The rate of whiting among all individuals was very high (98.85%) and the number of non-target species was found very low (23.35%). It is concluded that the deep water cast nets are beneficial for the ecosystem-based fisheries management due to high selectivity, less probability of ghost fishing and high survival rate of captured fish. New research studies should be performed on the selectivity and modification of fishing gear. This first specific snapshot regarding the deep-water cast net fishing benefits in Ordu can be a pathfinder for future studies in the Black Sea region.

Conflict of interests: The authors declare that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: Ethics committee approval was not required. All authors declare that this study does not include any experiments with human or animal subjects.

Funding: -

Acknowledgments: This article used data from a PhD thesis, which was completed by Uğur KARADURMUŞ.

Disclosure: -

REFERENCES

Avşar, D. (2005). *Balıkçılık biyolojisi ve populasyon dinamiği*. Adana: Nobel Kitabevi. ISBN: 9786059354264

Bilecenoğlu, M., Taşkavak, E., Mater, S. & Kaya, M. (2002). *Checklist of the marine fishes of Turkey*. Aucland: Magnolia Press. ISBN 1175-5326 [CrossRef]

Demirel, N., Zengin, M. & Ulman, A. (2020). First large-scale eastern Mediterranean and Black Sea stock assessment reveals a dramatic decline. *Frontiers in Marine Science*, [CrossRef]

Düzgüneş, O., Kesici, T. & Gürbüz, F. (1983). *Statistical methods*. Ankara: Ankara University Press. ISBN 1291

Emanet, M. & Ayaz, A. (2018). The catching efficiency of deep water cast net used for catching whiting (*Merlangius merlangus*) in Sürmene Coast, Trabzon (Black Sea) (In Turkish). *Ege Journal of Fisheries and Aquatic Sciences*, [CrossRef]

Emmanuel, E.B, Chukwu, L.O. & Azeez, L.O. (2008). Cast net design characteristics, catch composition and selectivity in tropical open lagoon Babatunde. *African Journal of Biotechnology*, [CrossRef]

Erdem, Y. (2018). Estimation of size at first maturity of Black Sea red mullet (*Mullus barbatus ponticus*). *Journal of Advances in VetBio Science and Techniques*, 3(2), 30-37. [CrossRef]

Karadurmuş, U., Düzgüneş, E. & Aydın, M. (2020). Identification of structural and operational characteristics of deep water cast nets

used for Whiting along the Turkish Coast of the Black Sea. *Turkish Journal of Maritime and Marine Sciences*, 6(1), 70-79.

Karadurmuş, U. (2019). Mezgit avcılığında kullanılan derin su serpmesinin yapısal-operasyonel özellikleri ve dip galsama ağları ile kıyaslanması (Doctoral dissertation). Retrieved from Turkish Council of Higher Education. (No: 543658)

Kasapoğlu, N. & Düzgüneş, E. (2017). The common problem in the Black Sea fisheries: By-catch and its effects on the fisheries economy. *Turkish Journal of Fisheries and Aquatic Sciences*, [CrossRef]

Malkoç, Y., Durukanoğlu, H.F., & Özer, F. (1995). Sea climate and aquatic products of the eastern Black Sea. *II. Su Ürünleri Sempozyumu*. Erzurum: Bildiriler Kitabı

Mater, S., Kaya, M. & Bilecenoğlu, M. (1989). *Türkiye deniz balıkları atlası*. Izmir: Ege Üniversity Press. ISBN 9789754835465

Meador, M.R. & Kelso, W.E. (1990). Physiological responses of Largemouth bass, *Micropterus salmoides*, exposed to salinity. *Canadian Journal of Fisheries and Aquatic Sciences*, [CrossRef]

Slastenenko, E. (1956). *Karadeniz havzası balıkları*. İstanbul: Et ve Balık Kurumu Umum Müdürlüğü Yayınları. Retrieved from https://www.zmo.org.tr/resimler/ekler/BALIK_BALIKCILIK/1968/68_02.pdf (accessed 08.10.20)

Smith, B.D., Tun, M.T., Chit, A.M., Winb, H. & Moeb, T. (2009). Catch composition and conservation management of a Human–dolphin cooperative cast-net fishery in the Ayeyarwady River, Myanmar. *Biological Conservation*, [CrossRef]

Sokal, R.R. & Rohlf, F.J. (1969). *Biometry. The principles and practises of in biological research*. San Francisco, William Freeman and Company. ISBN 0716724111

Sparre, P. & Venema, S.C. (1992). *Introduction to tropical fish stock assessment* (Report No. 306/1). Rome: Food and Agriculture Organization. ISBN 92-5-103996-8

Stein, W., Smith, P.W. & Smith, G. (2014). The cast net: An overlooked sampling gear. *Marine and Coastal Fisheries: Dynamics, Management and Ecosystem Science*, [CrossRef]

Tsikliras, A.C., Dinouli, A., Tsiros, V.Z. & Tsalkou, E. (2015). The Mediterranean and Black Sea fisheries at risk from overexploitation. *PloS one*, 10(3), e0121188. [CrossRef]

Turkish Statistical Institute. (01.12.2020). Fishery Statistics, 2015-219. Retrieved from https://biruni.tuik.gov.tr/medas/?kn=97&locale=tr (accessed 01.12.2020)

Türkmen, M. & Akyurt, İ. (2000). The population structure and growth properties of *Chalcalburnus mossulensis* (Heckel, 1843) caught from Aşkale Region of River Karasu. *Turkish Journal of Biology*, 24, 95-111.

Uçal, O., Cihangir, B., Tokaç, A., Önen, M., Tıraşın, M., Özkızılcık, S., Şıklar, K. & Samsaroğlu, M. (1986). *Orta Karadeniz (Sinop-Ünye) trol sahalarının hidrografisi ve verimliliği, Birinci dönem araştırmaları* (Report No. 185). İzmir: Dokuz Eylül Üniversitesi Deniz Bilimleri Teknolojisi Enstitüsü.

Vasilakopoulos, P., Maravelias, C.D. & Tserpes, G. (2014). The alarming decline of Mediterranean fish stocks. *Current Biology,* [CrossRef]

Zappes, C.A., Andriolo, A., Simoes Lopes, P.C. & Beneditto, A.P.M. (2011). Human-dolphin (*Tursiops truncatus* Montagu, 1821) cooperative fishery and its influence on cast net fishing activities in Barra de Imbé/Tramandaí, Southern Brazil. *Ocean and Coastal Management,* [CrossRef]

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2021; 36(3): 140-145 • DOI: https://doi.org/10.26650/ASE2020858534

Original Article

Some Aspects of Reproduction in *Amblypharyngodon mola* from Sylhet, Northeast Bangladesh

Mohammad Amzad Hossain[1]

Cite this article as: Hossain, M.A. (2021). Some aspects of reproduction in *Amblypharyngodon mola* from sylhet, Northeast Bangladesh. *Aquatic Sciences and Engineering, 36*(3), 140-145.

ABSTRACT

The mola carplet, *Amblypharyngodon mola* (Hamilton, 1822) is the most common small indigenous freshwater fish species in Bangladesh. The precise information addressing the reproduction in this species including breeding peak, ovarian histology, morphometry, and sex ration in nature is still insufficient. A one yearlong filed survey and different laboratory assays have been directed to disclose some key features of reproduction in female *A. mola* from wetland reservoirs in the greater Sylhet region in Bangladesh. The total length and the body weight have shown a strong correlation with gonad weight, occupying statistical r^2 values of 0.89 and 0.91, respectively. However, a Chi-square test at $P<0.05$ reported no significant deviation in sex ration of the wetland's stock. The maximum fecundity has been reported during the month of May as 12569+620 while the lowest is during the month of July as 9377+455. The highest values for gonadosomatic index have been reported in May while the lowest is in January with another peak in November. The histological study of ovarian cells resulted in the characterization of oocytes, previtellogenic oocytes, vitellogenic oocytes, perinuclear oocytes, and mature cells with yolk mass.

Keywords: *Amblypharyngodon mola*, sex ration, GSI, fecundity, morphometric regression analysis, gonadal histology

ORCID IDs of the author:
M.A.H. 0000-0001-9219-3628

[1]Department of Fish Biology and Genetics, Sylhet Agricultural University, Bangladesh

Submitted:
11.01.2021

Revision Requested:
28.02.2021

Last Revision Received:
03.03.2021

Accepted:
03.03.2021

Online Published:
13.04.2021

Correspondence:
Mohammad Amzad Hossain
E-mail:
mamzad.fbg@sau.ac.bd

INTRODUCTION

The mola, *Amblypharyngodon mola* is one of the most popular small indigenous fish (SIF) species in Bangladesh, which occupy almost all of the shallow freshwater habitats (Mondal & Kaviraj, 2013; Mondal et al., 2019). This fish has drawn significant commercial and cultural value due to its premium nutritional value (Abdulla et al., 2004; Alam et al., 2004; Shikha et al., 2019) and current adoption as ornamental fish in aquarium (Gupta & Banerjee, 2015). Currently this species is also subjected to coculturation with other cyprinid fish or in traditional pond culture technique to supplement domestic food supply in South Asian countries (Neetu & Seema, 2018). This fish is considered as one of the major species to meet the rural nutrition supplement and also constitutes the principle target of small scale artisanal fishing practices in Bangladesh (Bengal et al., 2017). Rich protein contents and the abundance of micronutrients make this species one of the thriving mediators for securing poor people's nutrition and the economy as well (Ahmed et al., 2012; Hossain et al., 2017; Kohinoor et al., 1998).

Information regarding the body parameters (Khalid et al., 2020), growth factors, and different reproductive indices, i.e., hepatosomatic index, gonadosomatic index and fecundity etc. (Jabed et al., 2020; Jannatul et al., 2015; Rahman et al., 2020), serves as key indicators of reproductive progression and breeding phase of fish (Amzad et al., 2015; Mian et al., 2020; Uddin et al., 2017), which are essential for implementing fishery strategies and conservation approaches (Iqbal & Naeem, 2018; Jannatul et al.,

2015; Mian et al., 2017). Biometric features of fish are a widely accepted form of data which bears necessary information about the growth, development and stock assessment of the fish (Muchlisin et al., 2010). The major quantitative features in fish (i.e., total length, body weight, gonad weight, and fecundity) provide actual biological modelling of species in a particular geographical area (Emre, 2020; Tharwat et al., 2018). The understanding on major aspects of wild breeding biology, annual reproductive peak, breeding season, ovarian maturation phases, and fecundity will certainly help in the future management of this species (Ahamed et al., 2017; Uddin et al., 2017).

MATERIALS AND METHODS

Study area and duration

Fresh and live random samples of *A. mola* have been collected from different fisher landing stations and local markets of the Greater Sylhet region of Bangladesh. They were transported in insulated ice boxes or in oval fiberglass tanks to bring into the Fish Biology and Genetics laboratory of Sylhet Agricultural University, Bangladesh. This research continued for a full-length year from July 2019 to June 2020.

Recording morphometric data

Shortly after bringing them back to the laboratory, all the fish were washed well with rinsing freshwater and placed on thick tissue towels to reduce the access of water and mucous content within them. Then, the total length and weight of each fish was calculated by using scale (a mm stainless-steel scale attached on a specially designed wooden structure) and electric balance (Ohaus corp. Pine Brook, NJ USA), respectively. Sexual recognition of each fish was confirmed by following the dissection of their gonad, which was weighted and recorded immediately.

Measurement of gonadosomatic index (GSI) and Fecundity

The value of gonadosomatic index for each fish was calculated by using the below formula (Brooks et al., 1997)

Gonadosomatic index (GSI) = (Weight of gonad (g)/ Total body weight (g)) ×100

Fishes collected during the early to late monsoon season was subjected to the calculation of fecundity. Gravid female fish were selected for fecundity, and Gilson's fluid was used to lessen the oocytes. Finally, absolute fecundity was calculated by using the description of Rahman & Samat, (2020).

The fecundity, F = n × G/ g, where "n" denoted the average number of eggs counted in sub-sample, "G" is net weight of the gonads, and "g" is the weight of the sub-sample.

Histology of gonad

The samples from the ovaries were chopped into small pieces and preserved in neutral buffered formalin. The standard protocol for gonad histology described by Van-Dyk & Pieterse, (2008) was followed for the preparation of histology slides. Tissues were embedded and infiltrated in Paraffin, and the resulting blocks were formatted into 3 micrometer slides. Finally, hematoxylin-Eosin staining accompanied the samples to view the cell with a Zeiss microscope (software version 3.0 pro).

Data analysis

The raw data were recorded on excel sheet before further analysis. Data were analyzed by using IBM SPSS Statistics v26 and a one-way ANOVA was tested at $P<0.05$ to measure the difference between mean values.

RESULTS AND DISCUSSIONS

The ratio between female and male fishes of *A. mola* in the wild natural wetland of the Greater Sylhet region does not vary significantly between months. However, the lowest male to female ratio was accounted in May (1:0.6), followed by January, July, and November (1:0.7), and by March, August, September, and December (1:0.8) (table 1). In contrast, the maximum value for the male to female ratio was observed during the month of October (1:2.03), followed by February, June (1:1.5) and April (1:1.3) (Table 1).

Table 1. Analysis (Chi-square test, $P<0.05$) of sex ration of *A. mola*

Month	Total	Female		Male		χ2-value	Ration (M: F)
	Sample	No.	%	No.	%		
Jan	20	12	60	08	40	1.2	01:00.7
Feb	25	10	40	15	60	1.2	01:01.5
Mar	30	16	53	14	47	0.14	01:00.8
Apr	30	13	43	17	57	0.54	01:01.3
May	30	19	63	11	37	2.13	01:00.6
Jun	25	10	40	15	60	1.2	01:01.5
Jul	20	12	60	08	40	1.2	01:00.7
Aug	30	16	53	14	47	0.13	01:00.8
Sep	30	16	53	14	47	0.13	01:00.8
Oct	30	10	33	20	67	3.33	01:2.03
Nov	30	17	57	13	43	0.53	01:00.7
Dec	30	16	53	14	47	0.13	01:00.8

The body weight (mg) data was plotted against the correspondent of total length (cm) data to disclose the regression analysis of above morphometry. The regression line shows a strong correlation between body weight and total length of *A. mola* in the natural wetlands of Northeast Bangladesh with a r^2 value of 0.89. It shows that about 89 percent of fish showed increasing weight in relation to total length or vice versa (Figure 1). Again, fecundity was also found to have a very strong correlation with increasing body weight of fish, occupying an estimated r^2 value of 0.91, showing that large fish tend to have more fecundity than smaller ones (Figure 2.A). However, the values of the total length show very minor and nonsignificant correlations with ovarian weight (Figure 2.B). The gonadosomatic index of *A. mola* in the natural wetlands of Sylhet, Northeast Bangladesh were reported to be very distinctive on a month-wise distribution. The highest GSI value was recorded in May (16.66) and then fell to 12.58 in June (Figure 3). The two peaks in GSI value were noticed once in May

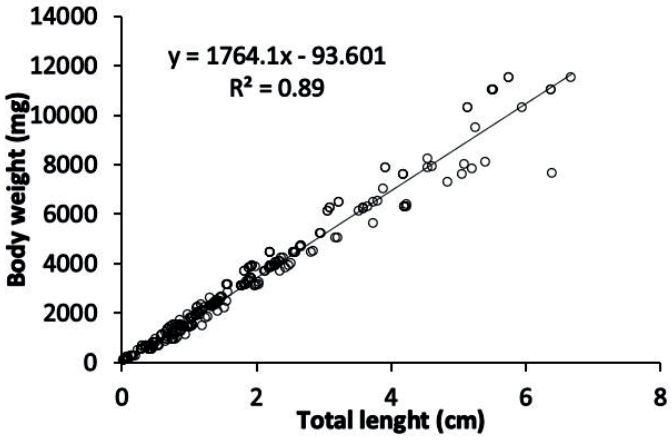


Figure 1. Length weight relationship of *A. mola* in Sylhet, Northeast Bangladesh.

