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Research Article

What Reference Genome Assemblies Tell Us and How to Detect the Best Available Version: A Case Study in Trout

Münevver Oral[1,2,3]

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ABSTRACT

Genomic studies have largely been accelerated by the advances of next generation sequencing technologies since the beginning of the millennium. This, in turn, has motivated the generation of more reference genome assemblies not only in model organisms but also in species of scientific interest. In the present study, we employed a comparison study between the two different reference genome assemblies available for the same species, *Salmo trutta*, in GenBank. The results indicated an overall 90% similarity index between the two assemblies. Furthermore, the inversion regions of which assembly needs corrections were detected. Taking into account the whole genome duplication origin of the *Salmonidae* family, both assemblies were of good quality. However, the updated version of the Wellcome Sanger Institute assembly (*fSalTru_1.2*) outperformed the Norwegian assembly and was detected as the best available reference genome assembly in *Salmo trutta*.

Keywords: Reference genome, genome comparison, brown trout, *Salmo trutta*

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INTRODUCTION

In the early 2000s, the world experienced one of the most important breakthrough events in genomic science. (Liu, 2011; Goodwin et al., 2016; Whibley et al., 2021). The development of Next Generation Sequencing (NGS) technologies was motivated by the Human Genome (HG) project. Although the consortium announced that the first draft would be ready in 2005, advances in NGS technologies accelerated the process to a speed that could not have been previously estimated and the first draft of the HG project was made available two years ahead of delivery time, in 2003 (Roushan et al., 2014; Wu et al., 2016). Since then, researchers have been motivated to generate more sequence data in a short period of time and in a cost-effective manner, while a significant effort has been made to not compromise accuracy (Jian & Schneeberger, 2017; Enguita & Leitão, 2022).

As predicted almost two decades ago by Mardis (2006), accessing personalised genome assemblies is becoming a reality for humans in 100 minutes at 99.9% accuracy and 30x high coverage (Chin & Khalak, 2019). Furthermore, the cost of sequencing is estimated to become even more affordable for personalised medicine in the near future. A review by Wu et al. (2016) highlights the collaborative efforts in human genomics that have significantly led the way towards impactful achievements in clinical and practical medical applications.

Over the past two decades, advances in sequencing platforms enabled reference genome assemblies to be generated not only in model organisms but also in non-model organisms, including economically important fish species, e.g., Atlantic salmon (Lien et al., 2016), brown trout (Hansen et al., 2021), rainbow trout (Berthelot et al., 2014), European seabass (Tine

et al., 2014), common carp (Xu et al., 2014) and zebrafish (Howe et al., 2013). The previously announced consortium Genome 10K aimed to sequence 10,000 vertebrate species' genomes corresponding almost one species for each genus (Koepfli et al., 2015). In addition, since 2017, the Vertebrate Genome Project (VGP) has aimed to sequence the whole genome of 71,657 vertebrate species, including agnatha, cartilaginous fishes, abmphibia, osteichthyes and reptilia (Rhie et al., 2021).

Genome assemblies serve as a starting point from which a catalogue of reference DNA sequence is provided in species of interest (Kersey, 2019). These are of particular interest for exploring genome-wide variations and evolutionary histories as well as understanding species biology, biodiversity and conservation (Whibley et al., 2021).

The present study was motivated by the availability of two different reference genome assemblies on the public server, which is uncommon and can be confusing for most researchers. Working as a part of the international research community on genetics, it is of critical importance to detect the best available version of the reference genomes in any species of interest. By doing so, intra- and interpopulation variation can be better captured from a better-quality catalogue sequence due to increased alignment rate. Therefore, taken all together, the aim of the present study was (i) to decide the best available version of the reference genome for *Salmo trutta* that will be of use for alignment of multiple NGS data generated by Illumina technologies and (ii) serve as a pilot study for researchers to detect the best available reference genomes in any species of interest.

MATERIALS AND METHODS

The National Center for Biotechnology Information (NCBI) was used to download the available reference genome assembly for brown trout (URL – 1). There were two available versions of the reference genome for species of interest. These were generated by (i) the Wellcome Sanger Institute and (ii) the Norwegian University of Life Science (see Table 1 for the details of the assemblies). Both reference genome assembly metrics were initially compared based on quality so as to decide the best available version; both assemblies were assessed by visualising similarities and flag ups.

Comparison of two reference genomes

Downloaded and zipped sequence FASTA files were uploaded to the online server (https://dgenies.toulouse.inra.fr/run) of D-genies version 1.4 (Cabanettes & Klopp, 2018). From the new alignment tool, the following parameters were chosen: (i) Target: GCA_901001165.2_fSalTru1.2_genomic and (ii) Query: GCA_931346615.1_Ssal_ARUN_Salmo_trutta_v1.0_genomic. Aligner and repeat options were kept as default for the analysis, and the task was submitted. The analysis time depends on the size of assemblies and it was completed in a day.

RESULTS AND DISCUSSION

The D plot generated has shown relatively high similarity between the two different genome assemblies being compared (Table 1). The alignment match was supported over 75% identity, indicated with continuous green dots (Fig 1). First, the noise was masked to visualise the higher percentage similarities. Overall, the summary identity resulted in over 36.36% similarity supported for >75%, followed by 54.37% for <75% similarity, 3.65% for <50% similarity, 0.45% for <25% similarity and 5.17% for no match at all between the two assemblies (Fig 2) in which, overall, the analysis supported statistically significant similarity over 90% (indicated as shades of green bars in Fig 2). When compared from the dropdown menu for each chromosomal region belonging to the target and query assemblies, respectively, this analysis also has resulted in high association supported with >75% identity index (Fig 3). There were flag ups indicating which regions of the assemblies need polishing using higher depth of coverage and longer sequencing for better quality. Furthermore, there were cases of inversions, structural rearrangement in the form of deletions, repeats and/or translocations, which were highlighted with opposite direction lines (see in details Fig 1 for such regions). These were end results of the same sequence but were represented in a different order. Similarly, these regions indicated a need for polishing to achieve a better-quality reference genome assembly for the available versions. The shaded grey bars on the upper right of the graph indicate sequences that are being merged in the form of a contig representing less than 0.2 % of the total assembly length. These regions signal that unassigned parts of the genome need to be assigned to the right positions in the genome.

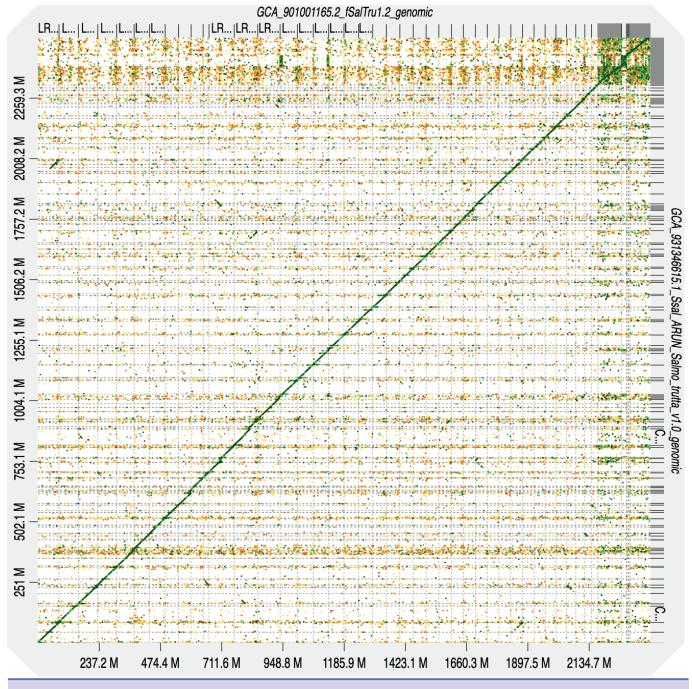

Figure 1. The D plot was generated indicating the similarity index of two reference genome assemblies compared for *S.trutta*. The bottom and left sides of the graph demonstrate the size of the sequence in nucleotide position while the right and upper sides of the graph indicate the comparison assemblies of query and target, respectively.

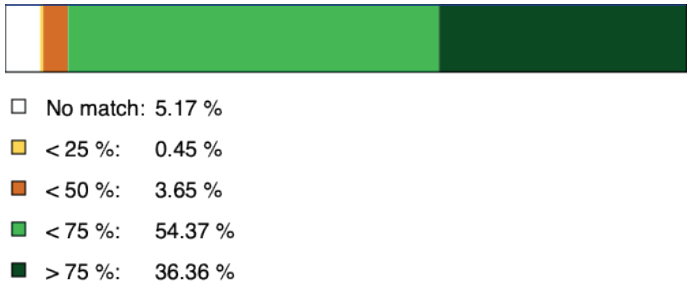

Figure 2. Similarity summary graph between the two reference genome assemblies.

Individual comparison between the target and query assemblies along the chromosome and matching contigs accordingly revealed high correspondence, thus a set of best matching D plot graphs are demonstrated in Fig 3. As the same colour code panel was applied to this analysis, the majority of these D plot graphs were supported over 75% similarity, indicated as dark green continuous lines, and in some cases, noise was detected, indicating assembly similarity less than 50% between the two assemblies, which appears as orange dots in the detailed graphs.

Reference genome assemblies have significantly improved the understanding of biological pathways and associations. However, genome assembly workload is an ongoing process which aims to serve the high-quality sequence archive for species of interest with gapless alignment (Whibley et al., 2021). While advances in next generation sequencing technologies multiply the capacities and the quantities of the high throughput data being generated in a short period of time, in parallel, the evolving era of bioinformatic analysis offers new tools to analyse such a large volume of data in an efficient manner (Jung et al., 2020). In the present study, we utilised an online tool developed for the comparison of two genomes available in GeneBank for *Salmo trutta*.

Salmo trutta is of scientific and economic interest as well as being popular among anglers. Thus, the species has been introduced to several countries (references therein Lobón-Cerviá, 2018). The phylogeny of *Salmo* has long been the subject of international debate due to the lack of understanding of the interaction between genotypic and phenotypic variation, and to its being regarded as a complex species, as opposed to designation of a single species (Ferguson 2004; Kottelat & Freyhof 2007). However, a recent opinion paper by Guinand, Oral and Thougard (2021) suggested a multiple species direction for the genus. The Salmonidae family has been though an additional round of whole genome duplication, which have caused one of the most complex genomes in vertebrates (Danzmann et al., 2008; Allendorf et al., 2015; Ohno et al., 1967). As indicated from the 2.37 Gb genome size and 41 chromosomes, *Salmo trutta* has a larger genome compared to most diploid fish species (Hansen et al., 2021). In the present study, D-genies enabled us to compare the similarity between the two large reference genomes of *Salmo trutta*. Both assemblies are of good quality, which is indicated with the quality metrics listed in Table 1 and a green line of correspondence demonstrating over 75% similarity in the colour coded identity panel (see Fig.1). The D-genies software utilises

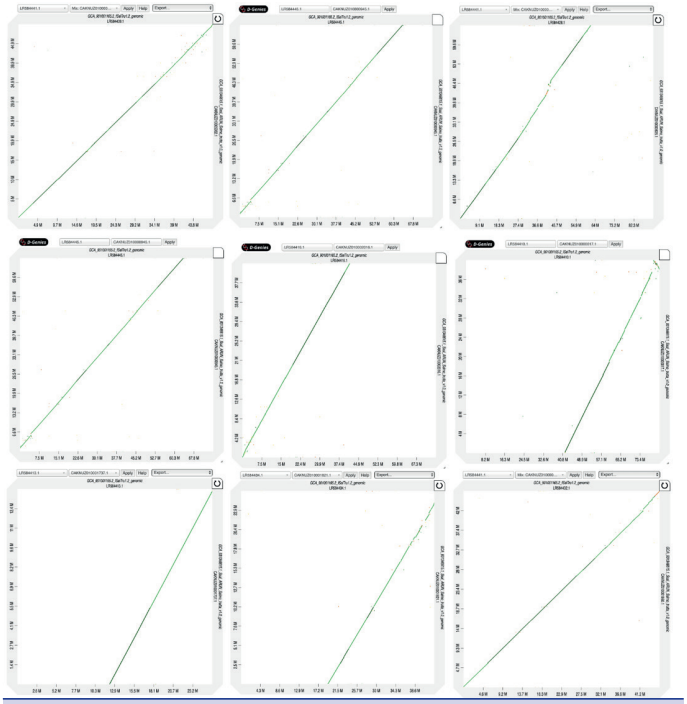

Figure 3. The best matching chromosome/contig graphs between the assemblies. The bottom and left sides of the graph demonstrate the size of the sequence in nucleotide position while the right and upper sides of the graph indicate the comparison assemblies of query and target, respectively.

a Blast-similar identity approach by applying the formula of I=M/N, in which I refers to Identity matrix, M indicates the quantity of matching nucleotides in the reference genome and N indicates the quantity of nucleotides, including gaps. Additionally, there were cases of inversions indicated as small chunks of opposite direction diagonal lines on the graph (Fig.1). These sequences exist in both assemblies, yet not in the same order. These are potential flag ups for reordering assemblies to improve quality. Fig 3 represents the best matching chromosomes/contigs between the assemblies, as follows: LR584441.1 versus CAKNUZ010005362.1, LR584445.1 versus CAKNUZ010000945.1, LR584416.1 versus CAKNUZ010002016.1, LR584413.1 versus CAKNUZ010001737.1 and LR584434.1 versus CAKNUZ010001821.1 in the Sanger Institute assembly versus the Norwegian assembly, respectively. Overall, taking into account the multisequence approach and high coverage of the Sanger Institute assembly (Table 1) as well as the chromosome level stage as opposed to the contig level unit in the Arun assembly, *SalTru_1.2*. generated by the Sanger Institute was selected for the downstream bioinformatic analysis of *Salmo trutta* sequenced via the Illumina platform.

One of the biggest limitations of the reference genome assemblies is the accuracy of the genomes and their annotations (Rhie et al., 2021). This is particularly the case in eukaryotic genomes, which contain high repeat content and duplication level (Elliot & Gregory, 2015). Regardless of the quantities of the reference ge-

Table 1. General metrics of both reference genome assemblies of brown trout

	Source: Wellcome Sanger Institute fSalTru		Source: Norwegian University of LifeScience
	Previous version_1.1	**Current version_1.2**	**Ssal_ARUN_Salmo_trutta_v1.0**
Accession number	GCA_901001165.1	GCA_901001165.2	GCA_931346615.1
Assembly level	Contig	Chromosome	Contig
Sample (tissue)	Female (spleen)	Female (spleen)	Male (n/a)
Sample diploidy	Doubled Haploid	Doubled Haploid	n/a
Total seq. length	2.371.880,186	2.371.880,186	2.510.277,823
Total ungapped length	2.298.279,497	2.298.279,497	2.510.277,823
Genome coverage	68x	68x	32x
Number of scaffolds	1.441	1.441	n/a*
Scaffold N50	52.209,666	52.209,666	n/a*
Scaffold L50	18	18	n/a*
**Contig count*	n/a	n/a	5,616
**Contig N50*	n/a	n/a	31.004,729
**Contig L50*	n/a	n/a	29
Number of contigs	5.378	5.378	5.616
Total number of chromosomes and plasmids	n/a	41	n/a
Number of component sequences (WGS or clone)	1.441	1.441	5.616
Registration date	02.06.2019	24.04.2021	19.02.2022

n/a: data is not available

nomes being available on public servers (1,348,815 genomes as of September 2022) the main focus should be directed towards improving the quality of the available reference genomes, including closing gaps with high coverage, as well as moving forward primary contig or scaffold level assemblies to the chromosome levels. To do so, hybrid sequencing approaches are applied by taking into consideration the advantages and disadvantages of the sequencing platforms. The Sanger Institute has applied the combination of the PacBio and 10X Genomics Chromium platform as well as BioNano and Hi-C data, achieving higher coverage, while the Norwegian assembly involved PromethION data from Oxford Nanopore technology combined with the Illumina platform. Given the quality metrics of both assemblies, the hybrid sequencing approach resulted in better quality assemblies as well as helping to close gaps while dealing with such an extended heterogenous genome.

The second most challenging task during reference genome assembly workload is dealing with the heterozygosity of the specimen being sequenced. In order to eliminate these cases, most assemblies utilise doubled haploid (DH) individuals, as these are theoretically 100% homozygotes thus helping eliminate the complications of duplicated genomes due to high heterozygosity (Whibley et al, 2021). Typically, eggs (n) of diploid female are fertilised using an irradiated sperm (n) from a diploid male. Although the genetic content of the sperm is inactive, the irradiated sperm is still motile and capable of initiating fertilisation. As there will be no genetic contribution from the sire, shock (chemical, physical or heat treatment) needs to be applied so as to ensure viability (2n) during the first mitosis. The resultant mitotic gynogenetics are produced by fully maternal genome transmission (Arai, 2001; Komen & Thorgaard, 2007; Oral, 2016; Manan et al., 2022). DH genomes offer the possibility of generating a more straightforward workflow, as fully a homozygous genome increases the chances of detecting any artefacts and/or sequencing errors in the form of variation. Therefore, DH individuals are preferred and have been utilised widely for reference genome assembly procedures in several aquatic species (Howe et al., 2013; Brawand et al., 2014; Berthelot et al., 2014; Xu et al., 2014; Lien et al., 2016; Hansen 2021). In the present study, the Sanger Institute's assembly was based on a DH female (Hansen et al., 2021) while no further information was provided in the Norwegian Arun assembly other than the sequencing of a male brown trout specimen (Table 1).

CONCLUSION

In an effort to determine the best reference genome assembly for *Salmo trutta* for downstream data analysis, we compared the two available genomes. Overall, the recent version from the Wellcome Sanger Institute (*fSalTru_1.2*) was determined to be the highest quality reference available for *Salmo trutta* in terms of the multisequence approach applied and coverage achieved, as well as consisting the fact that it contains the chromosome level assembly as opposed to the contig level in the Norwegian Arun assembly. Taken all together, *fSalTru_1.2* will be utilised for the upcoming downstream genomic analysis of *Salmo trutta*, which involves a short sequencing approach applied using Illumina technologies.

Conflict of Interest: The author declares no conflicts of interest.

Ethics committee approval: As the present study was carried out in silico, ethics committee approval was not necessary.

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Research Article

Benthic Macroinvertebrate Fauna (Clitellata and Chironomidae) of Lake Limni, Gümüşhane, Turkiye

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ABSTRACT

Benthic macroinvertebrate groups, which have adapted to life in a wide variety of aquatic habitats from fresh to saltwater, are often used as bioindicators to determine the status of aquatic ecosystems. Streams and lakes face the dangers of pollution due to anthropogenic impact, especially due to recreational uses. So far, a total of 262 protected nature parks have been declared in Türkiye, one of which is Lake Limni, the area under study. Lake Limni is located in the province of Gümüşhane in the Eastern Black Sea Basin. No studies were previously conducted to determine the macroinvertebrate fauna in the lake. To fill this gap, sampling was carried out from 2 stations in 2020 to determine the macroinvertebrate fauna of the lake. As a result of laboratory studies, 25 species belonging to 18 genera were identified. It was determined that the zoobenthic community of the lake consisted of Clitellata and Chironomidae individuals and that the dominant taxon of the lake was *Limnodrilus hoffmeisteri* from Oligochaeta with 14.71% dominancy. The high population density of Oligochaeta and Chironomidae individuals in the study area and the low species diversity indicate poor water quality. It is also possible to say that the water quality of Lake Limni has changed from eutrophic to hypereutrophic.

Keywords: Chironomidae, Oligochaeta, pollution

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INTRODUCTION

Aquatic habitats are home to a wide variety of species, which is one of the most essential aspects to preserve an aquatic ecosystem's resilience and stability. Biota in aquatic ecosystems is reliant on the water's physical, chemical, and biological properties which serve as direct controlling factors (Yaqoob & Pandit, 2009). Benthic communities, which make up a significant portion of the total biota in both lentic and lotic systems, are among these controlling factors and constitute an essential component of any aquatic ecosystem. By acting as grazers, collectors, shredders, or predators, they serve a variety of purposes (Pearson & Rosenberg, 2006). Benthic macroinvertebrates are extremely sensitive to physical and chemical disturbances (Furse et al., 2006; Jess-Crespo & Ramrez, 2011), as these disturbances lead to a decrease in the diversity and abundance of their assemblies and an increase in the dominance of species that can withstand challenging conditions (Wang et al., 2012). Their distribution, occurrence, and abundance are strongly influenced by the dominant environmental features. Their variability is typically attributed to abiotic factors, primarily substrate characteristics, such as temperature, depth, and food resources (Sanseverino & Nessimian, 2001; Chapman et al., 2010; Cesar & Henry, 2017). Additionally, they are sensitive to changes in the amount of dissolved oxygen (Hirabayashi & Hayashi, 1994).

Water resources are sensitive to variations in climatic patterns. It is believed that there will be changes in water resources due to climate change from the impacts of runoff, floods, drought, snowmelt and glacier melt, water quality, groundwater, transboundary problems

and agriculture (Singh et al., 2014). Globally, research into the preservation, restoration, and monitoring of these resources has grown (Türkmen & Kazancı, 2010). Türkiye is privileged to have a variety of lentic and lotic resources, including 135 globally significant wetlands, more than 120 natural lakes, 107 major rivers and 25 river basins. Türkiye's water resources and wetlands are starting to disappear, and some areas have also noticed deteriorating water quality and rising levels of water pollution (Türkmen & Kazancı, 2010).

In this article, Lake Limni located in Gümüşhane Province was chosen as the study area. To date, no studies have been conducted on the macroinvertebrate fauna of Lake Limni. While some studies have been conducted in the area, these discussed the lake's recreational potential (Birinci et al., 2016; Yeşil & Hacıoğlu, 2018; Çalık & Pir, 2019; Tozkoporan et al., 2020). According to the results of these studies, Limni Lake Natural Park has a high recreational potential due to factors such as its location near main transportation routes, flora and fauna, and fresh air. One study, however, has analyzed the species composition and diversity of epipelic algae and physicochemical characteristics in Lake Limni (Şahin, 2008). This study aims to determine the aquatic benthic macroinvertebrate fauna of Lake Limni. It, as such, addresses a major gap in the literature on Lake Limni and its biota, particularly its macroinvertebrate fauna.

MATERIAL AND METHODS

Lake Limni is located in the Gümüşhane province in Eastern Black Sea Basin of Türkiye. It has a surface area of about 72 ha. On average, the lake is 1 meter deep and 1850 m above sea level. The East Anatolian regional climate has an impact on the study area. This region experiences cold winters and hot, dry summers (seasonal average temperature of 16.2 °C, highest temperature of 39.5 °C, lowest temperature of -8.9 °C, and precipitation total of 39 mm) (Anonym, 1999; Şahin, 2008). On July 1, 2011, Lake Limni was designated as a nature park (Gümüşhane Çevre ve Şehircilik İl Müdürlüğü, 2020).

Benthic macroinvertebrate samples were collected using a grid frame hand net (according to standard of ISO 10870:2012) in Lake Limni at two stations in August 2020 (Figure 1). Samples were washed with sieves of decreasing mesh size and fixed in 70% ethyl alcohol. Moreover, some parameters (pH, temperature, dissolved oxygen and depth) were measured *in situ* from under the surface with Hach Lange DR40d. Benthic macroinvertebrate samples were taken to the laboratory and sorted under a stereomicroscope. Then, macrozoobenthic specimens were prepared with glycerin and identified to species level. Macroinvertebrate individuals were identified using different identification keys by Nilsson (1996), Brinkhurst and Jamieson (1971), Timm (2009), Thorp and Rogers (2019). Diversity indices were analyzed with ASTERICS 3.1 software (AQEM Consortium 2002). Additionally, dominancy values of macrozoobenthic samples were also calculated using Bellan-Santini's dominance index formula (Bellan-Santini 1969). A similarity diagram was created using the Wards method according to the abundances (using standard of ISO 10870:2012) and ecological requirements, considering measured physicochemical parameters of the detected species. In addition, cluster analysis was applied in Past program to create similarity dendrograms according to the abundance, distribution, and ecological requirements of the detected species (Hammer, 2001).

Figure 1. Geographical positions of sampling stations.

RESULTS AND DISCUSSION

This study determines the macroinvertebrate fauna of Lake Limni, which is a very small lake whose macroinvertebrate fauna has not been previously investigated. As a result of the research, 25 species belonging to 18 genera were identified. Their abundances as percentages of the general zoobenthic community and the taxonomic group they belong to are given in Table 1.

According to the indices results, the Lake Limni zoobenthos was quite poor in terms of taxonomic diversity. Oligochaeta (73.78%) individuals constituted the majority of the benthic community structure. This was followed by Chironomidae with a dominance rate of 24.76%. Members of both groups (with the exception of partially low-tolerant stenoecious species) are used as bioindicators in biomonitoring studies of surface waters as they contain species with a high tolerance to pollution (Bode et al., 1996).

Limnodrilus hoffmeisteri Claparède, 1862 was the dominant taxon of the lake, with a dominance rate of 14.71% from Oligochaeta (based on mean % abundance), followed by *Limnodrilus udekemianus* Claparède, 1862 (10.46%), *Stylaria lacustris* (Linnaeus, 1767) (11.82%), and *Nais elinguis* Müller, 1774 (13.22%) (Table 1). It has been shown that most Tubificin species include cosmopolitan species with a wide distribution worldwide (Wetzel et al., 2000). Species belonging to the genus *Limnodrilus* and *Tubifex* are especially capable of adapting to a wide range of environmental conditions. Moreover, they can live in very different habitats from α-β mesosaprobic environments to sewage waters (Kathman & Brinkhurst, 1998) and are also a rare species that can survive in the zoobenthic community in the face of changing environmental conditions and increasing pressure factors. Due to these features, they are used as indicators to determine the trophic levels of lakes (Langdon et al., 2006). The fact that these two species represented a quarter of the overall zoobenthic structure in Lake Limni (25.17% in total), as well as the low diversity of taxa in the community, indicates that the environmental conditions are no longer a suitable habitat for zoobenthic fauna elements.

Table 1. Taxon list of macrozoobenthic individuals which were determined in Lake Limni and their proportional as % (AIG: Abundance in group), water parameters and index values of sampling stations (individual numbers were given as in m^2).

	Sampling stations		Mean	AIG
Taxa	**1**	**2**		
Class: Clitellata				
Subclass: Oligochaeta				
Stylaria lacustris (Linnaeus, 1767)	14.98	8.67	11.82	16.61
Nais elinguis Müller, 1774	11.45	7.51	9.48	13.22
Nais pardalis Piguet, 1906	4.85	5.20	5.02	6.78
Nais communis (Piguet, 1906)	0.88	2.31	1.60	2.03
Dero digitata (Müller, 1773)	1.32	0.58	0.95	1.36
Dero furcatus (Müller, 1774)	0.44	0.00	0.22	0.34
Ophidonais serpentina (Müller, 1773)	3.52	3.47	3.50	4.75
Slavina appendiculata (d'Udekem, 1845)	0.88	0.00	0.44	0.68
Uncinais uncinata (Ørsted, 1842)	3.96	2.89	3.43	4.75
Tubifex tubifex (Müller, 1774)	3.08	6.36	4.72	6.10
Limnodrilus udekemianus Claparède, 1862	7.05	13.87	10.46	13.56
Limnodrilus hoffmeisteri Claparède, 1862	14.98	14.45	14.71	20.00
Potamothrix hammoniensis (Michaelsen, 1901)	6.17	4.62	5.40	7.46
Psammoryctides albicola (Michaelsen, 1901)	0.00	4.05	2.02	2.37
Hirudinea spp.	1.76	1.16	1.46	
Order: Diptera				
Familya: Chironomidae				
Tanypodinae				
Tanypus punctipennis Meigen, 1818	0.44	0.00	0.22	1.01
Chironominae	**0.00**	**0.00**	**0.00**	**0.00**
Cryptochironomus defectus (Kieffer, 1913)	0.44	0.00	0.22	1.01
Chironomus thummi (Kieffer, 1911)	7.93	3.47	5.70	24.24
Chironomus (Camptoch) tentans Fabricius, 1805	3.96	1.73	2.85	12.12
Chironomus plumosus (Linnaeus, 1758)	5.29	5.20	5.24	21.21
Chironomus anthracinus Zetterstedt, 1860	2.20	5.20	3.70	14.14
Polypedilum scalaenum (Schrank, 1803)	0.88	2.31	1.60	6.06
Tanytarsini				
Paratanytarsus lauterborni (Kieffer, 1909)	0.88	2.89	1.89	7.07
Cladotanytarsus mancus (Walker, 1856)	0.44	2.31	1.38	5.05
Virgotanytarsus arduensis (Kieffer, 1909)	1.76	1.16	1.46	6.06
Tanytarsus gregarius Kieffer, 1909	0.44	0.58	0.51	2.02
Water parameters	**1**	**2**		
pH	7.9	7.9		
Temperature (°C)	21	20		
Dissolved oxygen (mg/L)	2.0	2.6		
Depth (m)	0.5	0.6		
Indices	**1**	**2**		
Number of taxa	25	22		
Individuals	227	173		
Shannon_H	2.73	2.80		
Evenness_e^H/S	0.61	0.74		
Margalef	4.42	4.07		

Additionally, it is well-known that *Stylaria lacustris* and *Nais elinguis*, the other dominant Oligochaeta species in the lake can tolerate salinity even at levels lethal for many freshwater species, can live in brackish waters with salinity less than 7‰, even in the profundal zones of lakes, and can tolerate low oxygen concentrations and low temperatures (down to –8 °C) (Timm, 1970;

Dumnicka, 1978; Davis, 1982). Furthermore, *S. lacustris* is a phytophile species and this feature increases its adaptability (Schwank, 1982). It has also been reported that *N. elinguis*, which is less frequently detected than other *Nais* species, can increase in population in waters rich in organic matter and can easily tolerate environmental variables (cold, muddy-odorous areas, poorly oxygenated waters) (Timm, 2003). The high population density of *Potamothrix hammoniensis*, which has a 5.40% abundance in lake zoobenthos (Table 1), has been reported to indicate eutrophic conditions or organic pollution in lake systems (Milbrink, 1980). In line with all this information and the results of the research, it can be concluded that the trophic level of the lake has passed into a serious hypertrophic stage.

