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AQUATIC RESEARCH

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E-ISSN 2618-6365

Vol. 4 Issue 4 Page 304-394 (2021)

Content

RESEARCH ARTICLES

1. **Assessment of microbial community diversity in lakes of İğneada floodplain forest by metabarcoding approach** /304-312

   Emine Gözde ÖZBAYRAM İbrahim Halil MİRALOĞLU Bahar İNCE

2. **Determination of genetic variations by using mitochondrial DNA cyt b sequences in populations of Carasobarbus luteus (Cyprinidae)** /313-320

   Arif PARMAKSIZ

3. **Incubation temperatures, hatching success and congenital anomalies in green turtle nests from Guanahacabibes Peninsula, Cuba** / 321-330

   Randy CALDERÓN Julia AZANZA RICARDO

4. **İşlenmiş alabalık yumurtalarının raf ömrüne katkı maddelerinin etkisi** / 331-342

   Bibi Aaishah OLLEE Özkan ÖZDEN

5. **New fish species added to the ichthyofauna of Laguna Ojo de Liebre, Baja California Sur, México** / 343-350

   Laura CIVICO COLLADOS Jorge A. ROSALES-CASIAN

6. **Stress responses of Indian major carps cultured in the East Kolkata Wetland, West Bengal, India** / 351-362

   Anish DAS Talagunda NAGESH Sarita Kumarı DAS Thangapalam Jawahar ABRAHAM

7. **Monitoring of river health using aquatic insects: A study on Jatinga River, North East India** / 363-375

   Tanushree CHAKRAVARTY Susmita GUPTA

REVIEW ARTICLES

8. **Doğal arıtım sistemi: Yapay yüzen ada teknolojisinin Türkiye'deki göl, gölet ve baraj göllerinde uygulanma potansiyeli** / 376-394

   Mert MİNAZ Ayşegül KUBİLAY

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Aquat Res 4(4), 304-312 (2021) • https://doi.org/10.3153/AR21025 *Research Article*

Assessment of microbial community diversity in lakes of İğneada floodplain forest by metabarcoding approach

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ABSTRACT

This paper aims to contribute to the understanding of bacterial community patterns of the lakes of İğneada Floodplain Forest by metabarcoding approach. Within this scope, surface water samples were collected from three lakes located in the area namely Mert Lake, Hamam Lake, and Saka Lake, and the bacterial diversity was assessed by a high throughput sequencing of the 16S rRNA gene. Chao1 richness and Shannon diversity were higher in Saka Lake indicated a more diverse bacterial community. Proteobacteria was by far the most abundant phyla in all lakes. Although Bacteroidetes and Actinobacteria also dominated the community, their abundances differed in each lake. While the family Burkholderiaceae represented 25% of the bacterial community in Saka Lake, the abundances were 9% and 4% in Hamam Lake and Mert Lake, respectively. This study is one of the first investigations specifically focused on the bacterial communities in three lakes of İğneada Floodplain by next-generation sequencing platform and gave a prescreening of the bacterial diversity. Further studies are required to determine the biotechnological potential of this unique habitat.

Keywords: Amplicon Sequencing, Bacterial community, İğneada floodplain forest

Introduction

Floodplain forests, longos, are unique habitats mostly covered with freshwater seasonally or permanently and consisted of sand resulted from various streams. These ecosystems are like rainforests and have particular structures and functions (Arekhi et al., 2019). In the Thrace region in Turkey, İğneada has seasonally flooded forests which have critical importance at national and international levels (Arekhi et al., 2019) due to their high water purification potentials and diverse communities (Tecimen & Kavgaci, 2010). The area received a National Park status in 2007. Whereas, in the southern part of the National Park, there are Saka Lake, Deniz Lake, Hamam Lake, Pedina Lake, Mert Lake, the northern part of the National Park consists of Erikli Lake surrounding longos forests (URL 1). These unique habitats can be a major area of interest for biotechnological applications holding the potential to be a source for specific microbial communities, novel enzymes, and biologically important biomolecules.

Bacterial communities are at the center of ecosystem well-being, contribute to the biogeochemical cycles by breaking up organic matter, fixing nutrients, regulating carbon flux etc. (Diao et al., 2017; Ozbayram et al., 2021; Zhang et al., 2020). The community structures alter from one location to another due to a wide variety of factors such as the distinctive environmental conditions, land usage, human activities etc. (Ung et al., 2019). Besides their crucial roles in the ecosystem, only a minor portion of the microbial communities (<10%) can be determined by culture-dependent techniques which limit the revealing functions of the particular environments (Steen et al., 2019). Thus, using culture-independent tools is important to assess the community structures providing valuable insights into metabolic functions and genetic diversity (Riesenfeld et al., 2004; Lloyd et al., 2018). The emerged platforms using next-generation sequencing technology are powerful tools for revealing complex diverse community compositions along with advanced metagenomic databases and powerful bioinformatics and statistical tools (Michán et al., 2021). A considerable amount of literature has been published on bacterial diversity of different freshwater habitats using various next-generation sequencing platforms such as in eutrophic lakes (Ozbayram, et al., 2021; Zhang et al., 2020), flood-pulse tropical lake (Ung et al., 2019), alkaline lake (Kambura et al., 2016), alpine lakes (Llorens-Marès et al., 2020).

Although some researches on these lakes carried out for examining the biodiversity in the lakes of İğneada Floodplain (Altinsaçli, 2001; Güher, 1999, 2003), the bacterial diversity in the lakes has not been investigated. Recent developments in next-generation technology and lower analysis costs have facilitated to uncover the diversity of these ecosystems. Thus, the aim of this prospective study was to explore the bacterial community diversity of these lakes and reveal the unique community structures.

Material and Methods

Sample Collection & Physical Measurements

Sampling was performed in October 2020. One sampling point was determined in each lake and the samples were collected from the surface water of the shores (Figure 1). The water temperature, pH, and dissolved oxygen were measured by the multi-parameter analyzer (Hach Lange, Germany). The measurements were done in triplicates and the average results were presented in the study.

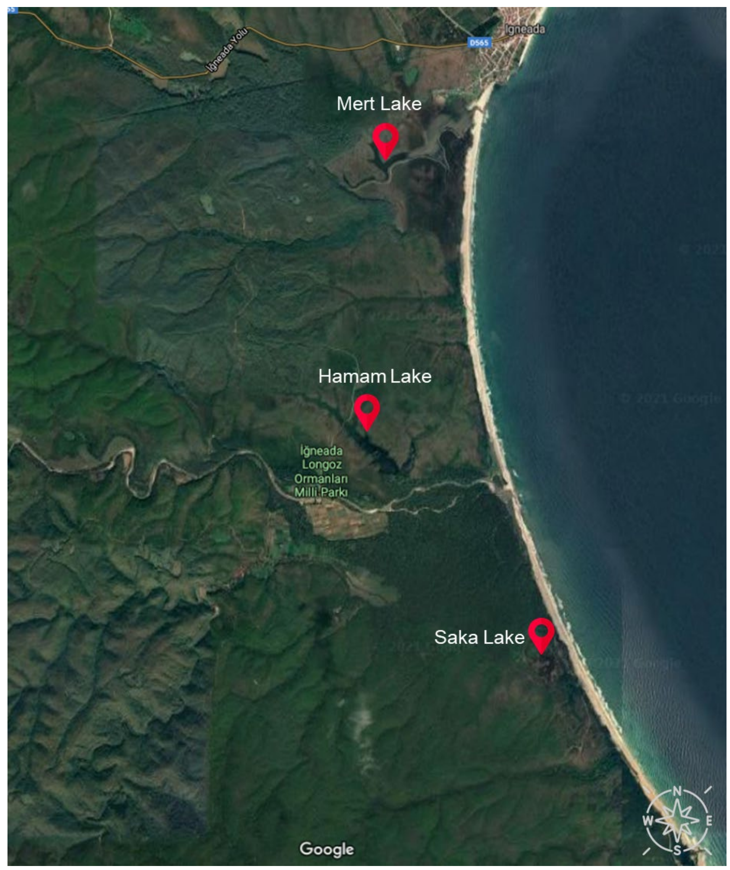

Figure 1. Study area and sampling points

DNA Extraction and Amplicon Sequencing

To concentrate the samples, waters were filtered from a 0.22 µm filter at the sampling site, and the filters were stored under cold conditions during their transfer to the lab. The DNAs were isolated from the filter paper using the NucleoSpin® Soil Kit (Macherey-Nagel, Germany) following the manufacturer's instructions and quantification was done using NanoDrop 1000 (Thermo Fisher Scientific, Inc., DE, USA).

The bacterial community diversity was assessed by 16S rRNA gene-targeted sequencing using Illumina® MiSeq™ platform. Amplicon sequencing library was prepared using bacteria-specific primers targeting the V3-V4 region of the 16S rRNA gene (341F (5'-CCTACGGGNGGCWGCAG-3) and 805R (5'-GACTACHVGGGTATCTAATCC-3'). Prepared libraries were purified, quantified, and further sequenced on the MiSeq instrument (Illumina, USA) using 300 bp paired-end chemistry. CASAVA data analysis software was used for demultiplexing and clipping of sequence adapters from raw sequences (Illumina, USA). The fragments with any mismatches to the barcodes or primers were excluded. Data analysis was performed through Quantitative Insight Into Microbial Ecology (QIIME2) v2020.2 (Bolyen et al., 2019). PCR primers were removed from sequences using cutadapt plugin (Martin, 2011). Paired-end reads were joined (vsearch join-pairs) and quality filtered (quality-filter q-score-joined). Then, sequences were denoised using deblur (deblur denoise-other) (Amir et al., 2017). Taxonomy was assigned to each amplicon sequence variant (ASV) using 'feature-classifier classify-sklearn' plugin against the pre-trained Naive Bayes classifier (classifier_silva_132_99_16S_V3.V4_341F_805R.qza) (Comeau, Douglas, & Langille, 2017). The final ASV table was used to calculate alpha diversity metrics. The bacterial diversity was visualized by Krona interactive metagenomic visualization (Ondov et al., 2011).

Results and Discussion

The physical characteristics of the lakes in the sampling period are presented in Table 1. While Mert Lake showed an alkaline characteristic, the lowest pH was determined for Hamam Lake. Water temperature was in the range of 20.1-24.0°C. The dissolved oxygen level in Mert Lake was quite high, in which the lake was covered by aquatic plants in the sampling period. The high oxygen level most probably resulted from the high photosynthesis rate of these aquatic plants. On the other hand, the dissolved oxygen concentrations were measured as 5.75 mg/L and 6.35 mg/L in Hamam Lake and Saka Lake, respectively. The results are in accord with the previous study obtained by (Güher, 2003) confirming the high pH and oxygen saturation in Mert Lake and similar profile for Hamam Lake. On the other hand, (Altinsaçli, 2001) reported different measurements for Saka Lake in the autumn (pH: 7.13; dissolved oxygen: 8.9 mg/L; water temperature: 16°C). Since the water temperature measured in this study was higher than that of (Altinsaçli, 2001), this contradiction may be due to the changing environmental conditions in the lake since 2001.

Table 1. Physical characteristics of the Lakes

Parameter	Mert Lake	Hamam Lake	Saka Lake
pH	9.10	7.30	7.72
Water temperature (°C)	24.0	23.2	20.1
Dissolved oxygen (mg/L)	13.75	5.75	6.35

The bacterial community pattern of Mert Lake is shown in Figure 2. The most dominant phyla were Proteobacteria, Bacteroidetes, and Actinobacteria, they represented 73% of the total reads. The highest abundance of the Cyanobacteria was detected in Mert Lake among the samples (11%). Together with Epsilonbacteraeota, Verrucomicrobia, and Patescibacteria represented 10% of the total sequences. In the community, Bacteroidia and Gammaproteobacteria were the most abundant classes. At the family level, 13% of the total reads were assigned to Nitriliruptoraceae, followed by Flavobacteriaceae (9%). These were the highest levels determined among the samples.

The results in Figure 3 show the bacterial community in Hamam Lake. The major share of the total reads was assigned to Proteobacteria species as in the Mert Lake. Actinobacteria was detected as the second dominant phyla representing 20% of the bacterial community. 7% of the total reads comprised of Bacteroidetes members followed by, Firmicutes (6%) and Verrrucomicrobia (5%). Proteobacteria classes Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria were the most abundant ones. On the other hand, Sporichthyaceae (11%) was by far the most abundant family followed by Burkholderiaceae (9%).

The bacterial community composition of Saka Lake is depicted in Figure 4. Proteobacteria was accounted as the most abundant phylum representing 60% of the bacterial community which was also the highest among samples. The majority of the Proteobacteria reads were assigned to Betaproteobacteria, especially the family Burkholderiaceae (25%). Whereas Firmicutes was the second dominant phylum (12%), Bacteroidetes comprised 11% of the total reads. Together with Bacteroidia, Clostridia species represented 20% of the sequences at the class level. Different from the other two lakes, 4% of the sequences were assigned to Rumminococcaceae species.

Figure 2. Bacterial community composition in Mert Lake

Figure 3. Bacterial community composition in Hamam Lake

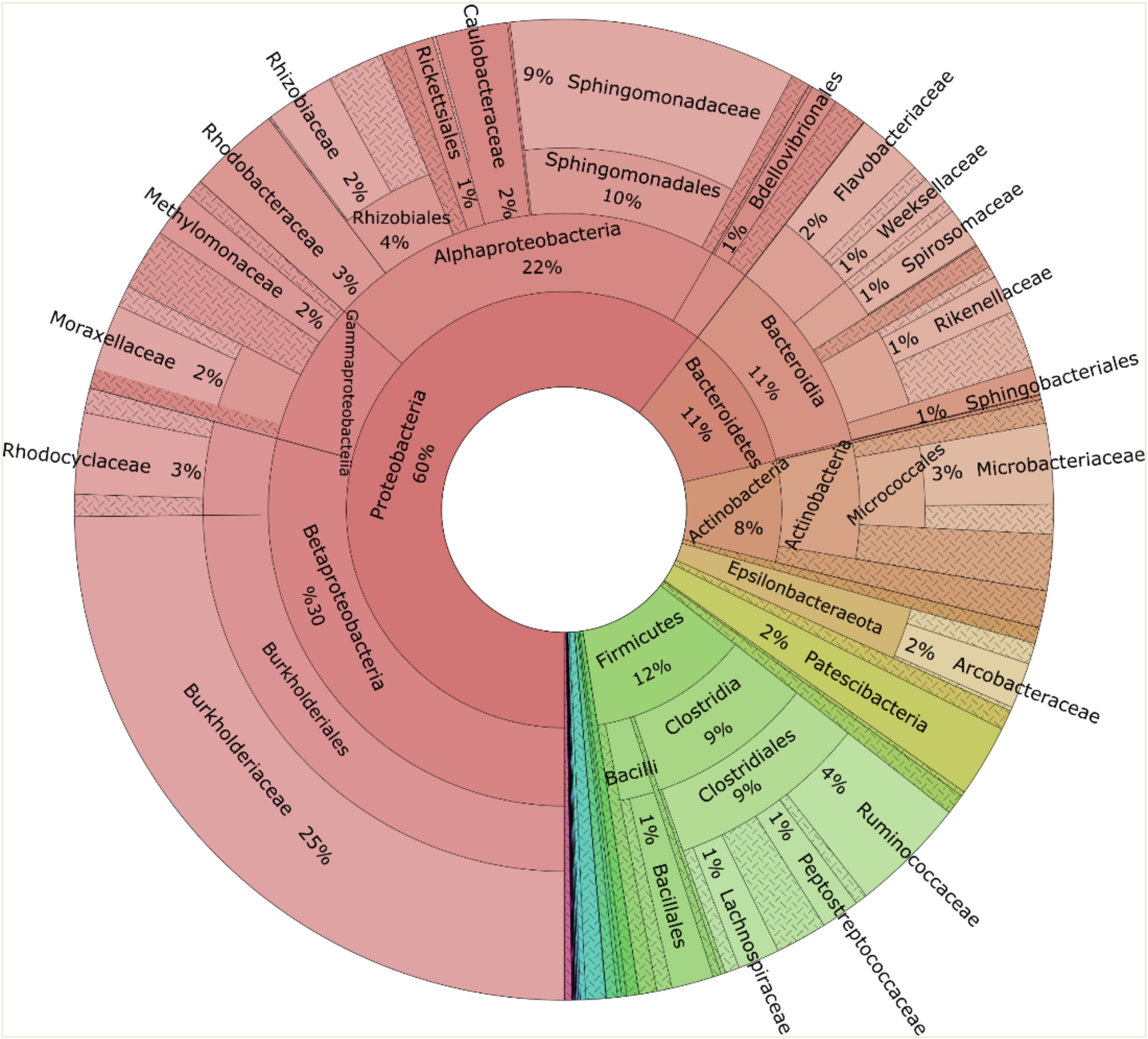

Figure 4. Bacterial community composition in Saka Lake

Table 2 provides an overview of the alpha diversity indices of the samples. Whereas the Chao index was used to reveal the richness of the bacterial community, the Shannon index was used for comparison of diversity. Although Saka Lake was represented with the least number of phyla, it has the highest number of OTUs. Moreover, Shannon and Chao1 indices pointed more diverse bacterial community and bacterial richness in Saka Lake. Among all three samples, Mert Lake had the lowest diversity and species richness.

Table 2. Alpha diversity indices of the samples

Sample	No. of OTUs	Shannon	Chao1
Mert Lake	915	8.2	918
Hamam Lake	1854	9.2	1863
Saka Lake	2309	9.7	2330

The taxonomic analysis revealed that Proteobacteria species were dominated the bacterial communities in the three lakes as well as Actinobacteria members. These results are in keeping with previous observational study, which show these two phyla are the most abundant ones in the freshwater ecosystems (Nakatsu et al., 2019). Betaproteobacteria, one of the well-studied taxonomic classes in freshwater habitats (Newton et al., 2011), was detected as the predominant class in Saka Lake. It was also abundant in Hamam Lake. Some members of this class are enriched at circumneutral to alkaline pH conditions and live in the surface part of the water bodies. This may explain their abundance on those lakes (Newton et al., 2011). Most of the Betaproteobacteria reads were assigned to the family Burkholderiaceae. This family comprises diverse organisms from aerobic and facultative anaerobic chemoorganotrophs to obligate and facultative chemolithotrophs (Coenye, 2014), occupying a diverse ecological niche. On the other hand, most of the sequences could not be accounted for any genera and the abundance of the assigned genus was lower than 1% of the total reads which was not representative. Whereas Flavobacteriales species were found abundant in Mert Lake, the abundance was lower in Hamam Lake and Saka Lake. The members of this order inhabit the freshwater where the phytoplankton blooms take place frequently (Newton et al., 2011). They can break up wide variety of macromolecules and carbohydrates as well as toxins produced during the phytoplankton proliferation (Ozbayram, et al., 2020). On the other hand, Actinobacteria was one of the dominant phyla in the bacterial communities in all lakes. However, at the family level, the dominant families differed in each lake. Whereas Mert Lake dominated by the heterotroph, alkaliphilic, Nitriliruptoraceae species which can use organic nitriles (Sorokin et al., 2009), facultative anaerobic Sporichthyaceae was abundant in Hamam Lake and Microbacteriaceae which includes mesophilic or psychrophilic bacteria (obligately aerobic/facultatively anaerobic) (Evtushenko & Takeuchi, 2006) was found in Saka Lake. The highest abundance of Firmicutes was determined in Saka Lake. Whereas most of the Firmicutes reads were assigned to Clostridia species, Bacilli was also detected. Firmicutes members are quite diverse and have a role in a wide variety of ecosystem functions. Moreover, they have various mechanisms to survive in different environments such as the formation of endospores. The endospore-forming Firmicutes members can be enriched in the environments where a single/multiple stressors occurred for other bacterial taxa (Filippidou et al., 2016). Thus, to better understand the reason for the abundance of Firmicutes members, periodic monitoring should be performed on this lake.

It is known that bacterial communities serve a function in aquatic ecosystems and have interaction between other components of the food web (Kiersztyn et al., 2019). However, to investigate these interactions a comprehensive study should be carried out considering the vertical diversity and other organisms in the foodwebs. Besides this multifarious relationship, the diversities are dependent on various factors including geographical settings and hydrological characteristics (Zwirglmaier et al., 2015) which give them specific microbial structures. Since the size and depth of these lakes are different, giving them particular characteristics, careful attention should be paid to the comparison of community diversity between these different aquatic environments.

The use of bacteria in the biotechnological field has attracted particular attention from the scholarly community for a long period and they have a wide range of application area such as wastewater treatment, green energy production, decomposition of hardly degradable substances, rehabilitation of contaminated sites etc. (Yong et al., 2021). Besides their direct application in the processes, the novel enzymes and bioactive compounds they produced have also great importance in the industry and commercial products. To contribute the technological innovations, the search for new species and novel metabolites are needed be investigated in the undiscovered areas.

Conclusion

In this study, pre-screening of the bacterial communities İğneada Floodplain Forest lakes, Mert Lake, Hamam Lake, and Saka Lake was completed by 16S rRNA targeted amplicon sequencing. Although the current study is based on a small number of samples, the findings suggest an overview of the bacterial diversity in these three lakes.

The results of this research will serve as a base for future studies. Further research should be undertaken to investigate the hidden biotechnological potential of these unique ecosystems.

Compliance with Ethical Standard

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 is not required.

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Research Article

Determination of genetic variations by using mitochondrial DNA cyt b sequences in populations of *Carasobarbus luteus* (Cyprinidae)

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ABSTRACT

As the human population increases, freshwater fish has become an important alternative source of protein to meet the need of protein. Especially numerous fish species from the family Cyprinidae are consumed by the people. Among these fish, *Carasobarbus luteus* (Heckel, 1843) is also one of the most preferred species thanks to its edible flesh as well as its low price. Since it is economically important, there has been the pressure of overfishing and invasive species on the populations of this species, resulting in decrease of the sources day by day. Management and conservation of the species have importance therefore it is need to know its genetic variations in the first place. The present study analyzed sequences of mtDNA cyt b locus and established the genetic variability following the collection of 65 individuals from five different localities in diverse river systems where *C. luteus* populations naturally inhabit. Sequence analysis revealed 13 polymorphic sites and 5 haplotypes. Birecik was the locality with the highest value in terms of both haplotype diversity and nucleotide diversity, Diyarbakır was the one with the lowest value. Tajima's D and Fu's Fs values were found to be statistically insignificant for all the localities. Population genetic diversity of this fish species was found to be low in terms of mtDNA cyt b marker. It is recommended to take measures to stop the loss of genetic diversity and to start conservation studies.

Keywords: *Carasobarbus luteus*, cyt b haplotypes, Genetic diversity, Euphrates River, Tigris River

Introduction

Development of urbanization and agriculture have a gradually increasing impact on both natural habitats and species, decrease the quantity of habitats which are appropriate for the wildlife species (Goudie, 2018; Zhang et. al., 2020). Destruction or change of habitats may lead to decline in the diversity of species and even to extinction of certain species. It has been estimated that genetic diversity has been decreasing faster than diversity of species under increasing number of threats, however its spatial distribution has not been adequately documented on a global scale yet (Manel et. al.,2020). Genetic diversity directly reflects the ability of species or population to adapt in environmental factors of alien habitats (Frankham et al., 2002; Spielman et al., 2004).

Populations in aquatic habitats are frequently under threat because of human pressure such as pollution, harvesting, fishing, alien species, tourism, and urban development (Cognetti and Maltagliati, 2000). *Carasobarbus luteus* which is the subject of our study and a species exposed to those threats is called Bizir or Common carp by the local people. *C. luteus* is a species from Cyprinidae family which is widely distributed in Euphrates, Tigris rivers, natural and artificial lakes in Mesopotamia (Kuru, 1979; Ünlü, 1991; Gökçek and Akyurt, 2008; Coad, 2010). Different studies have been done for this species, some of which are; investigation of reproductive organs and tissues (Rahemo and Al-Shatter, 2012), spermatologic characteristics (Aral et al., 2014), content of digestive system (Çelik and Saler, 2016), reproductive biology (Bilici et al., 2017), parasite studies (Mansoor et al., 2020).

Particularly fishing and dominant status of invasive species are the factors threatening this species most, and lead to decrease in the number of individuals in populations day by day. Decreased individuals in natural populations may result in the extinction of unique genotypes that are not found anywhere else (Parmaksız, 2020). When a genetic data is lost, it is almost impossible to bring it back (Parmaksız, 2020). Therefore, measurements are needed to stop loss of genetic data and to protect future of this species should be taken. For an effective conservation program, firstly there should be reliable genetic data. Analysis of population genetics is a useful tool to acquire knowledge about a species in order to protect it (Ryman, 1991; Ward, 2000). Mitochondrial DNA markers have been utilized in genetic studies on various species (Xia et al., 2016). As compared to nuclear DNA markers, mitochondrial DNA markers are preferred thanks to their unique characteristics such as maternal inheritance, lacking of fast evaluation and recombination. Diverse mtDNA gene sequences can be used to determine the variation in fish species (Saraswat et. al.,2014). Cyt b gene which is an effective molecular marker and has been used to analyze genetic data of several species (Maltagliati et al., 2010; Li et al., 2013; Deng et al., 2014). Variation in mtDNA cyt b gene is utilized for studies for genetic analysis of fish populations from order Cypriniformes (Fayazi et al., 2006). mtDNA cyt-b gene locus has been appeared to be a multipotent genetic marker that can be especially used for genetic variation analysis of fish (Saraswat et. al., 2014).

The goal of the present survey is to identify genetic variation in populations of *C. luteus* naturally inhabiting in river systems of Euphrates and Tigris via gene sequence analysis of mtDNA cyt b locus.

Material and Methods

65 fish samples used as the material of the present study were collected from 5 localities belonging to 2 different river systems. The map of these localities is given in Figure 1. Fish samples were included in the study by random sampling from fishes caught by fishermen at different times. Previous field surveys were utilized for selection of the localities where fish samples would be collected. Accordingly; localities on the river systems of Euphrates and Tigris were concluded as appropriate considering the number of samples in populations, convenience of the land conditions, presence of fishermen in adequate number, and closer distance to city center.

These samples which were purchased from fishermen were kept inside an ice bucket to transfer Zoology Laboratory of Harran University, Faculty of Science-Literature, Department of Biology. Following the identification of species, muscle tissue dissected from the samples was transferred into microcentrifuge tubes with a content of 90% of ethanol and kept at -20 C until DNA was extracted.

Total DNA was isolated from muscle tissue using GeneJET Genomic DNA Purification Kit (Thermo Scientific). To check the presence of DNA after the protocol, DNA samples from all individuals were run placing in the wells of 1% of agarose gel with addition of SYBR Green, monitorized under UV light device (Smart View Pro Imager System, Major Science).

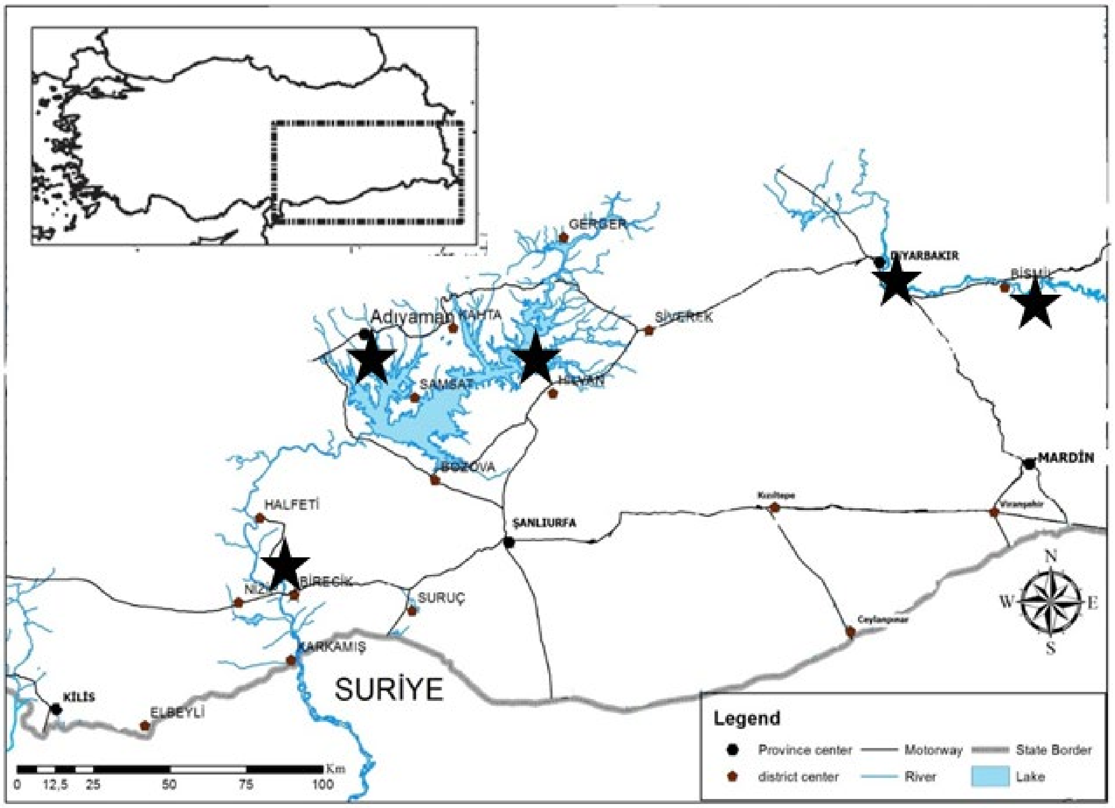

Figure 1. The map indicating the localities where *C. luteus* samples were collected

Target mtDNA cyt b gene locus was amplified by Polymerase Chain Reaction (PCR), the primers were referenced form the study by Briolay et. al., 1998 (L15267 F:5ˈ-GTT TGA TCC CGT TTC GTG TA-3ˈ; H15891 R:5ˈ-AAT GAC TTG AAG AAC CAC CGT-3; Gene bank Accession number: AY026411). Using Thermal Cycler device (BIO-RAD T100^{TM}), target mtDNA cyt b gene site was amplified by optimizing the PCR conditions, concentrations of the chemicals, and annealing temperatures of the primers according to the study of Parmaksız and Şeker (2018). 2% agarose gel was used in order to check the products occurring after PCR process. Agarose gel with addition of SYBR Green was placed in the tank with a content of 0.5x TBE solution, 4 µL of PCR products and 4 µL stain were loaded in the wells together, then were run at 120 V electricity currency for 25 minutes, and monitorized under UV light device. PCR products of target site were sent to a commercial company for sequence analysis with 3500 XL Genetic Analyzer (Thermo Fisher Scientific) device.

Raw data of mtDNA sequences were firstly converted in to FASTA format by evaluating with Chromas Pro v 2.0.1 (Technelysium Pty Ltd) and resulting sequences of all the samples were ranked utilizing BioEdit software version 7.2.5 program. The number of polymorphic sites and haplotypes, diversity of haplotypes and nucleotides, Tajima D and Fu's statistics for the populations were identified by using DNA SP5.10.01 program (Rozas et al., 2003). The phylogenetic relationship between haplotypes was identified via Network version 5.0 program.

Results and Discussion

Genetic Variation

From river systems of Euphrates and Tigris, variable sites and haplotypes were identified using sequence analysis of 586 bp locus on mtDNA cyt b (Figure 2) for a total number of 65 *C. luteus* samples. Variability of nucleotides by haplotypes from this gene site can be seen on Table 1.

Mean nucleotide, Cytosine (C), Timin (T), Adenine (A), and Guanin (G), content for all sequences was calculated as follows; 27.7%, 29%, 28.5%, and 14.8%, respectively.

13 polymorphic sites and 5 haplotypes were identified in a total number of 65 *C. luteus* samples collected from five diverse localities, variations of nucleotides by haplotype are shown on Table 1. Haplotype H1 was found in 54 samples and the most prevalent one. Haplotypes H3, H4, and H5 were identified in only one sample.