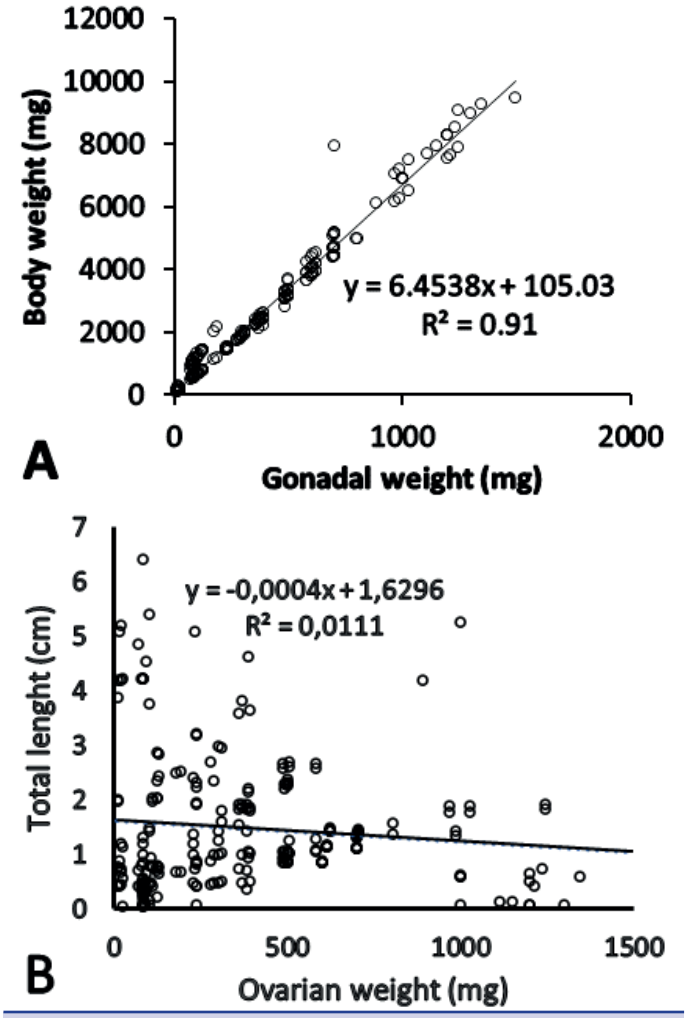


Figure 2. Relation between *A. body* weight and gonadal weight, B. total length and gonadal weight of *A. mola* in Sylhet, Northeast Bangladesh.

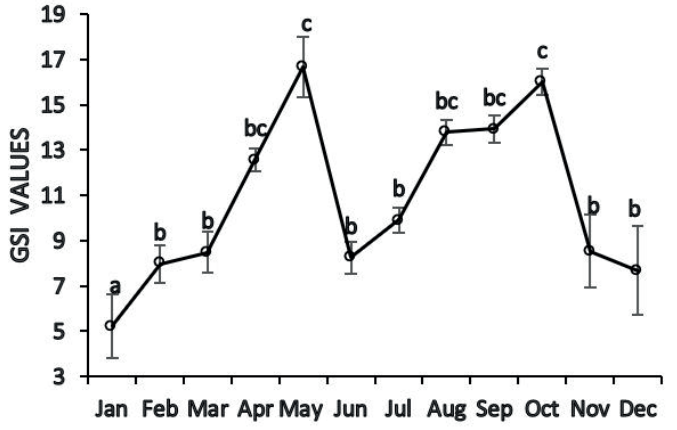


Figure 3. Gonado-somatic index of female *A. mola.*

and another in November 8.54 (Figure 3). The female *A. mola* in the natural wetlands of Sylhet, Northeast Bangladesh have been reported as an intermediate fecundated species. The highest fecundity was observed during the month of May as 125689+620, followed by 11172+160 in August and 10531+305 in June (Figure 4). However, the lowest fecundity was accounted during July 9377+455, followed by 9947+55 in September (Figure 4).

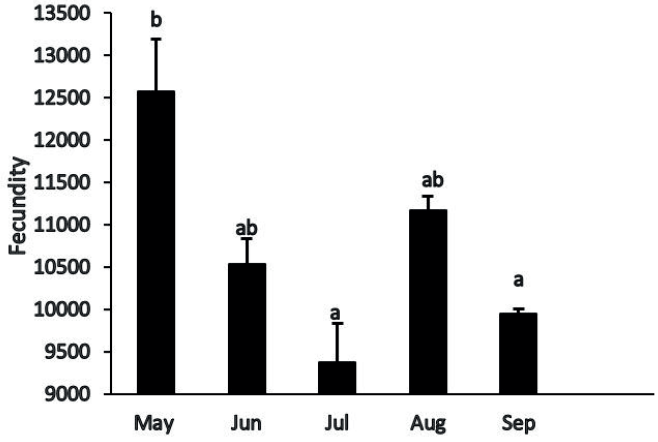


Figure 4. Variation in fecundity of *Amblypharyngodon mola* in Sylhet, Northeast Bangladesh.

The histological analysis of ovarian tissue resulted in the occurrence of oocytes and pre-vitellogenic oocytes as the initial phase of gonadal maturation (Figure 5.A). At the end of primary development phase, the nuclear mass of the cell tends to migrate toward the periphery, termed as perinuclear oocytes (Figure 5.B). Mature vitellogenin oocytes are characterized by the presence of yolk globules and yolk vesicles in the cell (Figure 5.C).

The sex ratio for a particular of the species would be different in response to habitat, fishing strategies, and harvesting method also (Oh et al., 2008). A study by Das et al., (2018) recorded sex ration of 1 : 2.05 for *A. mola* in Dekar Haor of Sunamganj, Bangladesh while a 1:3.04 male to female ratio was reported by Gupta

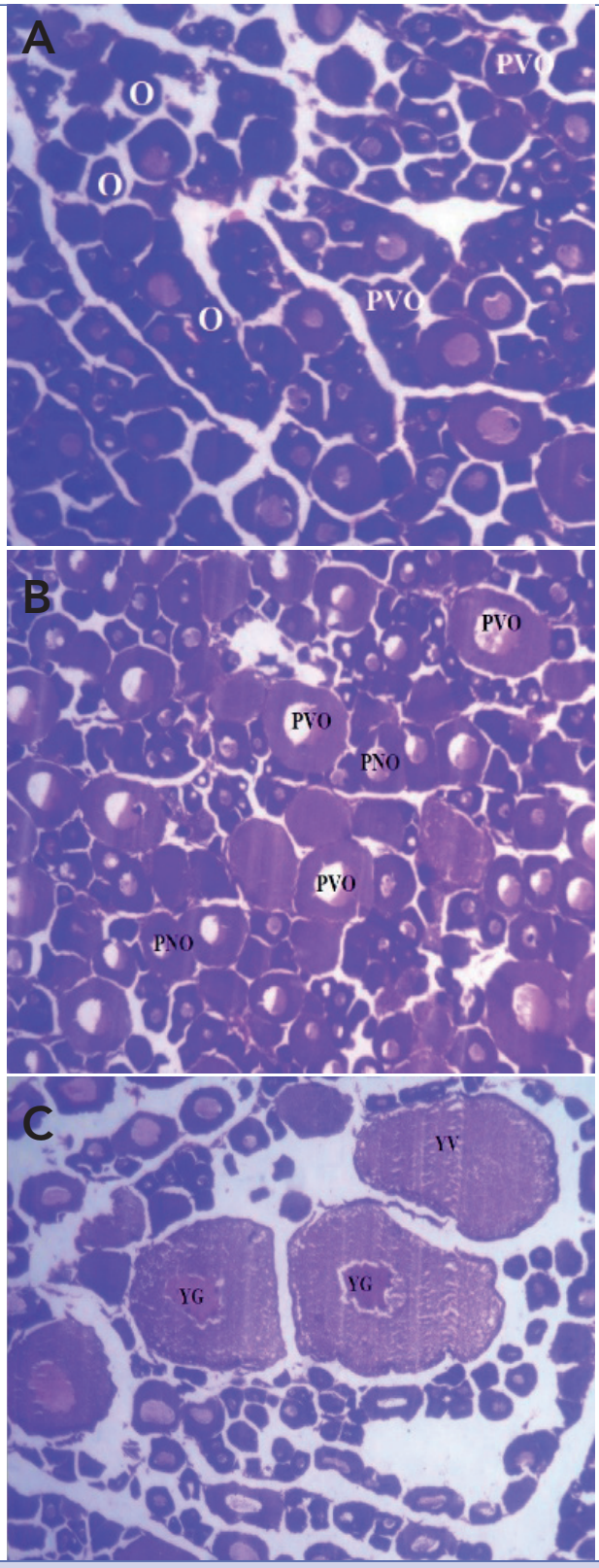


Figure 5. Different stages of development in ovarian cell of *A. mola* (O-oocytes stage, PVO-previtellogenic stage, PNO-perinuclear oocytes, YV-yolk vesicle, YG-yolk globules.

& Banerjee (2013) for the natural wetland of Baruipur, West Bengal, and 1.00:2.078 was recorded by Azadi & Mamun (2004) at the Kaptai Reservoir in Bangladesh. Current research is supportive of the previous study by Ahmed, et al., (2017), who had reported male to female ratio ranges between 1:1.14 to 1.32 in the Payra River of southern Bangladesh.

Morphometric studies of fish provide beneficial information towards the better understanding of the maturity and life history of particular species (Hossain et al., 2006). Previous research by Gupta & Banerjee (2015) observed a regression correlation value of 0.913 for female *A. mola* it freshwater from West Bengal, India, while Nawer et al. (2018) reported a r^2 value of 0.93-0.96 for the same species in the Ganges River, Northwestern Bangladesh. All the above studies closely stand with the present findings for *A. mola* in the Greater Sylhet region, Bangladesh. The fecundity of fish was reported to be boosted with fish size (Jannatul et al., 2015); the relation between fecundity and body weight might have shifted drastically during the onset of breeding season due to dramatic modification of egg size (Rai et al., 2018). A positive correlation between fecundity and body weight was reported for several, i.e., $r^2 = 0.77$ for *Xenontedon cancila* from Assam, India (Borthakur, 2018), $r^2 = 0.75$ for *Mystus cavasius* (Hamilton) from Brahmaputra and Kongsa river, Bangladesh (Islam & Das, 2006), $r^2 = 0.87$ for *Oreochromis Niloticus* from Balochistan (Kausar et al., 2019), and $r^2 = 0.79$ for cyprinid fish *Labeo calbasu* at Faridpur, Bangladesh (Kabir & Quddus, 2015). The present study also revealed a similar trend of morphometric correlation, and slight variations are raised because of differences in study location, season, and sampling strategies.

The values of gonadosomatic indices act as potential marker for addressing the gonadal development stage, maturity, and breeding peaks of individual fish species (Hasan et al., 2018). Gupta & Banerjee (2013) had identified two distinguished GSI peaks for *A. mola* in west Bengal, India, one in June and another one October, and reached the second peak in November while a single peak in July had been observed by Mondal & Kaviraj, (2013) in the floodplain lakes of India. The lowest GSI value had been observed in January while highest value was in June for the same species from Bengal, India (Pal & Mahapatra, 2016). Two peaks in June and November and the lowest value in January had been documented in South Bengal, India (Pal and Mahapatra, 2016). Multiple peaks revealed that this species might spawn several times within year. The deviation of peaks in the current research might vary due to the study's geography and sampling strategies. The fecundity of a species depends upon a variety of intrinsic factors, environmental features, and nutritional properties of the diet (Alam et al., 2004; Kohinoor et al., 1998; Mian et al., 2020), and accurate knowledge in fish fecundity helps in regulating harvesting quotas and also provides key roles in aquaculture and fishery management (Kant et al., 2016; Tracey et al., 2007). The fecundity of mature female of *A. mola* ranged between 3785 to 12590 oocytes in floodplain water of India (Mondal & Kaviraj, 2013), 1,548–4,020 oocytes in the natural waters of South Myanmar (Kulabtong, 2016), varied between 1,280-13,679 in the Kaptai Reservoir in Bangladesh (Azadi & Mamun, 2004), and fluctuated between 1652 to 15,985 in the Ganges River of Bangladesh (Rahman et al., 2018). The findings of present research are also strongly supported by above previous results.

Ovarian histology provides essential data for predicting the reproductive success and peak in several fish species (Alonso-Fernández et al., 2011; Emam & Abughrien, 2014; Jabed et al., 2020). Previous studies on different fish species revealed a characterization of oocytes and previtellogenic oocytes during the initiation of reproductive peak (Guraya et al., 1975; Viana et al., 2018). As the development process goes onward, the size of ovum and yolk globules increases (Murata et al., 2014; Quagio-Grassiotto et al., 2014). However, the simultaneous occurrence of different development stages also indicate the nature of several spawning peaks in this fish (Amzad et al., 2015; Jabed et al., 2020), which is also aligned with their multiple peaks in GSI values.

CONCLUSIONS AND RECOMMENDATIONS

This research has disclosed a set of necessary information regarding the reproductive aspects of *A. mola* from the natural wetlands of the Greater Sylhet region of Bangladesh. The above information might be used in the aquaculture development, wild stock management, and conservation of this fish in Bangladesh. However, broad studies with the collection of samples from different areas within the country would reflect actual trends of the reproduction in this fish.

Conflict of interests: No conflict of interest has been generated on this research work.

Ethics committee approval: The research has complied and essentially approved with all regional, national, and institutional ethical clearance.

Funding: -

Acknowledgments: -

Disclosure: -

REFERENCES

Abdulla, Al., Mondal, S., Wahab, A., & Barm, B. K. (2004). Food and feeding habits of *Amblypharyngodon mola* (Hamilton) from Kaptai Reservoir, Bangladesh. Pakistan Journal of Biological Sciences, 7(4), 584–588. [CrossRef]

Ahamed, F., Ahmed, Z. F., Hossain, M. Y., & Ohtomi, J. (2017). Growth and longevity of the mola carplet *Amblypharyngodon mola* (Cyprinidae) in the Payra River, southern Bangladesh. Egyptian Journal of Aquatic Research, 43(4), 291–295. [CrossRef]

Ahamed, F., Saha, N., Ara, M. G., Ahmed, F., & Hossain, Y. (2017). Biological aspects of the mola carplet *Amblypharyngodon mola* (Cyprinidae) in the Payra. International Journal of Fisheries and Aquatic Studies, 5(4), 336–339. [CrossRef]

Ahmed, Z., Hossain, Y., & Ohtomi, J. (2012). Modeling the growth of Silver hatchet chela *Chela cachius* (Cyprinidae) from the old Brahmaputra River in Bangladesh Using Multiple Functions. Zoological Studies, 51(3), 336–344.