The dominant Chironomidae species in the lake after Oligochaeta are *Chironomus thummi* (5.70%), *Chironomus plumosus* (5.24%), and *Chironomus anthracinus* (3.70%). It is known that *Chironomus* species in lakes and rivers are generally found in sediments, in polluted and turbid water or in waters rich in nitrogen and phosphorus and low in oxygen (Epler, 1995). Besides, they can live in muddy sediments where secondary aquatic plants are dense (Bat et al., 2000), even in puddles, and their abundance increases in the littoral zones of lakes, including brackish waters and the sublittoral zone, and among reeds. In addition, it has been reported that most *Chironomus* species are able to bind oxygen due to their high hemoglobin content and that they can easily compete with other species in the environment and survive by their gills (Pinder & Riess, 1983). The abundance of highly tolerant Chironomidae species followed by the highly tolerant Oligochaeta species in the lake and no individuals other than these tolerant groups identified in the lake are all natural warning signs for Lake Limni in ecological terms.

Although Lake Limni is a small lake, the index values calculated according to the data obtained from the two sampling stations are given in Table 1. In addition, the similarity diagram made using the Wards method according to the abundances and ecological requirements of the identified species is given in Figure 2 (introduced in 1963 by Joe H. Ward, the Ward clustering method aims to minimize the error squares between two merged clusters (Sharma, 1996; Xu & Wunsch, 2009)). This method, which is based on classical sum-of-squares criteria, is preferred among other cluster analysis methods because it is the only method that enables the formation of clusters by minimizing intragroup dispersion (Murtagh & Legendre, 2014).

The number of taxa detected in Lake Limni varied between 22 and 25, with the highest Shannon value at 2.8 while the Margalef value was 4.07 (Table 1). Both index values were higher than expected for an aquatic system with high trophic levels. Since these diversity indices work on the basis of the number of taxa and individuals detected in the area, these values were normal.

As can be seen in Figure 2, four specific species (*Limnodrilus udekemianus, Limnodrilus hoffmeisteri, Stylaria lacustris*, and *Nais elinguis*) of the taxa forming the zoobenthic community were in a different cluster from the other species. As explained in detail above, it was stated that although most of the species detected in the lake were highly tolerant, the four species in question have the ability to tolerate all kinds of environmental variables, including salinity. The tolerance of macrozoobenthic groups to changing environmental conditions prepared by Mandaville (2002) was examined and shown with values ranging from 1 to 10, from sensitive to tolerant species. Accordingly, the tolerance value of the Oligochaeta group was given as 8 without giving the species name. This value is not the upper limit, but Bode et al. (1996), in their species-based tolerance assessment, gave a maximum value of 10 for *Limnodrilus* species and *Nais elinguis*. It can be said that while the tolerance limits of the species in the other two main groups in the dendrogram are close to each other, these four species, which are the dominant species of the zoobenthic community, have a higher tolerance than the others.

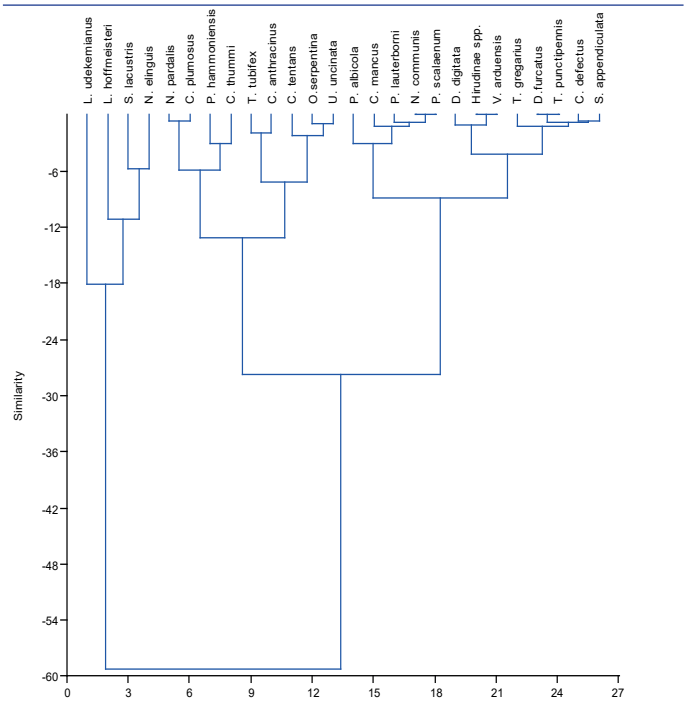

Figure 2. Similarity dendrogram of the species identified in Lake Limni according to their abundance, distribution, and ecological requirements.

High population densities of Oligochaeta and Chironomidae, which are known to be tolerant to increased organic and (or) inorganic pollution due to stressors in surface waters, and low species diversity of the overall zoobenthic community, are generally considered as indicators of poor water quality (Rosenberg and Resh, 1993). Such environments are generally known to have low dissolved oxygen and high nutrient concentrations (Langdon et al., 2006). Although very few water quality parameters were measured in this study, the low dissolved oxygen levels probably indicated water class IV. In a study conducted by Şahin (2008) in the same lake 17 years ago, the SO_4^{-2} value was determined as 1 mg/L, the NO_2-N value was 0.001 mg/L, the NO_3-N value was between 0.3 and 1.1 mg/L, and the o-PO_4 value was between 0.41 and 0.54 mg/L. In the study where epipelic algae were identified, Lake Limni was classified as an eutrophic lake with its morphometric struc-

ture, water parameters, and algal flora structure. In the same study, the dissolved oxygen value was between 8.3 and 10.9 mg/L. It is possible to say that this value has decreased significantly in the last 17 years and that the lake has passed from an eutrophic to hypereutrophic state, as stated by Şahin (2008), in terms of both water parameters and zoobenthic community structure.

CONCLUSION

Undoubtedly, a country's surface water is among the most important elements of its biological, cultural, and touristic heritage. Streams and lakes face the dangers of pollution from anthropogenic impact, especially due to their recreational use. So far, there are 262 protected nature parks in Türkiye. Lake Limni was declared the Lake Limni Nature Park by the General Directorate of Nature Conservation and National Parks in 2011. With this study, 25 species belonging to 18 genera were identified. It was determined that the zoobenthic community of the lake consisted of Clitellata and Chironomidae individuals and the dominant taxon of the lake was *Limnodrilus hoffmeisteri* from Oligochaeta. The high population density of Oligochaeta and Chironomidae individuals in the study area and the low species diversity of the general zoobenthic community indicate poor water quality. It is also possible to say that the water quality of Lake Limni has changed from eutrophic to hypereutrophic. It is necessary to take measures to protect the sustainability of the lake, inspect the surrounding businesses and picnic areas, and monitor the water quality parameters of the lake water and zoobenthic community diversity at regular intervals, since there is no river source that feeds the lake other than snow water and precipitation.

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Research Article

A Comprehensive Review on Microplastic Pollution in Aquatic Ecosystems and Their Effects on Aquatic Biota

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ABSTRACT

Plastic wastes released into the environment break down into fine particles due to exposure to meteorological events such as wind, precipitation, UV radiation, and abrasion. These smaller plastic particles, ranging between 1 µm and 5 mm, are called microplastics and they can be transported over longer distances with the aid of erosion, waste water discharges, winds, and currents. Aquatic habitats are the final sink for many pollutants including heavy metals, pesticides, nanoparticles, and microplastics released into environment. Thus, these pollutants are considered a major threat to aquatic life. In this study, we reviewed studies i: focusing on the type, size and the quantity of microplastics observed in freshwater and marine ecosystems, and ii: studies on the effects of microplastics on aquatic organisms. The data gathered clearly indicates that microplastics are quite abundant in freshwater and marine ecosystems. Furthermore, nearly in all studies reviewed, microplastic uptake and alterations in several biochemical parameters depending on microplastic exposure are recorded. The studies also point out that microplastics will become a global serious health concern both for human beings and aquatic organisms in the near future.

Keywords: Microplastic, polyethylene, PVC, plastic, pollution

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INTRODUCTION

The term "plastic" is derived from the Greek word "plastikos", which means one that can easily take the desired shape (Ivleva et al., 2017). After the discovery of bakelite, the first synthetic plastic, in 1907 and the synthesis of several other plastic polymers, a revolution has taken place in modern life (Shashoua, 2012). Plastics are flexible, easy to process, cost effective, have a low density, poor thermal and electrical conductivity, are easily accessible, and are resistant to corrosion and light, (Yurtsever, 2015; Frias & Nash, 2019). They have several uses in as agriculture, packaging, the textile and automotive industries, outdoor and indoor household goods, building elements and the construction sector, and toys (PlasticsEurope, 2016; Boucher & Friot, 2017; Kawecki et al., 2018; Meng et al., 2020).

Due to the increasing demand for plastic materials, plastic production is also increasing. Eventually plastic materials became one of the main wastes released into environment (Geyer et al., 2017). The annual global plastic production in 1960 increased from 5 to 359 million tons in 2019 (PlasticsEurope, 2019). The highest plastic production takes place in Asia with 51% of the global plastic production, 20% in Europe, 18% in North America, 7% in Africa, and 4% in South America (PlasticsEurope, 2019).

Plastic wastes discharged into terrestrial and aquatic habitats are considered a serious threat to biodiversity, due to their resistance to degradation (Gall & Thompson, 2015; Carbery et al., 2018;). Plastic wastes are classified into 4 groups depending on their size; macroplastic (2.5 to 100 cm), mesoplastic (5 mm to 2.5 cm), microplastic (1 µm to 5 mm) and nanoplastics (<1 µm) (GESAMP,

2015). The amount of larger plastic wastes form huge floating islands in the oceans and are considered the 7th continent due to their size. On the contrary, the nanoplastics, invisible to the naked eye, can be found everywhere in our daily lives, even in drinking water (Hidalgo-Ruz et al., 2012; Aydın, 2020). But the effects of nanoplastics on aquatic organisms have been studied only in a limited number of studies (Al-Thawadi, 2020; Amobonye et al., 2021).

Microplastics (1-5000 µm) are defined as synthetic solid plastic particles or polymeric matrices that have a regular or irregular shape (Frias & Nash, 2019). According to Cole et al. (2011) microplastics are divided in 2 groups depending on their origin; primary and secondary microplastics. Primary microplastics refer to microbeads in various sizes, and they are mainly produced for industrial purposes and in personal care products (Fendall & Sewell, 2009), in exfoliants (Leslie, 2014), sandblasting systems (Sundt et al., 2014) or plastic pellets (Browne et al., 2011) are used in the production. Seconder microplastics are formed from larger plastics due to exposure to UV radiation, or other photochemical processes, wind, heat, physical degradation, or various mechanical forces (Rocha-Santos & Duarte, 2015; Cole et al., 2011).

The earliest record of microplastic pollution is the observation of small plastic particles in the Atlantic Ocean during the 1970s (Schymanski et al., 2018; Wilcox et al., 2019). The first comprehensive study on the distribution of plastic particles in the oceans was conducted in 2014 by Richard Thompson and his team (Law & Thompson, 2014; Rochman, 2018). Unfortunately, nowadays, it is known that microplastics are present in the sediment and pelagic zones of oceans and seas worldwide (Thompson et al., 2004; Rochman et al., 2015; Kühn & Van Franeker, 2020). Microplastics, have a tendency to float in the surface layers of lakes or seas due to their low density. These particles are ingested by aquatic organisms including plankton, aquatic invertebrates, fish, aquatic birds and even mammals since they resemble an attractive and easy prey due to their color and shape. Eventually, they block the digestive tract of these animals which in turn may lead to death (Kumar et al., 2021).

Color, shape and the chemical structure of the microplastics are crucial for their fate in aquatic habitats (Zhang, 2017). Microplastics can be spherical, rectangular, cylindrical, disc or in forms of fiber, styrofoam, film and pellets (McDermid & McMullen, 2004; Abu-Hilal & Al-Najjar 2009; Free et al., 2014). They can be found in various colors such as crystalline, white, cream, orange, red, brown, blue, purple, opaque, gray, transparent, black, green, and yellow (Hidalgo-Ruz et al., 2012). Polyethylene (PE) is one of the most manufactured plastic polymer and composes 36% of the total plastic production, followed by polypropylene (PP) (21%), polystyrene (PS) (12%), polyester (PES) (10%), and polyvinyl chloride (PVC) (9%) (Obbard et al., 2014; Horton et al., 2017; PlasticsEurope, 2018). The density and uses of the most common microplastic polymer types observed in aquatic habitats are summarized in Table 1.

Microplastics are observed in the atmosphere (Gasperi et al., 2018), almost in all aquatic habitats and in all compartments of the water bodies (Eerkes-Medrano et al., 2015; Peeken et al., 2018), and even in the most remote regions of the world, such as the Arctic region and high mountain lakes (Woodall et al., 2014; Free et al., 2014; Lusher et al., 2015). Microplastics are also found in the sub-surface layers of soil. For example, 78 items/kg of microplastics were found in surface soils (0-3 cm) in agricultural fields in Shanghai (China), and 62.5 items/kg of microplastic particles were found in deep soils (3-6 cm) (Liu et al., 2018).

Rivers, ocean currents, turbulence (Ballent et al., 2012; Turra et al., 2014; Wagner et al., 2018), surface flows from agricultural areas (Nizzetto et al., 2016), waste water treatment plants (Murphy et al., 2016), wind and erosion (Zalasiewicz et al., 2016) are responsible in the transport of microplastics to different ecosystems. Wind is considered to play a key role in the transportation of microplastics to many different regions of the world. For example, the amount of microplastics at the sea ice was found to be 38-234 particles/m^3 in the Arctic Ocean and it was noted that this is primarily due to the transport of microplastics by the wind (Obbard et al., 2014).

Aquatic habitats are considered a final sink for many pollutants including microplastics, thus microplastic pollution is handled as a serious threat to aquatic life (Al-Thawadi, 2020). Therefore, in this study, studies focusing on microplastic pollution in aquatic ecosystems and the effects of microplastic pollution on plankton, invertebrate animals and fish living in these environments have been reviewed. We focused on the papers published in the past 5-6 years to get an up-to-date view of the current situation of microplastic pollution in freshwater and marine ecosystems.

Microplastic Pollution in Freshwater Ecosystem

The data including the examined water compartment, sampling and detection methods and sampling locality gathered from the studies focusing on the amount of microplastics observed in various freshwater bodies are given in Table 2. According to the data summarized, we found that the amount of microplastic particles found in surface water samples were between 0.051±0.036

Table 1. The density and uses of the most common microplastic polymer types observed in aquatic habitats (Yurtsever, 2015).

Microplastic type	Density	Usage area
High Density Polyethylene (HDPE)	0.94-0.96 g/cm^3	Bottles, stretch film
Low Density Polyethylene (LDPE)	0.91-0.93 g/cm^3	Plastic bags
Polypropylene (PP)	0.83-0.90 g/cm^3	Automotive industry, bottle caps, cooking utensils
Polystyrene (PS)	0.96-1.05 g/cm^3	Plastic sheets, paper, toys, houseware
Polyvinyl Chloride (PVC)	1.16-1.58 g/cm^3	Electric cables, packaging industry, pipe, and plumbing materials
Polyethylene Terephthalate (PET)	1.37-1.45 g/cm^3	Food and pharmaceutical industry, machine manufacturing

Table 2. The amount of microplastics observed in various freshwater bodies, the examined water compartment, sampling and detection methods, and sampling locality.

Locality and sampled compartment	Sampling and detection methods	Microplastic quantity	References
Water			
Antua River (Portugal)	Water pump FT-IR	58-1265 items/m³	Rodrigues et al., 2018
Teltow Canal (Germany)	Niskin bottle SW-IR	0.01-95.8 items/L	Schmidt et al., 2018
Citarum River (Indonesia)	125 µm manta trawl FT-IR	0.057±0.025 particles/m³	Sembiring et al., 2020
Surface Waters			
Hudson River (USA)	Metal bucket micro FT-IR	0.625-2.45 fibers/L	Miller et al., 2017
Sub-alpine Lake (Italy)	300 µm plankton net FT-IR	4000-57.000 particles/km²	Sighicelli et al., 2018
Saigon River (Vietnamese)	300 µm plankton net FT-IR	172.000-419.000 items/m³	Lahens et al., 2018
Pearly River (China)	Stainless steel sieve FT-IR	379-7924 items/m³	Lin et al., 2018
Hong Lake (China)	Water pump Stainless steel sieve Raman Spectroscopy	1250-4650 items/m³	Wang et al., 2018
Dongting Lake (China)	Water pump Stainless steel sieve Raman Spectroscopy	900-2800 items/m³	Wang et al., 2018
Rhine River (Germany)	300 µm manta trawl SEM FT-IR Raman Spectroscopy	0.05-8.3 particles/m³	Mani et al., 2019
Nakdong River (South Korea)	Stainless steel beaker FT-IR	293-4760 particles/m³	Eo et al., 2019
Pearl River Basin (China)	160 µm plankton net FT-IR Raman Spectroscopy	0.051±0.036 mg/L	Fan et al., 2019
Kallaandsi Lake (Finland)	20-100-300 and 333 µm Manta trawl Water pump Stereomicroscope FT-IR	0.27 particles/m³	Uurasjarvi et al., 2019
Carpathian Basin (Europe)	Water pump FT-IR	3.52-32.05 particles/m³	Bordos et al., 2019
Rainwater Pond (Denmark)	Glass bottle FPA-FT-IR	2.7x10⁵ items/m³	Olesen et al., 2019
Ofanto River (Italy)	Plankton net (333 µm) Py-GC-MS	0.9-13 particles/m³	Campanale et al., 2020
Manas River (Asia)	Stainless steel drum micro FT-IR, SEM	21-49 items/L	Wang et al., 2020a
Dutch River (Holland)	Water pump ATR-FT-IR	67-11532 MP/m³	Mintenig et al., 2020
Kızılırmak River Karasu River Yeşilırmak River Melet River Aksu River Değirmendere River Fırtına River	333 µm manta trawl Niskin bottles Stereomicroscope FT-IR SEM	1.783 and 40.03 particles/m³	Aytan et al., 2020
Vistula River (Poland)	55 µm plankton net SEM, FT-IR Raman Spectroscopy	1.6-2.55 particles/L	Sekudewicz et al., 2021
Saskatchewan River (Canada)	53 µm plankton net Stereomicroscope Raman Spectroscopy	4.6-88.3 particles/m³	Bujaczek et al., 2021

Table 2. Continue.

Locality and sampled compartment	Sampling and detection methods	Microplastic quantity	References
Kızılırmak River (Turkey)	200 µm steel sieve Stereomicroscope	Not available	Özkor, 2022
Asi River (Hatay, Turkey)	333 µm manta trawl Stereomicroscope	281 items	Şahutoğlu, 2022
Sediments			
Vembanad Lake (India)	Van-Veen grab Micro Raman Spectroscopy	252.80 particles/m²	Sruthy & Ramasamy, 2017
Shanghai River (China)	Spade micro FT-IR	802 items/kg (dry weight)	Peng et al., 2018a
Tame River (England)	Stainless steel scoop FT-IR	165 particles/kg (dry weight)	Tibbetts et al., 2018
Yangtze River Delta (China)	Stainless steel spatula micro FT-IR	35.76-3185.33 items/kg	Hu et al., 2018
Antua River (Portugal)	Van-Veen grab FT-IR	100-629 (March) items/kg 18-514 (October) items/kg	Rodrigues et al., 2018
Po River Delta (Italy)	Metal spatula ATR-FT-IR	2.92-23.30 particles/kg	Piehl et al., 2019
Kelvin River (Scotland)	Spade SEM-EDS Light and Electron microscope	161-432 particles/kg	Blair et al., 2019
Rainwater Pond (Denmark)	Glass corer with a diameter of 5 cm FPA-FT-IR	$9.5x10^5$ items/kg	Olesen et al., 2019
Carpathian Basin (Europe)	Van-Veen Grab FT-IR	0.46-1.62 particles/kg	Bordos et al., 2019
Pearl River Basin (China)	Bucket FT-IR Raman Spectroscopy	174±115 mg/kg	Fan et al., 2019
Bizerte River (Tunisia)	Stainless steel spatula ATR-FT-IR	2340-6920 items/kg (dry weight)	Toumi et al., 2019
Riva River Alacalı River Kumbala River Kurfalli River Ağva River	Metal spoons	20.7 particles/kg (dry weight)	Şener et al., 2019
Rhine River (Germany)	Steel spade micro FT-IR	0.26-11.07 particles/kg	Mani et al., 2019
Citarum River (Indonesia)	Ekman grab FT-IR	16.66±0.577 particles/100 g	Sembiring et al., 2020
Kızılırmak River Karasu River Yeşilırmak River Melet River Aksu River Değirmendere River Fırtına River	Box core FT-IR SEM	74.1 and 1778.8 particles/m²	Aytan et al., 2020
Brisbane River (Australia)	Stainless-steel grab sampler ATR-FT-IR	0.18-1290 mg/kg	He et al., 2020

Table 2. Continue.

Locality and sampled compartment	Sampling and detection methods	Microplastic quantity	References
Yongfeng River (China)	Peterson grab ATR-FT-IR FESEM, EDS	0.5-16.75 mg/kg	Rao et al., 2020
Shuangtaizi River (China)	Steel grab FT-IR	170±96 particles/kg	Xu et al., 2020
Daliao River (China)		237±129 particles/kg	
Solimoes, Negro and Amazon River (Brazil)	Van-Veen grab Stereomicroscope	417-8178 particles/kg	Gerolin et al., 2020
Lawrence River (Canada)	Ponar grab Fluorescent microscope	65-7562 particles/kg	Crew et al., 2020
Vistula River (Poland)	Stainless steel spade SEM FT-IR Raman Spectroscopy	190-580 particles/kg	Sekudewicz et al., 2021
Danube River (Romania)	Spade Microscope Py-GC-MS	87 particles/kg	Pojar et al., 2021

Py-GC-MS; Pyrolysis-Gas Chromatography-Mass Spectrometry, FT-IR; Fourier Transform Infrared Spectroscopy, FPA-FT-IR; Focal Plane Array Fourier-Transform Infrared Spectroscopy, ATR-FT-IR; Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy, micro FT-IR; micro Fourier Transform Infrared Spectroscopy, SW-IR; Short-wave Infrared, SEM; Scanning Electron Microscope, FESEM; Field Emission Scanning Electron Microscopy, EDS; Energy Dispersive Spectroscopy

mg/L to 172.000-419.000 items/m^3, and in the sediments it was between 0.18-1290 mg/kg to 9.5x10^5 items/kg. Clearly indicates that microplastic particles are observed almost in all sampling localities reviewed and were quite abundant both in water column and sediments.

Microplastic Pollution in the Marine Ecosystems

The data, including the examined water compartment sampling and detection methods and sampling locality, gathered from the studies focusing on the amount of microplastics observed in various marine habitats are given in Table 3. According to the data summarized, we found that the amount of microplastic particles found in surface water samples were between 4.38x10^4-1.46x10^6 particles/km^2 to 0.018 items/m^2, and in the sediments it was between 0.58 and 1154.4 items/kg. This data clearly indicates that the quantity of microplastic particles are quite high both in water column and sediments. On the contrary to freshwater habitats which are isolated to a certain degree, seas are under greater threat since they are exposed to microplastic entrance from various non-point sources.

The Effects of Microplastics on Aquatic Organisms

Several aquatic organisms, either freshwater or marine, including zooplankton, corals, lobsters, sea urchins, mussels, aquatic birds and aquatic mammals are exposed to microplastics (Browne et al., 2008). Those microplastic particles are either selectively ingested or mistaken as a prey or involuntarily ingested during respiration (Gregory, 1996; Derraik, 2002). Several studies demonstrated that many aquatic animals are usually not able to distinguish plastic from their natural food (Crawford & Quinn, 2017). The physicochemical characteristics of microplastics such as size, density and particularly color are important factors misleading aquatic animals (Wright et al., 2013; Alomar & Deudero, 2017). Once they enter an aquatic animal's digestive tract they can easily reach higher trophic levels through the food chain (Lusher et al., 2016).

Microplastics are observed in the digestive tract of several aquatic animals including zooplankton (Setala et al., 2014), macroinvertebrates (Van Cauwenberghe et al., 2015), fish (Foley et al., 2018), aquatic birds (Nelms et al., 2018), and even aquatic mammals (Lusher et al., 2015). There is also evidence that plastic microparticles can pass across biological barriers through endocytotic processes such as phagocytosis and persorption (Wright et al., 2013; Wright & Kelly, 2017). Persorption is a paracellular mechanical process in which particles penetrate into the underlying tissues through the epithelial layer (Wright & Kelly, 2017). These processes are dependent on the particle size; very small particles can passively pass through cell membranes, while large particles should be taken by endocytosis (Kettiger et al., 2013).

Microplastics ingested as a food particle can block the digestive tract of the animal and eventually may cause death, or lead to behavioral changes, or leading to a pseudo sense of satiety and thus leading to a weakening of the animal and making it an easy prey for other animals (Derraik, 2002; Wright et al., 2013; Jovanovic, 2017). In addition, microplastics can cause oxidative stress, cellular damage, damage to DNA, inflammation and also trigger various immune reactions (Yong et al., 2020).

Microplastics observed in freshwater aquatic organisms, the polymer type recorded, particle quantity and the detection methods are reviewed in Table 4. The studies focusing on the toxic effects of microplastic particles, the polymer type, particle size and quantity, and their effects on some freshwater organisms are summarized in Table 5.

Table 3. The amount of microplastics observed in various marine habitats, the examined water compartment, sampling and detection methods, and sampling locality.

Locality and sampled compartment	Sampling and detection methods	Microplastic quantity	References
Water			
Bandar Abbas Coastline	ATR-FT-IR	3252 particles/m^2	Nabizadeh et al., 2019
Antarctic Peninsula	330 µm manta trawl FT-IR	755-3524 items/km^2	Lacerda et al., 2019
Surface Waters			
Antarctic Ocean	Neuston net Stereomicroscope	3.1×10^{-2} particles/m^3	Isobe et al., 2017
Arabian Gulf	300 µm neuston net FT-IR	$4.38x10^4$-$1.46x10^6$ particles/km^2	Abayomi et al., 2017
Iskenderun Bay (Adana, Turkey)	333 µm manta trawl Stereomicroscope	1.067.120 items/ km^2	Gündoğdu, 2017
Mediterranean Coastal Water (Israel)	333 µm manta trawl Stereomicroscope	7.68 particles/m^3	Van der Hal et al., 2017
Marmara Sea	333 µm manta trawl Stereomicroscope ATR FT-IR	1.263 items/m^2	Tunçer et al., 2018
Faafu Coral Island (Maldives)	200 µm neuston net Stereomicroscope ATR FT-IR	0.32 particles/m^3	Saliu et al., 2018
Kingston Port (Jamaica)	330 µm manta trawl Stereomicroscope FT-IR	0-5.73 particles/m^3	Rose & Webber 2019
Tyrrhenian and Ligurian Seas	330 µm manta trawl Stereomicroscope ATR-FT-IR	1009-122817 particles/km^2	Caldwell et al., 2019
North Sea	100 µm neuston net ATR-FT-IR	0.1-245.4 particles/m^3	Lorenz et al., 2019
Pacific Ocean	330 µm manta trawl micro Raman Spectroscopy SEM	640-42000 items/km^2	Pan et al., 2019
White Sea, Barents Sea and Black Sea	330 µm manta trawl FT-IR	28000-963000 particles/km^2	Tošić et al., 2020
Macaronesia	200 µm manta trawl Stereomicroscope	15283-1007872 particles/km^2	Herrera et al., 2020
Greenland and Northern Canada	335 µm manta trawl Stereomicroscope FT-IR	0.018 items/m^2	Liboiron et al., 2021
Dalyan-Iztuzu Beach (East Mediterranean, Turkey)	Manta trawl Stereomicroscope	0.148±0.07 particles/m^2	Zilifli & Tunçer, 2021
Southern coast of the Black Sea	FT-IR	18.68±3.01 particles/m^3	Terzi et al., 2022
Sediments			
North Pole, Hausgarten observation station	Stereomicroscope micro FT-IR ATR FT-IR	4356 particles/kg	Bergmann et al., 2017
Terra Nova Bay (Antarctica)	Van-Veen grab FT-IR	1-90 items/m^2	Munari et al., 2017
Strait of Hormuz, Persian Gulf	Stainless steel spoon FT-IR	2-1258 particles/kg	Naji et al., 2017
North Bering and Chukchi Seas	Stainless steel box micro FT-IR	0-68.88 items/kg	Mu et al., 2018

Table 3. Continue.