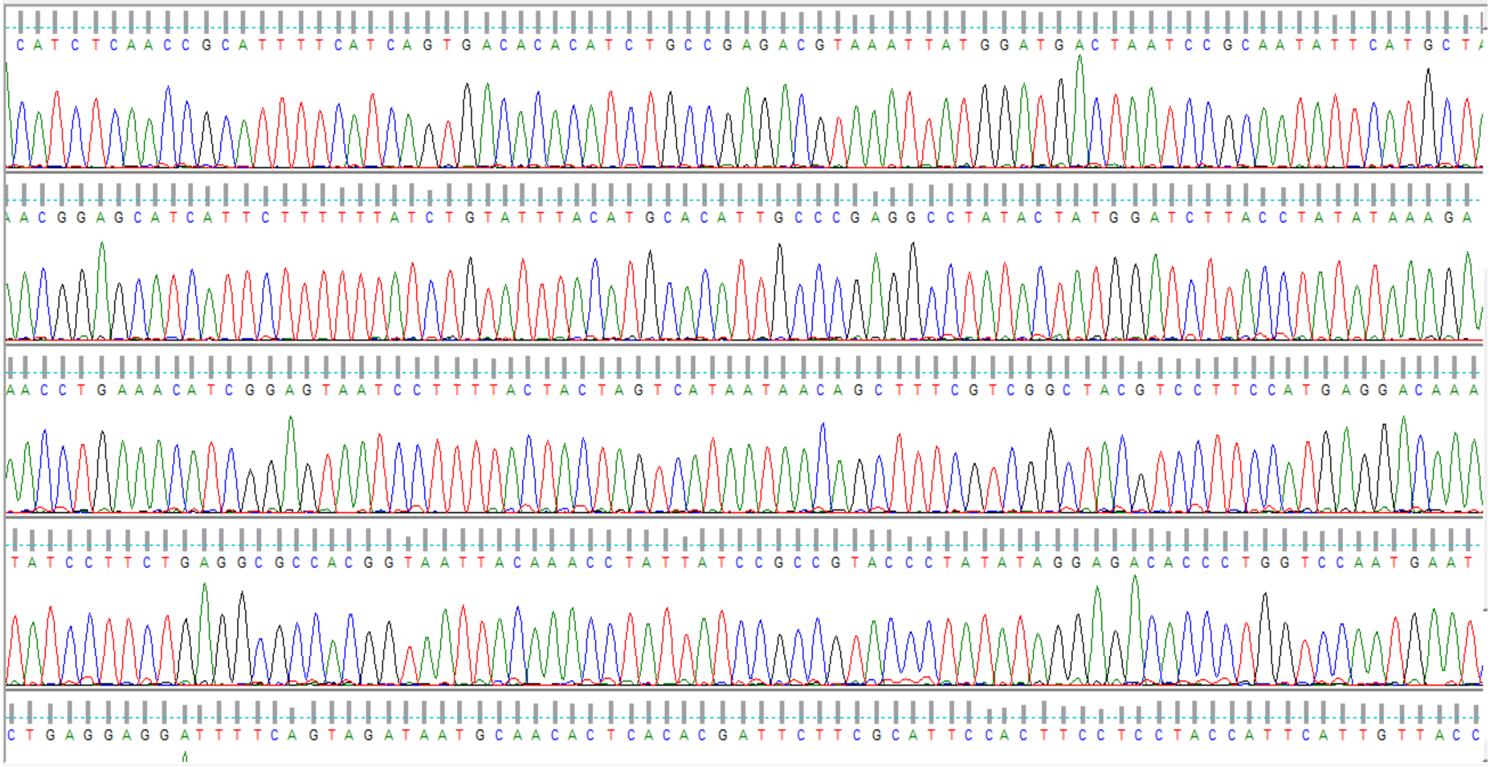

Figure 2. Chromatogram image of a sequence analysis from mtDNA cyt b site

Table 1. Nucleotide diversity and haplotypes of cyt b locus

Haplotype	Polymorphic Sites													Accession number
	117	174	303	324	345	408	424	444	456	474	493	537	541	
H1 (54 samples)	T	G	T	A	C	G	T	A	G	A	G	C	G	MW725236
H2 (8 samples)	C	A	C	G	T	.	.	G	A	G	.	T	A	MW725237
H3 (1 sample)	C	A	C	G	T	A	.	G	A	G	.	T	A	MW725238
H4 (1 sample)	.	.	.	.	.	.	C	.	.	.	.	.	.	MW725239
H5 (1 sample)	C	A	C	G	T	.	.	.	.	G	C	.	.	MW725240

Table 2. Genetic diversity and neutrality tests of localities (N: number of samples, Nh: number of haplotypes, Hd: haplotype diversity, π: nucleotide diversity)

River System	Locality	N	Nh and Haplotype distribution	Hd	π	Tajima's D	Fu's Fs
Euphrates	Adıyaman	16	4 (H1: 13) (H2: 1) (H3: 1) (H5: 1)	0,350	0,00536	-0,51025	2,810
Euphrates	Hilvan	13	2 (H1: 11) (H2: 2)	0,282	0,00481	-0,49831	5,847
Euphrates	Birecik	10	2 (H1: 7) (H2: 3)	0,467	0,00796	1,41919	7,272
Tigris	Diyarbakır	5	1 (H1: 5)	0000	000000	0000000	0000
Tigris	Bismil	21	3 (H1: 18) (H2: 2) (H4: 1)	0,267	0,00325	-1,32016	3,128

As seen on Table 2, haplotype H1 was the only one which was seen in all localities. Haplotype H2, on the other hand, was commonly found in all other localities, except Diyarbakır. H3 and H5 were identified only in Adıyaman, H4 only in Bismil locality. In Adıyaman, Hilvan, Birecik, Diyarbakır, and Bismil localities had; four, two, two and three different haplotypes respectively. The locality with the highest number in terms of both haplotype diversity and nucleotide diversity was Birecik, whereas Diyarbakır was the one with the lowest number. The results of Tajima's D and Fu's Fs neutrality tests were presented on Table 2. Neutrality tests are used to determine whether populations have been selected in the past. The Tajima D test is mostly used to determine natural selection from DNA polymorphism. Neutrality tests are used to determine whether populations have been selected in the past. The Tajima D test is mostly used to detect natural selection from DNA polymorphism and the Fu's Fs test is used to determine population expansion. Tajima's D and Fu's Fs values were determined to be statistically insignificant ($p>0.05$) for all localities.

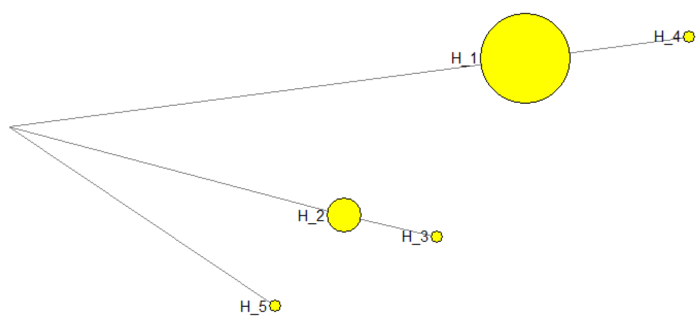

Figure 3. Median -Joining Network of haplotypes acquired after cyt b sequence analysis

Figure 3 shows totally five haplotypes joined on Median-Joining Network created for 65 *C. luteus* samples, resulting network indicates an evolutionary connection. It is also possible to speculate that haplotypes H2-H3 and H1-H4 were connected, haplotype H5 was different from these.

Euphrates and Tigris Rivers have been undergoing considerable change as the result of human activities. Several dams were constructed on these rivers in order to obtain energy and to provide irrigation to agricultural lands and surrounding cities. Thus changes occurring the river bed resulted in dramatic changes for physical, chemical, and biological combination of river. In addition, environmental factors such as industrial factors, intensive fishery, and destruction of habitats would lead to extinction of numerous species or reduction of their populations (Ünlü et al., 1997). Conservation of population size and genetic variability is needed for survival of species. Decrease in the population size results in reduced genetic variability and poses a threat regarding survival chance of population.

It is also known that *Carasius gibelio* and *C. auratus,* which are invasive species in the localities where the samples were collected, has become dominant and has had a negative impact on native species (Parmaksız et. al., 2017). As the result of observations, half of the fish caught in the nets of local fishermen were determined to be invasive species. This particularly puts a great pressure on the fish with economic importance. Because *C. luteus* is consumed by human, it is economically important, populations of this species are also influenced by this situation. Measurements should be taken in order to stop genetic loss of this species and to protect future of this species. To be able to apply an effective conservation program, there must be reliable genotypic data in the first place (Parmaksız and Altundağ, 2018).

Valuable data were provided by Parmaksız and Eskici (2018) using mtDNA COI to evaluate genetic variation of *C. luteus*, by Parmaksız (2020) using mtDNA D-loop sequences to enlighten genetic background of *C. luteus*. The use of multiple genetic marker systems increases the resolution power of genetic studies (Gruenthal et. al., 2007). The present study contributed to acquire further knowledge about genetic structure by analyzing cyt b sequences either. Some studies have been carried out with this marker (cyt b) in different fish species collected from similar localities and haplotypes have been determined (Parmaksız and Seker, 2018; Parmaksız and Altundag, 2018). For *Arabibarbus grypus* populations 5 polymorphic sites and 5 haplotypes were identified by Parmaksız and Seker (2018). For *Achantobrama marmid* populations 4 polymorphic sites and 5 haplotypes were identified Parmaksız and Altundag (2018).

In the present study 13 polymorphic sites and 5 haplotypes were identified following the assessment of cyt b sequences of 65 samples collected from five localities in two different river systems. While haplotypes H3, H4, and H5 were observed in one each sample, haplotype H1 was established as the common haplotype which was seen in 54 samples and all localities. Total haplotype diversity (Hd) was 0.299; Nucleotide diversity ($\boldsymbol{\pi}$) was 0,00453. Studies conducted in similar localities determined a total number of 7 haplotypes for D-loop locus, haplotype diversity (Hd) was 0.373; Nucleotide diversity ($\boldsymbol{\pi}$) was 0,00453 (Parmaksız, 2020); for haplotype were identified for COI locus haplotype diversity (Hd) was 0.534; Nucleotide diversity ($\boldsymbol{\pi}$) was 0,00367 (Parmaksız and Eskici, 2018). Results of the present study and other two surveys were in parallel. Haplotype and nucleotide diversity are important indicators of genetic variation, as higher values indicate higher genetic variation (Falush et. al., 2003; Liu, 2017). Higher levels of genetic diversity reflect strong adaptation and survival abilities of populations (Barrett and Schluter, 2008). As seen, haplotype and nucleotide diversity of this fish species were found to be lower with respect to mtDNA markers.

Conclusion

It is crucial to take measurements to stop loss of genetic diversity and to start conservation studies. Firstly, invasive species should be controlled and excessive fishing must be prevented. In case of failure to take measurements, the level of genetic diversity will decrease further, feeding, reproduction, competition, and adaptation abilities of populations will decline too and target organism will be under threat of extinction.

Compliance with Ethical Standard

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 is not required.

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Disclosure: -

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Research Article

Incubation temperatures, hatching success and congenital anomalies in green turtle nests from Guanahacabibes Peninsula, Cuba

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ABSTRACT

Elevated incubation temperatures of sea turtle nests decrease hatching success and alter the resulting hatchlings' morphology. There is an absence of studies assessing the relationships between temperature and hatching success in Cuba, even when they could improve understanding the limits of thermal tolerance in these species. This study evaluated the influence of incubation temperature on hatching success and phenotypic malformations in green turtle hatchlings (*Chelonia mydas*); and analyzed the temporal variation in hatching success on the studied beaches. In 48 green turtles nests distributed along two beaches, incubation temperature and hatching success were recorded between 2014 and 2019. Increasing incubation temperature caused a decrease in the hatching success and an increase in the frequency of supernumerary scutes. Despite the elevated temperatures (average > 30°C), hatching was higher than 80%. Significant differences in hatching success were only observed among seasons for nests in Antonio Beach (lower values in 2016 and 2019 compared to 2014).

Keywords: Marine turtles, Climate change, Temperatures, Hatching success, Phenotypic malformations

Introduction

In the last century, an excessive increase in the global temperature has been observed (IPCC, 2018). This increase endangers many species; including sea turtles, because it affects hatchlings' phenotype (Glen et al. 2003), hatching success (Broderick et al., 2001; Weber et al., 2012) and sex proportion (Laloë et al., 2016).

The maximum thermal limit for successful incubation is not adequately defined (Howard et al. 2014); assessing this value allows identifying sea turtle populations at risk of embryonic mortality due to possible increases in global temperatures. For green turtles, Miller (1997) defined a temperature of 33ºC as the maximum thermal limit for hatching to occur, while Weber et al. (2012) reported at a temperature of 33.4ºC a hatching success between 25 and 57%. However, Tilley et al. (2019) reported a hatching success of 71% at an average temperature of 33 ºC. Laloë et al. (2017), indicated for the Cape Verde rookery that by the year 2100, under the least favorable climate change scenario, hatchlings' emergence success would be reduced to 49.1%.

In addition, nest temperature influences hatchling' locomotor performance (Booth et al., 2012) and increase the frequency of congenital anomalies (Wyneken and Salmon, 2020). These morphological alterations can range from changes in the pattern of carapace scutes which are characteristic to each sea turtle species (Zimm et al., 2017), to other anomalies, such as the absence of a tail, deformations of the carapace, modifications or absence of eyes, and albinism (Wyneken & Salmon, 2020).

Monitoring nest temperatures and hatching success provides essential information for the conservation and management of sea turtles. Changes in hatching success may indicate variation occurring in some of the influencing factors (Miller et al., 2000). A significant decrease in hatching success over time would lead to reductions in population size (Saba et al., 2012; Santidrián et al., 2015).

Temperature studies in Cuba have been focused on rookeries in the Guanahacabibes Peninsula and the San Felipe Keys (Gerhartz et al., 2018 and Calderón et al., 2020). High temperatures in nests were found in both areas. Also, studies carried out by Azanza et al. (2008) with green turtle in the Guanahacabibes Peninsula, show low embryonic mortality and an emergence success rate over 80%, but with high levels of congenital anomalies. None of these studies attempted to investigate the relationship between nest temperatures and hatching success or phenotypic abnormalities in the hatchlings. Therefore, the objective of this study was to evaluate the influence of nest temperature on hatching success and the incidence of hatchling' phenotypic abnormalities.

Material and Methods

Study Area

The work was carried out from 2010 to 2019 during the months of June to September in the Guanahacabibes Peninsula (Fig. 1), one of the most important nesting colonies in southwestern Cuba (Moncada et al., 2011). Two of the index beaches located on the southern coast of the peninsula, Antonio (21.90 N; -84.66 W) and La Barca (21.85 W; -84.76 N), were selected (Fig. 1).

Data Collection

To monitor incubation temperatures, two types of dataloggers (HOBO U12 and Pendant® 8K-UA-001-08) were placed in the center of 14 nests on Antonio Beach and in 34 nests on La Barca Beach. HOBO U12 has an operating range from -20° to 70°C (temperature) and an accuracy of ± 0.35°C from 0° to 50°C and Pendant® 8K-UA-001-08 has an operating range from -20° to 70°C and an accuracy of ± 0.53°C from 0° to 50°C. They were introduced during the oviposition and programmed to register every two hours synchronously. All sensor information was downloaded using HOBOware Pro version 3.2.1 software (Onset Computer Corporation). Incubation duration was calculated according to the criteria stated in Calderón et al. (2020): the number of days between the night of laying and the night the first hatchlings emerged (Godley et al. 2002) minus the average number of days (four) that hatchlings spent in the egg chambers. Subsequently, average daily nest temperature and the average over the entire incubation period were calculated for each nest. In addition, each incubation period was divided into three equal thirds for analysis for which the average temperatures were also calculated.

Nest exhumation was performed three days after the emergence of the hatchlings and the number of empty eggs shells and unhatched eggs was counted. Hatching success was defined as the percentage of hatched eggs (assumed from the number of shells) with respect to the total number of eggs in the nest (sum of the number of shells and unhatched eggs). The number of nests that were evaluated for hatching success by seasons is reflected in Table 4.

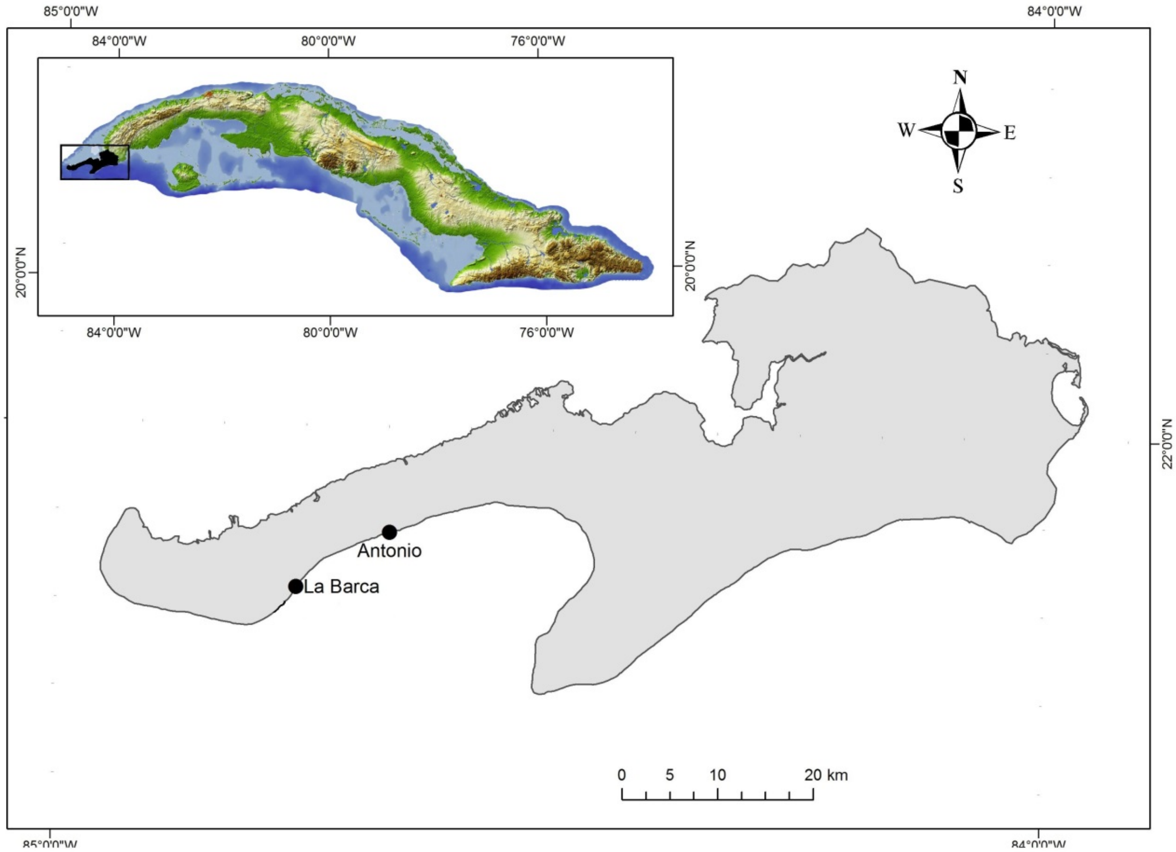

Figure 1. Location of sea turtle nesting beaches in the Guanahacabibes Peninsula National Park and Biosphere Reserve, Cuba. Antonio and La Barca, the index beaches where the study was carried out, are highlighted in black

In order to evaluate the influence of temperature on congenital anomalies, the number of hatchlings with supernumerary vertebral and costal shell scutes and carapace malformations (compressed carapace) was counted in 17 nests with sensors and another set of 109 nests without sensors at La Barca Beach. For each nest, the percentage of hatchlings with each malformation was calculated. These malformations were selected because they are the most frequent in the Guanahacabibes rookeries (Azanza et al., 2008). Hatchlings' morphological anomalies stated above were only related to the temperature of the last third of embryonic development, because according to Miller et al. (2017), it is during this stage that the formation of the shell and scales occur.

Statistical Analysis

Spearman correlation was used to evaluate the influence of average incubation temperature on hatching success for the entire period and during each incubation third. This test was also used to evaluate relationships between incubation temperature during the last third with the percentages of carapace malformations and supernumerary scutes. Differences in hatching success between seasons were determined with Kruskal-Wallis test followed by a non-parametric means' comparison test. Statistica version 8.0 software was used to carry out the statistical tests with a 0.05 value of significance.

Results and Discussion

According to Howard et al. (2014), the maximum limit for successful incubation is not adequately defined. In this study, the average temperature of the incubation period was 31.33 ±0.51°C (30.5 - 32.7°C) while average temperature during the last third of embryonic development was 33.23 ±0.76°C (32 - 35°C). Despite high temperatures, average hatching success per season was higher than 87%, with the exception of the nests of the 2016 in La Barca Beach, (Table 1). With temperature as high as 35°C during the last third of incubation, still, two nests in La Barca Beach presented hatching success higher than 94%.

Hatching success reported in this study is higher than in other nesting areas for a given temperature value. Table 2 shows the marked variation in hatching success between incubation temperatures, even in studies carried out on the same nesting beaches. A negative correlation was found between the average temperature during the entire incubation and during the last third with hatching success. At La Barca Beach, the relationship found between hatching success and temperature was low, while in Antonio was higher. The temperature of the second third was not related to hatching success on any of the beaches. At the same time, higher temperatures during the first third of embryonic development only caused a decrease in hatching success at Antonio Beach. In both beaches, the temperature of the last third was the one that best explained the decrease in hatching success (Table 3). This is the period of the development with the highest temperatures because of the metabolic heating (Sonmez, 2018).

Table 1. Temperature and hatching success of green turtle nests analyzed by seasons in the Guanahacabibes Peninsula, Cuba

Beach	Nesting season	N	TPI Mean ± SD	T3 Mean ± SD	HS Mean ± SD
La Barca	2014	6	31.04± 0.44	32.5 ± 0.54	93.03 ± 4.38
	2015	7	31.44± 0.60	33.51±0.91	95.51 ± 2.72
	2016	2	32.66 ±1.12	35.29± 1.52	82.61± 6.20
	2017	3	31.32± 0.47	33.12±0.71	90.93± 9.14
	2018	5	31.22± 0.31	32.97± 0.67	94.09 ± 3.34
	2019	11	31.87± 0.69	34.11±0.75	90.26± 6.18
	Total	34	31.59± 0.61	33.58± 0.85	91.07± 5.33
Antonio	2014	4	30.87± 0.35	32.43± 0.66	95.45± 3.74
	2015	1	30.96	32.64	100
	2016	2	31.36± 0.89	33.43± 1.33	90.55± 1.34
	2018	3	30.65± 0.28	32.22± 0.67	96.15± 3.16
	2019	4	31.34± 0.45	33.35± 0.60	87.96±4.76
	Total	14	31.04± 0.49	32.81± 0.81	94.02± 3.25

Temperature of the entire incubation period (TPI), incubation temperature during the last third (T3), number of nests (N), hatching success (HS), standard deviation (SD)

Table 2. Hatching success in green turtle nests, reported at different incubation temperatures on different nesting beaches

Reference	Location	TPI Mean ± SD (SEM) (°C)	Hatching Success Mean ± SD (%)
Broderick et al. (2001)	Long Beach	29.5 ±0.10	85 ±0.16
	NEB	32.2 ±0.18	57 ±0.23
Weber et al. (2012)	Long Beach	31	< 80
	NEB	33	< 60
Santidrián et al. (2017)	Cabuyal, Costa Rica	31.2 ±1.2	87 ±19
Tilley et al. (2019)	Long Beach	31 ±0.6	91 ±7
	NEB	33 ±0.9	71 ±18
Stewart et al. (2020)	Redang Island, Malaysia	31.9 ±0.2	80.2 ±6.4

Standard deviation (SD); Standard error of the mean (SEM); Temperature of the entire incubation period (TPI)

Table 3. Correlation between incubation temperature, during different stages of embryonic development, and hatching success in green turtle nests in the Guanahacabibes Peninsula, Cuba

Period	Antonio	La Barca
First third	r =-0.56*	r =-0.21 ns
Second third	r =-0.23 ns	r =-0.26 ns
Last third	r =-0.60*	r =-0.42*
Incubation temperature	r =-0.49 ns	r =-0.40*

*p<0.05

The decrease in hatching success with the increase in temperature during the first and last third found in Antonio Beach coincides with that found by Weber et al. (2012) in nests of *C. mydas* and by Kobayashi et al. (2017) in loggerhead (*Caretta caretta*) nests. These authors found no relationship between the temperature of the second third and the embryonic mortality recorded during this period. Bladow & Milton (2019) observed an increase in embryonic mortality in green turtle nests only in the final stages of embryonic development, similar to La Barca Beach.

The low relationship found between hatching success and nest temperature at La Barca Beach may be due to the fact that hatching success is influenced by other factors such as water availability (Erb et al., 2018), gas concentrations (Chen et al., 2010), presence of vegetation (Cabrera et al., 2019) and sand grain size (Ackerman, 1977; Stewart et al., 2020).

Hatching success did not show a difference between seasons at La Barca Beach ($H_{(9, N = 370)} = 14.99$ $p = 0.09$), but there was difference in Antonio Beach ($H_{(6; N= 93)} = 18.90$; $p = 0.004$). At this beach, only the year 2014 differed from 2016 and 2019. In most of the seasons, hatching success was higher than 85% (Table 4). Miller et al. (2000), suggests that temporary changes in hatching success could be related with changes in abiotic and biotic factors, therefore, lack of variation might be related with stable environment conditions during analyzed seasons. The fact that the increase in nesting temperatures observed in recent years by Calderón et al. (2020) at Guanahacabibes beaches is not equally reflected in hatching success, could be the result, not only of the thermal tolerance of embryos in nests with high temperatures, but the influence of other abiotic factors such as moisture, sand characteristics (Erb et al., 2018) and biotic factors like nest site selection (Weber et al. 2018) that might be compensating the effect of high temperatures.

Hatching success observed per season in Guanahacabibes is higher than reported in many nesting areas (Table 5). Wide ranges of beach characteristics have been evaluated in literature to determine their effect on nest site selection and hatching success (Turkozan et al., 2011; Ditmer & Stapleton, 2012). In the case of Guanahacabibes, it seems that slope (preventing erosion and nest flooding) together with sand characteristics (color and grain size) guarantee proper embryo development. Vegetation presence and species is also identified as a determinant factor for hatching success in this area (Cabrera et al., 2019).

Table 4. Hatching success per season in green turtle nests on Antonio and La Barca beaches

Beach	Nesting season	N(N *100/ total nests of the beach)	Hatching success Mean ± SD
La Barca	2010	40(38.84)	89.36 ± 13.6
	2011	44 (36.98)	76.06 ± 32.97
	2012	10 (9.74)	96.24 ± 4.21
	2013	18 (8.11)	91.02 ± 5.40
	2014	21 (34.4)	87.94 ± 9.21
	2015	100 (40.98)	89.59 ± 11.95
	2016	24 (21.42)	82.45 ± 21.06
	2017	32 (14.29)	87.86 ± 19.27
	2018	17 (21.79)	90.76 ± 7.53
	2019	64 (27.11)	86.32 ± 13.91
	Total	**370**	**87.76 ± 13.91**
Antonio	2010	11 (28.20)	86.39 ± 13.68
	2014	11 (52.38)	95.94 ± 3.55
	2015	14 (22.95)	88.71 ± 13.52
	2016	12 (20.33)	84.92 ± 10.58
	2017	15 (9.67)	87.99 ± 10.01
	2018	10 (22.73)	93.92 ± 4.51
	2019	20 (19.42)	81.14 ± 16.18
	Total	**93**	**88.43 ± 10.29**

Standard deviation (SD); Number of nests (N) in parentheses is the percentage that represents the number of nests analyzed with respect to the total nests of the season

Table 5. Hatching success in green turtle nests by nesting season in different study beaches. Some studies report hatching success and standard deviation (SD), others reflect standard error (SEM), and others only report hatching success

Reference	Location	Hatching success (%) Mean ± SD/ (SEM)	Years monitored
Cheng et al. (2008)	Lanyu Island, Taiwan	80.7 ±27.8	1997-2006
	Wan-an Island	72.2 ±30.2	1997-2006
Bellini et al. (2013)	Atol das Rocas, Brazil	72	1993
		78.6	1994
		74.1	1995
		70.1	1996
		70.1	1997
Zárate et al. (2013)	Galápagos Islands	46 ±33.4	2004-2007
Xavier et al. (2006)	Cucuyo, México	92	2002
		89	2003
		86	2004
Turkozan et al. (2011)	Akyatan, Turkey	58 - 67	2006-2009
Almeida et al. (2011)	Trindade Is, Brazil	84.4 ±21.5	---------
Brost et al. (2015)	Florida	54.9 ±(8.2)	2002- 2012
Bladow y Milton (2019)	Boca de ratón, Florida	54.82 ±(6.94)	2016
		55.02 ±(2.30)	2017

Effect of Temperature on Malformations

Many authors have evaluated the effect of biotic and abiotic factors on the morphology of hatchlings. Both the maternal origin and the conditions of the nest influence their morphology (Glen et al., 2003; Booth et al., 2012). On this matter, relocation of nests also impacts embryos morphology. Sonmez (2019) found significant differences in plastral scutes between natural and relocated nests while Sonmez et al. (2011) found significant differences in nuchal, costal and marginal scutes as well as in their straight carapace width, weight, and length of their fore limbs. Congenital malformations can reduce the emergence of hatchlings (Craven et al., 2019). According to Wyneken & Salmon (2020), if the young successfully emerge from the nest and reach the sea, their survival chances are lower due to the existence of anomalies or other defects caused by high temperatures.

As shown in Figure 2, a positive relationship was found between the temperature presented during the last third of embryonic development and the frequency of neonates with supernumerary scutes ($r = 0.70$; $p < 0.01$). However, the increase in temperature in the nests of this species did not seem to influence the malformations of the carapace in the neonates $r = -0.22$; $p > 0.05$.

The increase in the number of scutes due to the increase in temperature found in this study coincides with that described by Zimm et al. (2017). The increase in nest temperature increases the speed at which embryonic development occurs resulting in a decrease in the duration of incubation (Miller et al., 2017). This increase in the speed of embryonic development may be the cause of the appearance of errors in the process of formation of the scutes.

Of the 126 nests analyzed, malformations in the carapace were less frequent than supernumerary shells (Table 6). Barcenas-Ibarra et al. (2015) and Azanza et al. (2008) report supernumerary shells as the most frequent anomalies found in this species.

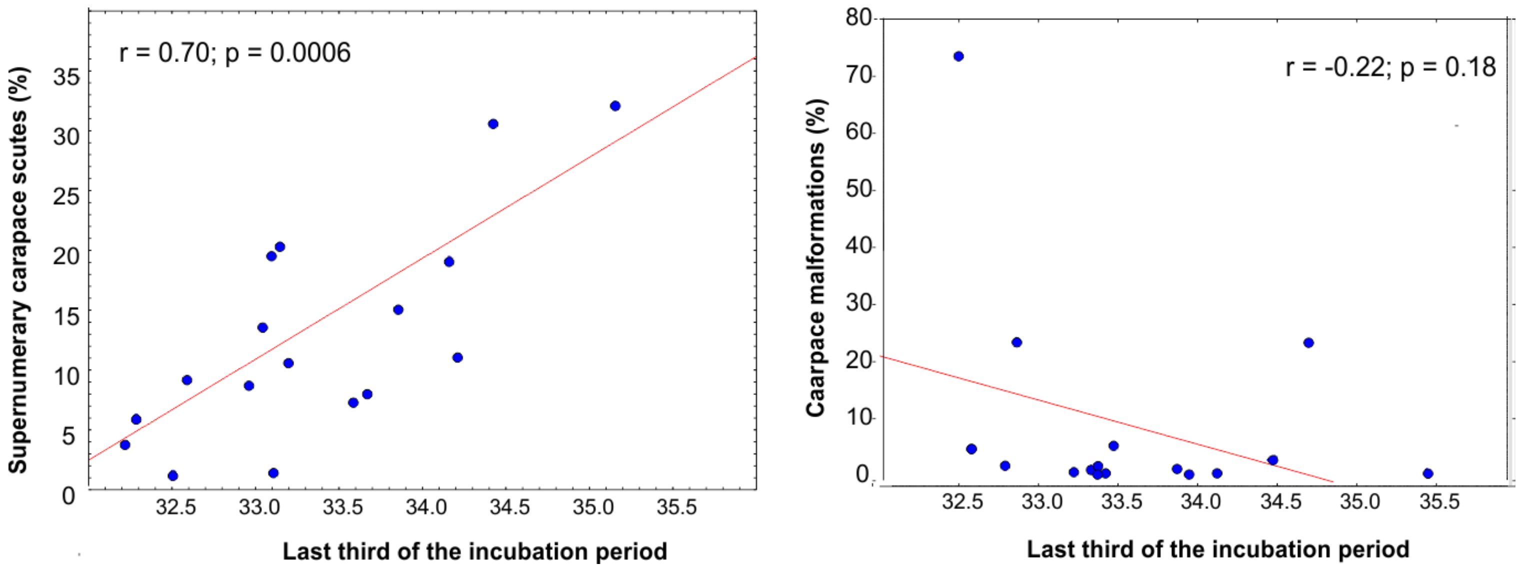

Figure 2. Relationship between the temperature of the last third of embryonic development and the main phenotypic anomalies reported in Guanahacabibes. Left: supernumerary scutes and right: malformations in the carapace

Table 6. Percentage of the most frequent malformations found in *Chelonia mydas* nests in the Guanahacabibes Peninsula, Cuba

	Mean ± SD	Min	Max
Carapace malformations (%)	2.11 ±5.99	0.00	41.67
Supernumerary scutes (%)	9.16 ±14.30	0.00	86.11

Standard deviation (SD)

Conclusion

High hatching success recorded in this study, despite high temperature; demonstrate the thermal resistance of green turtle hatchlings on the beaches analyzed. However, high temperatures, especially last third of embryonic development, did affect hatching success and caused alterations in the characteristic shell pattern of this species.

Compliance with Ethical Standard

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

Ethics committee approval: There was not experimentation with animals in this research since the study was observational. Authorization to access natural areas was provided by the Ministry of Science, Technology and Environment.

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Disclosure: -

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İşlenmiş alabalık yumurtalarının raf ömrüne katkı maddelerinin etkisi

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ÖZ

Yetiştiricilik alanındaki, tür çeşitliliğinin doğal kaynaklarla karşılaştırılmayacak kadar az olması, yetiştiriciliği yapılan balıklarda arz ve talebe bağlı bölgesel veya aşırı/dengesiz fiyat düşüşlerine sebep olmaktadır. Bu fiyat düşüşlerinin aksine porsiyonluk balık üretim maliyetleri ise yıldan yıla artmaktadır. Bu durum özellikle Avrupa'nın en büyük üreticisi olan Türkiye'de alabalık fiyatlarını etkilemektedir. Türkiye tüketim piyasası için uzun raf ömrüne sahip delikat (lezzetli-nefis) ürünlerin geliştirilmesi önemlidir. Bu çalışmada da işlenmiş gökkuşağı alabalık yumurtalarının daha uzun dayanım raf ömrünü belirlemek üzere katkı maddelerinin etkisi incelenmiştir. Çalışmada sağım yoluyla temin edilen gökkuşağı alabalığı yumurtaları %10'luk salamura ile temizlendikten sonra 40 saat boyunca kuru tuzlama (%7.5) yapılmıştır. Ardından %5'lik tuz salamurası ile yıkanmış, kuruma işleminden sonra muamelesiz (kontrol) bırakılan ve %1 oranlarında sıvı duman, kekik, limon, keten veya üzüm çekirdeği yağları ile muamele edilen toplam 6 gruba ayrılmıştır. Bu şekilde ayrılan balık yumurtaları cam kavanozlara yerleştirilmiş ve ısıl işlem uygulaması yapılmadan buzdolabı koşullarında depolanmıştır. Sıvı duman ve esansiyel yağ uygulanan ürünlerin duyusal analizleri sonucunda panelistler tarafından olumlu geri dönüşler alınmıştır. Sıvı duman uygulanan grup mikrobiyolojik açıdan en güvenli gruptur ve analiz süresi boyunca tüketilebilir özelliklerini kaybetmemiştir. Üzüm çekirdeği yağı uygulanan işlenmiş alabalık yumurtalarının raf ömrü 50 gün, kekik yağı uygulanan grubun 30 gün, keten ve limon yağı uygulanan grupların ise raf ömrü 10 gün olarak belirlenmiştir.

