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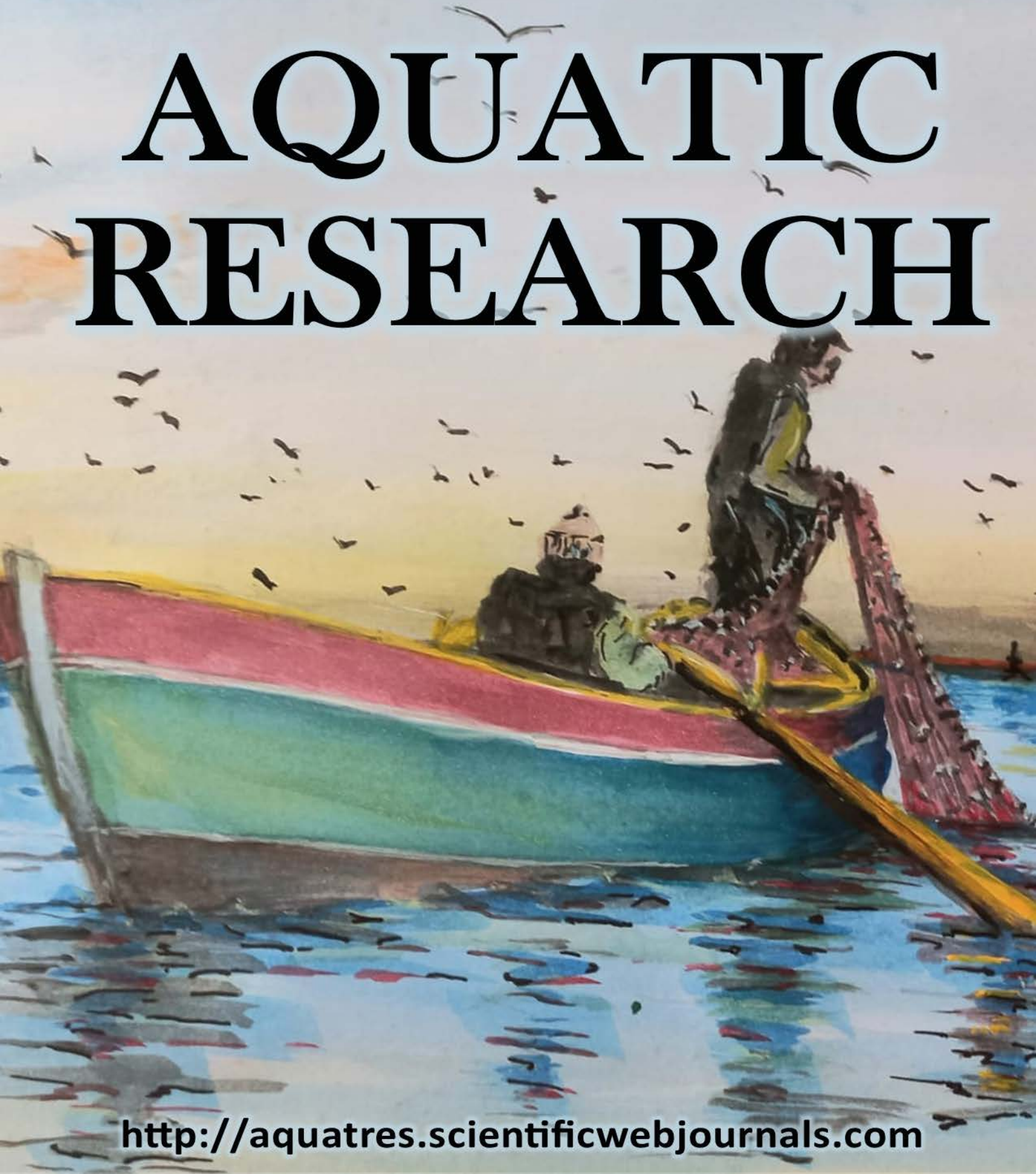

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Aquatic Biology, Aquatic Ecology, Aquatic Environment and Pollutants, Aquaculture, Conservation and Management of Aquatic Source, Economics and Management of Fisheries, Fish Diseases and Health, Fisheries Resources and Management, Genetics of Aquatic Organisms, Limnology, Maritime Sciences, Marine Accidents, Marine Navigation, and Safety, Marine and Coastal Ecology, Oceanography, Seafood Processing, and Quality Control, Seafood Safety Systems, Sustainability in Marine and Freshwater Systems The target audience of the journal includes specialists and professionals working and interested in all disciplines of marine and aquatic sciences.

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Short Communication

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Investigation of multiple resistance frequencies (antibiotic and heavy metal) of bacteria isolated from Gökçeada Island coastal marine sediment

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ABSTRACT

Marine sediments are important reservoirs for antibiotics and heavy metals. Bacteria play a key role in polluted sedimentary habitats. This study aimed to identify heavy metal and antibiotic resistance in marine sediment bacteria isolated from Gökçeada Island in Turkiye. The samples were collected seasonally from 10 different sampling stations in 2015. Ninety isolates determined by VITEK 2 were tested against seven antibiotics using the disk diffusion method. The minimum inhibitory concentration values were measured against four heavy metal salts. The antibiotic resistance frequency rates were ordered as sulphonamides compound (93.3%), cefotaxime (78.9), ampicillin (77.8%), oxacillin (67.8%), rifampicin (57.8%), imipenem (1.1%), and oxytetracycline (0%). The heavy metal resistance ratios against $ZnCl_2$, $CuSO_4$, $Pb(CH_3COO)_2$, and $HgCl_2$ were measured as 100%, 100%, 96.7%, and 73.3% respectively. The multiple heavy metal resistance index values were ranged from 0.75 (22.2%) to 1.0 (77.8%). The results show significant heavy metal and antibiotic contamination in the sediments of the Gökçeada Island. It is recommended that measures be taken against antibiotics and heavy metal pollution, as well as identifying and monitoring critical control points.

Keywords: Antibiotic resistance, Gökçeada island, Heavy metal resistance, Heterotrophic bacteria, Marine sediment

Introduction

Marine sediments are the important habitats of the islands. Biogeochemical processes determine the sediments' nutrient cycles and organic matter degradation (Arndt et al., 2013). The sediments are highly dynamic environments with rich microbial diversity. Microorganisms may grow in such rich environments by using different energy sources. Although approximately 70% of the earth's surface is formed of marine sediments, the diversity and features of bacteria in the marine sediments have not been understood entirely (Jørgensen & Boetius, 2007; Hoshino et al., 2020). Coastal marine sediments in aquatic ecosystems are potential reservoirs of various antibiotics and heavy metals (Buccolieri et al., 2006; Matyar et al., 2008; Vignaroli et al., 2018). Those ecosystems act as a pool of resistance genes, and marine sediment bacteria within the sediments may effectively exchange the resistance globally (Yang et al., 2013).

Antibiotics are the main group of pharmaceuticals. They have been used for several reasons, both for human and veterinary health, but the environmental risk of the antibiotics remains unclear. Resistance of bacteria against antibiotics in aquatic environments is still poorly studied (Kümmerer, 2009). However, antibiotic pollution threatens marine sediments' bacterial diversity and ecosystem health (Näslund et al., 2008). Environmental bacteria may transfer antibiotic resistance to human pathogens, which may cause serious health problems. Antibiotic discharges must be monitored consistently to define potential risks (Larsson, 2014).

Heavy metals are toxic pollutants and may be considered the major contaminants of marine sediments. Heavy metal contamination in marine environments details the long-term negative effects on the ecosystem (Bryan & Langston, 1992; Gillan et al., 2005). Bacteria metabolisms are highly sensitive to heavy metal contamination. Bioaccumulation of heavy metals in marine environments becomes a crucial issue. Some bacteria can survive in highly contaminated environments and tolerate certain concentrations of heavy metals. Heavy metal-resistant bacteria could prove valuable for detoxifying harmful heavy metals (Iyer et al., 2005; Nithya et al., 2011). Bacteria may be useful as bioindicators of pollution factors in marine environments (Dell'Anno et al., 2003).

It is established that heavy metal exposure and antibiotic resistance in bacteria are connected. Heavy metal contamination can be a significant factor in selecting antibiotic resistance traits, with implications for both environmental and clinical settings. However, thoroughly exploring the relationship between heavy metals and antibiotics is necessary to grasp the mechanisms underlying antibiotic resistance (Baker-Austin et al., 2006).

Antibiotic and heavy metal pollution pose serious threats to human health. However, bacterial resistances influenced by anthropogenic pollution have not been studied extensively enough to understand their effects on aquatic ecosystems fully (Baquero et al., 2008; Marti et al., 2014; Sharifuzzaman et al., 2016).

Gökçeada Island, which has an area of 285.5 $km^{2,}$ is the biggest in Turkiye, located between Samothrace Island, Lemnos Islands, and Gallipoli Peninsula (Balkıs et al., 2001). Previous studies have shown that Gökçeada Island has significant importance for marine life in terms of its rich biological diversity, and it has been suggested to set special regulations to protect against negative pollution effects (Keskin & Ünsal, 1998; Tarkan, 2000; Akmirza, 2013; Acarli et al., 2018; Altın & Ayyıldız, 2018; Gönülal & Güreşen, 2014; Güreşen et al., 2020).

Several studies have been conducted to assess the levels of heavy metal and antibiotic resistance among heterotrophic bacteria found in marine sediments in Turkiye (Altuğ ve Balkıs, 2009; Matyar et al., 2008; Kaçar et al., 2013; Kaçar & Koçyiğit, 2013; Tuncer & Bizsel, 2017; Altuğ et al., 2020). In previous studies, marine bacteria from both the seawater and sediment around Gökçeada Island were examined to investigate their metabolic characteristics and enzyme profiles (Altuğ et al., 2010; Cardak et al., 2013; Çiftçi-Türetken & Altuğ, 2016; Türetken, 2021). However, no research regarding antibiotic and heavy metal resistance in the vicinity of Gökçeada Island has been documented in the literature. The primary aim of this study was to analyse the prevalence of heavy metal and antibiotic resistance among marine heterotrophic bacteria isolated from coastal sediments to identify critical pollution sites around Gökçeada Island in Turkiye.

Materials and Methods

Sampling Area

The sediment samples were collected from 10 distinct sampling stations around the coastal regions of Gökçeada Island in Turkiye (see Figure 1). Stations have been chosen as recreational areas (St4-5-6-8-9), ports (St 2-3-7), bays (St1-5), and reference points (St 10) where there is no human activity. The study was conducted seasonally from April to November 2015. The collection of sediment samples was performed using an

Ekman Grab (Hydrobios), which was stored in sterile containers. Subsequently, the samples were refri-gerated and transported to the laboratory while maintaining a cold chain.

Identification of Bacterial Isolates

The sediment samples were aseptically weighed at one gram each. Serial dilutions were prepared using sterile phosphate-buffered distilled water. Marine Agar (Difco) served as the growth medium for heterotrophic bacteria. Petri dishes were then incubated at 22 ±0.1°C. Colonies that formed on the plates after 72 hours were monitored every 24 hours. Selected isolates were categorised based on gram reactions as GN (Gram-negative fermentative and non-fermentative rods), GP (Gram-positive cocci and non-spore-forming rod-shaped bacteria), and BCL (Gram-positive spore-forming rods). Colonies were identified using the VITEK 2 Compact 30 automatic micro-identification system (bioMerieux, France). Following identification, ninety bacterial isolates (comprising sixteen different species) were tested against various antibiotics and heavy metals (refer to Table 1).

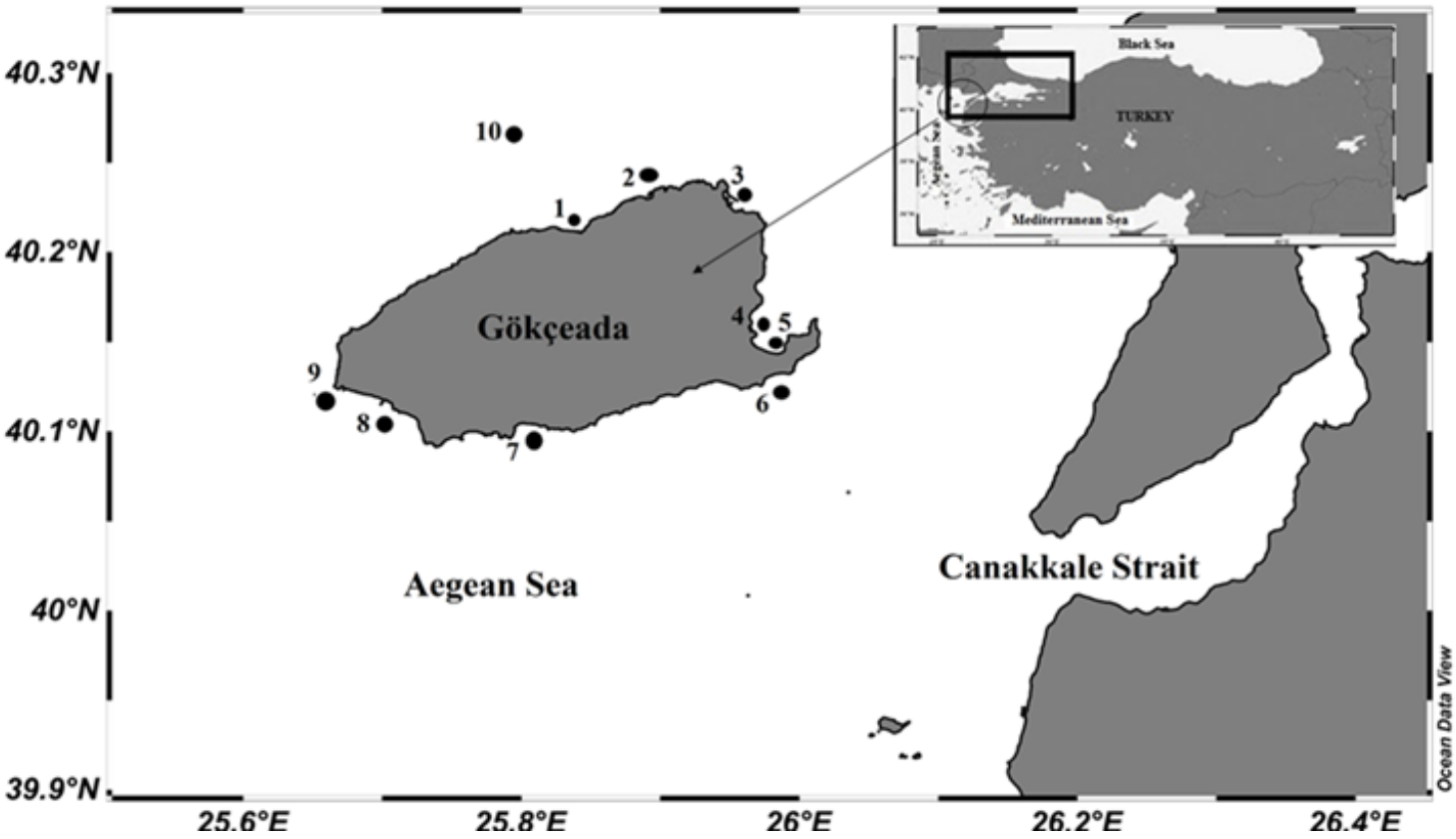

Figure 1. The sampling stations around the Gökçeada Island.

Table 1. The isolate codes and tested bacteria species.

Bacteria species	Isolation Frequency (%)	Station Code
Acinetobacter baumannii complex	2.2	St6 – St9
Acinetobacter calcoaceticus	3.3	St2 – St7 – St10
Acinetobacter haemolyticus	1.1	St5
Acinetobacter lwoffii	3.3	St3 – St6 – St8
Acinetobacter nosocomialis	2.2	St1 – St5
Acinetobacter pittii	2.2	St6 – St7
Aeromonas salmonicida	1.1	St5
Bacillus cereus/thuringiensis/mycoides	5.6	St3 – St4- St5 – St8- St9 – St10
Burkholderia mallei	3.3	St2 – St4 – St9
Enterococcus faecalis	1.1	St5
Kocuira kristinae	1.1	St5
Pasteurella canis	1.1	S4
Pseudomonas stutzeri	1.1	St4
Serratia marcescens	43.3	St1 – St2 – St3 – St4 – St5 – St6 – St7 – St8 – St9
Sphingomonas paucimobilis	15.6	St2 – St3 – St4 – St5 - St6 – St7 – St9
Stenotrophomonas maltophilia	4.4	St2 – St7 – St9 – St10

Antibiotic Resistance

As Bauer et al. (1966) described, the disk diffusion method used Mueller-Hinton agar to assess the antibiotic resistance of bacteria isolated from the sediment samples. Ninety different bacterial isolates were tested against seven distinct antibiotic discs, including Ampicillin 10 µg (AMP), cefotaxime 30 µg (CTX), imipenem 10 µg (IPM), rifampicin 2 µg (RD), oxacillin 5 µg (OX), oxytetracycline 30 µg (OT), and sulphonamides compound 300 µg (S3). Blank sterilised discs (Oxoid, UK) were also negative controls. After preparing pure cultures, the bacterial cell density was adjusted to 0.5 McFarland. Each isolate was spread onto Mueller-Hinton agar plates, and antibiotic discs were carefully placed. Incubation occurred for 24 hours (species-dependent) at 37 ±0.1°C. The diameters of the inhibition zones (in mm) were measured and interpreted by comparing them to the breakpoint diameters specified in The Clinical and Laboratory Standards Institute's (CLSI) tables. Tested isolates were categorised as resistant, intermediate, or susceptible. Quality control organisms recommended by CLSI were also tested to ensure the accuracy of the experiment (CLSI, 2018). The multiple antibiotic resistance (MAR) indexes were calculated based on the number of resistant isolates and the number of antibiotics tested, following the method outlined by Krumperman (1983).

Heavy Metal Resistance

The minimum inhibitory concentration (MIC) method assessed heavy metal resistance against four metal salts. Solutions of zinc sulfate ($ZnCl_2$), mercury chloride ($HgCl_2$), copper sulfate ($CuSO_4$), and lead (II) acetate ($Pb(CH_3COO)_2$) were prepared and sterilised using a 0.45 µm pore-sized filter (Sartorius). Fifty microliters of each heavy metal solution were inoculated into microplate wells using serial dilution with sterilised water. The initial and final molar concentrations ranged from 12 mM to 0.01 mM. The McFarland value for bacterial cell density was set at 0.5, and 50 µL of bacterial suspensions were added to each well. The control well consisted of bacterial solution and sterilised water. MIC values were determined as the lowest concentration of metal salt at which no visible bacterial growth occurred. *Escherichia coli* (ATCC 25922™) served as the reference strain. Isolates exhibiting higher resistance than the reference strain were classified as resistant strains (Matyar, 2012; Gillard et al., 2019). The multiple heavy metal resistance (MHMR) indexes were calculated based on the number of resistant isolates, similar to the MAR index (Krumperman, 1983).

Results and Discussion

Antibiotic Resistance

The antibiotic resistance frequencies of the bacterial isola-tes against 7 different antibiotic types were determined (Table 2). The maximum frequency percentage obtained from each group (R: resistant, S: susceptible, I: intermediate) is shown in bold in the table. The highest resistance frequency was detected at 93% against the sulphonamide compound (S3). There was no resistance against oxytetracycline among all isolates. The highest and the lowest intermediate frequencies were measured as 41% and %1 against rifampicin and imipenem, respectively. The highest susceptible frequency was recorded as 97% against imipenem. The lowest susceptible frequencies were calculated as 1% against cefotaxime, rifampicin, oxacillin, and sulphonamides compound.

Heavy Metal Resistance

The heavy metal resistance frequencies of the bacterial isolates against 4 different heavy metal salts were determined (Table 3). The minimum inhibitory concentrations ranged from 0.38 mM to 12 mM for $ZnCl_2$ and $CuSO_4$, from 0.05 mM to 12 mM for Pb $(CH_3COO)_2$, and 3 mM to 12 mM for $HgCl_2$. The most tolerated and toxic heavy metal salts were determined as zinc sulfate and mercury chloride, respectively. The heavy metal resistance ratios against $ZnCl_2$, $CuSO_4$, $Pb(CH_3COO)_2$, and $HgCl_2$ were measured as 100%, 100%, 96.7%, and 73.3% respectively.

Multiple Antibiotic Resistance and Multiple Heavy Metal Resistance

Figure 2 summarises bacterial isolates' multiple antibiotic resistance (MAR) and multiple heavy metal resistance (MHMR) indexes.

Table 2. The antibiotic resistance frequencies of the bacterial isolates.