Alam, M. J., Dewan, S., Rahman, M. R., Kunda, M., Khaleque, M.A., & Kader, M.A. (2004). Study on the cultural suitability of *Amblypharyngodon mola* with *Barbodes gonionotus* and *Cyprinus carpio* in a farmer`s rice fields. Pakistan Journal of Biological Sciences, 7(7), 1242–1248. [CrossRef]

Alonso-Fernández, A., Alós, J., Grau, A., Domínguez-Petit, R., & Saborido-Rey, F. (2011). The use of histological techniques to study the reproductive biology of the hermaphroditic Mediterranean fishes *Coris julis, Serranus scriba,* and *Diplodus annularis*. Marine and Coastal Fisheries, 3(1), 145–159. [CrossRef]

Amzad, H. M., Sohel, M., Mariya, A., Fazley, R. A., Marine, S. S., Rahman, M. A., Mahbub, I. M., Jakiul, I. M., Hassan, M. M., & Hossain, M. M. (2015). Ovarian biology of spotted snakehead (*Channa punctatus*) from natural wetlands of Sylhet, Bangladesh. Annals of Veterinary and Animal Science, 2(3), 64–76. http://naturepub.org/index.php/journal/navas

Azadi, M. A., & Mamun, A. (2004). Reproductive biology of the Cyprinid, *Amblypharyngodon mola* (Hamilton) from the Kaptai Reservoir, Bangladesh. Pakistan Journal of Biological Sciences, 7(10), 1727–1729. [CrossRef]

Bengal, W., Saha, D., Pal, S., Rahaman, H. S., Nandy, G., Chakraborty, A., Aditya, G., & Aditya, G. (2017). Exploitation pattern of small indigenous fish species: observations from fish markets of rural. AACL Bioflux, 10(2), 779–786. http://www.bioflux.com.ro/aacl

Borthakur, D. M. K. (2018). Study of gonadosomatic index and fecundity of freshwater fish *Xenontedon cancila*. Journal of Entomology and Zoology Studies, 6(3), 42–46. http://www.entomoljournal.com/archives/?year=2018&vol=6&issue=3&ArticleId=3548

Brooks, S., Tyler, C. R., & Sumpter, J. P. (1997). Egg quality in fish: what makes a good egg? Reviews in Fish Biology and Fisheries, 7(4),387–416. [CrossRef]

Das, P. R., Uddin, M. S., Islam, M. S., Biswas, M., & Mia, M. R. (2018). Length-weight relationship and sex ratio of *Amblypharyngodon mola* in Dekar haor of Sunamganj, Bangladesh. Journal of Asiatic Society, 44(2), 185–193. [CrossRef]

Emam, M. A., & Abughrien, B. (2014). Seasonal histological changes in gonads of the catfish (*Clarias lazera*). Fisheries and Aquaculture Journal, 05(01),3–6. [CrossRef]

Emre, N. (2020). Biometric relation between asteriscus otolith size and fish total length of seven cyprinid fish species from inland waters of Turkey. Turkish Journal of Fisheries and Aquatic Sciences, 20(3), 171–175. [CrossRef]

Gupta, S, & Banerjee, S. (2013). Studies on some aspects of Reproductive biology of *Amblypharyngodon mola*. International Research Journal of Biological Sciences, 2(2), 69–77.

Gupta, Sandipan, & Banerjee, S. (2015). Length-weight relationship of *Amblypharyngodon mola* (Ham.Buch., 1822), a freshwater cyprinid fish from West Bengal, India. Zoology and Ecology, 25(1), 54–58. [CrossRef]

Guraya, S. S., Kaur, R., & Saxena, P. K. (1975). Morphology of ovarian changes during the reproductive cycle of the fish, *Mystus tengara* (Ham.). Acta Anatomica, 91(2), 222–260. [CrossRef]

Hasan, T., Hossain, M. F., Mamun, M., Alam, M. J., Salam, M. A., & Rafiquzzaman, S. M. (2018). Reproductive Biology of *Puntius sophore* in Bangladesh. Fishes, 3 (2). [CrossRef]

Hossain, M. A., Akter, M., & Iqbal, M. M. (2017). Diversity of fish fauna in Kusiara river (Fenchungonj Upazilla), Northeast Bangladesh. Journal of Aquaculture in the Tropics, 32(1), 1–13.

Hossain, M. Y., Ahmed, Z. F., Leunda, P. M., Roksanul Islam, A. K. M., Jasmine, S., Oscoz, J., Miranda, R., & Ohtomi, J. (2006). Length–weight and length–length relationships of some small indigenous fish species from the Mathabhanga River, southwestern Bangladesh. Journal of Applied Ichthyology, 22(4), 301–303. [CrossRef]

Iqbal, M. J., & Naeem, M. (2018). Study of External morphometric variants and length-weight relationship of *Labeo rohita* (Hamilton-1822) fed with varying protein levels. Sarhad Journal of Agriculture, 34(4). [CrossRef]

Islam, M., & Das, M. (2006). Fecundity of Gulsha *Mystus cavasius* (Hamilton) from Brahmaputra and Kongsa rivers. Journal of the Bangladesh Agricultural University, 4(2), 347–355.

Jabed, M. N., Hossain, M. A., Mian, S., Kabir, M. A., Mazumder, S. K., & Iqbal, M. M. (2020). Some aspects of reproduction in long whiskered

catfish, *Sperata aor* (Hamilton 1822), from North-East Bangladesh. Aquaculture Studies, 21(2), 47–54. https://doi.org/10.4194/2618-6381-v21_2_01

Jannatul, H., Chandra, R. N., Jannatul, F. M., Amzad, H. M., Mehedi, H. M., Das, T. B., Sohel, M., Mahbub, I. M., Bodrul, M. M., & Mosarof, H. M. (2015). Reproductive biology of striped snakehead (*Channa striata*) from natural wetlands of Sylhet, Bangladesh. Annals of Veterinary and Animal Science, 2(6), 162–169.

Kabir, M., & Quddus, M. (2015). Fecundity and gonadosomatic index of *Labeo calbasu* (Hamilton) from a stocking pond of a hatchery at Faridpur, Bangladesh. Bangladesh Journal of Zoology, 41(1), 43–48. [CrossRef]

Kant, K. R., Gupta, K., & Langer, S. (2016). Fecundity in fish *Puntius sophore* and relationship of fecundity with fish length, fish weight and ovary weight from Jammu water. International Journal of Fisheries and Aquaculture Sciences., 6(2), 99–110. https://www.researchgate.net/publication/334162676

Kausar, R., Sadozai, S., Achakzai, W., Yar, A. A., Tariq, N., & Ali, H. (2019). Estimation of fecundity and gonado-somatic index of *Oreochromis Niloticus* (Linnaeus) from fish market of Quetta, Balochistan. Jacobs Journal of Entomology and Zoological Studies, 2(1), 1–8.

Khalid, M., Masud, S., Naeem, Z., Shahid, M. M., Naeem, A. D., Ishtiaq, A., & Naeem, M. (2020). Length-weight and length-length relationships of farmed *Catla catla* during winter season from Muzaffargarh, Pakistan. Sarhad Journal of Agriculture, 36(3). [CrossRef]

Kohinoor, A. H. M., Islam, M. L., Wahab, M. A., & Thilsted, S. H. (1998). Effect of mola (*Amblypharyngodon mola* Ham.) on the growth and production of carps in polyculture. Bangladesh Journal of Fisheries Research, 2(2), 119–126.

Kulabtong, S. (2016). Biological data of Burmese carplet *Amblypharyngodon atkinsonii* (Blyth, 1860) in South Myanmar (Cypriniformes Cyprinidae): a preliminary report. Biodiversity Journal, 7(2), 253–256.

Mian, S., Papree, S. D., Dey, T., Hossain, M. A., Iqbal, M. M., Abol-Munaf, A. B., & Islam, M. S. (2017). Some Reproductive Aspects of Freshwater Garfish, *Xenentodon cancila* (Hamilton, 1822) from North-East Bangladesh. Journal of Fisheries and Aquatic Science, 12(2), 82–89. [CrossRef]

Mian, S., Shah, A. W., Hossain, M. A., Hossain, M. S., Iqbal, M. M., & Debnath, P. (2020). Reproductive Biology of Captive reared spotted Snakehead, *Channa punctatus* (Bloch 1793) Channidae. Bulletin of Environment, Pharmacology and Life Sciences, 9(2), 8–15.

Mondal, D. K., & Kaviraj, A. (2013). Feeding and reproductive biology of *Amblypharyngodon mola* (Cyrpiniformes: Cyprinidae) from two floodplain lakes of India. International Journal of Aquatic Biology, 1(3), 125–131. https://doi.org/10.22034/ijab.v1i3.62

Mondal, S., Wahab, A., Barman, B. K., & Hossain, M. M. (2019). Advances in Bioresearch Reproductive Biology of Flying barb, Darkina, *Esomus danricus*. 10(1), 68–81. https://doi.org/10.15515/abr.0976-4585.10.2.6881

Muchlisin, Z. A., Musman, M., & Siti Azizah, M. N. (2010). Length-weight relationships and condition factors of two threatened fishes, *Rasbora tawarensis* and *Poropuntius tawarensis*, endemic to Lake Laut Tawar, Aceh Province, Indonesia. Journal of Applied Ichthyology, 26(6), 949–953. [CrossRef]

Murata, K., Conte, F. S., McInnis, E., Fong, T. H., & Cherr, G. N. (2014). Identification of the origin and localization of chorion (egg envelope) proteins in an ancient fish, the white sturgeon, *Acipenser transmontanus*. Biology of Reproduction, 90(6), 132. [CrossRef]

Nawer, F., Hossain, M. Y., Sarwar, M. G., Rahman, O., Khatun, D., Parvin, M. F., Jasmine, S., Ahmed, Z. F., Ahamed, F., & Ohtomi, J. (2018). Growth, maturity and form factor of Mola Carplet (*Amblypharyngodon mola*) from the Ganges River, Northwestern Bangladesh. Jordan Journal of Biological Sciences, 11(4), 375–380.

Neetu, N., & Seema, J. (2018). Otolith morphometry and fish length relation of *Amblypharyngodon mola* (Ham.) from Middle Ganga region (India). International Journal of Fisheries and Aquaculture, 10(10), 122–128. [CrossRef]

Oh, C.-W., Byun, J.-H., Choi, J. H., & Kim, H.-W. (2008). Reproductive Biology of *Pandalus gracilis* Stimpson, 1860 (Decapoda, Pandalidae) in the Southeastern Coastal Waters of Korea. Crustaceana, 81(7), 797–811. [CrossRef]

Pal, M., & Mahapatra, B. (2016). Fecundity and GSI of Mourala, *Amblypharyngodon mola* (Hamilton-Buchanan, 1822) from South Bengal district of West Bengal. International Journal of Fisheries and Aquatic Studies, 4(5), 47–53.

Pal, M., & Mahapatra, B. K. (2016). Fecundity and GSI of Mourala, *Amblypharyngodon mola* (Hamilton-Buchanan, 1822) from South Bengal district of West Bengal. International Journal of Fisheries and Aquatic Studies, 4(5), 47-53.

Quagio-Grassiotto, I., Wildner, D. D., & Guimarães-Bassoli, A. C. D. (2014). A cytochemical approach to describe oocyte development in the freshwater ostariophysan, *Serrasalmus maculatus* (Characiformes). Micron (Oxford, England: 1993), 60, 18–28. [CrossRef]

Rahman, M. A., Hossain, M. A., Ullah, M. R., & Iqbal, M. M. (2020). Reproductive biology of Gagora catfish (*Arius gagora*) at Meghna river system, Kushiara. International Journal of Aquatic Biology, 8(6), 383–395.

Rahman, M. M., Hossain, M. Y., Tumpa, A. Sen, Hossain, M. I., Billah, M. M., & Ohtomi, J. (2018). Size at sexual maturity and fecundity of the mola carplet *Amblypharyngodon mola* (Hamilton 1822) (Cyprinidae) in the Ganges River, Bangladesh. Zoology and Ecology, 28(4), 429–436. [CrossRef]

Rahman, Musrafizur M, & Samat, A. F. (2020). Reproductive cycle, sexual maturity and fecundity of *Nemipterus furcosus* (Valenciennes, 1830). Aquaculture and Fisheries. [CrossRef]

Rai, S., Yadav, K. K., & Trivedi, S. P. (2018). Ovarian Impairment in Fish, *Channa punctata* (Bloch) on Exposure to Mercuric Chloride, Caldan 50 SP and their Mixture. Journal of Ecophysiology and Occupational Health, 19(1&2), 65–70. [CrossRef]

Shikha, F. H., Hossain, M. I., & Shohan, S. (2019). Effect of low temperature on quality of Mola *(Amblypharyngodon mola)* fish pickle during storage and consumer preference towards. Bangladesh Journal of Fisheries Research, 31, 125–135.

Tharwat, A., Hemedan, A. A., Hassanien, A. E., & Gabel, T. (2018). A biometric-based model for fish species classification. Fisheries Research, *204*, 324–336. [CrossRef]

Tracey, S. R., Lyle, J. M., & Haddon, M. (2007). Reproductive biology and per-recruit analyses of striped trumpeter (*Latris lineata*) from Tasmania, Australia: Implications for management. Fisheries Research, 84(3), 358–367. [CrossRef]

Uddin, S., Hasan, M. H., Iqbal, M. M., & Hossain, M. A. (2017). Study on the reproductive biology of Vietnamese climbing perch (*Anabas testudineus*, Bloch). Punjab University Journal of Zoology, 32(1), 1–7.

Van-Dyk, J. C., & Pieterse, G. M. (2008). A histo-morphological study of the testis of the sharptooth catfish (*Clarias gariepinus*) as reference for future toxicological assessments. Journal of Applied Ichthyology, 24(4), 415–422. [CrossRef]

Viana, I. K. S., Gonçalves, L. A. B., Ferreira, M. A. P., Mendes, Y. A., & Rocha, R. M. (2018). Oocyte growth, follicular complex formation and extracellular-matrix remodeling in ovarian maturation of the imperial zebra pleco fish *Hypancistrus zebra*. Scientific Reports, 8(1), 13760. [CrossRef]

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2021; 36(3): 146-151 • DOI: https://doi.org/10.26650/ASE2020832579

Original Article

Upgrading Air Distribution System in the Thermal Units for Fish and Meat Products Aiming at Improving Efficiency of Heat-exchange Processes

Nina Usatenko[1], Sergii Verbytskyi[2]

Cite this article as: Usatenko, N., & Verbytskyi, S. (2021). Upgrading air distribution system in the thermal units for fish and meat products aiming at improving efficiency of heat-exchange processes. *Aquatic Sciences and Engineering, 36*(3), 146-151.

ABSTRACT

The issue of the equivalence of the physical parameters of the working medium in the heat-treatment zone of fish and meat products is solved by organizing uniform high-speed fields of the working medium in the working area, which is ensured by the corresponding design. The aerodynamic characteristics of a double-sided centrifugal blower fan with a vertical arrangement of the drive shaft and a spiral scroll with two outlet openings, the outlines of which were carried out according to the design square rule, provided that the opening and width of each outlet was equal to the half-opening and half-width of the outlet of the spiral case of the industrial prototype, were investigated. When testing the aerodynamic characteristics of a double-sided blower, the identities of the full and dynamic air pressure developed by the fan at all points of the measured cross-section of each of the two oppositely directed outlet pipes are established to be identical, which indicates the equalization of air flows. In this case, the dimensionless ratio of the cross-sectional areas of the equistatic pressure duct to the initial pressure is 0.20, and the cross-sectional area for the air passage of the heat exchanger, conditionally being the perforated base of the duct, was 0.0978 m^2. The test results confirmed the reliability of analytical calculations and the feasibility of using these fans, which reduces energy consumption, a specific quantity of metal, and the cost of equipment in general.