Anahtar Kelimeler: Alabalık, *Oncorhynchus mykiss,* Balık yumurtası, Sıvı duman, Esansiyel yağlar, Katkı maddeleri, Raf ömrü

ABSTRACT

The effect of additives on the shelf life of processed trout eggs

The fact that the diversity of species in the aquaculture sector is too low to compare with natural resources causes regional or excessive/unbalanced price decreases in aquaculture depending on supply and demand. In contrast to these price decreases, portion fish production costs are increasing year by year. This situation affects trout prices especially in Turkey, which is the biggest producer in Europe. It is important to develop delicate (tasty-delicate) products with a long shelf life for the Turkish consumer market. In this study, the effects of natural and synthetic additives were investigated to determine the longer shelf life of processed rainbow trout eggs. In this study, rainbow trout eggs obtained by stripping were cleaned with 10% brine, followed by dry salting (7.5%) for 40 hours. Then they were washed with 5% salt brine, left untreated (control) after drying, and divided into 6 groups, which were treated with 1% liquid smoke, thyme, lemon, flax, or grapeseed oils. The rainbow trout eggs were placed in glass jars and stored under refrigerator conditions without heat treatment. According to the sensory analysis results of the liquid smoke and essential oil applied products positive feedback has been received from the panelists. The liquid smoke applied group was the safest group in terms of microbiology and did not lose its consumable properties during the analysis period. The shelf life of processed trout eggs to which grapeseed oil was applied was 50 days, 30 days for the group treated with oregano oil, 10 days for the groups treated with flax and lemon oil.

Keywords: Trout, *Oncorhynchus mykiss,* Fish eggs, Essential oils, Liquid smoke, Additives, Shelf life

Giriş

Su ürünleri tüketiminde işlenmiş balık yumurtası besin bileşimi ve gastronomik özellikleri bakımından tüketici nezdinde talep gören besin kaynaklarından biridir. Geleneksel işlenmiş balık yumurtalarından yapılan ürünler (mersin havyarı, mumlu kefal yumurtası, tuzlanmış turna ve sazan yumurtaları vs.) dünyada olduğu gibi Türkiye' de de (ağırlıklı kıyı şeritlerinde) katma değeri yüksek ve gurme tercihler nezdinde tüketimi olan gıda maddeleridir. Giderek artan su ürünleri yetiştiricilik çalışmalarına bağlı olarak, bu tarz geleneksel ürünlere farklı balık yumurtalarının da işlenmesi eklenmiş ve pazara sunulmuştur.

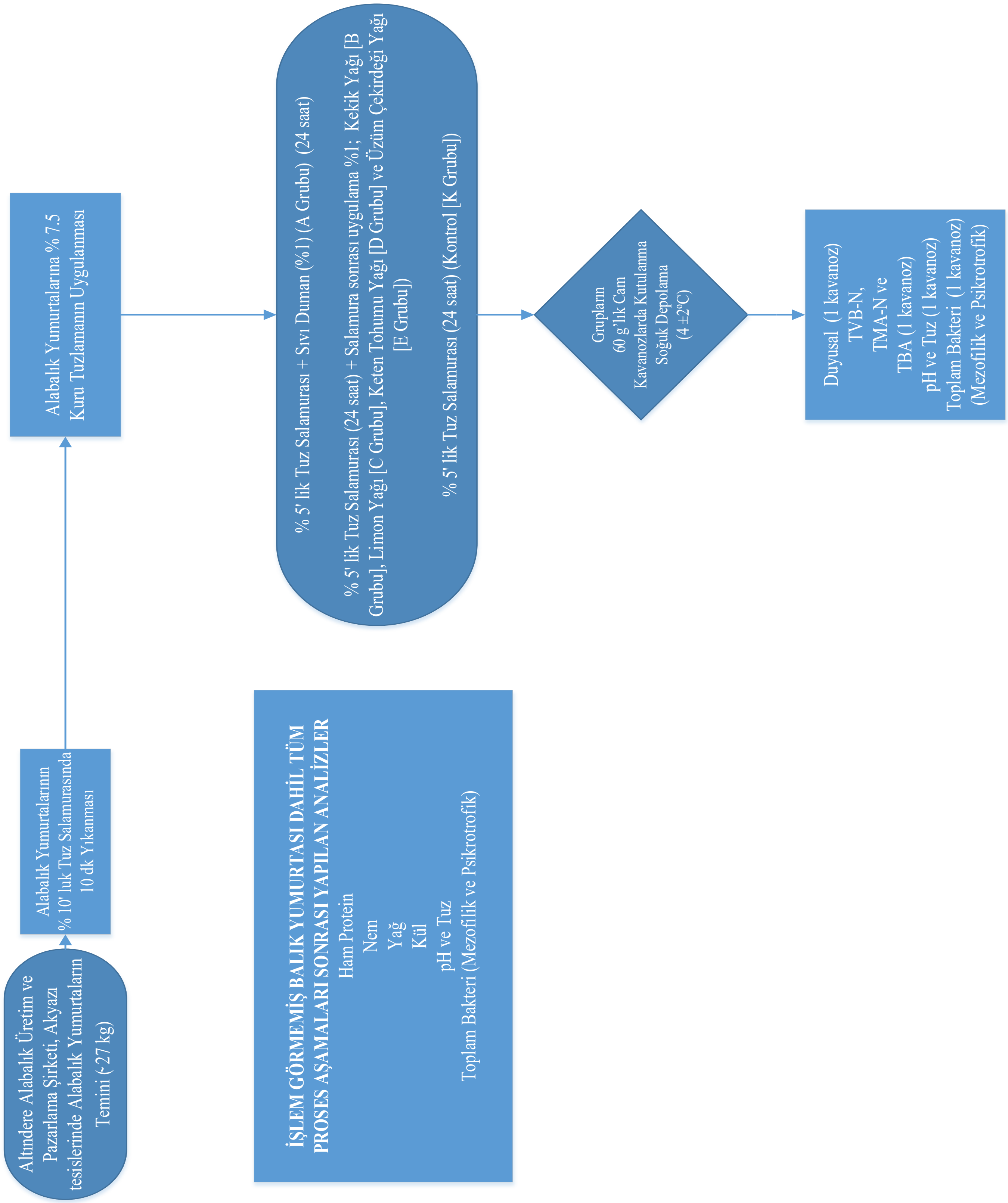
Salmo salar, Oncorhynchus kisutch ve *Oncorhynchus mykiss* yetiştiriciliği yapılan balıklar arasında en çok yumurtaları işlenen türlerdir. Bu anlamda mersin havyarı gibi popülarite kazanarak Rusya ve Japonya'da "Ikura" olarak adlandırılan özel ürünlere dönüşmüştür (Bledsoe ve Rasco, 2006). Aynı zamanda alabalık yumurtalarının işlenmesi mersin balığı türlerinin korunmasına yönelik bir faaliyet olarak değerlendirilmekte ve bu işlemle elde edilen ürün mersin balığından elde edilen havyara göre daha erişilebilir bir arza yardımcı olmaktadır (Machado ve diğ. 2015). Alabalıktan yapılan işlenmiş yumurta ürünleri, bölgesel üreticiler için ekonomik açıdan daha ucuz ve karlı olduğundan ürün çeşitliliğinin oluşumuna da katkı sağlamaktadır. Bazı kaynaklarda işlenmiş alabalık yumurtalarının mersin balığı havyarıyla mukayesesinde tane büyüklüğü bakımından daha büyük ve tat ve koku bakımından daha lezzetli olduğu da belirtilmektedir (Villegas, 2014). Salmonidae familyasından elde edilen bu ürünlerin renk skalası balığın beslenmesine bağlı olarak sarıdan-kırmızıya (somon rengi dahil) kadar değişik varyasyonlar sunmaktadır. Bu çeşitlilik ve son yıllardaki bu ürünlerin piyasalardaki arzı tüketici grupları tarafından benimsenmesine ve tüketim artışına katkı sağlamaktadır.

Güvenli olmayan gıda ve su tüketimi yaklaşık olarak 2 milyon insanın ölümüyle dünyada ilişkilendirilmektedir (WHO, 2015). Bu anlamda gıda güvenliğine yönelik doğal ve yapay gıda katkılarının kullanımı değerlendirilmektedir. Tüketicilerin genel tercihi ise ağırlıklı yapay koruyucu katkı maddeleri içermeyen gıdalara yönelik olmaktadır (Holley ve Patel, 2005; Erkan ve Bilen, 2010; Mastromatteo ve diğ. 2011). Tüketici nezdinde gıda güvenliğine yönelik istenen raf ömrü beklentilerinin karşılana bilmesi için engel teknolojileriyle birlikte kullanılabilir yeni alternatif yöntemlerin değerlendirilmesini gerektirmektedir (Burt, 2004). Bu ürünlerde pastörizasyon uygulaması ile daha uzun süreli raf ömrü kazandırılabilirken aroma/tat üzerindeki değişimlerde olumsuz etki söz konusu olabilmektedir. Ayrıca tuz konsantrasyonu ve depolamadaki sıcaklık, işlenmiş yumurtaların korunmasında her zaman etkili değildir (Fioretto ve diğ. 2005). Son dönemlerde ise gıdaları korumaya ve raf ömrü artışına yönelik bitkisel kökenli katkı maddeleri uygulamaları konusunda artan bir ilgi vardır (Holley ve Patel, 2005).

Yapılan bu çalışma ile ülkemizde yüksek üretim potansiyeline sahip olan ve sektör için yeni bir ihracat alternatifi oluşturabilecek gökkuşağı alabalığının (*Oncorhyncus mykiss Walbaum*, 1792) işlenmiş yumurtalarında katkı maddesi sıvı duman ile kekik, limon, keten ve üzüm çekirdeği esansiyel yağlarının kullanım etkilerinin araştırılması amaçlanmıştır.

Materyal ve Metot

Çalışmada kullanılan alabalık (*Oncorhynchus mykiss* Walbaum, 1792) yumurtaları Altındere Alabalık Üretim ve Pazarlama Şirketi'nden (Akyazı/Sakarya), toplam 41 balıktan sağım yoluyla 26.95 kg olarak temin edilmiştir. İlgili tesisten alınan yumurtalar İstanbul Üniversitesi Su Bilimleri Fakültesi İşleme Teknolojisi Anabilim Dalı Laboratuvarlarına soğuk zincir koşullarında nakledilerek ürün işleme ve analiz çalışmaları yapılmıştır.



Şekil 1. İşlenmiş alabalık yumurtalarının çalışma hazırlıkları ve tasarımı

Figure 1. Study preparations and design of processed trout eggs

Toplam tuz içeriği Varlık ve diğ. (2007), besin bileşimi analizlerinden protein tayini AOAC 928.08 Kjeldahl metoduyla (AOAC, 1998a), yağ analizi Weilmeier ve Regenstein (2004) tarafından önerilen metodun Özden ve diğ. (2007) tarafından modifiye edilmiş versiyonu kullanılarak, nem analizi AOAC 980.46 (1998b)' ya göre, kül analizi AOAC 938.08 (1998c) metoduna göre yapılmıştır. Antonacopoulas ve Vyncke (1989) tarafından verilen yönteme göre toplam uçucu bazik azot (TVB-N) analizi yapılmıştır. trimetilamin (TMA-N) ve tiyobarbütirik asit (TBA) analizi Erkan ve Özden'e (2008) göre yapılarak hesaplaması standartların eğrileri üzerinden oluşturulan denklem vasıtasıyla seyreltme faktörünün kullanımıyla yapılmıştır. pH Vyncke, 1981 göre gerçekleştirilmiştir. Toplam mezofilik ve psikrotrofik aerobik bakteri sayımı için FDA'nın (1984) dökme plak yöntemi kullanılmak suretiyle steril kabin içerisinde gerçekleştirilmiştir. Alabalık yumurtalarındaki mezofilik aerobik mikroorganizmaların tayini dökme plak yöntemi ile Plate Count Agar (PCA) (Merck, Almanya) besiyerine ekilip 37°C' de 24-48 saat inkübasyon sonrası sayılarak yapılmıştır. Psikrotrofik bakteri yükü dökme plak yöntemi kullanılmak suretiyle 7°C' da 10 gün inkübasyon yapılmak suretiyle gerçekleştirilmiştir. Tüm mikrobiyolojik sonuçlar log kob/g olarak verilmiştir. Duyusal analizlerde, Varlık ve diğ. (2007) tarafından önerilen duyusal skalanın, işlenmiş alabalık yumurtalarına modifiye edilmesi ile oluşturulan hedonik skala kullanılmıştır. Analizler raf ömrü süresince, 3 bay ve 7 bayan olmak üzere toplam 10 deneyimli panelistin (yaş aralığı 25-45) katılımıyla oda sıcaklığındaki laboratuvar koşullarında gerçekleştirilmiştir. Duyusal skalada 10-9 puan arası "mükemmel"; 8.9-8 puan "çok iyi"; "7.9-6" iyi; "5.9-5" Yeterli ve "<4.9" tüketilmez olarak değerlendirilmiştir.

Tüm çalışma boyunca deneyler 3 paralelli yapılmış, işlenmiş alabalık yumurtalarının deneysel sonuçlarının grupları arasındaki fark için tek yönlü varyans analizi (ANOVA) ile TUKEY istatistiksel değerlendirme yöntemi kullanılmıştır ($p<0,05$) (Sümbüloğlu ve Sümbüloğlu, 2002).

Bulgular ve Tartışma

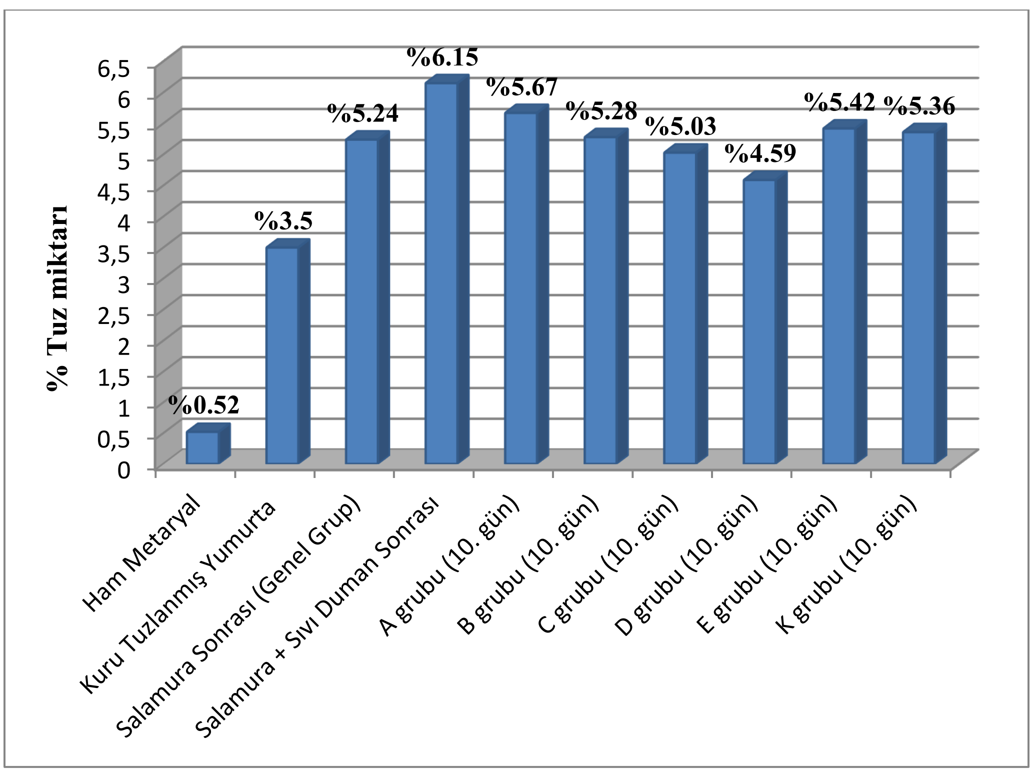
Havyar başta olmak üzere değişik balık yumurtaları, dünyada farklı konsantrasyonlardaki tuz ve koruyucu katkı maddelerinin çeşitli teknikler uygulanmasıyla yapılan, tüketiciler nezdinde yoğun bir talep gören "delikat" ürünlerdir. İşletmede sağımı yapılan alabalık yumurtalarının işlenmesi sırasında tuz geçişlerini ve son üründeki tuz miktarını belirlemek amacıyla tuz geçişi takip edilmiştir (Şekil 2). Ham materyal tuz miktarı %0.52 ±0.02 olarak tespit edilirken, kuru tuzlama (%7.5 tuz uygulaması) sonrası bu değer yumurtada %3.50 ±0.04'e yükselmiştir. Salamura ve salamura+sıvı duman uygulama aşamasında tuz geçişinde artış devam etmiştir. Ürünlerin gruplandırılması sürecinde ise soğuk depolamanın 10. gününde yapılan kontrollerde A, B, C, D, E ve K grubu için bu değerler sırasıyla %5.67 ±0.04, %5.71 ±0.16, %5.03 ±0.02, %4.59 ±0.00, %5.42 ±0.00 ve % 5.36 ±0.00 olarak bulunmuştur. İstatistiksel anlamda tuz miktarı bakımından A ve B grubu arasında fark bulunmazken ($p>0.05$) diğer gruplar arasında fark bulunmuştur ($p<0.05$). Besin bileşimleri yönünden değerlendirildiğinde ürünler diğer balık yumurta ürünlerinden farklı çıkmamış ve balık eti gibi yüksek oranda protein ile yağ içerdiği de tespit edilmiştir. Protein değerleri işlenme sürecinde bir miktar düşüş göstermiş, benzer sonuç yağ miktarlarında da tespit edilmiştir. Nem miktarı salamura öncesinde tuzlamaya bağlı olarak düşme eğilimi göstermekle birlikte salamura süresince başlangıç değerine göre artmıştır. Kül değerleri ise tüm bu süreçte istatistiksel olarak anlamlı artış göstermiştir ($p<0,05$) (Şekil 3).

Gökkuşağı alabalıklarında Mahmoud ve diğ. (2008) işlenmemiş yumurtaların besin bileşim içeriklerini %28,5 protein, %9.6 yağ, %57.0 nem ve %1.3 kül olarak tespit etmişlerdir. *Oncorhynchus tshawytscha* yumurtasının besin bileşimi ise %26.16 protein, %10.59 yağ, %55.60 nem ve %1.42 kül olarak belirlenmiştir (Bekhit ve diğ. 2009). İnanlı ve diğ. (2011) ise yapmış oldukları çalışmada gökkuşağı alabalığı yumurtasında bu değerleri sırasıyla %24.77, %11.65, %61 ve %2.15 olarak bulunmuştur. Bu araştırmada elde edilen sonuçlar, daha önce değişik araştırmacılar tarafından ortaya konan değerlerle uyumluluk içerisindedir. Yine de unutulmamalıdır ki balık yumurtaları ve havyarlarının kimyasal kompozisyonu balık türüne, yumurtanın olgunluğuna, yumurtalık içerisindeki yumurta miktarına, balığın beslenmesine, balık olgunluğuna, hasat mevsimine ve işleme şartları/tekniklerine bağlı değişkenlikler gösterebilmektedir (Bekhit ve diğ. 2009; Shirai ve diğ. 2006; Mahmoud ve diğ. 2008). İşlenmiş balık yumurtalarının kimyasal kompozisyonu balık türüne ve işleme tekniklerine göre değişmektedir (İnanlı *ve* diğ. 2010). Gessner ve diğ. (2002)' ne göre tuzlu salamura uygulanmış örneklerin su içeriğindeki artışdan dolayı protein, yağ ve kül miktarlarında bir miktar düşüş olmaktadır. Kül değerindeki benzer değişimden Pourashouri ve diğ. (2015) de bahsetmiş bunun da su emiliminden kaynaklandığını belirtmişlerdir. Martinez ve diğ. (2007) *Salmo salar* yumurtalarında sıvı duman uygulaması sonrası nem miktarının diğer gruplara göre daha düşük olduğunu ifade etmiştir. Himelbloom ve Crapo (1997) da *Oncorhynchus gorbuscha* yumurtalarını doygun salamurada tuzlamış ve besin bileşim (protein, yağ, nem ve kül) değerlerini %31.8, %11.0, %49.3 ve %7 olarak vermişlerdir. Sonuçlar literatür ile uyumlu bir şekilde işleme süreçlerine bağlı değişim göstermiştir. Bu değişimdeki etkenin su ve tuz arasındaki geçiş süreçlerine bağlı besin bileşiminde suyun azalmasıyla ortaya çıkan değişimin oranlara reel olmayan etkisinden kaynaklandığı tespit edilmiştir.

Bu çalışma ile işlenmiş balık yumurtalarında daha önce denenmemiş farklı katkı maddelerinin kullanımıyla çeşitli aroma ve tatlarda yeni ürünler geliştirilmiştir. Ayrıca yeni katkıların raf ömrü üzerine etkileri araştırılmış, bu araştırma süreçlerinde raf ömrü kimyasal, mikrobiyolojik ve duyusal analiz sonuçlarıyla değerlendirilmiştir. Sonuçlar bir bütün olarak değerlendirildiğinde özellikle kimyasal parametrelerin raf ömrünün ortaya konması açısından yetersiz kaldığı sonucuna varılmıştır. Tüm gruplar da kimyasal analiz sonuçlarına göre araştırmanın sürdürüldüğü 70 gün boyunca limit değerlerin aşılmadığı belirlenmiştir (Şekil 4).

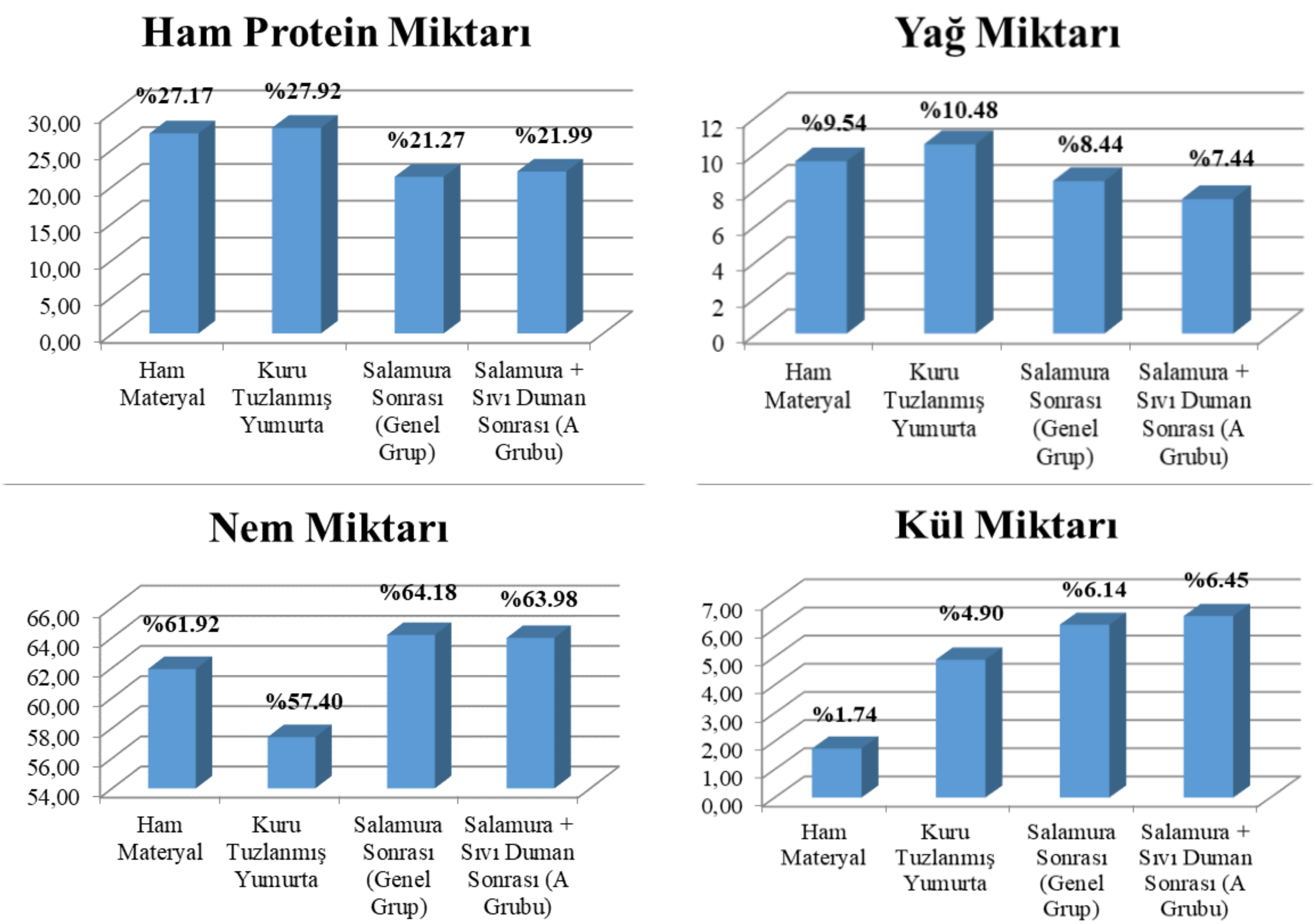
Gökkuşağı alabalığı yumurtalarının işlenmesine yönelik İnanlı ve diğ. tarafından 2010 yılında yapılan bir çalışmada ham yumurtanın TVB-N değeri 6.95 mg/100g olarak tespit edilirken, depolama başlangıcında %4'lük kuru tuzlama uygulanmış ürünlerde TVB-N miktarı 7.06 mg/100g ve %8 kuru tuzlama uygulanmış grupta 7.04 mg/100g olarak bildirilmiştir. Mirsadeghi ve diğ. (2015) ise işlenmemiş gökkuşağı alabalığı yumurtalarında TVB-N değerine 5.97 mg/100g, tuzlama işleminden sonra ise 6.05 mg/100g olarak tespit edildiği bildirilmiştir. Özpolat ve Patir (2010) tarafından yapılan diğer bir araştırmada ise alabalık yumurtalarının işleme sonrası 70. günde TVB-N değeri 6.78 mg/100g, 84. günde ise 6.64 mg/100g olarak belirlenmiştir. Bu çalışmada elde edilen değerler mevcut çalışmalardan daha düşük olarak tespit edilmiştir.

Basby (1997) tarafından *Cyclopterus lumpus* yumurtalarıyla 5°C' de yapılan depolama çalışmasında 2.5 aya kadar TMA-N konsantrasyonunun artmadığı tespit edilmiştir. Çelik ve diğ. (2012) balık yumurtasında yüksek düzeyde TMAO mevcut olmadığı ve buna bağlı kuru tuzlanmış *Mugil cephalus*'un yumurtalarında daha az miktar TMA-N bulunduğunu belirtmiştir. Taze balıkların TMA-N miktarı 1 mg/100g'a yakın olurken, bozulmuş balıkların TMA-N miktarı 8 mg/100g' ın üzerinde olması gerekmektedir (Varlık ve diğ, 1993). Basby ve diğ. (1997) *Cyclopterus lumpus* yumurtalarında düşük miktar TMAO ve buna bağlı TMA-N değeri (1.3 mg/100g ve ≤4.4 mg/100g) verilmiştir. Benzer şekilde yapılan bu çalışmada TMA-N miktarı depolama boyunca alabalık yumurtalarında da düşük değerler de bulunmuş, diğer literatür bilgilerinin desteği ile bu parametrenin kalite kriteri olarak değerlendirilemeyeceği sonucuna varılmıştır.



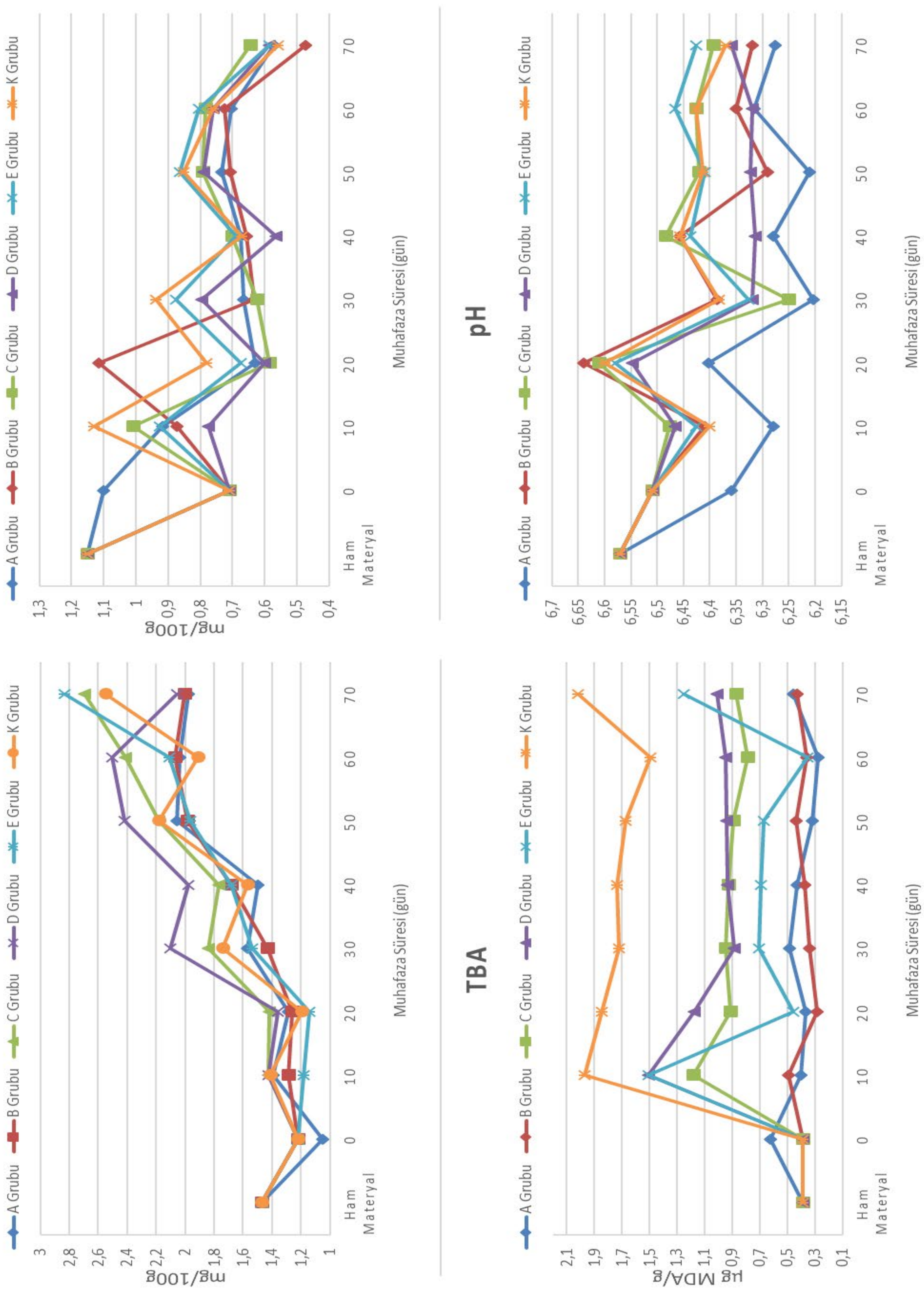
Şekil 2. Alabalık yumurtalarının işleme sürecindeki tuz geçişi

Figure 2. Salt transition in the processing of trout eggs



Şekil 3. Alabalık yumurtalarının besin bileşimlerindeki değişimler

Figure 3. Changes in nutritional components of trout eggs



Şekil 4. İşlenmiş alabalık yumurtalarının kimyasal parametreler yönünden değerlendirilmesi

Figure 4. Evaluation of processed trout eggs in terms of chemical parameters

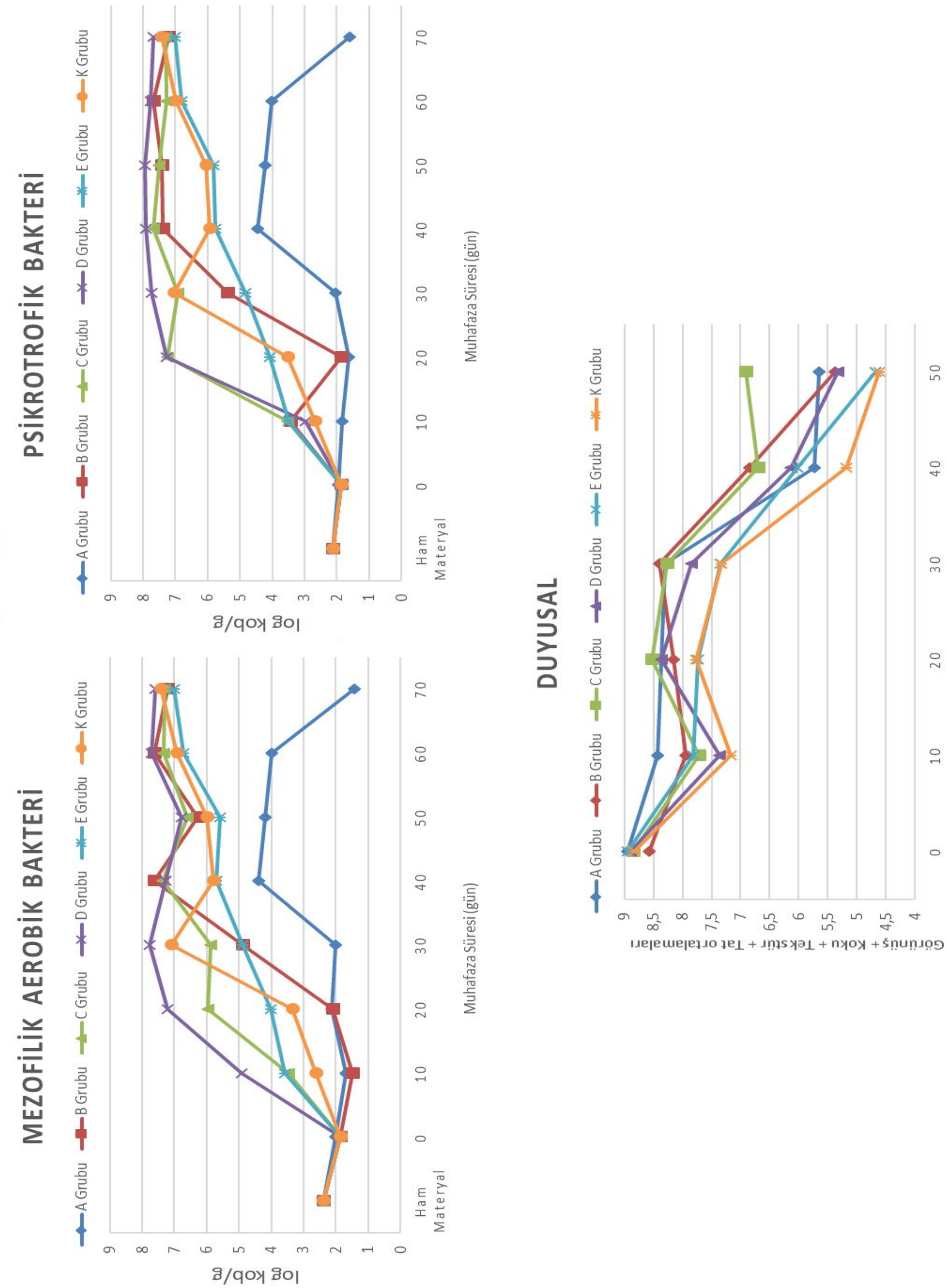
Yapılmış bir çalışmada, gökkuşağı alabalık yumurtalarında TBA miktarı 0.73 µgMDA/g olarak tespit edilmiş, tuzlama işleminden sonra bu değer 0.84 µgMDA/g olarak belirlenmiştir (Mirsadeği ve diğ. 2015). *Mugil cephalus*' un yumurtasının ise TBA miktarı 0.55 µgMDA/g iken, kuru tuzlama sonrası bu değer 3.23 µgMDA/g olarak bulunmuştur (Çelik *ve* diğ. 2012). Kaba ve diğ (2013)' lerinin mezgit balığı yumurta konservesi ile yaptıkları bir araştırmada ham materyalde 0.36 µgMDA/g, dumanlanmış balık yumurtalarında 1.28 µgMDA/g ve dumanlanmış konserve edilmiş mezgit yumurtasında 0.58 µgMDA/g olarak değişim gösterdiğini belirlemişlerdir. Bu çalışmada TBA değerleri depolama sürecinde tüm katkı uygulanmış gruplar da 1.5 µgMDA/g altında kalırken kontrol grubunda 10. gün itibariyle hızla artış göstererek 1.97 µgMDA/g değerine ulaşmıştır. Kontrol grubu örneklerinde bu değer depolama boyunca da diğer gruplardan yüksek kalmıştır. Oksidasyona karşı belirgin bir etki olarak en iyi sonuç sıvı duman ve kekik yağı uygulanan A ve B gruplarında görülmüş; TBA değerindeki değişim ise çok düşük düzeyde gerçekleşmiştir ($p>0,05$).