Locality and sampled compartment	Sampling and detection methods	Microplastic quantity	References
East Coast of Hong Kong	Stainless steel shovel ATR-FT-IR	0.58-2116 items/kg	Lo et al., 2018
Mariana Trench	Stereomicroscope Raman Spectroscopy	200-2200 particles/L	Peng et al., 2018b
Antarctica	Microscope FT-IR	31 particles/kg	Reed et al., 2018
Aegean Sea (Datça, Turkey)	Stainless steel shovel ATR-FT-IR	1154.4 items/kg	Yabanlı et al., 2019
Iskenderun Bay (Adana, Turkey)	Quadrate Stereomicroscope	3.4-658 items/kg 40.2-6354.1 items/m^2	Çevik & Gündoğdu, 2019
Sidi Mansour Port (Tunisia)	Core tube ATR-FT-IR	11242 particles/m^2	Chouchene et al., 2019
North Sea	Van-Veen grab ATR-FT-IR	2.8-1188.8 particles/kg	Lorenz et al., 2019
Tenerife Beach (Spain)	Stainless steel vessel ATR-FT-IR	2-115.5 items/m^2	Alvarez-Hernandez et al., 2019
Lanzarote Island	ATR-FT-IR Raman spectroscopy	36.3 g/m^2	Edo et al., 2019
Northwest Mediterranean	Steel trowel FT-IR	33-798 and 12-187 items/kg	Constant et al., 2019
New Zealand Coasts	FT-IR	459 particles/m^2	Bridson et al., 2020
Marmara Sea	Van-Veen grab FT-IR	0.3-85.6 g/kg	Baysal et al., 2020
Mediterranean (France, Toulon)	Remotely Operated Vehicle grab Stereomicroscope micro Raman Spectroscopy	80 particles/L	Cutroneo et al., 2022
Southern Coast of the Black Sea	FT-IR	64.06±8.95 particles/m^3	Terzi et al., 2022

FT-IR; Fourier Transform Infrared Spectroscopy, ATR-FT-IR; Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy, micro FT-IR; micro Fourier Transform Infrared Spectroscopy, SEM; Scanning Electron Microscope

Microplastics observed in marine organisms and the polymer type recorded, particle quantity and the detection methods are reviewed in Table 6. The studies focusing on the toxic effects of microplastic particles, the polymer type, particle size and quantity, and their effects on some marine organisms are summarized in Table 7.

CONCLUSIONS

The plastic wastes released, either intentionally or unintentionally, into the environment, break into smaller pieces when exposed to meteorological events such as wind, precipitation, UV radiation and abrasion. The recent studies indicated that the amount of microplastic particles are increasing in all compartments of the aquatic ecosystems. Thus, they are considered a serious health concern both for aquatic biodiversity and human health through the food chain. There are numerous studies demonstrating the toxic effects of various plastic polymers on several aquatic organisms. However, it is not easy to achieve a precise decision on the potential risks of microplastic pollution.

In this study, we reviewed the polymer type, particle size and quantity of microplastics observed in freshwaters and marine ecosystems. The data reviewed here clearly indicates that microplastics are present almost in all freshwater and marine ecosystems, both in surface waters and sediments. Studies on the toxic effects of microplastic particles on aquatic organisms demonstrate that alterations are common in the biochemical and behavioral characteristics of the organisms exposed to microplastics. Furthermore, mortality is reported to be a prevalent consequence of microplastic exposure.

The microplastic particles ingested have a great potential to reach higher trophic levels through the food chain. Therefore, future studies should focus on the mechanisms that can be responsible for the transmission of these particles through food chains and their potential risks for human beings and other organisms consuming fish and shellfish.

Conflict of Interest: The author has no conflicts of interest to declare.

Table 4. Microplastics observed in some freshwater aquatic organisms and the polymer type recorded, detection method and particle quantity.

Location	Species	Target tissue	Polymer type	Detection method	Particle quantity	References
Invertebrates						
Dutch River (Amsterdam)	*Gammarus* spp.	Digestive tract	Not available	NaOH 30% H_2O_2 FT-IR	11-105 particles/g	Leslie et al., 2017
Dutch River (Amsterdam)	*Carcinus maenas* *Littorina littorea* *Mytilus edulis* *Crassostrea gigas*	Stomach, intestine	Not available	NaOH 30% H_2O_2 FT-IR	11-105 particles/g	Leslie et al., 2017
Lawrence River (New York)	*Dreissena polymorpha* *D. bugensis*	Digestive tract	Not available	Dissection microscope	No microplastics were found.	Schessl et al., 2019
Melet River Yeşilırmak River	*Donax trunculus* *Chalelea gallina* *Abra alba* *Anadara inaequivalvis* *Pitar rudis*	Soft tissue	Not available	10% KOH Strereomicroscope	92 items	Şentürk et al., 2020
Grand River (Canada)	*Lasmigona costata*	Soft tissue	PP, PE	20% protease enzyme 70% ethanol Raman Spectroscopy	0-7 particles/ind.	Wardlaw & Prosser, 2020
Fish						
Citarum River (Indonesia)	*Chanos* sp.	Intestine, gill	PP, PE	30% H_2O_2 FT-IR	1.33±0.57 particles/fish	Sembiring et al., 2020
Akora River (Ghana)	*Oreochromis niloticus* *O. aureus* *O. mosssambicus* *Sarotherodon melanotheron* *Clarias anguillaris*	Digestive tract	PE, PS	KOH Light microscope	12-24 particles/m^3	Adu-Boahen et al., 2020
Fengshan River (Taiwan)	*Oreochromis niloticus,* *Pterygoplichthys pardalis* *Carassius auratus* *Leiognathus equulus* *Pomadasys argenteus*	Digestive tract	PE, PP, PS, PVC	KOH ATR-FT-IR	14-94 items/fish	Tien et al., 2020
Lijiang River (China)	*Cyprinus carpio* *Pelteobagrus fulvidraco* *Mystus macropterus* *Pelteobagrus vachelli*	Digestive tract	PVC, PA, PS, PP, PE, PET	30% H_2O_2 micro FT-IR	0.6±0.6 particles/fish	Zhang et al., 2021a

Table 4. Continue.

Location	Species	Target tissue	Polymer type	Detection method	Particle quantity	References
Other Aquatic Vertebrates						
Yangtze River (China)	*Microhyla ornata* *Rana limnochari* *Pelophylax nigromaculatus* *Bufo gargarizans*	Not available	PES, PP	1.2 g/cm^3 30% H_2O_2 ATR-FT-IR	0-2.73 items/individual	Hu et al., 2018
Ticino River (Italy)	*Alcedo atthis*	Pellet*	PE, PUR, PP	NaCl 0,0616 M Fe (II) micro FT-IR SEM-EDS	3 items/individual	Winkler et al., 2020

PP; Polypropylene, PE; Polyethylene, PET; Polyethylene Terephthalate, PVC; Polyvinyl Chloride, PA; Polyamide, PS; Polystyrene, PUR; Polyurethane, PES; Polyether sulfone) (FT-IR; Fourier Transform Infrared Spectroscopy, micro FT-IR; micro Fourier Transform Infrared Spectroscopy, ATR-FT-IR; Attenuated Total Reflection-Fourier Transform Infrared Spectroscopy, SEM-EDS; Scanning Electron Microscope-Energy Dispersive Spectroscopy
*Undigested parts of the food that the bird eats. These parts may include the exoskeleton of insects, claws, teeth, vegetable matter. The birds throw the pellet out by vomiting.

Table 5. Studies on the toxic effects of microplastics on some freshwater organisms.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Plankton						
Daphnia magna	PET	62-1400 µm (length) 31-528 µm (width) 1-21,5 µm (diameter)	12.5-25-50-100 mg/L	48 hours Added to water	Mortality was observed. Microplastic particles were observed in digestive tract.	Jemec et al., 2016
Brachionus koreanus	PS	0.05-0.5-6 µm	10 µg/ml	24-48 hours Added to water	Mortality was observed depending on the size and conc. of microplastics. Microplastics were found in gut. ROS conc. and GPx, GST, SOD activities increased.	Jeong et al., 2016
Ceriodaphnia dubai	PE PES	1-4 µm	0.5-1-2-4-8-16 mg/L PE 0.125-0.25-0.5-1-2-4 mg/L PES	48 hours 8 days Added to water	LC_{50} was found as 2,2 mg/L for PE and 1,5 mg/L for PES. Growth rate, number of juveniles and reproductive rates decreased. Abnormal swimming behavior was observed. Microplastics were found in gut.	Ziajahromi et al., 2017
Daphnia magna	PS PS-COOH	201.5 nm (PS) 191.3 nm (PS-COOH)	1-5-10-20-30 mg/L	48 hours Added to water	Immobilization was observed. PS-COOH type particles were found to be more toxic.	Kim et al., 2017

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Brachionus koreanus	Fluorescence microbead	0.05-0.5-6 µm	10 µg/ml	24 hours Added to water	Microplastics were found in gut. It was observed that microplastics can pass through the through cell membrane.	Jeong et al., 2018
Daphnia magna	Fluorescent PE microbead	63-75 µm	25-50-100 mg/L	5-21 days Added to water	Microplastics was observed in gut. No effect on mortality and reproductive rates.	Canniff & Hoang, 2018
Chlorella pyrenoidosa Microcystis flos-aquae	PP, PVC	64 µm PP 172 µm PP 236 µm PP 111 µm PVC 157 µm PVC 216 µm PVC	5-10-50-100-250-500 mg/L	11 days Added to water	Altered chlorophyll-a synthesis Microplastic internalization was observed.	Wu et al., 2019
Spirulina sp.	PP PET	1-2 µm	150 mg/500 ml 250 mg/500 ml 275 mg/500 ml	112 days Added to water	Decreased growth rate.	Khoironi & Anggoro, 2019
Daphnia magna	PS	1.25 µm	2-4-8 mg/L	10 days Added to water	The transcription of AK, TrxR and permease increased. No mortality was observed	Tang et al., 2019
Scenedesmus quadricauda	PS	1-2-3-4-5 µm	10 mg/L	24-48-72-96 hours Added to water	Microplastics internalization was observed. Population density decreased on a time dependent manner. No effect on photosystem II. Retarded growth.	Chen et al., 2020
Chlorella sorokiniana	PS	<70 µm	240 mg/L	30 days Added to water	ALA concentration decreased. Cell size decreased. Lipid accumulation, saturated myristic and palmitic acid levels increased.	Guschina et al., 2020
Chlamydomonas reinhardtii	PS	300-600 nm	5-25-50-100 mg/L	1-3-6-10 days Added to water	Decreased chlorophyll-a conc. Increased MDA conc.	Li et al., 2020
Chlamydomonas reinhardtii	PVC	50-100 µm	1-10-20-30-40-50 mg/L	24-48-72-96 hours Added to water	Decreased chlorophyll conc. Reduced growth and population density. Increased MDA and SOD activity.	Wang et al., 2020b

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Euglena gracilis	PS	0.1-5 µm	0.5-1-10-20-30-40-50 mg/L	24-96 hours Added to water	Reduced growth. Damage to cell membrane and chloroplasts. Decreased signal transduction and carbohydrate metabolism rate.	Xiao et al., 2020
Scenedesmus armatus *Microcystis aeruginosa*	PE	200 to 300 µm	250-500-1000 µg/ml	3-7-14-21-28 days Added to water	Decreased growth rate and photosynthetic activity.	Sanchez-Fortun et al., 2021
Acutodesmus obliquus	HDPE PP PVC	<100 µm	5-10-15-25-100-125-200-250 mg/L	21 days Added to water	Microplastics internalization was observed. Growth rate and photosynthetic activity decreased. Microplastics accumulation on the cell surface.	Ansari et al., 2021
Daphnia magna	PE	3.43±17.23 µm PE 13.09±34.43 µm PE 9.74±39.54 µm PE	5 mg/L	21 days Added to water	Microplastics was observed in gut. Body length, survival rates, reproductive rates, carbohydrate and protein reserves decreased.	An et al., 2021
Brachionus calyciflorus	PS	1 µm	0-1x10^4-1x10^5-1x10^6-1x10^7 particles/ml	2 days Added to water	Reproductive rates and PHGPx activity decreased. ROS conc. increased. SOD, MnSOD, CuZnSOD and CAT activities did not change.	Liang et al., 2021
Chlorella sp.	PE, PS, PP, PVC, PET	100-2000 µm	10-1000 mg/L	3 days Added to water	Microplastic internalization was observed. Retarded growth.	Miloloza et al., 2021
Brachionus calyciflorus	PE	10-22 µm	0.5x10^3-2.5x10^3-1.25x10^4 particles/ml	24 hours Added to water	NA^+-K^+-ATPase activity and SOD activity decreased GPx activity increased.	Xue et al., 2021
Euglena gracilis	PS	75-1000 nm	1-5-25 mg/L	4-8 days Added to water	Microplastics internalization was observed. No effect on growth mobility.	Sun et al., 2021
Spirulina sp.	PE PP	0.5-1 mm^2	500 mg/500 ml	30 days Added to water	Phycocyanin and protein conc. decreased. PES (extracellular polymeric substance) production rate increased.	Hadiyanto et al., 2021
Microcystis aeruginosa	Nylon	1-3 µm	25-50-100 mg/L	6-12-18-24-30 days Added to water	GPx and SOD activity increased. MDA conc. increased. Energy reserves, carbohydrate and lipid synthesis increased Regulation of *por*, *petE*, *petF*, *petH* and *cyt b6/f* genes decreased.	Zheng et al., 2022

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Daphnia magna	PVC	2±1 and 50±10 µm	10-20-40-80-160 mg/L	4-7-14-21 days Added to water	The reproductive rate, CAT activity and MDA conc. varied depending on the size of microplastic particles. SOD activity, GSH conc. and *vtg* gene regulation decreased.	Liu et al., 2022
Invertebrates						
Potamoryrgus antipodarum	PA, PET, PC, PS, PVC	118±105 µm	30%-70%	8 weeks Added to food	Mortality was observed. Number of juveniles did not change	Imhof & Laforsch, 2016
Gammarus fossarum	PS PA	500x20 µm PA 1.6 µm PS	100-540-2680-13380 PA fibers/cm^2 500-2500-12500-60000 PS beads/ml	0.5 hours to 28 days Added to food	Microplastics were found in the digestive tract. Mortality was observed.	Blarer & Burkhardt-Holm, 2016
Palaemonetes pugio	PS, PP, PE	30-34-35-59-75-83-93-116 µm	50000 particles/L	3 hours Added to water	Microplastics were found in intestines and gills. Mortality was observed.	Gray & Weinstein, 2017
Sphaerium corneum	PS	20-500 µm	0 to 40% dry weight of sediment	28 days Added to sediment	No mortality was observed.	Redondo Hasselerharm et al., 2018
Dreissena polymorpha	PS	1-10 µm	Mixture 1 (5x10^5 1 µm and 5x10^5 10 µm) Mixture 2 (2x10^6 1 µm and 2x10^6 10 µm)	6 days Added to water	Microplastics were present in intestinal lumens, hemolymph and on hemocyte surface. SOD, GPx activities did not change. Antioxidant conc. decreased.	Magni et al., 2018
Corbicula fluminea	Red fluorescent polymer	1-5 µm (ort 2 µm)	0.2 and 0.7 mg/L	96 hours Added to water	No mortality was observed. Microplastic were present in the digestive tract, gill surface and hemolymphatic sinuses.	Guilhermino et al., 2018
Corbicula fluminea	Fluorescent microspheres	1-5 µm	0.13 mg/L	8-14 days Added to water	No mortality was observed. Microplastics were found in the digestive tract. LPO conc. increased ChE activity decreased	Oliveira et al., 2018

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Lumbriculus variegatus Tubifex spp.	PS	20-500 µm	0 to 40% dry weight of sediment	28 days Added to sediment	Mortality was observed.	Redondo Hasselerharm et al., 2018
Gammarus pulex	PET	10-150 µm	0.8-4.000 particles/ml	24 hours to 48 days Added to water	Microplastics were present in gut. No changes were observed in feeding activities, energy reserves, and molting.	Weber et al., 2018
Gammarus pulex Hyalella azteca	PS	20-500 µm	0 to 40% dry weight of sediment (PS microplastics were added to the sediment mixture at the specified rates.)	28 days Added to sediment	No mortality was observed.	Redondo Hasselerharm et al., 2018
Hydra attenuata	PE	<400 µm	0-0.01-0.02-0.04-0.08 g/ml	3-24-48-96 hours Added to water	Microplastics were found in digestive tract. Morphological changes were observed.	Murphy & Quinn, 2018
Eriocheir sinensis	PS	5 µm	40-400-4000-400000 µg/L	7-21 days Added to water	Survival rates did not change, HSI level decreased. CAT, GPx, SOD and GST activities increased. *p38* gene expression increased Growth reduced. Microplastics were found in liver, gill and digestive tract.	Yu et al., 2018
Asellus aquaticus	PS	20-500 µm	0 to 40% dry weight of sediment	28 days Added to sediment	Mortality was observed.	Redondo Hasselerharm et al., 2018
Chironomus tepperi	PE	1-4 µm 10-27 µm 43-54 µm 100-126 µm	500 particles/kg	5-10 days Added to water	Survival rates decreased. The number of juveniles, larval growth rates and antenna length decreased. Microplastics were found in the digestive tract.	Ziajahromi et al., 2018
Eriocheir sinensis	PS	5 µm	0.04-0.4-4-40 mg/L	7-14-21 days Added to water	The hemocyanin conc. and AKP activity initially increased and then decreased. Lysozyme activity decreased. PO activity increased.	Liu et al., 2019

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Caenorhabditis elegans	PS	1 µm	0-0.1-1-10-100 µg/L	72 hours Added to water	Mobility decreased, growth rate reduced. ROS conc. increased. The expression of *daclk-1, ctk-1, ctk-2, ctk-3, gst-4, isp-1, skn-4, sod-1, sod-2, sod-3, sod-4* and *sod-5* genes increased. Microplastics were present in the intestinal lumen, intestinal cells and in the body cavity.	Yu et al., 2020
Dreissena bugensis	PE	10-45 µm	0.1-0.4-0.8 g/L	25 days Added to water	Microplastics were found in the intestines and gills. Oxygen consumption rate decreased. Mortality rate increased.	Pedersen et al., 2020
Dreissena polymorpha	PS	2-60 µm	6.4-160-4000-100000 particles/ml	14 days Added to water	No changes in energy reserves and lipid peroxidation rates. Mortality increased.	Weber et al., 2020
Chironomus riparius	PA	<80 µm <100 µm <160 µm <180 µm	10.100 particles/kg	28 days Added to water	Number of larvae increased. Altered egg shape.	Khosrovyan & Kahru, 2020
Chironomus riparius	PS microrubber	38.9±28.6 µm 82.3±40 µm	1-10 mg/L	36 hours Added to water	*glycoprotein93, hsp90, hsc70, hsp60, hsp40* and *hsp17* (heat shock proteins) gene expression rate increased.	Carrasco-Navarro et al., 2021
Corbicula fluminea	PS	5-10-45-90 µm	12 particles/ml (5-10-95 µm) 2 particles/ml	6-96 hours 28 days Added to water	Microplastics were present in gut. Energy reserves, oxidative stress level and reproductive rates did not change.	Weber et al., 2021b
Potamoryrgus antipodarum	PS	0.01-514 µm	100-500-1000 mg microplastics/kg (dry weight of sediment) 2000 and 4000 mg microplastics/kg (dry weight of sediment)	31 days Added to sediment	Mortality was observed. Reproductive rates decreased.	Romero-Blanco et al., 2021

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Lymnaea stagnalis	PS	5-10-45-90 µm	12 particles/ml (5-10-95 µm) 2 particles/ml	6-96 hours 28 days Added to water	Microplastics were present in gut. Energy reserves, oxidative stress level and reproductive rates did not change.	Weber et al., 2021b
Dreissena polymorpha	PS	5-10-45-90 µm	6.4-100000 particles/ml	6-96 hours 7-28 days Added to water	Microplastics was observed in gut. No changes in energy reserves and lipid peroxidation levels.	Weber et al., 2021a
Caenorhabditis elegans	PS	1.002±0.005 µm	0.1-10-100 µg/L	24 hours Added to water	ROS conc. increased. Microplastics were found in gut. Expression of oxidative stress-related genes (*clk-1, ctl-1, sod-3, sod-4* and *sod5*) increased.	Chen et al., 2021
Neocaridina palmata	PE PVC PS	41 and 87 µm PE <63 µm PVC 11 µm PS	20-200-2000-20000 particles/L	4-24 hours Added to food	Microplastics were present in gut. The reproductive rates decreased. Microplastic uptake decreased when exposed to higher concentrations.	Klein et al., 2021
Chironomus riparius	PET	50 µm	500-5000-50000 particles/kg dry weight of sediment	28 days Added to sediment	No mortality was observed. Microplastics were found in digestive tract.	Setyorini et al., 2021
Chironomus riparius	PE	32-63 µm 63-250 µm 125-500 µm	1.25-5-20 g/kg	48 hours Added to water	Microplastics were present in gut. ETS activity and lipid conc. decreased. Lipid peroxidation rates increased. No changes were observed in sugar and protein conc. There did not different seen in sugar and protein content. CAT and GST activities decreased.	Silva et al., 2021
Fish						
Danio rerio larvae	PS	0.7 µm	5 mg/ml	1-2-3-4 days Injected into embryos	Microplastics were found in digestive tract. Lipid metabolism rate and oxidative stress increased.	Veneman et al., 2017

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Danio rerio larvae	PE	10-45 µm	5-20 µg/L	2-7-14 days Added to water	No effect on brain, eye and embryo development. Oxidative stress, steroid synthesis and muscle development decreased.	LeMoine et al., 2018
Symphysodon aequifosciotus	PE	70-88 µm	0-200 µg/L	30 days Added to water	Survival rate, body length, ETC and lipase activities did not change. Trypsin, ALP and AChE activities decreased. Amylase, CS, COX and LDH activities increased.	Wen et al., 2018
Pimephales promelas larvae	PE (Green sphere) PE (White sphere)	425-500 µm (Green sphere) 180-212 µm (White sphere)	35.0-0.069-70.0-0.137-140.0-0.274 mg/L	7-14 days Added to water	No adverse effects were observed.	Malinich et al., 2018
Oreochromis niloticus	PS	0.1 µm	1-10-100 µg/L	0-1-3-6-10-14 days Added to water	Mortality was observed. AChE activity decreased. MDA conc. did not change.	Ding et al., 2018
Carassius auratus	EVA, PS, PA	<500 µm	0.96%-1.36%-1.94%-3.81%	6 weeks Added to food	Weight decreased Oral cavity damaged. Microgranuloma and inflammation were observed.	Jabeen et al., 2018
Barbodes gonionotus	PVC	0.1-1000 µm	0.2-0.5-1.0 mg/L	96 hours Added to water	Mortality has been observed. Microplastics were found in the intestine. Trypsin and chymotrypsin activities increased.	Romano et al., 2018
Acanthurus dussumieri	PE, PVC, PE, PS	9 particle PE (film), 5 particle PVC, 1 particle PE (pellet) and A mixture was prepared by mixing 1 particle PS. (1000-250 µm)	0.051 g	95 days Added to food	Mortality was observed. Microplastics were present in the digestive tract. Body length and weight decreased.	Naidoo & Glassom, 2019
Danio rerio larvae	PS	5-50 µm	100-1000 µg/L	7 days Added to water	Microplastics were found in digestive tract. Transcription of genes related to glycolysis and lipid metabolism decreased. GSH conc. and CAT activity decreased. SOD activity did not change.	Wan et al., 2019

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Danio rerio	PS	1 µm	0-10-100-1000 µg/L	120 hours Added to water	Microplastics were present in stomach and intestine Larvae development did not change, but the free swimming performance has decreased. Inflammation and oxidative stress (CAT, SOD activities) increased.	Qiang & Cheng, 2019
Danio rerio	PS	1 µm	10-100-1000 µg/L	21 days Added to water	Microplastics were found in intestines and gills. The reproductive rate, estradiol and testosterone conc. did not changed.	Qiang et al., 2020
Fundulus heteroclitus	Recycled rubber particles produced from scrap automotive tires	38-355 µm	0-0.3-1.9-6.0 g/L 0-0.1-0.33-1.0 g/L	7 days Added to water	Microplastic particles were found in the digestive tract. GST activity did not change.	Laplaca & van den Hurk, 2020
Prochilodus lineatus	PE	10-90 µm	20 µg/L PE	24-96 hours Added to water	No mortality was observed DNA damage was observed in liver cells. GSH conc. and AChE activities decreased.	Roda et al., 2020
Oncorhynchus mykiss	PE	180-212 µm 425-500 µm	50 particles/L 100 particles/L 500 particles/L	15-30 days Added to food	No mortality was observed. No microplastics were observed on the outside of the intestine, which indicated that microplastics were not transported from the intestine to other parts of the body.	Kim et al., 2020
Cyprinus carpio	PVC	Not available.	45.55-91.1-136.65 µg/L (10%-20%-30%)	30-60 days Added to food	Mortality was observed. MDA conc. decreased. SOD, CAT and GST activities decreased.	Xia et al., 2020
Carassius auratus	PS	5 µm	10-100-1000 µg/L	1-3-7 days Added to water	Retarded growth. Decreased CAT and GPx activities, increased SOD activity. Increased hearth beat rate. Enlarged intestinal cavity. Disturbance of the intestinal mucosa.	Yang et al., 2020

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Clarias gariepinus	PVC	95.41±4.23 µm	0.5% 1.5% 3.0%	45 days Add to food	Hb, MCH, RBC and LPO level increased. Neutrophil count, MCV and WBC counts decreased. GPx, SOD, AChE and CAT activities decreased. No changes were observed in monocyte and lymphocyte counts.	Iheanacho & Odo, 2020
Danio rerio larvae	Red fluorescent microspheres (MP)	1-5 µm	2 mg/L	2-6-10-14 days Added to water	Mortality was observed. Heart beat rates, GSH and ROS conc. and LDH, AChE, CAT activities increased.	Santos et al., 2021
Danio rerio	PS	1 µm	0-10-100-1000 µg/L	21 days Added to water	ROS conc. increased. Cell apoptosis rates increased in the testicle.	Qiang & Cheng, 2021
Danio rerio	PS	0.10-0.12 µm	10-100 µg/L	7-14-21-28-35 days Added to water	ROS production and lipid peroxidation increased. LDH, ALT and AST activities increased. GPx, CAT and SOD activities decreased. GST activity did not change. Inflammation, hepatic necrosis, eosinophilic granuloma, cytoplasmic degeneration were observed.	Umamaheswari et al., 2021
Danio rerio	PP	50-200 µm	10-100 µg/L	21 days Added to water	Microplastics were present in the intestine. Physical damage to the intestines was observed. Oxidative stress led to inflammation. Lipid conc. decreased.	Zhao et al., 2021
Danio rerio	50% PE 25% PP 15% PS 10% PVC	Not available.	5-100 mg/L	21 days Added to water	Circulation rate slowed. Swimming performances initially increased and then decreased. AChE activity decreased.	Hanslik et al., 2022
Danio rerio	PP PS	230 µm (PP) <100 µm (PS)	12.5-25-50-100 mg/L	24-48-72-96 hours Added to water	Mortality was observed. Heart beat rate decreased. Body length of the larvae reduced.	Prata et al., 2022

Table 5. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Danio rerio	PS	MPI MPII	$4x10^{6}$-$4x10^{4}$	5 days Added to water	GSH conc. increased. CAT, SOD and AChE activities increased.	Guimaraes et al., 2021
Oreochromis niloticus	PS	0.9-0.35 µm	5 mg/L	28 days Added to water	No mortality was observed. SOD and GPx activities decreased. MDA conc. decreased.	Ahmadifar et al., 2021
Cyprinus carpio	PVC	>100 µm 100-300 µm 300-1000 µm	1-10-100-1000 µg/L	10 days Added to water	Mortality was observed. Abnormal swimming behavior, irregular movements were noted. Mucus secretions increased. Structural abnormality in the intestinal wall and lesions in villi, liver and stomach inflammation were observed.	Ebrahimpour et al., 2021
Danio rerio	PE	146.20±8.86 µm	5-50 µg/L	10-20 days Added to water	CAT, GST, Na^{+}/K^{+}AT-Pase activities and LPO conc. increased.	Rangasamy et al., 2022

PP; Polypropylene, PE; Polyethylene, HDPE; High Density Polyethylene, PET; Polyethylene Terephthalate, PVC; Polyvinyl Chloride, PA; Polyamide, PS; Polystyrene, PS-COOH; Polystyrene, monocarboxy terminated, PUR; Polyurethane, PES; Polyether sulfone, PC; Polycarbonate, EVA; Ethylene-vinyl acetate copolymer). (SOD; Superoxide dismutase, MnSOD; Manganese superoxide dismutase, CuZnSOD; Copper and zinc superoxide dismutase, GPx; Glutathione peroxidase, CAT; Catalase, GSH; Glutathione, ROS; Reactive oxygen species, BaP; Benzo(a)piren, HSI; Hepatosomatic index, GSI; Gonadosomatic index, ETS; Electron transport system, Hb; Hemoglobin, MCH; Mean corpuscular hemoglobin, RBC; Red blood cell, LPO; Lipid peroxidation, MDA; Malondialdehyde, ALA; Alpha-lineoic acid, EPS; Extracellular polymeric substance, MCV; Mean corpuscular volume and WBC; White blood cell, AK; Arginine kinase, TrxR; Thioredoxin reductase, NA^{+}-K^{+}-ATPase; Sodium-potassium adenosine triphosphatase, GST; Glutathione S-transferase, AChE; Acetylcholinesterase, ChE; Cholinesterase, AKP; Alkaline phosphatase, PO; Phenoloxidase, ALT; Alanine aminotransferase, AST; Aspartate aminotransferase, LDH; Lactate dehydrogenase, ALP; Alkaline phosphatase, CS; Citrate synthase, COX; Cytochrome c oxidase, PHGPx; Phospholipid hydroperoxide glutathione peroxidase

Table 6. Microplastics observed in some marine organisms and the polymer type recorded, detection method and particle quantity.