Keywords: Fish and meat products, Aerodynamic characteristics, Double-sided blower fan, Equistatic pressure duct, Convection, Thermal unit

ORCID IDs of the author:
N.U. 0000-0002-0339-5189;
S.V. 0000-0002-4211-3789

[1]Pereiaslav-Khmelnutskyi Hryhoriy Skovoroda State Pedagogical University, Kyiv, Ukraine

[2]Institute of Food Resources of National Academy of Agrarian Sciences of Ukraine, Kyiv, Ukraine

Submitted:
28.11.2020

Revision Requested:
01.03.2021

Last Revision Received:
07.03.2021

Accepted:
08.03.2021

Online Published:
13.04.2021

Correspondence:
Sergii Verbytskyi
E-mail:
tk140@hotmail.com

INTRODUCTION

Despite the widespread use of equipment for the food industry at numerous enterprises of this profile, the issue of updating the machinery and equipment fleet and the technical improvement of such equipment has been rather outside the field of view of scientists and specialists for years, negatively affecting the development of the Ukrainian industry in the above-mentioned industrial sectors. Among the primary issues to be solved is the energy efficiency of said equipment (Kovalenko et al., 2020). The heat treatment process is among the principal operations of fish and meat products' manufacturing technology, said process being carried out by the method of convective heat supply to the product in modular type rooms (thermal units) (Ivashov, 2007; Belova et al., 2013, Urazov, 2015), thermally insulated from the external environment. However, literature sources on the processes that occur during this processing are rather rare and fragmented. In particular, the food safety issues of the process are disclosed in (Usatenko et al., 2019a), the issues of rationalizing the working area of thermal units are considered in (Pöhlmann et al., 2013), the influence of the working elements of the thermal unit on the temperature field inside it is described in (Pöhlmann et al., 2012), and the features of the effect of the design of the thermal unit on the nature of the ongoing heat exchange processes are described in

(Ledesma et al., 2016). The problems of the effective supply of the working medium to the working zone of a thermal unit, as well as the effective distribution of the specified medium in the space filled with the product being processed, are given in (Alakali et al., 2017). The structural elements of a thermal unit, which provide the dynamic process of formation of the smoke-air mixture during the smoking of food products of animal origin, are described in (Adeyeye, 2019). In particular, a properly selected fan plays an important role in ensuring uniform heat transfer and mass transfer in the working volume of the thermal unit during the heat treatment of the above-mentioned products (Adepoju et al., 2018). The importance of continuous effective temperature regulation and of maintaining a certain relative humidity of the working environment during the heat treatment is described in (Škaljac et al., 2018).

An obligatory element of the regulation of the technological process in the working area of a thermal unit is to check the uniformity of the air supply and its distribution, as well as temperature. The check should be carried out both at normal ambient and at elevated temperatures. When the air heats up, its volume increases, and thus, the circulation fan supplies the same amount of air (by volume) into the chamber but the amount is less by weight. Controlling the flow of hot air is much more complicated than controlling the flow of heavier cold air. For this reason, the balancing of air flows inside the chamber should be carried out both at a colder ambient temperature, as well as at an elevated temperature. Both temperature and air velocity are determined at various points inside the chamber. In case of significant deviations, it is necessary to adjust the distribution system of air flows in the working volume (Waters, 2010) in a proper way.

As a rule, one of the technological processes carried out in industrial universal thermal units is the smoking of fish and meat products, which involves processing the product with a smoke-air working environment. The nature of this process depends on the composition of the smoke-air mixture, its temperature, humidity, and density, as well as on the speed and technical implementation of its supply, the distribution efficiency in the working volume of the chamber, and the design of the units supplying the smoke-air mixture into the chamber (Kubiak et al., 2014; Kubiak & Jakubovski, 2013; Sikorski & Kołakowski, 2010). Factors that determine the achievement of the required sensorial and chemical properties of the smoked product are such parameters as temperature, density, and the intensity and rate of mixing smoke and air in the working area of the chamber during the entire process (Kubiak & Jakubowski, 2010a; Kubiak & Jakubowski, 2010b). The required regulation of the production cycle, in particular smoke flows – air mixture, when processing fish and meat in a heat chamber is facilitated by innovative microprocessor technology (Kubiak et al., 2014).

As can be seen from the above, the most important condition of the heat treatment process carried out in thermal units is the equivalence of the physical and chemical parameters of the working medium flowing around each product unit taken from the number of product units processed simultaneously (Kosoy et al., 2018; Bondarenko, 2013), which is possible with the uniformity of the velocity fields of the working medium in the heat treatment zone.

The solution of this problem, as a rule, narrows down to the development of an effective aerodynamic system composed of such basic elements as: a fan, air ducts, heat exchangers, and a control system, with the help of which the necessary physical and chemical parameters of the working medium recirculated in a turbulent mode within the volume of the heat chamber with the product are being maintained (Study, 1994). At the same time, along with the fundamental requirement for the process – ensuring high quality of finished fish and meat products, the economical component related to reducing energy consumption, metal consumption, and, accordingly, costs shall be considered.

Among the most important issues when solving the problem is the fan, forcing air to move in the confined space of the working chamber; the aerodynamic characteristics of the said fan substantiate the efficiency of the heat applied to the fish and meat products by means of convection. The fan motor, when overcoming the resistance of the elements of the aerodynamic system, consumes a significant amount of electricity in the operating mode. Therefore, the selection of a recirculation fan is usually carried out after conducting comprehensive studies of the effectiveness of convective heat transfer processes, allowing for the structural and functional features of all elements of the aerodynamic network. The design features of the network elements, their interaction and orientation relative to each other, are the basics to determine the preparation of the working medium flows and its uniform dispersal throughout the chamber filled with the product, in accordance with the given technological parameters.

The formation of the working medium flows identical in physical parameters in the working volume of the chamber begins directly after the recirculation fan, when the continuous working medium flowing from its discharge pipe should be divided into two, both being identical but opposite to the peripheral sides of the chamber. Further, using the duct system, the flow geometry is transformed to obtain free or limited flooded jets at the entrance to the heat treatment zone of the product, the said jets being equivalent in aerodynamic characteristics.

When procuring thermal units, taking into account the significant aerodynamic drag of the system, industrial centrifugal high-pressure fans are usually used, both with a case and, less often, without one. The number of the first ones, as a rule, is doubled on each module, using an equal number of left and right fans and organizing, at the same time, their work in a cyclic mode (Kubiak et al., 2014; Smyshlyaev, 2004). This allows the avoidance of the operation of dividing the flow into two oppositely directed flows. As practice shows, this negatively affects the longevity of fan motors – due to the presence of periodically repeating starting loads.

The disadvantage of the fans without cases consists in their low-pressure aerodynamic characteristics, and the fact they provide neither the efficiency of heat removal from heat exchangers nor the necessary kinematic parameters of the working medium in the working volume of the chamber (Study, 1994).

STUDY OBJECTS AND METHODS

This work aims at the development of a double-sided blower fan and equistatic pressure ducts to improve the air distribution system in the chambers intended for the heat treatment of fish and meat products.

The objects of study are the aerodynamic characteristics of a centrifugal double-sided blower fan and the geometric characteristics of air ducts of equal pressure as the main elements. determining the nature of the movement of air within the working volume of thermal units.

Taking into account sufficiently significant hydraulic resistances of the elements of the aerodynamic system (calculated data) and technological assumptions regarding the velocity of the working medium near the product (~ 2 m / s), the industrial centrifugal fan VTs 14-46-4 was adopted as a prototype when developing a double-sided blower, equipped with an electric motor of the AIR-100L6T1 series with a power of N = 2.2 kW and n = 1000 rpm. The rated capacity of the fan is V ≈ 6000 m^3 / h, pressure P ≈ 670 Pa.

The new fan unit was fitted up based on the use of the impeller and electric motor of the above-mentioned prototype fan. The unit casing was a double-sided spiral scroll specially designed for this case (Figure 1), the outlines of which were carried out according to the rule of design square [22]. Moreover, the opening and width of each of the two outlets was equal to the half-opening and half-width of the outlet opening of the VTs 14-46-4 fan spiral casing, and the total size of the measured cross-sectional areas of the outlet openings was equal to the measured cross-sectional area of the prototype outlet.

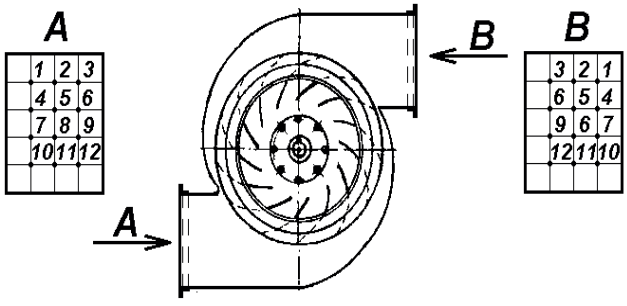


Figure 1. Diagram of a double-sided fan blower with the location of pressure measuring points in measured sections of outlet pipes: A – left; B – right.

When developing the design of the new fan blower, a vertical arrangement of the elongated drive shaft of the electric motor was provided in a special way, which allowed the latter to be moved outside the hot zone of the chamber (the temperature of the working medium is up to 100 °C), thereby avoiding the phenomenon of systematic overheating of bearings, significantly reducing their service life.

To conduct in situ tests of the objects of study, on the basis of analytical calculations – caloric and hydraulic resistances with a simultaneous outline study of the elements of the aerodynamic system (Solomakhova, 1975; Idelchik, 2013; Grimitlin, 1994), an experimental module of the heat treatment chamber was made, the diagram of which is shown in Figure 2.

The module was arranged according to the principle of symmetry with the axis of symmetry of the module coinciding with the central axis of the cylindrical suction pipe of the recirculation fan 1. In this case, the design of the right side of the module relative to the suction pipe of the fan was a mirror image of the structure of the left side. The outlet (pressure) pipes of the fan with welded cylindrical nipples 2 (Figure 2) are inextricably connected by means of connecting rotary air ducts 3 with equistatic pressure ducts 4 and bimetallic heat exchangers 5.

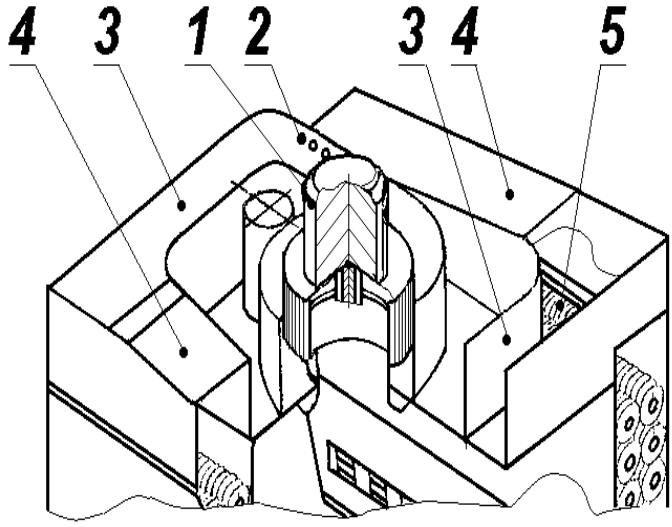


Figure 2. Scheme of the experimental setup module: 1 – centrifugal fan for double-sided blowing; 2 – cylindrical nipples with plugs; 3 – connecting turnable duct; 4 – equal pressure duct; 5 – finned bimetallic heat exchanger.

Each of the aforementioned elements of the aerodynamic network functionally complemented each other to obtain the uniform dispersion of the flow of the working medium along the sides of the module and, further, its uniform supply to the heat treatment zone of the product. So finned bimetallic heat exchangers, in addition to their direct function of heating the air, also served as the perforated base of the equistatic pressure ducts 4, or, in other words, as the distribution grid for the air flows flowing from these ducts. The total calculated aerodynamic drag of the system elements was approximately 550 Pa (Idelchik, 2013; Pozin et al., 1996), which corresponded to the certified value of the pressure developed by the analogue fan.

The uniformity of air flow distribution along the length of each equal pressure duct 4 (Figure 2) was established by changing the angle of inclination of its movable upper generatrix 2 (Figure 3) to the base.

The base of the air duct was a heat exchanger with a corridor arrangement of finned tubes 3 (Figure 3) and the cross-sectional area for air passage - 0.0978 m^2. The design of the heat exchange surface was created by casting molten aluminum onto a hot rolled steel pipe. With the height of the aluminum fin h = d_0 and its efficiency E = 0.77, the heat transfer coefficient, taking into account the deposition of smoke components on a bimetallic surface with a thickness of about 1 mm, amounted to k = 42.6 W / m^2 K, and the maximum aerodynamic resistance - 144 Pa. The high thermal characteristics of the heat exchanger significantly reduced its metal consumption, and the insignificant aerodynamic drag contributed to a decrease in the power of the recirculation fan electric motor. Equal pressure of air distributed along the length of air duct 1 (Figure 3), in the case of changing the type of heat exchange surface, is ensured by using a perforated bottom with the cross-section for air passage equal to the free section of the heat exchanger.

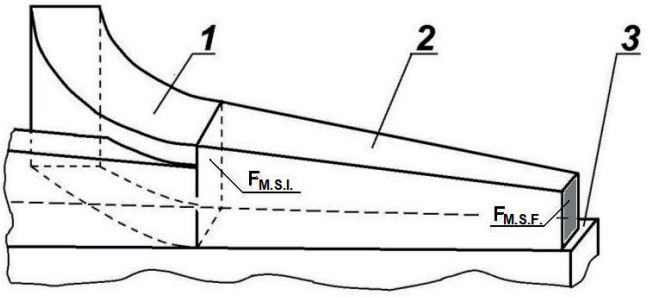


Figure 3. Diagram of the blowing ducts of the aerodynamic network: 1 – connecting turnable duct; 2 – the movable upper duct of the equal pressure duct; 3 – heat exchanger.

To determine the rational geometrical parameters of the equal pressure duct, a generalizing dimensionless parameter was used, the value of which was calculated as the ratio of the cross-sectional area of the equal pressure duct - the final (variable) area to the initial (unchanged and commensurate with the geometric parameters of the corresponding output section of the fan discharge pipe):

$$\overline{F} = \frac{F_{M.S.F.}}{F_{M.S.I.}} \quad (1)$$

where: $F_{M.S.F.}$ - the cross-sectional area of the duct at its end; $F_{M.S.I.}$ - the cross-sectional area of the duct at its beginning.

The rationality of the layout of the centrifugal double-sided fan of the applied electric motor was carried out in the operating mode by measuring the current consumption using the K-50 measuring set, the measurement limit was 1.0–600.0 A, accuracy class ± 0.5 A.

The measurement of the velocity of the air flow in the measured sections of the blow pipes of the fan and blow ducts was investigated using: hand induction anemometer ARI-49, measurement range 1.0 ÷ 30.0 m / s, accuracy ± 0.5 m / s; cup anemometer MS-13, measurement range 1.0 ÷ 20.0 m / s, accuracy ± (0.1 + 0.05) m / s and electrothermal anemometer - TA-LIOT, measurement range 0.0 ÷ 5.0 m / s, accuracy ± (0.1+0.05) m/s. The pressure was measured using a combined pneumometric fitting with a cylindrical head (correction coefficient of the bevel of the fitting during calibration of k_n = 0.95±1.0) and the differential alcohol micromanometer MMN-240 (5) -1.0, accuracy class 1.0.

The proportionality of the aerodynamic characteristics of the air flows generated in the double-sided blower was determined on the basis of a comprehensive comparative study of the total and dynamic pressure in the equal-sized and equal-distant from the impeller measured sections of and pressure ducts opposite the fan axis at the same temperature and humidity and avoiding leaks and suction from the outside. To fix the receiving part of the combined pressure measuring transducer perpendicularly to the flows of air leaving the pipes, cylindrical fittings with plugs 2 (Fig. 2) were welded at characteristic numbered measuring cross-section points (Figure 1 – A and B).

The average air pressure at the characteristic points of the measured sections was calculated by the formula:

$$P_{iavg.} = \frac{\left(\sqrt{P_{i1}} + \sqrt{P_{i2}} + \sqrt{P_{i3}} + \ldots + \sqrt{P_{in}}\right)^2}{n} \quad (2)$$

where: n is the number of measurements; i - type of measured pressure (static, dynamic, full) at the corresponding characteristic point.

The average air flow rate over the measured section of each pressure pipe of the fan was determined based on the average value of the dynamic pressure in the corresponding section:

$$W_{avg.} = \sqrt{\frac{2P_{davg}}{\rho}} \quad (3)$$

where: ρ is the air density, kg / m^2.