Literatürde verilen alabalık yumurtalarının pH değerleri ile yapılan bu çalışmada elde edilen veriler benzer aralıkta bulunmaktadır. Mirsadeghi ve diğ. (2015) pH değerini 6.47 ve Machado ve diğ. (2016) ise 6.45-6.49 olarak belirlemiştir. Su ürünleri için pH limit değerleri 6-8 ile 7,0 arasında verilmektedir (Özpolat ve Patir, 2010). Bu araştırmada 70 günlük depolama boyunca işlenmiş alabalık yumurtası gruplarında pH sonuçları limit değerlere ulaşmamıştır.

Mezofilik aerobik bakteri ve psikrotrofik bakteri gıda güvenliği bakımından ortak değerlendirildiğinde sıvı duman uygulamasının depolama boyunca en etkili sonucu verdiği mikrobiyolojik yük bakımından limit değeri aşmadığı tespit edilmiştir. İkinci sırada 50 günlük raf ömrü ile üzüm çekirdeği yağı uygulaması gelmektedir, kekik yağı uygulanmış işlenmiş alabalık yumurtalarının raf ömrü ise mikrobiyolojik kriterlere göre 30 gün olarak bulunmuştur. Limon ve keten yağı uygulanmış işlenmiş alabalık yumurtalarında mikrobiyolojik olarak raf ömrü ancak 10 gün ile sınırlı kalmıştır Hiçbir koruyucu uygulanmamış kontrol grubu örneklerinin ise 20 günlük bir raf ömrüne gıda güvenliği açısından sahip olduğu belirlenmiştir (Şekil 5; Tablo 1).

Goulas ve Kontaminas (2005)'a göre tuzlama su aktivitesini düşürmekte ve bunun sonucunda mikroorganizmaların gelişimi önlenmektedir. Su ürünlerinde tüketim açısından kabul edilebilir limit değer olarak mezofilik aerobik bakteri ve psikrotrofik bakteriler için 7 log kob/g olarak bildirilmekle birlikte gıda güvenliği açısından bu değer 6 log kob/g olarak değerlendirilmektedir (Olafsdóttir ve diğ. 1997). Himelbloom ve Crapo (1997)'nun çalışmalarında tuz ile kürlenmiş somon balığı (*Oncorhynchus gorbuscha*) yumurtasının 30. günden sonra mikrobiyolojik yükünün <2 1og kob/g'dan 7.65 1og kob/g'a yükseldiğini tespit etmişlerdir. *Cyclopterus lumpus* yumurtalarının 5°C' da yapılan raf ömrü çalışmasında mikrobiyal faaliyetlerden dolayı kötü koku oluşarak bozulduğu tespit edilirken, yeni işlenmiş az tuzlanmış yumurtaların toplam canlı sayısının <2-3 log kob/g' dan 3-4 haftalık depolama süresinde 7 log kob/g'a kadar yükseldiği tespit edilmiştir (Basby, 1997). Magnusson ve Martinsdottir (2006) ise 2. aydan sonra 4°C'de muhafaza edilen % 3,5'lik tuz içeriğine sahip *Cyclopterus lumpus* yumurtalarında toplam canlı sayısının 7 log'dan fazla olduğunu tespit etmişlerdir.

Tüketici ve değerlendirici konumundaki panelistler tarafından "Görünüş, Koku, Tekstür ve Tat" parametreleriyle ilişkili depolama süreç değerlendirmesi duyusal yönden yapılmış, aynı zamanda ürünler hakkında depolama öncesi tüketim tercihleri aroma ve tat bakımından olumlu geri dönüş yapılmıştır. Bu çalışmanın depolama başlangıcında tüm gruplarda yapılan Görünüş + Koku + Tekstür + Tat açısından panel testlerde en çok beğenilen grubun A (genel ortalama 8.95) grubu ve en az tercih edilen grubun B (genel ortalama 8,57) olduğu bulunmuştur. Parametrelerin ortalamaları değerlendirildiğinde ise üzüm çekirdeği yağlı ile kontrol grubunda 40 günlük raf ömrü tespit edilmiştir. Diğer gruplarda ortalama kriterler bakımından tüketim limit değerlerinin üzerinde yer almışlardır. Duyusal değerlendirmeler ürünün pazarda yer alabilmesi ve tüketiciler tarafından tercih edilmesi anlamında büyük önem taşımaktadır. Fakat raf ömrünün belirlenmesinde bu değerlendirmelerin aynı zamanda mikrobiyolojik yönden gıda güvenliği ile de ilişkilendirilmesi büyük önem taşımaktadır (Şekil 5 ve Tablo 1). Özpolat ve Patir (2010) balık yumurtalarının bozulmaya bağlı sert-elastik yapıdaki tektürünü kaybettiğini ve daha yumuşak-yapışık bir yapı oluşumunun gerçekleştiğini bildirmiştir. Benzer sonuçlar panelistlerin yazdıkları not kısımlarında bu çalışmada da belirtilerek depolama süresine bağlı artan yumuşak-yapışık yapı tespit edilmiştir.



Şekil 5. İşlenmiş alabalık yumurtalarının mikrobiyolojik ve duyusal parametreler yönünden değerlendirilmesi

Figure 5. Evaluation of processed trout eggs in terms of microbiological and sensory parameters

Tablo 1. İşlenmiş alabalık yumurtalarında tüketim limit değerlerinin aşımı ve günler

Table 1. Exceeding the consumption limit values and days for processed trout eggs

Gruplar	Mezofilik Bakteri Gelişimi (log kob/g)	Psikrotrofik Bakteri Gelişimi (log kob/g)	Mikrobiyolojik Güvenli Tüketim Raf Ömrü (Gün)
$A_{Sıvı\ Duman}$	Limit değer aşılmamıştır	Limit değer aşılmamıştır	**Raf ömrü sonlanmamıştır**
$B_{Kekik\ yağı}$	7.61 ±0.16	7.39±0.00	**30**
$C_{Limon\ yağı}$	5.98 ±0.71	7.25 ±0.04	**10**
$D_{Keten\ yağı}$	7.23 ±0.13	7.27 ±0.01	**10**
$E_{Üzüm\ Çekirdeği\ yağı}$	6.72 ±0.19	6.80 ±0.17	**50**
$K_{Kontrol}$	7.09 ±0.00	7.05 ±0.16	**20**

Sonuç

Balık yumurtalarındaki yüksek nem içeriği ve buna bağlı su aktivitesinin de yüksek olması mikrobiyal faaliyetlerin hızla gelişimini tetiklemekte ve buna yönelik önlemlerin alınmasını zorunlu kılmaktadır. Bu çalışmada uyguladığımız koruyucu katkıların bu anlamda etkileri de araştırılmış ve tüketim limitleri yönünden değerlendirilmiş, sıvı dumanın antimikrobiyal etkisi bir kez daha teyit edilmiştir. Bitkisel kökenli esansiyel yağlar arasında antimikrobiyal etki bakımından üzüm çekirdeğinin de bu tip ürünlerde rahatlıkla ve güvenle kullanılabileceği araştırma sonucunda belirlenmiştir.

İşlenmiş alabalık yumurtalarının raf ömrü gıda güvenliği yönünden değerlendirmesi yapıldığında sıvı duman uygulaması yapılan örneklerde çalışma süresi olan 70 gün içinde kabul edilebilir limit değerin aşılmadığı, ikinci sırada 50 günlük raf ömrü ile üzüm çekirdeği yağı uygulamasının geldiği, kekik yağı uygulanmış grubun ise 30 günlük bir raf ömrü gösterdiği bulunmuştur. Hiçbir koruyucu uygulanmamış Kontrol grubu örneklerinin ise 20 günlük bir raf ömrüne sahip olduğu belirlenmiş, limon ve keten yağı uygulamasının işlenmiş alabalık yumurtalarına kabul edilebilir aroma ve tat verme dışında bir etkisi olmadığı raf ömrüne bu uygulamanın negatif etki gösterdiği tespit edilen 10 günlük raf ömrü ile gözlemlenmiştir.

Çalışma verilerine göre aroma ve tat oluşumu yönünden sıvı duman, kekik ve üzüm çekirdeği yağları muamele edilmiş grupların rahatlıkla tüketime sunulabileceği belirlenirken, raf ömrü konusunda ise içerdiği antimikrobiyal etkiler bakımından da sıvı duman ilavesinin en etkili sonuç verdiği tespit edilmiştir. Aynı zamanda antimikrobiyal katkı kullanımında doğal bileşenlerin tercih edilme eğilimi işlenmiş alabalık yumurtalarında sıvı duman uygulaması üzerine daha derin ve detaylı çalışmaların yapılarak bu ürün grubunun su ürünleri üretim sektörüne kazandırılması gerekliliğini ortaya koymaktadır.

Etik Standart ile Uyumluluk

Çıkar çatışması: Yazarlar herhangi bir çıkar çatışmasının olmadığını beyan eder.

Etik kurul izni: Araştırma niteliği bakımından etik izin gerektirmemektedir.

Finansal destek: Bu çalışma İstanbul Üniversitesi Bilimsel Araştırma Projeleri Yürütücü Sekreterliğinin FYL-2016-20974 numaralı projesi ile desteklenmiştir.

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Açıklama: Bu çalışma "İşlenmiş alabalık yumurtalarının raf ömrüne farklı katkı maddelerinin etkisi" başlıklı yüksek lisans tezinin özetidir.

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New fish species added to the ichthyofauna of Laguna Ojo de Liebre, Baja California Sur, México

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ABSTRACT

The Laguna Ojo de Liebre (Scammon's lagoon) is the iconic sanctuary of the Pacific gray whale and belongs to the El Vizcaino Biosphere Reserve in Baja California, México. From June 2015 to August 2016, six seasonal visits were conducted on the ichthyofauna in seven sites of the lagoon. By diving, trapping, hook & line, and gillnet commercial fishing, a total number of 39 fish species was identified belonging to 25 families. In this study a total number of eight fish species is added to the first two existing 20-year-old lists: the *Gymnothorax mordax (Ayres, 1859*), *Apogon* sp. *Pomacanthus zonipectus (Gill, 1862*), *Balistes polylepis (Steindachner, 1876*), *Pareques viola* (Gilbert 1898), *Caranx* sp., *Sphoeroides lobatus (Steindachner, 1870*), and the *Icelinus* sp. During 2015-2016, two anomalous events warmed the lagoon, and possibly, it contributed to the fish species movement from the adjacent tropical or subtropical zones. Ichthyofauna from Laguna Ojo de Liebre is reported here before the installation of reef modules as a refuge for red lobster and fish aggregation.

Keywords: Species occurrence, Scammon's lagoon, The Blob, El Niño, Warming conditions

Introduction

Laguna Ojo de Liebre (Scammon's lagoon) with 57,100 hectares is a coastal lagoon on the Pacific of Baja California Sur (Mexico) and considered of great importance for conservation since it serves as refuge, feeding and breeding area for fish species (Acevedo-Cervantes, 1997). As a sanctuary for the gray whale, the lagoon has been declared a World Heritage Site by UNESCO as part of the Man and Biosphere program (De la Cruz-Agüero, Gómez, and Arellano-Martinez, 1996). Such protected areas are scarce in the Pacific of Baja California and, therefore, essential as a nursery for commercial fish species or of ecological interest due to sparsely populated areas with low development pressure (Rosales-Casián, 1997). This lagoon is of particular interest as a local cooperative of Guerrero Negro, B.C.S. (Mexico) will install artificial reefs made with concrete (1.5m long, 1.15m wide, and 0.25 m high) as a refuge for lobster and fish for commercial capture.

Several studies have been carried out in Laguna Ojo de Liebre to characterize longshore sediments transport (Marinone, 1982; Zamora-Salvador, 2015), environmental conditions (Alvarado *et al*., 1986; Álvarez-Borrego and Granados-Guzman, 1992; Gutiérrez de Velasco, 2000; Rodríguez-Padilla, 2013), commercial bivalves (Arellano-Martinez *et al*., 2004; Hernández-Olalde *et al*., 2007; Quiñones-Arreola, 2003), and the green turtle population *Chelonia mydas* status (Hernández-Cruz, 2013). Despite it is importance as a refuge and feeding area for different organisms, there are few studies on the fish species that live there. De la Cruz-Agüero, Gómez, and Arellano-Martinez (1996) published a first systematic list of fish species from Ojo de Liebre and Guerrero Negro lagoons, and Acevedo-Cervantes (1997) characterized the community of Laguna Ojo de Liebre, concluding that it is a low diversity site compared to other lagoon systems in Mexico, with small size specimens, and seasonally influenced by the entry of species from the adjacent Sebastian Vizcaino Bay to complete their life cycle.

Motivated by the planned artificial reef deployment by a local cooperative of Laguna Ojo de Liebre, the present study aimed to identify the fish species inhabitants at sites considered suitable for artificial reef enhancement, for future comparisons of the fish community after installation.

Material and Methods

Study Area

Laguna Ojo de Liebre is located at the Pacific half of Baja California peninsula, close to the town of Guerrero Negro, Baja California Sur, Mexico (Figure 1), between 27° 36 'and 27° 55' North latitude and 113° 55 'and 114° 19' West latitude. It is part of the Ojo de Liebre lagoon complex comprised of Laguna Manuela (north), Laguna Guerrero Negro (middle), and to the south the Laguna Ojo de Liebre (Eberhardt, 1966). The lagoon area is 480 km^2, 40 km length, and a mean of 6 km width. It is connected to the Sebastián Vizcaino Bay through a 3.7 km wide of mouth and has four main islands in its interior: Conchas, Brosas, Piedras, and Choya. The lagoon topography is shallow (average 5-6 meters depth) and includes a relatively deep channel (up to 30 m maximum depth) surrounded by intertidal flats (Rodríguez-Padilla, 2013).

Tide is an important component in the Laguna Ojo de Liebre dynamics, with an interval from 1.20 to 2.70 m, and predominant currents up to 1.18 m/s (Gutiérrez de Velasco, 2000). Maximum tidal speeds are reached during rising and falling, weaker in the ridges and valleys, and most intense and turbulent currents appear at mouth and channels (Zamora-Salvador, 2015).

The present study was carried out in a section with an influence of seawater from the adjacent Sebastian Vizcaino Bay during high tide (Rodríguez-Padilla, 2013). Seven sites were chosen after the fishermen experience as the best sites for catching lobster and fish, and therefore feasible in depth for the artificial reefs deployment (Table 1), and all the work carried out was fully supported by the staff of the Cooperative "Luis Gómez Z." of Guerrero Negro, B.C.S. (Mexico).

Ojo de Liebre lagoon Fish Collections and observations

The fish survey began in June 2015 (spring), followed by September (summer) and November (autumn) 2015, and February (winter), May (spring), and August (summer) 2016, for a total of six seasonal visits. We consider winter from January to March, spring (April-June), summer (July-September) and autumn (October-December).

Trap collections: Because the lagoon is a marine reserve, two fish monitoring methods were selected to avoid disturbing the bottom; we used commercial traps and underwater diving visual census during two days. The traps were built by the fishermen with pieces of wire mesh covered with a plastic layer, dimensions 88 cm wide, 122 cm long, and 43 cm high, with an entrance 30 cm in diameter and a 7 cm mesh. The traps were prepared with buoys, ropes, and sardines as bait. A total of six sites within the lagoon were selected for fish collections with traps because they are usually fishing spots with the possibility of improvement with artificial reefs: El Borbollón, El Conchalito, La Ventana, Isla Concha, El Esterito, and Carros Viejos (Figure 1).

Table 1. Monitoring sites and coordinates in the Laguna Ojo de Liebre, BCS, Mexico, 2015-2016.

Sampling sites	North latitude	West longitude	Survey	Substrate
El Borbollón	27°51’28.14”	114°14’2.40”	Diving / Traps	Rocky / Sandy
El Conchalito	27°47’1.14”	114°16’28.68”	Diving / Traps	Limestone
La Ventana	27°47’18.04”	114°18’11.40”	Diving / Traps	Limestone
La Ventanita	27°46’48.18”	114°17’7.62”	Diving	Sandy
Isla Concha	27°49’15.70”	114°13’54.40”	Traps	Shell / Sandy
Carros Viejos	27°50’26.80”	114°16’59.80”	Traps	Sandy
El Esterito	27°48’24.10”	114°17’49.50”	Traps	Sandy

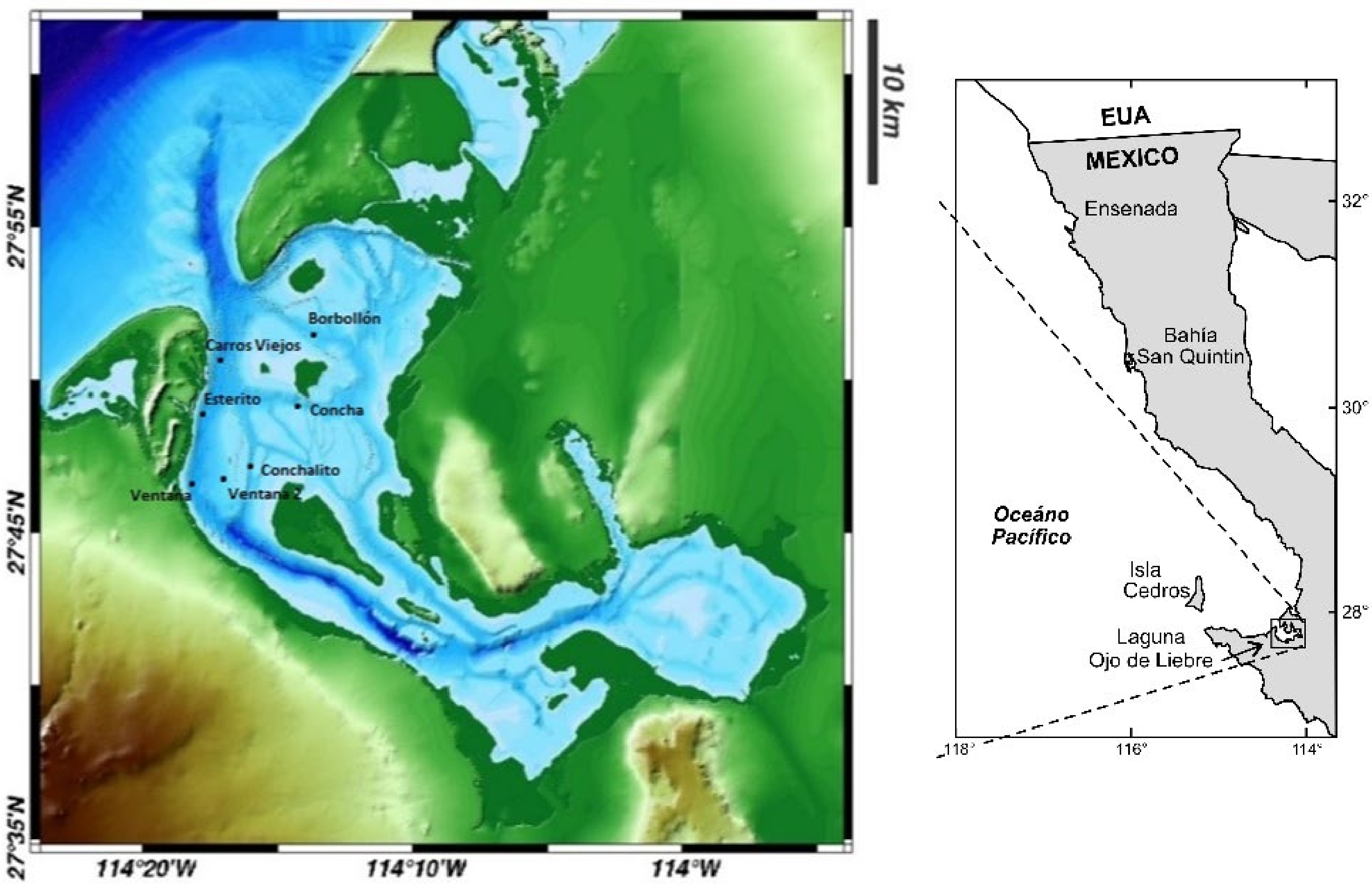

Figure 1. Localization of the Laguna Ojo de Liebre, BCS, Mexico, and sampling sites.

For the seasonal catch of fish, four traps as repetitions were launched separated by 50-100 m in each selected site, and remained at the bottom during 45 minutes following the local fishermen's technique (30 to 60 minutes); their total length (TL, mm) of captured fish was measured, intervals for each fish species are reported here.

Diving observations: Because diving is limited in time by the tidal current, four sites were selected: El Borbollón, El Conchalito, La Ventana, and a new site La Ventanita. We select the neap low tide from the monitoring day to avoid strong tidal currents. In each visited site, three diving transects in parallel of 30 m long, 2 m wide, 2 m high, and separated by 5 m between transects were made (Freiwald *et al*., 2015). Each transect was performed in a maximum time of seven minutes. One diver identified the fish species, determined the fish counts of each species and logged the approximate size. A second diver photographed the fish species. A third diver photographed the lagoon bottom and invertebrates. Trap collections and underwater diving census were carried out on boats separately in order to not interfere.

During our lagoon visits and the periods between the trap catches, fishing with hook-and-line was done, and also fish from commercial catches with gillnets also were identified, and added to the list without their lengths.

Fish species identification: All the species were identified using the keys for the Pacific of Baja California, Mexico, and California, USA (Miller and Lea, 1972), and for warm fish species, the work of Humann and Deloach (2004) was used. Arrangement of the classes, orders, and families follows the work of Nelson (2006) and Fricke *et al*., (2021). The biogeographic affinities of the fish species are presented according to Horn, Allen, and Lea (2006).

Results and Discussion

During the sampling period in Laguna Ojo de Liebre (June 2015 to August 2016), a total of 138 traps launches was made, for a total capture time of 138 hours trap and 48 minutes. Regarding the underwater visual census, a total of 72 transects was made for 360 minutes of diving.

The taxonomic list in Laguna Ojo de Liebre included a total number of 39 fish species belonging to 25 families. Underwater visual census showed 28 fish species, and traps showed 13 fish species, with nine common species in both methods (Table 2). Hook-and-line captured seven of the fish species. From a total of 21 species identified in the commercial catch, six species were not found with traps and diving (Table 2). A total of 31 species were teleost, and eight were elasmobranchs (Table 2). A total of 2,724 fish was counted, 893 individuals in the trap collections, and 1,179 individuals observed during diving. In the first ichthyofaunal list of Laguna Ojo de Liebre, De la Cruz Agüero *et al*., (1996) identified 59 species from 36 families, however they included the adjacent Guerrero Negro Lagoon without discriminating the fish species from each lagoon, and used five collection gears with hook-and-line as the only coincident with the present study. In the second study by Acevedo-Cervantes (1997) he also identified 59 fish species and none of the three collecting gears used coincided with those of the present study; a species not included in the first study and mentioned in the second was the kelp bass *Paralabrax clathratus*, while in the present study this species was collected frequently in traps and observed in diving.

In the present study, families that contributed with the largest number of individuals were Sparidae with 1,227 individuals (one species), Serranidae with 1,165 individuals (three species), and Labridae with 153 individuals (two species). Most abundant fish species were the Pacific porgy, *Calamus brachysomus* (Lockington, 1880) with 1,227 individuals (45.1%), the barred sand bass, *Paralabrax nebulifer* (Girard, 1854) with 768 individuals (28.2%), the spotted sand bass, *Paralabrax maculatofasciatus* (Steindachner, 1868) with 302 individuals (11.1%,) the rock wrasse, *Halichoeres semicinctus* (Ayres, 1859) with 142 individuals (5.2%), and the kelp bass, *Paralabrax clathratus* (Girard, 1854) with 95 individuals (3.5%), which together contributed 93% of the total abundance (Table 2).

Elasmobranchs *Hypanus dipterurus* (Jordan & Gilbert, 1880) (130 cm TL) and *Zapteryx exasperata* (Jordan & Gilbert, 1880) (100cm TL) were the bigger fish, while larger teleost was the California moray *Gymnothorax mordax* (Ayres, 1859) (98cm TL), the kelp bass *P. clathratus* (65cm TL), and the broomtail grouper *Mycteroperca xenarcha* Jordan, 1888 with 60cm TL (Table 2). Small fish were the California killifish *Fundulus parvipinnis* Girard, *1854,* the spotted sand bass *P. maculatofasciatus*, the barred sand bass *P. nebulifer* (all 4cm TL), and the cardenalfish *Apogon* sp., the rock wrasse *H. semicinctus*, and the bullseye puffer *Sphoeroides annulatus (*Jenyns, *1842)* with 5cm TL (Table 2).

One fish species showed a cold affinity to the Aleutian Province, seven species with northern distribution to the Oregon Province, 26 species show affinity to the Province of San Diego (68.4%), and three to the Cortez Province. The major percentage (86.8%), comprising 32 species, that extends south to the Provinces of Cortez, Mexican province, or to Panamian Province. (Table 2).

Table 2. Fish species from Laguna Ojo de Liebre, BCS, Mexico, 2015-2016; lengths (cm); H&L: hook and line; CF: commercial fishing; BA: Biogeographic affinity. Distribution by provinces after Horn et al. (2006): AP: Aleutian province; OR: Oregonian province; SD: San Diego province; Cortez province; MX: Mexican province; PP: Panamian province.

Class/Order	Family	Fish species	Number	Dive	Trap	Size (cm)	H&L	CF	BA
Chondrichthyes									
Heterondontiformes	Heterodontidae	*Heterodontus francisci* (Girard, 1855)	11	X	X	30-80		X	OR-CZ
Torpediniformes	Narcinidae	*Narcine entemedor* Jordan and Starks, 1895	1	X		75			SD-PP
Rhinopristiformes	Rhinobatidae	*Pseudobatos leucorhynchus* (Günther, 1867)	4	X		70-76			CZ-PP
Rajiformes		*Pseudobatos productus* (Ayres, 1854)	2	X		60-70		X	SD-MX
		Zapteryx exasperata (Jordan & Gilbert, 1880)	2	X		100		X	SD-MX
Myliobatiformes	Urotrygonidae	*Urobatis halleri* (Cooper, 1863)	13	X	X	20-30			SD-PP
		Urobatis maculatus Garman, 1913	2	X		40			SD-CZ
	Dasyatidae	*Hypanus dipterurus* (Jordan & Gilbert, 1880)	2	X		130			SD-PP
Osteichthyes									
Anguilliformes	Muraenidae	*Gymnothorax mordax* (Ayres, 1859)	1		X	98			SD
Cyprinodontiformes	Fundulidae	*Fundulus parvipinnis* Girard, 1854	29	X		4-6			SD
Gasterosteiformes	Syngnathidae	*Hippocampus ingens* Girard, 1858	4	X		15-20			SD-PP
Scorpaeniformes	Scorpaenidae	*Scorpaena guttata* Girard, 1854					X	X	SD-CZ
	Cottidae	*Icelinus* sp.	1	X		10			
Perciformes	Epinephelidae	*Mycteroperca xenarcha* Jordan, 1888	2	X		60	X	X	SD-PP
	Serranidae	*Paralabrax clathratus* (Girard, 1854)	95	X	X	10-65	X	X	OR-SD
		Paralabrax maculatofasciatus (Steindachner, 1868)	302	X	X	4-42	X	X	OR-CZ
		Paralabrax nebulifer (Girard, 1854)	768	X	X	4-53	X	X	OR-MX
	Apogonidae	*Apogon* sp.	10	X		5-7			SD
	Carangidae	*Caranx* sp.	1	X		70			SD-PP
		Caranx caninus Günther, 1867						X	SD-PP
	Haemulidae	*Anisotremus davidsonii* (Steindachner, 1875)	40	X	X	20-33		X	SD-CZ
	Sparidae	*Calamus brachysomus* (Lockington, 1880)	1227	X	X	9-47	X	X	SD-PP
	Sciaenidae	*Atractoscion nobilis* (Ayres, 1860)	1		X	74		X	AP-CZ
		Cynoscion parvipinnis Ayres, 1861						X	SD-CZ
		Cynoscion xanthulus Jordan & Gilbert, 1882						X	CZ-MX
		Menticirrhus undulatus (Girard, 1854)						X	SD-PP
		Pareques viola (Gilbert, 1898)	8	X		5-8			SD-PP
		Roncador stearnsii (Steindachner, 1875)						X	SD
		Umbrina roncador Jordan and Gilbert, 1882						X	SD-CZ
	Pomacanthidae	*Pomacanthus zonipectus* (Gill, 1862)	1	X		25			CZ
	Pomacentridae	*Hypsypops rubicundus* (Girard, 1854)	2	X		25			SD-CZ
	Labridae	*Halichoeres semicinctus* (Ayres, 1859)	142	X		5-26			SD-CZ
		Semicossyphus pulcher (Ayres, 1854)	11		X	33-48		X	OR-CZ
	Blenniidae	*Hypsoblennius gentilis* (Girard, 1854)	1	X		7			SD-CZ
Pleuronectiformes	Paralichthyidae	*Paralichthys californicus* (Ayres, 1859)	1	X		11		X	OR-SD
	Pleuronectidae	*Pleuronichthys guttulatus* (Girard, 1856)	1	X		10		X	OR-CZ
Tetraodontiformes	Balistidae	*Balistes polylepis* Steindachner, 1876	4		X	24-31		X	SD-CZ
	Tetraodontidae	*Sphoeroides annulatus* (Jenyns, 1842)	31	X	X	5-38			SD-PP
		Sphoeroides lobatus (Steindachner, 1870)	4	X	X	6-34			SD-PP
		Total	2724						

Laguna Ojo de Liebre is influenced by tidal currents and oceanic events such as El Niño/La Niña (Gutierrez de Velasco, 2000). During our monitoring in 2015-2016, the tidal currents limited the time of the diving census, and the selected neap lowest tides allowed only one hour for the transects. This lagoon is adjacent to Vizcaino Bay which has been considered a center of fish larvae production (Moser *et al*., 1993), and during El Niño events there was a high proportion of tropical and subtropical taxa that contributed to the highest abundance of fish larvae (Jimenez-Rosenberg *et al*., 2007).

The lagoon was influenced by the warm water "The Blob" formed in the Gulf of Alaska in 2013 that extended south to 2016, and El Niño 2015-2016 (Dorantes-Gilardi and Rivas, 2019). The El Niño was intense with anomalies of more than 2°C for the Eastern Pacific at Northern Hemisphere, began in January 2015 up to winter 2016 to weaken at Spring and to finish in June (NOAA's El Niño, available at http://www.elnino.noaa.gov/, last accessed 10 August, 2019).

The fish monitoring was focused on potential sites for the deployment of artificial reefs, and this may explain the lesser number of registered fish species compared to other studies which monitored a larger area of the lagoon identifying a total number of 59 fish species (De la Cruz-Agüero, Gómez, and Arellano-Martinez, 1996). Notably, in the present study, a total of eight new species are added to the two systematic lists of fish species in the Laguna Ojo de Liebre: The California moray (*G. mordax*), the sculpin (*Icelinus* sp.), the cardenalfish (*Apogon* sp.), the jack (*Caranx* sp.), the gungo highhat (*Pareques viola*), the Cortez angelfish (*Pomacanthus zonipectus*), the finescale triggerfish (*Balistes polylepis*), and the longnose puffer (*Sphoeroides lobatus*). In the present study, of the eight added fish species, six were identified by diving, and two collected with traps: the California moray, and the finescale triggerfish.