Antibiotic derives	Number of isolates			Frequency (%)		
	R	S	I	R	S	I
Ampicillin (10 µg)	70	2	18	77.8	2.2	20.0
Cefotaxime (30 µg)	72	1	17	78.9	1.1	18.9
Imipenem (10 µg)	1	88	1	1.1	**97.8**	1.1
Rifampicin (2 µg)	52	1	37	57.8	1.11	**41.1**
Oxytetracycline (30 µg)	0	63	27	0.00	70	30.0
Oxacillin (5 µg)	61	1	28	67.8	1.1	31.1
Sulphonamides compound (300 µg)	84	1	5	**93.3**	1.1	5.6

R: resistant, S: susceptible, I: intermediate

Table 3. The heavy metal resistance frequencies of the bacterial isolates.

	Metal concentrations (mM)												
Heavy metal salts	**12**	**6**	**3**	**1.5**	**0.75**	**0.38**	**0.19**	**0.09**	**0.05**	**0.02**	**0.01**	**Resistant Isolates**	**%**
$ZnCl_2$ (Reference strain)	61	18	3	6	1	1	-	-	-	- *	-	**90**	**100**
$CuSO_4$ (Reference strain)	5	1	4	28	41	11	-	-	-	- *	-	90	**100**
Pb $(CH_3COO)_2$ (Reference strain)	14	9	1	41	14	4	2	2	3 *	-	-	87	**96.7**
$HgCl_2$ (Reference strain)	5	6	3	-	-	-	-	-	11	41	20 *	66	**73.3**

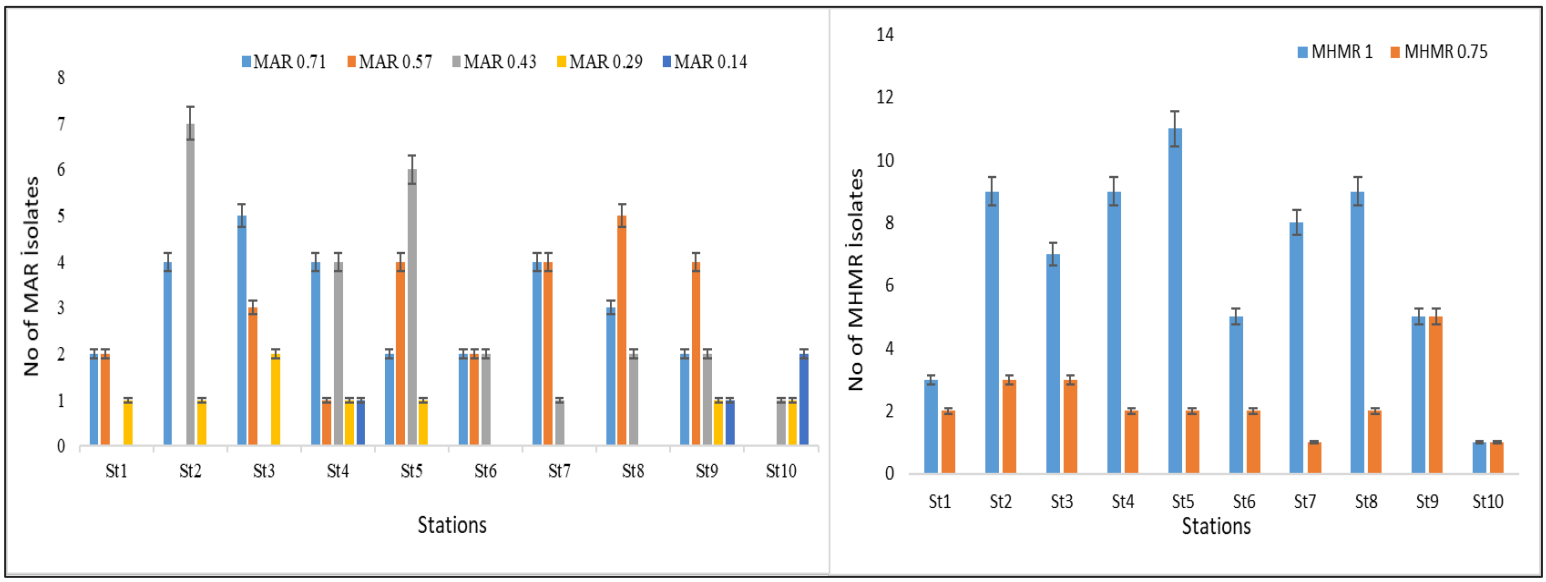

Figure 2. Distribution of multi-resistant bacterial isolates according to stations

The multiple antibiotic resistance (MAR) index values of the bacterial isolates were recorded as 0.71 (33.1%), 0.57 (33.3%), 0.43 (22.2%), 0.29 (8.9%) and 0.14 (4.4%). The multiple heavy metal resistance (MHMR) index values of the bacterial isolates ranged from 0.75 (22.2%) to 1.0 (77.8%). It was noted that 75.5% of the isolates were resistant to the concentrations of all heavy metal salts.

Studies aimed at determining bacterial diversity in marine ecosystems are crucial for comprehending ecosystem dynamics and identifying pollution types. The highly dynamic nature of marine environments, in contrast to terrestrial ones, leads to the development of resistance mechanisms in bacteria as they adapt to environmental conditions. The identification of bacterial isolates exhibiting resistance to encountered pollutants indicates ongoing exposure of the environment to these contaminants (Zeglin, 2015; Delgado-Baquerizo, 2016).

The misuse of antibiotics poses a significant threat to human and ecosystem health. Aquatic environments, heavily impacted by anthropogenic activities, serve as ideal settings for disseminating antibiotic resistance. The emergence of antibiotic resistance has spurred the search for new classes of antibiotics. However, developing novel antibiotic derivatives is economically unfeasible for the pharmaceutical industry. Therefore, it is imperative to regulate and control the unnecessary use of antibiotics through important regulatory measures. Antibiotics enter marine environments via anthropogenic, industrial, and clinical pathways, facilitating the spread of resistance mechanisms among bacteria through horizontal gene transfer. Given bacteria's affinity for surface attachment, the littoral region acts as a dynamic environment conducive to the exchange of resistance traits developed against pollutants such as antibiotics and heavy metals (Sabatino et al., 2020; Zhang et al., 2020; Marti et al., 2014).

Several researchers have documented that marine sediment bacteria isolated from Turkish marine environments exhibit a high antibiotic and heavy metal resistance prevalence. Matyar et al. (2008) reported that bacteria isolated from Iskenderun Bay sediments displayed resistance to ampicillin (94.4%) and imipenem (4.4%). Altuğ et al. (2020) found that sediment bacteria from Güllük Bay exhibited resistance to sulfonamide (100%), rifampicin (100%), tetracycline (100%), ampicillin (100%), nitrofurantoin (98%), and oxytetracycline (98%). Kacar and Kocyigit (2013) demonstrated that bacteria isolated from sediment in the Aliaga ship dismantling zone in the Eastern Aegean Sea were resistant to gentamicin and tobramycin. Additionally, Çardak et al. (2016) indicated that bacterial isolates from the Marmara Sea and the Turkish Straits displayed resistance to kanamycin (82%), vancomycin (78%), and ampicillin (60%). The findings of the present study also reveal high resistance rates among bacterial isolates against commonly used antibiotics, such as sulphonamides compound (93.3%), cefotaxime (78.9%), ampicillin (77.8%), oxacillin (67.8%), rifampicin (57.8%), imipenem (1.1%), and oxytetracycline (0%). These results suggest that islands typically considered less polluted, such as Gökçeada Island, may be more contaminated than anticipated concerning antibiotics. Therefore, monitoring studies are recommended, especially at ports and recreational areas.

Bacteria isolated from the marine environment play crucial roles in ecosystem functioning and biotechnological applications. They acquire various characteristics through adaptation to pollutants, making them valuable for human health and environmental management. Heavy metals, elements naturally occurring in the earth's crust with high weight-to-volume ratios, are present in the marine environment due to both natural processes (such as river runoff, erosion, atmospheric and volcanic activity) and human activities (including the use of fossil fuels, pesticides, mining, wastewater discharge, and the disposal of domestic and industrial waste).

While limited research specifically compares heavy metal resistance among sediment bacteria in Gökçeada Island, several studies have focused on heavy metal levels and environmental contamination in the region. Kahraman et al. (2009) found elevated levels of zinc (Zn), lead (Pb), and other heavy metals in various lichen species. Aslan et al. (2021) reported higher levels of Zn, Pb, and copper (Cu) in sediment samples from the Salt Lake lagoon of Gökçeada Island, attributing this to environmental issues linked to population growth, anthropogenic waste, and sewage. Yılmaz and Tuncer (2021) identified elevated concentrations of heavy metals, with iron (Fe), Zn, Cu, Pb, and cadmium (Cd) being ranked highest in sea urchin species, recommended as pollution biomonitors. Belivermis et al. (2019) found high mercury (Hg) concentrations in cephalopod muscular tissues. Sarı and Cagatay (2001) investigated heavy metal concentrations in surface sediments near Gökçeada Island, reporting it as comparatively unpolluted but affected by anthropogenic and natural inputs, especially Cu and Pb. In the present study, bacterial resistance against heavy metals was found to be highest for Zn, followed by Cu, Pb, and Hg, with mercury being the most toxic. These findings suggest that Gökçeada Island is exposed to Zn, Cu, Pb, and Hg, with heavy metal effects in sediments expected to increase due to rising maritime traffic and pollution levels.

It is recognised that heavy metals' toxicity varies depending on their cycling between components such as water, sediment, flora, and fauna in the marine ecosystem. Heavy metals that remain without dissolution or bacterial degradation accumulate in sediment, persisting as fixed pollutants.

The Multiple Antibiotic Resistance (MAR) and Multiple Heavy Metal Resistance (MHMR) index ratios exceeding a value of 0.2 indicate a potential risk of antibiotic and heavy metal pollution in environments (Krumperman, 1983). Matyar et al. (2008) found that the MAR index of sediment bacteria from İskenderun Bay exceeded the 0.2 thresholds. Conversely, Vignaroli et al. (2018) reported that the MAR index of enterococci isolated from the sediment of the Adriatic Sea remained below the 0.2 threshold, indicating a lower risk to public health. Ab Rahman et al. (2015) demonstrated that a high percentage of sediment bacteria isolated from coastal waters in Malaysia had MAR index values above 0.2.

In the present study, 95.5% of the MAR index values for antibiotic resistance exceeded the threshold of 0.2. This evidence suggests significant antibiotic contamination in the sediment of Gökçeada Island.

Conclusion

With this study, we aimed to elucidate the antibiotic and heavy metal resistance levels among bacteria isolated from sediment samples collected from Gökçeada Island. The high resistance against common antibiotics may signal significant anthropogenic impacts and excessive antibiotic pollution in the sediments. The findings of this study will be instrumental in highlighting the risks associated with antibiotics in the marine environments of Gökçeada Island. Strong measures to control antibiotic pollution and the need for further advanced studies are advised.

Moreover, the high resistance frequencies against heavy metal salts indicate that bacteria have adapted to cope with these metals over a prolonged period, suggesting longstanding heavy metal contamination in the sediment structure of Gökçeada Island. Other studies conducted in the area also indicate heavy metal contamination. Further analysis is warranted to detect heavy metal accumulation in sediment using advanced methods precisely. Additionally, certain bacterial species may have evolved to thrive in high concentrations of heavy metals. Their abilities could be harnessed through experimental methods, including proteomic and transcriptomic studies, for applications in wastewater treatment and other biotechnological endeavours.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require ethics committee permission or any special permission.

Data availability: Data will be made available on request.

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Disclosure: -

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Investigation of benthic macroinvertable fauna and some environmental variables in Sızır Waterfall (Gemerek-Sivas)

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ABSTRACT

In this study, the benthic macroinvertebrate fauna of Sızır Waterfall, which is located in Sivas Province (Türkiye) and has an important place in recreational activities, and some environmental variables (velocity speed, water temperature, pH, conductivity, dissolved oxygen, total hardness of water, Ca, Mg, Cl, salinity, total amount of dissolved matter, PO_4, SO_4, NO_2-N, NO_3-N contents) that may be effective in their distribution were investigated. Also, some elements (Li, B, Na, Al, K, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Sr, Cd, Sb, Ba, Tl, Pb) and a total of 181 types of pesticides were investigated in the studied area. During the wet and dry seasons of 2022, samplings were made from a total of 3 stations: at the beginning of the waterfall (upstream), inside the waterfall (waterfall) and at the exit of the waterfall (downstream). While individuals belonging to Oligochaeta (*Potamothrix* sp.), Gastropoda (*Physa* sp.), Amphipoda (*Gammarus pseudosyriacus*), Ephemeroptera (*Baetis* sp.), Plecoptera, Trichoptera, Coleoptera, Diptera and Odonata were determined. Also the physicochemical analysis results were evaluated in terms of water quality. The physicochemical and biological data of the sampling stations were examined statistically, and based on the similarity obtained, the effects of environmental variables on the distribution of macroinvertebrates were evaluated. The relationship between physicochemical data was analyzed using Pearson Correlation Analysis.

Keywords: Waterfall, Running water, Water quality, Benthic macroinvertebrate, Similarity

Introduction

Waterfalls are morphological formations occurring in slope breaks that may be present along stream beds. These formations, with different shapes and sizes, can differ from each other in terms of their ecological characteristics and biological content.

Aquatic environments must maintain healthy ecological conditions by balancing their physical, chemical, and biological components. Water quality consists of a set of parameters or variables that describe a water body's physical and chemical properties and biological components, which sustain various uses or processes. (Hussen et al., 2018).

Waterfalls are often overlooked in inland water studies because they give the impression of a lifeless area due to the pressure created by gravity (Hussen et al., 2018). For this reason, research on waterfall systems is generally limited to examining their hydrological and geological features regarding their ecotourism potential and their use for drinking and irrigation water purposes (Hussen et al., 2018). However, as an aquatic ecosystem, waterfalls have various functions such as providing clean water, controlling pollution, and supplying some critical chemicals to the ecosystem (Hussen et al., 2018). In nature-based tourism activities, habitat destruction and pollution elements entering the ecosystem damage the natural ecosystem and biodiversity. Therefore, evaluating biological elements and physical and chemical parameters in aquatic ecosystems is important. In addition to the effects of anthropogenic activities, it is reported that climate change and soil type characterised by wet/dry seasons also affect water quality (Hussen et al., 2018). Additionally, any change in physical and chemical parameters in aquatic ecosystems can affect aquatic biota in various ways.

Studies on the water quality of waterfalls and benthic macroinvertebrate groups are also included in limnological research. Nyamangara et al. (2008) investigated some heavy metals in water and sediment samples taken from a waterfall in Zimbabwe, while Hussen et al. (2018) examined physical and chemical parameters in water samples taken from a waterfall in Indonesia from three locations (upstream, waterfall, and downstream) during the wet season. In the previous studies performed in Türkiye, Çağlar & Saler (2014) examined the water quality of Koçan Waterfall and Saplıoğlu et al. (2017) evaluated the water quality of Karpuz Stream, Düden Stream and Kurşunlu Waterfall.

In addition to taxonomic studies investigating benthic macroinvertebrate groups in waterfalls, there are also studies evaluating the regional distributions of these groups and ecological factors that may be effective in their distribution (Sites & Vitheepradit, 2007; Gregoric et al., 2010; Sites et al., 2011; Prommi et al., 2012; Rackemann et al., 2013; Sharifah Aisyah et al., 2015; Clayton & Pearson, 2016; Tavares et al., 1998; Baker et al., 2017; Andrade et al., 2020; Thamsenanupap et al., 2021; Mello & Abessa, 2021; Zakiah et al., 2022). Findings of benthic macroinvertebrate groups are also reported in the studies conducted in some waterfalls in Türkiye. In the study carried out by Kum (2018) in Ilıca Waterfall (Kastamonu), members belonging to the Trichoptera group, one of the benthic macroinvertebrates, were recorded, while Demir (2020) reported the findings obtained from waterfalls in the Black Sea and Mediterranean Regions.

Sızır Waterfall, which is the study area, was declared a second-degree natural protected area by the Sivas Regional Directorate for the Protection of Cultural and Natural Heritage in 2001. There are two studies performed in the previous study area: one of these was published with the title "Hydrogeological and hydrogeochemical properties of the Sızır Springs aquifer" by Aydın & Ekmekçi (2005) and the other with the title "Recreational potential of Sızır Waterfall and its surroundings within the scope of sustainability" by Karadeniz (2013). Also, Aydın & Ekmekçi (2005) presented a study that included data on some chemical analyses from the studied area. Up to now, no study has evaluated the physicochemical and biological properties of Sızır Waterfall together.

Materials and Methods

The Sızır Waterfall is situated between 39°18' north latitude and 35°56' east longitude, within the borders of the Sızır town in the Gemerek (39º11′ N, 36º04′ E) district of Sivas province, located in the Upper Kızılırmak Section of the Central Anatolia Region of Türkiye (Karadeniz, 2013). The waterfall was formed by the pouring of the Göksu Stream (also known as Sızır Suyu), a tributary of the Kızılırmak River, over travertine steps. The height of the waterfall varies according to seasonal water flow, with water falling from approximately 22 meters at peak capacity (Karadeniz, 2013). Some of the waters coming out of the springs (Ayanözü Stream formed by the merger of Çatalkaya, Bağırsak, Kurudere and Erikli streams and Kırkgöz waters coming out of the karstic springs in the town of Sızır) that come out of Akdağ and feed Göksu Stream are transferred to Sızır Dam, while some flow by forming the Sızır Waterfall and mix with the Kızılırmak River (İzbırak, 1971; Saraçoğlu, 1990). It is reported that the flow rate of Sızır Waterfall is the highest in the spring season due to increased precipitation and melting snow. The flow rate is the lowest in the summer due to drought, the amount

of water supplied to the dam, and the use of agricultural irrigation water (Karadeniz, 2013).

In the study area, water and sediment samples were taken from a total of 3 stations (upstream, waterfall, downstream) in the wet season (May 2022) and dry season (August 2022). The sampling periods were determined by taking into account the seasonal characteristics of the waterfall according to the literature. The location called "upstream", where the waterfall's water first comes out, was chosen as station number 1, while the location called “waterfall”, where the waterfall falls, was chosen as station number 2, and the location called “downstream”, the part just before where the waterfall merges with the Kızılırmak River was determined as station number 3. The locations of the waterfall and sampling stations are shown in Figure 1. The coordinates and bottom structures of the sampling stations are given in Table 1.