The volume of air flowing over the measured cross-section of each pressure pipe of the fan was calculated by the formula:

$$V = F \cdot W_{avg}, m^3 / s \quad (4)$$

where: F is the measured cross-sectional area equal to 0.0375 m^2 for this particular case.

RESULTS AND DISCUSSION

The results of field and analytical studies of the aerodynamic characteristics of a centrifugal double-sided fan are shown in Table 1.

An analysis of the results of analytical and full-scale studies of the aerodynamic characteristics of the developed double-sided blower (Table 1) indicates a fairly complete identity of the significant parameters characterizing the alignment of air flows and a decrease in the effect of turbulence in the output measured sections of each of the two oppositely directed blow pipes. The discrepancy between the pressure indicators (full and dynamic) in the left and right outlet openings of the blow pipes was within the accuracy limits of measuring instruments and can be explained by an insufficiently perfect impeller balancing quality.

Table 1. The results of analytical and full-scale studies of aerodynamic characteristics of a double-sided centrifugal fan.

Aerodynamic characteristics of fan	Measurement points numbers in measured sections												Average value
	1	2	3	4	5	6	7	8	9	10	11	12	
Pressure in measured section of the left blow pipe, Pa													
full, P_f	372	321	372	372	321	310	372	321	310	321	321	310	340.0
dynamic, P_d	319	292	319	319	292	266	319	292	266	292	292	266	301.0
Pressure in measured section of the right blow pipe, Pa													
full, P_f	372	321	371	371	321	310	372	321	310	321	321	310	339.8
dynamic, P_d	319	292	318	318	292	266	319	292	266	292	292	266	300.8
Average air flow velocity in measured section of the left blow pipe, m / s													22.40
Average air flow velocity in measured section of the right blow pipe, m / s													22.38
Calculated value of air volume flowing in measured section of the left blow pipe, m^3 / s													0.842
Calculated value of air volume flowing in measured section of the right blow pipe, m^3 / s													0.841
Average calculated performance of the double-sided blowing fan, m^3 / s													1.68
Average current consumed by fan electric motor, A													5.4

The results of measuring the current consumed by the electric motor in the operating mode of the current showed that its actual value corresponded to the nominal value - 5.4 A, and the fan performance was 1.68 m^3 / s (6059.0 m^3 / h), which is comparable with the certified technical characteristics of the centrifugal industrial fan VTs 14-46-4, taken as a prototype.

The averaged data on the study of the physical characteristics and uniformity of the distribution of air flows along the length of each of the two equal pressure ducts 4 (Figure 1), depending on the value of the generalizing parameter, are shown in Table 2. The measurements were carried out in measured sections at the outlet of the heat exchangers 5 (after straightening the flows) every 0.1 m with the length of each duct and, accordingly, the heat exchanger equal to 1.0 m

Based on an analysis of the results of studying air flow velocities along the length of air ducts for different values of the generalizing parameter, it was found that the best uniformity of air distribution and, as a result, more efficient heat removal from the heat exchanger is achieved with the value of the generalizing parameter:

$$F = \frac{F_{M.S.F.}}{F_{M.S.I.}} = 0.20 \quad (5)$$

The data obtained served as the basis for halving the number of fans when completing thermal units: replacing the two left and right VTs 14-46-4 twin industrial centrifugal fans, alternately switched on during operation, with one double-sided fan. At the same time, to improve the uniformity of the dispersion of air flows along the sides of the chamber, it was envisaged to tie the fans with equistatic pressure ducts, structurally made in accordance with the calculated value of the generalizing dimensionless parameter equal to 0.20 and the use of finned heat exchangers that functionally perform the role of the perforated bottom of the duct, and heat source for heating the flowing air.

The successful implementation of these technical solutions took place when creating the designs of universal heat chambers and cooling chambers for food products that are produced by the machine-building company "VI-VA LTD" (Ukraine) and resulted in a rather significant reduction in metal consumption and equipment and operating costs in general (Usatenko et al., 2019b).

CONCLUSION

Thus, oppositely directed compact flows of the working medium with the same aerodynamic characteristics were obtained using the developed double-sided fan with a vertical drive shaft and a spiral scroll with two outlet openings, the outlines of which were made according to the design square rule, provided that the opening and width of each outlet were equal to the half-opening

Table 2. Averaged data of the study of physical characteristics and uniformity of distribution of air flows formed in equistatic pressure ducts depending on the value of the generalizing parameter.

Generalizing parameter $\overline{F} = \frac{F_{M.S.F.}}{F_{M.S.I.}}$	The volume of air pumped by the fan over a measured cross-sectional area $F_{m.s.i.}$ m^3/s	The average air velocity in a measured cross-sectional area $F_{m.s.i.}$, m/s	The average air velocity in a measured section at the outlet of the finned heat exchanger, m/s	The uniformity of the distribution of air flows along the length of the duct of equistatic pressure, %
0.15	0.84 ± 0,01	21.0 ± 0,1	8.6 ± 0,1	2.3
0.20	0.84 ± 0,01	21.0 ± 0,1	8.6 ± 0,1	1.0
0.25	0.84 ± 0,01	21.0 ± 0,1	8.6 ± 0,1	4.5

and half-width of the outlet of the spiral casing of an industrial centrifugal prototype fan. This allowed to halve the number of recirculation fans equipped with each module of the thermal unit.

The uniform distribution of the working medium flows along the side walls of the module, before it enters the product heat treatment zone, was achieved due to the design features and the relative positioning of the elements of the aerodynamic system – equistatic pressure ducts and finned bimetallic heat exchangers.

It was established that the configuration of the heat chamber with the created elements of the aerodynamic system contributes to the improvement of the kinematic component of the process of bringing heat to fish and meat products by forced convection while reducing the metal consumption, energy consumption, and the cost of the equipment as a whole.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethics committee approval: -

Funding: -

REFERENCES

Adepoju, M. A., Omitoyin, B. O., Ajani, E. K., & Asha, K. (2018). Effect of Smoking Time and Temperature on the Proximate Composition and Quality of Milkfish Steaks. *Journal of aquatic food product technology, 27*(3), 369-378. [CrossRef]

Adeyeye, S. A. O. (2019). Smoking of fish: a critical review. *Journal of Culinary Science & Technology, 17*(6), 559-575. [CrossRef]

Alakali, J. S., Adekoyeni, O. O., Alaka, I. C., Faasema, J., & Torvor, T. (2017). Fabrication and Performance Evaluation of A Hybrid Fish Smoking Kiln. *Journal of Food Processing and Preservation, 41*(3), e12935. [CrossRef]

Belova, M. A., Aleksandrova, G. A., Poruchikov, D. V., & Novikova, G. V. (2013). Processing equipment for heat treatment of agricultural raw materials. *Herald of Chuvashia State Pedagogical University named after I. Ya. Yakovlev, 2*(78), 12-15.

Bondarenko, N. V. (2013). Research of heat treatment of boiled sausages in advanced thermal chambers. *Food Science and Technology, 2*(23), 92-93.

Erdoğdu, F. (2005). Mathematical approaches for use of analytical solutions in experimental determination of heat and mass transfer parameters. *Food Eng., 68*(2), 233-238. [CrossRef]

Grimitlin, M. I. (1994). Indoor air distribution. St. Petersburg: NPO Ehkoyurus-Vento, 316.

Idelchik, I. E. (2013). Reference book on fluid resistance. Moscow: Ripol Classic.

Ivashov, V. I. (2007). Technological equipment of the enterprises of meat industry, equipment for meat processing, part 2, 310. St. Petersburg: GIORD; ISBN 5-93879-023-2.

Kosoy, V. D., Ryzhov, S. A. & Dorokhov, V. P. (2018). Improving the process of sausages production (theoretical bases, processes, equipment, formulations and quality control). Moscow: DeLi, 682; ISBN: 978-5-6041606-1-9.

Kovalenko, O., Verbytskyi, S., Yashchenko, L., & Lysenko, H. (2020). Peculiarities of technical means of meat processing industry in Ukraine. *The Scientific Journal of Cahul State University "Bogdan Petriceicu Hasdeu" Economic and Engineering Studies, 1*(7), 66-72.

Kubiak, M. S., & Jakubowski, M., (2010a). Model symulacyjny warunków przepływu w komorze wędzarniczej. *Postępy Techniki Przetwórstwa Spożywczego*, 1, 55–57.

Kubiak, M. S., Jakubowski, M., (2010b). Trójwymiarowa analiza symulacyjna CFD rozkładu pola prędkości przepływu mieszaniny dwufazowej w komorze wędzarniczej. *Nauka Przyroda Technologie, 4*, 5-66.

Kubiak, M. S.; & Jakubowski, M. (2013). CFD simulations as a supporting tool of process and construction optimization in food industry production practice: the case study of a single truck smoking chamber. *Italian Journal of Food Science, 25*(3), 251-254.

Kubiak, M. S., Jakubowski, M., & Dolata, W. (2014). Computational Fluid Dynamics (CFD) innowacyjne narzędzie do symulowania procesu wędzenia oraz modelowania elementów konstrukcji komory wędzarniczo-parzelniczej. *Agricultural Engineering*, 18.

Ledesma, E., Laca, A., Rendueles, M., & Díaz, M. (2016). Texture, colour and optical characteristics of a meat product depending on smoking time and casing type. *LWT – Food Science and Technology, 65*, 164-172. [CrossRef]

Pozin, G. M., Belyaev, K. B., Nikulin, D. A., & Strelets, M. Kh. (1996). Issues of improving methods of calculation of air exchange and air distribution basing on approximate and precise mathematic models. Materials of 5th Congress of AVOK, 165-170.

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K., & Jira, W. (2012). Contents of polycyclic aromatic hydrocarbons (PAH) and phenolic substances in Frankfurter-type sausages depending on smoking conditions using glow smoke. *Meat Science, 90*(1). [CrossRef]

Pöhlmann, M., Hitzel, A., Schwägele, F., Speer, K., & Jira, W. (2013). Polycyclic aromatic hydrocarbons (PAH) and phenolic substances in smoked Frankfurtertype sausages depending on type of casing and fat content. *Food Control*, 31, 136-144. [CrossRef]

Sikorski, Z. E.; & Kołakowski, E. (2010). Smoking, in Handbook of Meat Processing (Ed Toldrá F.), Chapter 12. Wiley-Blackwell, Oxford, UK, 231-245; ISBN 978-0-9138-2182-5.

Škaljac, S., Jokanović, M., Tomović, V., Ivić, M., Tasić, T., Ikonić, P., ... & Petrović, L. (2018). Influence of smoking in traditional and industrial conditions on colour and content of polycyclic aromatic hydrocarbons in dry fermented sausage "Petrovská klobása". *LWT – Food Science and Technology, 87*, 158-162. [CrossRef]

Smyshlyaev, P. V. (2004). Optimal control system of thermal chambers. PhD Thesis, 139.

Solomakhova, G. S. (1975). Centrifugal blower fans. Moscow: Machine-building, 415.

Study of heat-and-mass exchange processes in the thermal processing of meat products to optimize technology and design of equipment. (1994). Scientific Report No 3.91, Kyiv: TIMM UAAN, 102.

Usatenko, N., Kalashnik, M., Dobroskok, S., & Verbitskyi, S. (2019a). Prerequisites to improve the safety of smoked foodstuffs. *Scientific Horizons, 6*(79), 29-35. [CrossRef]

Usatenko, N. F., Shchesyuk, O. V., Verbytskyi, S. B., Kalashnik, M. G., Dobroskok, S. S. (2019b) Efficiency improvement of heat and mass exchange processes in the production of dry sausages. *Food Industry: Science and Technologies, 12*(4), 20-27.

Urazov, D. Yu. (2015). Scientific support of the process of thermal and humid processing of sausage products. PhD Thesis, 186.

Waters, E. (2010). Appendix A: Objectives and critical elements of thermal processing of ready-to-eat meat products. Processing of Ready-to-eat Meat Products; ISBN 978-0-8138-0148-3.

İSTANBUL UNIVERSITY PRESS

EISSN 2602-473X

AQUATIC SCIENCES AND ENGINEERING

Aquat Sci Eng 2021; 36(3): 152-158 • DOI: https://doi.org/10.26650/ASE2020793132

Original Article

The changes in Alkaline, Neutral and Acid Protease Activities of *Artemia* Enriched with Commercial Emulsion and Different Additive Combinations

Mehmet Naz[1], Gürkan Diken[2], Metin Yazıcı[3], Yavuz Mazlum[4], Selin Sayın[5], Oktay Söyler[6]

Cite this article as: Naz, M., Diken, G., Yazici, M., Mazlum, Y., Sayin, S., & Soyler, O. (2021). The changes in alkaline, neutral and acid protease activities of Artemia enriched with commercial emulsion and different additive combinations. *Aquatic Sciences and Engineering, 36*(3), 152-158.

ABSTRACT

The biochemical compositions and the changes observed in alkaline, neutral and acid protease activities of *Artemia* enriched with commercial emulsion and different additive combinations were determined. Artemia nauplii (ArtN), GroBiotic-A (GA), Red Algamac (RA), Red Algamac:GroBiotic-A (50:50) (RA:GA (50:50)), Spirulina (SP), Spirulina:Red Algamac (50:50) (SP:RA (50:50)), Spirulina:GroBiotic-A (50:50) (SP:GA (50:50)) and Spirulina:Red Algamac:GroBiotic-A (33:33:33) (SP:RA:GA (33:33:33)) were tested in the study. The lowest and highest protein contents after the enrichment of Artemia were 40.74±1.02% (RA) and 55.03±1.26% (SP:RA:GA (33:33:33)), respectively. The lowest lipid contents of tested groups were found in 5.63±0.47% (GA) and 5.63±0.84% (RA:GA (50:50)). The highest lipid value after the enrichment were observed in 16.98±1.15 % (RA). The lowest and highest ash values observed after the enrichment were 4.51±0.27% (SP:GA (50:50)) and 6.07±0.35% (RA) ($p<0.05$). The lowest and highest protease activities of the pH=3, pH=4, pH=5, pH=6, pH=7 and pH=8.5 values were 18.18±0.37 U/mg protein (GA), 31.04±0.38 U/mg protein (RA), 9.1±0.32 U/mg protein (SP), 9.66±0.19 U/mg protein (SP), 16.94±0.61 U/mg protein (SP), 63.09±0.75 U/mg protein (SP) and 33.77±0.59 U/mg protein (RA:GA (50:50)), 57,54±0.34 U/mg protein (RA:GA (50:50)), 23.75±0.28 U/mg protein (GA), 40.82±0.49 U/mg protein (GA), 69.94±0.65 U/mg protein (GA), and 286.14±8.2 U/mg protein (GA) ($p<0.05$). In conclusion, GA and SP:RA:GA (33:33:33) enrichment combinations are recommended as an alternative to enrichment products. On the other hand, SP and RA should not be used alone due to the disadvantages such as the biochemical composition and proteolytic enzyme activities of *Artemia* observed in the present study.