Location	Species	Target tissue	Polymer type	Detection method	Particle quantity	References
Invertebrates						
Black Sea, Marmara and Aegean Sea Coasts	*Mytilus galloprovincialis*	Soft tissue	PET, EVA, PA, PAC, PC, PE, PAN, PS, PP, PVC, PVF, CA	30% H_2O_2 Stereomicroscope FT-IR	0.06 and 2.47 items/mussels	Gedik & Eryaşar, 2020
Izmir Bay (Aegean Sea)	*Mytilus galloprovincialis* *Ruditapes decussatus*	Soft tissue		30% H_2O_2 1.2 g/cm^3 NaCl Stereomicroscope	1682 items	Yozukmaz, 2021
Çesme/Ildır (Izmir, Aegean Sea)	*Pinctada imbricata radiata*	Digestive tract	PET, PE, PP	10% KOH 1.2 g/ml NaCl micro FT-IR	65 items	Aksakal et al., 2021
Terengganu (Malaysia)	Calanoida Cladocera Cyclopoida Harpacticoida Mysids Decapoda	Digestive tract	Not available	65% HNO_3 Stereo microscope	Microplastics were found in 47%, 2%, 11%, 6%, 2%, 1% of the samples, respectively.	Taha et al., 2021
Liaohe Estuary (China)	*Mactra andneriformis* *Sinonovacula constricta* *Neandrita didyma* *Rapana andnosa* *Oratosquilla oratoria* *Portunus trituberculatus*	Digestive tract	PE, LDPE, HDPE, PET	30% KOH micro FT-IR	0.83±1.97 items/individual	Wang et al., 2021
Fish						
Tokyo Bay (Japan)	*Engraulis japonicus*	Digestive tract	PE, PP, PS	FT-IR	2.3±2.5 items/individual	Tanaka & Takada, 2016
Kwazulu-Natal (Dayey Africa)	*Mugil cephalus*	Digestive tract	Not available	Dissection microscope	3.8±4.7 items/individual	Naidoo et al., 2016
Mallorca Island (Spain)	*Galeus melastomus*	Digestive tract	PET, PE, PP, PA, Cellophane, PAN, Polyacrylate	Stereomicroscope FT-IR	0.34±0.07 items/individual	Alomar & Deudero, 2017

Table 6. Continue.

Location	Species	Target tissue	Polymer type	Detection method	Particle quantity	References
South China Sea	*Rexea solandri* *Synagrops japonicus* *Centroneryx lineatus* *Malakichthys griseus* *Lepidotrigla guentheri* *Antigonia capros* *Chlorophthalmus agassizi* *Diaphus watasei* *Benthodesmus tenuis* *Polymetme elongata* *Neoscopelus microchir* *Borostomias pacificus* *Chlorophthalmus albatrossis*	Stomach and intestine	PARA, PA, PET PAE	69% HNO_3 Optical microscope micro FT-IR	1.96±1.12 items/individual (stomach) 1.77±0.73 items/individual (intestine)	Zhu et al., 2019
Bosphorus and Golden Horn (Marmara Sea) Izmir Bay (Aegean Sea) Iskenderun Bay (Mediterranean Sea)	*Chelon saliens* *Mullus barbatus barbatus* *Mullus surmuletus* *Trachurus mediterraneus* *Lithognathus mormyrus*	Digestive track	PP, PE	KOH: NaClO KI Stereomicroscope Micro-Raman Spectroscopy	283 items	Gündoğdu et al., 2020
Rize (Black Sea)	*Engraulis encrasicolus* *Trachurus mediterraneus* *Sarda sarda* *Belone belone* *Pomatotus saltatrix* *Merlangius merlangus* *Mullus barbatus barbatus*	Digestive tracks	PAN, PP, PET, PE, PS	63% HNO_3 Stereomicroscope ATR-FT-IR	352 items	Aytan et al., 2022
Other Aquatic Vertebrates						
New Zeland	*Delphinus delphis*	Stomach	PP, ABS, PET, Nylon,	Enzymatic digestion FT-IR	117 items	Stockin et al., 2021
Qaqaluit and Akpait Islands (Canada)	*Uria lomvia* *Fulmarus glacialis*	Digestive tract	PE, PA, PES	10% KOH micro Raman Spectroscopy	61 particles/individual	Bourdages et al., 2021

ABS: Acrylonitrile Butadiene Styrene, HDPE: High Density Polyethylene, LDPE: Low Density Polyethylene, PA: Polyamide, PAN: Polyacrylonitrile, PE: Polyethylene, PES: Polyester, PET: Polyethylene Terephthalate, PARA: Polyacrylamide, PAE Polyarylether PP: Polypropylene, PS: Polystyrene, PVC: Polyvinyl Chloride FT-IR: Fourier Transform Infrared Spectroscopy, micro FT-IR: micro Fourier Transform Infrared Spectroscopy

Table 7. Studies on the toxic effects of microplastics on some marine organisms.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Bacteria						
Halomonas alkaliphila	PS	55-nm and 1 µm	20-40-80-160-320 mg/L	2 hours Added to water	Mortality increased depending on microplastic quantity. ROS conc. was higher when exposed to nanoplastics.	Sun et al., 2018
Uronema marinum	PS	0.5, 1.07, 2.14 and 5 µm	2×10^5, 4×105, 6×10^5, 8×10^5, and 1×10^6 microplastic/L	96 hours Added to water	Number of individuals, body size and biomass decreased. Decrease in biomass is not dependent on the size of microplastics.	Zhang et al. 2021b
Plankton						
Amphibalanus amphitrite *Artemia franciscana*	PS	0.1 µm	0.001-0.01-0.1-1-10 mg/L	24-48 hours Added to water	No mortality was observed. Swimming performances decreased with increasing conc. Neurotoxic effects were observed.	Gambardella et al., 2017
Skeletonema costatum	PVC	1 µm and 1 mm	1-5-10-50 mg/L	96 hours Added to water	Growth decreased. Photosynthesis rate and chlorophyll conc. decreased.	Zhang et al., 2017
Brachionus plicatilis *Tigriopus fulvus* *Acartia clausi*	PE	1-6 µm	0-30 mg/L	48 hours Added to water	LOEC= 1 LOEC>10 LOEC>30	Beiras et al., 2018
Calanus finmarchicus	Nylon granule Nylon fibers	10-30 µm 10x30 µm	50 microplastic/ml	6 days Added to water	Algae consumption decreased and molting rate increased when exposed to microplastic fibers.	Cole et al., 2019
Artemia parthenogenetica	PS	10 µm	0.1-1-10-100-1000-10000 microplastic/ml	24 hours and 14 days Added to water	Anomalies were observed in intestinal epithelial tissue.	Wang et al., 2019
Acartia tonsa	PE	7.73 µm	20-200-2000 microplastic/L	24-48 hours Added to water	No effect on the mortality, nutrition, egg production and incubation period.	Bellas & Gil 2020
Tigriopus japonicus	PS	50 nm and 10 µm	For 50 nm particle: 2.9×10^{11} particles/ml, For 10 µm particle: 3.6×10^4 particle /ml	24-48 hours Added to water	ROS production showed an inverse relationship with the size of microplastics and a linear relationship with exposure duration. Expression of oxidative stress related genes and enzymes activities increased.	Choi et al., 2020
Calanus finmarchicus *C. glacialis* *C. hypolietilenrboreus*	PE	20.7 µm	200-20000 particles/L	6 days Added to water	No effect on the defecation rate. Fecundity increased.	Rodríguez-Torres et al., 2020

Table 7. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Phaeodactylum tricornutum	PE	5-60 µm 61-499 µm 500-3000 µm	0.1 mg/ml	72 hours Added to water	The species was found to be tolerant to microplastic exposure.	Piccardo et al., 2020
Karenia mikimotoi	PS	For PS 65 nm, 100 nm and 1 µm	10 mg/L	3-13 days Added to water	Nanoplastics had worse effects on cell mortality, cell membrane integrity and DNA conc. compared to microplastics.	Zhao et al., 2020
Brachionus rotundiformis	PS	5 µm	10-100-1000 µg/L	24 hours Added to water	Number of individuals decreased. MDA conc. and SOD activity increased.	Chen et al., 2022
Artemia salina	PP	11.86–44.62 µm	1-25-50-75-100 µg/ml	2-7-14 days Added to water	LC_{50} was found as 40.95 µg/ml and 51.95 µg/ml for nauplius and meta nauplius larvae respectively. SOD, CAT, GST and AChE activities increased. Epithelial cells damaged.	Jeyavani et al., 2022
Invertebrates						
Mytilus galloprovincialis	PS	2 and 6 µm	32 µg/L/day	7 days Added to water	ROS production increased. Antioxidant conc. and GR activity increased. Mortality was observed in hemocytes.	Paul- Pont et al., 2016
Perna sp.	New and PP collected from the beach	Not available	2 ml	48 hours Added to water	Embryonic development altered.	de Silva et al., 2016
Crassostrea gigas	PS	2 µm	0.023 mg/L	2 months Added to water	Oxidative activity increased in hemocytes. Number and diameter of oocytes decreased.	Sussarellu et al., 2016
Nephrops norvegicus	PP	0.3 mm length, 0.2 mm diameter	360 piece	8 months Added to food	Feeding activity, body weight, the protein and stored lipid conc. in the blood decreased with increasing exposure duration.	Welden & Cowie 2016
Scrobicularia plana	PS	20 µm	1 mg/L	14 days Added to water	Microplastic particles were found in the digestive gland, gill and hemolymph. LPO conc., SOD, CAT, GPx and GST activities increased in the gills and digestive gland. CAT, GST and AChE activities decreased in digestive gland.	Ribeiro et al., 2017

Table 7. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Paracentrotus lividus	PE	1–40 µm	0-100 mg/L	48 hours Added to water	NOEC=30 LOEC=100	Beiras et al., 2018
Scrobicularia plana	PE	11-13 µm	1 mg/L	3-7-14 days Added to water	BaP concentrations, hemocyte size and CAT activity increased. No changes on AChE activity	O'Donovan et al., 2018
Mytilus galloprovincialis	PE	1-40 µm	0-100 mg/L	48 hours Added to water	LOEC>100	Beiras et al., 2018
Mytilus galloprovincialis	HDPE	1-50 µm	4.6E+5 microbeads/L	18 days-64 days Added to water	Retarded growth. Microplastic particles were observed in gut.	Detree & Gallardo-Escarate, 2018
Mytilus galloprovincialis	PS	3 µm	50-10000 particles/ml	24-48 hours 3-6-9 days Added to water	Microplastic uptake rate increased with increasing particle conc.	Capolupo et al., 2018
Mytilus galloprovincialis	PS	1-10-90 µm	Not available.	3 hours-40 days Added to water	Microplastics were observed in gut. Microplastics were also found in feces.	Kinjo et al., 2019
Paracentrotus lividus	PE	5-60 µm 61-499 µm 500-3000 µm	0.1 mg/ml	72 hours Added to water	Anomalies observed during larval development increased with increasing particle size.	Piccardo et al., 2020
Mytilus galloprovincialis	PE, PS	20 and 75 µm	10^4 particles/L and 5×10^4 particles/L	7 days Added to water	Amylase and xylanase activities decreased, cellulose activity increased, with PS being more prominent.	Trestrail et al., 2021
Mytilus galloprovincialis	PE	40-48 µm	1-10-100-1000 µg/L	7-14 days Added to water	GST and CAT activity decreased. LPO level decreased.	Abidli et al., 2021
Perna viridis	PE	<32 µm 32-43 µm	1 µg/L 2 µg/L 3 µg/L	30 days Added to water	Byssus production decreased. CAT activity decreased. LPO conc. increased.	Hariharan et al., 2021
Crassostrea gigas	PE PET	36.72±24 µm (PE) 31.11±14.36 µm (PET)	10-1000 µg/L	21 days Added to water	SOD activity increased in digestive glands. MDA conc. did not change. CAT activity decreased in the digestive gland.	Teng et al., 2021

Table 7. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Fish						
Dicentrarchus labrax	PVC	<0.3 mm	0.1% (w/w)	30-60-90 days Added to food	Morphological changes and hyperplasia observed	Peda et al., 2016
Pomatoschistus microps	PE	1-5 µm	0.184 mg/L	48 hours Added to water	Lipid oxidation was observed. AChE activity was altered.	Ferreira et al., 2016
Sparus aurata	PVC	40–150 µm	100 and 500 mg/kg	15 and 30 days Added to food	Creatine kinase, aspartate aminotransferase, albumin and globulin conc. in serum decreased. IgM conc. in the skin mucosa increased. *prdx5* gene expression decreased, *prdx1* and *prdx3* genes.	Espinosa et al., 2017
Dicentrarchus labrax	Thermoset amino formaldehyde polymer	1–5 µm	0.26 mg/L 0.69 mg/L	96 hours Added to water	AChE activity and lipid peroxidation in brain tissue showed alterations. ChE activity, LPO, LDH and LDH conc. in the muscle tissue changed	Barboza et al., 2018
Oryzias melastigma	PE	4-6 µm	0-1-10 mg/L	12 days Added to water	LOEC>10	Berias et al., 2018
Sparus aurata *Dicentrarchus labrax*	PVC, PE	40-150 µm	1-10-100 mg/ml	1-24 hours Added to water	Phagocytosis rate of the anterior kidney lymphocytes and respiratory burst rate increased. Expression of the *nrf2* gene increased.	Espinosa et al., 2018
Sebastes schlegelii	PE	15µm	10^6 particles/L	14 days Added to water	Weight gain and specific growth rate decreased.	Yin et al., 2018
Pomatoschistus microps	PE	1-5 µm	0.18 mg/L	96 hours Added to water	AChE and GST activities decreased.	Miranda et al., 2019
Sparus aurata	LDPE	Not available	10%	21 days Added to food	CAT, SOD, GST, GPx activities increased in liver. CAT and SOD activities increased in brain. No changes were observed in MDA conc.	Rios-Fuster et al., 2021
Sparus aurata	PE	10-20 µm	5±1 µg	35 days Added to food	Survival rates decreased. Alanine, glucose, mannose, inosine phenyl alanine, valine, ATP, N-asetlaspartat, creatine, glycine, taurine, GABA, glutamate, asetamid and glutamine conc. increased.	Jacob et al., 2021

Table 7. Continue.

Target organism	Microplastic			Experimental duration and exposure method	Effects	References
	Polymer type	Size	Concentration			
Oryzias melastigma	PS	2-µm	2-20-200 µg/L	28 days Added to water	Diversity and quantity of the intestinal microflora decreased.	Wang et al., 2022
Oryzias melastigma	Primary PVC Secondary PVC	53–106 µm	10^3 particles/L and 10^6 particles/L	25 days Added to water	Anomalies observed during embryonal development.	Xia et al., 2022
Pagellus bogaraando	Irradiated microspheres	1–5 µm	0.3 mg/L	9 days Added to water	ROS production increased. AChE activity decreased.	Santos et al., 2022

HDPE: High Density Polyethylene, LDPE: Low Density Polyethylene, PE: Polyethylene, PP: Polypropylene , PS: Polystyrene , PVC: Polyinyl Chloride , UPVC: Unplasticised Polyvinyl Chloride) AChE: Acetylcholinesterase, CAT: Catalase, ChE: Cholinesterase, COX-2:Cyclooxygenase-2, GPx: Glutathione Peroxidase, GR: Glutathione Reductase, GRd: Glutathione-disulfide Reductase, GSH: Glutathione, GST: Glutathione S-Transferase, HIF-1α: Hypoxia-Induced Factor-1, IDH: Isocitrate dehydrogenase, IgM: Immunoglobulin M, LC_{50}: Lethal concentration, LDH: Lactate dehydrogenase, LOEC: Lowest observed effect concentration, LPO: Lactoperoxidase, MDA: Malondialdehyde, NOEC: No observed effect concentration, nrf2: Nuclear factor erythroid 2-associated factor 2, prdx1: Peroxiredoxin-1, prdx3: Peroxiredoxin-3, prdx5: Peroxiredoxin-5, SOD: Superoxide Dismutase.

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Research Article

Effects of Size Grading on Growth Performance, Survival Rate and Cannibalism in Russian Sturgeon (*Acipenser gueldenstaedtii*) Larvae Under Small-Scale Hatchery Conditions

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ABSTRACT

Sturgeon aquaculture is important due to the value of their caviar and meat as well as its ecological importance. The current study focused on the effects of different size groups on the final mean weight, specific growth rate, survival rate and cannibalism of Russian sturgeon (*Acipenser gueldenstaedtii*) larvae. The groups were graded with homogeneous small-size larvae in the first group, 50% small and 50% large-size heterogeneous larvae in the second group, and homogeneous large-size larvae in the third group. The highest specific growth rates in each group occurred between days 28-35. After 35 days, the specific growth rate (SGR) in all groups dramatically reduced compared to the first week. The SGR of the larvae were not significantly affected by size ($p>0.05$). Mortalitiy were high during the first week in all groups, but, decreased as the larvae grew larger during the fourth weeks. At the end of the study, the survival rates were 30% for the small-size, 53% heterogeneous-size, and 64% for the large-size groups. The highest cannibalism rate in the present study occurred in heterogeneous-size group. However, the literature shows cannibalism rates to not be high for any groups of sturgeon. Size grading in the early period may negatively affect the survival rate and growth performance of larvae. Therefore, maintaining optimum larval rearing conditions such as stocking density, and feeding strategy may support higher survival and growth performance, in larvae that are newly acclimated to exogenous feeding.

Keywords: Sturgeon, aquaculture, larvae, size heterogeneity, survival

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INTRODUCTION

Russian sturgeon (*Acipenser gueldenstaedtii)* are distributed throughout the Azov Sea, Black Sea and Caspian Sea as well as in the large rivers that flow into these seas (Hurvitz et al., 2008). Sturgeon populations in the world have decreased due to dams along migration routes, destruction of breeding grounds, and overfishing (Gisbert & Williot, 2002). Russian sturgeon are a critically endangered species on the International Union for Conservation of Nature Red List of Endagered Species (IUCN, 2020). The rapid decline in the sturgeon population has increased interest in their aquaculture, especially in areas where sturgeonare naturally common. In addition, the increases in fish and caviar prices have also increased the interest in sturgeon farming (Hurvitz et al., 2008). The stable improvement of sturgeon farming is extremely important, both in terms of supporting of their natural stocks as well as commercial production.

Caviar production, is the main focus of sturgeon farming, and reached 364 tons annually in 2017, with the forecast for the next 10 years increasing from 500 to 2,000 tons (Tavakoli et al., 2021; Bronzi et al. 2019). The most cultivated species for caviar production in the world were

Acipenser baerii (31%), *Acipenser gueldenstaedtii* (20%) and the *Huso dauricus* x *Acipenser schrenckii* (13%) hybrid, respectively. The top caviar producing countries in order are China, Russia, Italy, France and Poland, (Bronzi et al., 2019).

For the sturgeon aquaculture in Türkiye, Siberian sturgeon (*A. baerii*) fry from France were brought to Ankara University to conduct experimental studies in 1997 (Köksal, Rad, & Kindir, 2000). Afterwards, fertilized eggs of Russian sturgeon (*A. gueldenstaedtii*) were brought from Russian federation, Krasnodar Fisheries Research Institute in 2001. Eggs were hatched in the University of Istanbul, Faculty of Fisheries of the Sapanca Inland Fisheries Production Research and Application Unit (Çelikkale, Timur, Memiş, & Ercan, 2002). In recent years, experimental and conservation studies on sturgeon culture have increased (Akbulut et al., 2011; Kayiş, Er, Kangel, & Kurtoğlu, 2017; Ak, Kurtoğlu, Serezli, Kayış, &Yandı, 2019; Memiş, Yamaner, Tosun, Tunçelli, & Tınkır, 2020). Although Russian sturgeon are a species native to Türkiye, their commercial production has been very limited. Aquaculture techniques should be developed in order for Türkiye gain its share from the increasing sturgeon production in the world.

Larval feeding and fingerling production have been stated as two of the most difficult aspects of sturgeon aquaculture. Managing the transition to nutrition after the larvae have consumed the yolk sac directly affects their survival rate and growth performance (Gisbert et al., 2018). Underfeeding and overstocking can increase competition among larvae, which can then lead to cannibalistic behavior (Manley et al., 2014). Falahatkar & Roosta (2022) stated cannibalism to not occur in sturgeons' normal behavior but to be only observable under unsuitable cultivation conditions such as starvation or high stocking density. In addition, size differences in larvae due to genetic and environmental factors also affect cannibalism rates (Kestemont et al., 2003; Baras & Jobling, 2002; Baras et al., 2003). Larvae have a larger mouth in proportion to their body size compared to adult individuals. Therefore, the larvae may tend to consume smaller individuals (Baras, 1998). In general, having different size groups coexist in aquaculture prevents larger individuals from pressuring smaller ones (Tidwell et al., 2003), as well as the uniform use feed appropriate for the larvae's size. Jobling (2010), stated regular fish-grading to increase survival rate and growth performance. Fish-grading has also been found to positively impact weight gain, feed conversion rate, and specific growth rates (SGR) in juvenile Nile tilapia (*Oreochromis niloticus*; Dikel, 2011). However, fish-grading has also been reported not enhance survival or growth rate among sea bass *(Dicentrarchus labrax)* larvae (Kestemont et al., 2003). Studies in the literature have shown the effects of fish-grading on growth performance and survival rate to vary for several species (Jobling, 2010; Dikel, 2011; Kestemont et al., 2003).

This study, investigates the efficacies of size homogeneity in larvae on survival rate, growth performance and cannibalism in order to increase production regarding Russian sturgeon hatchery management. In addition, this study aims to determine the growth and survival rates of Russian sturgeon larvae under aquacultural conditions.

MATERIAL AND METHODS

Experimental design and fish maintenance

The trial was carried out at the Aquaculture Application and Research Center at Recep Tayyip Erdoğan University in Rize, Türkiye in June 2022. Larvae were obtained by incubating eggs obtained from the research center's broodstock of Russian sturgeon. First feeding of the larvae was initiated seven days post-hatching with *Artemia nauplii* six times a day. The sturgeon larvae were acclimatized to the commercial salmonid starter feed 21 days after having absorbed their yolk. After the exogenous feeding (28 days post-hatching, [DPH]), three separate groups (S, M, and L) were arranged in which Russian sturgeon of different initial body weights (BWi) were reared (0.096 g, 0.273 g, and 0.433 g). The sturgeon larvae were put into nine rectangular tanks. Fish were placed in experimental tanks 3 days before the trial in order to adapt to the conditions. The experimental groups were graded with small-size homogeneous larvae in the first group (S), 50% small- and 50% large-size heterogeneous larvae in the second group (M), and large-size homogeneous larvae in the third group (L). The artificial feeding rate was 15% of total body weight per day. Every seven days, the dose of feed was adjusted after checking their measurements. The water level was about 20 cm deep. The initial stock density of the larvae was 15 gr/L. The total number of fish in each tank was determined according to the stock density. In the trials, aerated well water was fed to the tanks at 0.5 L/min. The pH, water temperature, and dissolved oxygen levels were measured using a multi-parameter water quality measuring device (Hach HQ40d 58,258–00, Loveland, CO). Water pH was measured between 7.18–7.46. Water temperature at 18±0.8 °C, and dissolved oxygen between 6.8 -7.4 mg/L. During the trial, the photoperiod regime was 14L:10D. Three replications were performed for each group.

Growth performance and survival rate

The trial was continued for 5 weeks, with the measurements performed weekly. The mean live weight of each group was measured using a laboratory scale (0.01 mg). The total lengths of the fish were measured using a computer program (TPSdig). Dead sturgeon larvae were removed daily, and this daily count was used to determine the survival rate. The SGR of the larvae in each group was calculated in accordance with Gisbert & Williot (1997) as follows:

$$SGR\ (\%day^{-1}) = 100 \times (\ln W_f - \ln W_0) / t, \quad (1)$$

where W_f and W_0 are the final and initial mean weights in grams, and t is the growth period in days .

The survival rate of the fish over the 5 weeks was calculated from the weekly number of dead larvae in the tanks.

Cannibalism rates were determined over the 28 days according to the formula from Falahatkar & Roosta (2022) as follows:

$$\% = 100 \times (\text{bitten larvae/initially number of larvae}) \quad (2)$$

Statistical analyses

All data are presented as a mean ± standard deviation (SD). One-way analysis of variance (ANOVA), and Tukey tests were used to

identify any significant differences among the groups. Differences were considered statistically significant at a $p \leq 0.05$. Data sets were analyzed using the package program SPSS 25 for Windows (version 25, IBM Corp., Armonk, New York, USA).

RESULTS AND DISCUSSION

The effects from the grading were investigated with respect to the larvae's weight gain (WG), SGR, survival, and cannibalism (Table 1). The mean total length of the 56-DPH Russian sturgeon in the S, M, and L groups were respectively 3.5 ± 0.1 cm, 6.7 ± 0,3 cm, and 8.2 ± 0.4 cm, (Figure 1). In addition, the mean weights of the S, M, and L groups were 0.96 ± 0.05 g, 2.65 ± 0.369 g, and 4.56 ± 0.4 g, respectively (Figure 1). Memiş et al. (2009) found the mean weight of *A. gueldenstaedtii* for 22-DPH and 41-DPH larvae to be 0.095 ±0.008 g and 1.18 ±0.36 g, respectively. Similarly, hybrid sturgeon ([*A. baeri* × *A. gueldenstaedtii*] × *A. gueldenstaedtii*) larvae reached a mean body weight of 3.4± 1.3 g on 57-DPH (Szczepkowsk & Kolman, 2002).

Biotic and abiotic factors can cause size heterogeneity between sibling larvae (Kestemont et al., 2003; Dammerman et al., 2015). In Asian catfish larvae, the temperature producing the highest growth also appeared in regard to the lowest size heterogeneity. Furthermore, size heterogeneity of larvae in the same group may have been due to individual growth potentials or the effect of more active larvae on other larvae (Baras et al., 2011).

During the larval stage, sturgeon develop very quickly, when reared under optimum conditions (Deng et al., 2003). The highest specific growth rates for each group occurred between the28th-35th day after day 35. The SGR dramatically decreased in all groups compared to the SGR for the first week (Figure 2). In the first week, the highest growth rate was obtained in the M group (18.9 % day^{-1}) and the lowest was in the L group (11.2% day^{-1};$p < 0.05$). At the end of the trial, the SGRs did not differ significantly among the groups ($p > 0.05$). Similarly, Memiş et al. (2009) declared an SGR of 13.26 %day^{-1} between the 22nd-41st days post-hatching for *A. gueldenstaedtii* larvae, . Similar high SGRs have been described for white sturgeon larvae (9.6–13.0%day^{-1};Deng et al., 2003) and *A. medirostris* fry (7.1%day^{-1} ;Zheng et al., 2015). Falahatkar et al. (2017) found the mean SGR in live artemia feeds to range between 2.27-2.13% day^{-1} for Persian sturgeon (*A. persicus*) 21 days after first feeding. The SGR of larvae was not significantly affected by size ($p > 0.05$). However, the high SGR in the M group during the first period may have been due to the larger larvae consumting the small larvae's feed. Small-size larvae in the M group were detected to have the lowest survival rate during the first period which appears to have increased the M group's SGR due to the large larvae surviving.