The species-specific zoogeographic affinities in the present study were grouped into five zoogeographic provinces: Aleutian, Oregonian, San Diego, Cortez, Mexican and Panamian (Horn, Allen, and Lea, 2006); species considered from temperate zones are represented by those distributed in the province of San Diego and in this study made up 68.4%; those from cold environments (Oregonian and Aleutian provinces) accumulated 23.7%, while three species (7.9%) presented subtropical and tropical affinity, only. However, of the total species, 86.8% have a southern distribution corresponding to the subtropical and tropical environment, from the provinces of Cortez, Mexican to Panamian. Those species affinities can be attributed to the southern boundary of the Southern California Current and the transition to the subtropical province at the latitude (27º5'43" N, 115º08'15" W) of Punta Eugenia (Hubbs, 1960, Jiménez-Rosenberg *et al*., 2007). The presence of a warming events that began at end of 2013 and finished in 2016 (Dorantes and Rivas, 2019; Robinson, 2016) may have caused movements of fish from the tropical or subtropical zone to the Laguna Ojo de Liebre, like the *Cortez Angelfish*, the gungo highhat, and the jack which contributed to the increased list of fish species.

Although the artificial reef modules will be built and deposited in the bottoms of the Ojo de Liebre lagoon as a refuge for red lobster, they present an excellent opportunity to study for the fish aggregation that take refuge in the structures.

Conclusion

This new study on the ichthyofauna of Laguna Ojo de Liebre identified 39 fish species, with eight new species that are added to the first lists of fish in the lagoon. Combining of traps and mainly diving was essential to identify the new species, also as non-destructive methods it avoided the alteration of the lagoon bottom. The presence of two warming events (The Blob and El Niño) during the 2015-2016 study possibly promoted the movement of subtropical or tropical species to the North. This work will be compared with the next survey on the possible changes when a local cooperative installs the artificial reef modules in the lagoon.

Compliance with Ethical Standard

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

Ethics committee approval: This study was conducted in accordance with ethics committee procedures of animal experiments.

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Research Article

Stress responses of Indian major carps cultured in the East Kolkata Wetland, West Bengal, India

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ABSTRACT

Fish are continuously exposed to multiple environmental stressors that work cumulatively and synergistically. This study assessed the stress responses of Indian major carps (IMCs) cultured in a sewage-fed pond (SP) in the East Kolkata Wetland (EKW), India and compared with the normal carps *in situ*. The experiment was conducted in two farms that cultured *Labeo rohita, Catla catla* and *Cirrhinus mrigala* for seven months, covering the summer and winter periods. Serum biomarkers of primary (cortisol) and secondary (glucose, total protein, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH)) stress responses, and oxidative stress (superoxide dismutase (SOD)) were quantified using standard kits. The health status of carps was evaluated as a tertiary response. The biological oxygen demand, hardness, total dissolved solids, ammonia and phosphate levels of both ponds exhibited marked variations. The SP carps had significantly low haemoglobin and total protein, and high serum glucose, creatinine, ALT, AST and LDH levels. The SOD and cortisol levels were comparatively low in SP carps. The winter temperature had a significant effect on serum glucose, cortisol, SOD, creatinine, ALT and AST. Carps had a high degree of ectoparasitic infestation during the winter. *Cirrhinus mrigala* of the SP had significantly high serum creatinine levels. The increasing levels of serum glucose, creatinine, ALT and AST suggested that these indices, which were more pronounced in the carps of EKW in conjunction with winter temperature, could be useful biomarkers of stress, kidney and liver functioning in carps, respectively.

Keywords: Indian major carps, Fish health, Serum biomarkers, Oxidative stress, Ectoparasites

Introduction

Carp culture is the backbone of Indian aquaculture, which has evolved from the level of traditionally backyard activity to viable commercial farming practice. Application of sewage and other wastes in carp culture to augment fish production is in vogue in India since the 1930s (Bhowmik, 2011). Though the Indian major carps (IMCs) are the principal species cultured in the system, other fish groups such as exotic carps, minor carps, tilapia, etc. are also being cultured along with them (Abraham et al., 2010; 2020). Under farm conditions, the inappropriate water quality, inadequate or high stocking density, feed and unscientific management practices that quantitatively or qualitatively not suitable, cause physiological variations. Long-term exposure to these stressors can negatively affect fish growth, health and ultimately productivity (Relić et al., 2010). Among the various stressors, water quality parameters are one of the most influencing ones in fish welfare assessment including fish health, behaviour and productivity (Boyd, 1979; Banerjee et al., 2015). The effects that multiple stressors exhibit on organisms can be synergistic or antagonistic (Petitjean et al., 2019).

The city of Kolkata, India diverts about 60–70% of its sewage to the East Kolkata Wetlands (EKW). The EKW, a Ramsar site, sustains the world's largest and perhaps oldest integrated resource recovery practice based on a combination of agriculture and aquaculture. It provides livelihood support to a large, economically underprivileged population of around 20,000 families, which depend upon the various wetland products, primarily fish and vegetables for sustenance. The wetlands consist of about 264 sewage-fed fisheries that cover about 12,500 ha (EKWMA, 2010). This massive biological cleaning system is highly productive, and carp aquaculture is quite popular. A considerable amount of fish consumed in Kolkata is produced from this system (SWRE, 2007; Bhowmik, 2011; Banerjee et al., 2015). Fish living in the wastewater-fed ponds are constantly being exposed to varying levels of physicochemical parameters, which may disturb the normal physiological functions of fish leading to stress and infection (Banerjee et al., 2017). The exposure of fish to various biotic and abiotic agents may induce haematological changes. The haematological and biochemical parameters, although highly variable, can provide prognostic information, depending on the fish species, age, the cycle of sexual maturity and health condition (Bernet et al., 2000, 2001, Rey-Vázquez and Guerrero, 2007). Organ alterations, water quality induced changes in blood chemistry, increased prevalence of infectious diseases, temperature fluctuations and evidence for endocrine disrupting substances were all found to be indicative of an environmentally influenced health condition (Wahli, 2002).

The most comprehensive and effective assessments of fish health are achieved if both (i) short- and long-duration responses, (ii) slow- and fast responding and (iii) contaminant-specific and unspecific biomarkers are conjointly included in environments subjected to complex contaminants, which act synergistically or cumulatively (Sopinka et al., 2016; Petitjean et al., 2019). The alterations in biochemical (glucose, cortisol and protein), enzymological (aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), superoxide dismutase (SOD)) and other biomarkers are widely used to assess the toxic stress, pollution level, immune status and tissue damage (Kavitha et al., 2010; Chitra-Pakira et al., 2015). There are reports on the carp culture in the sewage-fed ponds (Abraham *et al.,* 2010), accumulation of heavy metals and toxicants in water, sediment and fish (SWRE, 2007; Chatterjee et al., 2010; Sarkar et al., 2011) and increasing incidence of infectious diseases of fish from such systems (Banerjee et al., 2015, 2017). Nevertheless, *in situ* studies related to serum biomarkers of fish from such an ecosystem are lacking. The present study assessed the primary, secondary and tertiary stress responses of Indian major carps (IMCs) namely *Labeo rohita* (Hamiton, 1822), *Catla catla* (Hamilton, 1822) and *Cirrhinus mrigala* (Hamiton, 1822) cultured in the EKW that received sewage and compared with the normal carps *in situ.*

Material and Methods

Site Description and Experimental Design

The present experiment was carried out in two fish ponds of varied culture conditions. The experiment was conducted in a 2 (farms) × 3 (species) × 2 (seasons) factorial design with two replications in each pond from December to June. The normal pond (NP) of size 0.33 ha was taken from a rain-fed farm situated at the Faculty of Fishery Sciences, Chakgaria, Kolkata (Lat. 22° 82'N; Long. 88° 20'E). The sewage-fed pond (SP) of size 18.67 ha was from the EKW situated at Haripota, Bamanghata, South 24 Parganas district, West Bengal, India (Lat. 22° 29'N; Long. 88°29'E). Six nylon net-cages (2.43 m × 1.37 m × 1.37 m) were erected in each pond with the support of bamboo poles. Individual net-cage, a fixed and fine-meshed nylon net enclosure, was fixed in such a way that one-third portion of the net-cage remained above the water surface to allow the surfacing of fish. The healthy and active IMCs, viz., *C. catla* (22.03±0.91 cm and 150.61±16.38 g), *L. rohita* (19.73±1.46 cm and 101.40±16.60 g) and *C. mrigala* (22.46±1.03 cm and 121.64±24.82 g) were stocked in each net-cage with 6 individuals of each species in the ratio 1:1:1, i.e., 18 fish/net-cage. The experimental fish were procured from Gangajoara, South 24 Parganas district, about five kilometres from the experimental site and acclimated for ten days in fibreglass reinforced plastic (500-L capacity) tanks containing 400-L chlorine-free tap water. They were then transferred to experimental net cages and conditioned for 15 days. An additional net cage was also maintained with few individuals of each species in both the ponds for an emergency requirement, if any, during the conditioning. The fish were fed with commercial pellet feed containing 30% protein, 10% fat, 9% moisture, 32% carbohydrate and 9.5% ash at 3% of the bodyweight twice daily.

Analysis of Water Quality Parameters

The temperature of the pond water was recorded by a centigrade thermometer at the time of sampling usually between 8.00 and 9.00 am. The pH of water samples was estimated by pH meter (Eutech Instruments Pte Ltd). Total dissolved solids (TDS) were measured by TDS meter (HiMedia, India) and expressed as mg/L. The water quality parameters such as total hardness, dissolved oxygen (DO), free carbon dioxide (CO_2), biological oxygen demand (BOD), phosphate-phosphorous and ammonia-nitrogen were measured fortnightly following the standard methods (APHA/AWWA/WEF 2005).

Collection of Fish Blood and Serum

For blood collection, the fish (n=6) from each net-cage were randomly sampled during the winter (December–February) and summer (March–June) months, transferred to plastic containers of 100-L capacity containing water of the same temperature with added clove oil (30 µL/L) to reduce the activity of fish. The experimental fish were starved for 24 h before the blood collection to minimize the physiological stress during sampling. The blood was drawn using a 2 mL sterile disposable plastic syringe by the caudal venous puncture. For serum collection, the blood (min: 0.5 mL and max: 1.5 mL/fish) was collected from six individual fish of each replicate for each species of the NP and SP. The blood was allowed to clot overnight at 4ºC by keeping the syringes at a 15º angle. Serum from the syringes was then carefully poured out into Eppendorf tubes and centrifuged in a microfuge at 2500 × g for 10 min. The serum samples of six fish from each of the replicate were pooled separately (≥1.0 mL serum/tube), labelled and stored at -20ºC until use. Simultaneously, a portion of the blood sample from each species was transferred to 1.5 mL Eppendorf tubes rinsed with an anticoagulant (trisodium citrate, 3.8% w/v) to prevent clotting. It was used for the estimation of haemoglobin by the acid-haematin method using Sahli's haemoglobinometer (Wintrobe, 1975) as a secondary stress response.

Serum Biomarkers as Stress Responses

The details on the analysis of various serum biomarkers such as glucose, cortisol, total protein, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and superoxide dismutase (SOD) are summarized in Table 1. The serum cortisol, as a primary stress response, was determined by using an ELISA based cortisol test kit (AccuBind Elisa Microwells, Cortisol test system, Monobind Inc, Lake Forest, USA). As secondary stress responses, parameters such as serum glucose, total protein, creatinine, ALT, AST, and LDH were determined by using standard kits in a Photometer (Model: 5010 v5+, Robert Riele KG, Berlin). The SOD levels as an indicator of oxidative stress were determined by using the SOD assay kit (Cayman Chemical, USA). All kits were used as per the manufacturer's protocol.

Assessment of Fish Health

The health status of experimental fish was evaluated as a tertiary response. On the day of routine monthly sampling or harvesting time during the winter and summer seasons, live IMCs from the SP (n=327) and NP (n=225) were also assessed for the frequency of ectoparasitic infection due to *Lernaea, Argulus,* myxosporeans and skin and gill flukes (Banerjee et al., 2015). The gills from each carp were removed with the least damage and placed for examination in separate Petri dishes containing filtered water. The gills were examined under a microscope for the presence of gill flukes or various types of plasmodia for myxosporean infection. Likewise, the skin scrapings from the caudal peduncle or tail, under the chin and behind the fins were placed on the slide and microscopically examined. The presence of cutaneous haemorrhages on each of the sampled fish was also noted visually. The parasitic frequency index (PFI) in percentage was calculated by taking the number of carps infected with at least one of the individual ectoparasites, against the total number of carps examined. The severity of infection was determined using the following scale: 0 = no signs of ectoparasites, 0.5 = a very few scattered signs of ectoparasitic infection; 1 = low ectoparasitic infection; 2 = low to moderate ectoparasitic infection, 3 = moderate ectoparasitic infection, 4 = severe ectoparasitic infection (Banerjee et al., 2015).

Table 1. Kits and methods followed for the analysis of fish serum biomarkers

Parameters	Kits used and methods followed
Alanine aminotransferase (ALT)*	Modified UV (IFCC) and kinetic assay methods, DiaSys Diagnostics Systems, GmbH, Germany
Aspartate aminotransferase (AST)*	Modified UV (IFCC) and kinetic assay methods, DiaSys Diagnostics Systems, GmbH, Germany
Cortisol**	AccuBind Elisa Microwells, Cortisol test system, Monobind Inc, Lake Forest, USA
Creatinine*	Modified Jaffe's reaction, Initial rate assay method, DiaSys Diagnostics Systems, GmbH, Germany
Glucose*	GOD-FS method, DiaSys Diagnostics Systems, GmbH, Germany
Lactate dehydrogenase (LDH)*	Modified IFCC method, DiaSys Diagnostics Systems, GmbH, Germany
Superoxide dismutase (SOD)**	Elisa Microwells, SOD test system, Cayman Chemical, USA
Total protein*	Biuret method, DiaSys Diagnostics Systems, GmbH, Germany

***:** Analysed using Photometer 5010 v5+, Robert Riele -KG, Berlin.
**: Analysed using Microplate Reader, HaloMPR-96, Dynamica, Australia

Statistical Analyses

A 2 × 3 × 2 three-way analysis of variance (ANOVA) was performed by R statistical software version 3.4.1 to assess the effect of three factors namely ponds, species and seasons, and their interaction followed by Duncan's new multiple range test (DMRT). Data on PFI and cutaneous haemorrhages were analyzed by two-way ANOVA and DMRT. Student 't' test was used to find out the significance of the difference between the water quality parameters of normal and sewage fed ponds in the Microsoft Excel package.

Results and Discussion

Water Quality Parameters

From the farm records, the minimum and maximum water temperatures during the study period were noted as 8 and 32ºC, respectively with a mean of 12.70 ±2.10ºC in winter and 28.20 ±2.70ºC in summer. The results of the physicochemical parameters of the SP and NP water samples are presented in Table 2. The water quality parameters such as pH, temperature, DO and free CO_2 did not vary much between the normal and sewage-fed ponds. Although the level of free CO_2 was high in the SP, the differences were insignificant ($p>0.05$). On the other hand, the levels of total hardness, total dissolved solids and phosphate-phosphorous of the NP and SP exhibited significant differences ($p>0.05$). The differences between the BOD as well as ammonia-nitrogen of the two systems were significant only at $p<0.09$.

Primary Stress Response

The IMCs of SP recorded mean serum cortisol levels of 50.43 ±6.00 µg/dL; while those of NP had 51.70 ±3.23 µg/dL during the study period. There existed insignificant differences in the serum cortisol levels among the carps and ponds ($p>0.05$). The mean serum cortisol levels of the carps were significantly higher in winter (52.49 ±3.43 µg/dL) than in summer (49.65 ±5.60 µg/dL). The mean serum cortisol levels of *C. mrigala* were insignificantly higher ($p>0.05$) than *L. rohita* and *C. catla* (Table 3).

Table 2. Ranges (mean±standard deviation (SD)) of physicochemical parameters of sewage-fed and normal fish pond waters

Water parameters	Normal pond: Range (Mean ±SD)	Sewage-fed pond: Range (Mean ±SD)
pH	7.20-7.90 (7.57 ±0.18)	7.20-7.80 (7.51 ±0.18)
Temperature (°C)	13.00–32.00 (23.17 ±3.27)	13.00–32.00 (22.54 ±3.43)
Dissolved oxygen (mg/L)	4.00-8.80 (7.69 ±1.31)	6.00-8.54 (7.09 ±0.68)
Free carbon dioxide (mg/L)	0.00-16.00 (8.08 ±6.75)	8.00-20.00 (13.27 ±5.21)
Total hardness (mg/ L)	180.00-360.00 (294.63 ±55.81)^a	140.00-300.00 (250.50 ±54.77)^a
Total dissolved solids (mg/L)	296.00-604.00 (465.08 ±107.89)^a	200.00-351.00 (264.58 ±41.08)^a
Biological oxygen demand (mg/L)	4.00-7.20 (5.68 ±0.97)^b	4.10-8.30 (6.95 ±1.46)^b
Phosphate-phosphorous (mg/L)	0.03-0.24 (0.10 ±0.08)^a	0.17-0.61 (0.35 ±0.17)^a
Ammonia-nitrogen (mg/L)	0.15-0.35 (0.25 ±0.06)^b	0.30-0.93 (0.48 ±0.20)^b

Values are the average of 10 observations recorded during the study period. a: Values sharing common alphabets within the row for each of the parameters differ significantly ($p<0.05$); b: Significant at $p<0.09$.

Table 3. Mean values of blood and serum biomarkers of the cultured Indian major carps

Parameter	Carps			Ponds		Seasons	
	Catla catla	*Labeo rohita*	*Cirrhinus mrigala*	Sewage-fed	Normal	Summer	Winter
Cortisol (µg/dL)	49.55 ±6.34^{1}	51.23 ±4.40^{1}	52.43 ±2.85^{1}	51.70 ±3.23^{a}	50.43 ±6.00^{a}	49.65 ±5.60^{A}	52.49 ±3.43^{B}
Haemoglobin (g/*dL*)	5.14 ±0.83^{1}	4.56 ±0.83^{2}	5.47 ±0.96^{1}	5.31 ±0.83^{a}	4.80 ±.99^{b}	5.39 ±0.93^{A}	4.73 ±0.84^{B}
Glucose (mg/dL)	48.61 ±14.06^{1}	48.94 ±20.72^{1}	46.75 ±17.77^{1}	39.12 ±12.28^{a}	57.08 ±17.38^{b}	41.85 ±12.71^{A}	54.35 ±19.42^{B}
Total protein (g/dL)	3.47 ±0.60^{1}	3.62 ±1.01^{1}	3.57 ±0.74^{1}	3.91 ±0.80^{a}	3.20 ±0.59^{b}	3.72 ±0.76^{A}	3.39 ±0.79^{A}
Creatinine (mg/dL)	0.40 ±0.08^{1}	0.41 ±0.06^{1}	0.53 ±0.24^{2}	0.40 ±0.07^{a}	0.48 ±0.21^{b}	0.38 ±0.07^{A}	0.51 ±0.21^{B}
Alanine aminotransferase (IU/L)	15.60 ±8.19^{1}	13.75 ±7.93^{1}	11.55 ±5.94^{1}	11.86 ±6.95^{a}	15.40 ±7.69^{b}	10.13 ±2.66^{A}	17.13 ±9.03^{B}
Aspartate aminotransferase (IU/L)	84.35 ±33.59^{1}	97.35 ±27.55^{2}	96.00 ±29.84^{12}	80.83 ±30.38^{a}	104.30 ±26.12^{b}	75.31 ±24.07^{A}	109.81 ±26.37^{B}
Lactate dehydrogenase (U/L)	68.12 ±10.18^{1}	68.75 ±12.40^{1}	76.60 ±13.26^{2}	71.35 ±9.35^{a}	70.96 ±15.09^{a}	68.25 ±12.86^{A}	74.07 ±11.51^{A}
Superoxide dismutase (U/mL)	49.41 ±16.57^{1}	49.15 ±7.91^{1}	56.31 ±16.20^{1}	53.93 ±10.64^{a}	49.31 ±17.07^{a}	47.51 ±15.25^{A}	55.73 ±12.17^{B}

a,b; 1,2; A,B: Parameters with uncommon alphabets ornumerals within the row for each of the parameters among the ponds, fish species and seasons differed significantly (p<*0.05*). Values are the average of 10 observations recorded during the study period.

Secondary Stress Responses

The results of the biomarkers of secondary stress responses are presented in Table 3. *Catla catla, L. rohita* and *C. mrigala* recorded mean haemoglobin levels of 5.14 ±0.83, 4.56 ±0.83 and 5.47 ±0.96 g/dL, respectively. There existed significant differences (p>*0.05*) in the mean haemoglobin levels among the carps, seasons and ponds. The carps of the SP had significantly lower haemoglobin levels (*p>0.05*). The haemoglobin levels of carps were significantly high in summer (p>*0.05*). Among the carps, *L. rohita* had significantly lower (p>*0.05*) haemoglobin levels. Serum glucose levels of *C. catla, L. rohita* and *C. mrigala* were 48.61 ±14.06, 48.94 ±20.72 and 46.75 ±17.77 mg/dL. Significant differences existed in the glucose levels among the seasons and ponds (p<*0.05*), but not among the carps (p>*0.05*). The serum glucose levels of the carps of the SP (57.08 ±17.38 mg/dL) as well as the winter season (54.35 ±19.42 mg/dL) were significantly higher (p<*0.05*). The serum total protein levels of the IMCs were in the range of 3.47 ±0.60-3.67 ±1.01 g/dL. The differences in the total serum protein levels among the carps as well as seasons were, however, insignificant (p>*0.05*). The carps of the NP had significantly higher (p<*0.05*) serum total protein levels. *Labeo rohita* recorded the highest mean serum total protein values. The serum creatinine values of IMCs ranged from 0.40 ±0.08 mg/dL in *C. catla* to 0.53 ±0.24 mg/dL in *C. mrigala*. The carps of the SP (0.48 ±0.21 mg/dL) and those sampled during the winter (0.51 ±0.21 mg/dL) recorded significantly higher creatinine levels (p<*0.05*). There existed significant differences in the serum creatinine levels among the carps, seasons and ponds (p<*0.05*). *Cirrhinus mrigala* recorded significantly higher serum creatinine levels (p<*0.05*). The serum ALT levels of the IMCs ranged from 11.55 ±5.94 IU/L in *C. mrigala* to 15.60 ±8.19 IU/L in *C. catla.* The carps of the SP (15.40 ±7.69 mg/dL) and those sampled during the winter (17.13 ±9.03 mg/dL) recorded significantly higher ALT levels (p<*0.05*). There existed significant differences in the serum ALT levels among the seasons and ponds (p<*0.05*), but not among the carps (p>*0.05*). The IMCs *C. catla, L. rohita* and *C. mrigala* recorded the serum AST levels of 84.35 ±33.59, 97.35 ±27.55 and 96.00±29.84 IU/L, respectively. The serum AST levels of the carps of the SP (104.30 ±26.12 IU/L) as well as the winter season (109.81 ±26.37 IU/L) were significantly higher (p<*0.05*). There existed significant differences in the serum AST levels among the carps, seasons and ponds (p<*0.05*). *Labeo rohita* recorded the highest mean serum AST levels. The mean serum LDH levels of the IMCs ranged from 68.12±10.18 U/L in *C. catla* to 76.60 ±13.26 U/L in *C. mrigala*. Significant differences existed in the serum LDH levels among the carps (p<*0.05*), but not among the seasons and ponds (*p>0.05*). The carps of the NP and those sampled during the winter recorded high LDH levels, but the differences were insignificant (p>*0.05*).

Oxidative Stress

The serum SOD levels recorded in *C. catla, L. rohita* and *C. mrigala* were 49.41±16.57, 49.15±7.91 and 56.31±16.20 U/mL, respectively (Table 3). The differences in the serum SOD levels among the carps and ponds were insignificant (p>*0.05*). *Cirrhinus mrigala* recorded the highest mean serum SOD levels. The carps recorded significantly higher serum SOD levels (55.73±12.17 U/mL) during the winter (p<*0.05*).

Tertiary Stress Responses

The results of the PFI and incidence of cutaneous haemorrhages in IMCs are presented in Figures 1 and 2, respectively. The PFI was more in *L. rohita* followed by *C. catla* and *C.*

mrigala but the differences were insignificant (p>*0.05*). The differences in the PFI among the ponds and seasons were, however, significant (Figure 1; p<*0.05*). The frequency of infection and incidence of cutaneous haemorrhages was significantly more in the carps of the SP particularly during the winter (Figure 2; p<*0.05*). The severity of ectoparasitic infection was 0-2.0 in winter and 0-0.5 in summer in both the ponds.

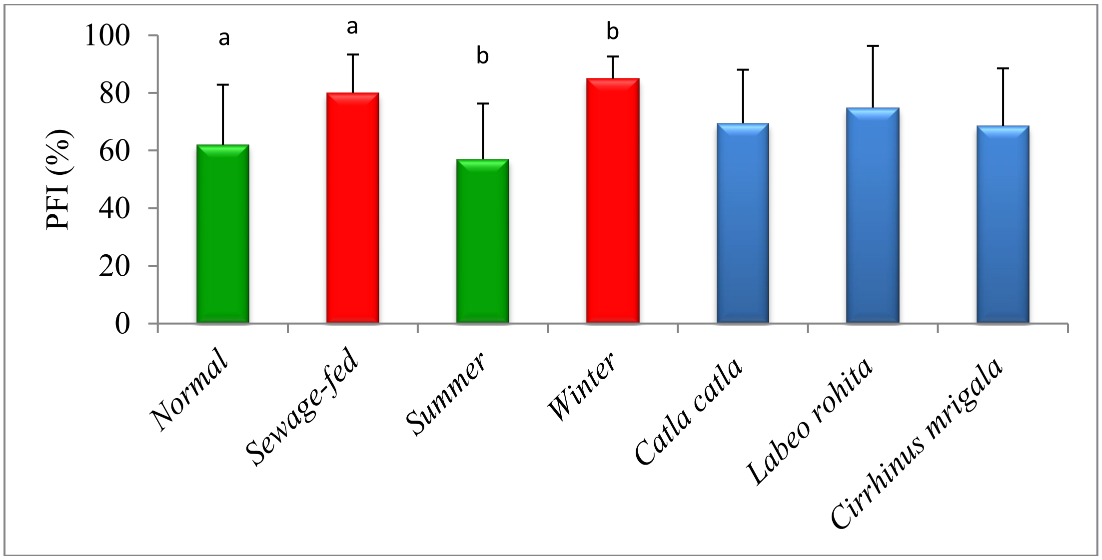
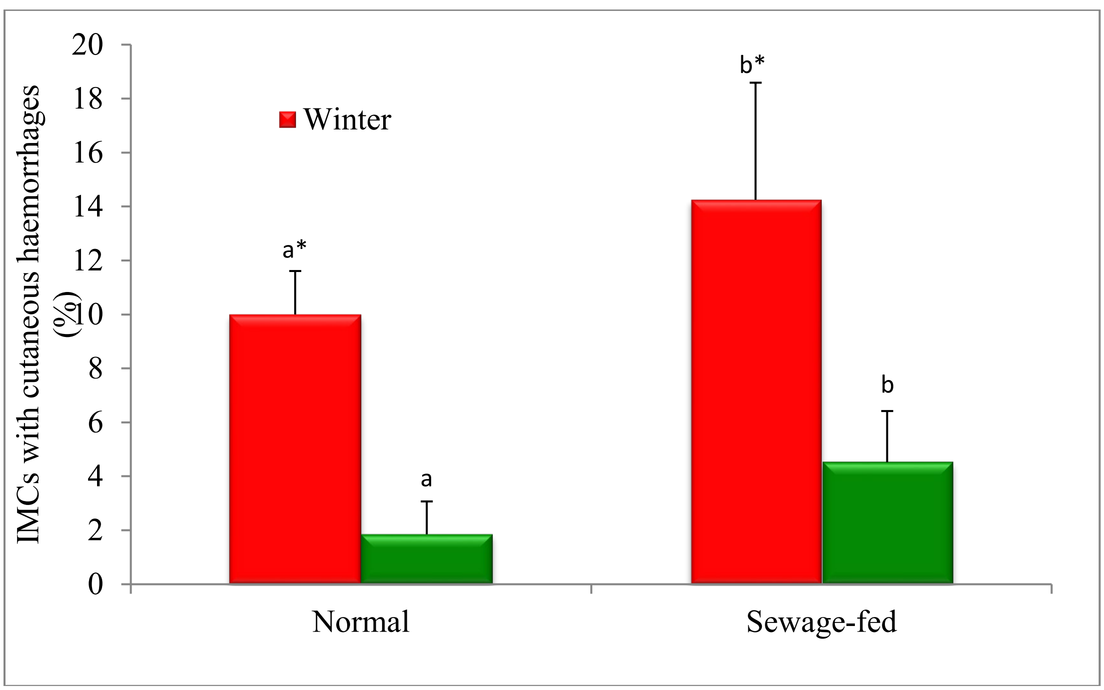


Figure 1. Parasitic frequency index (PFI) in Indian major carps cultured in sewage-fed and normal ponds. a,b: Bars sharing common alphabets differed significantly (*p*< 0.05).

$$\text{PFI} = \frac{\text{The number of carps infected with at least one of the individual ectoparasites}}{\text{The total number of carps examined}} \, x \, 100$$



a,b,*: Bars sharing common alphabets or the asterisk (*) differed significantly (*p*< 0.05).

Figure 2. Indian major carps (IMCs) with cutaneous haemorrhages (in %) from the sewage-fed and normal ponds.

The treatment of Kolkata metropolitan liquid waste is done by natural means through a network of canals in the peri-urban areas. This naturally treated wastewater is used for aquaculture in the EKW and the peri-urban areas without any further treatment (SWRE, 2007; Chatterjee et al., 2010; Sarkar et al., 2011; Banerjee et al., 2015). The untreated sewage from the feeder canal of the SP area was reportedly had pH 7.01-7.40, BOD 61-84 mg/L, TDS 370-415 mg/L, phosphate 1.28-1.98 mg/L, ammonia 3.10-3.90 mg/L and nitrate 0.93-3.90 mg/L (SWRE, 2007). The fish caught in these water bodies were found to accumulate chromium, copper, lead and other toxic elements in their flesh (Chatterjee et al., 2010; Sarkar et al., 2011). It is widely acknowledged that the physicochemical characteristics of the water influence the biological and physiological functions of fish and unsuitable water quality results in the activation of the stress responses (Bernet et al., 2000, 2001**)**. In the present study, the water quality parameters recorded in the NP were well within the optimum range, except for the total hardness and BOD recommended for carps (Boyd, 1979). On the other hand, the water quality parameters of the SP fluctuated greatly and inconsistently, though few of them had higher levels. The SP of the present study also received untreated tannery waste, which possibly led to poor water quality, mainly due to the increase in ionic levels and organic pollution denoted by higher levels of BOD (6.95±1.46 mg/L), ammonia-nitrogen (0.48±0.20 mg/L), phosphate-phosphorous (0.35±0.17 mg/L) and free CO_2 (13.27±5.21 mg/L). Compared to the levels recorded in the sewage feeder canal (SWRE 2007), the water quality parameters of the SP were quite low by about 6-12 folds, possibly due to the dilution of sewage in the pond environment. The observed high ammonia, phosphate-phosphorous and BOD levels in the water of the SP are an indication of eutrophic condition, which further hampers the fish homeostasis as multiple stressors.

In the present study, the carps of the SP had significantly higher haemoglobin levels. The highest haemoglobin level was observed in *C. mrigala* and the least in *L. rohita*. The higher haemoglobin content in the carps of the SP might be due to the increased demand for oxygen necessitated by the use of untreated sewage in the pond with high BOD as well as the use of tannery waste as feed. It infers that a small quantity of DO was utilized by the carps in the presence of high ammonia in the surrounding medium. Furthermore, significant differences in the haemoglobin levels of carps during the summer and winter seasons were noted, which corroborate the earlier study (Pradhan et al., 2014). They recorded a higher concentration of haemoglobin in *L. rohita* (8.50 g/dL) in summer and the least (5.50 g/dL) in winter. Likewise, higher haemoglobin levels were recorded in carps exposed to chemical stressors (Prusty *et al.,* 2011), possibly due to the replacement of oxidized denatured haemoglobin by the toxic elements and to supply more oxygen to oxygen-deficient tissues (Nussey et al., 1995). The increase may also be attributed to the catalyzing action of toxicants on the incorporation of body iron stores into haemoglobin or increased erythropoiesis and haemoglobin synthesis (Prusty et al., 2011).

It is well known that the responses of fish to stress are cumulative. Under stressful conditions, the fish body release stress hormones, viz., cortisol and catecholamines into the bloodstream by the endocrine system as a primary response and produce increased levels of glucose as a secondary response (Martínez-Porchas et al., 2009; Sopinka et al., 2016). The results on the marked differences in the levels of serum cortisol among the carps corroborate the earlier observations (Dutta et al., 2005), who observed significant variations in the plasma cortisol (90.0-377.0 ng/mL) in fish reared in a sewage-fed farm than those reared in a farm having optimum water quality. Significant differences in the serum cortisol levels of carps, particularly *L. rohita* of the SP, were recorded between the seasons. The carps during the low-temperature period recorded significantly higher levels of serum cortisol, which, more or less, corroborate the previous observations (Das et al., 2009) made in carps with the increase in acclimation temperature from the optimal (26°C) to 36°C. In contrast, fish could display a "metabolic conservation" strategy with no cortisol increase, when the dose, duration and/or the number of stressors is high (Petitjean et al., 2019). The suitability of using circulating cortisol levels in the blood of chronically stressed fish has, therefore, been questioned (van Weerd and Komen, 1998). Our results on the serum cortisol suggested that the carps were chronically stressed, which was more pronounced during the winter season when grown in the SP. Besides, our insignificant results on the serum cortisol levels as stress biomarker of carp from the NP (51.70±3.23 μg/dL) and SP (50.43±6.00 μg/dL) provided supportive evidence to earlier reports (van Weerd and Komen, 1998). The results of the present study and the past studies (Martínez-Porchas et al., 2009; Petitjean et al., 2019) amply revealed that serum cortisol response would not be sufficient, but rather a less reliable tool to examine the stress status of chronically stressed carps.

The present study recorded significantly high levels of serum glucose in carps reared in the SP as well as winter season. The levels of glucose may vary in ecologically distinct species and are influenced by environmental and non-environmental factors (Sopinka et al., 2016). The differences in the serum glucose levels among the carps of the present study were insignificant, though the IMCs occupy different ecological niches of the pond. It has been reported that under stressful conditions the glucose production is mostly mediated by the action of cortisol (Sopinka et al., 2016). Several earlier workers also reported a rise in glucose levels of carps exposed to various kinds of stressors such as ammonia (Das et al., 2004a) and temperature (Das et al., 2009). Though there were differences in the glucose levels of carps between the ponds, the differences in the cortisol levels of carps from these systems were insignificant. These results indicated that there was no correlation between serum glucose and cortisol levels in chronically stressed carps exposed to sewage in EKW.