Keywords: *Artemia*, enrichment, GroBiotic-A, biochemical compositions, enzymes

ORCID IDs of the author:
M.N. 0000-0002-5129-8498;
G.D. 0000-0002-3386-3676;
M.Y. 0000-0002-7011-886X;
Y.M. 0000-0002-9547-0966;
S.S. 0000-0002-7497-388X;
O.S. 0000-0003-4342-3209

[1]Iskenderun Technical University, Faculty of Marine Science and Technology, Iskenderun, Hatay, Turkey

[2]Isparta University of Applied Sciences, Faculty of Eğirdir Fisheries, Isparta, Turkey

[3]Iskenderun Technical University, Faculty of Marine Science and Technology, Iskenderun, Hatay, Turkey

[4]Iskenderun Technical University, Faculty of Marine Science and Technology, Iskenderun, Hatay, Turkey

[5]Iskenderun Technical University, Faculty of Marine Science and Technology, Iskenderun, Hatay, Turkey

[6]Iskenderun Technical University, Iskenderun Vocational School of Higher Education, Iskenderun, Hatay, Turkey

Submitted:
10.09.2020

Revision Requested:
23.11.2020

Last Revision Received:
30.11.2020

Accepted:
15.12.2020

Online Published:
20.04.2021

Correspondence:
Yavuz Mazlum
E-mail:
yavuz.mazlum@iste.edu.tr

INTRODUCTION

Fish larvae are strongly dependent on the exogenous enzymes of live foods used in the feeding protocols due to a poor development of the digestive system. The contribution of exogenous enzymes to the digestive system of larvae is an important issue in aquaculture. When the larvae begin to feed, their digestive enzyme activities are alkaline. Acid proteases towards the end of the critical larval stage are important in digestion. This information about the digestive physiology of larvae is important for the utilization of microdiets in the digestive system. According to the mentioned data, protease activities are indicators for determining the digestive ability of larvae (Diken, Demir & Naz, 2019).

Feeding protocols are of critical importance for aquaculture. The larval stages need live food such as *Artemia* due to the digestive enzymes contributed to the digestion of nutrients. Although *Artemia* is not a natural food such as copepods for marine fish larvae, it is an important food source in the larval stages of aquaculture. The alkaline enzyme activity of Artemia metanauplii (ArtMn) is higher than that of Arte-

mia naupli (ArtN). This shows that live food exogenous enzyme contributions will change according to the developmental periods of live foods and larvae. Therefore, the exogenous enzyme contributions by the live foods offered to the fish needs to be considered in combination with their dietary nutrition and/or enrichments used in their culture (Naz & Yufera, 2012).

Live food enrichment should be done to meet the nutritional requirements of fish larvae (McEvoy et al., 1998). It is known that enriching products contain high amounts of essential ingredients for larval feeding. However, these products exhibit large differences in primary nutrients, such as proteins, lipids and ash. In addition, the different forms of enrichment products could effect the biochemical composition of live food. The enrichment process of *Artemia* is one of the most important applications in hatcheries. The enrichment techniques for *Artemia* are well developed in the aquaculture sector. Efficient enrichment techniques contribute to the production of high-quality live food.

Nakagawa & Montgomery (2007) reported that *Spirulina* is one of the most frequently used algae in diets due to valuable ingredients such as high protein and fatty acid content. Several studies have been carried out using dried *Spirulina* as feed additives (Jaime-Ceballos, Villarreal, Garcia, Perez-Jar & Alfonso, 2005; Hanel, Broekman, de Graaf & Schnack, 2007; Dernekbasi, Unal, Karavucel & Aral, 2010; Ghaeni, Matinfar, Soltani, Rabbani & Vosoughi, 2011). Cho, Hur & Jo (2001) showed the effect of nutritional enrichment of live foods by w-yeast, *Spirulina* powder and Super Selco TM on survival and growth rates of rockfish larvae. Rocha, Garcia & Henriques (2003) indicated that microalgae are recognized as excellent sources of proteins, carbohydrates, lipids and vitamins. Senthil, MaruthuPandi, Kumar, Devi & Balasubramanian (2012) used algae such as Nannochloropsis sp., Chlorella sp. and Spirulina sp. and commercial A1 DHA Selco media for the enrichment of Artemia salina. Arumugam, Inbakandan, Ramasamy & Murugan (2013) tested liposome encapsulated Spirulina to enrich Artemia. Eryalcin, (2018) determined the effects of enrichments on growth, biochemical and fatty acid composition of L-type rotifer and Artemia franciscana nauplii.

Prebiotics are defined as non-digestible carbohydrates that cannot be digested by the host, but can be metabolized by normal gastrointestinal (GIT) microflora (Manning & Gibson, 2004). Although prebiotics have beneficial effects on the survival, growth and immune system of some species in aquaculture studies, there are limited studies on the effects of prebiotic encapsulation in live feed. (Yazıcı et al., 2020; Daniels et al., 2010; Hoseinifar, Zare & Miandare, 2015; Azimirad, Meshkini, Ahmadifard & Hoseinifar, 2016; Azimirad & Meshkini, 2017; Widanarni, Yuhana & Ekasari, 2018).

García-Ortega, Verreth, Coutteau, Segner, Huisman & Sorgeloos (1998) determined the biochemical and enzymatic characterization of decapsulated cysts and ArtN at different developmental stages. Naz (2008) revealed the changes observed in the biochemical compositions and the digestive enzymes such as trypsine, aminopeptidase N and alkaline phosphates at the different developmental stages of Artemia. Yenmis & Naz (2019) showed the changes in protease activities (alkaline, neutral and acid) of Artemia during starvation, enrichment and 4°C storage.

Until now, protease activities of Artemia were determined in pH 8.0-8.5 substrate (Naz, 2008; Naz et al., 2011; Naz & Yufera, 2012; Haközü, 2014; Diken, Demir & Naz, 2016a; Diken, Demir & Naz, 2016b; Diken, Demir & Naz, 2019) except for Garcia-Ortega et al. (1998) and Yenmis & Naz (2019). There is no available combined study on protease activities (alkaline, neutral and acid) and the biochemical compositions of Artemia enriched with the commercial emulsion (Red Algamac (RA)) and different additive combinations such as GroBiotic-A (GA), and Spirulina (SP). Considering this information, the aims of this study were (1) to determine the biochemical compositions such as proteins, lipids and ash of Artemia enriched with commercial emulsion and different additive combinations and also, (2) to reveal the changes in alkaline, neutral and acid protease activities of Artemia enriched with commercial emulsion and different additive combinations. The results will provide important knowledge to the aquaculture sector on the biochemical compositions and enzyme contributions of Artemia enriched with commercial emulsion and different additive enrichment products.

MATERIALS AND METHODS

Artemia hatching and enrichment process

Artemia cysts (1 g L^{-1}) (Artemia SepArt EG >250000 np/g, INVE Aquaculture Inc.) were incubated in a 150 L tank (approximately 35-36 ppt) at 30°C under continuous aeration and illumination. After 24 h, the Artemia nauplii (ArtN) were collected and washed with tap water. Enrichment was performed in a 5 L glass jar. ArtN were added to give a density of 400 nauplii mL^{-1}. The nauplii were enriched with GroBiotic-A (GA), Red Algamac (RA), Red Algamac:GroBiotic-A (50:50) (RA:GA (50:50)), Spirulina (SP), Spirulina:Red Algamac (50:50) (SP:RA (50:50)), Spirulina:GroBiotic-A (50:50) (SP:GA (50:50)) and Spirulina:Red Algamac:GroBiotic-A (33:33:33) (SP:RA:GA (33:33:33)). The proximate composition of Grobiotic A, Red Algamac and Spirulina is shown in Table 1.

The enrichment diet was added after hatching (time 0) and again 12 h. The experimental groups were performed in triplicate. Samples for the proximate composition and protease (alkali, neutral and acid) analyses were taken and washed with distilled water, transferred to cryotubes and stored immediately at -80 °C until the analysis stage.

Table 1. Proximate compositions of ingredients used for the enrichment periods of *Artemia*

	GroBiotic-A	Red Algamac	Spirulina
Ash	6%	8%	8.65%
Crude Lipid	0.1-2% min	50%	8.86%
Crude Protein	30-32%	18%	51.82%
Crude Fiber	2-3% max	-	-
Moisture	5%	5%	-
Carbohydrate	53%	19%	-

ANALYSES

Biochemical compositions

Biochemical compositions such as dry matter, ash and protein of samples taken from the experimental groups were tested according to the AOAC (2000) procedures and also lipid analyses were performed according to the chloroform-methanol extraction method described by Bligh & Dyer (1959).

Live food extracts

Artemia metanauplii (ArtMn) sampled after the enrichment period from the experimental groups were analyzed. The samples were rinsed in distilled water after thawing and then, the extracts of live foods were homogenized and centrifuged (16,000 g, 30 minute 4°C).

Determination of alkaline, neutral and acid protease activities of live foods

Protease activities were assayed at pH=3, 4, 5, 6, 7 and pH=8.5. Hemoglobin (1%) was used as the substrate in acid pH (3–6) and casein (1%) in neutral and alkaline pH (7–8.5). Protease activities were evaluated according to Walter (1984) and Anson (1938) as follows: The mixtures including extracts of live food-substrate were incubated at 37°C and then the reaction was stopped by addition of 500 µL trichloroacetic acid (TCA) (120 g/L). One unit of enzyme activity was defined as 1 µg of tyrosine release per minute. The soluble protein concentrations of live foods were determined according to Bradford (1976).

Statistical methods

All measurements were carried out in triplicate. The experimental data were subjected to one-way (ANOVA) and mean±standard error (SE) differences were calculated by Duncan test at $p<0.05$ content level by using SPSS 15.0 statistical package (SPSS, 2006).

RESULTS AND DISCUSSION

In the present study, the biochemical compositions and the changes observed in alkaline, neutral and acid protease activities of *Artemia* enriched with commercial emulsion and different additive combinations were determined. The biochemical compositions of *Artemia* tested in the current study are given in Table 2.

The changes observed in the ash, lipid and protein values were statistically significant ($p<0.05$). According to Table 2, ash, lipid and protein values tended to decrease at the end of enrichment. A decrease in protein value was observed at the end of the enrichment process, except for SP: RA: GA (33:33:33). The lowest and highest protein contents after the enrichment of ArtN were 40.74±1.02% (RA) and 55.03±1.26% (SP:RA:GA (33:33:33)), respectively. Lipid contents after the enrichment period of ArtN decreased ($p<0.05$). The lowest lipid contents of tested groups were found in GA (5.63±0.47%) and RA:GA (50:50) (5.63±0.84%). The highest lipid value after the enrichment were observed in RA (16.98±1.15%). The lowest and highest ash values observed after the enrichment were SP:GA (50:50) (4.51±0.27%) and RA (6.07±0.35%).

Changes in the biochemical compositions and enzyme contributions of live foods are the main factors that affect weaning success. Westelmajer (2008) and Beyhan (2011) reported that the biochemical compositions of ArtN changed depending on the enrichment process. The current study data is important because hatchery practices often involve an enrichment process of ArtN before it is given to the larvae.

Naz (2008) showed that the ash levels of ArtN and ArtMn ranged from 11.52 ±0.33% to 12.30±0.15%. Yenmis & Naz (2018) reported that ash levels of ArtN and ArtMn ranged from 6.95±0.26% to 9.66±0.02%. Aktaş, Genç, Bozkurt, Genç & Naz (2019) determined that ash levels of ArtN and ArtMn ranged from 5.84±0.05% to 9.32±0.48%. In the current study, ash contents of ArtN and ArtMn values were lower than Naz (2008) and similar to Yenmis & Naz (2019) and Aktaş et al. (2019).

Naz (2008) determined that the lipid contents of ArtN and ArtMn were 20.97±0.15% and 24.80±0.48%, respectively. Yenmis & Naz (2019) reported that lipid levels of ArtN and ArtMn ranged from 19.99±0.13% to 25.28±0.07%. Aktaş et al. (2019) determined that lipid levels of ArtN and ArtMn were 30.19±0.65 and 16.59±0.53%, respectively. In the present study the lipid contents for ArtN was higher than Naz (2008) and Yenmis & Naz (2019) and lower than Aktaş et al. (2019) while lipid content of ArtMn was lower than Naz (2008), Yenmis & Naz (2019) and lower than Aktaş et al. (2019) except for the RA group.

Naz (2008) revealed that the protein levels of ArtN and ArtMn ranged from 62.66±0.47% and 49.10±0.32%. Yenmis & Naz (2019) reported that protein levels of ArtN and ArtMn ranged from 60.61±0.04% to 49.28±0.07%. Aktaş et al. (2019) determined that protein levels of ArtN and ArtMn ranged from 61.55±0.28% and 64.4±0.78%, respectively. In the present study the protein con-

Table 2. Biochemical compositions of *Artemia* enriched with different enrichment emulsions (%).

Groups	Ash	Lipid	Protein
Artemia nauplii	7.54±0.29^{e}	27.93±1.04^{e}	58.42±2.40^{c}
GroBiotic-A	5.71±0.20^{cd}	5.63±0.47^{a}	46.70±0.55^{b}
Red Algamac	6.07±0.35^{d}	16.98±1.15^{d}	40.74±1.02^{a}
Red Algamac:GroBiotic-A(50:50)	5.30±0.12^{bc}	5.63±0.84^{a}	45.93±0.54^{b}
Spirulina	4.98±0.12^{ab}	9.99±0.41^{c}	49.78±0.62^{b}
Spirulina:Red Algamac (50:50)	6.23±0.22^{d}	7.73±0.53^{abc}	49.75±1.67^{b}
Spirulina:GroBiotic-A (50:50)	4.51±0.27^{a}	7.23±0.89^{ab}	48.97±2.49^{b}
Spirulina:Red Algamac:GroBiotic-A (33:33:33)	5.88±0.13^{cd}	9.07±0.89^{bc}	55.03±1.26^{c}

tents for ArtN were similar to Naz (2008), Yenmis & Naz (2019) and Aktaş et al. (2019) while protein contents of ArtMn were similar to Naz (2008) and Yenmis & Naz (2019) except for RA and SP-RA-GA groups and lower than Aktaş et al. (2019).

Navarro et al (1999) revealed that the digestive tract of ArtN during the enrichment period were differentiating and the differences observed in enrichment products could be effected on their ontogenesis, absorption efficiency and the nutritional value of live food. In addition, a combination of the proximate composition and form of the enrichment products, and the lipid pathways of ArtN could be responsible for the biochemical composition of live food. In this study, differences were observed in protein, ash, and lipid values due to the use of enrichment products and combinations. When the results were compared with previous studies, ash contents of ArtN and ArtMn values were lower than Naz (2008) and similar to Yenmis & Naz (2019) and Aktaş et al. (2019).

It is known that the protein and lipid contents of commercial RA, GA and SP used for ArtN enrichment are 18%, 30%, 51.82% and 50%, 0.1%, 8.86%, respectively. Ash levels are similar to other enhancers (8-9%), although RA has a higher lipid content (50%) and a lower protein content (18%) than the others. On the other hand, the protein and lipid contents of SP were found to be higher than that of GA. Thus, it is not surprising that the groups tested after enrichment had a tendency to decrease in protein and lipid contents. The post-enrichment RA group has the highest lipid value due to its high lipid composition compared to other enrichment emulsions. Biochemical data showed that proteins and lipids were used as energy sources after enriching with the emulsion used in this study. Our results were supported by Naz (2008) and Yenmis & Naz (2019).

Table 3 shows the changes observed in the different pH values such as alkaline (pH=8.5), neutral (pH=7) and acid protease activities (pH=6, pH=5, pH=4, pH=3) of ArtN and ArtMn. The changes observed in the protease activities (alkaline, neutral and acid) of the tested groups were statistically significant ($p<0.05$). The products, which used as enricher, showed alteration in the developmental periods of Artemia, depending on the pH on the protease activities. The lowest protease activities after enrichment according to pH = 3, pH = 4, pH = 5, pH = 6, pH = 7 and pH = 8.5 values were given as; 18.18±0.37 U / mg protein (GA), 31.04±0.38 U / mg protein (RA), 9.1±0.32 U / mg protein (SP), 9.66±0.19 U / mg protein (SP), 16.94±0.61 U / mg protein (SP), and 63.09±0.75 U / mg protein (SP), respectively. In addition, the highest protease activities after enrichment according to pH = 3, pH = 4, pH = 5, pH = 6, pH = 7 and pH = 8.5 values were determined as 33.77±0.59 U / mg protein (RA: GA (50:50)), 57.54±0.34 U / mg protein (RA:GA (50:50)), 23.75±0.28 U / mg protein (GA), 40.82±0.49 U / mg protein (GA), 69.94±0.65 U / mg protein (GA) and 286.14±8.2 U / mg protein (GA), respectively. The lowest and highest alkaline, neutral and acid protease activities after the enrichment were 63.09±0.75 U/mg protein (SP), 16.94±0.61 U/mg protein (SP), 86.57±0.38 U/mg protein (RA) and 286.14±8.2 U/mg protein (GA), 69.94±0.65 U/mg protein (GA), 140.14±0.38 U/mg protein (RA:GA (50:50)), respectively.