Mortalities were high during the first week in all groups; however, this reduced as the larvae grew larger during the first four weeks (Figure 2). The small-size group had the lowest survival rate (30%), while the large size group had the highest (64%;$p < 0.05$). The size grading of the sturgeon larvae showed the large fish to have reached a higher survival rate compared to the small fish (Figure 2). The highest cannibalism rate in the present study typically occurred in group M (Table 1). However, the literature

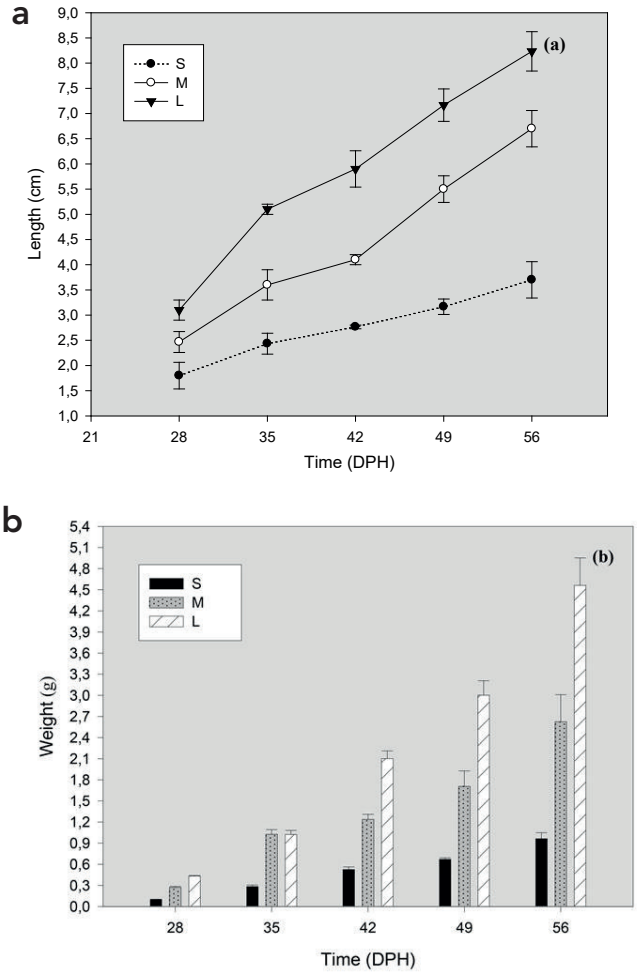

Figure 1. Mean (±SD) individual length (a) and weight (b) of Russian sturgeon larvae during the experiment. S, M and L assigned as small size larvae, heterogeneous size larvae and large size larvae, respectively. DPH; Day Post Hatching.

shows low cannibalism rates for all size groups . Falahatkar & Roosta (2022) observed cannibalism rates in Persian sturgeon larvae that were starved and kept under different stocking densities, determining the highest cannibalism rate of Persian sturgeon larvae to have occured in the starved small size group (40%). Memiş et al. (2009) reported mortality rate to be affected by cannibalism and feeding antagonism among the *A. gueldenstaedtii* larvae between the14th-22nd DPH. Szczepkowski & Kolman (2002) previously reported mortality rates for two hybrid sturgeon larvae. The researchers noted that the species had different rates of cannibalism due to their behavioral differences. The cannibalism is influenced by behavioral and environments parameters such as food availability, stocking density, water quality, light and feeding frequency (Li & Mathias, 1982; Braid & Shell, 1981; Hecht & Pienaar, 1993; Khan, et al., 2021).

Mortality was noted in all groups, though mainly among weaker individuals that did not feed. These weak larvae showed poor

swimming activity, which triggered the cannibalistic behavior of the fast-growing larvae. However, cannibalism-based mortality showed no significant percentage difference compared to the overall mortality rate. Low cannibalism rates may have been ensured due to the selected feeding rate and frequency in the heterogeneous-size group compared to previous studies. Krol et al. (2014) reported their cannibalism rate to be the main contributor to the mortality of European catfish (*Silurus glanis*) larvae. Similarly, studies are found to have stated cannibalism to be an important cause of mortality in *Sander lucioperca* (Hamza et al., , 2007), *Perca fluviatilis* (Babiak et al., 2004), and *Lates calcarifer* (Khan et al., 2021). The cannibalism rate of Russian sturgeon larvae as observed in this study was quite low compared to these other species.

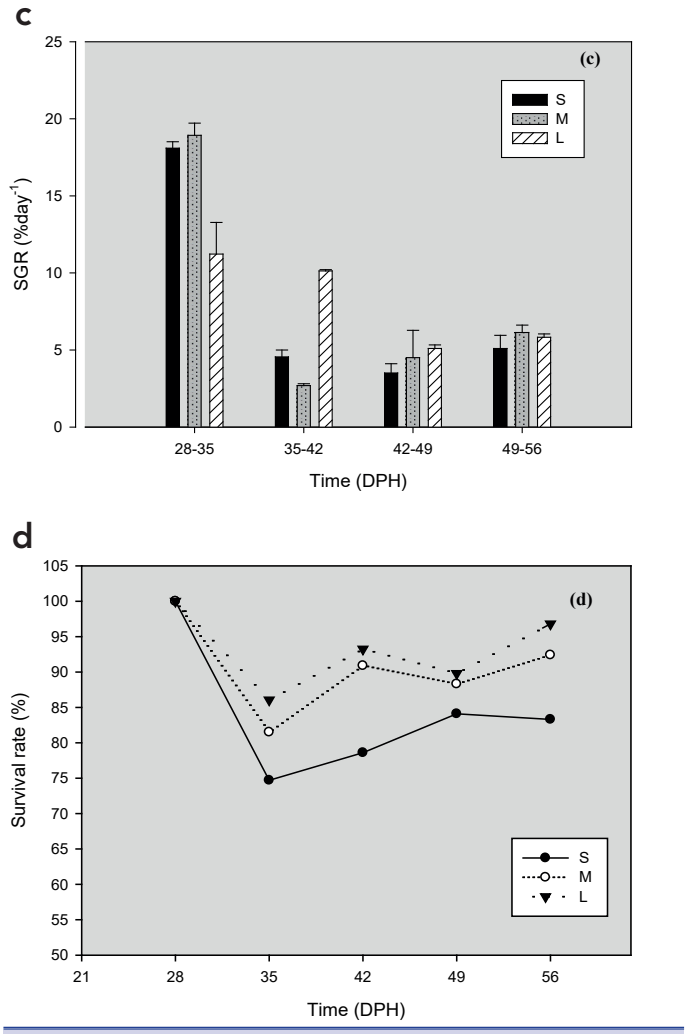

Figure 2. Specific growth rate (c) and survival rate (d) of Russian sturgeon larvae during the experiment. S, M and L assigned as small size larvae, heterogeneous size larvae and large size larvae, respectively. DPH; Day Post Hatching.

This study, observed the small-size larvae to bite large-size larvae from the tail direction as a form of cannibalistic behavior. This behavior not only caused the death of the prey larva but also the death of the predator larvae. This type of predation is known type-I cannibalism (Cuff, 1980). Normally in type-I cannibalism, the head is discarded after the tail is digested (Baras & Jobling, 2002). However, this study observed the predator Russian sturgeon larvae to have died in with their prey in their mouths. This may be sturgeon-specific cause due tosturgeon larvae have much larger heads than their bodies. In brief, the high mortality rate among theRussian sturgeon larvae appears to have resulted based on body weight, larval size and difficulties in accepting artificial feed at the start of feeding. For this reason, revealing the factors that cause size difference in Russian sturgeon is important for larval culture.

CONCLUSIONS

Overall, this study confirmed that size grading did not significantly improve the survival or specific growth rates of the small larvae group regarding Russian sturgeon larvae; however, it did influence the cannibalism rate. Size grading in the early DPHmay negatively affect the survival rate and growth performance of the larvae because of their weak tolerances. Therefore, maintaining optimal rearing conditions such as stock density, and feeding strategy may support higher survival and growth performance rates, especially in larvae that are newly acclimated to exogenous feeding. Increasing the number of daily meals, collecting the dead larvae in a timely manner, and may contribute to the reduction of cannibalism in Russian sturgeon fry pre-feeding, thus increasing the survival rate.

The fact that, the natural distribution areas of sturgeon are in Türkiye makes the aquaculture of this species important. Developing effective culture methods for Russian sturgeon larvae is crucial both for conservation and commercial production.

Table 1. Total Length, Weight, SGR, Survival, and Cannibalism Rates of Russian Sturgeon Larvae at the End of the Experiment.

Group	W0 (g)	Wf (g)	L0 (cm)	Lf (cm)	SGR (%day-1)	SR (%)	Cannibalism
S	0.108±0.006	0.964±0.05	1.5±0.1	3.5±0.3	7.5[a]	30[a]	%2[a]
M	0.273±0.015	2.612±0.35	2.2±0.2	6.7±0.4	8.0[a]	53[b]	%9.3[b]
L	0.433±0.009	4.563±0.4	3.1±0.2	8.2±0.4	8.42[a]	64[c]	%5.1[c]

Note: Significant differences of SGR, survival rate and cannibalism among treatments assigned by different letters (a, b, c) ($P < 0.05$). W_0, initial weight; W_f, final weight; L_0, initial length; L_f, final length; SGR, specific growth rate, SR, survival rate. Values are the mean ± standard deviations of three replicates.

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Research Article

Catch Composition of Different Bottom Trawl Cod-ends in the Western Black Sea

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ABSTRACT

Across the Mediterranean and the Black Sea, an improvement in technological adoption which optimizes the environmental benefits of fisheries is needed. The testing of quality standards in trawl fishing is one of the essential components. In an experiment, we tested four bottom trawl cod-ends in the western Black Sea to determine the characteristics of the catch composition. Fishing trials were conducted by 40 mm diamond (40D), 44 mm diamond (44D), 40 mm square (the 40S), and 40 mm 90-degree turned (40T) mesh cod-ends for 31 bottom trawling hauls. The multivariate analysis of catch composition indicated a significantly higher differentiation between 40D and 40T cod-ends, mainly characterized by five species: *Merlangius merlangus, Mytilus galloprovincialis*, *Trachinus draco, Mullus barbatus,* and *Uranuscopus scaber*. The difference in the shape of cod-end meshes reflected the variation in the catchability and catch composition. However, the 40S and 40T showed 80% similarity in catch composition. Among cod-ends, 40T yielded in lowest catch per unit effort for both commercial and other species. Adoption of gear specially made to catch more target species can help bottom trawl fisheries further improve their ecological and economic sustainability.

Keywords: Black Sea, Bottom trawl, catch composition, cod-end

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INTRODUCTION

Bottom trawling, globally, is an essential fishing technique used today. Approximately 22% of the landed catch in the world originates from trawl fishery (Kelleher, 2005). However, it has always been the controversial fishing gear in the Black Sea and the Mediterranean Basin. This fishing method has used cutting-edge technology to boost its efficacy ever since the beginning of the industrial era (Sacchi, 2008). The trawl technique has an important position in Turkish demersal fishery and there is a bottom trawler fleet including 820 trawlers in suitable shelf areas except for the eastern Black Sea (TÜİK, 2022). In Turkish waters, the most suitable areas for trawling in the Black Sea are between İğneada and Kefken in the western part and between the provinces of Sinop, Samsun, and Ordu in the eastern part. The littoral zone outside these areas is highly fractured and unsuitable for trawling (Kutaygil & Bilecik, 1973; Kara, 1980). On the Turkish Black Sea coast, the Central and Western Black Sea coasts are significant trawling areas (Kaykaç, Zengin, Özcan-Akpınar, & Tosunoğlu, 2014; Sağlam & Samsun, 2018).

The bottom trawl fishery is one of the essential fishing activities in the western Black Sea, regarding the volume of landings and the economic value and demersal resources have traditionally provided economically significant catches for human consumption (Yildiz, 2016; Yıldız & Karakulak, 2018a). The demersal resources in the Black Sea were exploited by 196

trawl vessels fleet (TÜİK, 2022). In the Black Sea, the catch composition of bottom trawl nets is composed of a complex of fishes and invertebrates (Yildiz & Karakulak, 2017; Öğreden & Yağlıoğlu, 2017). Whiting (*Merlangius merlangus*), red mullet (*Mullus barbatus*), and one flatfish species turbot (*Scophthalmus maximus*) are the main target species (Başkaya, 2012; Yildiz, 2016). About 96% of annual landings of demersal fish stocks have been made up of whiting and red mullet, and bottom trawlers account for the majority (90%) of those catches in the western Black Sea (TÜİK, 2022).

As a result of management failures, the stock size of many demersal resources has decreased in the last decades. The overcapacity of the bottom trawl vessels, together with management problems with the illegal design (using two cod-ends-Yildiz, 2016) and functioning of the bottom trawls are the major contributor to the discarding of the demersal fish resources in the Black Sea (Ceylan, Şahin, & Kalaycı., 2014; Yildiz & Karakulak, 2017). High discarding rates (Başkaya, 2012; Ceylan et al., 2014; Yildiz, 2016; Öğreden & Yağlıoğlu; Yıldız and Karakulak, 2018b) and landing of small-sized specimens (Başkaya, 2012; Yildiz, 2016; Yıldız & Karakulak, 2018b) by bottom trawling have led the governments and regional commissions to apply more selective mesh sizes and mesh shapes (GFCM, 2009). According to the current management, the mesh size of the trawl cod-end in the Black Sea cannot be less than 40 mm square mesh, and after 1 September 2024, the 44 mm rhombic mesh cod-end will be permitted to use (BSGM, 2020) On the other hand, since 2008, European Union members in the Mediterranean Basin have been allowed to use and onboard only one of the 40 mm square mesh or 50 mm rhombic mesh cod-ends (COUNCIL REGULATION (EC) 1967/2006; GFCM/33/2009/2).

In Turkey and all over the world, demersal resources have been fished less than pelagic stocks, however, these species have a relatively high economic return (Genç, 2000). All species also have a significant impact on the resiliency and evolution of the ecosystem. Hence, to make optimum use of and protect fish stocks, management actions should be determined by scientific research. However, there are selectivity studies for different mesh sizes in the Black Sea (Özdemir, Erdem & Erdem, 2012; Zengin, Akpınar, İ., Kaykaç, & Tosunoğlu, 2019), but no study evaluates the catch composition of the different codends. Catch composition means species diversity is defined as species' variety and relative abundance (Winston B et al., 2019). Investigating how fishing affects marine ecosystems involves whole systems. For the first time in the Black Sea, this study provides new insights into the catch composition of the bottom trawl nets equipped with different mesh cod-ends configurations as 40 mm diamond (40D), 44 mm diamond (44D), 40 mm square (the 40S) and 40 mm 90-degree turned (40T).

MATERIAL AND METHODS

Study plan and data collection

This study was conducted in the coastal area between Kıyıköy/Kırklareli and Kefken/Kocaeli (Figure 1). Operations were carried out between 21 and 28 July 2019 with the R/V Yunus S research vessel, 32 m long, and 500 HP engine power. The coordinates

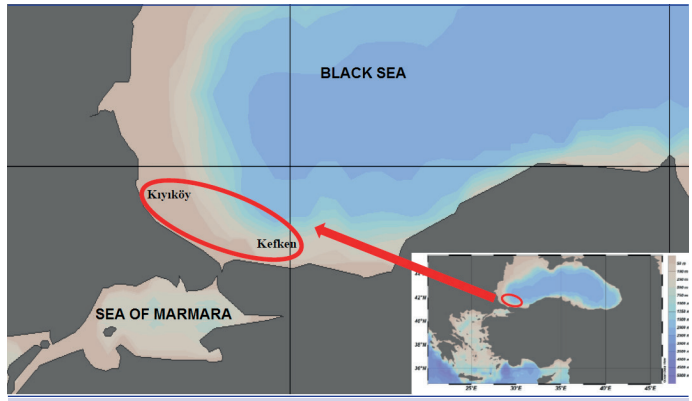

Figure 1. A general map of the sampling area.

and depth (m) data of the areas for each trawl operation were recorded. Haul durations were kept short of adequate sampling by four test cod-ends as 30 minutes and a constant speed of 3 miles/hour. The depth ranges of the stations varied between 19 m and 61 m.

A 900-mesh bottom trawl net, widely used by a commercial trawler, was employed. A 40 mm diamond (40D) mesh, legally used by trawlers, and three alternative cod-ends were tested. A 44 mm diamond (44D) mesh size was allowed for the Black Sea in bottom trawl fishing in fishery management (BSGM, 2020). Cod-ends have traditionally been constructed using diamond netting oriented with its normal direction in-line with the towing direction, also known as the T0 orientation - oriented 0° in the transversal or towing direction. However, this same netting can be installed in such a way that the mesh is rotated 45° (T45) or 90° (T90) in the transversal direction and improving size selectivity (Einarsson, Cheng, Bayse, Herrmann & Winger, 2021). In addition, the 40 mm mesh size of the square (the 40S) and 90-degree turned mesh (40T) cod-end were assessed. The length of all trawl cod-ends used in the study is 5 m. With the 40D, 44D, and 40S trawls, eight hauls were made; the 40T trawl cod-end produced seven hauls. For each species maintained, the total weights for each haul were recorded. The total weights and numbers were directly recorded when the catch was low; otherwise, a subsample was taken and sorted into the lowest taxonomic level.

Data analysis

The average catch per unit effort for the species caught in the four-trawl cod-ends was calculated using the following formula (Phiri & Shrikihara, 1999). The amount of catch per hour (kg. hour^{1}) was calculated and standardized for each trawling.

$$CPUE = \frac{\Sigma\, Ci/Nh}{\mathrm{t/Nh}}$$

Ci: The amount of catch per trawling (kg), t: Haul duration, Nh: Number of operations

The statistical significance of the difference between the CPUE values was checked with the One-Way ANOVA Test by using the "aov" function in the"ggpubr" package through R Studio ver-

sion 4.2.1 (R Core Team, 2022). Before this test, it was checked whether the data were in the normal distribution or not and whether the variances were homogeneous with the Levene test. Log(x+1) transformations were applied if data didn't fit the normal distribution (Zar, 1999; Ozdamar, 2009).

CPUE data were matrixed by the Bray-Curtis Similarity matrix for similarity tests in various cod-ends, and cluster analysis was compared (Bray & Curtis, 1957; Everitt, 1980). The data were transformed using the logx+1 transformation before the cluster analysis (Field et al., 1982). Transformed data were used for cluster analysis and nonparametric multidimensional scaling (nMDS). The "analysis of similarity percentages (SIMPER)" test was used to determine the species that caused the difference in trawl cod-ends by using the Primer 6 statistical package program (Clarke & Warwick, 2001; Quinn & Keough, 2002). Species number (S), species richness (d), species diversity, Shannon-Weaver diversity index (H'log2), Pielou's equation index (J'), and Simpson Dominance-Diversity index (1-λ) were calculated by using abundance values.

RESULTS

Catch composition

64104 individuals (701.13 kg) belonging to 33 species were obtained by 31 bottom trawling operations. The catch composition of four different trawl cod-ends was given in Table 1. While the highest number of species was taken in the 40D (28 species) and

Table 1. Species and catches (kg) in examined cod-ends.

			Cod-ends					
	Species	**Value**	**44D**	**40D**	**40S**	**40T**	**TOTAL**	**%**
Chordata	*Dasyatis pastinaca*	NC	14.75	0.50	1.50	0	16.75	2.39
Chordata	*Raja clavata*	NC	0.55	0	1.50	0	2.05	0.29
Chordata	*Arnoglossus kessleri*	NC	0.03	0.12	0.04	0	0.18	0.03
Chordata	*Callionymus risso*	NC	0	0.10	0	0	0.10	0.01
Chordata	*Chelidonichthys lucerna*	C	0	0.15	0.08	0.13	0.35	0.05
Chordata	*Gaidropsarus mediterraneus*	NC	0.17	0	0	0	0.17	0.02
Chordata	*Gobius niger*	NC	0.50	4.72	0.96	0.61	6.78	0.97
Chordata	*M. merlangius euxinus*	C	64.76	114.98	20.12	12.10	211.96	30.23
Chordata	*Mesogobius batrachocephalus*	NC	1.95	0.64	0.05	0.05	2.69	0.38
Chordata	*Mullus barbatus*	C	19.30	6.40	0.33	0.12	26.14	3.73
Chordata	*Neogobius melanostomus*	NC	0.23	4.25	0.96	0.42	5.85	0.83
Chordata	*Parablennius tentacularis*	NC	0	0.05	0.02	0	0.07	0.01
Chordata	*Pegusa nasuta*	C	0.98	0	0.37	0	1.35	0.19
Chordata	*Platichthys flesus*	C	0.02	0.06	0.14	0.18	0.40	0.06
Chordata	*Scophthalmus maximus*	C	1.25	4.14	1.06	0.43	6.87	0.98
Chordata	*Scorpaena porcus*	NC	0.88	0.19	0.12	0.01	1.19	0.17
Chordata	*Sprattus sprattus*	C	0.30	4.06	0.54	1.34	6.22	0.89
Chordata	*Syngnathus abaster*	NC	0	0.06	0.01	0	0.07	0.01
Chordata	*Trachinus draco*	NC	0.82	7.46	1.59	0.26	10.12	1.44
Chordata	*Trachurus mediterraneus*	C	0.24	0.03	0	0	0.27	0.04
Chordata	*Uranoscopus scaber*	NC	7.56	15.02	12.24	4.75	39.57	5.64
Arthropoda	*Crangon crangon*	NC	0	0.04	0	0	0.04	0.01
Arthropoda	*Liocarcinus depurator*	NC	42.45	93.00	74.60	44.36	254.40	36.28
Arthropoda	*Liocarcinus navigator*	NC	0.05	0.36	0.11	0.25	0.77	0.11
Arthropoda	*Eriphia verrucosa*	NC	0.50	1.63	1.56	1.17	4.86	0.69
Mollusca	*Anadara inaequivalvis*	NC	0	0.08	0	0	0.08	0.01
Mollusca	*Mytilus galloprovincialis*	C	23.80	0.42	39.51	21.93	85.66	12.22
Mollusca	*Chamelea gallina*	C	0	0.13	0	0	0.13	0.02
Mollusca	*Rapana venosa*	C	0.98	0.21	0.35	0.12	1.66	0.24
Mollusca	*Acanthocardia deshayesii*	NC	0.01	0.01	0	0.05	0.07	0.01
Echinodermata	*Stereoderma kirschbergi*	NC	0.01	0	0	0	0.01	0.00
Tunicata	*Corella eumyota*	NC	0	7.90	3.17	3.12	14.19	2.02
Porifera	*Suberites domuncula*	NC	0.14	0	0.01	0.04	0.19	0.03
	TOTAL		182.20	266.64	160.88	91.41	701.13	

C:Commercial; NC: Non-commercial

the lowest was in the T90 (20 species). The maximum number of individuals was taken from 40D with 25199, followed by 44D with 15879, the 40S with 13865, and 40T with 9161. The same trend was observed in terms of catch amount.

The most dominant species were *Liocarcinus depurator* (55.5 %), *Merlangius merlangus euxinus* (23.42%), *Mytilus galloprovincialis* (% 8.78), *Mullus barbatus* (3.63%), *Sprattus sprattus* (3.02%), *Uranoscopus scaber* (1.46%) by numbers (Figure 2). The most dominant species by weight were *Liocarcinus depurator* (36.28%), *Merlangius merlangus euxinus* (30.23%), *Mytilus galloprovincialis* (12.22%), *Uranoscopus scaber* (5.64%), *Mullus barbatus* (3.73%) and *Dasyatis pastinaca* (2.39%) (Table 1).

Catch per unit effort (CPUE)

The minimum, maximum, mean, standard deviation, and standard error values of CPUE were given in Table 2 and Figure 3. The p-value of the cod-end variable is low ($p<0.05$), so it appears that the type of cod-end used has a real impact on the CPUE. The highest CPUE value was obtained in the 40D as 66.66±13.00 kg. hour^{-1}and followed by 44D with 45.55±7.34 kg. hour^{-1}, 40S with 40.22±6.04 kg. hour^{-1} and 40T with 26.12±3.60 kg. hour^{-1}. Among commercial fish (Table 1), whiting had the highest CPUE value of 28.75±15.56 kg. hour^{-1} with 40D cod-end ($p<0.05$) and the highest CPUE of red mullet was caught in 44D cod-end with 4.83±2.62 kg. hour^{-1} ($p<0.05$).

The similarity in catch composition

According to the Bray-Curtis similarity index, the 40D formed a single group (Fig 4 and 5), while the other three cod-ends formed a separate group. The 40S and 40T were at 83% similarity level, while 44D was 67% and 40D was at 60% similarity levels. Table 3 lists the species that contribute to the difference and their rates of contribution according to SIMPER analysis. Table 3 demonstrates that the 40D and 40T had the most significant difference (mean dissimilarity = 42.40).

Ecological indexes

The number of species ranged from 22 to 26, the species richness ranged from 2.668 to 3.017, and the Shannon-Wiener diversity index ranged from 1.171 to 1.327 (Table 4). The highest number of species was observed in 40D, while the lowest was in 40T.

DISCUSSION

This study compared the catch composition, CPUE, and species diversity of 40D, used in commercial bottom trawling in the Black Sea, and alternative cod-ends (44D, the 40S, and 40T).

Bottom trawlers are used extensively to catch demersal species on the Turkish coast. The number of species caught in Turkish waters by bottom trawlers is more than 50 (Tosunoğlu, Özbilgin & Özbilgin, 2003), even 84 species were recorded in the Aegean Sea (Sokyan et al., 2016). Another study revealed that as depth climbed, the number of species rose to 200. (Soykan et al., 2019). However, commercial trawlers in the Black Sea with 40 mm diamond mesh have captured anywhere between 18 and 34 species (Aksu, 2012; Başkaya, 2012; Yıldız, 2016). Bony fishes were the main category, according to Aksu (2012), Başkaya (2012), and Yıldız (2016), who identified 11, 25, and 22 species, respectively. According to Yıldız, Zengin, Karakulak, Uzer & Özcan Akpınar

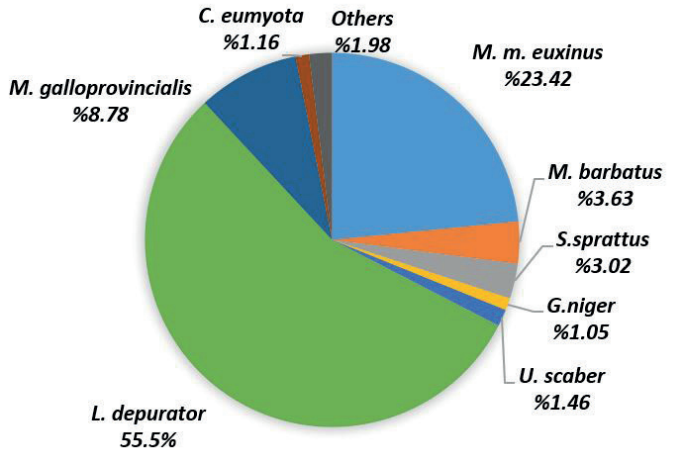

Figure 2. Numerical distribution of species obtained from bottom trawl trials.

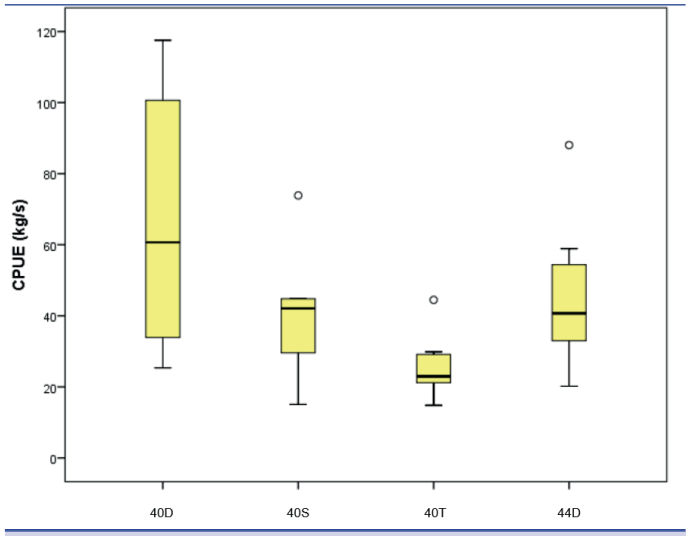

Figure 3. Variation of CPUE values obtained in trawl cod-ends.

Table 2. CPUE values of examined codends

Cod-ends	No. hauls	Min.	Max.	Mean	SD	SE
44D	8	20.20	88.05	45.55	20.76	7.34
40D	8	25.35	117.55	66.66	36.78	13.00
40S	8	15.05	73.86	40.22	17.08	6.04
40T	7	14.81	44.47	26.12	9.51	3.60

Table 3. Average dissimilarity rates between trawl cod-ends and the species that contributed to the difference.