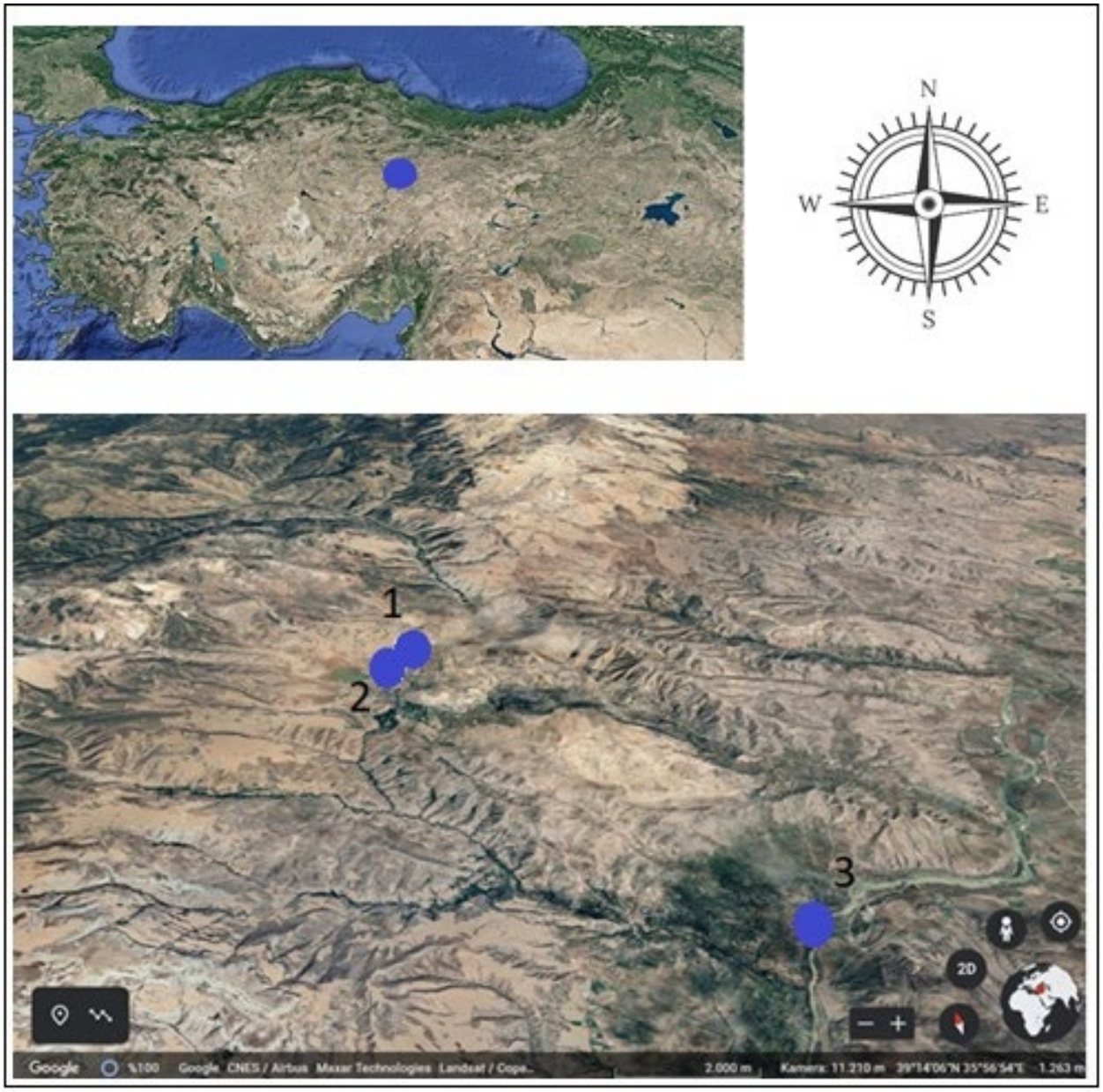

Figure 1. Location of Sızır Waterfall and the sampling stations

Table 1. The coordinates of the sampling stations and their bottom structures

Stations	Coordinates	Locations	Bottom structures
St. 1 (upstream)	39°18′47″ N 35°57′13″ E	Kalebaşı, Sızır / Gemerek	Pebbles, secondary aquatic plants
St. 2 (waterfall)	39°18′19″ N 35°56′49″ E	Köprübaşı, Sızır / Gemerek	Pebbles
St. 3 (downstream)	39°13′03″ N 35º59′14″ E	Tekmen village, Gemerek	Pebbles, sand, secondary aquatic plants

During the field studies, water temperature (°C), pH, electrical conductivity (µS/cm) and total dissolved solids (mg/L) contents were measured by using a portable TE-200 meter. In contrast, the flow rate of the water (m/sec.) was measured using a portable YSI Flow Tracker 2 Handheld-type device. Water samples from the surface were transported to the laboratory using 2L dark-coloured glass bottles by cold chain method and analysed without delay. In the laboratory, dissolved oxygen (mg/L by Winkler method), calcium and magnesium (mg/L by EDTA titration), salinity (‰ by Mohr-Knudsen method) and chloride content (mg/L by titration) were measured by titrimetric methods. The macronutrients (phosphate, nitrite nitrogen, nitrate nitrogen and sulphate) were measured and recorded at mg/L values by spectrophotometric methods (Egemen & Sunlu, 1999). In addition, water samples taken from each sampling station during wet and dry seasons were placed in 50 ml polythene bottles, brought to the laboratory by cold chain, acidified by adding 150 µl (1+1) HNO3, and preserved. For elemental analyses, Agilent Technologies 7700 model inductively coupled plasma mass spectrometry (ICP-MS) device was used in TÜTAGEM (Trakya University Technology Research and Development Centre) laboratories according to U.S. Environmental Protection Agency (EPA) 200.7 and 200.8 methods and Aluminum (Al), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Selenium (Se), Strontium (Sr), Cadmium (Cd), Antimony (Sb), Barium (Ba), Thallium (Tl), Lead (Pb), Lithium (Li), Boron (B), Sodium (Na), Potassium (K) contents were measured as ppb. In addition, a total of 181 pesticides were analysed in water samples using liquid chromatography-mass/mass spectrometry (LC-MS/MS) (Agilent 1260 infinity liquid chromatography, Agilent 6460 Triple Quadrupole MS/MS System, Jet Stream Electrospray ion source) (Table 2). For pesticide analyses in the water samples, the QUECHERS method developed for multiple pesticide residue determination in the extraction of pesticides in the sample was applied. In the chromatographic conditions applied, mobile phase gradient programme 0-1 min / A phase 95%, 7-10 min / A phase 5%, 10.10-12 min / 0% A phase, 12.10-13 min / 95% A phase was used (Mobile Phase A; UPW, 5mM Ammonium Formate, 0.1% Formic acid, Mobile Phase B; Acetonitrile, 0. 1% Formic acid, Column; Poroshell 120 EC-C18, 3.0X50mm (2.7 Micron), Flow rate; 0.5 mL/min, Injection volume; 5 µL, Column Temperature; 40°C, Ionisation Mode; ESI(+), Mode; Dynamic MRM, Gas temperature and flow; 325°C and 10 L/min, Nebulizer; 40 psi, Capillary; 3 kV). The chromatographic operating conditions for pesticide analysis are given in Table 3.

Sediment samples were taken from the sampling stations using a simple mud-grab, and benthic macroinvertebrate samples were collected by sifting the sediment through sieves. Sediment samples were taken from the sampling stations using a simple mud-grab, and benthic macroinvertebrate samples were collected by sifting the sediment through sieves. Benthic macroinvertebrates were also collected from submerged parts of aquatic plants and under stones in water. The obtained materials were put into polyethene bottles, which included 70% ethanol. Benthic macroinvertebrates were sorted under a stereo binocular microscope in the laboratory. Timm (1999), Wetzel et al. (2000), and Karaman and Pinkster (1977) were consulted to determine the taxonomical identification of the groups.

Table 2. List of pesticides measured at the water samples

Acephate	Cycluron	Fluometuron	Mevinphos	Siduron
Acetamiprid	Cymoxanil	Fluoxastrobin-698	Mexacarbate	Simetryn
Acibenzolar-S-Methyl	Cymoxanil	Fluquinconazole -699	Monocrotophos	Spinetoram-741
Aldicarb	Cyprodinil	Flusilazole	Monolinuron	Spinosad A
Aldicarb sulfone	Cyromazine	Flutolanil-703	Myclobutanil	Spirodiclofen
Aldicarb sulfoxide	Desmedipham	Flutriafol	Neburon	Spiromesifen
Ametryne	Dicrotophos	Forchlorfenuron-706	Novaluron	Spirotetramat
Aminocarb	Diethofencarb	Formetanate-hydrochloride	Nuarimol	Spiroxamine
Amitraz	Difenoconazol	Fuberidazole-707	Omethoate	Tebuconazole
Azoxystrobin	Diflubenzuron	Furalaxyl	Oxadixyl	Tebufenozide
Benalaxyl-M	Dimethoate	Furathiocarb	Oxamyl	Tebufenpyrad
Bendiocarb	Dimoxystrobin-688	Hexaconazole	Paclobutrazol	Tebuthiuron
Benfurocarb	Diniconazole	Hexaflumuron	Penconazole	Teflubenzuron
Benzoximate	Dinotefuran	Hexythiazox	Pencycuron	Terbumeton
Bifenazate	Diuron	Hydramethylnon	Phenmedipham	Terbutryn
Bitertanol	Emamectin-Benzoate	Imazalil	Picoxystrobin	Tetraconazole
Boscalid	Epoxiconazole	Indoxacarb	Piperonyl butoxide	Thiabendazole
Bromuconazole	Etaconazole	Ipconazole-713	Pirimicarb	Thiacloprid
Bupirimate	Ethiofencarb	Iprovalicarb	Prochloraz	Thiamethoxam
Buprofezin	Ethirimol	Isoprocarb	Promecarb	Thidiazuron-747
Butocarboxim	Ethofumasate	Isoproturon	Prometon	Thiobencarb-748
Butoxycarboxim	Etoxazole	Kresoxim-methyl	Prometryn	Thiofanox
Carbaryl	Famoxadone	Linuron	Propamocarb-hydrochloride	Thiophonate Methyl
Carbendazim	Fenamidone	Lufenuron	Propargite	Triadimefon
Carbetamide	Fenarimol	Mandopropamid	Propham	Triadimenol
Carbofuran	Fenazaquin	Mefenacet	Propiconazole	Trichlorfon
Carbofuran-3-hydroxy	Fenbuconazole	Mepronil	Propoxur	Tricyclazole-753
Carboxin	Fenhexamid	Mesotrione	Prothioconazole -734	Trifloxystrobin
Carfentrazone Ethyl	Fenobucarb	Metalaxyl	Pymetrozine	Triflumizole
Chlorantraniliprole	Fenproprimorph	Metconazole -718	pyracarbolid	Triflumuron
Chlorfluazuron	Fenuron	Methabenzthiazuron-719	Pyraclostrobin	Triticonazole
Chlorotoluron	Fibronil	Methamidophos	Pyridaben	Vamidathion
Chloroxuron	Fluazinam	Methiocarb	Pyrimethanil	Zoxamide
Clethodim -682	Flubendiamide -695	Methoprotryne	Pyriproxyfen	
Clofentezine	Fludioxonil	Methoxifenozide	Quinoxyfen	
Clothianidin	Flufenacet	Metobromuron	Rotenone-739	
Cyazofamid	Flufenoxuron	Metribuzin	Secbumeton	

Table 3. Chromatographic examination conditions used in pesticide analyses

Mobile Phase		A: UPW, 5mM Ammonium Formate, 0.1% Formic Acid B: Acetonitrile, 0.1% Formic Acid	
Column		Poroshell 120 EC-C18, 3.0x50mm, 2.7 Micron	
Flow speed		0,5 mL/minute	
Injection volume		5 µL	
Column Temperature		40°C	
Ionisation Mode		ESI(+)	
Gas temperature and flow		325°C and 10 L/minute	
Nebulizer		40 psi	
Capillary		3 kV	
Timing	Time (min)	A (%)	B (%)
	0.00	95.0	5.0
	1.00	95.0	5.0
	7.00	5.0	95.0
	10.00	5.0	95.0
	10.10	0.0	100.0
	12.00	0.0	100.0
	12.10	95.0	5.0

Results and Discussion

Table 4 provides data on some environmental parameters measured during the wet and dry seasons at 3 stations selected from Sızır Waterfall. The data show that the flow rate, which varied between 0.66-1.1 m/sec in the wet season, decreased to 0.1-0.45 m/sec in the dry season, and this decrease was quite evident at station 3 (St.3).

It was determined that the water temperature, an average of 25 °C in the wet season, increased to 30 °C in the dry season. The water hardness measured at the sampling stations varied between 22-34 °FS and showed hard water characteristics, possibly due to the high Ca ion values measured. Sızır Waterfall exhibits a completely freshwater characteristic according to the measured salinity values and does not exceed the expected value in freshwater resources in measured conductivity values. However, at St.3, this value increases during the dry season (1966 µS/cm).

The other measured environmental variables were compared with the values in the Water Pollution Control Regulation of Türkiye (SKKY, 2008). According to this, it was found that the measured TDS (total dissolved matter) values decreased to Class II Water Quality level at St.3 during the dry season; the pH values range from 7.5 to 8.2 and indicate Class I Water Quality level; the dissolved oxygen value remained low (signs Class III Water Quality Level), with a maximum of 5.71 mg/L (in the wet season at St.2) and a minimum of 2.85 mg/L (in the dry season at St.3); chloride ions was found as Class I Water Quality level; the macronutrient salt (phosphate, nitrite nitrogen, nitrate nitrogen) values were found to exceed the Class I Water quality values, except for the sulphate value (in Class I Water Quality in terms of sulphate).

The relationship between physicochemical parameters was evaluated by applying the Pearson Correlation Analysis (Krebs, 1999). Accordingly, a positive correlation was found between NO_2-N and PO_4; NO_3-N and pH; Cl and EC; Cl and TDS; EC and TDS. Correlation coefficients are given in Table 5.

The data on measured elements are presented in Table 6. The obtained values were compared with the water quality classes in the Surface Water Resources Control Regulation of Türkiye (YSKKY, 2016). According to this, it was determined that B, Al, As, Cu, Ba, Ba, Zn, Fe, Cd, Co, Cr, Pb, Ni and Se values did not exceed the Class I water quality level, but Mn value exceeded (<100 µg/L) only in the dry season at St.2. V and Sb values were found below the Maximum Permissible Environmental Quality Standard values (MAK-ÇKS) given in YSKKY (2016), and the measured Li ratio was found below the values found in the lakes of Türkiye (Helvacı, 2018). Na ratio, which is stated to be a maximum of 100 mg/L in natural inland waters, and K ratio, which is stated to be a maximum of 10 mg/L in natural waters, were not found to exceed these values in this study (Boyd, 1998; Tepe et al., 2006).

Sampling locations were also compared for physicochemical and elemental contents by Bray-Curtis Cluster Analysis (Fig 2). Accordingly, while there was no significant difference between the sampling stations in terms of both physicochemical and element contents in the wet season, St.3 exhibited different characteristics from the other stations in the dry season (similarity < 50%). Especially the fact that the B, Na, Al, K, V, Co, Ni, Cu, Sr, Cd and Ba values measured in the dry season at station number 3 are higher than the other stations significantly reduces the similarity rate (similarity < 20%) (Fig 3). Although a total of 181 types of pesticides were analysed, no pesticides were found.

Benthic macroinvertebrates collected from the sampling stations were identified taxonomically. In the identification of taxa, the smallest possible taxonomic category was used. *Potamothrix* sp. from Oligochaeta, *Physa* sp. from Gastropoda, *Gammarus pseudosyriacus* from Amphipoda, *Baetis* sp. nymphs from Ephemeroptera, and nymphs and larvae from Plecoptera, Trichoptera, Coleoptera, Diptera and Odonata were found (Table 7).

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Table 4. Data on some environmental variables in Sızır Waterfall

		St. 1	St. 2	St. 3
Wet Season	Velocity (m/sec)	0.66	0.8	1.1
	Water Temperature (°C)	26	25	24
	pH	7.59	7.91	7.85
	Conductivity (µS/cm)	451	440	635
	Dissolved Oxygen (mg/L)	4.95	5.71	5.52
	Total Hardness (°FS)	22.4	33.4	22
	Ca (mg/L)	89.77	92.9	88.17
	Mg (mg/L)	2.72	5.30	16.09
	Chloride (mg/L)	2.99	3.99	4.99
	Salinity (‰)	0.01	0.02	0.01
	TDS (ppm)	225	187	220
	PO_4 (mg/L)	0.220	0.91	0.234
	SO_4 (mg/L)	0.417	0.277	1.988
	NO_3-N (mg/L)	21.79	33.18	33.62
	NO_2-N (mg/L)	0.019	0.19	0.019
		St. 1	**St. 2**	**St. 3**
Dry Season	Velocity (m/sec)	0.32	0.45	0.12
	Water Temperature (°C)	29	31	30
	pH	7.82	7.95	8.23
	Conductivity (µS/cm)	547	530	1966
	Dissolved Oxygen (mg/L)	4.76	3.80	2.85
	Total Hardness (°FS)	31	25	34
	Ca (mg/L)	9.37	33.66	104.28
	Mg (mg/L)	4.69	5.05	5.10
	Chloride (mg/L)	1.99	0.99	26.99
	Salinity (‰)	0.02	0.01	0.01
	TDS (ppm)	273	265	1013
	PO_4 (mg/L)	0.386	0.261	0.400
	SO_4 (mg/L)	0.922	0.660	2.592
	NO_3-N (mg/L)	33.85	43.70	55.53
	NO_2-N (mg/L)	0.047	0.041	0.028

Table 5. Pearson correlation analysis

	NO_2-N	NO_3-N	pH	PO_4	Cl	EC	TDS
NO_2-N	1						
NO_3-N	-,110	1					
pH	,080	,970**	1				
PO_4	,969**	,031	,234	1			
Cl	-,185	,750	,778	,033	1		
EC	-,282	,818*	,813*	-,067	,986**	1	
TDS	-,276	,816*	,798	-,063	,974**	,992**	1

*: correlation significant at 0.05 level ($p < 0.05$);
****: correlation is significant at 0.01 level ($p < 0.01$);**
-: No statistically significant correlation was detected

Table 6. Data on some elements in Sızır Waterfall (ppb)

	Wet Season				Dry Season		
	St. 1	St. 2	St. 3		St. 1	St. 2	St. 3
Li	1.84	1.65	1.18		1.08	0.66	1.37
B	4.80	3.90	3.80		2.70	1.60	14.11
Na	505.42	607.45	853.64		401.93	383.00	24206.83
Al	3.76	7.93	12.59		4.24	6.49	24.08
K	395.66	625.14	439.35		360.67	452.75	1317.89
V	1.80	1.92	2.16		1.51	1.65	3.18
Cr	3.11	3.91	20.91		2.12	2.21	4.80
Mn	0.48	0.84	4.87		19.49	136.96	44.50
Fe	32.49	41.51	88.69		28.38	36.11	92.67
Co	0.21	0.17	0.20		0.13	0.12	0.32
Ni	1.08	1.17	1.26		1.56	1.01	4.17
Cu	5.16	4.74	5.90		3.12	3.02	8.50
Zn	30.89	15.11	14.54		8.93	8.06	13.03
As	3.10	6.04	43.48		4.92	9.11	12.13
Se	0.73	0.79	1.10		1.71	1.22	1.22
Sr	252.49	267.91	294.41		286.65	255.05	2449.07
Cd	0.04	0.03	0.02		0.03	0.02	0.05
Sb	0.40	0.38	0.28		0.26	0.23	0.38
Ba	20.67	31.89	33.56		98.03	120.92	204.24
Tl	0.010	0.005	0.005		0.005	0.004	0.005
Pb	1.24	1.30	1.87		4.65	4.51	4.66

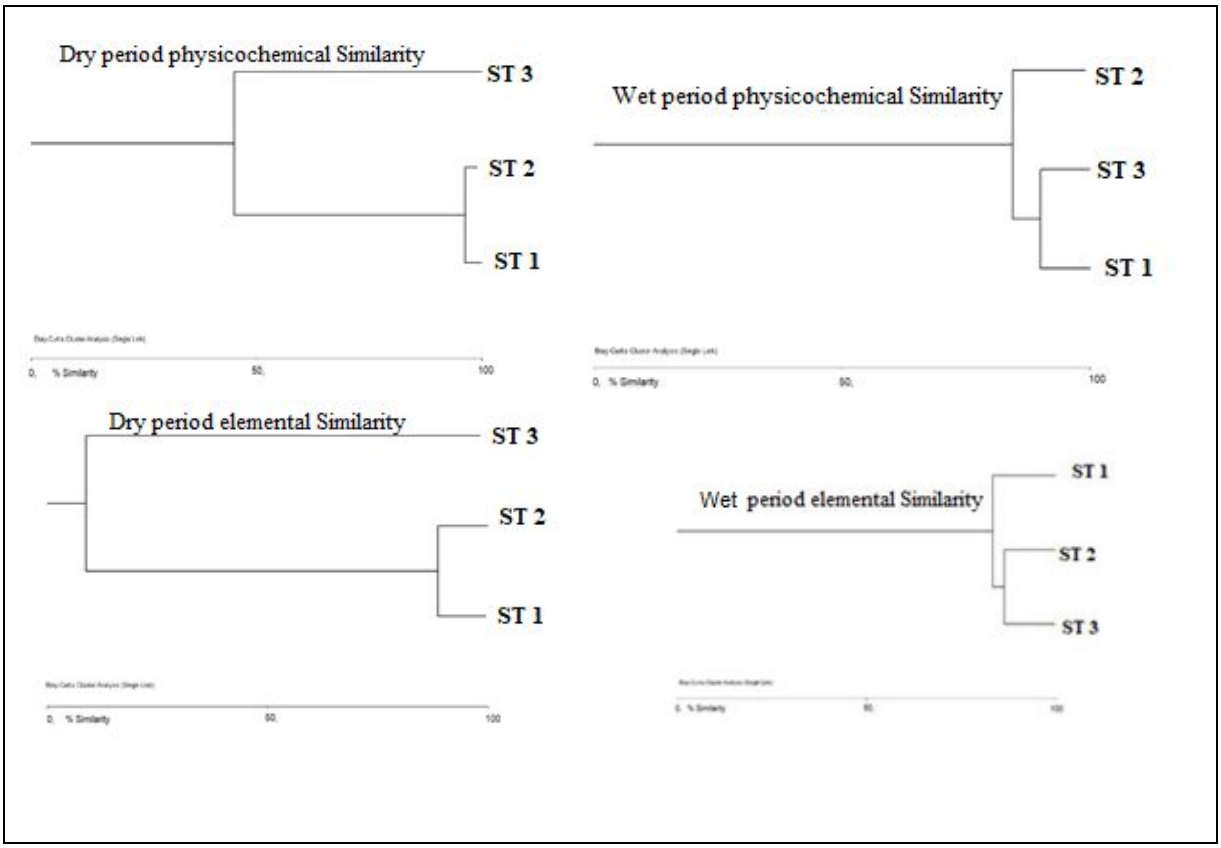
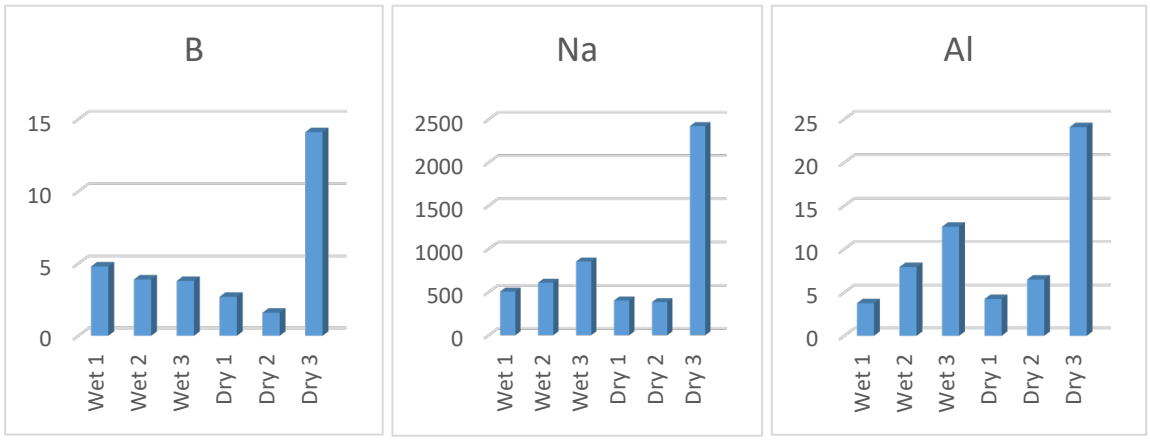