Munilla-Moran, Stark & Barbour (1990) indicated that the enzymatic activity of *Artemia* varies depending on the nutritional status and developmental stage. Differences in protease activities of ArtN and ArtMn due to *Artemia* enrichment were reported by Bonnie, Lan & Hung (1991). Naz (2008) revealed that the maximum enzyme contributions from live food to fish larvae were at the end of the enrichment process. The protease activity value of ArtN (253.48±6.54 U/mg) and ArtMn (481.31±22.10 U/mg protein) were found to be higher than the results of the present study. Protease activity values of ArtMn (414.5±0.41 U / mg protein) determined by Naz and Yúfera (2012) were higher than the current study. Haközü (2014) reported that ArtN and ArtMn protease activity values were found to be lower (34.67±0.88 U/mg protein) and higher (317.16±2.67 U / mg protein) than the current study, respectively. Diken et al. (2016a) revealed that protease activity value of ArtMn was 338.02±4.65 U/mg protein and higher than the present study. The differences observed in enzyme activities of tested groups could be related to developmental periods, the age of the live food, and also, enrichment applications. Depending on the enrichment process, *Artemia*'s enzyme contributions are more attractive in neutral (pH 7) and alkaline (pH 8.5) conditions. It is known that the digestive enzymes of larvae are

Table 3. The protease activities observed at different pH values of Artemia enriched with different enrichment emulsions.

	Protease Activities (U/mg protein)					
	Acid				Neutral	Alkaline
Groups	**pH=3**	**pH=4**	**pH=5**	**pH=6**	**pH=7**	**pH=8.5**
AN	28.77±0.13^{c}	52.04±0.40^{f}	31.61±0.68^{f}	32.82±0.42^{g}	75.92±0.20^{g}	185.22±0.53^{e}
GA	18.18±0.37^{a}	34.47±0.41^{b}	23.75±0.28^{e}	40.82±0.49^{h}	69.94±0.65^{f}	286.14±8.2^{g}
RA	26.15±0.41^{b}	31.04±0.38^{a}	12.45±0.40^{c}	16.93±0.34^{d}	28.56±0.34^{c}	115.20±0.65^{c}
RA:GA (50:50)	33.77±0.59^{e}	57.54±0.34^{g}	23.27±0.60^{e}	25.56±0.45^{e}	53.29±0.45^{e}	158.44±0.68^{d}
SP	31.95±0.66^{d}	45.65±0.37^{d}	9.10±0.32^{a}	9.66±0.19^{a}	16.94±0.61^{a}	63.09±0.75^{a}
SP: RA (50:50)	27.52±0.22^{bc}	47.77±0.22^{e}	11.06±0.07^{b}	13.95±0.67^{c}	18.27±0.46^{a}	82.97±0.53^{b}
SP:GA (50:50)	31.96±0.82^{d}	52.16±0.52^{f}	11.79±0.32^{bc}	12.61±0.44^{b}	25.63±0.14^{b}	81.52±0.15^{b}
SP:RA:GA (33:33:33)	18.43±0.53^{a}	42.14±0.73^{c}	14.98±0.07^{d}	29.14±0.23^{f}	43.83±0.46^{d}	196.97±0.97^{f}

alkaline due to a poor development of the digestive system and then, acid proteases are important in digestion at the end of the larval period.

Simpson (2000) revealed that fish acid proteases showed high activity from pH=2 and to pH=4. In the present study, the highest acid proteolytic activities were detected at pH=4 except for GA group (pH=6). Similarly, the current study findings showed that ArtN and ArtMn have significant levels of acid proteolytic activity between pH 3-6. The potential activities of proteases depend on the pH of the larval digestive system. Warner & Shridhar (1985) indicated that acid proteases are not active at alkaline pH. This process may be neutralized when acid proteases are in contact with the alkaline conditions of the larval digestive system. Therefore, the contribution of acid proteases might be meaningless due to the pH in the larval digestive system being around 8.

It is known that the importance of the contribution of proteolytic enzymes from *Artemia* is decreased due to the differences in the digestive system of larvae. Cahu, Zambonino Infante, Le Gall & Quazuguel (1995) and Kurokawa, Shiraishi & Suzuki (1998) showed that live food proteases provided only a low contribution to the protease activities observed in sea bass and sardine larvae. Simpson (2000) indicated that alkaline proteases are most active between pH=8 and pH=10. Yufera, Fernandez-Diaz, Vidaurreta, Cara & Moyano (2004) showed that sea bream larvae digest mainly by the action of alkaline proteases due to the gastrointestinal pH being alkaline. The present data indicated that the GA group exhibited good performance due to the highest alkaline proteolytic activities between tested groups, followed by SP:RA:GA (33:33:33).

Figure 1 reveals the observed changes in alkaline, neutral and acid protease activities of *Artemia* enriched with commercial emulsion and different additive combinations. Total alkaline and neutral protease activities obtained from the tested groups were found to be higher than those of the total of acid protease activities**.** The combination of ArtN enrichment with the RA-GA (50:50) group tended to decrease to alkaline proteolytic activities, while the enrichment with the GA group of ArtN increased to alkaline proteolytic activities. On the other hand, the enrichment with RA group of ArtN had higher alkaline proteolytic activity than those of the enrichment with SP group. The enrichment with SP group of ArtN showed the lowest alkaline proteolytic activity. The alkaline proteolytic activities of the SP-GA (50:50) and SP-RA (50:50) groups were found to be similar, but not lower than that of the SP group. The alkaline proteolytic activity of the SP-RA-GA (33:33:33) group was lower than those of the GA group, but not lower than the other experimental groups. The highest acid proteolytic activity in tested groups was detected in the RA-GA (50:50) group following the GA group, the lowest acid proteolytic activity was observed in the RA group. Acid protease/Total protease (Alkaline + Neutral + Acid proteases) ratios observed in the tested groups ranged from 54.62% (SP) to 24.76% (GA).

According to the results of the study, alkaline and neutral protease activities were higher than those of acid protease activities. ArtN and ArtMn acid protease contents should be taken into account for optimum growth and survival rate, together with functional differences in the digestive system of the larvae. Overall, the acid protease/Total protease ratios of the SP containing groups were high except for SP:RA:GA (33:33:33). The results revealed that the enrichment with SP of ArtN was not effective due to high acid proteolytic activities. Warner & Shridhar (1985) showed that over 90% of the protease activity in Artemia embryos and ArtN was associated with a cysteine protease. Our study findings confirmed the presence of a significant level of cysteine proteases of ArtN and ArtMn.

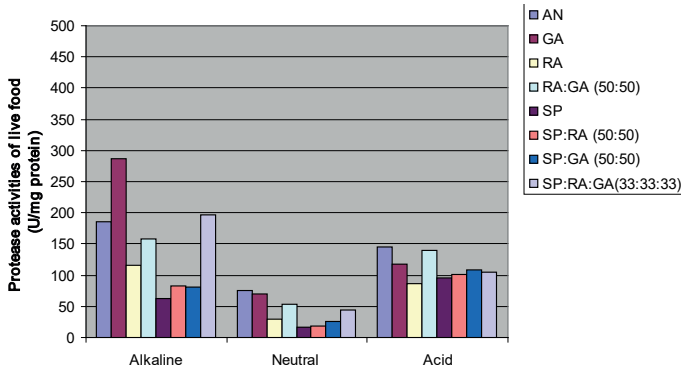


Figure 1. The changes observed in alkaline, neutral and acid protease activities of Artemia enriched with different enrichment emulsions (AN: Artemia nauplii; GA: GroBiotic-A; RA: Red Algamac; RA:GA(50:50): Red Algamac:GroBiotic-A(50:50); SP: Spirulina; SP:RA (50:50): Spirulina:Red Algamac (50:50); SP:GA (50:50): Spirulina:GroBiotic-A (50:50); SP:RA:GA (33:33:33): Spirulina:Red Algamac:GroBiotic-A (33:33:33).

During the critical periods of marine larvae, the digestive system is not yet developed, and alkaline digestion is present. For this reason, proteolytic enzymes working in the acid digestive system do not contribute significantly during feeding. However, proteolytic enzymes working in an alkaline digestive system have important contributions to digestion. The results in the present study showed that commercial emulsion and different additive enrichment groups have significant effects on acid, neutral and alkaline proteolytic enzymes.

CONCLUSIONS

In this study, which was carried out considering biochemical values and proteolytic enzyme activities, it is recommended to use the enrichment combinations GA and SP: RA: GA (33:33:33) as an alternative to enrichment products. On the other hand, SP and RA should not be used alone due to the disadvantages such as the biochemical compositions and protease activities of ArtMn observed in the present study.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical statement: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

Funding: -

Acknowledgments: We would like to thank Prof. Dr. Delbert M. Gatlin and the International Ingredient Corporation (St. Louis, MO) for kindly providing GroBiotic®-A for our study.

Disclosure: -

REFERENCES

Aktaş, M., Genç, M.A., Bozkurt, A., Genç, E. & Naz, M. (2019). The changes in the molecular weight profiles and biochemical compositions of potential feed ingredients for sustainable aquaculture. *Journal of Aquaculture Engineering and Fisheries Research,* 5(1), 1-11

Anson, M. L. (1938). "The estimation of pepsin, trypsin, papain, and cathepsin with hemoglobin." *Journal of General Physiology,* 22, 79–89. [CrossRef]

AOAC. (2000). "official methods of analysis of AOAC international." In *Official Methods of Analysis.,* USA: Gaithersburg, Md. : AOAC International.

Arumugam, P., Inbakandan, D., Ramasamy, M. S. & Murugan, M. (2013). "Encapsulated Spirulina Powder Feed for the Nutritional Enrichment of Adult Brine Shrimp (*Artemia salina*)." *Journal of Applied Aquaculture,* 25(3), 265–70. [CrossRef]

Azimirad, M., Meshkini, S., Ahmadifard, N. & Hoseinifar, S. H. (2016). The study of enrichment capability of adult *artemia franciscana* with singular or combined administration of *pediococcus acidilactici* and fructooligosaccharide. *International Journal of Aquatic Biology,* 4(2), 96–101.

Azimirad, M. & Meshkini, S. (2017). Determination of the Optimal Enrichment Artemia Franciscana with a Synbiotic Combination of Probiotics *Pediococcus acidilactici* and Prebiotic Fructooligosaccharide. *Veterinary research forum : an international quarterly journal,* 8(1), 49–54.

Beyhan, T. (2012). Bazı Zenginleştirici Ürünlerin Artemia (*Artemia salina* Linneaus, 1758)'nın Besinsel İçeriğine ve Yağ Asidi Kompozisyonu Üzerine Etkileri. Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü, Isparta, Türkiye.

Bligh, E. G. & Dyer, W. J. (1959). A Rapid Method of Total Lipid Extraction and Purification. *Canadian Journal of Biochemistry and Physiology,* 37(8), 911–17. [CrossRef]

Bonnie, S. P., Lan, C. C. & Hung. T. Y. (1991). Changes in Composition and Proteolytic Enzyme Activities of Artemia during Early Development. *Comparative Biochemistry and Physiology -- Part A: Physiology,* 100(3), 725–730. [CrossRef]

Bradford, M. M. (1976). Interactive Effects of Drought Stress and Chitosan Application on Physiological Characteristics and Essential Oil Yield of Thymus Daenensis Celak. *Analytical Biochemistry,* 72, 248–54. [CrossRef]

Cahu, C. L., Zambonino Infante J. L., Le Gall M. M. & Quazuguel, P. (1995). Early weaning of seabass: are digestive enzymes limiting? In: Larvi '95-Fish and Shellfish Larviculture Symposium (eds. Lavens P, Jaspers E & Roelants I), European Aquaculture Society, Special Publication No. 24, Gent, Belgium, pp. 268– 271.

Cho, S. H., Hur, S. B. & Jo, J. Y. (2001). "Effect of Enriched Live Feeds on Survival and Growth Rates in Larval Korean Rockfish, Sebastes Schlegeli Hilgendorf." *Aquaculture Research,* 32(3), 199–208. [CrossRef]

Daniels, C. L. Merrifield, D. L., Boothroyd, D. P., Davies, S. J., Factor, J. R. & Arnold, K. E. (2010). Effect of Dietary Bacillus Spp. and Mannan Oligosaccharides (MOS) on European Lobster (*Homarus gammarus* L.) Larvae Growth Performance, Gut Morphology and Gut Microbiota. *Aquaculture,* 304, 49–57. [CrossRef]

Dernekbasi, S., Unal, H., Karayucel, I. & Aral, O. (2010). Effect of Dietary Supplementation of Different Rates of Spirulina *(Spirulina platensis*) on Growth and Feed Conversion in Guppy. *Journal of Animal and Veterinary Advances,* 9(9), 1395–99. [CrossRef]

Diken, G., Demir, O. & Naz, M. (2016a). The Potential Effects of Commercial Feeding Protocol on Protease Activities and Cortisol Stress Responses of Meagre (*Argyrosomus regius*). *Journal of International Scientific Publications,* 4, 460–72.

Diken, G., Demir, O. & Naz, M. (2016b). Sörvaj Dönemi Çipura (*Sparus aurata* Linnaeus, 1758) ve Levrek (*Dicentrarchus labrax* Linnaeus, 1758) Balığı Larvalarının Proteaz Aktiviteleri Üzerine Besinlerin Etkileri ve Fizyolojik Durumlarının Belirlenmesi. In *IV. Balık Besleme ve Yem Teknolojileri Sempozyumu*, Adana, Türkiyre, 1–4.

Diken, G., Demir, O. & Naz, M. (2019). The Inhibitory Effects and Positive Contributions of Live Foods on Protease Activities of Meagre, *Argyrosomus regius* (Asso 1801) Larvae in Vitro Assay. *Fishes,* 4(5), 1–12. [CrossRef]

Eryalcin, K. M. (2018). Effects of Different Commercial Feeds and Enrichments on Biochemical Composition and Fatty Acid Profile of Rotifer (*Brachionus plicatilis*, Müller 1786) and *Artemia franciscana. Turkish Journal of Fisheries and Aquatic Sciences,* 18, 81–90.

Garcia-Ortega, Verreth, A. J., Coutteau, P., Segner, H., Huisman, E. A. & Sorgeloos, P. (1998). Biochemical and Enzymatic Characterization of Decapsulated Cysts and Nauplii of the Brine Shrimp Artemia at Different Developmental Stages. *Aquaculture,* 161(1–4), 501–14. [CrossRef]

Ghaeni, M., Matinfar, A., Soltani, M., Rabbani, M. & Vosoughi, A. (2011). Comparative Effects of Pure Spirulina Powder and Other Diets on Larval Growth and Survival of Green Tiger Shrimp, *Peneaus semisulcatus. Iranian Journal of Fisheries Sciences,* 10(2), 208–217.

Haközü, G. (2014). Çipura (*Sparus aurata,* Linneaus 1758) Larvalarının Kortizol Seviyeleri Üzerine Ticari Besleme Prosedürünün Etkileri. Mustafa Kemal Üniversitesi Fen Bilimleri Enstitüsü, Hatay, Türkiye.