The levels of antioxidant enzymes including SOD have been extensively used as an early warning indicator of aquatic pollution (Birnie-Gauvin et al., 2017). SOD is the only antioxidant that detoxifies superoxide, dismutating it to hydrogen peroxide and oxygen (Kammer et al., 2011). The estimated antioxidant activity of SOD in carp blood was in the range of 3.20±0.01-5.02±0.30 U/mL (Valon et al., 2013), which was almost 12-18 times lower than the present study. The significant increase in the SOD activity of carp blood particularly during the winter season may be explained as a compensation mechanism against the stress factors. According to Kammer et al. (2011), the SOD activity may increase during low temperature to offset the depressive effects on the catalytic rate of the enzyme, rather than to defend against elevated levels of reactive oxygen species (ROS) production. Several earlier studies also reported elevated SOD activities in fish exposed to low temperatures (Pavlović et al., 2013; Birnie-Gauvin et al., 2017), possibly due to increased polyunsaturation of mitochondrial membranes and elevated mitochondrial respiration. *Cirrhinus mrigala* recorded the highest mean serum SOD activity (56.31±16.20 U/mL), by its bottom-dwelling behaviour and exposure to unhealthy pond bottom conditions. Also, the higher SOD activity in *C. mrigala* from the SP indicated that the enhanced production of superoxide radicals created oxidative stress in this species. It means that *C. mrigala* has better adaptive responses to protect its physiological system, counteract the oxidative effect of ROS and resist the pollutants on the pond bottom. Earlier studies also demonstrated that SOD activity increased after exposure to pollution in various fish (Zikić et al., 2002; Sopinka et al., 2016). In general, the results of the stress biomarkers such as serum cortisol and SOD provided inconclusive evidence on the effect of long-term exposure of sewage on carps. On the other hand, the effect of seasonal pond water temperature on the stress biomarkers such as glucose, cortisol and SOD was more evident, where their levels were always and significantly higher during the winter.

The carps of the NP had significantly higher serum total protein levels. Similar to the present study, many researchers recorded a reduction in total protein levels when the carps were subjected to stressors (Das et al., 2004a,b). The plasma protein may decrease due to alteration in enzymatic activity involved in protein biosynthesis (Sopinka et al., 2016), a high degree of haemodilution under the stress conditions (Burgos-Aceves et al., 2019), shrinkage and lysis of RBC causing plasma dilution and/or protein catabolism (Das et al., 2004b), increased effects on the fish immune system, and liver disorders (Nayak et al., 2004). The observed reduction in fish serum protein levels of the present study may be attributed to their possible utilization for energy fulfilment. The carps of the NP and those sampled during the summer recorded significantly lower serum creatinine levels. The results of the present study on elevated serum creatinine levels of carps cultured in the SP or during the winter corroborate the observations of the earlier studies upon exposure to various stressors and effluent from the sewage treatment units (Bernet et al., 2000, 2001). They reported *Salmo trutta* exposed to effluent from the sewage treatment unit had significantly different alkaline phosphatase, blood urea nitrogen, creatinine and bilirubin values than the fish kept in river water. Increased level of serum creatinine is an indication of kidney dysfunction, and physical exertion of organisms (Bernet *et al.,* 2001; Outtandy et al., 2019), which was more pronounced in the bottom-dwelling *C. mrigala* from the SP. Possibly, the unfavourable pond bottom condition might have favoured dysfunction of *C. mrigala* kidney. The farmers of the sewage-fed farms of the surrounding area expressed that the growth of *C. mrigala* was poor compared to other carps.

Liver enzymes (ALT, AST and LDH) are the most suitable variables in fish as indicators of water with a high organic load like sewage water. Acceptable reference intervals of cyprinids as 9-23 IU/L for ALT (Nicula et al., 2010) corresponded well with the carps of the NP or in summer. While the carps of the SP recorded significantly high levels of ALT. The results of the present study corroborate several earlier observations recorded on carps (Prusty et al., 2011), goldfish (Simmons et al., 2017) and trout (Bernet et al., 2001) exposed to chemical stressors and sewage effluent. The levels of AST recorded in the carps of the present study were almost 2-4 times the acceptable reference level of cyprinid, i.e., 26-54 IU/L (Nicula et al., 2010). Teleosts are known for their ability to convert amino acids into glucose (Rodwell, 1988). Therefore, the higher activities of AST and ALT indicated the mobilization of aspartate and alanine via gluconeogenesis for glucose production to cope with stress. It may be noted here that glucose and protein levels recorded in the present study differed significantly due to ponds and seasons. Under the effect of oxidative stress, which was confirmed by high SOD levels, there may be an increase in AST and ALT activities and a greater degree of hepatic dysfunction (Nayak et al., 2004). Generally, the levels of ALT and AST were high in carps of the SP, which correlated well with the decrease in serum protein level, thus confirming the role of protein in metabolism, possibly as a compensatory mechanism to the impaired metabolism (Simmons *et al.,* 2017). Further, it is noteworthy to mention here that dysfunction of the carp liver has been noticed in the form of elevated ALT and AST levels both in the SP and NP, most likely due to the stress induced by the winter temperature and/or microbial infection. In contrast, some researchers reported a significant decrease in AST and ALT activities during acute and sublethal treatment of pollutant, which they have attributed to severe damage of hepatocytes that are no longer capable of synthesizing AST protein. Renal failure may also contribute to a significant decrease in ALT activity (Kavitha et al., 2010).

In the present study, the highest LDH values were observed in *C. mrigala* of the SP followed by *L rohita* and *C. catla*. The carps during the winter season recorded higher serum LDH levels. Likewise, a significant increase in the LDH activity was observed in carps exposed to different stressors such as sewage (Bernet et al., 2000, 2001), suboptimal temperature (Chatterjee et al., 2004) and high ammonia (Das et al., 2004a). Interestingly, *C. catla* and *L. rohita* of the SP recorded slightly lower LDH levels than those from the NP. Similar to this study, a reduced LDH activity in fish exposed to toxicants was noted (Mishra and Shukla, 2003), possibly due to the higher glycolysis rate under stressful conditions. Nevertheless, the observed insignificant differences in the serum LDH levels among the seasons and ponds suggested that the LDH is a less reliable biomarker to assess the stress status of chronically stressed IMCs. The application of serum chemistry variables as indicators of histological lesions in case of chronic exposure is questionable (Bernet et al., 2001).

During the culture period, infections due to ectoparasites (*Lernaea, Argulus*, skin flukes and myxosporeans), and cutaneous haemorrhages were observed in IMCs of both the ponds in January, February and the first week of March, which coincided with the mid and late winter. *Labeo rohita* was the most susceptible to parasitic infection followed by *C. catla* and *C. mrigala*. The carps of the SP had a high degree of ectoparasitic infection. Effluents from the sewage treatment units affect fish health, causing histological lesions and higher susceptibility to infectious diseases has been well demonstrated (Petitjean et al., 2019; Bernet et al., 2000, 2001) which also corroborates with the present study. Our results also indicated that the sewage-fed aquaculture in EKW had a detrimental impact on the health of carps by enhancing the stress responses and their susceptibility to microbial infection. The infection due to the microbial agents and the temperature stress during the winter season could be the probable reason for the insignificant differences observed in many of the serum biochemical parameters. Due to low water temperature and microbial infection, fish mortalities in net-cages ranging from nil in summer to 11% in winter were recorded. The increased susceptibility to disease could also be attributed to the confinement in the net-cages and the multiple stressors. Besides, growth retardation and production loss were recorded in the SP so also in an earlier study to the tune of 13 ±4 % (Banerjee et al., 2015, 2017). Also, the involvement of heavy metals and other toxicants present in the sewage (SWRE, 2007, Chatterjee et al., 2010; Sarkar et al., 2011) on the elevated levels of serum biochemical indices could not be ruled out. In West Bengal aquaculture, the factors like the use of sewage water, poor pond bottom conditions and low temperature were identified as putative as they increased the chance of occurrence of infectious diseases (Abraham et al., 2020).

Conclusion

In general, the present study revealed that the winter temperature (12.70±2.10 ºC) and the chronic exposure of IMCs to sewage in EKW elevated the stress levels of carps. The results of the stress biomarkers such as cortisol and SOD provided inconclusive evidence for their usefulness as suitable biomarkers on the effect of long-term exposure of sewage on carps. On the other hand, the effect of seasonal water temperature on the stress biomarkers such as glucose, cortisol and SOD was more evident, where their levels were always and significantly high during the winter. Our results on the increasing levels of serum glucose, creatinine, ALT and/or AST suggested that these indices, which were more pronounced in a sewage-fed pond in EKW in conjunction with low water temperature, could be useful biomarkers of stress, kidney and liver functioning of carps, respectively. Although these *in situ* studies provided some insights into the stress responses and serum biomarkers of IMCs cultured in the EKW, further investigations on the fish immune status, carp-microbial interactions, genetic/resistance markers and epigenetic responses of carps to environmental stressors are needed to evolve suitable management strategies for the sustainable aquaculture activities in such system.

Compliance with Ethical Standard

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

Ethics committee approval: All applicable guidelines of the "Committee for the Purpose of Control and Supervision of Experiments on Animals" (CPCSEA), Government of India, New Delhi were followed by the authors. The conventional regulatory framework may not be applied regarding the use of experimental animals in agricultural production research as per the guidelines of the

"Committee for the Purpose of Control and Supervision of Experiments on Animals", Government of India and, hence, Ethical Committee approval wasn't needed. All efforts were also made to minimize the suffering of the animals during the experimentation.

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Research Article

Monitoring of river health using aquatic insects: A study on Jatinga River, North East India

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ABSTRACT
A biomonitoring study of River Jatinga located in south Assam, north east India was conducted for the first time. The study aimed to evaluate water quality of the river in different stretches using aquatic insect as bioindicators. Insect samples were collected from selected sites of midstream and downstream of the river during monsoon- post monsoon, 2018 and winter- pre monsoon, 2019. A total of 25 families of aquatic insects recorded from 8 orders; Gerridae was eudominant in mid-stream across all the seasons with highest percentage in winter. Hemiptera, the most diverse group was represented by six families with highest relative abundance. The study found that although both the sites were represented by same functional feeding groups, there were seasonal and spatial variations in the families of insects and their percentage occurrences. All the biotic indices, $BMWP^{THAI}$, SIGNAL2 scores, EPT % and HFBI have shown relatively better quality of water of the River Jatinga in the midstream than that in the downstream during monsoon and post monsoon seasons. Presence/absence and abundance of certain insect groups can provide information about health of the river.

Keywords: Jatinga River, Bioindicators, Eudominant, Gerridae, Functional feeding group

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Introduction

Freshwater systems are rich in biological diversity but these days a majority of the aquatic fauna is under threat because of human activities (Meyer *et al*., 1999). Millions of people depend on the goods and services of freshwater systems like, rivers, streams, lakes, wetlands etc. and daily supply of clean fresh water is needed in every human existence (Barathy *et al*., 2021). Unfortunately, in some areas people still consume polluted and contaminated water without any treatment (Arthington *et al*., 2010). Currently, surface water pollution is increasing and as a result human and ecosystem health is deteriorating gradually (Aazami *et al*., 2020). Hence assessment of river health is very essential as organic and inorganic pollution is on rise affecting human health as well as freshwater faunal diversity. Biomonitoring is the "systematic use of living organisms or their responses to determine the condition or changes of the environment" (Oertel and Salanki, 2003). Many countries have a long history of using macroinvertebrates to monitor the ecological status of river ecosystems (Hellawell, 1986; Li et al., 2010; Birk et al., 2012; Carter et al., 2017; Musonge et al., 2020; Akyildiz and Duran, 2021; Eriksen et al., 2021). Studies on the potential use of benthic macroinvertebrates as bioindicators for river ecosystems also have been broadly reported in literature (Rosenberg and Resh, 1993; Mustow, 2002; Ganguly *et al*., 2018; Aazami *et al*., 2019; Mahmoud and Riad, 2020).

Aquatic insects are important component of benthic macroinvertebrates who spend their life or some part of their life in water. Being highly specialized they represent less than 1% of the total animal diversity (Pennak, 1978). They are known to play a very significant role in the processing and cycling of nutrients as they belong to several specialized feeding groups such as shredders, filter feeders, deposit collectors, and predators (Rosenberg and Resh, 1993). Some of the aquatic insects are very sensitive to pollution, while others are tolerant and many species are very susceptible to pollution or alteration of their habitat (Merritt and Cummins, 1996). Macroinvertebrates are vital indicators of the changes in freshwater habitats and family-level identification can be valuable in evaluation of water quality (Aazami *et al*., 2020). In northeast India though surface water pollution is on rise, a few studies on biomonitoring of streams and rivers have been recorded (Takhelmayum *et al*., 2013; Barman and Gupta, 2015; Marwein and Gupta, 2018).

The River Jatinga originating from the Jatinga village, Dima Hasao district of Assam flows all the way through the western boundary of the Barail Wildlife Sanctuary, Assam and joins River Barak, the second largest river of Assam (Figure 1). The district comprises geographic area of 3786 sq. km and 57.86 % of it is under forest cover (FSI, 2019). The Tea based agroforestry at the riverbank of midstream provides employment opportunities to the local people to sustain their livelihoods. Cultivation of seasonal crops at the bank and catchment area of the river strengthen the economic status of the riverine villagers. Since there is paucity of data on this important river of south Assam, we have attempted to generate baseline information on the aquatic insect community of the river and monitor water quality using aquatic insects as bioindicators. Various biotic indices such as Biological Monitoring Working Party Score ($BMWP^{THAI}$), Average Score per Taxon ($ASPT^{THAI}$), percentage of Ephemeroptera, Plecoptera, Trichoptera (%EPT), Hilsenhoff family biotic index (HFBI) and Stream Invertebrate Grade Number Average Level (SIGNAL2) were used. It is expected that this study would give insight of the water quality of the river, which is supposed to be pristine.

Material and Methods

Study Area and Collection of Aquatic Insects

The River Jatinga flows through evergreen and deciduous forest area with varied flora and fauna. The selected study sites of River Jatinga are midstream of the river at Damcherra in Cachar district and downstream at Borkhola in Cachar district (Table 1). The selected study sites, midstream and downstream of the river (Figure 2), on both the sites have hillocks and there are small villages in the foothills. Villagers are mostly dependent on the river as a source of their revenue generation and enjoy the ecosystem services of the river. River bank is mostly utilized for agricultural practices in downstream. Jhum cultivation is the usual practice in the surrounding hilly region of midstream area and also a few tea gardens nearby. Midstream site is also rich in riparian vegetation.

Aquatic insects were collected by using "kick-net method" and "all out search method" from the midstream and downstream of River Jatinga during monsoon, post monsoon 2018 and winter, pre monsoon 2019. Sampling was done by taking three, 1-minute kick-net samples (mesh opening: 180µm; area $1m^2$). The kick-net is held against water current and an area of ($1m^2$) in front of the net is disturbed for one minute (Brittain, 1974; Subramanian and Sivaramakrishnan, 2007). All out search method is used when water flows through boulder and cobble with high turbulence. Aquatic insects were collected from 10 square meter area for one hour. Within the sampling area, aquatic insects were searched in all the possible substrata such as boulders, cobbles, leaf litter and dead wood. A sable hairbrush or forcep was used for collection of all samples (Subramanian and Sivaramakrishnan, 2007). After collection, insects were preserved in 70% ethanol. Collected samples were examined under a motic stereozoom microscope and identified upto family using standard taxonomic literature (Merritt and Cummins, 1996; Subramanian and Sivaramakrishnan, 2007; Thirumalai, 1999; Choate, 2003; Bouchard, 2009; Boonsoong and Braasch, 2013).

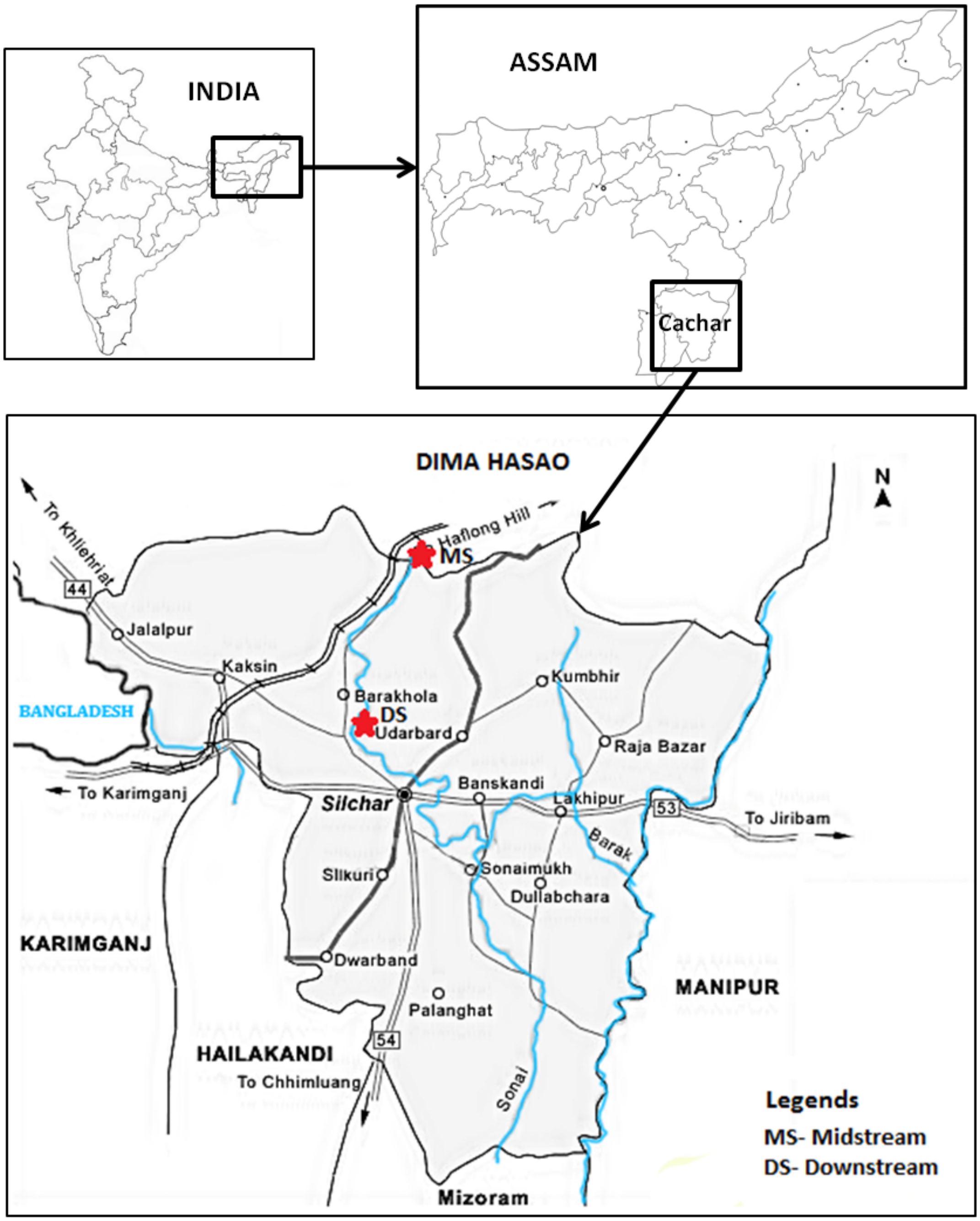

Figure 1. Map showing midstream and downstream of River Jatinga. Map not to scale.

Figure 2. Photographs of midstream and downstream of River Jatinga. [A] Midstream, [B] Downstream.

Table 1. Description of midstream and downstream of River Jatinga

Site	Lattitude, longitude and altitude	Riparian vegetation	Habitat types (mm)	Riverine settlement type
Midstream (Damcherra, Cachar District)	N 25̊ 00′ 6.67″ E 92̊ 45′ 0.70″. 28 m MSL.	*Camellia sinensis*, *Polygonum* sp., *Melia azadirach*, *Colocasia* sp., Fern sp., various types of orchid species, various grass species, *Bambusa* sp.etc	Cobbles (≤82 mm), pebbles (≤34 mm), granule (≤3 mm, sand (≤ 2mm), silt (≤0.06 mm), clay (≤0.004 mm) and few boulder (≤390mm)	Hillock, Tea garden, home garden, river bank agricultural cultivation (Cucurbitaceae, Solanaceae, Liguminosae etc.)
Downstream (Borkhola, Cachar district)	N 24̊ 55′ 52.1″ E 92̊ 45′ 15.2″. 18 m MSL.	*Bambusa* sp., various types of agricultural crops, various types of herbs, shrubs and trees.	Sand, silt and clay (≤2mm)	River bank agricultural cultivation (Cucurbitaceae, Solanaceae, Liguminosae, Brassicaceae, leafy greens, root crop etc.), home garden, grazing land, wet paddy cultivation.

Data Analysis

The dominance status of different families was worked out following Engelmann's scale based on relative abundance (Engelmann, 1978). Diversity indices such as Shannon-wiener diversity index and Margalef's richness index were carried out using PAST 3.14 software version for Windows 10 (Adu and Oyeniyi, 2019).

The biological monitoring working party score ($BMWP^{THAI}$) can be obtained by summing the individual scores of all families present. Score values for individual families reflect their pollution tolerance (Mandaville, 2002). The Average Score per Taxon ($ASPT^{THAI}$) is calculated by dividing the score by the total number of scoring taxa (Mandaville, 2002). A high $ASPT^{THAI}$ usually characterizes clean sites with relatively large numbers of high scoring taxa. Stream Invertebrate Grade Number-Average Level (SIGNAL 2) was calculated by total grade of aquatic insect families multiplied by the weight factor divided by total weight factor of aquatic insect family. A weight factor was determined for each type of macroinvertebrate, considering the number of specimens collected (Chessman, 2003). The percentage occurrence of Ephemeroptera, Plecoptera and Trichoptera (% EPT) were also calculated (Subramanian and Sivaramakrishnan, 2007). Hillsenhoff family biotic index (HFBI) was developed by Hilsenhoff (Hilsenhoff, 1988) to summarize the various tolerances of the benthic arthropod community with a single value. Tolerance values for families range from 0–10. Value increases as water quality decreases (Table 2).

Results and Discussion

A total of 25 families of aquatic insects belonging to 8 orders were recorded in both midstream and downstream during the study period (Table 3). The number of families recorded in midstream and downstream is 20 and 14 respectively. Aphelocheiridae, Notonectidae, Gyrinidae, Psephenidae, Ephemeridae, Lepidostomatidae, Perlidae, Gomphidae, Euphaeidae, Tipulidae and Corydalidae were found only in midstream and Caenidae, Elmidae, Dytiscidae and Veliidae were recorded only in downstream. The common aquatic insect families found in both midstream and downstream are Gyrinidae, Corixidae, Dytiscidae, Hydrophilidae, Baetidae, Heptageniidae, Hydropsychidae, Libellulidae, Culicidae and Chironomidae. Hemiptera, the most diverse group represented by six families was followed by Coleoptera, Ephemeroptera and Odonata. Highest number of Hemipteran families and their highest relative abundance were also recorded in Moirang River of Manipur, India (Takhelmayum *et al*., 2013)

Table 2. Evaluation of water quality using family level biotic index (Hilsenhoff 1988)

HFBI	Water quality	Degree of organic pollution
0.00-3.75	Excellent	Organic pollution unlikely
3.76-4.25	Very good	Possible slight organic pollution
4.26-5.00	Good	Some organic pollution probable
5.01-5.75	Fair	Fairly substantial pollution likely
5.76-6.50	Fairly poor	Substantial pollution likely
6.51-7.25	Poor	Very substantial pollution likely
7.26-10.00	Very poor	Severe organic pollution likely

During monsoon season, 7 orders, 8 families and 46 individuals of aquatic insects were found in midstream and 3 orders, 4 families and 15 individuals in downstream. Again, during post monsoon a total of 6 orders, 7 families and 102 individuals were found in midstream and 4 orders, 5 families and 34 individuals in downstream. In winter, 4 orders, 7 families and 76 individuals were found in midstream while 4 orders, 6 families and 98 individuals found in downstream. During pre monsoon, 4 orders, 6 families and 36 individuals were found in midstream and 5 orders, 8 families and 40 individuals in downstream (Figure 3). Both highest and lowest number of order and family were found in monsoon, highest in midstream and lowest in downstream. Density was found highest in midstream during post monsoon and lowest in downstream during monsoon season (Figure 3). Takhelmayum *et al.* (2013) in a study on River Moirang found that highest density was directly proportional to the seasonal fluctuations of water level and availability of food resources. According to Arunachalam *et al*. (1991), leaf packs and algal biomass are the high-quality food resources for most of the benthic macroinvertebrates and very important energy source for stream communities. The highest insect density in the post monsoon in midstream could be attributed to high algal biomass in the stream in the same season. High temperature of water and air during post monsoon might have also played a role. The seasonal differences in the abundance of aquatic insects in streams are largely governed by temperature (Gupta and Michael, 1983). The distribution, abundance and diversity of the aquatic insects are affected by intra and inter specific competition plus tolerance capacity of organisms to changing environmental variables of water (Habib and Yousuf, 2012).

Shannon-Wiener diversity index of both the midstream and downstream ranged between 1.02–1.47 during the study period (Figure 5). In the midstream, it ranged from 1.05–1.31, where highest value recorded during pre monsoon and lowest recorded in post monsoon. In the downstream, Shannon-Wiener diversity index (H′) ranged from 1.02–1.47 where, highest value recorded during pre monsoon and lowest recorded in the monsoon season. Values above 3.0 indicate stable habitat and values under 1.0 indicate that there is pollution of habitat structure (Turkmen and Kazanci, 2010). The diversity index value greater than 1 in both the sites during the study period indicated moderate pollution of the river as seen in other studies (Takhelmayum *et al*., 2013; Sandin and Johnson, 2000). Margalef's richness index (Figure 5) ranged from 2.99–5.25 where highest score recorded during winter and lowest score recorded in the post monsoon season in the midstream while in the downstream it ranged from 2.51–4.37 where, highest score recorded during pre monsoon and lowest in the winter season. Score more than 3 indicates 'clean' condition; score less than 1 indicates 'severe' pollution and intermediate score indicates 'moderate' pollution of water (Lenat *et al*., 1980).

Based on Engelmann scale (Engelmann, 1978) (Table 3), in all the four seasons in the midstream Gerridae was eudominant in all the seasons ranging from 58.33% to 72.5% followed by Heptageniidae (13.04%) while Corixidae recorded eudominant in the downstream followed by Veliidae as dominant during monsoon. Eudominance of Gerridae in the midstream in all the seasons is related to their wide preference of laying and attaching eggs to the vegetation and stone surface etc. Moreover, during hibernation, Gerridae usually use leaf litter, rocks and other sheltered sites near water (Stonedahl and Lattin, 1982). Thus, Gerridae preferred midstream because of the substratum type and rich riparian vegetation. Although Corixidae was eudominant in the monsoon in the downstream, in the post monsoon it was replaced by Chironomidae as eudominant group followed by Corixidae as dominant. However, in the winter season Corixidae regained its eudominant status in the downstream (Table 3).

Aquatic insects were classified in to functional feeding groups (FFG) on the basis of ecosystem functioning, as they belong to several specialized feeding groups and from their relative abundance, we assess the impact of anthropogenic activity on freshwater ecosystem (Marsese *et al*., 2014). The FFG of the stream insects show deviation across habitat (Subramanian and Sivaramakrishnan, 2005). In the midstream, the main FFG was predators (67.65%–91.25%) followed by scrapers (13.04%–22.55%) whereas in the downstream piercers-herbivores, predators/scrapers (17.65%–68.37%) was the main functional feeding groups followed by collectors-gatherers and filterers, predators (8.16%–64.71%) during study periods (Table 4). The predators were represented by Gerridae, Notonectidae, Mesoveliidae, Veliidae, Aphelocheiridae, Dytiscidae, Gyrinidae, Perlidae, Gomphidae, Libellulidae, Euphaeidae, Corydalidae and scrapers were represented by Psephenidae and Heptageniidae. Although both the sites were represented by same FFGs, there were seasonal and spatial variations in their percentage occurrences. Thus, meager percentage occurrence of scrapers (6.67%) (Table 4) in the downstream in monsoon and absence in other seasons can be explained by the fact that downstream habitat is always dominated by sand and their mouthparts are not equipped for collection of food from such habitat. According to Brasil *et al.* (2014), their mouthparts are specialized for removing materials adhered to the substrate like stones/boulders found in the upper stretches. The study found that the relative abundance of the families of order Hemiptera was found highest and mostly represented by predators as revealed in other studies also in northeast India (Takhelmayum *et al*., 2013; Barman and Gupta, 2015). Hemipterans are successful in all the possible aquatic environments as they possess oar like hind legs for swimming, can walk on surface water, breathe by the means of an air store and have prominent eyes (Barman and Gupta, 2015).

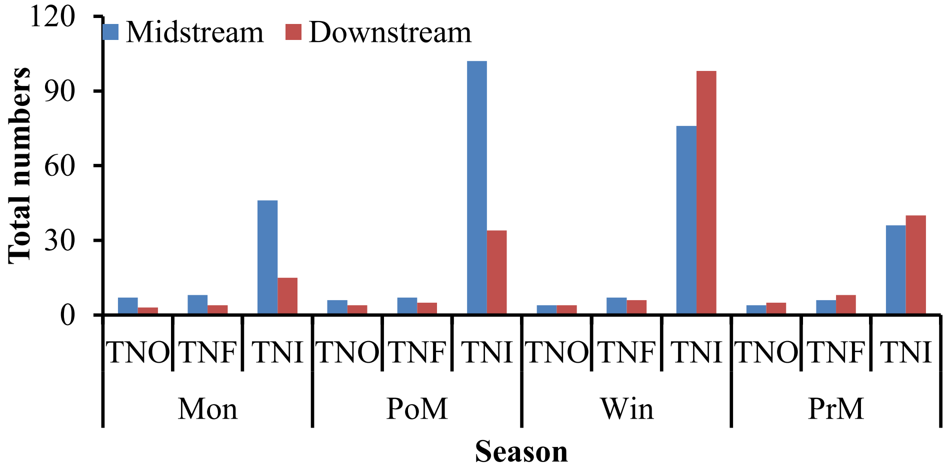

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Figure 3. Total number of order, family and individuals of aquatic insects of River Jatinga during 2018-2019; Mon- Monsoon, PoM- Post monsoon, Win- Winter, PrM- Pre monsoon. TNO- Total number of order, TNF- Total number of family and TNI- Total number of individual.

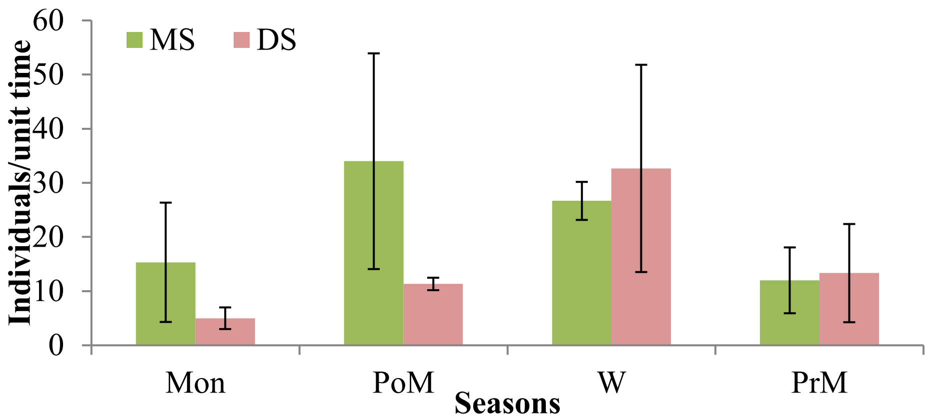

Figure 4. Seasonal insect density (mean±SD) in midstream and downstream of Jatinga River during 2018-2019.

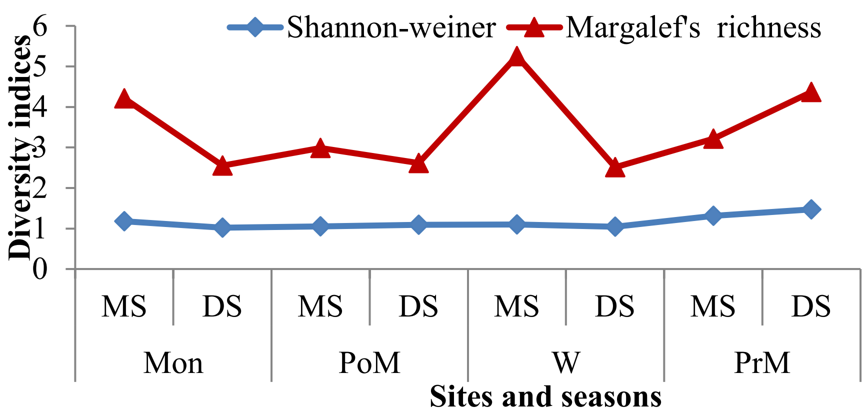

Figure 5. Seasonal variations of diversity indices of aquatic insects of the midstream and downstream of Jatinga River during 2018-2019.

$BMWP^{THAI}$ score indicated 'moderate' water condition in midstream and 'poor' condition at downstream during monsoon, post monsoon and winter season whereas, 'poor' water condition recorded at midstream and 'moderate' condition recorded in downstream during the pre monsoon season (Table 5). Presence of good number of EPT groups in midstream revealed good condition of water quality during study period, as a result midstream found high $BMWP^{THAI}$ score than downstream (Chaw *et al*., 2018). The $BMWP^{THAI}$ score provides single value, at the family level of the aquatic insect tolerance to pollution. The greater their tolerance towards pollution the lower is their $BMWP^{THAI}$ score (Mustow, 2002; Mandaville, 2002).