Figure 2. Bray-Curtis cluster analysis results for the physicochemical and elemental contents of the sampling stations



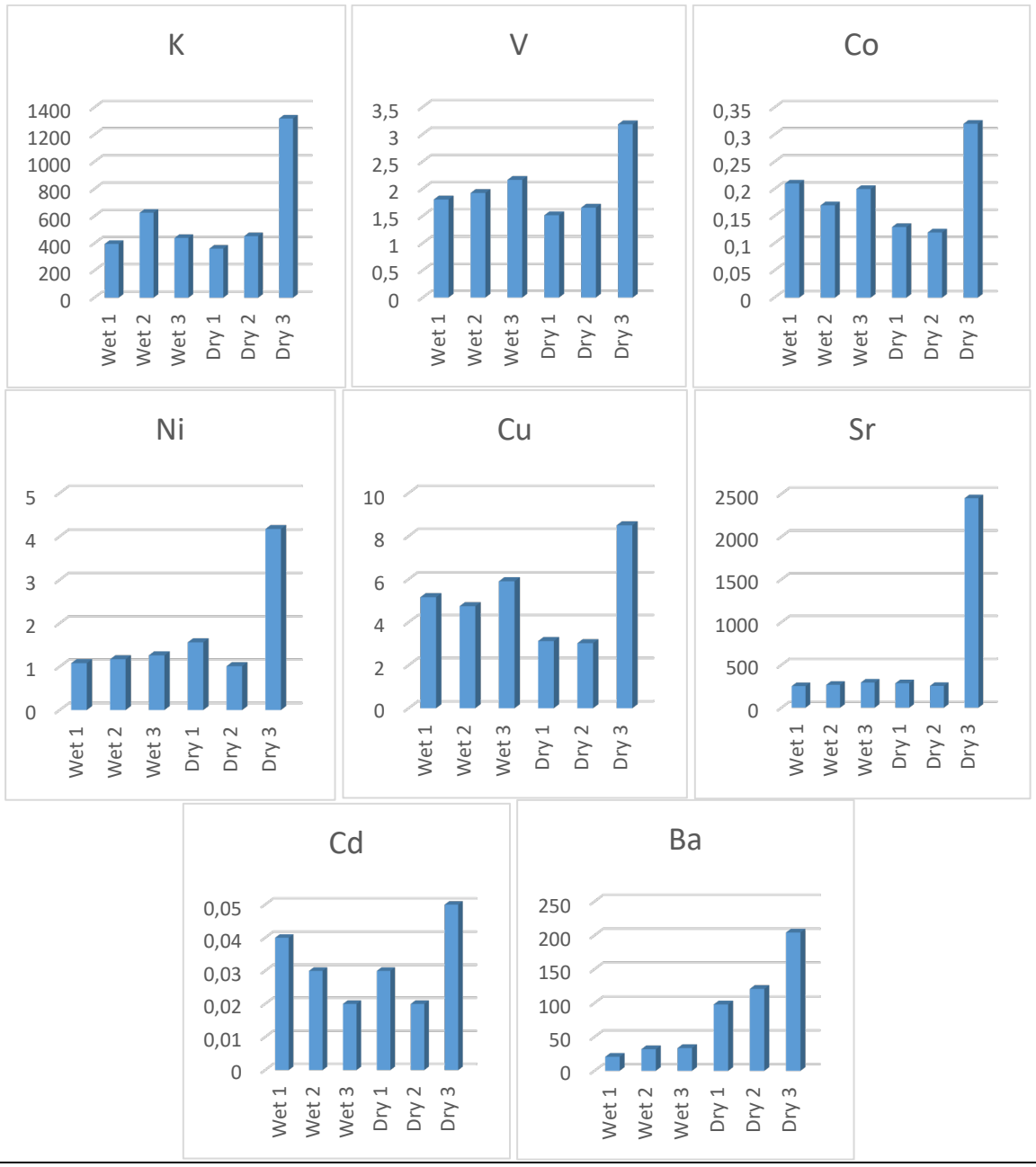


Figure 3. Comparison of data on some elements measured at sampling stations (1,2,3: station numbers)

Table 7. Benthic macroinvertebrates determined in the sampled stations of Sızır Waterfall

	Taxa	St. 1	St. 2	St. 3
Wet Season	Oligochaeta	-	-	*Potamothrix* sp.
	Amphipoda	*G.pseudosyriacus*	*G.pseudosyriacus*	-
	Ephemeroptera	-	*Baetis* sp.	*Baetis* sp.
	Trichoptera	-	larvae	-
	Coleoptera	-	larvae	-
Dry Season	Oligochaeta	*Potamothrix* sp.	-	-
	Gastropoda	*Physa* sp.	-	*Physa* sp.
	Amphipoda	*G. pseudosyriacus*	-	-
	Ephemeroptera	-	*Baetis* sp.	-
	Plecoptera	-	-	nymph
	Odonata	-	-	Zygoptera nymph
	Diptera	-	-	Chaoboridae larvae

At St. 1, *G. pseudosyriacus* was determined during the wet season and *Potamothrix* sp., *Physa* sp., and *G. pseudosyriacus* during the dry season. At the St. 2, *G. pseudosyriacus*, *Baetis* sp., Trichoptera and Coleoptera larvae were determined in the wet season and *Baetis* sp. in the dry season. At St. 3, *Potamothrix* sp. and *Baetis* sp. were found in the wet season; *Physa* sp., Plecoptera nymph, Zygoptera nymph and Chaoboridae larvae were found in the dry season.

Hussen et al. (2018) used water samples taken from three locations: upstream, waterfall, and downstream during the rainy season to analyse the physical and chemical parameters (pH, conductivity, turbidity, water and air temperatures, velocity, Biochemical oxygen demand, Dissolved oxygen, nitrate, orthophosphate, COD, and phosphate) of a waterfall in Indonesia. They reported that the water from the waterfall does not comply with drinking water standards but is suitable for tourism, fishing and irrigation. This study chose three locations (upstream, waterfall and downstream) to analyse the environmental variables in Sızır Waterfall during the wet and dry seasons. The high hardness values in Sızır Waterfall, in particular, affect the water's drinkable quality negatively. Sızır Waterfall waters are more suitable for agricultural irrigation due to their high nutritional salt content.

When some physicochemical findings obtained from the previous studies conducted in waterfalls in Türkiye are examined, Çağlar & Saler (2014) recorded that the water temperature ranges between 12.0-24.4 ºC, pH values as 8.0-8.8, dissolved oxygen as 8.1-9.6 mg/L, chloride value as 0.90-1.11 mg/L in Koçan Waterfall where the water was classified as "medium hard" in terms of total hardness. Saplıoğlu et al. (2017) evaluated the water quality data of Karpuz Stream, Düden Stream and Kurşunlu Waterfall and they reported that pH values as 7.4-7.8, sulphate value as 20.8-58.9 mg/L and hardness as 5.3-9.97 °FS. In this study, pH values in Sızır Waterfall varied between 7.5 and 8.2; oxygen value was between 2.85-5.71 mg/L, chloride value was between 0.99 mg/L (St.2, waterfall station) and 26.99 mg/L (St.3, downstream), sulphate value was between 0.2-2.5 mg/L and water hardness was between 22-34 °FS.

In a previous study performed by Aydın & Ekmekçi (2005), the pH value was reported as 7.3 on average in the physicochemical measurements of the location specified as the Sızır source. The pH values were determined in our study's range of 7.5-8.2. Conductivity values measured between 440 and 635 µS/cm in our study were in parallel with the average value of 796 µS/cm determined by Aydın & Ekmekçi (2005). However, it was observed that it exceeded these values (1966 µS/cm) at St. 3 during the dry season.

Nyamangara et al. (2008) investigated the effects of sewage and industrial wastewater on Harare Falls and the lower Mukuvisi River (Zimbabwe). They determined the concentrations of Zn, Cu, Pb, and Cd in water and sediment samples and reported that the upstream sampling sites contained the highest concentrations of all metals compared to the other locations. Zn and Cd values determined in our study were compared to the findings of Nyamangara et al. (2008) and were found to be similar.

Sharifah Aisyah et al. (2015) recorded the presence of sensitive organisms such as Ephemeroptera, Plecoptera and Trichoptera in a waterfall in Malaysia. They reported that benthic macroinvertebrate communities were more abundant downstream than upstream. In our study, specimens belonging to these three sensitive groups were found. It was also observed that the benthic macroinvertebrate findings were

similar to their findings (3 taxa upstream, four taxa at the waterfall, and six taxa downstream).

In a study performed by Baker et al. (2017) on benthic sampling from the upper and lower parts of a waterfall in Brunei and another study performed by Andrade et al. (2020) on EPT (Ephemeroptera, Plecoptera, Trichoptera) diversity in the upper and lower parts of a waterfall in Brazil, close similarities between the lower and upper parts of rivers were reported. In a study performed by Demir (2020) including some waterfalls in Türkiye, Ephemeroptera (in Manavgat Waterfall) and Trichoptera (in Ilıca Waterfall) individuals were found with the highest and lowest rates, respectively. Zakiah et al. (2022) reported that in the study performed in different waterfalls in Malaysia, individuals belonging to Ephemeroptera were found at the highest rate in the upstream and individuals belonging to Trichoptera in the downstream.

Mello and Abessa (2021) evaluated a waterfall's physical-chemical parameters and macrobenthic organisms in Brazil. They observed a higher density of Diptera than other groups due to their wide tolerance range. Clayton and Pearson (2016) reported, in a study performed in 5 different waterfalls in Australia, that the highest number of samples were found to belong to the Diptera group. However, the waterfalls differed significantly regarding general invertebrate abundance and diversity. Our study found Diptera samples only at St. 3 (downstream) in Sızır Waterfall. This station had high values, especially regarding conductivity, chloride and sulphate. *G. pseudosyriacus* reported from freshwater sources with conductivities of 120-1015 µS/cm (Zamanpoore et al., 2011) was observed at St.1 (upstream) and St.2 (waterfall) in our study. The high conductivity value (1966 µS/cm) measured at St.3 (downstream) may be an environmental factor limiting the presence of this species.

Rackemann et al. (2013) studied taxonomic diversity in 12 waterfalls in Australia. They selected three stations from each waterfall and sampled the autumn and winter seasons. They reported that taxonomical diversity increased in waterfalls covered with density moss; these ecosystems were used as shelters for rheophilic species, especially during low-flow seasons. Our study observed the highest diversity in St.3, which has the lowest flow velocity.

Conclusion

To ensure the sustainable use of aquatic ecosystems, these environments' physical, chemical and biological components must interact in a balanced manner. The healthy ecological structure of special ecosystems such as waterfalls also depends on these three important components. However, with the entry of some pollutants into the ecosystem, the environmental components in the water change, thus disrupting the balance in the aquatic environment and causing damage to biodiversity.

In conclusion, waterfalls are important aquatic ecosystems with unique characteristics. Therefore, it is recommended that studies on waterfalls be evaluated from a biological point of view and that physical and chemical properties be determined.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require ethics committee permission or any special permission.

Data availability: Data will be made available on request.

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Ballast operations at ports: Nemrut Bay analysis

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ABSTRACT

Ballast water operations are one of the most essential activities of commercial ships whose purpose of existence is to carry cargo between ports. With their growing ballast capacities, ships become an important vector that carries the origin species of the sea waters they took into their ballast tanks. Foreign species were transported between ports with ballast water in these operations, which have been going on since the mid-1800s. Although various guidelines were published by the International Maritime Organization (IMO) regarding ballast water, whose adverse effects on the marine environment began to be noticed in the international arena in the late 1980s, the desired positive effect could not be achieved. As a result of technological developments and R&D studies, ballast water treatment systems (D-2 Standard), which have completed the type approval process, have started to be applied on ships. The deadline for applying treatment systems, which have various types in terms of method and capacity, on ships is September 8, 2024. In this study, an application was made on ships calling Aliağa Port Nemrut Bay to investigate compliance with the International Ballast Water Management Convention and ship ballast operations' by port state control measures. This study showed that most ballast water at Aliağa Port (73%) was treated and discharged. Mechanical filter + UV treatment systems are also the preferred type (63%) among other systems. Also, according to this study's data set, the Adriatic Sea is the majority (44%) among other origins for Aliağa Port.

Keywords: Ballast water, Ship ballast water operations, Ballast water treatment, International ballast water management convention

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Introduction

To ensure positive stability, increase the draft, adjust the trim and keep the stresses on the ship within appropriate limits, ships often fill solid objects such as sand, gravel and stones in the port where they discharge their cargo, and this concept is called ballast. In the mid-1850s, seawater began to serve as ballast on ships, especially in England, when bulk cargo ships were built that used seawater instead of useless dry ballast in the coal trade (Carlton, 1985; NRC, 1996).

Different microorganisms in various numbers and species are transferred with ballast water in international maritime trade and can change the ecosystem of the place by clinging to the places they are transported to (Medcof, 1975; Hayes ve Sliwa, 2003). Ballast water systems are fundamental for commercial ships today and are critical in their functionality. Regardless of the ship's type, purpose and size, ballast tanks are considered an integral part of the ship design (David et al., 2018).

Intake or discharge times are very important in ballast water operations. The most critical factor determining this period is the hourly capacity rate of the ballast pumps installed on the ship. Smooth and timely ballast operations are extremely important for ships taking full cargo. One of the most important factors that negatively affects biodiversity is the introduction of alien species. When we look at the adverse effects of alien species, we can list the changes in food webs, the spread of new diseases and the struggle for food with native species. Foreign invasive species can interact with native species and cause the gene pool to change (Elton, 1958; Occhipinti Ambrogi, 2001).

The global impact of alien species transported through ships' ballast water is a significant concern (Carlton, 1985; Davidson & Simkanin, 2012). This method of transfer has led to notable changes in marine environments worldwide. Numerous negative effects exist, including the Japanese single-celled flagellated animal in Australia, the European zebra mussel in Canada, and the American carnivorous jellyfish in the Black Sea (NRC, 1996).

Turkish coasts have also been affected by invasive species. In a study conducted in the Marmara Sea, samples taken from the ballast water of 21 different ships were examined, and 38 different bacterial species foreign to the Marmara Sea marine fauna were detected (Altug et al., 2012). In 2014, high numbers of pathogenic bacteria were detected as a result of experiments conducted on samples taken from 5 different regions of the Marmara Sea (Tuzla, Tuzla shipyards region, Kartal, Derince, Zeytinburnu) (Elçiçek, 2014). In another scientific study, it was determined that ballast waters carried 122 different alien species detected on the coasts of Türkiye. It has been revealed that the spinoid polychaetes polydora cornuta, streblospio gynobranchiata and pseudopolydora paucibranchiata, which are among the invasive species, were carried to the Izmir Bay by ballast waters (Çınar et al., 2005). Another study determined that the number of alien species detected on Türkiye's Aegean Sea coast was 165. Compared to the scientific study conducted in 2005, an increase of 69% was recorded. This increase was shown to be due to the increase in detections due to the increasing number of scientific studies (Çınar et al., 2011).

David et al. (2018) analysed and compared the ballast water discharged in the Ports of Hamburg and Tallinn. In this study, where ship data from 2012 was used, the discharge estimation method obtained the amount of discharged ballast water data. Ballast water source port data is also considered the ship's previous port.

Chen et al. (2022) conducted a ballast water risk analysis study in Latvia's Riga and Taiwan's Kaohsiung Ports. In this study, ballast water risk analysis was made with factors determined using the characteristic data of incoming ships, and the aim was to determine priorities for port state control inspections. Data such as ship name, IMO number, ship flag, ship type, ballast source port, last port of the ship, next port of destination and gross tonnage were obtained from the ships arriving at Riga and Kaohsiung Ports between 2013 and 2015. The identified risk factors are specific to the ship: ship type, ship flag performance and number of voyages. This study ignored the ships' ballast notations (D-1 or D-2 compliance).

In the ballast water risk analysis conducted by Hasanspahic et al. (2022) in the Ploce Port of Croatia, previous risk analysis studies in the literature were examined based on the G7 Guideline of the International Ballast Water Management Convention, and factors were determined for the analysis of possible risks posed by ballast water. Accordingly, ship age, voyage duration, ship type, voyage frequency, flag of the ship discharging ballast, salinity rate and water temperature of the source and presence of invasive species in the source port were determined. Data on ships arriving at the Port of Ploce between July 2013 and January 2022 were obtained and analysed through the port management information system of the Croatian Maritime Administration. This study ignored the ships' ballast notations (D-1 or D-2 compliance).

International Ballast Water Management Convention

The aim of the Convention, which entered into force 13 years ago, is to minimise the spread of invasive/alien species

through ballast water. The Convention has many technical requirements for both the control and management of ballast water and sediment, which requires the application of different methods to achieve this. The Convention includes 5 Sections, 23 Rules, and 14 Guidelines.

For all ships over 500 gross tons embarking on international voyages, possessing a 'Ballast Water Management Certificate ' and a 'Ballast Water Record Book ' is not just a recommendation but a legal requirement (IMO, 2004). In practical terms, ships must treat their ballast water to the standards set by the Convention. These standards, categorised as D-1 (Ballast water replacement) and D-2 (Ballast water treatment), are not mere suggestions but stringent guidelines that must be followed.

In outline, Standard D-1 requires ships to replace existing ballast water in an area at least 200 nautical miles offshore and with a water depth of at least 200 meters. D-2 Standard means reducing the number of organisms in the existing ballast water with various equipment and systems on the ship (IMO, 2004).

Ballast Water Treatment Standard (D-2 Notation)

The D-2 Standard plays a crucial role in safeguarding marine ecosystems by limiting the number of harmful microorganisms that a ship can discharge into the sea in ballast water. It sets the minimum requirements for systems likely to be installed on ships, emphasising the importance of compliance with these restrictions to preserve our oceans.

According to the Convention,

- Less than 10 microorganisms greater than or equal to 50 micrometres per cubic meter,
- Less than 10 microorganisms between 10 micrometres and 50 micrometres per millimetre,
- Less than 1 colony forming unit (Cfu) per 100 millilitres of Toxicogenic Vibrio cholerae,
- Less than 250 cfu per 100 mililitres of Escherichia coli,
- Determined as less than 100 cfu per 100 millilitres of intestinal Enterococci (IMO, 2019).

Systems used in ballast water treatment must be tested by G8 and G9 guidelines and have received a "Type Approval Certificate" from the administrations or authorised classification societies. The requirements for a ballast water treatment system to have a type approval certificate are included in the G8 (Guide for Approval of Ballast Water Management Systems) and G9 (Guide for Approval of Ballast Water Management Systems Using Active Substances) guidelines. To confirm compliance with the D-2 standard, the specified tests must be carried out at the land facility and ship (Prabovo, 2018; Top, 2019). All ships constructed after the date on which the Convention came into force must comply with the D-2 Standard. A transition plan has been determined for existing ships, as many detailed processes are required, such as the physical installation of ballast water treatment systems on ships, as required by the standard. This transition plan has been determined according to the International Oil Pollution Prevention Certificate (IOPPC) renewal inspection dates of existing ships. According to this plan, the mandatory deadline for compliance with the D-2 Standard is 08.09.2024 (Bilgin Güney, 2022; IRClass, 2017).