Hanel, R., Broekman, D., De Graaf, S. & Schnack, D. (2007). Partial Replacement of Fishmeal by Lyophylized Powder of the Microalgae. *The Open Marine Biology Journal,* 1, 1–5. [CrossRef]

Hoseinifar, S. H., Zare, P. & Miandare, H. K. (2015). The Effects of Different Routes of Inulin Administration on Gut Microbiota and Survival Rate of Indian White Shrimp Post-Larvae (*Fenneropenaeus indicus). Veterinary research forum : an international quarterly journal,* 6(4), 331–35.

Jaime-Ceballos, Villarreal, H., Garcia, T., Perez–Jar, L. 1 & Alfonso, E. (2005). Effect of *Spirulina platensis* Meal as Feed Additive on Growth, Survival and Development in *Litopenaeus schmitti* Shimp Larvae. *Revista de investigaciones marinas,* 26(3), 235–241.

Kurokawa, T., Shiraishi, M. & Suzuki, T. (1998). Quantification of Exogenous Protease Derived from Zooplankton in the Intestine of Japanese Sardine (*Sardinops melanotictus*) Larvae. *Aquaculture,* 161(1–4), 491–499. [CrossRef]

Manning, T. S. & Gibson, G. R. (2004). Prebiotics. *Best Practice & Research Clinical Gastroenterology,* 18(2), 287–98. [CrossRef]

McEvoy, L. A., Estevez, A., Bell, J. G., Shields, R. J., Gara, B. & Sargent, J. R. (1998). Influence of dietary levels of eicosapentaenoic and arachidonic acid on the pigmentation success of turbot (*Scophtalmus maximus*) and halibut (*Hippoglossus hippoglossus). Bulletin of the Aquaculture Association of Canada,* 98, 17-20.

Munilla-Moran, R., Stark, J. R. & Barbour. A. (1990). The Role of Exogenous Enzymes in Digestion in Cultured Turbot Larvae (*Scophthalmus maximus* L.). *Aquaculture,* 88(3–4), 337–350. [CrossRef]

Nakagawa, H. & Montgomery W. L. (2007). Algae. In: Nakagawa, H., Sato, S & Gatlin III, D. M., editors. Dietary supplements for the health and quality of cultured fish. Cambridge, MA: CABI North American Office; p. 133–168. [CrossRef]

Naz, M. (2008). The Changes in the Biochemical Compositions and Enzymatic Activities of Rotifer (*Brachionus plicatilis*, Müller) and

Artemia during the Enrichment and Starvation Periods. *Fish Physiology and Biochemistry,* 34(4), 391–404. [CrossRef]

Naz, M. & Yufera, M. (2012). The Potential Inhibitory Effects of The Commercial Diets on Protease Activities of *Dentex dentex* Larvae and Live Foods. *Journal of Fisheries Science.* 6(3), 224-231. [CrossRef]

Navarro, J. C. Henderson, R. J., McEvoy, L. A., Bel, M. V. & Amat, F. (1999). Lipid Conversions during Enrichment of Artemia. *Aquaculture,* 174(1–2), 155–66. [CrossRef]

Rocha, J. M. S., Garcia, J. E. C. & Henriques. M. H. F. (2003). Growth Aspects of the Marine Microalga *Nannochloropsis gaditana*. *Biomolecular Engineering,* 20(4–6), 237–42. [CrossRef]

Senthil, S. L., MaruthuPandi, T., Kumar, T. A., Devi, K. N., & Balasubramanian, T. (2012). Exigent of Micro Algae for the Enrichment of *Artemia salina*. *Journal of Aquaculture Feed Science and Nutrition,* 4(2), 34–38. [CrossRef]

Simpson, B. K. (2000). Digestive proteases from marine animals. In : Haard, N. F. & Simpson B. K. (eds.), Seafood enzymes (pp. 191– 213). New York, NY: Marcel Dekker, Inc.

SPSS, 2006. SPSS Base 15.0 user's guide for Windows. Chicago: SPSS; 591 p.

Walter, H. E. (1984). Proteinases: methods with haemoglobin, casein and azocoll as substrates. In: Methods of enzymatic analysis (ed. Bergmeyer H U), Weinheim, Verlag, p. 270-277.

Warner, A. H. & Shridhar V. (1985). Purification and Characterization of a Cytosol Protease from Dormant Cysts of the Brine Shrimp Artemia. *Journal of Biological Chemistry,* 260(11), 7008–14. [CrossRef]

Westelmajer, S. K. M. (2008). Ontogeny of the corticosteroid stress response and effect of differentially enriched live feed on growth, lipid composition and acute stress tolerance of larval Atlantic cod, *Gadus morhua*. MScThesis, Memorial University of Newfoundland, Canada, 128P.

Widanarni, W., Taufik, A., Yuhana, M. & Ekasari, J. (2019). Dietary Mannan Oligosaccharides Positively Affect the Growth, Digestive Enzyme Activity, Immunity and Resistance against *Vibrio harveyi* of Pacific White Shrimp (*Litopenaeus vannamei*) Larvae. *Turkish Journal of Fisheries and Aquatic Sciences,* 19(4), 271–278. [CrossRef]

Yazıcı, M., Mazlum, Y., Naz, M., Sayın, S., Ürkü Ç. & Akaylı. T. (2020). Effects of GroBiotic®-A supplementation on growth performance, body composition and liver and intestine histological changes in European Seabass (*Dicentrarchus labrax*) juveniles. *Ege Journal of Fisheries and Aquatic Sciences* 37(2). [CrossRef]

Yenmis, A.M. & Naz, M. (2019). The changes in alkaline, neutral and acid protease activities of Artemia during the starvation, enrichment and stored at 4 °C temperature. *Journal of Experimental Zoology, India,* 22 (1), 201-207.

Yufera, M., Fernandez-Diaz, C., Vidaurreta A., Cara, J. B. & Moyano, F. J. (2004). Gastrointestinal PH and Development of the Acid Digestion in Larvae and Early Juveniles of *Sparus aurata* (Pisces: Teleostei). *Marine Biology,* 144(5), 863–869. [CrossRef]

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Instructions to Authors

Aquatic Sciences and Engineering is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of İstanbul University Faculty of Aquatic Sciences and it is published quarterly on January, April, July, and October. The publication language of the journal is English and continues publication since 1987.

Aquatic Sciences and Engineering aims to contribute to the literature by publishing manuscripts at the highest scientific level on all fields of aquatic sciences. The journal publishes original research and review articles that are prepared in accordance with the ethical guidelines.

The scope of the journal includes but not limited to; aquaculture science, aquaculture diseases, feeds, and genetics, ecological interactions, sustainable systems, fisheries development, fisheries science, fishery hydrography, aquatic ecosystem, fisheries management, fishery biology, wild fisheries, ocean fisheries, biology, taxonomy, stock identification, functional morphology freshwater, brackish and marine environment, marine biology, water conservation and sustainability, inland waters protection and management, seafood technology and safety.

The target audience of the journal includes specialists and professionals working and interested in all disciplines of aquatic sciences.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the Committee on Publication Ethics (COPE), the European Association of Science Editors (EASE), the International Council of Medical Journal Editors (ICMJE), and National Information Standards Organization (NISO). The journal conforms to the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization.

Manuscripts submitted to Aquatic Sciences and Engineering will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. The Editor in Chief is the final authority in the decision-making process for all submissions.

An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects," amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies. If required, ethics committee reports or an equivalent official document will be requested from the authors.

For manuscripts concerning experimental research on humans, a statement should be included that shows the written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect the patients' anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

Aquatic Sciences and Engineering requires experimental research studies on vertebrates or any regulated invertebrates to comply with relevant institutional, national and/or international guidelines. The journal supports the principles of Basel Declaration (basel-declaration.org) and the guidelines published by International Council for Laboratory Animal Science (ICLAS) (iclas.org). Authors are advised to clearly state their compliance with relevant guidelines.

Aquatic Sciences and Engineering advises authors to comply with IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora for research involving plants.

All submissions are screened by a similarity detection software (iThenticate by CrossCheck).

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

Each individual listed as an author should fulfil the authorship criteria recommended by the ICMJE. The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he/she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged in the title page of the manuscript.

Aquatic Sciences and Engineering requires corresponding authors to submit a signed and scanned version of the authorship contribution form (available for download through istanbul.dergipark.gov.tr/ase) during the initial submission process in order to act appropriately on authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of "gift authorship," the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all the responsibility for authorship during the submission and review stages of the manuscript.

Aquatic Sciences and Engineering requires and encourages the authors and the individuals involved in the evaluation process of submitted manuscripts to disclose any existing or potential conflicts of interests, including financial, consultant, and institutional, that might lead to potential bias or a conflict of interest. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. To disclose a potential conflict of interest, the ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by all contributing authors. Cases of a potential conflict of interest of the editors, authors, or reviewers are resolved by the journal's Editorial Board within the scope of COPE and ICMJE guidelines.

The Editorial Board of the journal handles all appeal and complaint cases within the scope of COPE guidelines. In such cases, authors should get in direct contact with the editorial office regarding their appeals and complaints. When needed, an ombudsperson may be assigned to resolve cases that cannot be resolved internally. The Editor in Chief is the final authority in the decision-making process for all appeals and complaints.

When submitting a manuscript to Aquatic Sciences and Engineering, authors accept to assign the copyright of their manuscript to İstanbul University Faculty of Aquatic Sciences. If rejected for publication, the copyright of the manuscript will be assigned back to the authors. Aquatic Sciences and Engineering requires each submission to be accompanied by a Copyright Agreement Form (available for download at istanbul.dergipark.gov.tr/ase). When using previously published content, including figures, tables, or any other material in both print and electronic formats, authors must obtain permission from the copyright holder. Legal, financial and criminal liabilities in this regard belong to the author(s).

Statements or opinions expressed in the manuscripts published in Aquatic Sciences and Engineering reflect the views of the author(s) and not the opinions of the editors, the editorial board, or the publisher; the editors, the editorial board, and the publisher disclaim any responsibility or liability for such materials. The final responsibility in regard to the published content rests with the authors.

MANUSCRIPT PREPARATION

The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2017 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, TREND guidelines for non-randomized studies, and COREQ guidelines for qualitative studies.

Manuscripts can only be submitted through the journal's online manuscript submission and evaluation system, available at istanbul.dergipark.gov.tr/ase. Manuscripts submitted via any other medium will not be processed.

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following forms during the initial submission. These are available for download at istanbul.dergipark.gov.tr/ase

- Copyright Agreement Form,
- Author Contributions Form, and
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors)

Preparation of the Manuscript

Title page: A separate title page should be submitted with all submissions and this page should include:

- The full title of the manuscript as well as a short title (running head) of no more than 50 characters,
- Name(s), affiliations, and highest academic degree(s) of the author(s) and ORCID ID (orcid.org)
- Grant information and detailed information on the other sources of financial support,
- Name, address, telephone (including the mobile phone number) and fax numbers, and email address of the corresponding author,
- Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfil the authorship criteria.

Abstract: A Turkish and an English abstract should be submitted with all submissions except for Letters to the Editor. Submitting a Turkish abstract is not compulsory for international authors. Please check Table 1 below for word count specifications.

Keywords: Each submission must be accompanied by a minimum of three to a maximum of six keywords for subject indexing at the end of the abstract.

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text should contain Introduction, "Materials and Methods", "Result and Discussion", "Conclusion" and "References" sections.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

After the Conclusion section and before references list, information regarding conflict of interest, financial disclosure, ethics committee approval and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

Conflict of interests: When you (or your employer or sponsor) have a financial, commercial, legal or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit and Service Evaluations" document and evaluate the study at the editorial board and decide whether or not it needs approval.

Financial disclosure: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Review Articles: Reviews prepared by authors who have extensive knowledge on a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. These authors may even be invited by the journal. Reviews should describe, discuss, and evaluate the current level of knowledge of a topic in researches and should guide future studies. The main text should start with Introduction and end with "Conclusion" and "References" sections. Authors may choose to use any subheading in between those sections.

After the Conclusion section and before references list, information regarding conflict of interest and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

Conflict of interests: When you (or your employer or sponsor) have a financial, commercial, legal or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Short Communication" Readers can also present their comments on the published manuscripts in the form of a "Short Communication". The main text should contain Introduction, "Materials and Methods", "Result and Discussion", "Conclusion" and "References" sections.

After the Conclusion section and before references list, information regarding conflict of interest, financial disclosure, ethics committee approval and acknowledgement are given. These information are to be provided in the author form which must be submitted togather with the manuscript.

Conflict of interests: When you (or your employer or sponsor) have a financial, commercial, legal or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.

Ethics committee approval: Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the approval of the ethics committee, and the authors may argue that they do not need the approval of their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit and Service Evaluations" document and evaluate the study at the editorial board and decide whether or not it needs approval.

Financial disclosure: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤20	250	40
Review Article	≤25	250	60
Short Communication	≤5	250	20

Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

References

While citing publications, preference should be given to the latest, most up-to-date publications. If an ahead-of-print publication is cited, the DOI number should be provided. Authors are responsible for the accuracy of references. List references in alphabetical order. Each listed reference should be cited in text, and each text citation should be listed in the References section. The reference styles for different types of publications are presented in the following examples.

Reference Style and Format

Aquatic Sciences and Engineering complies with APA (American Psychological Association) style 6th Edition for referencing and quoting. For more information:

- American Psychological Association. (2010). Publication manual of the American Psychological Association (6th ed.). Washington, DC: APA.
- http://www.apastyle.org

Accuracy of citation is the author's responsibility. All references should be cited in text. Reference list must be in alphabetical order. Type references in the style shown below.

Citations in the Text

Citations must be indicated with the author surname and publication year within the parenthesis.

If more than one citation is made within the same paranthesis, separate them with (;).

Samples:

More than one citation;
(Esin et al., 2002; Karasar, 1995)
Citation with one author;
(Akyolcu, 2007)
Citation with two authors;
(Sayıner & Demirci, 2007)
Citation with three, four, five authors;
First citation in the text: (Ailen, Ciambrune, & Welch, 2000)
Subsequent citations in the text: (Ailen et al., 2000)
Citations with more than six authors;
(Çavdar et al., 2003)

Major Citations for a Reference List

Note: All second and third lines in the APA Bibliography should be indented.

- **A book in print:** Baxter, C. (1997). *Race equality in health care and education.* Philadelphia: Ballière Tindall. ISBN 4546465465
- **A book chapter, print version:** Haybron, D. M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (pp. 17-43). New York, NY: Guilford Press. ISBN 4546469999
- **An eBook:** Millbower, L. (2003). *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song.* Retrieved from http://www.amacombooks.org/ (accessed 10.10.15)
- **An article in a print journal:** Carter, S. & Dunbar-Odom, D. (2009). The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy, 14*(1), 38-48.
- **An article with DOI:** Gaudio, J. L. & Snowdon, C. T. (2008). Spatial cues more salient than color cues in cotton-top tamarins (saguinus oedipus) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
- **Websites - professional or personal sites:** The World Famous Hot Dog Site. (1999, July 7). Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.15)
- **Websites - online government publications:** U.S. Department of Justice. (2006, September 10). Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.15)
- **Photograph (from book, magazine or webpage):** Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.15)
- **Artwork - from library database:** Clark, L. (c.a. 1960's). *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
- **Artwork - from website:** Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.15)

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

AQUATIC
SCIENCES and ENGINEERING

İSTANBUL UNIVERSITY PRESS

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

Editor in Chief: Prof. Devrim MEMİŞ
Address: İstanbul Üniversitesi Su Bilimleri Fakültesi Yetiştiricilik Anabilim Dalı Ordu Cad. No:8 34134 Laleli / İstanbul, Türkiye
Phone: +90 212 4555700/16448
Fax: +90 212 5140379
E-mail: mdevrim@istanbul.edu.tr