44D & 40D (Average dissimilarity = 40.45)					
	44D	**40D**			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Cont. %**	**Cum.%**
M. galloprovincialis	1.94	0.10	6.32	15.63	15.63
D. pastinaca	1.55	0.12	4.90	12.12	27.75
C. eumyota	0	1.09	3.75	9.27	37.02
T. draco	0.18	1.05	2.99	7.40	44.42
M. barbatus	1.76	0.96	2.78	6.88	51.30
L. depurator	2.45	3.19	2.54	6.27	57.58
G. niger	0.11	0.78	2.30	5.67	63.25
N. melanostomus	0.06	0.72	2.29	5.66	68.91
S. sprattus	0.07	0.70	2.17	5.37	74.28
M. m. euxinus	2.84	3.39	1.89	4.67	78.95
U. scaber	1.06	1.56	1.71	4.23	83.19
S. maximus	0.27	0.71	1.53	3.77	86.96
M. batrachocephalus	0.40	0.15	0.86	2.13	89.09
P. nasuta	0.22	0	0.77	1.90	90.99
44D & 40S (Average dissimilarity = 30.71)					
	44D	**40S**			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Cont. %**	**Cum.%**
M. barbatus	1.76	0.08	6.64	21.63	21.63
D. pastinaca	1.55	0.32	4.82	15.69	37.32
M. m. euxinus	2.84	1.80	4.13	13.44	50.76
C. eumyota	0	0.58	2.29	7.47	58.23
L. depurator	2.45	2.98	2.07	6.75	64.98
M. galloprovincialis	1.94	2.39	1.77	5.75	70.73
M. batrachocephalus	0.40	0.01	1.53	4.99	75.72
U. scaber	1.06	1.40	1.34	4.36	80.08
E. verrucosa	0.12	0.33	0.82	2.66	82.73
R. clavata	0.13	0.32	0.75	2.45	85.19
S. porcus	0.20	0.03	0.67	2.17	87.36
N. melanostomus	0.06	0.22	0.62	2.01	89.37
T. draco	0.18	0.34	0.61	1.98	91.35
40D & 40mmS (Average dissimilarity = 35.51)					
	40D	**40S**			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Cont. %**	**Cum.%**
M. galloprovincialis	0.10	2.39	8.49	23.91	23.91
M. m. euxinus	3.39	1.80	5.94	16.72	40.63
M. barbatus	0.96	0.08	3.27	9.20	49.84
T. draco	1.05	0.34	2.66	7.48	57.32
S. sprattus	0.70	0.12	2.14	6.03	63.35
G. niger	0.78	0.22	2.10	5.91	69.26
N. melanostomus	0.72	0.22	1.89	5.32	74.58
C. eumyota	1.09	0.58	1.88	5.30	79.88
S. maximus	0.71	0.23	1.79	5.05	84.93
R. clavata	0	0.32	1.20	3.37	88.31
L. depurator	3.19	2.98	0.78	2.20	90.51

Table 3. Continue.

44D & 40T (Average dissimilarity = 34.79)					
	44D	**40T**			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Cont. %**	**Cum.%**
M. barbatus	1.76	0.03	7.65	22.00	22.00
D. pastinaca	1.55	0	6.82	19.61	41.61
M. m. euxinus	2.84	1.50	5.96	17.12	58.74
C. eumyota	0	0.64	2.81	8.08	66.81
M. batrachocephalus	0.40	0.01	1.72	4.93	71.75
S. sprattus	0.07	0.32	1.12	3.23	74.98
P. nasuta	0.22	0	0.99	2.83	77.81
U. scaber	1.06	0.86	0.89	2.57	80.38
S. porcus	0.20	0	0.88	2.52	82.90
R. venosa	0.22	0.03	0.85	2.46	85.36
L. depurator	2.45	2.62	0.72	2.07	87.43
E. verrucosa	0.12	0.29	0.72	2.07	89.50
S. maximus	0.27	0.11	0.69	1.99	91.49
40D & 40T (Average dissimilarity = 42.40)					
	40D	**40T**			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Cont. %**	**Cum.%**
M. m. euxinus	3.39	1.50	7.86	18.53	18.53
M. galloprovincialis	0.10	1.98	7.78	18.34	36.87
T. draco	1.05	0.07	4.07	9.60	46.48
M. barbatus	0.96	0.03	3.83	9.04	55.52
U. scaber	1.56	0.86	2.90	6.83	62.35
G. niger	0.78	0.16	2.58	6.08	68.43
N. melanostomus	0.72	0.11	2.52	5.95	74.38
S. maximus	0.71	0.11	2.48	5.86	80.24
L. depurator	3.19	2.62	2.37	5.60	85.83
C. eumyota	1.09	0.64	1.87	4.41	90.25
40S & 40T (Average dissimilarity = 16.66)					
	40S	**40T**			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Cont. %**	**Cum.%**
U. scaber	1.40	0.86	2.64	15.88	15.88
M. galloprovincialis	2.39	1.98	1.97	11.84	27.71
L. depurator	2.98	2.62	1.77	10.62	38.33
D. pastinaca	0.32	0	1.57	9.43	47.76
R. clavata	0.32	0	1.57	9.43	57.18
M. m. euxinus	1.80	1.50	1.47	8.83	66.01
T. draco	0.34	0.07	1.31	7.87	73.88
S. sprattus	0.12	0.32	0.97	5.85	79.72
S. maximus	0.23	0.11	0.57	3.45	83.17
N. melanostomus	0.22	0.11	0.50	2.98	86.15
P. nasuta	0.09	0	0.42	2.52	88.67
G. niger	0.22	0.16	0.28	1.70	90.37

Av.Abund: Avearge abundance; Av.Diss: Avearge Dissimilarity; Cont. %: Contribution percentage; Cum.%: Cumulative contribution percentage

Table 4. Ecological index values for the examined trawl codends.

Cod-ends	S	d	J'	H'(log$_e$)	1-λ
44D	26	3.017	0.3865	1.259	0.616
40D	28	3.086	0.3952	1.317	0.6186
40S	25	2.944	0.3636	1.171	0.5636
40T	22	2.668	0.4294	1.327	0.6256

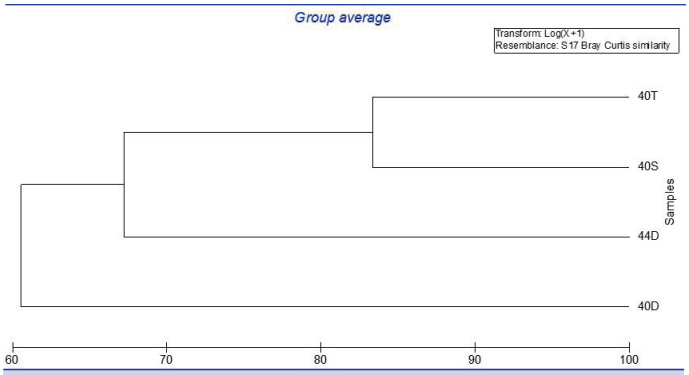

Figure 4. Clustering dendrogram based on Bray-Curtis similarity analysis of species caught in examined trawl cod-ends.

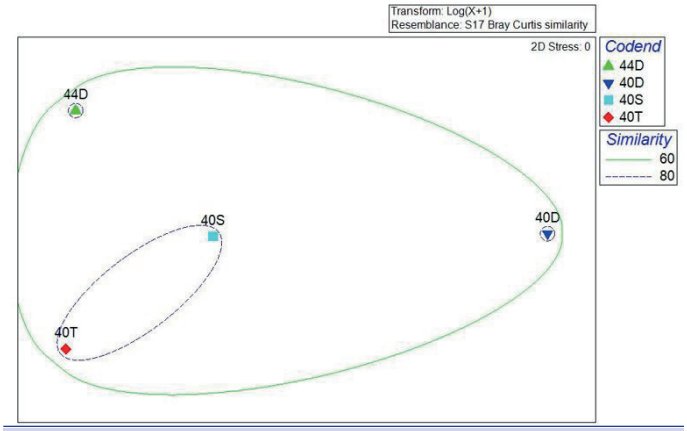

Figure 5. nMDS (Multidimensional scaling) analysis of species caught in examined trawl cod-ends.

(2019), depth is statistically the most influential factor determining the ordination of the faunal zonation in the study area. The species richness ranged from 2.668 to 3.017, and the Shannon-Wiener diversity index ranged from 1.171 to 1.327 during the study period. The species richness index value calculated by previous studies in the Western Black Sea Region was found between 0.177 and 2.156 in 2016 (ÇŞB, TUBITAK MAM, 2017) and between 0.691 and 1.809 in 2019 (ÇŞB, TUBITAK MAM, 2021). The Black Sea species diversity is relatively low compared to the Aegean Sea and the Mediterranean Sea. The species diversity varies according to the season, depth, behavior of the species, the duration of light during the day, the experiment period and time, the bottom structure, and the water's physical characteristics. The presence of fish or other species in the prey-predator status and their abundance are two additional dependent and independent variables that can alter the composition of the catch (Özbilgin & Ferro 1997; Özbilgin & Wardle 2002).

Gönener and Bilgin (2010) reported that red mullet, whiting, and turbot rank first in stock sizes, according to the data they obtained with their calculations covering two fishing seasons in the Black Sea. They noted that the changes in fish stock density in commercial trawling fishing areas might vary daily, monthly, or seasonally. Catch density of other species in the fishing area, and bioecological and physicochemical properties of waters can also affect this change.

CPUE is a proximal measure of a target species' abundance. To determine changes in the target species' abundance, CPUE changes were calculated. Overfishing is indicated by a decrease in CPUE values, whereas sustainable fishing is shown by a constant CPUE value (Puertas & Bodmer, 2004; Skalski, Ryding & Millspaugh, 2005). CPUE values were calculated as 241.63 kg. hour^{-1} in autumn and 29.64 kg. hour^{-1} in spring in 2010/2011 fishing season in bottom trawl fisheries in the Western Black Sea (Başkaya, 2012). Yıldız (2016) found that CPUE values as 100.4 kg. hour^{-1}in the autumn and 27.9 kg. hour^{-1} in the spring in the fisheries seasons of 2012/2013 and 2013/2014. Similarly, in other studies conducted in the Mediterranean and Black Seas, it has been reported that CPUE values are higher at the beginning of the trawling season, while CPUE values decrease throughout the season (Machias et al., 2001; Çiçek, 2006; Aksu, 2012). In this summer study, the average CPUE value was calculated for 44D as 40.22±6.04 kg. hour^{-1}, for 40D as 66.66±13.00 kg. hour^{-1}, for 40S as 45.55±7.34 kg. hour^{-1}, for 40T as 26.12±3.60 kg. hour^{-1}. The highest CPUE value of whiting, one of the commercial fish, was 28.75±15.56 kg. hour^{-1} 40D and the highest CPUE value of red mullet was detected in 44D as 4.83±2.62 kg. hour^{-1}. The difference between CPUE values 40D for whiting between 40D -the 40S and 40D - 40T was significant. In addition, the difference between 44D-40T and 40D-40T for red mullet and the total catch was significant.

The most efficient trawl cod-end in terms of catch rates, and the number of species obtained in this study is the diamond mesh with 40D. When we examine the ratios and CPUE values of red mullet and whiting determined as the target species in the catch composition, these ratios were found to be higher for 44D and 40D but relatively low for the 40S and 40T. It was found that the amount of whiting decreases by half when the mesh size increases from 40 mm to 44 mm for the diamond mesh shape. Considering the economic concerns (profits) of the fishers, the catch efficiency of square mesh and T90 trawl cod-ends in the Western Black Sea region is relatively low. It is clear from these results that there are pros and cons to each of these cod-ends. To prevent illegal fishing, new fisheries management policies need to be more moderate and accepted by fishermen. Better regulation of the double cod-end usage ban is required for a sustainable fishery.

The Mediterranean General Fisheries Commission (GFCM), responsible for the Mediterranean fisheries management, has decided to apply 40 mm square nets or 50 mm diamond mesh cod-

ends for all trawling activities in the Mediterranean basin since 2009. The immediate implementation of the square mesh types advised by the scientific committee (GFCM/33/2009/2) will improve the protection of juveniles of different species and develop the selectivity of 40 mm diamond mesh cod-ends in bottom trawling fishing vessels and multi-species fisheries. Similarly, the European Union has made it mandatory to use 40 mm square or 50 mm diamond mesh cod-ends instead of 40 mm rhombic for bottom trawl nets used in the European Union waters of the Mediterranean basin. According to Turkish fisheries legislation, 44 mm diamond mesh is used in bottom trawl nets in the Aegean Sea and the Mediterranean Sea, and 40 mm diamond is employed in the Black Sea. It is stated that after 1 September 2020, rhombic-eyed trawl nets will be applied as 44 mm in the Black Sea in the communiqué, Fisheries No. 2016/35 (BSGM, 2016). Since the fishermen's organizations stated that this practice would adversely affect the fishing activities in the Black Sea, it has not yet entered into force (BSGM, 2020) and is to be after 1 September 2024.

It has been reported that fish in 40 mm square mesh trawl cod-end were injured during the removal process from the net, and therefore fishers were not satisfied with square mesh nets (Dogru, 2014). It is also known that square mesh cod-ends are not used in bottom trawl fishing in Turkey. The reasons for this are the economic loss of fish, no production of rhombic mesh nets by the fishing net factories, and the need for more labor as species are compressed and crushed in the cod end meshes.

It has been stated that 28mmD trawl cod-end is harmful in terms of species diversity, demersal stocks, and commercial/non-commercial species (Stergiou, Politou, Christou & Petrakis, 1997). In Greek waters, 40mm diamond mesh cod-end is more effective than 40mm square mesh with regard to commercial/non-commercial species ratios (Stergiou et al., 1997). It has been determined in the Western Black Sea that the 40D trawl cod-end is more efficient than the other trawl cod-ends from the fishers' perspective.

Comparing the Mediterranean and the Black Sea in terms of biodiversity, they are two very different seas. Only two commercial fish species—red mullet and whiting—are caught frequently in bottom trawl fisheries, despite the Black Sea's low biodiversity. The high-body-height fish species that are frequently found in bottom trawling in the Aegean and Mediterranean are missing from the Black Sea. Therefore, it is essential for sustainable fishing to adjust the characteristics of the fishing gear used according to the characteristics of the fish in the region. Decision-makers should take appropriate management steps for this region by taking into account the results of the present study and the outputs of socioeconomic research.

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Research Article

Dose Selection for Induced Breeding and Larval Development of Indigenous Ornamental Fish *Puntius chola* (Hamilton, 1822)

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ABSTRACT

Puntius chola (Hamilton, 1822) is a small freshwater indigenous fish with food and ornamental value belonging to the family Cyprinidae found in Pakistan, India, Nepal, Bangladesh, Myanmar, and Sri Lanka. Due to the selective exotic species and the culture of Indian big carp, the species have gradually diminished in India. Evaluating *P. chola*'s optimum selection, induced breeding, embryonic, and larval development is the goal of the current study. The optimum dose was selected through the trial-and-error method applied 5 doses (0.25 ml/kg, 0.50 ml/kg, 1 ml/kg, 1.5 ml/kg, 2 ml/kg body weight) of synthetic hormone, ovatide to both sexes. The study reveals that the optimum dose of synthetic hormone ovatide @ 1.5 ml/kg body weight for females and males is effective for induced breeding of *Puntius chola*. At the optimum dose, fecundity, fertilization, and hatching rates were 106308±3075, 79.28±0.589%, and 78.03±0.495%, respectively. The physicochemical parameters of water have been enlisted for proper induced breeding. The outcomes of this research will enable P. chola to have a more decorative design and assist in its protection by encouraging it to reproduce and survive on its own in the wild. Additionally, the study will aid in community members' economic development.

Keywords: Optimum dose, Induced breeding, *Puntius chola*, Ornamental, Conservation

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INTRODUCTION

Ornamental fish are calm, beautiful fish that are kept as pets in small aquariums or backyard swimming pools so that their beauty can be appreciated. Because of their color, shape, and behavior, ornamental fish are often referred to as "living jewels" (Das et al., 2005). The number of people who keep ornamental fish is growing every day because it is a great way to start a business and make money. Aquaculture's fastest-growing branch could be ornamental fish breeding and rearing and the ornamental fish trade. Even though indigenous ornamental fish contribute less to the ornamental fish trade generally, they have the potential to generate indigenous ornamental fish. Breeding and aquarium technology improvements have given the ornamental fish industry a replacement dimension. India has a huge market opportunity in the production of ornamental fish, which are much sought after both domestically and internationally (Elamparithy, 1996). *Puntius* has become popular as a freshwater aquarium fish due to their striking coloration; several species are traded internationally as ornamentals (Collins et al., 2012). *Puntius chola* (Hamilton, 1822), also known as the "swamp barb" is a freshwater indigenous ornamental fish species belonging to the family Cyprinidae. It is distributed in Bangladesh, India, Pakistan, Nepal, Myanmar, and Si Lanka (Pethiyagoda, 1991), where it can be found in lakes, rivers, streams, ponds, ditches, and inundated water bodies.

Induced breeding is a technique that uses artificial stimulation to breed economically valu-

able fish that do not usually reproduce in captivity. By injecting pituitary hormone or similar synthetic hormone, ripe fish breeders can be encouraged to breed in captivity using the procedure known as "induced breeding" (Mohapatra et al., 2016). The stimulation encourages the release of sperm and eggs on time. Udit et al. (2014) reported 0.3 ml for 232-240 g females and 0.2 ml for 180 g males for induced breeding and larval development on *Puntius sarana*, with male and female ratios of 2:1 and 1:2. Sarma S. (2015) studied the induced breeding of *Pethia gelius* where they applied Ovaprim 0.5-0.4 ml/kg (F), 0.4-0.3 ml/kg (M) with a sex ratio of 2: 1 in the Bihar. Motilan *et al.* (2014) applied WOVA-FH, 0.2-0.4 ml/kg body weight of both males and females with a sex ratio of 2:1 for induced breeding of *Pethia manipurensis*. Saha & Saha (2010) reported some biological aspects of *P. chola* (Hamilton, 1822) but did not work on induced breeding. Sit *et al*. (2020) studied only the diversity of Puntius species but did not study on induced breeding of these species. Vincent and Thomas (2008) observed courtship behavior and nuptial coloration during the breeding of the *P. chola*. There was no clear study on egg release and larval development of *P. chola*. It is clear from the literature already in existence that P. chola from Asia in general and India in particular have not yet been the focus of a thorough investigation of induced breeding. Due to the scarcity and significance of small indigenous fish *P. chola* (Hamilton,1822), we worked on induced breeding to increase the species' availability. Therefore, the present study obtained management of captive broodstock, dose selection for artificial breeding, and larval development of *P. chola* (Hamilton,1822)

MATERIAL AND METHODS

Brooder's collection

The collection of ornamental fish has been carried out covering the period commencing from April 2020 to March 2022 from different ponds and rivers of the Paschim Medinipur district of India using a gill net and a cast net and brought to the ornamental fish rearing and breeding center at Raja Narendra Lal Khan Women's College (A), Midnapore (Figure 1a). Before the brooders were put in their new homes for further research, their length and weight were measured and written down (Figure 1c-d).

Acclimatization and feeding of the brooders

The brooders were acclimated in customized aquarium tanks constructed of glass that featured aquatic plants and artificial hideouts (Figure 1 b). The rearing tanks were sanitized with a 5 percent KMnO4 solution before the fish were introduced. During the acclimatization period, the fish were fed live feed such as mosquito larvae, phytoplankton, zooplankton, and high-protein commercial feed at 4–5 $g.kg^{-1}$ body weight daily. The brooders were fed commercial feed containing 47% protein, 5 % crude fat, 10 % moisture, 17 % ash content, 1 % phosphorous, vitamin A (2,500 IU/kg), vitamin D (2,500 IU/kg), vitamin E (2,000 IU/kg), and ascorbic acid (510 mg/kg).

Hormone injection

During the present study, brooders (6 - 8 g) were injected by using the synthetic hormone ovatide (M/s. Hemmo Pharma, Mumbai) at 5 doses (0.25 g/kg, 0.5 g/kg, 1g/kg, 1.5 g/kg, 2 g/kg) to optimize the ideal dose in both sexes of *P. chola*. Using a 1ml graduated syringe, appropriate doses were administered intramuscularly at a 45° angle on each pair's dorsal side of the caudal peduncle (Figure 1 e). Use a breeding hapa (a mosquito net made for breeding purposes) for each set (Figure 1 f). An emerged portion of the hapa measures 4 feet x 2 feet x 2 feet in surface volume.

Fecundity

After the release of the eggs, the eggs are counted across an area of one square foot on the surface area of hapa and then multiplied by the total surface area of an emerged portion of hapa. Fecundity is measured by the following formula

$$\text{Fecundity} = \frac{\text{Number of egg in one squre feet net} \times \text{Total squre feet of surface area}}{\text{Weight of fish}} \times 1000$$

Hatching rate and fertilization rate

Inside clear egg cells, fertilized eggs had intact nuclei. A sample of approximately 100 eggs was tanked at random in a glass petri dish to determine the fertilization rate of eggs. The rate of fertilization is calculated using the formula below.

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs}}{\text{total number of eggs counted}} \times 100$$

Spent fish were collected from each breeding hapa after spawning. After that, the fertilized eggs were placed in hatching trays and left to hatch. The hatching rate is calculated using the formula below.

$$\text{Hatching rate} = \frac{\text{Number of spawn}}{\text{total number of fertilized eggs}} \times 100$$

Embryonic and larval development observed

For further research, samples of eggs were taken before fertilization and after fertilization at 10-minute intervals. The embryonic stage takes place within the chorion and culminates in hatching. When the larva can ingest food from outside its body, the larval stage, which is defined by the yolk sac's nutritional contribution, comes to an end. The post-larval stage begins as soon as the yolk sac is absorbed and is characterized by self-feeding. With the help of a light microscope (Olympus CX31), the development of embryos, larvae, and spawn was watched, as well as how long each stage lasted and how it changed in shape (Figure 1 k-l).

Water quality measure

The water quality in the brood, spawning, and larval rearing tanks was regulated at an optimal throughout the experiment, as required for *P. chola*. In accordance with APHA guidelines, the water parameters were monitored weekly during rearing using water analyzer 371 (Systronics). Some of the physical and chemical parameters that were measured were the surface temperature, water temperature, precipitation, pH, dissolved oxygen, hardness, and alkalinity (Figure 1i).

Care for New-borns

The yolk was reduced after 70 - 75 hours of hatching, and then the new glass aquarium used for spawn developed. In general,

Figure 1. Some working photographs; a-Specimen's collection, b-Broder fish in an aquarium, c-Measurement of length, d-Measurement of Weight, e-Hormone injection, f- Breeding hapa, g-Colour changes after injecting hormone, h- Egg on a net of hapa, i- Egg on a petri dish, j-Water parameter analysis, k-egg under the microscope, l-observed embryonic development.

conditions should be similar to those in the main aquarium. The aquarium should not be over-filtered to the point that the fry is being drawn in by the pump. They give them diluted yolk (chicken) for the initial three days. Then they use the dust of dry commercial tubifex, which has crude protein (58 %), crude fiber (12%), crude fat (7 %), crude ash (5%), and moisture for their better growth and development.

Data analysis:
Finally, data were analyzed with the help of Microsoft Excel 2019 and the SPSS-2021 software system.

RESULTS AND DISCUSSION

Optimum Dose selection: To find the best dose for induced breeding, mature brooders (6 -7 g) were using the artificial hormone ovatide at five different doses. The changes in brooders in respect of applied doses are represented in Table 1.

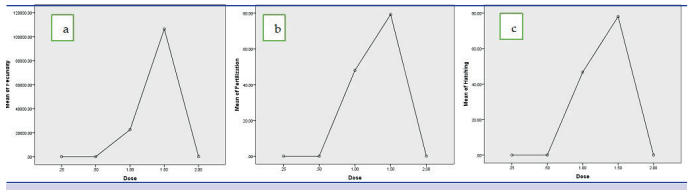

Figure 2. Dose wise Fecundity, Fertilization rate, and Hatching rate.

Breeding behavior
Active spawning was observed hours after the injecting dose. The breeding behavior is enlisted in Table 1.

Water quality
Water quality metrics are important in captive breeding because they duplicate water quality as closely as possible to the natural environment. This makes breeding much easier. In all of the experiments during rearing and breeding time, the parameters of water were shown in Table 3.

Embryo and Larva: The characteristics of the following embryonic developmental phases were observed and noted (Figure 3-5)

Morula
In the morula stage, cleavage begins after 30 minutes of fertilization. The cleavage furrow of cleavage gradually narrows to the animal pole of the cytoplasm. A huge number of cells formed a clump at the animal pole after multiple consecutive cleavages. 'Morula' is the name given to this stage of embryonic development (Figure 3 a).

Blastula
After the morula stage, the growing embryo is separated into multiple cells and organized into a layer called the blastoderm. The blastodisc, or extra cell division, is what causes the blastoderm to gradually develop into a vast number of layers. A gap called a blastocoel develops between the yolk and the blastoderm at this stage. This stage is known as the 'blastula' (Fig. 3 b).

Gastrula
During the early gastrula stage, the blastoderm began invading and spreading in a thin layer over the yolk. This is the start of the gastrulation process. During the mid-gastrula stage, a ring-like structure formed (Figure 3 c). The ring is known as a "germinal ring". The blastoderm covers 80% of the yolk in the late gastrula stage. The embryonic shield could be seen plainly. The rudiment of optics was abundantly obvious.

Organogenesis
The embryo was stretched due to organogenesis. The heartbeat was heard and the head and tail ends could be distinguished from the yolk. The yolk sac could be seen plainly. In this period, the rudiments of many body organs were developed (Figure 3 d).

Embryo in C-Shape
The embryo lengthened and gradually transformed into a head and tail, resulting in a C-shaped embryo. The body took on the shape of a C. Here, between the tail and the head, the yolk was connected. The growth of myotomes has been noticed. The embryo began to move on its own (Figure- 3 e).

Twitching
The tail was freed from the yolk at this point. The yolk sac was only found in the cranium. Myotomes grew in numbers. The embryo grew active and began to twitch continuously (Figure 3 f).

Hatching of the embryo
After completing embryonic growth, the twitching movement became more pronounced, and finally, the embryo was released

Table 1. Dose selection and observation during experimental breeding of *P. chola*.

Set	Dose (ml/kg)	Sex ratio (M: F)	W	Observation	R	F	S	I	Fr (%)	H (%)
1	0.25	02:01	M-6; F-7	No Change	No egg	-	-	-	-	-
2	0.5	02:01	M-7; F-8	Body color change	No egg	-	-	-	-	-
3	1	02:01	M-6; F-7	Aggressive behavior Body color change	Egg release	22447± 1331.19	15 - 17	8	47.97 ± 0.685	46.70 ± 0.39
4	1.5	02:01	M-6; F-7	Aggressive behavior Body color change Aggressive behavior	Egg release	106308± 3075.04	15 - 17	8	79.28 ± 0.589	78.03 ± 0.495
5	2	02:01	M-7; F-8	Dead within Half an hour	No egg	-	-	-	-	-

W=Weight (g); F=Fecundity (Egg/Kg); S=Spawning time (hr); I=Incubation period; Fr=Fertilization rate; H=Hatching rate

Table 2. ANOVA test.

		Sum of Squares	df	Mean Square	F	Sig.
Fecundity	Between Groups	42448578024.400	4	10612144506.100	4725.756	.001
	Within Groups	44911941.600	20	2245597.080		
	Total	42493489966.000	24			
Fertilization	Between Groups	26744.841	4	6686.210	40874.253	.001
	Within Groups	3.272	20	.164		
	Total	26748.113	24			
Hatching	Between Groups	25795.399	4	6448.850	81076.813	.001
	Within Groups	1.591	20	.080		
	Total	25796.990	24			

Table 3. Physico-chemical parameters in water.

Optimum range		
Parameter	**Aquarium**	**Hapa**
Temperature (°C)	26.3±5.22	27±3.2°C
pH	7.6±.84	7.6±9.1
Ammonia (ppm)	0.1±.002	0.03-±0.01
Nitrate (ppm)	80±2.5	80±2.8
Nitrite (ppm)	0.25±0.014	0.3±0.12
DO (ppm)	6.0±.94	6±2.5

within 15–17 hours with a temperature of 27 ± 3.2 ℃ (Figure-3g). The forceful rotation of a fully formed embryo shattered the egg-shell, allowing the larva to emerge. The length of the hatched larva was 0.17 mm and gradually developed to 0.29 mm (Figure 4). The yolk absorbent fry was plucked and put in a stocking tank for continued growth. In 18 days, the fry had developed to a size of 12 to 15 mm (Figure 5).

Sarma (2015) studied the induced breeding of *Pethia gelius* (Hamilton, 1822) where they applied Ovaprim 0.5-0.4 ml/kg (F), 0.4-0.3 ml/kg (M) with a sex ratio of 2: 1 in the Bihar. Motilan et al. (2014) applied WOVA-FH, 0.2-0.4 ml/kg body weight of both males and females with a sex ratio of 2:1 for induced breeding of *Pethia manipurensis* (Menon, Rema Devi & Vishwanath 2000). In this study, ovatide doses of 0.25 ml/kg, 0.5 ml/kg, 1 ml/kg, 1.5 ml/kg, and 2 ml/kg body weight were administered to both male and female *P. chola* with a ratio of 2:1 during the pre-monsoon season. Here, observed only 1.5 ml/kg ovatide was the optimum dose for induced breeding of *P. chola* because this dose was responsible for high fecundity (106308 ± 3075 kg/body weight), spawning, and hatching. *P. chola* required a higher concentration of a synthetic hormone than other species to reproduce.