Ballast Water Treatment Methods

It is determined that three main methods are used among existing type-approved ballast water treatment systems. Ballast water treatment systems are developed by creating mixed systems using these 3 methods: Mechanical, Physical and Chemical. The systems seen on ships have two stages. The first stage is the Mechanical Filter. The second stage is mostly physical or chemical. Seawater passing through a mechanical filter is treated by a preferred physical or chemical method (Bilgin Güney, 2018). Treatment of ballast water means the elimination of aquatic organisms. Ballast treatment methods can be explained in four main ways: mechanical methods, physical methods, chemical methods, and alternative methods. This section will share alternative methods, such as scientific studies and concept designs. Table 1 shows four main ballast treatment methods and their details.

Upon reviewing the current list of type-approved systems announced by IMO and USCG, it becomes evident that most ballast water treatment systems for ships are intricate models, often necessitating the combined use of at least two different systems. A closer look at the USCG's current list of type-approved ballast water treatment systems reveals that 46 out of the 52 systems are ballast water treatment systems used in conjunction with mixed systems (IMO, 2023; USCG, 2023a). This data underscores the preference for ballast water treatment systems that employ multiple methods, further emphasising the complexity.

Let us delve into the mechanical Filter + UV System. Regarding its functioning, the seawater, drawn from the sea chest valve during ballast intake, is meticulously guided through a pump and a mechanical filter of the treatment system. It then passes through the UV chamber, ensuring thorough treatment, before being directed to the ballast tank. Similarly, during ballast discharge, the water from the tank is precisely pumped through the UV chamber and discharged into the sea, maintaining the system's efficiency and control (AlfaLaval, 2023).

Table 1. Ballast water treatment methods

BALLAST WATER TREATMENT METHODS	
Mechanical Methods	Filtration, Hydrocyclone, Electro Mechanical Separation
Physical Methods	Heat, Ultraviolet, Deoxygenation, Ultrasound and Cavitation, Magnetic
Chemical Methods	Disinfecting Biocides, Non-Disinfecting Biocides
Alternative Methods	Port-Based System, Ballastless/Zero Discharge (Continuous Ballastless Concept, Bad (Heavy) Air Ballast Method, Fixed Internal Ballast Methods, Potable Ballast Water Concept), Continuous Flow Concepts (Longitudinal Main Bodies Concept, Buoyancy Control Compartments Concept, Advanced Ballast Water Exchange concepts)

Mechanical Filter + Electrochemical System: During the ballast intake to the ship, the seawater from the sea chest valve passes through the pump filter and reaches the mechanical filter of the treatment system. After the seawater passes through the mechanical filter through the flow meter on the line, the chemical used is added to the seawater via the chemical dosage pump. Afterwards, ballast water, whose chemical content is measured in the ballast water control unit, is taken into the tank. In ballast discharge, after the water taken from the ballast tank passes through the flow meter via the pump, the neutralising agent required according to the type of chemical substance previously introduced into the system for purification is added to the water to ensure that the active substance is at the allowed limit values. After chemical measurements of the water passed through the control unit are made, it is pumped into the sea (AlfaLaval, 2023; Jang & Cha, 2020).

In the Mechanical Filter + Electrolysis system, seawater passed through the pump filter during ballast water intake to the ship is taken into the electrolysis chamber. While microorganisms are eliminated by the electric current given to the water in this chamber, sodium hypochlorite is also formed due to the electrolysis process. Ballast water passed through the electrolysis chamber is taken into the ballast tank, and the purification process continues until it is in the tank. During ballast discharge from the ship, the water waiting with the active substance in the tank is taken with a pump and passed through the control unit. According to the measurement result in the control unit, the neutralising agent is added to the water with the dosage pump to ensure the active substance is within the allowed limit values (Bilgin Güney, 2017).

The Ozone/Inert Gas method offers several advantages. This system introduces ozone or oxygen-free inert gas into the ballast water as it passes through the pump filter. This method also included in the USCG-type approval list, is particularly effective as the presence of solids has a negligible impact on the purification process. The oxygen cylinders are filled via the air compressor and then transferred to the ozone generator chamber. The ozone gas produced in the ozone generator is then added to the ballast water line (Yang & Tong, 2021).

Control and Management Project of Harmful Aquatic Organisms Carried by Ballast Water in Türkiye

Türkiye's national project was launched in 2002 under the responsibility of the Undersecretariat of Maritime Affairs of the Republic of Türkiye under the name "Control and Management Project of Harmful Aquatic Organisms Carried by Ballast Water". In this project, where Tübitak Marmara Research Center is the implementing agency, data such as the amount of ballast water carried by ships arriving at Turkish ports between 2002 and 2006 and ballast water resources were collected. In the light of this data collected in the developed ballast water risk analysis software, the adverse effects of invasive species on Turkish coasts were evaluated. In addition, a geographical information system has been created on the Turkish coasts within the scope of the current legislation, and sensitive coastal areas have been determined. In the project's first phase, there are Ballast Water Reporting Forms from all ships calling at Turkish ports in the 5-year data pool. A web-based Ballast Water Risk Assessment System has been created by TÜBİTAK to be used in the risk assessments envisaged to be carried out within the scope of the GloBallast Project. In this system, environmental risk analysis methods have been applied to the requirements of the Convention, taking into account the G7 Guideline of the Convention (Olgun, 2011).

In the project's second stage, a case analysis study was conducted as a pilot application in the Botaş Port between 2011 and 2012. This study, a significant part of the project, explained port state control inspections of 206 ships and analysed their ballast water management plans. The alarming findings were that alarming-37 of the ships were evaluated as high risk, and ballast water samples were taken from these ships and analysed (GEF-UNDP-IMO, 2017). Two different risk assessment methodologies were applied for Turkish ports in the project. When examined with the applied Globallast Risk Assessment Method and the Advanced HELCOM Method, the results revealed that an average of 23 million

tons of ballast water was discharged to the Turkish coasts annually. The report further stated that 66 species were transported to Turkish coasts, and 19 were described as harmful organisms. Of these, three in particular (Mnemiopsis leidyii, Rapana Venosa, Beroe ovata) were found to cause a decrease in fish stocks and economic losses in the Black Sea (Republic of Türkiye Prime Ministry Undersecretariat of Maritime Affairs, 2010).

Risk analysis and evaluation of ballast water was carried out at Botaş Ceyhan Terminal using the ballast water risk assessment method and software developed within the scope of the GloBallast Project, in which Türkiye is also a stakeholder. When the results of the study, whose data were collected between June 2006 and June 2010, are examined, it is seen that 45,551,876 tons of ballast water were discharged in a total of 1126 ship operations, and the Mediterranean Sea comes first in terms of source, with 69%. When source ports were examined in detail, it was stated that 17 of all 133 source ports were Mediterranean ports and included in the highest risk group (Olgun, 2011, Republic of Türkiye Prime Ministry Undersecretariat of Maritime Affairs, 2010).

2010, an economic evaluation was conducted for Türkiye's ballast water management. The report used market price and travel cost analysis methods for quantitative estimates. It revealed that the total value of sectors such as fishing, aquaculture, and coastal tourism was 1 billion dollars, 323 million dollars, and 18 billion dollars, respectively (at the exchange rate dated 30.06.2010). The report also highlighted a significant finding: In the worst-case scenario, the total potential cost of invasive, harmful species to these sectors could reach 8.16 billion dollars. This underscores the critical need for effective ballast water management. The ballast water management convention's national implementation cost was 822 million dollars (Interwies & Knuchua, 2017). The 2010 report also analysed the source ports of ballast water discharged in Turkish ports and their regions, with the results presented in Table 2 below.

Table 2. Analysis of ballast water discharged on Türkiye Coasts (GEF-UNDP-IMO, 2017)

Source Region	Ballast Water (ton)	Percent
Mediterranean Sea	12.794.422	54%
Black Sea	6.271.615	27%
Northeast Atlantic	1.332.463	6%
Northwest Atlantic	755.201	3%
Indian Ocean	582.168	3%
South Atlantic Ocean	493.292	2%
Northwest Pacific Ocean	465.468	2%
Eastern Pacific Ocean	261.882	1%
Red Sea	250.398	1%
Persian Gulf	223.239	1%
Other	160.771	0%
Total	**23.590.920**	**%100**

Deficiencies Detected in Ballast Water Under Port State Control

The Ballast Water Management Convention Guidelines, a pivotal document published by the Marine Environment Protection Committee on 17 October 2014, play a crucial role in determining the scope and implementation method of inspections. The guide's second part outlines a comprehensive four-stage inspection format for ballast water management systems and components on ships (IMO, 2014). During port state control inspections, it is important to note that there could be 16 deficiencies across 3 distinct areas related to ballast operations (Med MoU, 2022).

Port State Control Inspection Procedures (Resoultion A.1155 (32)) by IMO aims to ensure uniformity in practice worldwide. The International Ballast Water Management Convention and inspection methods of its applications onboard ships are methodised. Annual reports of various MoU are analysed in this section. US Coast Guard 2022 Annual PSC Report stated that a concentrated inspection campaign was implemented on ballast water-related issues in 2022 and that the deficiencies detected in ships within the scope of ballast increased by 25% compared to 2021. When we look at the statistical analysis of ballast water deficiencies detected on ships during the year, "Ballast Water Management Systems" comes first with 118 deficiencies. In second place, there is "Reporting of Defective Systems" with 44 deficiencies, and in third place is "National Ballast Information Office Reporting" with 30 deficiencies (USCG, 2023b). When the Paris MoU 2022 Annual Report is examined, it is seen that the number of "Ballast Water Management" deficiencies detected on ships is recorded as 528 in 2020, 706 in 2021 and 892 in 2022. "Ballast Water Management" deficiencies, which are observed to increase in number every year, are examined under the title "Main Deficiency Categories" in the report (Paris MoU, 2023b).

Materials and Methods

This study was applied to ships arriving at Aliağa Port in 2023 under port or flag state control. This study aims to investigate current practices and determine Convention compliance progress on ships within the scope of the International

Ballast Water Management Convention requirements, to which Türkiye is also a party, without distinguishing between ships whose transition phase is ongoing or completed within the scope of the Convention. Quantitative research techniques were used. Primary and secondary data were collected to ensure that the study progressed by its purpose. The data contained in the Ballast Water Reporting Forms and International Ballast Management Certificate were obtained on ships during PSC inspections and as pre-arrival information declarations from the ships. These were considered primary data, and the port state control data regarding the ships obtained from the open-source EQUASIS website were considered secondary data. The data was analysed using descriptive statistics and Microsoft Office Excel program graphs.

Table 3 shows details of the data set used in this research. Primary data contains five topics deriving from ballast water reporting forms and international ballast water management certificates. Secondary data contains seven topics regarding ship characteristics.

Table 3. Primary and Secondary Data Used in the Study.

Data Type	Data Name
Primary Data (Data obtained from Ballast Water Reporting Form and International Ballast Water Management Certificate.)	• Type of ballast operation (receiving or discharging) carried out by the ship in the port • Amount of ballast discharged by the ship • Ship's ballast notation (compliance with D-1 or D-2 Standard) • Type of ballast water treatment system on board • Source port of the ballast discharged by the ship
Secondary Data (Open-source data from EQUASIS website.)	• Ship type • Ship's flag • Gross tonnage of the ship • Age of the ship • Deficiency in ballast detected on the ship as of the date of entry into force of the contract • High, medium or low status in the Paris MoU performance list of the organisation Authorised on behalf of the flag for certification of the ship (Paris MoU, 2022a).

Results and Discussion

Data was collected from 50 ships called Aliağa Port (Nemrut Bay) between March and June 2023, which were subject to port state control or flag state control inspection within the scope of the Convention. These data were transferred to the Microsoft Excel program, and frequency and percentage information were calculated using descriptive statistics.

Analysis was made according to the ships' ballast operations in the port, and ships discharging ballast were examined according to their ballast notations. At the same time, the amounts of discharged ballast water were analysed according to their treatment status. It is understood that 76% of the ballast discharged at Aliağa Port is treated and discharged into the sea. It is seen that 74% of these are passed through the Mechanical Filter and UV Purification System.

Figure 1 shows ballast notations of all ships' in a data set of this research.

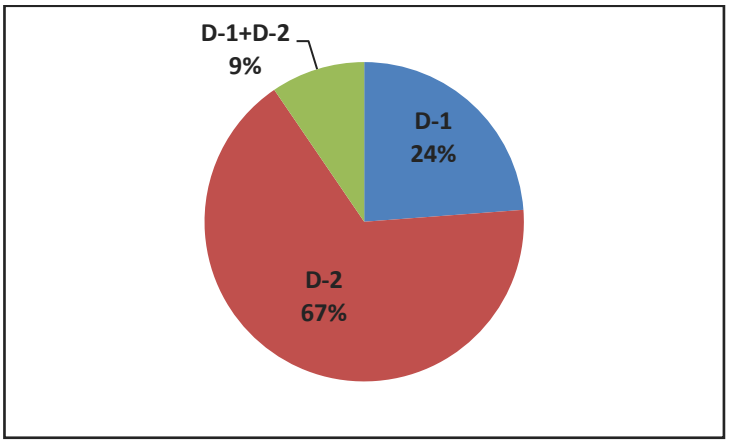

Figure 1. Ships' Ballast Notations Analysis

The amounts of ballast water discharged in the port were examined according to ship types. Crude Oil/Product Tankers were in first place.

Figure 2 explains the types of ships which constitute this research's data set.

In Figure 3, the ballast notations of the ships arriving at Aliağa Port were examined according to whether they were D-1, D-1+D-2, or D-2. According to the notation, the systems on ships with ballast treatment systems were analysed in terms of type, and mechanical filters and UV treatment systems were used first.

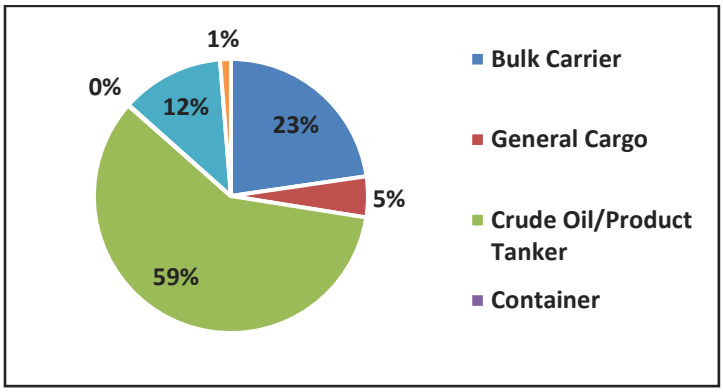

Figure 2. Ship Type Analysis of Ballast Discharged Ships

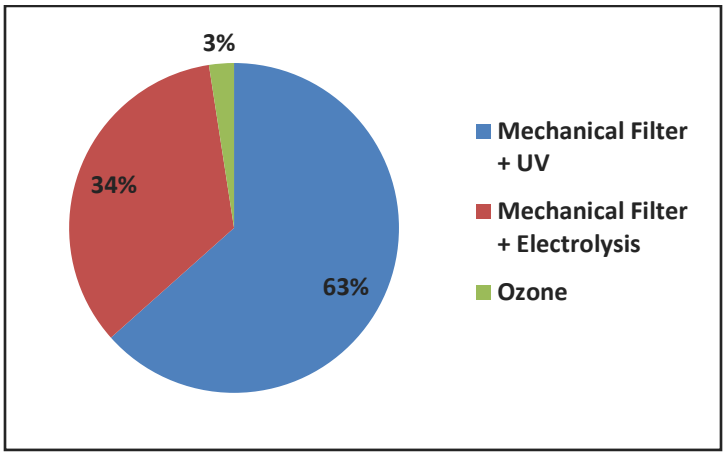

Figure 3. Treatment Equipment Analysis

The amount of discharged ballast water was first examined according to the regions where the source ports are located in Figure 4. Subsequently, a local analysis was made of the Mediterranean, which had the highest ballast water discharge amount, 66%. According to this analysis, the Adriatic Sea has the highest rate as a resource in the Mediterranean, 44%.

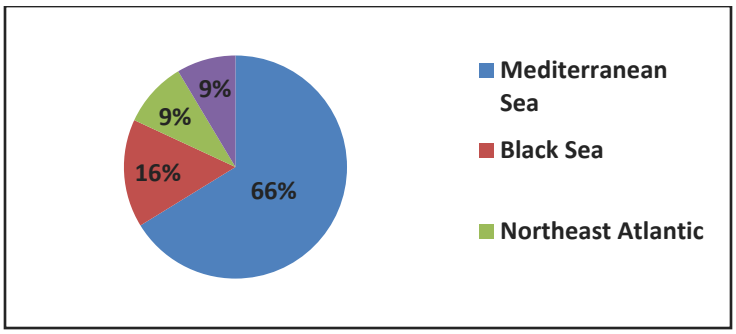

Figure 4. Discharged Ballast Origins

Figure 5 shows Mediterranean ballast water origins discharged at Nemrut Bay. Adriatic Sea (44%) is the major donor for Nemrut Bay regarding ballast waters. Alboran Sea (20%) and Ionian Sea (14%) are other important donor ports.

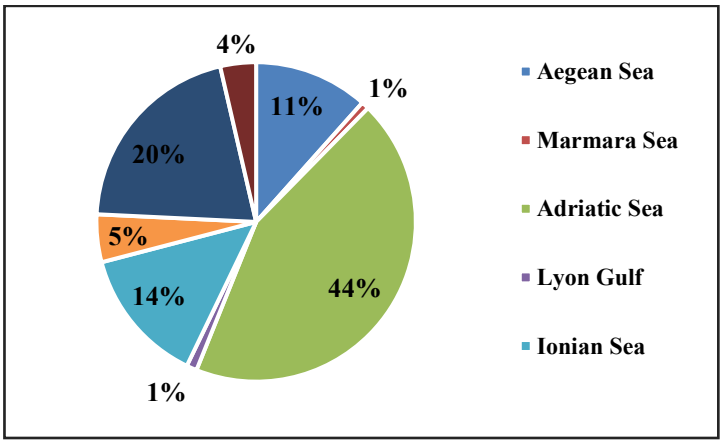

Figure 5. Discharged Ballast Origins in the Mediterranean Sea

Bulk carriers are the most frequent ship type with 36%, and general cargo ships are the second most frequent with 22% of this study. It has been observed that Mechanical Filter + UV Treatment Systems are the most preferred ballast water treatment systems in bulk cargo ships and general cargo ships according to ship types in the research data set. Figure 6 shows the treatment equipment types of bulk carriers and general cargo ships.

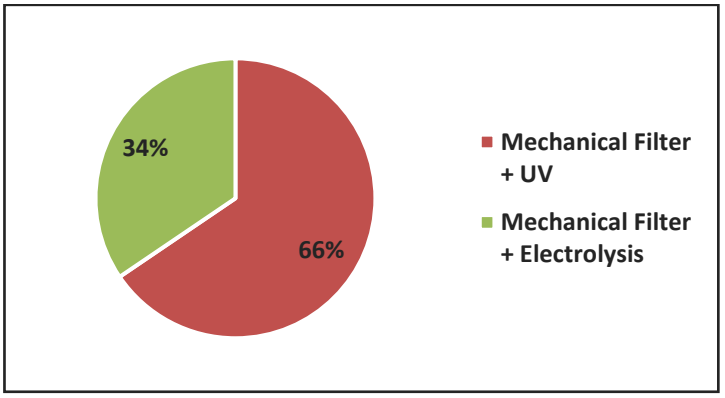

Figure 6. Treatment Equipment of Solid Bulk Carriers

Crude oil/product tankers are the third most frequent ship type, accounting for 20% of this study. Figure 7 shows the ballast treatment equipment of these tankers in this research data set. The mechanical filter + Electrolysis method is the most preferred treatment system (50%) for these tankers.