'Clean water' recorded at both the midstream and downstream during monsoon season as per $ASPT^{THAI}$ score. During post monsoon and winter season, midstream and downstream indicated 'doubtful quality' and 'probable moderate pollution' respectively. But in the pre monsoon season midstream recorded 'clean water' and downstream recorded 'severe pollution' (Table 5). If $ASPT^{THAI}$ score is greater than 6 then 'clean water', 5–6 'doubtful quality', 4–5 'probable moderate pollution' and less than 4 then 'probable severe pollution' (Mandaville, 2002). SIGNAL2 score indicated 'healthy habitat' for midstream and 'severe pollution' for downstream in both monsoon and post monsoon season. Similarly, it indicated 'Moderate' and 'severe pollution' at midstream and downstream during winter and pre monsoon, respectively (Table 5). A SIGNAL2 score gives an indication of water quality in the river from which the sample was collected. Each type of macroinvertebrate has a grade number between 1 and 10, low-grade number indicates macroinvertebrate is tolerant towards pollution and higher the number, the greater the average sensitivity (Chessman, 2003).

The % of EPT recorded 'moderate' condition of the water, 26.09% in monsoon and 29.41% in post monsoon in the midstream while 'poor' condition 6.7% in monsoon and 5.9% in post monsoon in the downstream (Table 5). Studies on three streams of Terengganu, Malaysia also revealed good quality of river water by the presence of major aquatic insect taxa i.e. Ephemeroptera, Plecoptera and Trichoptera (Wahizatul *et al*., 2011; Azmi *et al*., 2018). Presence of high % of EPT communities represent a good quality stream as EPT communities are prevalent in undisturbed streams and exhibit low tolerance towards water pollution (Chaw *et al*., 2018). In the present study, % of EPT in the midstream is low though compared to downstream it is higher indicating relatively better status of water quality in the midstream. HFBI recorded 'good' condition of water quality in the midstream during monsoon and post monsoon season (4.46 and 4.83), while 'fairly poor' and 'poor' (6 and 7.12) water quality recorded in downstream during the monsoon and post monsoon season respectively. 'Fair' (5.06–5.5) water condition recorded at both the midstream and downstream in the winter and pre monsoon season respectively (Table 5).

All the biotic indices such as $BMWP^{THAI}$ and SIGNAL2 scores, EPT % and HFBI have shown relatively better quality of water of the River Jatinga in the midstream than that in the downstream during monsoon and post monsoon seasons. Sand mining activity nearly 5 kilometers upwards from the downstream might have impacted the water quality of the downstream. Sand mining not only deteriorate water quality but also change the richness and diversity of aquatic community (Bhattacharya *et al*., 2019). Other anthropogenic activities of the villagers include domestic work, fishing, recreational activities, input of agricultural waste and pesticide from the riverbank agricultural activities. In the winter and pre monsoon, water quality was seen to have further deteriorated in both the stretches, which may be due to lack of rainfall and thus nutrients concentrated.

Table 3. Seasonal variations in aquatic insect families, functional feeding groups and dominance status (Engelmann, 1978) of the midstream (MS) and downstream (DS) of River Jatinga during 2018-2019

Order	Family	FFG	Midstream				Downstream			
			Mon	PoM	W	PrM	Mon	PoM	W	PrM
Hemiptera	Gerridae	Pr	67.39% (ED)	65.69% (ED)	72.5% (ED)	58.33% ED)	-	5.88% (SD)	-	5% (SD)
	Notonectidae	Pr	-	-	1.25% (R)	8.33% (SD)	-	-	-	-
	Mesoveliidae	Pr	-	-	-	-	-	-	-	2.50% (R)
	Veliidae	Pr	-	-	-	-	26.67% (D)	-	-	-
	Aphelocheiridae	Pr	-	-	2.5% (R)	8.33% (SD)	-	-	-	-
	Corixidae	Pc-Hb, Pr/Sc	-	-	2.5% (R)	-	60% (ED)	17.65% (D)	68.37% (ED)	50% (ED)
Coleoptera	Dytiscidae	Pr (L and A)	-	-	-	-	-	-	4.08% (SD)	-
	Gyrinidae	Pr (L and A)	-	-	12.5% (D)	-	-	-	-	-
	Psephenidae	Sc (L)	-	0.98% (SR)	-	-	-	-	-	-
	Elmidae	CG, Sc, Sh-Hb (L and A)	-	-	-	-	-	-	-	2.50% (R)
	Hydrophilidae	Pr (L), CG (A)	2.17% (R)	-	-	-	6.67% (SD)	-	15.31% (D)	20% (SD)
Ephemeroptera	Baetidae	CG	4.35% (SD)	5.88% (SD)	-	13.89% (D)	-	-	-	2.50% (R)
	Ephemeridae	CG	-	-	1.25% (R)	-	-	-	-	-
	Caenidae	CG	-	-	-	-	-	-	2.04%(R)	-
	Heptageniidae	Sc	13.04% (D)	21.57% (D)	-	-	6.67% (SD)	-	-	-
Trichoptera	Hydropsychidae	Ft, Pr and Sc (S)	2.17%(R)	1.96%(R)	-	-	-	5.88% (SD)	-	-
	Lepidosto-matidae	Sh-Dt	-	-	-	2.78% (R)	-	-	-	-
Plecoptera	Perlidae	Pr	6.52% (SD)	-	-	-	-	-	-	-
Odonata	Gomphidae	Pr	-	-	1.25%(R)	-	-	-	-	-
	Libellulidae	Pr	-	1.96% (R)	1.25%(R)	8.33% (SD)	-	5.88% (SD)	-	2.50% (R)
	Euphaeidae	Pr	2.17% (R)	-	-	-	-	-	-	-
Megaloptera	Corydalidae	Pr	2.17% (R)	-	-	-	-	-	-	-
Diptera	Tipulidae	Sh-Dt, CG	-	-	2.5% (R)	-	-	-	-	-
	Culicidae	Ft and CG	-	-	1.25% (R)	-	-	-	2.04% (R)	-
	Chironomidae	CG and Ft, Pr	-	1.96% (R)	1.25% (R)	-	-	64.71% (ED)	8.16% (SD)	15% (D)

Mon- Monsoon, PoM- Postmonsoon, W-Winter, PrM-Premonsoon, FFG- Functional Feeding Group, RA <1.0% = Subrecedent (SR);1.1% – 3.1% = Recedent (R);3.2% – 10.0% = Subdominant (SD); 10.1% – 31.6% = Dominant (D); 31.7% – 100% = Eudominant (ED), Pr- Predators, Pc- Piercers, Hb- Herbivores, Sc- Scrapers, CG- Collectors-Gatherers, L- Larvae, A- Adult, Sh- Shredders, Ft- Filterers, Dt- Detritivores

Table 4. Functional feeding groups (%) of midstream and downstream during study period (2018-2019)

FFG	Mon		PoM		W		PrM	
	MS	DS	MS	DS	MS	DS	MS	DS
Pr	78.26	26.67	67.65	11.76	91.25	4.08	83.33	10.00
Pc-hb, Pr/Sc	0.00	60.00	0.00	17.65	2.50	68.37	0.00	50.00
Sc	13.04	6.67	22.55	0.00	0.00	0.00	0.00	0.00
CG, Sc, Sh- Hb	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.50
Pr, CG	2.17	6.67	0.00	0.00	0.00	0.00	0.00	20.00
CG	4.35	0.00	5.88	0.00	1.25	17.35	13.89	2.50
Ft, Pr and Sc	2.17	0.00	1.96	5.88	0.00	0.00	0.00	0.00
Sh-Dt	0.00	0.00	0.00	0.00	0.00	0.00	2.78	0.00
Sh-Dt, CG	0.00	0.00	0.00	0.00	2.50	0.00	0.00	0.00
Ft and CG	0.00	0.00	0.00	0.00	1.25	2.04	0.00	0.00
CG and Ft, Pr	0.00	0.00	1.96	64.71	1.25	8.16	0.00	15.00

Mon- Monsoon, PoM- Postmonsoon, W-Winter, PrM-Premonsoon, FFG- Functional Feeding Group, Pr- Predators, Pc- Piercers, Hb- Herbivores, Sc- Scrapers, CG- Collectors-Gatherers, Sh- Shredders, Ft- Filterers, Dt- Detritivores. Midstream (MS), Downstream (DS).

Table 5. Seasonal variations in biomonitoring scores in midstream (MS) and downstream (DS) of River Jatinga during 2018-2019

Season	Site	BMWP^{THAI}		ASPT^{THAI}		SIGNAL 2		%EPT		HFBI		
		Score	Biological class/Remark	Score	Biological class/Remark	Score	Remark	Score	Remark	Score	Water quality	Degree of organic pollution
Monsoon	MS	58	Moderate	7.3	Clean water	6.6	Healthy habitat	26.09	Moderate	4.46	Good	Some organic pollution probable
	DS	30	Poor	7.5	Clean water	3.4	Severe pollution	6.7	Poor	6	Fairly poor	Substantial pollution likely
Post monsoon	MS	42	Moderate	6	Doubtful quality	6.1	Healthy habitat	29.41	Moderate	4.83	Good	Some organic pollution probable
	DS	25	Poor	5	Probable moderate pollution	3.2	Severe pollution	5.9	Poor	7.12	Poor	Very substantial pollution likely
Winter	MS	61	Moderate	5.5	Doubtful quality	4.3	Moderate pollution	1.25	Poor	5.06	Fair	Fairly substantial pollution likely
	DS	25	Poor	4.2	Probable Moderate pollution	3	Severe pollution	17.35	Poor	5.15	Fair	Fairly substantial pollution likely
Pre monsoon	MS	40	Poor	6.7	Clean water	5	Moderate pollution	14.63	Poor	5.5	Fair	Fairly substantial pollution likely
	DS	42	Moderate	5.3	Doubtful quality	3	Severe pollution	2.5	Poor	5.5	Fair	Fairly substantial pollution likely

Biological Monitoring Working Party (BMWP) score: 0–10 = very poor, 11–40 = poor, 41–70 =moderate, 71–100 = good, > 100 = very good. Average Score Per Taxon (ASPT) score: > 6 = clean water, 5–6 = doubtful quality,4–5 = probable moderate pollution, < 4 = probable severe pollution. Stream Invertebrate Grade Number-Average Level (SIGNAL) score: > 6 = healthy habitat, 5–6 = mild pollution, 4–5 = moderate pollution, < 4 = severe pollution. %EPT: ≤1 = poor, 2-5 = moderate, > 5 = good. Midstream (MS), Downstream (DS).

Conclusions

This is a first study on aquatic insect community of River Jatinga and their role in biomonitoring of river health. Computation of different biotic indices revealed that water quality is mostly moderate in the midstream while poor in the downstream of the river. The study further confirmed the role of aquatic insects as bioindicators in the lotic systems and the significance of use of different biotic indices in discerning water quality. It is recommended that for water quality management of rivers or streams, distribution of aquatic insect communities in different sites and seasons can be used in biomonitoring study. Future studies on this river would pinpoint the sources of pollution and also provide insight to the biodiversity of the river.

Compliance with Ethical Standard

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

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Doğal arıtım sistemi: Yapay yüzen ada teknolojisinin Türkiye'deki göl, gölet ve baraj göllerinde uygulanma potansiyeli

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ÖZ

Bu çalışmada bir fitoremedasyon stratejisi olan yapay yüzen ada (YYA) teknolojisinin Türkiye göl, gölet ve baraj göllerinde uygulanma potansiyeli incelenmiştir. Küresel ısınma ve buna bağlı olarak meydana gelen iklim değişikliği ile birlikte su kaynakları üzerine olan baskı her geçen gün daha da artmaktadır. Ayrıca artan çevre kirliliği, doğadaki canlılara doğrudan ve/veya dolaylı olarak zarar vermekte ve yaşamlarını olumsuz yönde etkilemektedir. Bu kapsamda ülkemizde bulunan göl, gölet ve baraj göllerinin olumsuz küresel ve çevresel koşullara karşı önem değeri son yıllarda artmıştır. İçme suyu kaynağı, tarımsal alanları sulama, rekreasyon amacıyla kullanımı ve içerisinde bulunan ekosistem dikkate alındığında göl, gölet ve baraj göllerinin iyileştirilmesi için yenilikçi, sürdürülebilir ve ekonomik çözüm önerileri gerekmektedir. Ülkemizde var olan doğal suların mevcut trofik durumu dikkate alındığında ötrofikasyon potansiyeline sahip birçok göl, gölet veya baraj gölü tespit edilmiştir. Bu organik kirlilik yükünü iyileştirmek için dünya üzerinde yaygın olarak uygulanan YYA teknolojisi oldukça ekonomik bir alternatif doğal arıtım sistemidir. Literatürde var olan çalışmalar YYA teknolojisinin genellikle laboratuvar ve pilot ölçekli uygulanmasına odaklanmıştır. İlerleyen süreçte YYA teknolojisinin gerçek ölçekli uygulanması hem bilimsel hem de ülke ekonomisi için önemli bir katma değer sağlayacaktır.

Anahtar Kelimeler: Yapay yüzen adalar, Fitoremedasyon, Ötrofikasyon, Trofik durum

ABSTRACT

Natural water treatment system: the potential of applying artificial floating island technology in lakes, ponds and dam lakes in Turkey

In this study, the implementation potential of artificial floating island (AFI) technology, which is a phytoremediation strategy, was investigated in Turkish lakes, ponds and reservoirs. The pressure on water resources is increasing ever with the global warming as well as the effect of climate change. In addition, increasing environmental pollution directly and/or indirectly harms the organisms thus has negative effects on their lives. In this context, the importance of lakes, ponds and dam lakes in Turkey against adverse global and environmental conditions has increased in recent years. Innovative, sustainable and economical solutions are required for the improvement of lakes, ponds and dam lakes, considering that these are main resources of drinking water, irrigation of agricultural lands, and recreational activities. Considering the current trophic state of natural waters in our country, many lakes, ponds or dam lakes have been identified with eutrophication potential. AFI technology, which is widely established globally to remediate such organic pollution load, is a highly economical alternative treatment system. Previous studies have generally focused on the laboratory and pilot scale implementation of AFI technology. In the future, full-scale establishment of AFI technology will provide significant added value for both the scientific and the national economy.

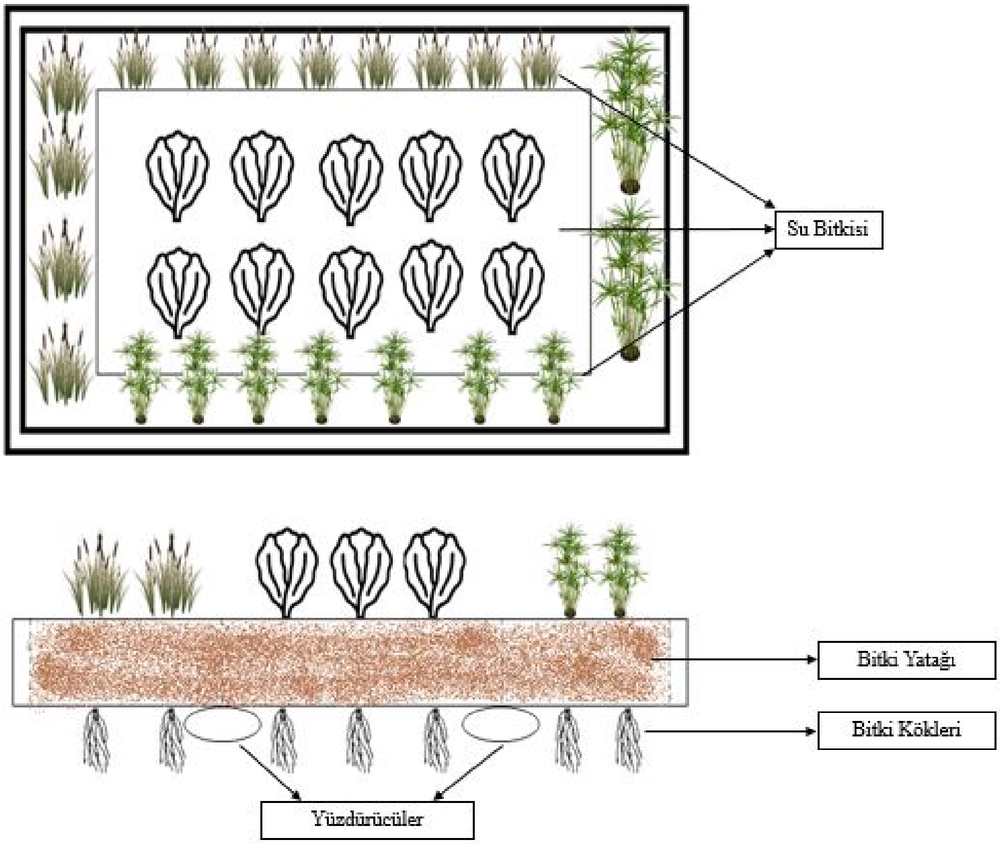
Keywords: Artificial floating islands, Phytoremediation, Eutrophication, Trophic state

Giriş

Hayat için gerekli ve değerli olan su kaynakları, çeşitli nedenlerle günümüzde tehdit altındadır. Son dönemlerdeki nüfusun hızlı artışı, çarpık kentleşme, sanayileşme, gıda arzının artması su talebini ve su kirliliğini artırmıştır (Vadde vd., 2018). Artan nüfusun taleplerini karşılamak ve bunun yanında rekreasyon, sulama, su ürünleri yetiştiriciliği ve habitatların korunması için güvenilir ve temiz bir tatlı su temininin sürdürebilirliği gereklidir. Fakat insan kaynaklı atıklardan dolayı hava, su ve toprağın kirlenmesi son yıllarda ciddi bir sorun haline gelmiştir (Şener vd., 2014). Dünya Sağlık Örgütü (WHO) ve Birleşmiş milletler Çocuklara Yardım Fonu (UNICEF) tarafından yayınlanan son raporlara göre, dünya nüfusunun %90'ı içme suyu kaynağına ulaşımı olsa dahi, dünya çapında iki milyardan fazla insanın güvenli bir şekilde yönetilen içme suyu hizmetlerine erişimi yoktur (WHO, 2019; WHO/UNICEF 2019). Dünya Bankası raporuna göre ise 2030 yılında öngörülen kullanılabilir su talebinin, mevcut su arzına göre %40 oranında daha fazla olması beklenmektedir (World Bank, 2019). Tarımsal, endüstriyel ve kentsel faaliyetler, sucul ekosistemlerde hem besin hem de kimyasal kirliliğin önemli kaynaklarıdır (Ouyang vd., 2006; Chen vd., 2020). Doğal su kaynaklarının kirletilmesinde üretimin etkisi oldukça fazladır. Son dönemde COVİD-19 pandemisi birçok ülkede uluslararası üretim ve ticaret zinciri olumsuz etkilenmiş, fakat buna karşın küresel çapta çevresel pozitif değişimler gözlenmiştir (Kanniah vd., 2021; Rupani vd., 2020; Shrestha vd., 2020; Wang ve Su, 2020). Fakat insan ihtiyaçlarının karşılanması için üretimin sürekli olarak devam etmesi de tartışılmaz bir gerçektir. Sürdürülebilir ve çevreci üretime ek olarak doğal kaynakların iyileştirilmesi politikası dikkate alınmalıdır (Colares vd., 2020).

Su kaynaklarının kirlenmesi, telafisi imkansız problemlerin oluşmasına zemin hazırlamaktadır (Ahmet, 2006). Gelişen teknoloji ve endüstrileşmenin insan hayatını kolaylaştıran olumlu etkilerine karşın çevre üzerindeki olumsuz etkileri göz ardı edilemezdir (Sun vd., 2019). Çevre kirliliği 20. yüzyılda insanlığın en önemli sorunu olmuştur (Gönülol ve Obalı, 1986). Sosyo-ekonomik gelişmenin su kaynakları üzerindeki etkileri, özellikle su kirliliği göz önüne alındığında, özel bir ilgi odağı haline gelmiştir (Fan ve Fang, 2020). Su kirliliği canlıları yapısını direkt olarak etkilediği için kirliliğin tespit edilmesi temelde biyolojik bir olgudur. Sucul ortamlardan daha verimli bir şekilde yararlanmak ve bunların sürekliliğini sağlamak için, su kaynaklarının sahip olduğu potansiyelin ve özelliklerinin detaylı bir şekilde incelenmesi gerekmektedir. Bu nedenle sucul kaynakların fizikokimyasal özelliklerinin ortaya konması gerekmektedir (Yüce, 1999).

Göllerin biyolojik çeşitlilik, balıkçılık, rekreasyon, turizm ve hidrolojik döngüdeki rolü ekosistemdeki önemini ortaya koymaktadır (Dodson vd., 2000; Kristensen ve Hansen, 1994). Doğal ve yapay göller, insan ihtiyaçlarını karşılamak için toplam tatlı su kaynaklarının büyük bir bölümünü oluşturmaktadır (Beyhan ve Kaçıkoç, 2014). Kötü su kalitesi ötrofikasyona yol açabilir ve toplu balık ölümleri gibi, insanlar ve suda yaşayan canlılar üzerinde bir dizi olumsuz etkiye sahip olabilir (Flynn ve Suplee, 2011). Ötrofikasyona uğramış göletler, göller ve barajlarda meydana gelen siyanobakteriyel çiçeklenmeler, su kaynaklarının etkin kullanımı açısından ciddi sorunlara yol açmıştır (Nakai vd., 2008). Ötrofikasyon sonucu su kalitesindeki bozukluk, tarım ve sanayi gibi endüstrilerle ekonomik kalkınmayı olumsuz etkileyebilir (Liu vd., 2014; Ma vd., 2010). Doğal su kaynaklarında su kalitesinin iyileştirilmesi sadece su ekosistemini pozitif etkilemekle kalmaz, aynı zamanda insan tüketimi ve sulama için güvenli tatlı su arzını sağlar (Bui vd., 2020). Özellikle hastalık etkeni taşıyan içme suyu güvenliğinin havzadan tüketiciye kadar en aza indirilmesi için evsel suların arıtımı, güvenli depolama ve su kaynaklı patojenlerin azaltılması oldukça önemli bir mekanizmadır (Xiao vd., 2020). Dünya genelinde su kirliliğinin olumsuz çevre etkisini önlemek için birtakım sürdürülebilir uygulamalarla da tedbirler alınmaktadır. Bunlar arasında su ortamının yapay yüzen ada (YYA) teknolojisi ile arıtımı, ekonomiklik ve basit uygulanabilirliği açısından yaygın olarak kullanılmaktadır (Sun vd., 2009; Dotro vd., 2017). Bazı çalışmalarda YYA'ların, su kalitesinin bozulmasına karşı bir tampon görevi üslendiği (Egertson vd., 2004; McGill vd., 2010), su içerisindeki besin konsantrasyonlarını azalttığı (Palombo vd., 2013) ve trofik değişimleri etkilediği bildirilmiştir (Pelicice ve Agostinho, 2006). Bu bilgiler ışında YYA'lar, yaklaşık 40 yıllık geçmişe sahip ötrofik bir su arıtma teknolojisidir (Chang vd., 2017). Bu çalışmada, dünya genelinde uygulanan bir teknoloji olan yapay yüzen adaların Türkiye'deki göl, gölet ve baraj göllerinde uygulanma potansiyeli incelenmiştir.



Şekil 1. Yapay yüzen ada tasarımı

Figure 1. The design of artificial floating island

Yapay Yüzen Ada Teknolojisi

YYA'lar hedef su kaynakları üzerinde özellikle endüstriyel, su ürünleri yetiştiriciliği ve tarım arazisi atık sularının dekontaminasyonu ve ekolojik iyileştirmesi için sürdürülebilir bir teknolojidir (Nahlik vd., 2006; R. K. Hubbard vd., 2004). Genel bir tanımlama ile YYA'lar (Şekil 1), yüzen matlar, yüzen sulak alan bitkileri ve algler, biyofilmler, zooplanktonlar ve küçük omurgasızlar gibi ilgili ekolojik topluluklardan oluşan topraksız bir ekim yapısıdır (Hu vd., 2010; Sun vd., 2009). Wolverton ve McDonald, 1975 tarafından yapılan ilk çalışmada YYA'lar ile kirli sudan gümüş (Ag), kobalt (Co) ve stronsiyum (Sr) giderimi yapılmıştır. 40 yıllık geçmişi bulunan YYA'lar günümüzde geleneksel YYA'lar, kompleks YYA'lar ve taşınabilir YYA'lar olacak şekilde 3 farklı teknoloji olarak geliştirilmiştir (Chang vd., 2017).

YYA'ların dört ana işlevi olduğu bildirilmektedir: Bunlar, (1) su arıtma, (2) belirli hayvanlar için habitat sağlama, (3) kıyı bölgesini koruma ve (4) peyzaj özelliklerinin iyileştirilmesidir (Nakamura ve Shimatani, 1997). Bunlara ek olarak Hoeger, (1988) tarafından biyolojik dezenfeksiyon etkisi de bildirilmiştir. Su üzerindeki bitkiler etkili bir rüzgâr kırılmasına, kök sistemleriyle kıyı bölgelerdeki akıntıları zayıflatmaya ve böylece toprak hareketini önemli ölçüde azaltmaya yardımcı olur. Ağırlıkları ile dalgaların yörüngesel hareketini yavaşlatır, böylece YYA'lar ile kıyı arasında sakin bir su bölgesi oluşturur. YYA'lar habitat adaları olarak konuşlandırıldığında, belirli bir su kütlesindeki birçok hayvan ve bitki türü için yaşam alanı görevi görür. Ada üzerinde su kuşları, memeliler ve sürüngenler için yaşam alanı oluştururken, ada altında balık yavruları ve balıklar için yaşam alanı, dönemsel olarak yumurtlama alanları oluşturur. Böcekler, böcek larvaları, kerevitler ve yumuşakçalar gibi çok sayıda omurgasız hayvanın yanı sıra tek hücreliler, bakteriler ve algler, besin zincirindeki ayrıştırıcılar, yırtıcılar ve diğer elementler de bu adalar üzerinde yerlerini alırlar. Bir peyzaj aracı olarak özellikle ötrofik göllerde alglerle kaplı su görüntüsünü ortadan kaldırarak farklı tropikal çiçeklendirmeler ile görsel ihtiyaca cevap verir. En önemli işlevi olarak düşünüldüğünde ise yüzen adalara dikilen bitkilerin kök sistemleri, su kütlesine asılır ve büyük, üç boyutlu bir kök labirenti oluşturur. Bu ağ, yalnızca çözünmüş maddeleri doğrudan sudan uzaklaştırmakla kalmaz, aynı zamanda mekanik olarak yüzen parçacıkları filtreler ve tutar. YYA'lar, özel olarak tasarlanmış deneysel alanlara veya enstrümantasyonlara ihtiyaç duymadıklarından atık suyu arıtmak için uygun maliyetli yöntemlerdir. Böylece proses enerjisinde %80'e ve malzeme girdisinde %50'ye kadar tasarruf sağlayabilir (Luederitz vd., 2001). Türkiye'de özellikle ağır metal içeren atıksuların iyileştirilmesi için uygulanan biyoremediasyon çalışmaları mevcuttur (Ayas vd., 2019; Nassouhi vd., 2018; Tatar, 2014). Ekvatorda arsenik ve demir içeriği bulunan bir rezervuarda yapılan iyileştirme çalışmasında 3,6 m^2 kapasiteli YYA kullanılmıştır (Largo vd., 2020). Çalışma sonucunda YYA'nın sudaki ve sedimentteki areseniği sırasıyla %97 ve %84 oranında azalttığı gözlenirken, demir ise sedimentte ortalama %87 oranında giderilmiştir. Ayrıca çalışma sonunda makrofitlerin hayatta kalma oranı ise %92 olarak bulunmuştur. Ek olarak, rizosfer, faaliyetleri ile su arıtma sürecini geliştiren sayısız bakteri barındırır. Biyolojik dezenfeksiyonu üzerine çok fazla çalışma bulunmamasına rağmen büyük sümbül (*Scirpus zacustris*) bitkisinin özellikle ilkbahar ve yaz aylarında salgılamış olduğu mikrobiyal maddeler, sazlarla bitkilendirilen alanlarda *koliform* ve *Salmonella* gibi patojenleri etkisiz hale getirmektedir. Daha da önemlisi, kirlenmiş deniz kırlangıcı ve deniz martılarının, bu tür yerlerde yuva yapmaya başladıktan sonraki günler içinde *Salmonella* enfeksiyonlarından kurtulmaları mümkündür (Hoeger, 1988). Bununla birlikte yanlış seçilen ve yönetilen YYA'lar, istilacı türler nedeniyle yerel tarım, su ürünleri yetiştiriciliği ve biyolojik çeşitlilik üzerinde istenmeyen etkilere neden olabilir (Vera vd., 2010). Ayrıca, çürümüş, uzun süre ıslatılmış yüzen malzemeler de bir kirlilik kaynağı olabilir (Yeh vd., 2015).

Yapay Yüzen Adaların Tasarımında Kullanılan Malzemeler

YYA'lar üç katmanlı olarak inşa edilir. Bunlar; ada üzerinde bulunan bitkilerin yaprağı ve gövdesini içeren bölge, adanın yüzmesini sağlayan yüzdürücü ekipmanlar ve su içerisindeki mikro ve makro canlılarla etkileşim halinde olan kök sistemleridir (Chang vd., 2017). YYA tasarımında öncelikle yüzdürücü bir iskelet sistem ve suya dayanıklı bitkiler, gerekli görüldüğü takdirde ise bir bitki yatağı ve su yüzeyinde sabit noktada kalmasını sağlayacak çapa veya iple kıyıya bağlama sistemi bulunur. Bitki yatağı kullanımı çok yaygın olmasa da kullanılması durumunda genellikle kokopit kullanılan çalışmalar bulunmaktadır (Billore vd., 2009; Van Acker vd., 2005). Genellikle toprak ikamesi olarak kullanılan kokopit, su tutma özelliği ile bitkinin sürekli olarak su ile temasını sağlarken aynı zamanda bitkinin sabit kalmasını da sağlamaktadır (Billore vd., 2009). Yüzdürücü iskelet olarak bir çok farklı tasarım söz konusudur (Alberto vd., 2021). Geleneksel sistemlerde genellikle PVC borular veya koruge drenaj boruları kullanılırken bunların dışında strafor, plastik dubalar, yüzdürücü matlar, bambular, polietilen yüzdürücüler ve su şişeleri kullanılmaktadır. Fakat kullanılan bu yüzdürücülerin son dönemlerde önem seviyesi oldukça fazla olan mikroplastik kirliliğine katkı sağlayabileceği gerçeğine dikkat edilmelidir. Su ortamında yaşayan canlılar için endokrin bozucu etkiye sahip fenolik grupların canlılar üzerinde birikimi (Faheem vd., 2016; Molina vd., 2018; Nane vd., 2021) YYA'ların olumsuz etkileri olmamalıdır. Bu kapsamda farklı üreticiler tarafından çevre dostu ve daha dayanıklı sistemler (BioHaven ve Tech-IA) üretilmiştir. BioHaven adaların gövdeleri, oldukça gözenekli ve çevresel faktörlere dirençli üç boyutlu bir matris sağlamak için iç içe geçmiş ve bağlanmış ince (0,007 inç çaplı) polimer şeritleri içerir (Stewart vd., 2008). Ayrı polimer şeritleri, mikrobiyal biyofilmler tarafından kolonizasyon için ideal bir substrat sağlar ve matris ayrıca su, nehir kenarı ve kara bitkilerinin kökleri için mükemmel bir büyüme ortamı sağlar. Tech-IA, ise yüksek mekanik, kimyasal, biyolojik şartlara ve hava koşullarına dayanıklı, geri dönüştürülebilir ve toksik olmayan bir formül olan etilen vinil asetattan yapılmıştır (Alberto vd., 2021). Bitkilerin sabitlenebilmesi için ızgara sistemi bulunan bu tasarımlar dikdörtgen şeklinde sekiz bölme içermektedir. YYA'ların tasarımında kullanılan malzemelerin listesi Tablo 1'de gösterilmektedir.

Yapay Yüzen Adaların Tasarımında Kullanılan Bitkiler

YYA sistemlerindeki bitki türleri, büyüme hızı ve kök türleri gibi spesifik biyolojik özellikleri nedeniyle farklı kirletici uzaklaştırma kapasitelerine sahiptir (Chang vd., 2017). Doğal suların trofik durumunu ortaya koyan organik kirleticiler genellikle azot ve fosfordur. YYA teknolojisi için kullanılan bitkilerin de daha çok bu kirleticiler için etkinliği önem arz etmektedir. Ortaya çıkan çok sayıda su bitkisi türü, doğada yüzen adalar oluşturma potansiyeline sahiptir ve bunların çoğu, su kalitesini iyileştirmek amacıyla kendi kendine büyüyebilir (Chen vd., 2016). *Scirpus validus*'un kullanıldığı bir sistemde toplam azotun (TN) giderimi başarılı bulunurken, *Canna generalis* kullanılan sistemde ise büyük ölçüde nitrat azotu giderimi görülmüştür (Zhang vd., 2014). Bir başka çalışmada ise toplam fosfor (TP) ve TN giderimi için *Ipomoea aquatica* bitkisi kullanılmıp YYA teknolojisi önerilmiştir (Chen vd., 2010). Tech-IA yüzer sistemlerle *Poaceae, Asteraceae, Cyperaceae, Iridaceae* ve *Thypaceae* ailelerine ait 28 farklı bitki türünün büyüme performansları ve uyarlanabilirliği incelenmiştir (Alberto vd., 2021).

YYA sistemlerinde kullanılan bitkilerin kökleri lifli veya kalın olabilir (Lai vd., 2011). Kök gelişimi, bitki türü, bitki yaşı, besin konsantrasyonları, suyun redoks koşulları ve bazı durumlarda destekleyici hasırlar veya sallar gibi birçok faktörden etkilenir. Yağmur suyu arıtımı üzerine yapılan bir çalışmada, *Carex dipsacea, Carex virgata, Cyperus ustilatus, Eleocharis acutis* ve *Schoenoplectus tabernaemontani* için ortalama kök uzunlukları 24 ve 48 cm arasında, *Juncus edgariae* için maksimum 87 cm olarak bildirilmiştir (Tanner & Headley, 2008). Farklı bitki türlerinin araştırıldığı bir çalışmada *Calamagrostis epigejos, Phragmites australis, Typha latifolia* ve *Juncus maritimus*'un kök gelişimleri dikkat çekmiştir (Pavan vd., 2015). Bir başka çalışmada ise benzen, metil tert-butil eter ve amonyum içeren bir kirli su için YYA tenolojisi uygulanmış ve *P. australis*'in kök uzunluğu üç yıl içinde 25 cm'ye ulaşmıştır (Chen vd., 2012). Literatürde genellikle kullanılan bitkiler Tablo 1'de gösterilmiştir.