Vincent & Thomas (2008) observed courtship behavior and nuptial coloration during induced breeding of the *P. chola*; during the present study, the same observation was shown. The significance of the effects of ovatide on fecundity, fertilization, and hatching was observed through the ANOVA with a 0.05 level of significance (Table 2). The dose-wise fecundity, fertilization, and hatching rates

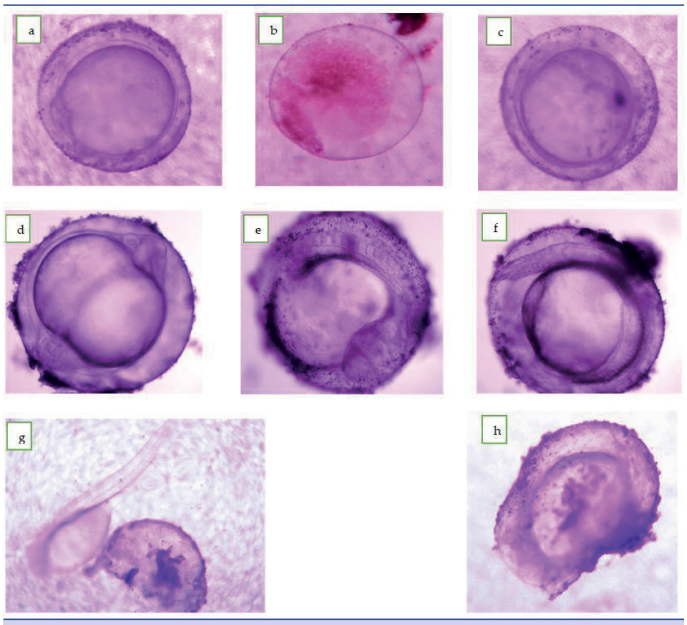

Figure 3. Embryonic development of *Puntius chola*; a-Morula, b-Blastula, c-Gastrula, d-Organogenesis, e-C-shaped larva, f-twitching, g-Hatching, h-Egg brust

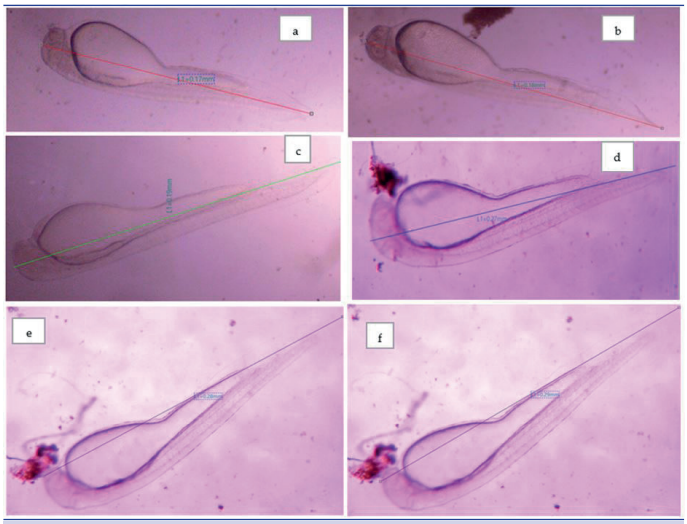

Figure 4. Larval development; a-0.17 mm, b-0.18mm, c-0.19mm, d-0.27mm, e-0.28mm, f-0.29 mm.

have fluctuated; only 1.5 ml/kg ovatide shows a high rate of fecundity, fertilization, and hatching; 1 ml/kgn is very low and the other three doses are not effective for breeding (Figure 2).

In this study, the average fertilization rate (%) was 79.28 ± 0.589, and the hatching rate (%) was 78.03 ± 0.495 through the optimum dose. The hatching and fertilization rate were almost identical to those in the major carp, where Hossain *et al.* (2007) achieved those eggs to early fry (spawn) and could achieve up to 80 % survival (Chaudhuri *et al.*, 1984). In this study, the incubation of the egg lasted 8 hours at 27 ± 3.2℃, and the observed hatching period was between 10 - 11 hours. According to the results of the

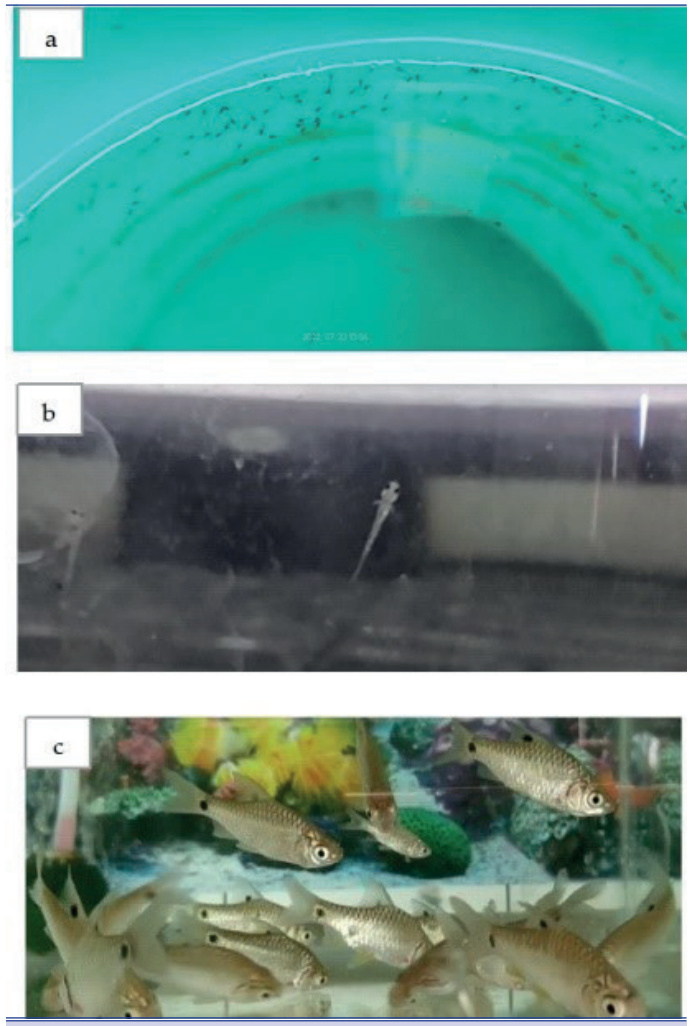

Figure 5. Spawn (a), fry (b), and fingerling (c) of *P. chola.*

current study, fertilized eggs were initially translucent and turned creamy as embryonic development progressed. The size of the fertilized eggs ranged from 0.09 to 0.1 mm. The unfertilized eggs were opaque and white, whereas the fertilized ones were transparent. Common carp's swollen, fertilized eggs range in size from 1.5 to 2.5 mm (Woynarovich et al., 1984). Mumtazuddin et al.(1982) found that the quantity and rate of growth of carp spawn are significantly influenced by the availability of high-quality living food species, especially zooplankton. Early spawn was removed from the hatching tank and stockpiled in a well-prepared tank for continued rearing in the current investigation. The fries were given chicken yolk that had been diluted with water. From day 5 to day 10, the fry was fed newly hatched Artemia nauplii, which is important for fish growth and survival. After 10 to 15 days, it will be able to feed on natural as well as Pilate's artificial feed. Freshly hatched *P. chola* larvae were between 0.16 mm and 0.17 mm in size, compared to 4.8 to 5.0 mm for common carp (Woynarovich et al., 1984). At a water temperature of 27 ± 3.2 °C in the present work, hatchlings of *Puntius chola* reduced the yolk in 65 to 70 hours. However, at temperatures between 24 and 31 °C, the Indian major carp's yolk absorption duration was 3 to 4 days (Woynarovich et al., 1984). The findings show that the length

of time it takes for the yolk to absorb depends on the water's temperature as well as the number and size of the yolk sacs. The yolk sac of P. chola was depleted more quickly because it was smaller compared to that of Indian major carp.

CONCLUSION

The maximum spawning, egg production, and hatching rates in *P. chola* are achieved at an optimum dose of 1.5 ml/kg (ovatide) of body weight. For commercial seed production and the restoration and conservation of species, the male and female responses to a single dose of ovatide are crucial.

Conflict of Interest: The authors declare no conflict of interest.

Ethics Committee Approval: Ethical clearance from IAEC, Approval no. 18/IAEC (05)/RNLKWC/2019, dated 27/07/2019

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Research Article

The Effect of Improper Packaging on Moisture and Fatty Acid Composition in Frozen Bluefin Tuna (*Thynnus Thynnus*)

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ABSTRACT

This article investigates the effect of improper packaging and freezer burn on the moisture content and fatty acid profile of frozen bluefin tuna. Improper packaging caused serious freezer burn on the surface of a bluefin tuna slice during frozen storage. The moisture content of the surface affected from freezer burn and an inner part of the tuna slice was analysed. Visual examination showed that the surface of the tuna slices were dried and different from normal flesh colour. Moisture content of the frozen tuna slices dropped significantly on the surface compared to the inner part unaffected by freezer burn. Direct methylation method was successfully achieved on the sample without any lipid extraction. Separation of fatty acid methyl esters of the bluefin tuna was successfully achieved by using GC-FID 100 m column in 65 minutes. Significant changes were observed in saturated and polyunsaturated fattyacids, whereas monounsaturated fatty acids remained the same. The level of polyunsaturated fatty acids reduced by half on the surface of the flesh compared to the inner part. Among the PUFA, n3 and n6 fatty acids were greatly reduced, but more intense in n3 fatty acids.

Keywords: Bluefin tuna, lipid oxidation, fatty acid, moisture, improper packaging

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INTRODUCTION

The Atlantic (or Northern) bluefin tuna (*Thunnus thynnus*, ABT) is one of the tuna species with the greatest commercial interest (Zohar et al., 2016). Storage condition of tuna is important for both commercial interest and nutritional quality. Frozen storage is generally chosen to extend the shelf life of ABT (Martinez et al., 2022). Normally, when tuna is caught, it is bled immediately to minimize deterioration in the muscle, and to obtain high quality raw material for further processing. Tuna fish is frozen at much lower temperatures than other seafood to reduce freezing time due to its size. It must be frozen as quickly as possible and conserved at temperatures under −50°C (C. Chow et al., 1989). Some processors operate freezers for tuna up to as low as -70°C to freeze the whole tuna. Correct storage temperatures are important in order to prevent the deterioration of marine product quality. Various researchers have showed the chemical changes in fish from different freezing storage periods and temperatures. During shelf-life lipid and hemoglobin oxidations, protein coagulation and color changes are the main alteration (Ayala et al., 2005; Chow et al., 2004; Tanaka et al., 2016; Torrieri et al., 2011). These changes were also related to freezing time. During slow freezing, conformation of large extracellular ice crystals creates higher injury to the cells than the small intracellular ice crystals that form in quick freezing (Badii & Howell, 2002; García et al., 1999). The loss of color during storage is also slower in quickly frozen tuna than in gradually frozen tuna (Bito, 1968). The formation of large ice crystals during freezing reduces the quality of fish, for instance, rupturing the membrane

structure. For this reason, it is important to carefully manage marine products in terms of both freezing speed and temperature.

Marine products contain more unsaturated lipids than other organisms and the oxidation of these unsaturated lipids results in deterioration of texture, appearance, flavor, and consistency. Especially, polyunsaturated fatty acids (PUFA) are prone to oxidation and formation into lipid hydro peroxides (L-OOHs) during the early stages of storage. L-OOHs generate a variety of volatile compounds, such as ketones, alcohols, and aldehydes (Frankel, 1984) These products cause the rancid odor in rotten fish (Swoboda & Peers, 1977). Moreover, the L-OOHs alter other unoxidized lipids, proteins (Lenz et al., 1990), carbohydrates (Ravussin et al., 1986) and nucleic acids (Lewis et al., 1986). Differently, the modification in the meat color is correlated to the amount of oxidized myoglobin (Kannan et al., 2001). The amount of the myoglobin content to hemoglobin content in the meat influences the color of the fish (Richards & Hultin, 2003). Changes in fish color depend on various components, such as light exposure, storage temperature, redox potential, muscle pH, and susceptibility to lipid oxidation. The accumulation of hydro peroxide during the initial phase of lipid oxidation differs between fish species and between ordinary and dark muscles (Sohn et al., 2005).

Frozen fish that has been improperly packaged is a severe issue during the frozen storage. Over time, the unfavourable moisture loss and the presence of air cause the oxidation of the fish lipids, especially the long-chain unsaturated fatty acids. The oxidation of these nutritionally important unsaturated fatty acids is the major problem in frozen fatty fish. Moreover, undesirable flavours and colours changes occur as a result of the oxidative deterioration. The aim of the study is to determine the impact of improper packaging on the moisture loss and the severity of the long chain unsaturated fatty acid loss of frozen bluefin tuna.

MATERIAL AND METHODS

Bluefin tuna fillet was purchased from the fish market in Konya. The total weight of the tuna was 70 kg. The fish were sliced at about 2 cm thicknesses and wrapped with cling film, frozen with a home type freezer at -24°C and then stored at -18°C for a year. Visual examination of the fish slices showed some freezer burn on the surface of the slices. The freezer-burned surface of the slice and its underneath, which seemed to be free from freezer burn, was gently cut in a flake-like manner with a sharp knife while still frozen.

Moisture analysis

The moisture content of the frozen bluefin tuna was determined according to oven drying method (Official Methods of Analysis, AOAC, 2000). The drying process was done by holding the sample at 105°C for eight hours and a constant weight was obtained. Dried samples were cooled in a desiccator for 30 minutes. The moisture content was calculated based on the weight loss after drying.

Preparation of fatty acid methyl esters

Fatty acid methyl esters of the samples were prepared according to Joseph & Ackman (1992) with a minor modification. Approximately 1 g of the fish flesh (instead of fish oil) was cut finely while it was frozen and then transferred into a screw capped glass tube and 2 ml of 0.5 M methanolic NaOH was added, and then capped tightly. Tubes were heated up at boiling temperature for 7 minutes, and then cooled down to room temperature. After addition of 1.5 ml methanolic BF_3 (%14), the tubes were capped tightly and then heated for a further 5 minutes. Tubes were cooled to room temperature and fatty acid methyl esters were extracted with 2 ml iso-octane using a vortex mixer. After phase separation, the upper phase was taken into a 2 ml amber vial via a glass Pasteur pipette, and then immediately injected into GC-FID (Shimadzu GC-2025) to determine fatty acid composition.

Separation of fatty acids methyl esters

Fatty acid methyl esters were separated with Shimadzu Gas Chromatography (GC-2025) equipped with auto injector (AOC-20I). The injection temperature was maintained at 230° C and detector (FID) temperature was set to 250°C Column specification and column temperature program as follows: Teknokroma TR-CN100, TR-882192 100% biscyanopropyl polysiloxane, column length 100 m, id 0.25 mm and film thickness 20 mm. Initial column temperature was hold at 45°C for 3 minutes, then raised to 225°C with a ramp rate 5°C per minute and held at this temperature for 26 minutes. Fatty acid methyl esters were separated in 65 minutes.

Injection volume 1 µL Injection mode split 1:25 total flow 68.3 mL, column flow 1.28 mL/min. purge flow 3 mL/min. Identification of fatty acid methyl esters was done comparing their retention time with standards of fatty acid methyl ester (Restek Food Industry FAME Mix 37 and PUFA Mix No 3 Supelco-47085u). The results were expressed as a percentage area of individual fatty acid.

Statistical analysis

All biochemical analyses were done in triplicate and presented as mean values ± SD, after controlling for the normality and homogeneity of the data. The statistical analysis was performed with SPSS 27 package program by using Student's t test. The significance level of the values were determined at 95% level where $p<0.05$ was considered to be significantly different.

RESULTS AND DISCUSSION

Moisture content of the frozen tuna slice is shown on Table 1. It was found that the moisture level of the fish slice changed from 42.6% on the surface to the inner part with a level of 59%. This result indicates that significant moisture loss occurred on the surface of the frozen tuna slice compared to inner part. Moisture content of bluefin tuna may vary in the body part depending on their lipid content. Loss of moisture in the slice has an undesirable effect on the sensory and texture properties of the flesh. It

Table 1. Moisture content of frozen Bluefin tuna.

Tuna Slice	Moisture (%)	Ref
Surface	42.64±0.98*	61.1[a] ; 62.9[b];59.9[c]; 61[d]
Inner part	59.17±1.46*	

*significance level at 0.05; (Öksüz, 2017[a]; Parisi et al., 2007[b]; Roy et al., 2010[c]; Topic Popovic et al., 2012[d])

becomes dry and takes a cotton-like texture. Even further dryness may occur when they are cooked. Therefore, it is crucial to prevent moisture loss during frozen storage in order to maintain the quality of the flesh. Moisture content of fresh Bluefin tuna ordinary muscle was reported to be 61.1%, having a level of 20.3 % lipid (Oksuz, 2017). Similarly, bluefin tuna moisture content was stated by different researchers as 58.96 to 60.08% in female and male bluefin tuna (Parisi et al., 2007). Our findings in moisture content of inner part of the bluefin tuna slices are close to the literature values. However, the most prominent moisture loss was observed on the surface compared to the moisture content of the inner part of the slice and stated in the literature. This value was almost 16.5 % lower than inner part of the tuna slices. Moisture loss results in undesirable changes to the flesh quality, and the loss of moisture on the surface causes degradation and dryness in the flesh texture, reducing sensory characteristics.

On the freeze burned flesh surface, lipid concentration increases on dry weight basis, and lipid become more available to atmospheric oxygen to initiate oxidation. Therefore, PUFA in the fish flesh are oxidised rapidly, which lowers the nutritional quality of fish lipid. Oxidation of lipid is considered to be a major problem in fatty fish, such as tuna, mackerel and sardine. Polyunsaturated fatty acids were highly affected from the lipid oxidation in fish muscle during the frozen storage. As a consequence, fish muscle becomes rancid, inedible and loses its nutritional value.

Oxidation of PUFAs is a multistep process that occurs after exposure to atmospheric oxygen during the production stage. At the initial step of oxidation, formation of peroxides and dienes occurs and with the aid of oxygen in the cold store, secondary oxidation products such as carbonyl and aldehydes are produced. These secondary oxidation products results in an undesirable odour and colour changes (Mason & Sherratt, 2017). In our personal experience, a yellowish colour formation was observed in the oil rich mackerel skin around the belly in prolonged frozen storage. Prolonged exposure to oxygen produces secondary oxidation products carbonyl and aldehyde (Albert et al., 2013).

Lipid oxidation may be retarded by rapid freezing along with proper packaging and preventing fluctuation in cold store temperature.

The distribution of fatty acid composition of bluefin tuna muscles are presented in Table 2 and 3. Total saturated fatty acids (SFA) on the oxidised surface of the slice compromised more than half of (54.5%) the total lipid. However, the inner part of the slice contained only 31.5% of total saturates of lipid. Among the saturated fatty acids, palmitic acid (C18:0) was the most prominent fatty acid followed by stearic acid (C16:0), and myristic acid (C14:0). The monounsaturated fatty acid (MUFA) contents of oxidised surface and inner part were similar. Nevertheless, the level of polyunsaturated fatty acid (PUFA) in the inner part (37.0%) was significantly higher than the oxidised surface (17.9%). PUFAs have multiple unsaturated bonds, and are therefore more susceptible to oxidation (Tao, 2015). Hydrolysis of lipids during storage is one of the causes of lipid oxidation in fish meat (Tanaka et al., 2016). Lipid oxidation causes quality losses, production of unpalatable flavour and odour, shortening of shelf life, loss of nutritional quality, and possible production of unhealthy molecules (malondialdehyde) (Secci & Parisi, 2016). Past studies have shown the positive effects of n-3 fatty acids in fish meat and fish oil on health. Especially eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) are crucial because synthesis of EPA and DHA in mammalians is slow and show significant genetic variation (Nowicki et al., 2020). Therefore, regular consumption of fish and seafood is important for adequate intake of EPA and DHA. In this study ratio of total n-3, EPA and DHA in oxidised part was significantly two times lower than inner part (Table 2 and Table 3). Although the bluefin tuna slice was stored at -24 ^{0}C, oxidation on the surface caused loss of PUFA.

Moreover, 4-hydroxy-2-alkenals are the main aldehyde substances produced during the peroxidation of PUFAs. During peroxidation of n-3 PUFAs, 4-hydroxy-2-hexenal (HHE) is formed (Yamada et al., 2004). Pathological conditions in relation to HHE in humans and mammals have been reported by previous researchers. HHE is a toxic end-product of lipid peroxidation and is shown to be involved in the pathogenesis of several degenerative dis-

Table 2. Fatty acid composition (in percentage) of oxidised surface and unoxidized part in Bluefin tuna.

Compound Name	Oxidised surface	Inner part	t	p
C14:0	9.53±0.95	4.62±0.09	8.933	0.001*
C16:0	32.94±4.25	18.62±0.64	5.773	0.004*
C17:0	1.15±0.17	0.69±0.02	4.531	0.011
C18:0	10.31±1.63	7.06±0.10	3.448	0.074
C20:0	0.61±0.07	0.55±0.02	1.488	0.211
C22:0	0.22±0.03	0.24±0.02	-.941	0.400
C14:1 n5	1.05±0.13	0.55±0.04	6.203	0.003
C16:1 n7	8.35±0.82	6.84±0.17	3.138	0.035
C17:1 n7	0.30±0.01	0.63±0.01	-44.505	<0.001*
C18:1 n9c	10.87±0.79	11.24±0.34	-.745	0.498
C18:1 n7c	4.04±0.23	3.93±0.11	.776	0.481
C20:1 n9	1.39±0.09	1.57±0.11	-2.335	0.080
C20:3 n3	0.53±0.02	0.54±0.03	-.658	0.547
C18:2 n6	1.83±0.45	2.50±0.00	-2.560	0.125
C20:2 n6	0.30±0.05	1.41±0.01	-37.904	<0.001*
C20:3 n6	0.22±0.03	0.24±0.02	-0.941	0.400
C20:4 n6	0.65±0.20	1.36±0.10	-5.549	0.005*
C22:4 n6	0.27±0.10	0.56±0.04	-4.735	0.009*
C22:5 n6	0.51±0.03	0.56±0.01	-3.785	0.019
C18:3 n3	0.63±0.14	0.90±0.03	-3.232	0.032
C20:4 n3	0.49±0.09	0.71±0.02	-4.027	0.016
C20:5 n3	6.69±2.45	13.53±0.13	-4.824	0.040
C22:5 n3	0.92±0.29	2.14±0.02	-7.257	0.018
C22:6 n3	5.42±1.84	13.10±1.42	-5.719	0.005*

*significance level at 0.01

Table 3. Total SFA, MUFA and PUFA content of oxidised surface and unoxidized part surface in Bluefin tuna.

Fatty acid	Oxidised surface	Inner part	t	p
Tot SFA (%)	54.53±7.06	31.54±0.88	5.600	0.005*
Tot MUFA (%)	26.52±1.77	25.31±0.79	1.079	0.341
Tot. PUFA (%)	17.94±5.60	37.01±1.53	-5.691	0.005*
n3	14.16±4.82	30.38±1.49	-5.572	0.005*
n6	3.78±0.78	6.63±0.04	-6.292	0.024
n6:n3	0.28±0.04	0.22±0.01	2.688	0.055
EPA:DHA	1.23±0.03	1.04±0.11	2.981	0.041

*significance level at 0.01

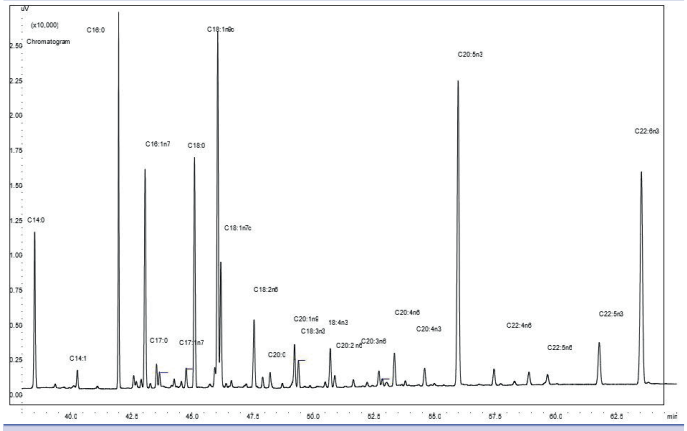

Figure 1. Fatty acid profile of bluefin tuna unaffected from freezer burn.

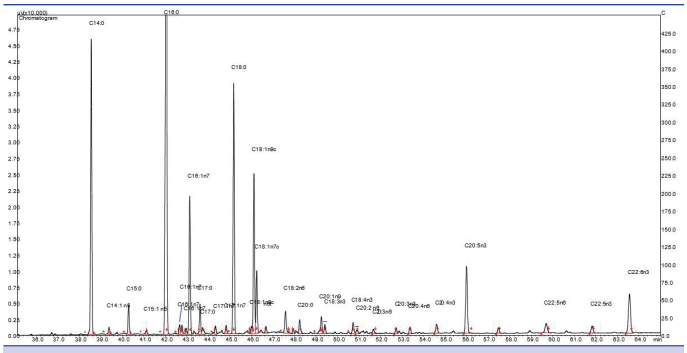

Figure 2. Fatty acid profile of bluefin tuna affected from freezer burn.

eases (Leonarduzzi et al., 2012; Long & Picklo, 2010; Yamada et al., 2004). Therefore, the level of HHE could be a useful indicator for quality assessment of marine products. In literature, various studies have shown several agents inhibiting lipid oxidation. Salt, plants extracts, chitosan, chitooligosaccharide, bacteriocins, antimicrobial and antioxidant peptides, and essential oils were commonly used (Kaewprachu et al., 2017; Mariutti & Bragagnolo,

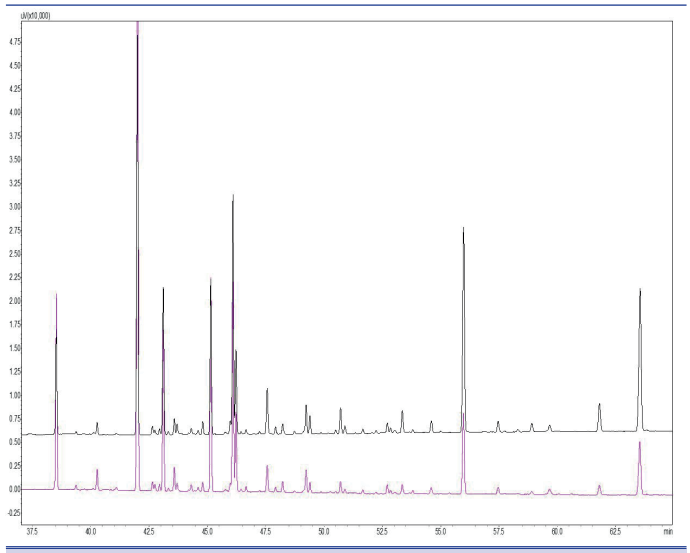

Figure 3. A comparative illustration of fatty acid profile of normal and freeze burned muscle.

2017; Olatunde & Benjakul, 2018). Lipid oxidation can be influenced by different factors, such as freezing temperature, storage temperature, fatty acid composition, pro-oxidants, myoglobin, pH, and oxygen consumption (Arab-Tehrany et al., 2012). If fish is frozen quickly, proper storage temperatures are maintained and thawed in the best manner, it can retain the same quality as when fresh (Cappeln et al., 1999).

Freezer burn manifests in whitish or yellow brown, dry, tree-like areas on the fish flesh, and has thus a major impact on the appearance and the sensory quality of the product (Pham & Mawson, 1997). High levels of water evaporation may also accelerate protein denaturation, resulting in a tough texture and lipid oxidation causing off-flavour production (Hyldig et al., 2012). Glazing is often used to protect the surface of both lean and fatty fish from oxidation and dehydration. The frozen product is either sprayed with or dipped in water, thereby, forming an 'ice cap' around the product. When cold storage is prolonged, it might be necessary to renew or reapply the glazing layer. Trials have shown that drip loss can be reduced by dipping the fish in a salt solution before freezing. However, this treatment has also been shown to accelerate the development of a rancid freezer taste due to the presence of cis-4-Heptenal formed by oxidation of n-3 fatty acids (Hyldig et al., 2012).

However, dietary LC n-3 PUFA are highly vulnerable to oxidation (Awada et al., 2012). Volatile secondary oxidation products formed as a result of the oxidation of PUFAs cause off-flavors (Let et al., 2005). Beyond sensory changes, the oxidation process can also result in the formation of substances that have negative effects on health. One of these potentially deleterious substances is 4-hydroxy-2-alkenals. It has been shown that the levels of serum 4-hydroxy-2-alkenals and inflammation biomarkers increase in rats fed with oxidized n-3 PUFA. It was emphasized that consumption of oxidized n-3 PUFA results in 4-HHE accumulation in blood and triggers oxidative stress and inflammation in the upper intestine (Awada et al., 2012).

Fish meat tends to oxidize easily due to its rich polyunsaturated fatty acid content. During storage oxidized lipids or secondary breakdown products interact with the proteins in fish meat. It causes insolubilization, polymerization, loss of enzymatic activity in protein and formation of lipid–protein complexes (Hematyar et al., 2019). It is crucial to prevent lipid oxidation in fish muscle in order to maintain protein quality. Nutritional loss in freezer burned fish may occur with other frozen meat and poultry products if similarly mishandled during transport and storage.

CONCLUSION

In summary, this research acknowledged that a tuna slice wrapped with cling film did not prevent moisture losses efficiently in extended storage. Therefore, it is advised that tuna slices stored in freezers should be vacuum-packaged when possible. In fish frozen without proper packaging, it was observed that the surface became freezer burned and took a yellowish dull colour. The fatty acid profile of the tuna slices showed that the level of omega3 PUFA's were significantly lower on the surface compared to inner part. Although the ratio of n3: n6 did not change significantly, the percentage of total n6 and n3 lowered by half in the freezer burn samples compared to their counterparts. Lipid oxidation in fish causes three problems: it reduces the nutritional value of lipid-containing fish, it increases the formation of off-flavors, and it gives rise to free radicals that may participate in the development of diseases like atherosclerosis. Moreover, the loss of nutritional quality and moisture are strictly connected with an economic loss. In order to avoid all these problems, it is strongly recommended to pack tuna slices in an air tight packaging materials such as vacuum packaging.