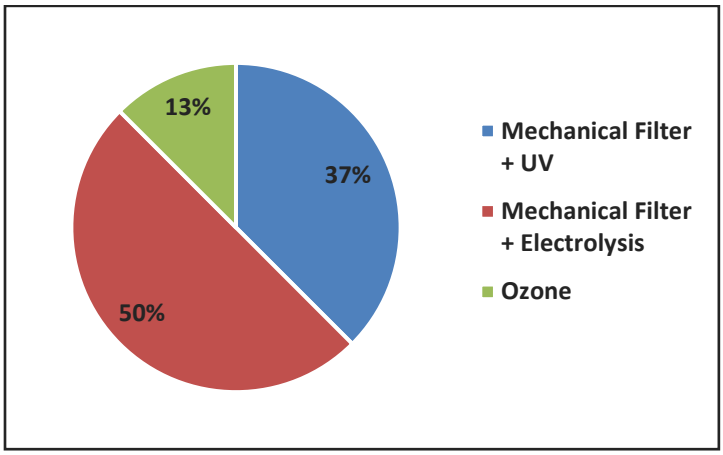

Figure 7. Ballast Treatment Equipment of Crude Oil/Product Tankers

In this context, ballast-related deficiencies detected during port state control inspections carried out on ships as of the date of entry into force of the Convention were also analysed and detailed in Figure 8. It was observed that no PSC deficiency regarding ballast was detected in 74% of the ships in the data set. An analysis of the average age of ships with ballast-related PSC deficiencies compared to ships without any deficiencies was also conducted. Accordingly, it was revealed that the average age (18,07) of the ships whose ballast deficiencies were detected during the PSC inspection was higher than the others (10,62) without deficiencies.

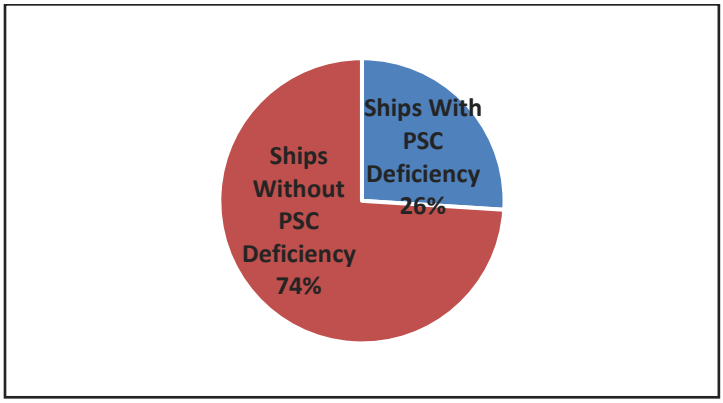

Figure 8. Ballast-Related PSC Deficiency Analysis

Figure 9 shows ship types and ballast-related PSC deficiency correlations. Based on this research data set, ships with ballast-related PSC deficiencies are analysed according to their ship types. General cargo (31%) and bulk carrier (31%) ships are the most frequent ship types with ballast-related PSC deficiencies.

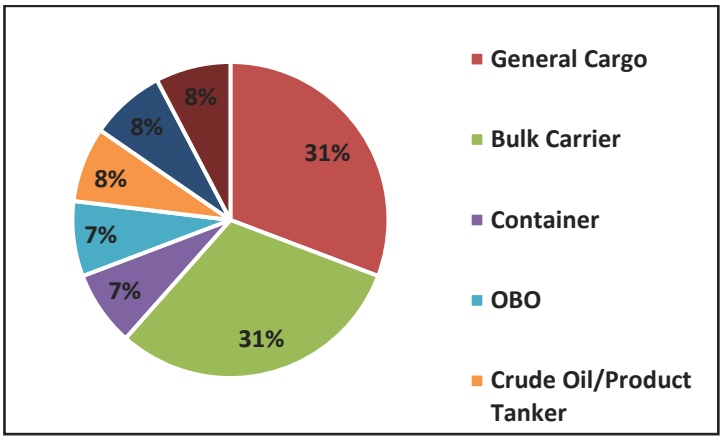

Figure 9. Ship Type Analysis with Ballast-Related PSC Deficiency

In her study, Kara (2022) revealed that the port state inspection results of the flags on the Paris MoU White List are compatible with each other regarding their performance. In this research, the fact that the ships on the Paris MoU White List (92%) are more than the others in terms of flags in which ballast deficiencies were not detected during port state inspections supports this. Figure 10 shows the flag performance of ships without ballast-related PSC deficiency.

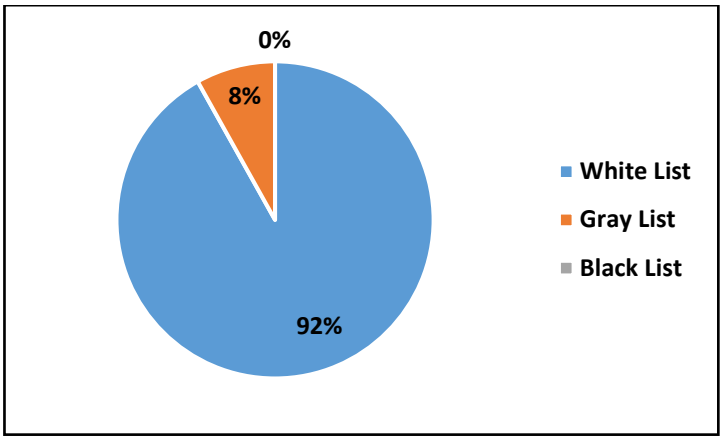

Figure 10. Flag Performance Analysis of Ships Without Ballast-Related PSC Deficiency

Chuah et al. (2023) revealed in their study that classification society performance is an essential factor in the ship's port state control inspections. Few deficiencies were detected in port state inspections of ships authorised by high-performance classification societies. This research supports this, as ships with no deficiencies in ballast during port state inspections are found to be high performers in the Paris MoU Performance List regarding classification societies (95%). Details are shown in Figure 11.

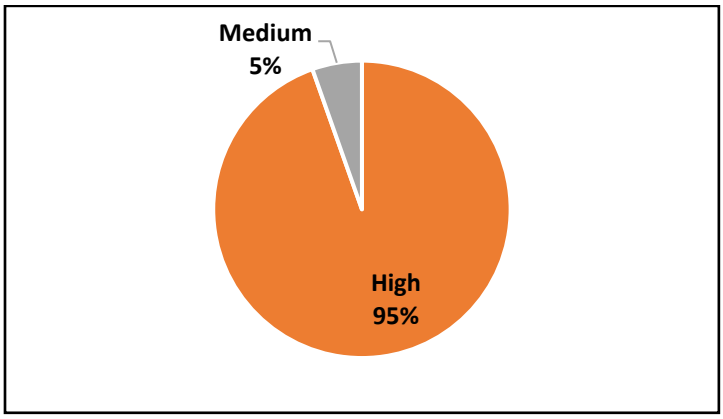

Figure 11. Recognised Organization Performance Analysis of Ships Without Ballast Related PSC Deficiency

In their study, Fu et al. (2020) revealed that the deficiencies detected in port state inspections increase as ships' service periods increase. The average age of the ships in this research's data set, where ballast deficiencies were detected during port state inspections (18.07), is higher than the average age for no deficiencies (10.62). This confirms that the risk of detecting deficiencies increases in older ships.

Limitations of the Research

- The research was geographically conducted only in Aliağa Port (Nemrut Bay).
- In the research, data could only be collected in 3 months between March and June 2023.
- In the research, data could only be collected from ships subject to port and flag state control instead of all ships calling Aliağa Port.

Suggestions for Future Studies

- Considering that there is no need to inspect every ship for the data to be collected specifically, the scope of the research can be expanded, and the implementation period can be extended with the information in the ships' ballast water reporting forms and international ballast water management certificates through the Port Management Information System,
- Continuity in practice can be ensured by integrating the ballast risk analysis format previously developed within the scope of Türkiye's national project with the updates that can be made to the Port Management Information System,
- If there is an updated port-specific ballast water risk analysis, ships with higher risk factors can also be included in the port state control targeting system. For all that, ballast water samples can be taken from high-risk ships, and detailed laboratory analysis can be carried out,
- Invasive alien species in ballast water source ports can be compared with invasive alien species detected in the local port where the research is carried out,
- Ballast operation analyses to be carried out on a port basis can be carried out by taking into account international and regional agreements,
- Ballast-related PSC deficiencies of the ships calling Aliağa Port can be analysed entirely, and a concentrated inspection campaign can be implemented in flag state inspections,
- Comparative analysis of countries' national ballast risk analysis can be made,
- Analyzing ballast water operations in all ports with heavy ship traffic in Türkiye can ensure the protection of the marine environment.

Conclusion

It has been observed that three different systems (Mechanical et al. System, Mechanical Filter and Electrolysis Treatment System and Ozone Treatment System) are preferred in terms of ballast water treatment systems on ships. It has been revealed that almost all (97%) of the treatment systems used are systems that include more than one method. Mechanical Filter and UV Treatment Systems (63%) were preferred. When we look at the current type approval lists of USCG and IMO, it is significant that most systems include more than one method. Among all systems, the majority use Mechanical Filters and UV Treatment methods.

Considering the source ports of the ballast water discharged by ships, Mediterranean Ports come first, Black Sea Ports come second, and Northeast Atlantic ports come third. When compared with the data of the Ballast Water Assessment Report for Türkiye prepared by the Prime Ministry Undersecretariat of Maritime Affairs of the Republic of Türkiye in 2010, it is seen that the results are compatible with each other, and the ranking is the same. When we look at the details on a regional basis within the Mediterranean, it turns out that the highest amount of ballast originates from the Adriatic Sea (44%). This situation reveals the need to examine ports' ballast water risk analysis, especially in the Adriatic Sea, to conduct water and ballast water risk analyses in Aliağa Port.

When the flags of the ships subject to this research were examined in terms of performance, it was understood that those in the Paris MoU White List were more than those in the Gray and Black Lists (75%). This situation provides suitable conditions for ships to meet the requirements regarding compli-

ance with the Convention. Again, in this study, the high compliance with the D-2 Standard (82%) confirms the existence of high-performance flags.

When the performance of the ships' classification societies is examined, the majority (94%) are high-performance. This demonstrates that ships certified by high-performance classification societies are important for complying with the Convention requirements.

The International Ballast Water Management Convention is one of the international maritime agreements that took the longest time to adopt and come into force. After the Convention had entered into force, too many decisions were made by the Marine Environmental Protection Committee, and the guidelines were published and updated continuously. A severe amount of rules and regulations confirmed the literature, which can be described as the convention itself, its annexes and manuals. Although ballast water is a fundamental operational issue for ships, it also serves as a vector for transporting many living creatures that negatively affect the marine environment. Over the years, scientific studies have observed and revealed the negative effects of creatures carried by this vector on the regions where ballast water is discharged.

The most important project carried out by IMO regarding ballast is the GloBallast Program. This project lasted until the date of acceptance of the Convention on 8 September 2017, and it aimed to provide global readiness until then. Priority was given to field studies in different regions of the world, and expert groups formed with national support carried out risk analyses of ports. Türkiye also became one of the major stakeholders in this important project, and a national risk analysis was conducted.

Port state control is an essential tool in the maritime field where international conventions and national regulations are inspected on ships. All decisions taken and guidelines published by the governing body regarding international Conventions, including the transition periods between the adoption and entry into force of each international convention, must be considered at each inspection. It is one of the most effective ways to track the current implementation of any international convention on ships in maritime.

This study investigated the current practices of the International Ballast Water Management Convention on ships arriving at Aliağa Port. A data set was created based on international literature and the Convention's requirements.

When the research results are evaluated, it is revealed that the vast majority of ships are at a reasonable level of compliance with the Convention. However, it should always be taken into consideration that the maritime sector is one of the most dynamic fields of expertise by its nature. It is thought that it would be beneficial to carry out port state control inspections in the form of a concentrated inspection campaign and to examine only the Convention requirements within a specified period. In addition, it is thought that the concentrated inspection campaign, which will be implemented in flag state inspections carried out on Turkish Flagships, will ensure that the port state inspection performances of the ships in question remain high and, thus, the Turkish Flag`s White List performance remains.

As stated in the Ballast Water Assessment Report for Türkiye (2010), Aliağa Port (also known as Nemrut Bay) is the second port with the most ballast discharge. According to TÜRKLİM's 2022 data, Aliağa Port is the second largest port in Türkiye in terms of cargo handling (TÜRKLİM, 2024). This situation reveals the intensity of ship traffic and, therefore, ballast operations in Aliağa Port. It is thought that it would be beneficial to develop the specific risk analysis format for Aliağa Port within the scope of previous research in the Control and Management of Harmful Aquatic Organisms Carried by Ballast Water Project in Türkiye.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require ethics committee permission or any special permission.

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Studies on organogenesis of common carp *Cyprinus carpio* var. *koi* with reference to histological perspectives

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ABSTRACT

The objective of the current study was to investigate the histological characteristics of larval organogenesis in *Cyprinus carpio* var. *koi*. The eggs and several developmental stages of *C. carpio* var. *koi* larvae were preserved in Bouin's fluid. Histological examinations were conducted daily from the first day after fertilisation (DAF) to the 12th DAF and the 16th, 24th, 32nd, and 40th DAF. Histological examinations of *C. carpio* var. *koi* eggs revealed the development of an embryonic disc, followed by the formation of the neural tube and embryonic eyes on the second day after fertilisation (DAF). The just-emerged larvae have distinct eyes and a neural tube that has undergone differentiation into the brain. Following that, an examination was conducted on the development of the digestive system, swim bladder, gills, skin, and fins. The ontogeny developments were categorised into five distinct stages. During larval development, the initial two stages have exhibited variations in their organogenesis. The larvae head was aligned and devoid of the yolk, but their body was connected to the yolk sac and gill arches, which were visible between the 3rd and 5th days after fertilisation (DAF), and their total length range was 4.35-6.83mm. The digestive system had a linear configuration, with an enlargement in both the dorsal and ventral regions of the body on the ninth day after fertilisation (DAF). During the 10th to 17th developmental stages, larvae have a total length range of 11.23-14.35mm. The operculum, gill lamella, and dorsal fin were developed in this stage. Between the 24th and 40th days after fertilisation (DAF), the larvae acquired a fin, a coiled gut, and well-developed accessory glands and their total length was measured between 15.01 and 23.68mm.

Keywords: *C. carpio*, Ontogeny, Histology, Larval rearing

Introduction

The embryonic development and organogenesis of common carp (*Cyprinus carpio* var. *koi*) are vital processes that significantly impact the growth and maturity of this economically valuable fish species. Gaining a comprehensive understanding of the complexities involved in the development of organs and the growth of embryos in common carp is crucial for maximising the efficiency of aquaculture production and guaranteeing the long-term viability of fish populations. Ługowska & Kondera (2018) provide a detailed account of the initial growth stages of vimba under varying temperature conditions. Muthupriya et al. (2022) studied the account of the ontogenic development of Carassius auratus in detail. This data enhances our comprehension of the various stages of growth in this particular species. In addition, the research conducted by Burggren and Pinder (1991) emphasised the significant growth in size that occurs throughout the development of lesser vertebrates such as common carp. This study showed the astonishing metamorphosis from a small larva to a fully mature adult. In their study, Milan et al. (2006) examined the zebrafish as a prominent model organism for studying development, particularly about the formation of organs. Common carp's embryonic development and organogenesis are intricate processes impacted by genetic, environmental, and ecological variables. By deciphering the intricacies of these developmental processes, scientists can improve aquaculture methods, preserve fish populations, and minimise the effects of invasive species such as common carp on aquatic environments. The stages of embryonic development, ontogenesis, organogenesis, and early larval stages play a vital role in the life cycle of fishes, affecting their growth, survival, and ecological interactions. Understanding these processes is crucial for knowing fish biology's intricacies and enhancing aquaculture methods. The study conducted by China and Holzman (2014) focused on the difficulties larval fish encounter when they experience hydrodynamic famine during the initial feeding stage. The research highlighted the crucial shift from limited success in capturing prey to enhanced eating behaviour throughout early growth.

In addition, Zimmer et al. (2017) conducted studies that examined the molecular and transcriptional characteristics associated with the processing of ammonia and the development of larvae in fish. These studies offer valuable information about the physiological adjustments that occur throughout the early stages of life. Nowlin & Drenner (2000) and Nebeker et al. (1985) studies emphasise the intricate relationship between fish development, environmental conditions, and species interactions in aquatic habitats. The initial phases of fish development encompass a variety of processes that impact their growth, behaviour, and ecological functions. By clarifying the processes involved in embryonic, ontogenic, and organogenetic events, scientists can improve conservation initiatives, aquaculture administration, and our comprehensive comprehension of fish biology.

Organogenesis is a crucial step in the development of organisms that significantly impacts the structure and function of different organs. When studying fish, having knowledge of the histological aspects of organogenesis helps us gain a significant understanding of the complex cellular and tissue-level transformations that take place during the development of embryos and larvae. Boulhic & Gabaudan (1992) studied the histological examination of organogenesis in the digestive system and swim bladder of the Dover sole (*Solea solea*). This research provided insights into the structural growth of these crucial organs in fish. It explored the intricate aspects of tissue development, providing a thorough understanding of how organs are formed in a particular fish species. In addition, Behrouz et al. (2014) studied the histological aspects of larval organogenesis in *Schizothorax zarudnyi*. Their research provided an in-depth understanding of the development of important organs like the gills, heart, kidney, bladder, and spleen during the initial phases of life. This study provided significant insights into the structural condition of these organs during their development. Histological examinations, as shown in this research, are crucial for understanding the cellular mechanisms and tissue structure involved in fish organ development. By analysing minute alterations that occur throughout growth, scientists can better understand the processes by which organs are shaped and operate in various fish species. This knowledge contributes to the broader field of developmental biology and aids in advancing aquaculture operations. The present study examined many stages of organogenesis in *C. carpio* var. *koi*, specifically focusing on the development of various organs.

Materials and Methods

Breeding of Common Carp C. carpio var. *Koi*

The experiment was conducted at the School of Aquaculture, Department of Zoology, The New College, Chennai, Tamil Nadu, India, in the laboratory, breeding and spawning of the common carp. The broodstock of this species was obtained from a Tamil Nadu hatchery located in Poondi, Tamil Nadu, India. The organisms were housed in concrete tanks and nourished with live feed consisting of tubifex worms and chironomids larvae. The breeding and spawning tanks, measuring 10 x 4 x 5 ft, were thoroughly cleaned and then filled with tap water that had been filtered. For optimal breeding conditions,

it is preferable to have water with a pH of 6.8 and a temperature ranging from 28.67±1.92C. A continual aeration process is necessary for water. Aquatic weed (*Chara* sp.) was supplied in the breeding tanks. The breeders were introduced into the breeding tanks during the late evening hours. The typical male-to-female ratio of introduced fish was 4:1 (Mohale et al., 2020).

The female swam rapidly through the plants and laid the eggs directly on the leaves. The male then releases milk to fertilise the eggs. Spawning in fish often occurs in the early morning hours and lasts 2 to 4 hours. Once the spawning process was over, the breeders were delicately relocated to a different tank. The aquatic weed with attached eggs was delicately extracted from the breeding tank and evenly dispersed into a hatching hapa, measuring 2.5 x 1 x 1.5 meters, constructed from thin fabric. The hapa was then placed in water with qualities comparable to the breeding tanks. The hapa containing developing eggs was undisturbed until the eggs hatched into larvae (Sivakumar, 2005).

Process of Larval Rearing

We chose newly born larvae that were just one day old for larval rearing. The larval rearing of *C. carpio var. koi* was nourished with pelletised feed and a combination of live feed consisting of cladoceran (*Moina micrura*) and cyclopoid (*Thermocyclops decipiens)*. A group of 100 newly hatched larvae of *C. carpio* var. *koi*, all of the same size, were placed into a concrete experimental tank of 75 cm in length and 40 cm in diameter, containing 35 litres of water. The larval tank was aerated and kept without food during the non-feeding stage of the larvae, following the natural light-dark cycle. The larvae of *C. carpio* var. *koi* began feeding on external food sources on the seventh day after fertilisation. The larvae were provided unlimited food, and studies were carried out for 40 days. Each morning, the larval rearing tank was cleaned by removing faecal matter and surplus feed, and 50% of the water was replaced. The dimensions of eggs and larvae were assessed using a micrometre (Qin & Fast, 1997; Sivakumar, 2005).

In order to conduct histological analysis, *C. carpio* var. *koi* eggs and larvae were chosen from the earliest stage of freshly hatched eggs to larvae that had reached a maturity of 40 days. The larvae were rendered unconscious using a concentration of 50 mg/l of benzocaine and preserved daily from the first day after fertilisation (DAF) to the 12th DAF. They were also preserved on the 16^{th}, 24^{th}, 32^{nd}, and 40^{th} DAF. The eggs and larvae were euthanasia (0.4 ml of clove oil/litre) and immersed in aqueous Bouin's fluid for 12 hours. They were rinsed in flowing tap water until the yellow hue of picric acid was eliminated.