Yapay Yüzen Adalar ile İlgili Yapılmış Çalışmalar

Laboratuvar Ölçekli Çalışmalar

Nakai vd. (2008) tarafından yapılan bir çalışmada YYA'lar üzerinde kullanılan Afrika ararotı (*C. generalis*) ve Japon şemsiyesi (*Cyperus alternifolius*) bitkilerinin anti-siyanobakteriyel alelokimyasalları serbest bırakıp bırakmadığı incelenmiştir. 3 anti-siyanobakteriyel fenolik bileşik (VA, PCA ve GA), *C. alternifolius*'un alelopatik etkisine katkıda bulunabilse dahi, bunların kültür çözeltisindeki görünen miktarları, *Micrococcus aeruginosa*'nın büyüme inhibisyonuna neden olmak için yeterli olmadığı gözlenmiştir. Pekin'de yapılan bir başka çalışmada ise YYA'lar için dört farklı bitki kullanılmıştır (Yao vd., 2011). Bunlar; *S. validus, Lythrum salicaria, Iris wilsonii ve Typha minima* olarak belirlenmiştir. Bitkilere ait büyüme performansları da incelenen çalışmada en yüksek kök büyümesini *T. minima* gerçekleştirmiştir. Bitkilerin su üzerindeki büyümesi su altındakinden daha yüksek bulunmuştur. Kimyasal oksijen ihtiyacı (KOİ) tüm gruplar için net bir eğilim göstermese dahi *T. minima* bulunan grupta zamana bağlı azalma göstermiştir. TP, TN ve PO_4 tüm gruplarda zamana bağlı azalan trend göstermiştir. Laboratuvar ölçekli yapılan diğer bir çalışmada iki adet geleneksel YYA sistemine ek olarak iki adet geliştirilmiş YYA sistemi kullanılmıştır (Kong vd., 2019). Geleneksel YYA sistemi PVC boru ile yüzdürülen kutu sistemlerinden oluşurken geliştirilmiş YYA sistemlerinin bitki yataklarını luffa süngeri ve mısır koçanı oluşturmaktadır. Çalışma sonunda bitki gelişimi açısından en çok mısır koçanı kullanılan sistemdeki bitkilerin büyüdüğü buna karşılık geleneksel sistemdeki bitkilerin ise net bir gelişim göstermediği gözlenmiştir. Ayrıca su kalitesi incelendiğinde geliştirilmiş YYA'nın TP, TN ve nitrat gideriminin sırasıyla %92,8, %90,3 ve %96 olarak geleneksel gruptan yüksek olduğu bildirilmiştir. Çin'de Shahu Gölü'nden alınan su numunelerinin farklı seyreltme oranları uygulanması ile elde edilen farklı kirlilik konsantrasyonlarına sahip numuneler üzerinde YYA teknolojisinin etkisi incelenmiştir (Chen vd., 2020). Ham göl suyuna ek olarak 5, 7 ve 9 kat seyreltilmiş numuneler üzerinde *I. aquatica*'nın statik testi uygulanmıştır. Toplam 60 gün süren çalışmanın sonunda YYA teknolojisinin TN konsantrasyonunu anlamlı derecede azalttığı ve su kalitesini iyileştirdiği gözlenirken su ıspanağının büyüme performansı da olumlu yönde etkilenmiştir. Benzer bir çalışmada göl suyu ve musluk suyu karışımı olan bir ötrofik numune üzerinde dört farklı süs bitkisi *(Spathiphyllum floribundum, Hydrocotyle sibthorpioids, Chlorophytum comosum, Peperomia obtusifolia*) içeren YYA teknolojisinin etkisi incelenmiştir (Zhang vd., 2021). Sonuçlar tüm süs bitkilerinin hayatta kalma oranlarının yüksek olduğunu ve biyokütlelerinin zamana göre arttığını göstermiştir. Toplam organik karbon ve amonyum giderim veriminin sırasıyla %85 ve %97 olduğu belirtilmiştir. Bitkiler arasındaki giderim ve büyüme performansı incelendiğinde ise *H. sibthorpioids*'in diğer gruplara göre daha faydalı olduğu gözlenmiştir.

Atıksular üzerine yapılan laboratuvar ölçekli bir çalışmada yüksek konsantrasyona sahip biyogaz ve düşük konsantrasyona sahip çökeltme havuzlarından gelen atıksuyun üç farklı bitki (çavdar otu, hindiba ve tere) içeren YYA'lar ile arıtımı incelenmiştir (Huang vd, 2021). Düşük konsantrasyona sahip atıksuların arıtımı için çavdar otu grubunun diğer gruplara göre daha yüksek KOİ, TN ve TP giderdiği gözlenmiştir. Benzer şekilde yüksek konsantrasyona sahip atıksu için biyolojik oksijen ihtiyacı (BOİ), KOİ, TN ve bulanıklık çavdar otu grubunda anlamlı olarak daha düşük bulunmuştur. Bitkiler arasında büyüme performansı incelendiğinde çavdar otunun hindiba ve tereye göre anlamlı olarak daha iyi geliştiği görülmüştür. Sonuç olarak çavdar otu YYA teknolojisi olarak atıksuların arıtımı için dikkat çekicidir. Güney Brezilya'da yapılan bir başka çalışmada ise mezozom YYA teknolojisinin üniversite atıksu kalitesini iyileştirme potansiyeli incelenmiştir (Bauer vd., 2021). Bitki olarak *Typha domingensis* kullanıldığı yüzen adanın fizikokimyasal, besinsel ve ağır metal açısından etkileri araştırılmıştır. Sonuçlar YYA'nın giriş suyu ile karşılaştırıldığında çıkış suyunda su kalitesini arttırdığını göstermiştir. Fakat TP ve çinko parametrelerinde çıkış suyunda anlamlı bir farklılık gözlenmemiştir.

Pilot Ölçekli Çalışmalar

Tayvan'daki Lize Gölü'nden alınan su ile doldurulan bir tank ve üniversite yurdundan gelen atıksu ile doldurulan bir başka tank üzerine kurulan YYA sistemi için su kalitesini temsil eden biyolojik indikatör türler incelenmiştir (Chang vd., 2014a). Sonuçlar YYA'ların su katmanlaşmasını ve alg büyümesini önlediğini ve suyun homojenleşmesine katkı sağladığını göstermiştir. Aynı göl ve atıksudan alınan benzer bir çalışmada üç aylık izleme sonucunda su kalitesi üzerindeki etkiler incelenmiştir (Chang vd; 2014b). YYA'lar göl suyu için iletkenliği %30 azaltırken, çözünmüş oksijeni (ÇO) 2,8 kat arttırmıştır. Atık su üzerinde ise iletkenliği %34 azaltacak ve ÇO'yu 982 kat arttıracak şekilde olumlu etki göstermiştir. Devamındaki çalışmada ise YYA, göl suyu ve atıksuda sırasıyla TN'yi %66,6 ve %100; TP'yi %74,4 ve %62,2; PO^4'ü %64 ve %71; KOİ'yi %100 ve askıda katı maddeyi ise %80 ve %86 oranında gidermiştir (Lu vd., 2015). Su ürünleri çıkış sularının iyileştirilme motivasyonu ile Brezilya'da yapılan bir çalışmada tilapia yetiştiricilik çıkış suyunun YYA ile arıtımı hedeflenmiştir (Osti vd., 2020). YYA üzerinde bitki olarak *Eichhornia crassipes* kullanılmıştır. Yarı yoğun yetiştiricilik yapılan balık kültüründen gelen atıksuyun TN ve TP konsantrasyonları üzerinde YYA sistemlerinin pozitif etkisi dikkat çekmiştir.

Amonyum, nitrat ve nitrit giderimleri için de YYA'ların etkisi dikkat çekmiştir. Bir başka çalışmada iki farklı su bitkisi (*L. salicaria* ve *I. wilsonii*) kullanılarak 40 günlük bir deneme ile su kalitesi izlenmiştir (Liu vd., 2016). Sonuçlar, *L. salicaria* bulunan grubun KOİ, TN ve TP için sırasıyla %75, %57 ve %71 giderim yaptığını, *I. wilsonii* için ise sırasıyla %60, %49 ve %58 giderim yaptığını göstermiştir. Çin'in Yangcheng Gölü'nde Çin mitten yengecinin (*Eriocheir sinensis*) yetiştirilmesi sırasında kirlilik kontrolünü ve yerinde biyoremediasyonu test etmek için pilot ölçekli bir çalışma yapılmıştır (Ni vd., 2018). Bu çalışmada YYA'lar ile birlikte su altına inen biyolojik filtreler kombin edilmiştir. Toplam beş farklı su bitkisinin (*L. salicaria, Thalia dealbata, Pontederia cordata, Iris tectorum, I. wilsonii,* ve *Canna warscewiezii*) bulunduğu iyileştirme bölgesinde su kalitesi yetiştirme bölgesine göre hafif bir iyileşme göstermiştir. Biyofiltrede oluşan biyofilm, organik kirleticilerin ve azotun uzaklaştırılmasında büyük rol oynamıştır.

Gerçek Ölçekli Çalışmalar

Çin'in Honghu Şehri'nde bulunan bir nehir üzerinde su kalitesini arttırmak için yedi farklı bitkiden (*Oenanthe javanica* (O), *Gypsophila* sp. (G), *Rohdea japonica* (R), *Dracaena sanderiana* (D); shrubs: *Gardenia jasminoides grandiflora* (Gg), *Gardenia jasminoidesprostrata* (Gp), ve *Salix babylonica* (S)) oluşan YYA teknolojisi uygulanmıştır (Zhu vd., 2011). 130 günlük çalışmanın sonunda bu türler arasında bitkilerin kuru ağırlıkları sırasıyla S>G>O>D>Gg>Gp>R şeklinde olmuştur. Bitki gövdesindeki N konsantrasyonu için O>D>G>Gp>S>R>Gg ve P konsantrasyonu için ise R> Gp>O>Gg>G>S>D gibi bir ilişki gözlenmiştir. Bitki biyokütle artışı ile N ve P birikimleri arasında pozitif doğrusal bir ilişki gözlenmiştir. Bu durum bitki hasatının su içerisindeki N ve P yükünü azaltan bir göstergedir. Benzer şekilde Çin'de Taizhou Üniversitesi tarafından gerçekleştirilen bir çalışma ile beş farklı su bitkisini (*C. generalis, S. validus, Alternanthera philoxeroides, C. alternifolius* ve *Thalia geniculata*) içeren on beş YYA kurulmuştur (Zhang vd., 2014). Sırasıyla *A. philoxeroides* veya *C. alternifolius* ekilen YYA'larda $BOİ_5$, KOİ, TP ve amonyumun daha fazla uzaklaştırıldığı gözlenmiştir. *S. validus* ekilen adalarda, TN'nin ve *C. generalis* ekilen adada ise nitratın büyük ölçüde uzaklaştırıldığı bildirilmiştir. Hindistan'da bulunan Khipra Nehri üzerinde kurulan YYA'nın toplam katı (TS), amonyum, nitrat ve BOİ üzerine etkileri incelenmiştir (Billore vd., 2009). 200 m^2 YYA üzerine bölge için yerel bir bitki olan *Phragmites karka* dikilmişir. Çalışmanın sonunda TS için %55-60, amonyum için %45-55, nitrat için %33-45 ve BOİ için ise %40-50 arasında iyileşme olduğu bildirilmiştir. Aynı nehir üzerinde benzer bir senaryo ile kurulan bir başka çalışma daha gerçekleştirilmiştir (Prashant ve Billore, 2020). Çalışmanın sonunda YYA teknolojisinin TS'yi %46, bulanıklığı %51, toplam Kjeldahl azotunu %37 ve BOİ'yi %39 iyileştirdiği gözlenmiştir. Ayrıca YYA, su içerisinde bulunan makro omurgasızlar için ilave yüzen bir niş oluşturmuştur. Hindistan'ın Mula ve Mutha nehirleri bölgede bulunan arıtıma tabi tutulmamış atıkların deşarj noktasıdır (Kamble ve Patil, 2012). Bu kirliliğin önüne geçilebilmesi için sürdürülebilir bir teknoloji olan YYA'lar önerilmiştir.

Brezilya'da bulunan bir üniversitenin 400 m^2'lik kentsel rezervuarında gerçekleştirilen çalışmada üç farklı YYA (32,5 m^2, 4,7 m^2 ve 40 m^2) sistemi kurulmuştur (Rocha vd., 2021). Geçici olarak bulanıklık, elektriksel iletkenlik ve toplam, sabit ve uçucu çözünmüş katılar azalma eğilimi göstermiştir. Çalışma alanı üzerindeki 13 örnekleme noktası dikkate alındığında toplam çözünmüş katılarda, uçucu çözünmüş katılarda ve elektriksel iletkenlikte azalma gözlenmiştir. Ek olarak KOİ ve TP gibi istatistiksel eğilimleri göstermeyen değişkenlerde ara sıra önemli azalmalar görülmüştür. Endonezya'da Maninjau Gölü'nde bulunan bir ağ kafes yetiştiricilik sisteminden göle karışan organik kirlilik yükünü azaltmak için YYA teknolojisi uygulanmıştır (Henny vd., 2020). *Echinodorus palaefolius* bitkisi bulunan YYA sisteminin organik kirlilik ve klorofil-a konsantrasyonunu azalttığı tespit edilmiştir. Mevcut çalışmada YYA teknolojisinin ötrofikasyon potansiyelini azaltan bir doğal arıtım stratejisi olduğu belirtilmiştir.

Türkiye'deki Doğal Su Kaynakları İçin YYA Potansiyeli

Küresel iklim değişikliği dünya genelinde su kütlelerini olumsuz etkilerken kullanılabilir su kaynaklarının kirlilik yükünü de arttırmaktadır. Azalan su miktarı nedeniyle güncel olarak Türkiye'de kişi başına düşen yıllık su miktarı 1.519 m^3'tür. Yıllara göre azalan su miktarı ile Türkiye, mevcut dönem içerisinde "su azlığı" çeken bir ülke konumuna gelmiş ve ilerleyen süreçlerde ise "su kıtlığı" çekme potansiyeline sahiptir (Aksay vd., 2005). Toplam 25 su havzası bulunan Türkiye'nin 2 tanesi (Fırat ve Dicle) toplam su akış hızının %30'unu oluşturmaktadır (Aküzüm vd., 2010). Bu dengesiz su dağılımı sonucu su problemi çeken diğer bölgelerde doğal su kaynaklarının kirlilik kontrolü dikkat çekmektedir. Bu amaçlar yenilikçi, sürdürülebilir, ekonomik ve ekolojik doğal arıtım sistemleri geliştirilmelidir.

Kullanılabilir doğal su kaynakları içerisinde önemli bir paya sahip göller sürekli akımlı tam karışımlı reaktörlerdir. Göllerde evsel atık suların deşarjı, tarımsal arazilerden gelen yüzeysel sular ve hayvancılık gibi antropojenik faaliyetler sonucu ötrofikasyon oluşmaktadır (Jeppesen vd., 1998). Ötrofikasyon, su kalitesini olumsuz etkilerken suda yaşayan canlılarının ve su kuşlarının yok olmasına neden olmaktadır (Scheffer vd., 1993). Çünkü ötrofikasyon suyun bulanıklığını arttırırken ışık geçirgenliği ve çözünmüş oksijen miktarını azaltmaktadır. Bir su ortamının ötrofikasyon açısından ele alınması için öncelikle trofik durumun tespit edilmesi gereklidir (Topkara, 2011). Trofik durum indeksini belirlemek için, üç indeks değişkeni (secchi diski, klorofil-a ve toplam fosfor) arasındaki ilişkiler kullanılır. Bu faktörler doğrultusunda göllere ait trofik durum oligotrofik, mezotrofik, ötrofik veya hiperötrofik şeklinde sınıflandırılmaktadır (Carlson ve Simpson, 1996). Ek olarak su kaynağı içerisinde bulunan fitoplankton ve zooplankton kompozisyonları takip edilerek trofik durum açıklanabilir.

A grubu sulak alan ve SİT alanı kapsamında Türkiye'nin en büyük tatlı su kaynağı olan Beyşehir Gölü üzerinde iki farklı dönemde trofik durum belirleme çalışması yapılmıştır. Türkiye için oldukça önemli bir tatlı su kaynağı olan Beyşehir Gölü için 1985-1986 yılları arasında yapılan ölçümler sonucu gölün oligotrofik yapıda olduğu ve içme suyu veya su ürünleri açısından temiz ve uygun olduğu tespit edilmiştir (Altındağ ve Yiğit, 2004). Fakat 2007 yılında yapılan Ankara Üniversitesi tarafından gerçekleştirilen bir proje doğrultusunda 0,40 ila 6,43 mg/L arasında değişen fitoplankton biyokütlesine dayanarak gölün mezotrofik yapıda olduğu sonucuna ulaşılmıştır (Demir, 2008). Buradan yola çıkarak zamana bağlı göl kalitesinin bozulduğu, yeniden yapılacak çalışmalar ile trofik durumun takip edilmesi gerektiği dikkat çekmektedir. Göller bölgesinde yer alan ve Türkiye'nin ikinci en büyük tatlı su gölü olan Eğirdir gölü üzerinde trofik durumun incelenmesi adına yapılmış çalışmalar mevcuttur (Şener vd., 2010; Cicek vd., 2017; Bulut ve Kubilay, 2018). Eğirdir gölü içme suyu, tarım arazilerinde sulama suyu, turizm ve rekreasyon olmak üzere farklı amaçlarla kullanılan oldukça önemli bir tatlı su kaynağıdır. Bundan dolayı göl üzerinde farklı dönemlerde yapılan trofik durum incelemesi daha da dikkat çekmektedir. Şener vd. (2010) tarafından yapılan çalışmada gölün %47'lik bir bölümünün ötrofik olduğu gözlenmiştir. Fitoplankton taksonları ile yapılan diğer çalışmada ise su kalitesinin iyi olduğu gözlenmesine rağmen N ve P bakımından zengin göllerde yaşayan taksonların olması bentik üzerinde bir yoğunluk olabileceğini düşündürtmektedir (Cicek vd., 2017). Gölün oligotrofik özellikte olduğu belirlenmiş olmasına rağmen çeşitli değişkenler için mezotrofik düzeyde olduğu da belirtilmiştir. Bulut ve Kubilay, (2018) tarafından yapılan çalışmada, Eğirdir Gölü'nde TP, seki disk derinliği, klorofil-a ve TN analizleri yapılmıştır. Çalışmanın sonuçları Eğirdir Gölü'nün trofik durumunun Yerüstü Su Kalitesi Yönetimi Yönetmeliği ve OECD trofik indekslerine göre mezotrofik karakterde olduğunu göstermiştir. İçme suyu kaynağı olan Eğirdir Gölü'ndeki azot ve fosfor düzeylerinin günümüzde normal seviyelerinde seyrediyor olmasına rağmen ileriki süreçlerde dikkatle takip edilmesi gerekmektedir (Bulut ve Kubilay, 2019). Türkiye'nin önemli bir milli parkı olan Abant Gölü heyelan sonucu oluşan doğal bir set gölüdür (Tosun, 2014). Yıl içerisinde çok fazla ziyaretçi alan Abant Gölü'nde tespit edilen bentik makroomurgasız faunasında ötrofik göllerde bulunan indikatör türlere rastlanmıştır (Tereshenko, 2019). Gölün genel trofik durumunun mezotrofik olduğu belirlenmiştir. Türkiye'deki birçok göl için yapılmış trofik durum belirlenmesi çalışmaları Tablo 2'de gösterilmektedir.

Tablo 2'ye göre Türkiye'de bulunan ve mevcut çalışma içerisinde incelenen göl, gölet ve baraj göllerinin yaklaşın %54'ü hipertorfik, ötrofik veya ötrofik olma durumu içerisindedir. Göllerin yaklaşık olarak %22'si oligotrofik karaktere sahiptir. Genel anlamda göllerdeki bu yüksek organik nitrüent birikimi, ötrofikasyon oluşumuna sebep olabilir. Bu olumsuz şartların oluşmasını önlemek için sürdürülebilir, ekonomik ve ekolojik çözüm önerileri gerekmektedir. Bu amaçla agronomik ve mühendislik teknolojisi olan YYA'ların kullanılması, ötrofikasyon potansiyeline sahip göllerin iyileştirilmesine destek sağlayacaktır.

Tablo 1. YYA çalışmalarında çoğunlukla kullanılan malzeme ve bitkiler (Alberto vd., 2021)

Table 1. Materials and plants mostly used in the AFI studies (Alberto vd., 2021)

	Yüzen Mat	Yüzen Çayır/Sazlık	Plastik Malzeme	PVC Borular	Polietilen Yüzdürücü	Bambu	Strafor Köpük	Tech-IA	BioHaven
Canna spp..	Sun vd. (2009)		Chang vd. (2012)	Saeed vd. (2014); Zhao vd. (2012)	Zhang vd. (2016)	Zhao vd. (2012)	Hartshorn vd. (2016); White ve Cousins (2013)		
Carex spp.	Borne vd. (2014)		Van de Moortel, (2011); Van De Moortel vd. (2010)	Winston vd. (2013)	Ladislas vd. (2013); Van Acker vd. (2005)			de Stefani vd. (2011; Pappalardo vd. (2017); Winston vd. (2013)	Tanner ve Headley (2008)
Iris pseudacorus			Van de Moortel, (2011); Van De Moortel vd. (2010)		Van Acker vd. (2005)		Hartshorn vd. (2016); Keizer-Vlek vd. (2014)	Barco ve Borin (2020); De Stefani (2012); Mietto vd. (2013); Pappalardo vd. (2017); Pavan vd. (2015)	
Juncus effusus			Chang vd. (2012); Van de Moortel, (2011); Van De Moortel vd. (2010)	Hubbard vd. (2004); Winston vd. (2013)	Ladislas vd. (2013)		Ebrahimi, (2015); Hartshorn vd. (2016); White ve Cousins, (2013); Winston vd. (2013)	de Stefani vd. (2011); Pappalardo vd. (2017)	Chang vd. (2013)
Lolium spp.	Li vd. (2012)				Xian vd. (2010)				
Lythrum salicaria			Van De Moortel vd. (2010)					Pappalardo vd. (2017)	
Mikroplar									Stewart vd. (2008)
Oenanthe javanica	Zhou ve Wang (2010)						Yang vd. (2008)		
Phragmites australis	Rehman vd. (2018); Saleem vd. (2019)	Garbett, (2005); Lakatos vd. (2014), (1997); Revitt vd. (2001); Richter, (2004)	Revitt vd. (1997)	Saeed vd. (2014)	Tara vd. (2019); Van Acker vd. (2005)			Barco ve Borin, (2020); de Stefani vd. (2011); De Stefani, (2012); Mietto vd. (2013); Pavan vd. (2015)	
Phragmites karka				Billore vd. (2008)		Billore vd. (2008)			
Pontederia cordata				Winston vd. (2013); Zhao vd. (2012)		Zhao vd. (2012)	Winston vd. (2013)		Chang vd. (2013)
Typha spp.			Boutwell, (2002); Di Luca vd. (2019)	Hubbard vd. (2004)	Shahid vd. (2019; Van Acker vd. (2005)		Keizer-Vlek vd. (2014)	de Stefani vd. (2011); De Stefani, (2012); Pavan vd. (2015)	
Vetiveria zizanioides						Zhao, Yang,vd. (2012)	Boonsong ve Chansiri (2008)		

Tablo 2. Türkiye'de trofik durum çalışması yapılan göl, gölet ve baraj gölleri

Table 2. Lakes, ponds and dam lakes for which trophic state studies were conducted in Turkey

Göl	Metot	Trofik Durum	Referans
Sarımsaklı Baraj Gölü	Carlson Trofik İndeks	Ötrofik	Sezen (2008)
Kemer Baraj Gölü	Carlson Trofik İndeks	Mezotrofik	Özyalin ve Ustaoglu (2008)
Sarımsaklı Baraj Gölü	Seki Disk, Zooplankton Kompozisyonu	Ötrofik	Aydin ve Ahiska (2009)
Kralkızı Baraj Gölü Dicle Baraj Gölü Batman Baraj Gölü	Carlson Trofik İndeks	Oligomezotrofik Oligomezotrofik Mezotrofik-Ötrofik	Varol (2010)
Üçpınar Baraj Gölü	Zooplankton Kompozisyonu	Ötrofik	Ertosun vd. (2010)
Afşar Baraj Gölü	Carlson Trofik İndeks	Ötrofik	Ayvaz vd. (2011)
Almus Gölü	Toplam P, Seki Disk	Mezotrofik	Polat ve Özmen (2011)
Çambaşı Göleti	Carlson Trofik İndeks	Oligomezotrofik	Topkara (2011)
Gaga Gölü	Carlson Trofik İndeks	Oligomezotrofik	Taş (2011)
Karamuk Gölü	Seki Disk	Hipertrofik	Kıvrak (2011)
İznik Gölü	Zooplankton Kompozisyonu	Oligotrofik	Apaydin Yağci ve Ustaoğlu (2012)
Yeniçağa Gölü	Zooplankton Kompozisyonu	Ötrofik	Döver (2012)
Cernek Gölü	Zooplankton Kompozisyonu	Ötrofik	Can ve Taş (2012)
Büyük Akgöl Gölü	Fitoplankton Kompozisyonu	Hipertrofik	Şahin vd. (2013)
Işıktepe Baraj Gölü	Toplam P	Ötrofik	Küçükyılmaz vd. (2014)
Küçük Lota Gölü	Fitoplankton Kompozisyonu	Oligotrofik	Kasaka (2014)
Süloğlu Baraj Gölü	Zooplankton Kompozisyonu	Oligomezotrofik	Güher ve Çolak (2015)
Bayındır Baraj Gölü	Carlson Trofik İndeks	Ötrofik	Erdoğan (2015)
Burdur Gölü	Carlson Trofik İndeks	Ötrofik	Kocasari vd. (2015)
Saklıgöl Gölü Gökçeova Göleti Kartal Gölü Karagöl Gölü	Fitoplankton Kompozisyonu	Oligotrofik	Sömek ve Ustaoğlu (2016)
Demirköprü Baraj Gölü	Carlson Trofik İndeks	Ötrofik-Hipertrofik	Erdogus (2016)
Kovada Gölü	Trofik Seviye Sınıflaması	Ötrofik	Şener ve Şener (2016)
Ikizdere Baraj Gölü	Toplam P, Seki Disk, Fitoplankton Kompozisyonu	Oligomezotrofik	Akar (2017)
Cernek Gölü	Carlson Trofik İndeks	Ötrofik	Cüce ve Bakan (2017)
Uzunçayır Baraj Gölü	Carlson Trofik İndeks	Oligotrofik	Kutlu vd. (2017)
Çiğ Gölü	Carlson Trofik İndeks	Mezo-ötrofik	Karakaya (2018)
Taşmanlı Göleti	Göl Trofik Diatom İndeksi	Ötrofik	Gümüş ve Gönülol (2018)
Ulugöl Gölü	Carlson Trofik İndeks	Mezotrofik-Ötrofik	Taş vd. (2018)
Mamasın Baraj Gölü	Carlson Trofik İndeks	Hipertrofik	Işık (2018)
Karkamış Baraj Gölü	Carlson Trofik İndeks	Mezotrofik	Tepe vd. (2018)
Suat Uğurlu Baraj Gölü	Carlson Trofik İndeks	Ötrofik	Orak (2019)
Balık Gölü	Carlson Trofik İndeks	Hipertrofik	Ariman ve Koyuncu (2019)
İznik Gölü Manyas Gölü	Carlson Trofik İndeks	Mezotrofik Hipertrofik	Ozbayram vd. (2020)
Karagöl Gölü	Carlson Trofik İndeks	Oligotrofik	Taş ve Hamzaçelebi (2020)
Dedeyolu Göleti	Zooplankton Kompozisyonu	Oligotrofik	Saler (2020)

Dünya üzerindeki tüm canlılar için en temel ihtiyaçlardan biri olan kullanılabilir su kaynakları birçok doğal ve antropojenik nedenlerden dolayı kirlenmektedir. Artan dünya nüfusu ve küresel iklim değişikliği, su kaynaklarının azalmasına ve buna bağlı olarak kirlilik yüklerinin artmasına neden olmaktadır. Dünya genelinde bu problemlerin üstesinden gelecek yenilikçi ve sürdürülebilir çözüm önerileri aranmaktadır. Bu kapsamda incelenen yapay yüzen ada teknolojisi organik atıkların su bitkileri tarafından kullanıldığı bir fitoremedasyon tekniğidir. YYA teknolojisi sadece su içerisindeki organik atıkların tüketimi değil aynı zamanda su ortamında doğal yaşam alanı oluşturma, görsel zenginlik kazandırma ve kıyı şeridini koruma gibi farklı biyolojik ve fiziksel avantajlara da sahiptir. Tüm bu avantajları dikkate alındığında literatürde bulunan birçok laboratuvar, pilot ve gerçek ölçekli uygulaması ile faydalı sonuçlar gösterdiği tespit edilmiştir. Genel olarak incelendiğinde laboratuvar ve pilot ölçekli çalışmaların, gerçek ölçekli çalışmalara göre daha fazla olduğu gözlenmiştir. İlerleyen dönemlerde bilimsel anlamda gerçek ölçekli çalışmalara yoğunlaşılması gerektiği dikkat çekmektedir.

Sonuç

Türkiye üç tarafı denizlerle çevrili ve doğal su kaynaklarının bulunduğu bir ülke olmasına rağmen gelecekte su kıtlığı çekme riski ile karşı karşıyadır. Bu riski minimum seviyeye indirmek için öncelikle mevcut su kaynaklarının korunması ve yerinde arıtılması gerekmektedir. Mevcut çalışmada da gösterildiği gibi Türkiye'de bulunan göl, gölet ve baraj göllerinin büyük bir bölümünün trofik durumu hipertrofik, ötrofik ve ötrofik olma yolundadır. Bu kapsamda doğal sulardaki organik yükün azaltılması için biyoteknolojik çalışmalara ihtiyaç vardır. Bu çalışmada su kalitesini iyileştirmek için yurt dışında birçok örneği bulunan YYA teknolojisi önerilmiştir. Gerçek ölçekli çalışmalar incelendiğinde genellikle Çin ve Hindistan gibi nüfus yoğunluğunun fazla olduğu ülkelerde yapıldığı görülmektedir. Bunun temel sebebi insan kaynaklı etkiler sonucunda doğal suların direkt ya da dolaylı olarak kirletilmesi ve küresel iklim değişikliğinden kaynaklı kullanılabilir su kaynaklarının azalmasıdır. Türkiye'nin gelecek projeksiyonu, trofik durumu kötüye giden doğal su kaynaklarının iyileştirilmesine odaklanacaktır. Bu kapsamda YYA teknolojisinin daha küçük su kütleleri olan göletler üzerinde pilot ve gerçek ölçekli olarak araştırılması önem arz edecektir.

Etik Standart ile Uyumluluk

Çıkar çatışması: Yazarlar herhangi bir çıkar çatışmasının olmadığını beyan eder.

Etik kurul izni: Yazarlar, bu çalışmanın etik izin gerektirmediğini beyan etmişlerdir.

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Instructions to Authors

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 (https://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 Research**" 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 Research**" journal 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 (https://www.basel-declaration.org/) and the guidelines published by International Council for Laboratory Animal Science (ICLAS) (http://iclas.org/). Authors are advised to clearly state their compliance with relevant guidelines.

"**Aquatic Research**" journal 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.

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 Research**" journal requires corresponding authors to submit a signed and scanned version of the authorship contribution form (available for download at

https://dergipark.org.tr/en/download/journal-file/19583)

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

AQUATIC RESEARCH
E-ISSN 2618-6365

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.

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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.

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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 http://dergipark.gov.tr/journal/2277/submission/start

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.

- Copyright Transfer Form,
- Author Contributions Form (one form for copyright and contributions available in **https://dergipark.org.tr/en/download/journal-file/19583**)
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors) Download this form from **http://www.icmje.org/conflicts-of-interest/** fill and save. Send this to the journal with your other files.

Preparation of the Manuscript

Manuscripts prepared in Microsoft Word must be converted into a single file before submission. Please start with the title page and insert your graphics (schemes, figures, *etc.*), tables in the main text.

Title (should be clear, descriptive and not too long)

Full Name(s) and Surname (s) of author(s)

ORCID ID for all author (s) (http://orcid.org/)

Address (es) of affiliations and e-mail (s)

Complete correspondence address and e-mail

Abstract

Key words (indexing terms), normally 3-6 items

Introduction

Material and Methods

Results and Discussion

Conclusion

Compliance with Ethical Standard

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.

AQUATIC RESEARCH

AQUATIC RESEARCH
E-ISSN 2618-6365

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.

Funding: 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.

Disclosure: Explanations about your scientific / article work that you consider ethically important.

References

Tables (all tables give in the main text)

Figures (all figures/photos give in the main text)

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", "Results and Discussion" and "Conclusion" 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).

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 sections. Authors may choose to use any subheading in between those sections.

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", "Results and Discussion" and "Conclusion" sections.**

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤25	180	40
Review Article	no limits	180	60
Short Communication	≤5	150	20

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.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted in main document WORD files (in JPEG or PNG format) through the submission system. 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 (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 RESEARCH

AQUATIC RESEARCH
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References

Reference System is APA 6th Edition

In-text Citation with APA

The APA style calls for three kinds of information to be included in in-text citations. The **author's last name** and the work's **date of publication** must always appear, and these items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Crockatt, 1995).

Direct quote from the text

"The potentially contradictory nature of Moscow's priorities surfaced first in its policies towards East Germany and Yugoslavia," (Crockatt, 1995, p. 1).

Major Citations for a Reference List in Table 2.

Note: All second and third lines in the APA Bibliography should be indented.

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 15 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 cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-day period is over.

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.

Table 2. Major Citations for a Reference List

Material Type	Reference List/Bibliography
A book in print	**Baxter, C. (1997).** *Race equality in health care and education.* Philadelphia: Ballière Tindall, p. 110-115, 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* (p. 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*. p. 92-90. 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.
Preview article in a journal 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 10.10.2015)
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 10.10.2015)
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 10.10.2015)
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 10.10.2015)