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Research Article

Green Synthesized Nanoceria Applied as a Fenton-Like Catalyst for Degrading Methylene Blue

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ABSTRACT

Nanomaterials are preferred for scientific studies due to their spectral properties and perfect surface appearance. This study aims to introduce a novel, environmentally friendly, photocatalytic method for degrading methylene blue (MB) in aqueous solutions. With this purpose in mind, the study synthesizes nanoceria particles and coats them with zahter (*Thymbra spicata*; zahter-coated nanoceria, ZCNC) following the main outlines of green chemistry as characterized by SEM and FTIR analyses. The study proposes this new nanoparticle (with the aid of H_2O_2 and UV combinations) as an alternative to iron in Fenton-type reactions for enabling MB degradation. The maximum efficiency was observed through the ternary combination of zahter-coated nanoceria, UV light, and H_2O_2 at 63% concentration. The degradation of the MB solution was achieved by installing a small amount of ZCNC (0.1g), after which the absorbance values were measured at 664 nm. According to the possible reaction kinetics discussed within the study, the reaction rate was calculated at 1.49×10^{-2} min $^{-1}$, thus enabling a faster reaction for a better evaluation of the reaction mechanism compared to other degradation processes that have been previously investigated..

Keywords: Hydroxyl radicals, nanoceria, *Thymbra spicata*, methylene blue, advanced oxidation process, photocatalytic degradation

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INTRODUCTION

Green chemistry is considered to be the clean approach to processing, synthesizing, and employing chemical products for eliminating chemical hazards. The main purpose of green chemistry is to provide efficient routes for synthesizing and reducing waste with regard to non-biodegradable stabilizing agents by preferring safer chemicals (Anastas et al., 1998). To serve this purpose, even small amounts of toxic pollutants should be removed using cost-effective, novel, and easy-to-use technologies. Nanostructured materials have been applied in many areas (Zaera, 2013; Ozdemir Olgun et al., 2018) and are still a focus of interest by providing unique mechanical and physicochemical properties (Yu et al., 2007). As for nano-oxides, cerium oxide is a preferable material for use in nanotechnology studies due to the ease with which it can switch between the Ce(III) and Ce(IV) oxidation states (Kamer et al., 2019). Cerium oxide nanoparticles (CeONPs), also known as nanoceria, belong to the metal oxide class of nanomaterials (Wei et al., 2019). Nanoceria has a high oxygen-transfer capacity and large surface area, which gives rise to better catalytic properties. As observed through X-ray spectroscopic analyses (Xu et al., 2014; Ni et al., 2015), both Ce(III) and Ce(IV) ions are found on the nanoceria surface and may be employed as redox couples. By taking advantage of these properties, nanoceria can be a reasonable alternative to iron compounds in Fenton-like reactions. Moreover, nanoceria's versatile pH range, it may be superior to iron salts, which show a narrower more-acidic pH range for Fenton reactions.

Dyes are problematic organic compounds in wastewaters and contribute largely to environ-

mental pollution as a result of their persistent (hard-to-decompose) molecular structures (Joshi et al., 2004; Jo et al. 2014; Shinde et al., 2014; Nguyen et al., 2015; Reddy et al., 2015; Natarajan et al. 2018). Approximately $7x10^5$ tons of commercial products are known to require dyes during their production, and 10-15% of these dyes end up in wastewater (Natarajan et al., 2013). Due to textiles being the leading industry in terms of production, it is majorly responsible for dye pollution all over the world. Methylene blue is a cationic dye that causes vomiting, hypertension, and anemia as a result of long-term exposure. MB has a reduction potential close to that of oxygen and can be reduced by components of the electron transport chain; it can further be used in singlet oxygen production (Solano et al., 1987; Tüfekçi et al., 2007; Hameed, 2009; Foo et al., 2012; Ghaly et al., 2014; Ghaedi et al., 2014, 2015; Pathania et al., 2017; Safardoust-Hojaghan & Salavati-Niasari, 2017; Zidan et al., 2018). The absorbance maximum of methylene blue is known to be measurable at 664 nm (depending on the solution conditions) and to change color in the presence of electron donors. Thus, the concentration of MB remaining in a solution after advanced oxidation processes (AOPs) and photocatalysis can be monitored with the aid of spectrophotometry (Hong et al., 1999; Maezawa et al., 2007; Reza et al., 2017; Natarajan et al., 2018). AOPs using UV or IR light were favored in the past as they were regarded as ecofriendly and inexpensive methods (Ni et al., 2015). Hydrogen peroxide can also be used to increase the decomposition of dye through the production of reactive oxygen species (ROS) in the mixture (Srivastava et al., 2013; Rao et al., 2009; Sobana & Swaminathan, 2007; Aleboyeh et al., 2012). As an ROS, hydroxyl radicals (•OHs) are strong oxidants that are produced in the presence of a catalyst and/or UV light for removing color from dye solutions (Aguedach et al., 2005). Hydroxyl radicals are the strongest oxidizing agents (i.e., have the highest standard reduction potential) among all ROS, only second to molecular fluorine (F_2) in oxidizing capacity and able to act almost 10^{12} times faster than ozone (Munter, 2001; Choe & Min, 2006; Babuponnusami & Muthukumar, 2014). •OHs are also known to effectively degrade dyes (Jin et al., 2014; Navgire et al., 2016). In preference to catalytic wet peroxide oxidations and AOPs for wastewater treatment, •OH may be desirable for employing a Fenton-like heterogeneous catalyst, where the homogeneous Fenton reagent is basically a mixture of aqueous Fe^{2+} and H_2O_2, in order to produce •OH (Martinez et al., 2011; Yang et al., 2013). The main advantage of the heterogeneous catalyst is that it can be removed at a desired stage of controlled oxidation and be regenerated for further use. The more •OH is generated, the more the absorbance value of the MB solution decreases, as demonstrated by Gogoi and Sarma (2017); however, these authors used a relatively high concentration of H_2O_2 along with β-cyclodextrin-supported nanoceria catalyst for the oxidative degradation of MB, which increased the cost of treatment. The existence of the Ce^{3+} and Ce^{4+} redox cycle on the surface of the nanoceria catalyst may play a favorable role in heterogeneous Fenton-like reactions (Kamer et al., 2019). In the photocatalytic process, the catalyst's band-gap energy is important as it is responsible for the redox power of the substance; therefore, a band-gap energy in the range of 2.0 to 3.3 eV is suggested as ideal for a semiconductor (Ni et al., 2015). Metal oxide semiconductors have more positive valence band potentials compared to semiconductors and also have the ability to produce •OH in suspensions (Chan et al., 2011). Cerium oxide has a band-gap energy of 3.2 eV and is the only lanthanide with two stable oxidation states (+3 and +4; Lu et al., 2011; Channei et al., 2014: Mohammad et al., 2014; Zheng et al., 2017).

This study synthesizes zahter-coated nanoceria (ZCNC) using the procedure recently introduced by our research group (Kamer et al., 2019) following the major outlines of green chemistry and uses the ZCNC as a Fenton-like catalyst for MB degradation. During the degradation of the dye, •OHs were produced both in the catalytic (ZCNC + H_2O_2) and photocatalytic (ZCNC + H_2O_2 + UV) processes. We measured MB's decolorization at 664 nm and recorded the decrease in MB's absorbance values. We also studied the reaction kinetics and evaluated the catalytic efficiency of nanoceria using the calculated *k* (rate constant) values.

MATERIAL AND METHODS

Chemicals and materials

All chemicals were of an analytical grade and used without further purification. The methylene blue, cerium (III) nitrate hexahydrate, hydrogen peroxide (H_2O_2, 30% w/w), and ethyl alcohol (EtOH) were purchased from Merck and Sigma Aldrich. *Thymbra spicata* (zahter) was supplied from Malatya Pazarı Kuruyemiscilik Sanayı ve Ticaret, A.S., Istanbul. Nanoceria particles were synthesized and coated with zahter according to the procedure proposed by Kamer et al. (2019), with deionized water (Simplicity UV Millipore) being used throughout.

Spectral measurements were made with the Varian Cary 100 Bio UV-VIS spectrophotometer. The Bandelin Sonorex ultrasonic bath, Hermle Centrifuge Z 206 A, Heidolph vortex, and Chiltern magnetic stirrer were used for the equilibration and extraction tests. For the photocatalytic experiments, the Kerman UV 6/14 reactor with 6-Watt UV lamps were used as the source of radiation, emitting light at 254 nm.

Synthesis of Nanoparticles

The ZCNC particles were synthesized using an eco-friendly approach with coprecipitation in the basic medium. The *Thymbra spicata* plant extract is called zahter, a Middle Eastern herb commonly found in the Antakya region of Türkiye, and was first used for the green synthesis of the cerium oxide nanoparticles due to its high antioxidant capacity. The plant extract was used because it helps the reduction of metal oxides and nanoparticle aggregation in the environment.

Ten grams of dried zahter was extracted in 100 mL of deionized water at 60°C. After 15 min of agitation and incubation, the mixture was filtered off and 15 g of solid $Ce(NO_3)_3$ were added; the temperature of the mixture was then increased from 60°C to 80°C. In order to adjust the pH value to 9-10, a 1.0 M Na_2CO_3 solution was added drop by drop. Due to the low solubility (K_{sp} = 7.10^{-21}) of the $Ce(OH)_3$ complex, light brown precipitates suddenly formed. As the reaction progressed, the precipitate color changed to purple. Then at around pH 9.5, the color of the precipitate changed to a dirty yellow, with $Ce(OH)_4$ also being formed in the oxidation of $Ce(OH)_3$. The mixture was allowed to stand at 80°C under constant stirring for 4–6 hours. In order to re-

move impurities, the particles were washed, filtered, and dried at 50°C (Kamer et al., 2019). The SEM image of the synthesized ceria nanoparticles (CeONPs) was taken and is shown in Fig.1.

Preparing the solutions

The MB solution (10 mg/L) was prepared using deionized water and homogenized in an ultrasonic bath. A 10 mM H_2O_2 solution was prepared using 100 µL of a 30% (w/w) H_2O_2 solution diluted with 1 L of deionized water.

Methods

Photocatalytic degradation of methylene blue

Ten milliliters of 1.1×10^{-3} M MB were mixed with deionized water and diluted to 80 mL. The mixture was allowed to stand under UV radiation, with samples being analyzed at regular time intervals. Absorbance values were recorded at 664 nm, which is MB's maximum absorption wavelength.

Degradation of methylene blue by H_2O_2 interaction

Five milliliters of a 30% (w/w) H_2O_2 solution was added to 15 mL of 1.1×10^{-4} M MB and diluted to 80 mL using deionized water. Changes in absorbance values were measured at 664 nm.

Photocatalytic degradation of methylene blue by h_2o_2 interaction

Fifteen milliliters of 1.1×10^{-4} M MB were mixed with 5 mL of a 30% (w/w) H_2O_2 solution, and the final volume was adjusted to 80 mL using deionized water. Absorbance values were measured at 664 nm and time intervals of 0.5, 10, 15, 20, 30, 45, 60 and 90 minutes.

Degradation of methylene blue by nanoceria interaction

A mass of 0.1 g of nanoceria was allowed to contact 15 mL of 1.1×10^{-4} M MB for 10 min, then the total volume was adjusted to 80 mL using deionized water. The absorbance values of the prepared solutions were measured over time intervals of 0-90 min.

Photocatalytic degradation of methylene blue by nanoceria interaction

A mass of 0.1 g of nanoceria was mixed with 15 mL of 1.1×10^{-4} M MB, then diluted to 80 mL using deionized water. The absorbance values were measured over time intervals of 0-90 min under UV radiation.

Photocatalytic degradation of methylene blue by both nanoceria and H_2O_2 interaction

A mass of 0.1 g of nanoceria was mixed with 15 mL of 1.1×10^{-4} M MB and 5 mL of 30% (w/w) H_2O_2, then diluted to 80 mL using deionized water. The absorbance values were measured over time intervals of 0-90 min under UV radiation.

Kinetic study of methylene blue degradation

The catalytic activity of the zahter-coated nanoceria was investigated for the degradation of methylene blue. The initial and final concentration values of the solutions were calculated with the aid of a methylene blue calibration graph. Graphs of the *Ln (Ci / Ct)* values over time were drawn for each method (C_i and C_t denoting the initial and instantaneous concentrations, respectively) using the integrated rate expression, and the rate constants (k) were calculated from the slopes of the lines represented by Eq. 1. In this case, Eq. 1 can be simplified to an apparent first-order kinetic model as follows (Saien & Khezrianjoo, 2008):

$$ln\frac{[Ci]}{[Ct]} = kt \quad (1)$$

where C_i is the initial concentration, C_t is the remaining concentration after time t and k is the first-order rate constant. The calculated rate constants have been tabulated and evaluated.

RESULTS AND DISCUSSIONS

Characterization of nanoparticles

For the characterization of the synthesized nanoceria nanoparticles (CeONPs), SEM images were taken, with the average particle size being shown to vary between 20 and 35 nm (Figure 1).

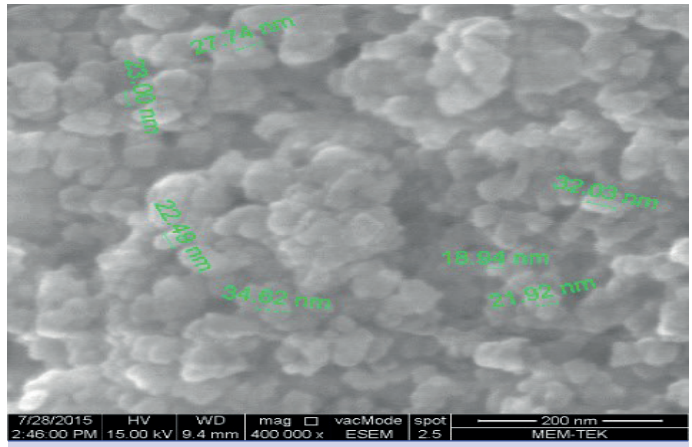

Figure 1. SEM image of zahter-coated nanoceria particles..

In order to define the functional groups of the zahter-coated nanoceria, a FTIR spectrum was obtained by scanning in the range of 650 – 4,000 cm^{-1} (Figure 2). The peaks at 3,425 cm^{-1} from the zahter-coated nanoceria were attributed to the O–H stretching vibration in the OH^- groups. The peak around 1,476 cm^{-1} was assigned to the bending vibration of the C-H stretching in the polyphenols. The bands located at around 723, 750, and 1,072 cm^{-1} have been attributed to the CO_2 asymmetric stretching vibration, carbonate bending vibration, and C-O stretching vibration, respectively. All these band assignments indicate that the plant polyphenols may be the components in powdered nanoparticles, in addition to them clinging on the surface of the particle capping. This agrees with the proposed molecular structure.

The observed spectrum of the sample also exhibited a strong broad band below 650 cm^{-1}, which may be due to the δ (Ce-O-C) mode of CeO_2.

Absorption Spectra of the MB Solution Alone as well as Degraded with Nanoceia, UV Light, and H_2O_2

Figure 3 shows the collection of the absorption spectra of the seven solutions, which include 1.1×10^{-4} M MB solution (alone),

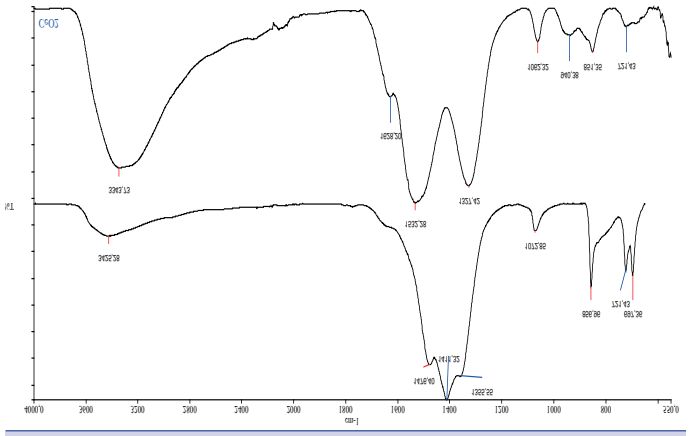

Figure 2. FT-IR spectra of the zahter-coated nanoceria particles (lower spectrum), along with that of uncoated nanoceria (upper spectrum).

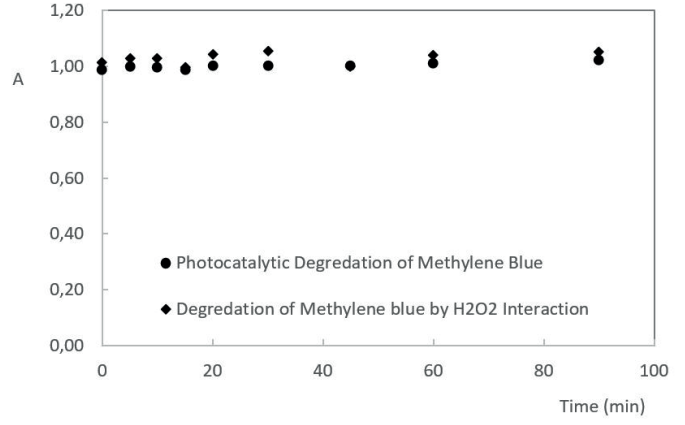

Figure 3. The absorption spectra of methylene blue solution alone as well as degraded with all possible binary and ternary combinations of nanoceria, UV, and H_2O_2.

MB degraded individually with nanoceria, UV, or H_2O_2, MB degraded with the possible binary combinations of these agents, and finally MB degraded with the ternary combination of (nanoceria + UV + H_2O_2).

Photocatalytic and H_2O_2–Induced Degradation of MB

In order to observe the degradation of methylene blue (i) under UV radiation and (ii) after allowing the 1.1×10^{-4} M MB solution to interact with 5 mL of 30% (w/w) H_2O_2, the absorbance values of the MB solution were recorded for 90 min at 664 nm. This analytical wavelength of 664 nm specifically corresponds to the monomeric form of MB (Gogoi & Sarma, 2017). As long as ZCNC was excluded from the reaction as a catalyst, both degradation attempts gave rise to a stable absorbance with no significant difference over time when compared to the initial value (Figure 4).

Because MB shows high stability in acidic and neutral solutions, the absorbance values of the initial MB solution did not show any dramatic decrease (Mills & Wang, 1999).

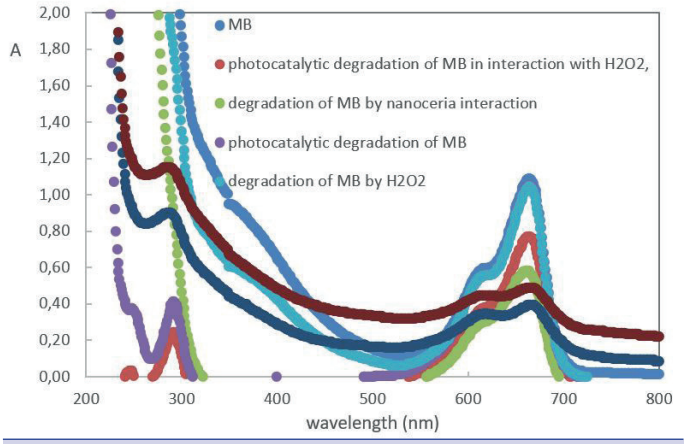

Figure 4. Absorbance values of the 1.1×10^{-4} M MB solution under UV radiation and after H_2O_2 interaction.

Photocatalytic Degradation of MB by H_2O_2 Interaction and MB Degradation by ZCNC Catalyst Interaction

Figure 5 shows the degradation of 1.1×10^{-4} M MB with H_2O_2 and after allowing 0.1 g of ZCNC to contact 15 mL of 1.1×10^{-4} M MB. As seen in Figure 5, MB degradation by H_2O_2 interaction caused a 36% decrease in absorbance, whereas the initial absorbance of 1.1×10^{-4} M MB had decreased by 52.5% with ZCNC alone. This experiment shows the importance of reactive species adsorbed on ZCNC and displaying exceptional surface properties with regard to MB degradation.

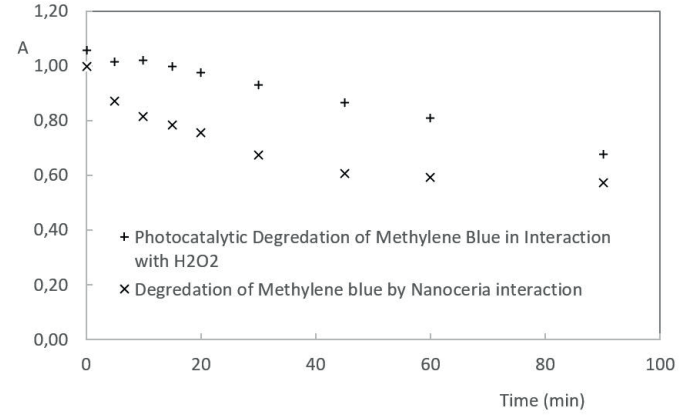

Figure 5. The change in absorbance of 1.1×10^{-4} M MB by H_2O_2 interaction under UV radiation and by ZCNC interaction.

Photocatalytic Degradation of MB by ZCNC Interaction and Photocatalytic Degradation of MB by both ZCNC and H_2O_2 Interaction

A mass of 0.1 g ZCNC was allowed to contact 1.1×10^{-4} M MB, and the absorbance values recorded over 90 min under UV radiation (Figure 6). The absorbance values decreased by 60%. Meanwhile, the same amount of ZCNC was mixed with 15 mL of 1.1×10^{-4} M MB and 5 mL of 30% (w/w) H_2O_2 diluted to 80 mL us-

ing deionized water, and the absorbances measured over 90 min under UV radiation. With respect to the initial absorbance of the MB solution, a decrement of 63% was observed (slightly better than 60%), indicating that even a binary combination of ZCNC + UV would suffice to bring about a significant degradation of MB.

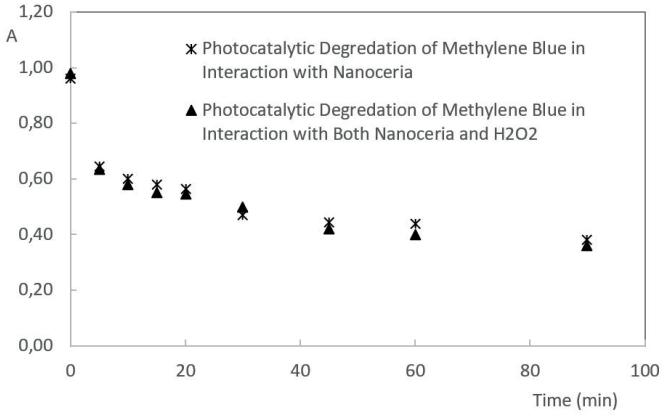

Figure 6. The change in absorbance values of 1.1×10^{-4} M MB with ZCNC under UV radiation and in interaction with both H_2O_2 and ZCNC under UV radiation.

Kinetic study of MB degradation associated with nanoceria properties

The integrated rate expression has been used to determine the rate constant values of the reactions. The rate constant values of the reactions were calculated by equation 1., and are displayed in Table 1. Figure 7 shows the maximum degradation rate to have been obtained using the zahter-coated nanoceria (ZCNC) catalyst with H_2O_2 under UV radiation; the obtained rate constant, however, does not significantly differ from that for the binary combination of ZCNC + UV light. In other words, just nanoceria (even without using hydrogen peroxide) may be an effective photooxidation catalyst, as demonstrated in the oxidative degradation of methylene blue. Also, UV light may provide the necessary band gap energy for the generally accepted $O_{2p}\rightarrow Ce_{4f}$ (Corma et al., 2004) or the strongly mixed energy-level 4f→5d intervalence band transitions of nanoceria. The redox properties of nanoceria are known to be affected by particle size, shape, surface chemistry, and many other factors such as local pH, as well as by the types of additives and ligands attached to surface coatings, resulting in quite different surface energies and reactivities for each specified preparate of nanoceria (Grulke et al., 2014). Because the Ce(III):Ce(IV) ratio is an important element of nanoceria's oxidative catalytic ability, the use of ZCNC in this study may bear a higher proportion of Ce_2O_3-to-CeO_2 due to the reducing power of zahter phenolics, thus enabling a stronger catalytic efficiency for the reduction of molecular oxygen in order to produce surface-adsorbed reactive species, as proposed by Liu and Sun (2015). In other words, this is done without requiring the more expensive chemicals,

H_2O_2, for the oxidative conversion of MB.

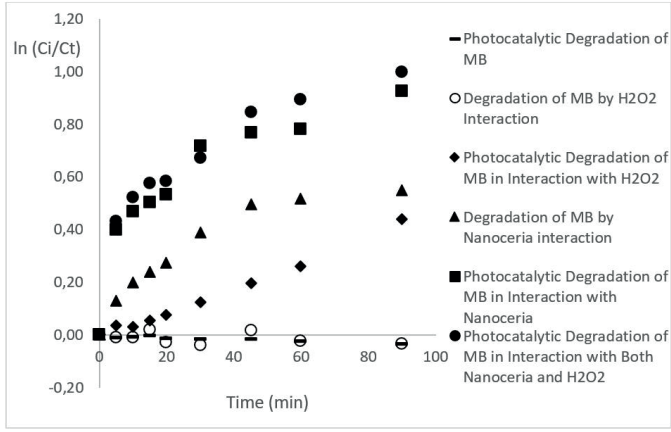

Figure 7. Reaction kinetics curve for the MB degradation with the proposed degradation process. (- = photocatalytic degradation of MB; O = degradation of MB by H_2O_2; ◆ = photocatalytic degradation of MB in interaction by H_2O_2; ▲ = degradation of MB by ZCNC catalyst; ■ = photocatalytic degradation of MB by ZCNC catalyst; ● = photocatalytic degradation of MB by both H_2O_2 and ZCNC catalyst).

Table 1. Calculated Rate Constants for Different Degradation Procedures of MB.

Degradation Process	Rate Constant (min^{-1})
Photocatalytic degradation of MB	4.0×10^{-4}
Degradation of MB by H_2O_2	3.0×10^{-4}
Photocatalytic degradation of MB by H_2O_2	4.6×10^{-3}
Degradation of MB by ZCNC catalyst	8.2×10^{-3}
Photocatalytic degradation of MB by ZCNC catalyst	1.37×10^{-2}
Photocatalytic degradation of MB by both H_2O_2 and ZCNC catalyst	1.49×10^{-2}

CONCLUSION

This study has been designed to synthesize and employ an environmentally friendly catalyst of nanoceria (ZCNC) for the Fenton-like oxidation of dyes (as represented by MB) in wastewater by considering the main goals of green chemistry. For the synthesis of ZCNC, the combination of precipitation and sol-gel method were found to be operationally easy and effective. The synthesized catalyst was characterized by SEM, with the zahter coating onto the surface being confirmed by FTIR analyses. Coating nanoceria with zahter enhanced the favorable surface properties of the nanoceria, thus increasing the number of active sites and minimizing the particle size while also ensuring a better degradation of the organic dye. ZCNC proved to have im-

proved surface properties compared to that of the parent metal oxide. MB degradation was performed using ZCNC at room temperature in a Fenton-like reaction where it acted as a heterogeneous catalyst in the presence of H_2O_2 under UV radiation. The results from the kinetic experiments revealed the photocatalytic degradation of MB by both ZCNC and H_2O_2 interaction to have been more rapid than the other degradation procedures this study tested; however, this result did resemble the performance of the photocatalytic degradation of MB by ZCNC interaction. This was also reflected in the kinetic rate constants. When referring to a methylene blue-hydrogen peroxide system, H_2O_2 is not an effective oxidant of MB in acidic media, even at high concentration levels. However, employing a catalyst with strong oxidative properties such as nanoceria may cause MB decomposition through N-demethylation (Katafias et al., 2010). Fenton-like oxidative degradation of MB had earlier been claimed to produce small molecules (not totally identified) that resulted from the destruction of the aromatic rings (Yang et al., 2009). To synthesize and employ such a heterogenous catalyst is more applicable and advantageous compared to Fenton reactions with a limited pH range. Normally, nanoceria is well known for its photo-inactivity due to the large band-gap energy of 3.2 eV for the $O_{2p} \rightarrow Ce_{4f}$ electronic transition. Thus, this study may be the first of its kind to show that undoped nanoceria (simply prepared with zahter plant extract) may show photocatalytic degradation ability toward a redox-active dye (MB) without significantly necessitating H_2O_2. This low-cost nanoceria may prove to be a good photocatalyst for the degradation of recalcitrant organic dyes in wastewater and industrial effluents.

Many metal oxides such as cobalt, copper, and manganese are used as catalysts in place of iron in Fenton-like systems (Dong et al., 2021). However, the literature is limited with regard to MV degradation due its challenging structure needing two aromatic groups to disable its degradation. Many degradation applications still exist that use different dye molecules alongside CeO_2 (Zang et al., 2017; Xiazhang et al., 2012); however, this study shows superiority at the reaction kinetics for the first time in the literature by installing green synthesized nanoceria as catalyst for the degradation of MB and achieving a rate constant of 1.49 10^{-2} min^{-1}, thus enhancing a remarkable degradation percentage.

Conflict of Interest: The authors declare the article to have no conflicts of interest.

Ethics committee approval: This article has required no ethics committee approval.

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