Histological Study

The samples were dehydrated using a progressive sequence of ethyl alcohol concentrations (30%, 50%, 70%, 90%, and absolute alcohol), followed by clearing with xylene. The specimens were immersed in paraffin wax at a temperature of 52ºC. The specimens were sliced into longitudinal sections (L.S.) and cross sections (C.S.) at a thickness of 8 µm using an Erma rotatory microtome. Tissue sections were stained using the hematoxylin and eosin staining method. The sections underwent deparaffinisation in xylene and were then hydrated in a declining ethanol series. The specimens underwent water treatment and a 30-minute staining process using hematoxylin. The specimens were rinsed using tap water and subsequently treated with 30%, 50%, and 70% ethanol. Following this, they were stained with alcoholic eosin for around 5 minutes. Additionally, dehydration was conducted by subjecting them to 90% absolute alcohol. After drying, the sections were treated with xylene to remove any remaining moisture. Subsequently, permanent mounts were created using Canadian balsam (Martins et al., 2018).

Observations were made on the structure of the egg and the development of larvae, including the eye, nervous system, digestive system, gills, pigmentation, and fins. Photomicrography captured the specimens using a Samsung (CCD) camera connected to a Leica ATC 2000 microscope. The images were taken at various levels of magnification.

Results and Discussion

Fertilised Eggs

The fertilised eggs of *C. carpio* var. *koi* have a sticky surface, and at some particular points, they have adhesive material consisting of hexagonal compartments. The fertilised eggs are attached to the leaves of the aquatic plants. The fertilised eggs of koi carp are either spherical or oval, with a light yellowish colouration, while unfertilised eggs appear whitish. Scanning electron microscopy of the eggs (Fig. 1) reveals irregular folding of the outer membrane with depressed regions that aid in attaching the eggs to vegetation. The breeding tank water temperature for koi carp should be maintained between 25ºC and 30ºC to facilitate breeding (Wu et al., 2007). Regarding aquaculture, koi carp are raised globally, especially in Japan, as an ornamental variety of common carp. During further development, embryonic tissue rises from the yolk surface, constricting the broad connection between the body of the embryo and the yolk into a narrow zone. Through this narrow zone, yolk material, after enzymatic breakdown, is transported to the embryo. As the embryo grows, the connection between the body and the yolk is further constricted,

forming a stalk with which the embryo proper is connected to the yolk sac.

When hatching, the larva has a small yolk sac attached to it ventrally. The newly hatched-out larva is transparent and has a laterally compressed body. The yolk sac is oval. Within two days after hatching, the yolk is completely utilised, and the yolk sac is absorbed ventrally. The eyes are dark, and there is a faint black pigmentation near the lateral line of the anterior part of the larva. As growth proceeds, the larva shows morphological changes such as the formation of distinct head and body regions, the development of eyes, the appearance of buccal invagination, and the formation of upper and lower jaws. During further development, the caudal fin is fully distinct from the embryonic fin fold, and the pectoral fins appear as flaps just behind the operculum. The morphological structures of the head and body of the larva resemble those of the juvenile koi carp. The larva starts surfacing and swims actively in the water column. It feeds voraciously on zooplankton and grows into a juvenile, showing most adult characteristics (Table 1).

Histological Aspects of Eggs and Larvae of C. carpio var. Koi

Eggs

The incubation period of koi carp eggs typically ranges from four to seven days, a duration influenced by the temperature of the medium. Approximately 80 to 90% of these eggs hatch into larvae, with the fertilised eggs having a diameter ranging from 1.15 to 1.51 mm. Unlike some species, koi carp eggs do not contain oil globules. The development of fertilised koi carp eggs begins with the accumulation of active cytoplasm towards the animal pole, followed by cleavage (Adamek et al., 2017). During this process, the yolk granules and plates get more compactly arranged at the centre of the egg. Blastoderm is formed at the cytoplasmic cap, and the yolk and a thin layer of cytoplasm surrounding it remain unaffected. The periblast did not contribute to the formation of the embryo. The blastoderm cells undergo epibolic and embolic movement to form a gastrula. Further, development leads to the blastodisc formation, which thickens to become the embryonic shield (Fig. 2a). The primary organ rudiments, such as the neural plate, neural tube, notochord, and lateral somites, are formed in the embryonic shield. The formation of primary organ rudiments starts at the anteriormost part and progresses backward (Fig. 2b). The lateral edges of the blastodisc develop progressively to form the embryo's body. The lateral edges of the blastodisc are drawn towards the midline, forming more posterior parts of the body and the tail. At this stage, the body is formed completely around the yolk sac, and the head and eyes are visible (Fig. 2c).

Histology of the egg undergoing embryonic development shows granular yolk, borderer cells, and active cytoplasm (Fig. 2d). The development of the fertilised egg commences with the accumulation of cytoplasm at the animal pole of the egg. In this species, the active cytoplasm forms a mount on the yolk and undergoes meroblastic cleavage. The active cytoplasm stains blue with hematoxylin and eosin, while yolk granules stain pink and bluish-brown with these dyes. The border cells are large and highly acidophilic, appearing bright pink with haematoxylin and eosin. These cells have a centrally located, prominent nucleus. After cleavage, a blastodisc is formed, which lies over the yolk at the animal pole. Gastrulation proceeds with the epibolic and embolic movement of the blastomeres in the spreading of the embryo over the yolk and the commencement of organogenesis. At this stage, the vitelline envelope is formed, leading to the formation of the yolk sac. The embryo now has a curved body distinguished into a head and trunk (Fig. 2e). As development proceeds, the embryo grows over the yolk sac to the extent that the head and tail end come into proximity (Fig. 2f). Initially, the head region of the embryo is acidophilic, and the trunk appears to be basophilic. When embryonic development is complete, the entire embryo becomes basophilic. At the end of embryonic development, the tail region gradually detaches from the yolk sac and moves away; however, the head is directed downwards and still attached to the yolk. The release of the embryo's body from the yolk and its straightening narrow the contact point between the embryo and the yolk sac. Yolk material is broken down at this contact point, and nutrients are transferred to the embryo. Sections passing through the head region of the embryo indicate differentiation of the anterior part of the neural tube into the cephalon. At this stage, the optic vesicles are differentiated; however, the eyes are not pigmented. The duration of embryonic development is about three days, and the larva with an elongated yolk sac hatches out.

Nervous System

During gastrulation, neural cells differentiate from blastomeres to form the neural plate, which subsequently folds to give rise to the neural tube. By the second day of embryonic development, the neural tube differentiates into the brain and spinal cord (Fig. 3a), with histological sections indicating the division of the brain into the forebrain, midbrain, and hindbrain (Fig. 3b). The process of neural tube formation and subsequent brain and spinal cord development is crucial for the proper functioning of the central nervous system. An interplay between molecules like Robo and N-cadherin plays a role in sorting spinal commissural axons within the spinal cord, facilitating their targeting to the brain (Sakai et al., 2012). In the hatched-out larva, differentiation of the brain

into the telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon is evident (Figure 3c). In fifth-day-old larvae, olfactory bulbs and olfactory lobes are formed. The diencephalon shows the formation of the thalamus and hypothalamus. The optic lobes, cerebellum, and medulla oblongata take shape in this stage. The pineal and pituitary glands are observed in the tenth-day-old larval olfactory tract. In twelfth-day-old larvae, brain structures are fully formed. The spinal cord is enclosed in the vertebral canal.

Eye

During the second day of embryonic development, a critical stage in eye formation, a pair of optic vesicles emerges in the anterior region of the embryo. These optic vesicles are spherical and darker than other regions of the embryo. As the larva hatches, the eyes are located dorso-laterally as dark spherical structures, increasing their size the following day. Pigmentation is not initially observed during these early stages of eye development (Fig. 4a). By the time the larva is two days old, differentiation of eye structures such as the sclera, choroids, cornea, and lens become apparent. Subsequent stages show intensified pigmentation and differentiation of the cornea. By the fourth day, distinct layers of the retina are distinguishable, including the pigment layer, visual cell layer, outer plexiform layer, nuclear layer, inner plexiform layer, ganglionic cell layer, and nerve fibre layer (Fig. 4b). Further development leads to the formation of a compact retina lining the choroid coat, with the completion of lens formation dividing the eye into aqueous and vitreous chambers. By the fifth day, eye development is complete. The development of the eye has highlighted the crucial roles of various signalling molecules and transcription factors. Bone Morphogenetic Protein 7 (BMP-7) has been identified as an inducer of nephrogenesis and is required for eye development and skeletal patterning, emphasising its significance in early eye development in both flies and vertebrates (Luo et al., 1995). Proper patterning of the optic fissure has been shown to require the sequential activity of BMP7 and Sonic Hedgehog (SHH), underscoring the importance of lens-derived signalling in the regionalisation of the optic vesicle (Morcillo et al., 2006).

Furthermore, the differentiation of the optic vesicle into distinct eye structures involves complex molecular interactions. Studies have indicated that dorsal and ventral specification in the early optic vesicle is crucial in proper eye development (Uemonsa et al., 2002). The transcription factor Six3 has been identified as necessary for neuroretinal specification by regulating cell signalling and survival, particularly in a small population of progenitors during early eye formation (Liu & Cvekl, 2017). Moreover, retinoic acid signalling, generated from the optic vesicles and retina, has been implicated in eye development, highlighting the intricate molecular mechanisms involved (Duester, 2022).

Digestive System

The development in larval fish is a critical process involving transforming simple structures into fully functional organs. During the early stages of larval development, the digestive tract consists of a basic tubular structure without a mouth or anal opening. The oral cavity, pharynx, and oesophagus are lined with squamous epithelium, and the stomach and intestine exhibit infoldings along their inner surfaces. As the larva progresses, the formation of the mouth and anal opening occurs, with the appearance of an oral invagination on the first day and the development of a buccal opening by the second day. Exogenous feeding becomes evident as the larva consumes cladocerans and copepods, showing the initiation of feeding behaviour. Histological studies have revealed the differentiation of various digestive organs around the stomach region, including the liver and pancreas. The stomach transitions into a sac-like structure with deep infoldings, while the intestine becomes coiled with a wider lumen. Taste buds are observed in the oral cavity and pharyngeal region, indicating sensory development (Fig. 5). By the fourth day, the stomach transforms into a round muscular structure, and the liver becomes well-developed with vacuolated hepatocytes. The pancreas forms acini, contributing to digestive enzyme production. By the twelfth day, the digestive system is fully formed, with taste buds in the pharynx and well-developed gastric glands in the stomach. The intestine differentiates into anterior and posterior regions, and mucous cells line the inner surface of the digestive tract. Understanding the ontogeny of the digestive system in larval fish is crucial for optimising feeding practices and rearing protocols to enhance larval survival and growth. Research on the histological development of the digestive system in various fish species provides valuable insights into the maturation of digestive organs and enzymatic activities during larval development, aiding in the formulation of appropriate diets and feeding strategies tailored to larval fish's nutritional requirements and digestive capacities.

Swim Bladder

The development in larval fish is a complex process that involves the differentiation of epithelial cells from the anterior part of the stomach to form the primordial cells of the swim bladder. As the larva progresses, these cells develop into a swim bladder, enclosing a cavity with one or two layers of epithelial cells. The swim bladder exhibits thickened regions at the anterior and posterior ends, with the thickened region at the anterior end constituting the gas gland. The swim bladder inflates at the onset of the exclusively exogenous feeding

stage, and its size increases during further larval development. By the twelfth day, the swim bladder takes on a round and large shape anteriorly and an elongated and narrow shape posteriorly. Research on swim bladder morphology and development in fish species has provided valuable insights into the functional significance of swim bladders (Fig. 6). Studies have shown that swim bladder morphology can influence hearing sensitivity in cichlid species, highlighting the relationship between swim bladder structure and auditory abilities (Schulz-Mirbach et al., 2012). Additionally, the swim bladder has been implicated in expanding the frequency range of sound detection in sciaenid fishes with different swim bladder-inner ear configurations, underscoring the importance of swim bladder morphology in auditory functions (Ramcharitar et al., 2006). Understanding the ontogeny of the swim bladder in larval fish is crucial for elucidating its role in buoyancy regulation and sound detection. Studies have demonstrated that swim bladder inflation affects larval density and buoyancy, with larvae exhibiting diel changes in swim bladder volume to adjust their vertical distribution in the water column (Leis, 2007). Furthermore, the swim bladder has been linked to larval survival and growth, with swim bladder inflation influencing larval behaviour and feeding activity (Witeska et al., 2013).

Gill

The development of gill structures in larval fish is a crucial process that involves the differentiation of primordial gill arches into functional respiratory organs. In freshly hatched larvae, primordial gill arches appear on either side of the branchial region. By the second day, these gill arches differentiate into conical structures within the branchial cavity (Fig. 7a). Subsequent stages show the formation of undifferentiated cells at the base of the gill arches, which give rise to gill filaments in later stages. The development of gill filaments involves the formation of lamellae composed of pillar cells, with chloride cells observed at the base of the lamellae. Blood supply becomes evident in the gill filaments of five-day-old larvae, with further development leading to an increase in the length of gill lamellae and filaments (Fig. 7b). The gill development in fish species has provided insights into the morphological and physiological adaptations of gills for respiratory and ionoregulatory functions. Studies have shown that neuroepithelial cells in the gills of zebrafish play a role in oxygen sensing, contributing to the detection of changes in oxygen tension in embryos and larvae (Jonz & Nurse, 2003; 2005). Additionally, chloride cells in gill structures have been linked to ion regulation and osmoregulation in fish larvae, highlighting the importance of gill function in maintaining internal homeostasis (Saltys et al., 2005).

Heart

During embryonic development, the heart transforms from primordial cells located ventrally beneath the pharyngeal region to a fully functional organ. The initial organisation of these cells into a simple tube marks the onset of heart development, with the observation of the heartbeat preceding hatching. The heart exhibits a slightly broader posterior and narrow anterior tube in newly hatched larvae. As larval development progresses, the tubular heart undergoes intricate foldings, leading to the formation of distinct cardiac structures, including the sinus venosus, atrium, ventricle, and bulbous arteriosus (Fig. 8). Subsequent development involves the formation of blood vessels that initially vascularise the gills, highlighting the intricate process of heart development and vascularisation in larval fish. The study of heart organogenesis in vertebrates has shed light on the regulatory mechanisms and morphogenetic processes that drive the formation of cardiac structures. Studies have shown that regulated patterns of gene expression and proliferation within the embryonic heart play a crucial role in the morphogenesis of atrial and ventricular chambers. At the same time, the formation of cardiac cushions contributes to the development of definitive valves in the heart (Miquerol & Kelly, 2012). The developmental processes of the heart in larval fish are essential for elucidating the molecular and cellular events that govern cardiac morphogenesis and function. Research on heart development in fish species provides valuable insights into the evolutionary conservation of cardiac developmental pathways and the adaptive mechanisms that enable efficient cardiovascular function in aquatic environments.

Kidney

Kidney development in larval fish involves a series of intricate processes leading to distinct renal structures. In a freshly hatched larva, the kidney appears as an elongated structure below the notochord, distinguishable into the head kidney and trunk kidney, consisting of pronephros, internal lymphoid, and hematopoietic tissue. By the fifth day, renal corpuscles and tubules become distinguishable, and the urinary bladder opens directly to the exterior. By the twelfth day, the mesonephros are well developed, marking the maturation of the kidney in the larval fish (Figs. 9a and 9b). Research on kidney development in various species has provided insights into the molecular and cellular mechanisms underlying nephrogenesis and renal maturation. Studies have shown that mesonephric nephrons in teleosts are derived from precursor cells within the nephrogenic zone, with the nephrogenic capacity of cells maintained throughout life, allowing for neonephrogenesis (Zhou et al., 2010). Understanding the developmental processes of the kidney in larval fish is essential for

elucidating the evolutionary conservation of renal structures and their functional adaptations in aquatic environments. Furthermore, investigations into the ontogeny of the kidney in fish larvae have implications for understanding renal physiology and osmoregulation in early life stages.

Muscle

The skeletal muscle in larval fish is a complex structure composed of muscle bundles, or myotomes, formed of myofibers. These myofibers exhibit striations and contain multiple nuclei within them. The muscle bundles are separated by the myoseptum, contributing to the organisation and segmentation of the skeletal muscle. In a 7-day-old larva, the notochord is well flexed, indicating the ongoing musculoskeletal development in the larval fish (Figs. 10a and 10b). The development of skeletal muscle in vertebrates has highlighted the role of signalling pathways and transcription factors in regulating myogenesis and muscle fibre differentiation. Studies have shown that hedgehog signalling plays a crucial role in the regulation of muscle fibre types, including slow-twitch and fast-twitch fibres, contributing to the diversity of muscle function in vertebrates (Grimaldi et al., 2004; Du et al., 1997). Establishing the epaxial-hypaxial boundary in the myotome also segregates trunk skeletal muscles into distinct regions, reflecting the spatial organisation of muscle groups in the larval fish (Ahmed et al., 2006). The morphological and functional aspects of skeletal muscle development in larval fish are essential for elucidating the mechanisms underlying muscle growth and locomotor abilities. The myotome organisation, myoseptum structure, and muscle fibre differentiation provide valuable insights into the evolutionary conservation of musculoskeletal systems and their adaptations to diverse environmental conditions.

Fin

The development in larval fish is a dynamic process that involves the differentiation and morphogenesis of various fin structures. In the hatched-out larva, the presence of marginal fin folds and pectoral fins marks the initial stages of fin development. By the second day after hatching, differentiation of the caudal fin is observed, leading to the formation of a homocercal caudal fin in five-day-old larvae, which subsequently differentiates into a homocercal caudal fin. By the twelfth day, the larva exhibits a well-developed caudal fin, with fin rays formed in the pectoral, pelvic, and caudal fins (Figs. 11a–11b). The dorsal fin begins its differentiation around the tenth day and is fully formed by the twentieth day in larval fish. The fin development in fish species has provided insights into the genetic and molecular mechanisms that govern fin morphogenesis and patterning. Investigations into the ontogeny of fins in larval fish have implications for understanding the evolutionary origins and functional adaptations of fins in aquatic organisms (Thorsen & Hale, 2007; Cajado et al., 2021). The developmental processes of fins in larval fish are essential for elucidating fins' structural diversity and functional roles in locomotion, stability, and manoeuvrability. Research on fin differentiation, fin ray formation, and fin morphology provides valuable insights into fins' evolutionary and ecological significance in fish species.

Skin

Histologically, the skin of larval fish is composed of three main layers: the epidermis, dermis, and hypodermis. The epithelium of the skin consists of layers of squamous and columnar cells, with club-shaped cells and goblet cells interspersed between them. In the dermis, two distinct layers, the stratum spongiosum and stratum compactum, are observed, with scales originating from this layer. The hypodermis is present as a thin layer. In a two-day-old larva, pigment cells are observed in the dermis and hypodermis, with the intensity of pigment cells and pigments progressively increasing during further larval development. The histological characteristics of the skin in larval fish are essential for elucidating the skin's structural composition and functional adaptations in early life stages. Research on skin histology provides insights into the cellular organisation, pigment distribution, and developmental changes in the skin of larval fish, contributing to our understanding of skin biology and physiology in aquatic organisms.

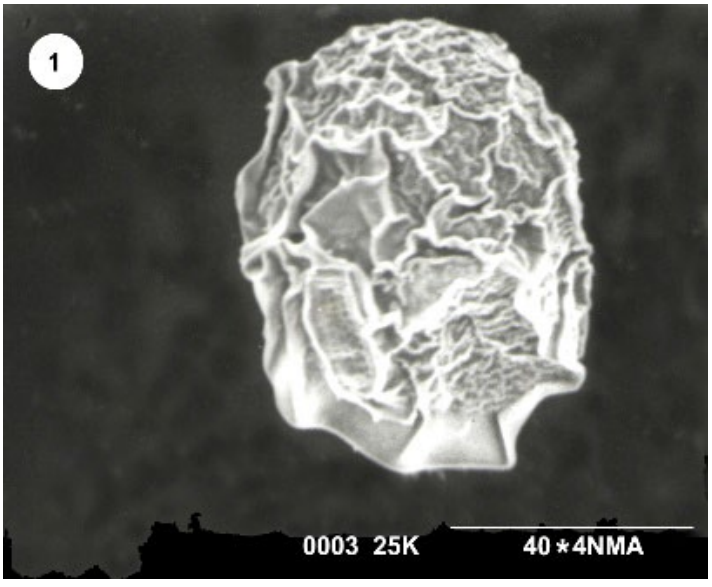
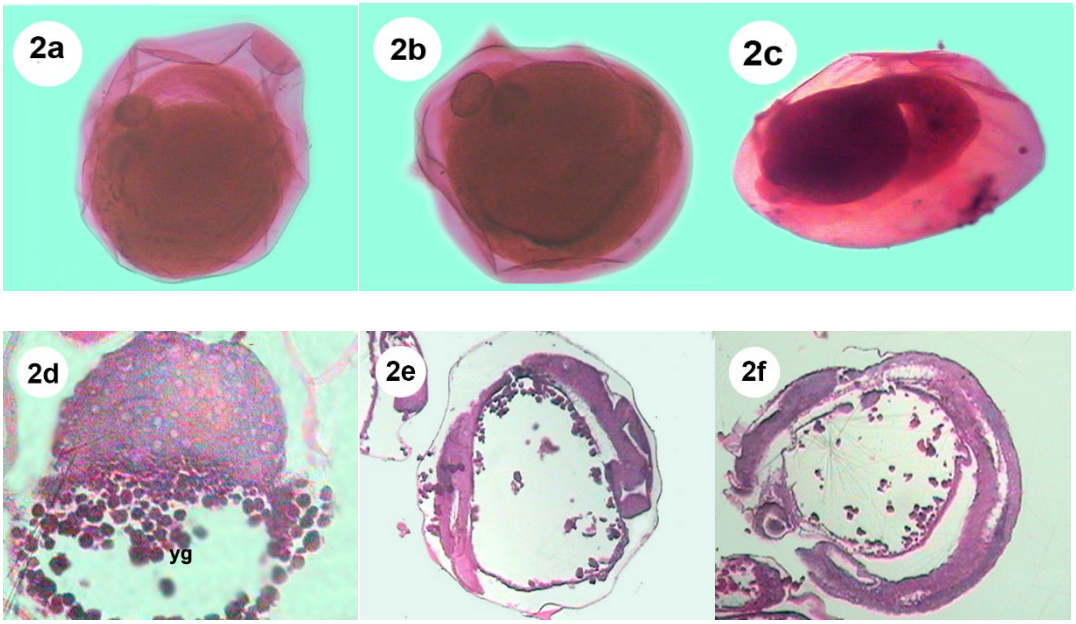
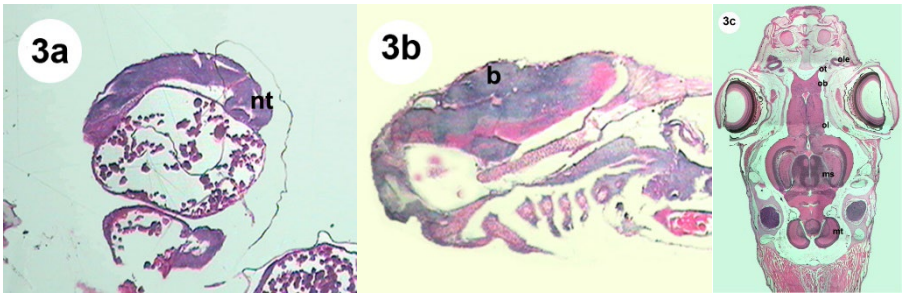


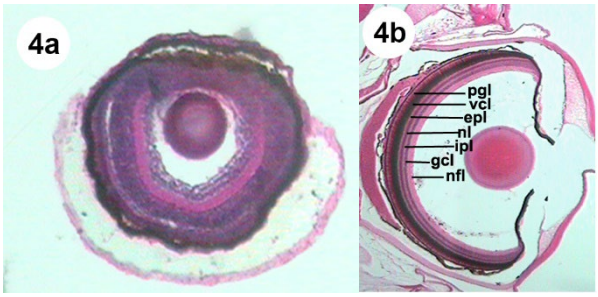
Figure 1. SEM of *C. carpio var. koi*



Figures 2. a. Fertilized egg of *C. carpio var. koi;* b. Egg showing the development of head and eye; c. Advanced stage embryo; d. C.S. of egg showing cleavage; e. C.S. of egg showing the formation of the head; f. C.S. of egg showing fully formed embryo



Figures 3. a. Section of the egg showing neural tube (nt- noto cord); b. L.S. of an embryo showing brain (b- brain); c. V.S. of the brain showing differentiation of different regions of forebrain, midbrain and hindbrain (ole- olfactory epithelium, ot- olfactory tract, ob- olfactory bulb, ol- olfactory lobe, ms- mesencephalon, mt- metencephalon)



Figures 4. a. Histology of embryonic eye; b. 168 Histology of the eye showing different layers of the retina (PGL- pigment layer, vcl- visual layer, epl- external plexiform layer, nl- nuclear layer, ipl- internal plexiform layer, gcl- ganglion cell layer, nfl- nerve fibre layer)

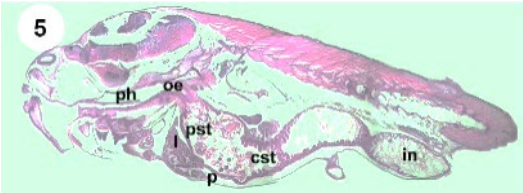


Figure 5. L.S. of larva showing differentiation of stomach (ph- pharynx, oe- oesophagus, l- liver, pst- pyloric stomach, cst- cardiac stomach, in- intestine, p- pancreas)

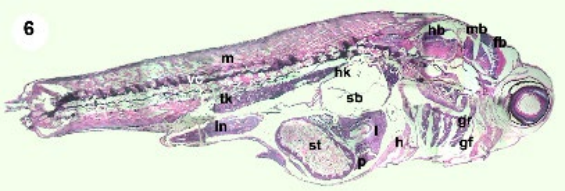
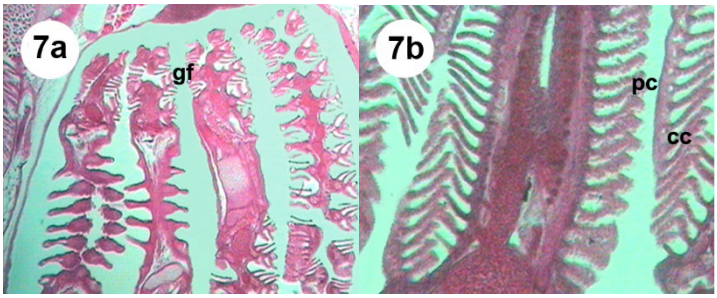


Figure 6. 153 L.S. of larva showing exclusively exogenous feeding (fb- forebrain, mb- midbrain, hb- hindbrain, hk- head kidney, tk- trunk kidney, gr- gill racker, gf- gill filament, h- heart, l- liver, p- pancreas, st- stomach, sb- swim bladder, in- intestine)



Figures 7. a. C.S. of the branchial region showing differentiation of gill filaments (gf- gill filament); b. Higher magnification of gill lamellae showing pillar cells and chloride cells (cc- chloride cells, pc- pillar cells)

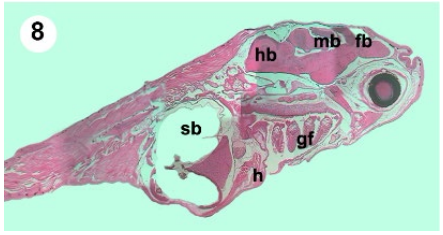
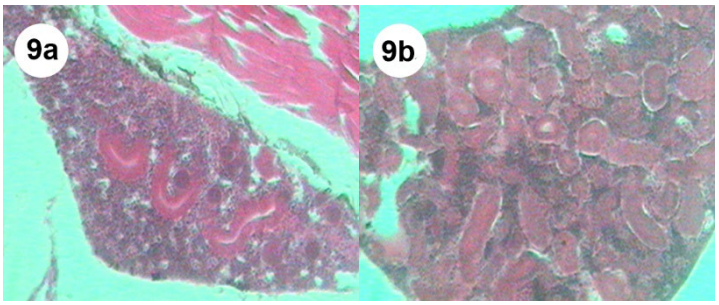
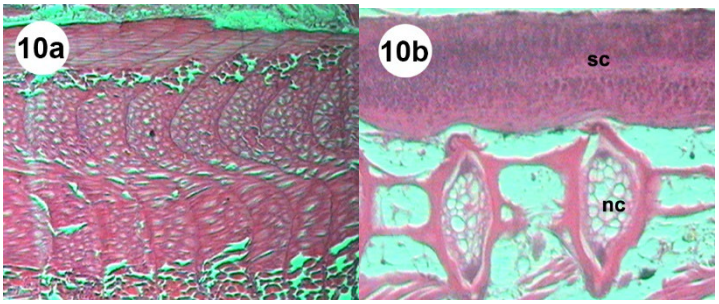


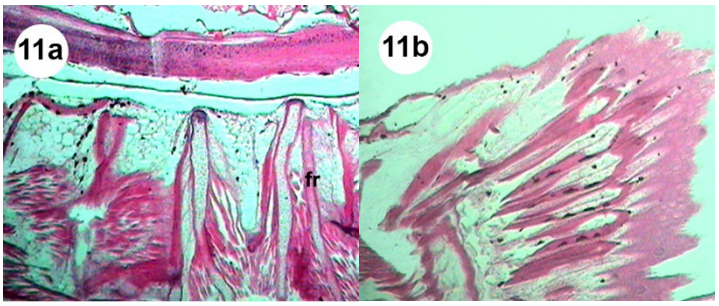
Figure: 8. L.S. of larva showing differentiation of brain lobe, gill filament and caudal fin lobe (fb- forebrain, mb- midbrain, h- heart, hb- hindbrain, gf- gill filament, sb- swim bladder)



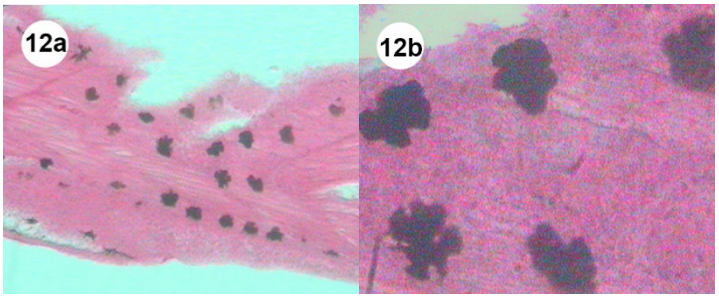
Figures 9. a & b. L.S. of larva showing mesonephric kidney



Figures 10 a. L.S. of longitudinal and circular muscle; L.S. of larva showing notochord (nc- noto cord, sc- spinal cord)



Figures 11. a. L.S. of larva showing fin rays; L.S. of larva showing differentiation of caudal fin



Figures 12. a & b. The skin of different body regions of a larva shows pigmentation

Conclusion

The current study on larval organogenesis in *C. carpio var koi* demonstrates species-specific variation in the incubation duration and distinct ontogenic processes occurring in larvae at different trophic levels. Key developmental milestones include the maturation of the brain, digestive tract, liver, pancreas, gills, kidney, muscles, skin, and fins. Coordinated development is crucial for achieving the functions of eating, breathing, osmoregulation, and behaviour. The transition from an internal to an external energy source was recognised as a crucial phase that could result in significant mortality rates throughout the early stages of life. Investigating the variation in the timing of start feeding among different fish species, the yolk sac larvae that exhibit successful prey consumption are more likely to survive than those that commence feeding later. The transition phase in which tropical fishes switch from relying on internal food sources to external food sources for energy is brief. A wait of more than 24 hours in initiating feeding, either after eye pigmentation or after yolk absorption, is considered crucial for the survival of these species. The larvae are well adapted for this transition in the energy source since they can be raised with a greater survival rate when provided with suitable live feed.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The experiments were conducted in accordance with the guidelines and regulations established by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India and the experimental protocol was approved by Institutional Animal Ethical Committee (Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Tamil Nadu, India) (No: 181GO/ERE/S/15/CPCSEA dated 04.12.2018).

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Disclosure: -

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AQUATIC RESEARCH

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Preliminary studies on the population dynamics of African Sicklefish (*Drepane africana,* Osorio 1892) from the coast of Ghana

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ABSTRACT

Individuals of African Sicklefish (*Drepane africana*), one of Ghana's most commercially and significant marine fish species, are declining in abundance. Therefore, the study aimed to provide the first estimates of growth and mortality parameters for sustainable management of the species from the coast of Ghana. The total length (TL) of 515 individuals of African Sicklefish sampled from June 2020 to July 2021 was measured and analyzed using the FISAT II software to determine the growth, mortality, and exploitation rates. The growth equation was Lt = 27.3 (1 - exp 1.80 (t + 0.09)). The size at first capture (Lc) and maturity (Lm) were 12.3 cm and 16.3 cm TL, respectively. The total mortality rate (Z), natural mortality rate (M), and fishing mortality rate (F) were 7.34, 2.73, and 4.61 per year, respectively. The exploitation rate was assessed at 0.63, indicating that the stock is currently overexploited. The fishery risks collapsing if sustainable management measures are not implemented since the maximum sustainable yield (Emax) slightly exceeds the current exploitation rate.

Keywords: Fisheries management, Growth parameters, Length at capture, Length at maturity, Mortality parameters

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Introduction

Drepane africana, characterised by a compressed body structure, has a small head, short snout, and small mouth with fleshy lips. Its upper jaw contains thin and sharp teeth in bands (Bauchot, 2003). The body of *D. africana* is coloured in silvery-grey, with a darker back and an almost white belly (Bauchot, 2003). This fish species is a benthopelagic, commonly found inhabiting the coastal waters of Canaries, Cape Verde, Senegal, Angola, and Mauritania (Desoutter, 1990).

In Ghana, this species is mostly harvested with fishing gears such as bottom trawl nets, beach seine nets, and hook and line (Segbefia et al., 2013). According to Edwards et al. (2003), the fishing season for this species spans from July to November and January to April. They are highly valued for their flesh, which is of excellent quality and greatly contributes to the nutritional security of many fishing households (Kwei & Ofori-Adu, 2005). Furthermore, the fishery of African Sicklefish contributes significantly to the economic growth of Ghana, generating approximately 1300 tonnes annually (Edwards et al., 2003).

Despite their importance to the local economy and food security, the landings of these species have decreased over time. According to FAO (2019), the landings of *D. africana* in Ghana have plummeted from 6.740 tonnes in 2008 to 32 tonnes in 2019. This alarming decline has dire consequences for the economic welfare of the communities, the livelihoods of dependent households, and food security in rural fishing communities of Ghana. Moreover, there is a significant lack of studies on the population parameters of this species from the coast of Ghana, which could lead to poor fishery management, consequently reducing its resilience in the face of overcapacity of fishing efforts (Aoki et al., 2008). This study aimed to investigate the growth, mortality, and biological reference points that could serve as indicators for sustainable management of the sampled fish species in Ghana. The information gathered from this study will also be used as a resource for future studies in Ghana since it is the first study on the population dynamics of this species.

Materials and Methods

Study Area

The study was carried out in four fishing communities along the coast of Ghana: Sekondi (4°55'45.74"N, 1°43'22.75"W), Sakumono (5°36'40.50"N, 0°2'41.13"W), Keta (5°53'34.41"N, 0°59'36.22"E) and Apam (5°16'59.24"N, 0°44'9.96"W). The main livelihoods of the people in the selected study sites include fishing and farming.

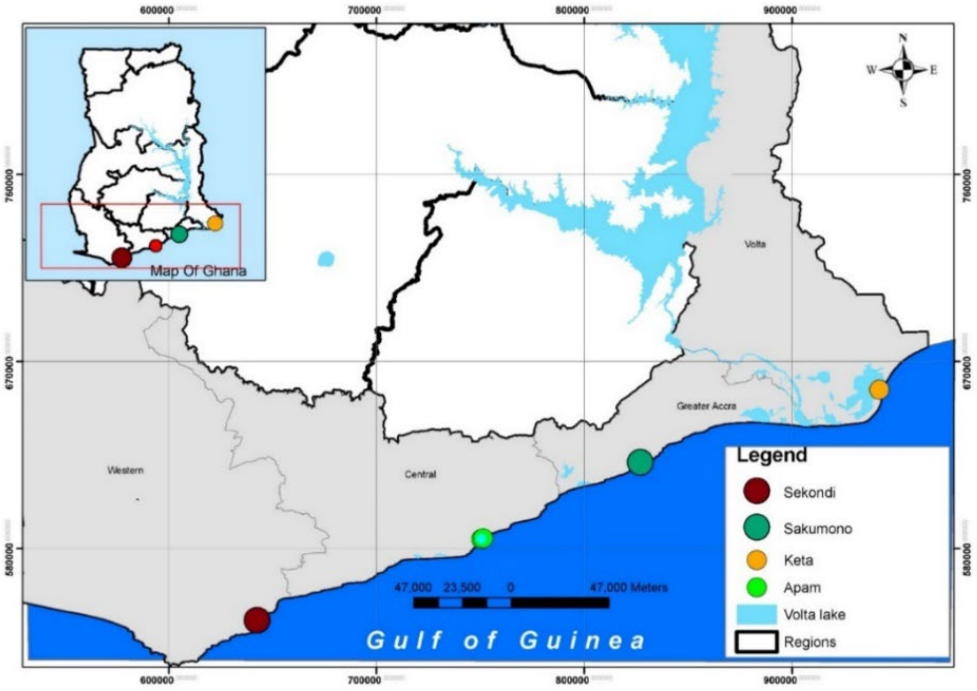

Figure 1. Map showing the sampling locations for the study

Data Collection

Between June 2020 and July 2021, 515 samples of *D. africana* from the coast of Ghana were purchased monthly from local fishermen. Samples obtained were measured to the nearest centimetres for total length (TL) with a wooden measuring board and weighed to the nearest gram using an electronic balance. The samples were identified to the species level using identification keys (Kwei & Ofori-Adu, 2005).

Growth Parameters

Electronic Length Frequency Analysis (ELEFAN) option of FiSAT II Tool, was used to estimate the growth parameters following the Von Bertalanffy Growth Function (VBGF) by Pauly (1980): $TL_t = TL_\infty\left(1 - e^{-K(t-t_o)}\right)$,

L_t is the average length at the time (or age), L∞ is the asymptotic length, K is the growth rate, and t_o represents the age when the average length was zero.

The longevity (T_{max}) was determined as T_{max}=3/K (Pauly, 1983).

The growth performance index was estimated as 2logL∞ + log K (Pauly & Munro, 1984).

The theoretical age at length zero (t_0) was calculated as Log_{10} (-t0) = -0.3922 – 0.2752 log_{10} L∞ —1.038 log_{10} K (Pauly, 1979).

Length at First Capture

The downward left portion of the length-converted catch curve was applied to calculate the lengths at capture. These include Lc_{25}, Lc_{50}, and Lc_{75}, which correlate with the cumulative probability at 25%, 50%, and 75%, respectively (Pauly, 1984).

Length at First Maturity

The length at first maturity (Lm_{50}) was determined as Log Lm_{50} = 0.8979*Log_{10} (L∞) - 0.0782 (Froese & Binohlan, 2000).

Mortality Parameters

The total mortality rate (Z) was determined from the length converted catch curve (LCC). The natural mortality rate (M) at a temperature of 28.9ºC was computed using the empirical formula by Pauly (1980): ln M = – 0.0152 - 0.279 * ln L∞ + 0.6543 * ln k + 0.463 * ln T

where M is natural mortality in a given stock and the value of T is the seawater's annual mean temperature (in ºC).

The fishing mortality coefficient (F) was computed as F = Z–M (Pauly, 1983).

The exploitation rate (E) was estimated as E= F/Z (Pauly, 1983).

Relative Yield Per Recruit (Y/R)' and Relative Biomass Per Recruit (B/R)'

The data of Lc/Linf and M/K values were used to estimate exploitation at maximum yield (Emax), 10% of yield ($E_{0.1}$), and 50% of yield ($E_{0.5}$).

Data Analysis

Length measurement data was pooled together at 5 cm intervals and analysed for population parameters using FAO-ICLARM Stock Assessment Tool (FiSAT) II software (Gayanilo et al., 1988).

Results and Discussion

Length Distribution

The mean length of 515 individuals of *D. africana* obtained during the study was 14.5 ±0.18 cm (Figure 2). The minimum and maximum lengths obtained were 3.90 cm and 27.0 cm, respectively.

Growth Parameters

The calculated Von Bertalanffy growth function (VGBF) parameters of *D. africana* were L∞= 27.3 cm, K = 1.80 $year^{-1}$ (Figure 3), t_0 = -0.09, and Ø' = 3.128, while the estimated goodness of fit of model was Rn = 0.36.

Mortality Parameters

The total, natural and fishing mortality rates for *D. africana* were Z = 7.34 $year^{-1}$ (Figure 4), 2.73 $year^{-1}$ and 4.61 $year^{-1}$ respectively. The exploitation rate (E) was 0.63.

Probability of Capture

The capture probability was 11.3 cm, 12.3 cm, and 13.3 cm at 25%, 50%, and 75%, respectively (Figure 5). Therefore, the length at first capture was 12.3 cm. The length at first sexual maturity was estimated at 16.3 cm.

Yield Per Recruit Analysis

The exploitation rates at the 10 and 50 %, and maximum levels were 0.567, 0.348, and 0.657, respectively (Figure 7).

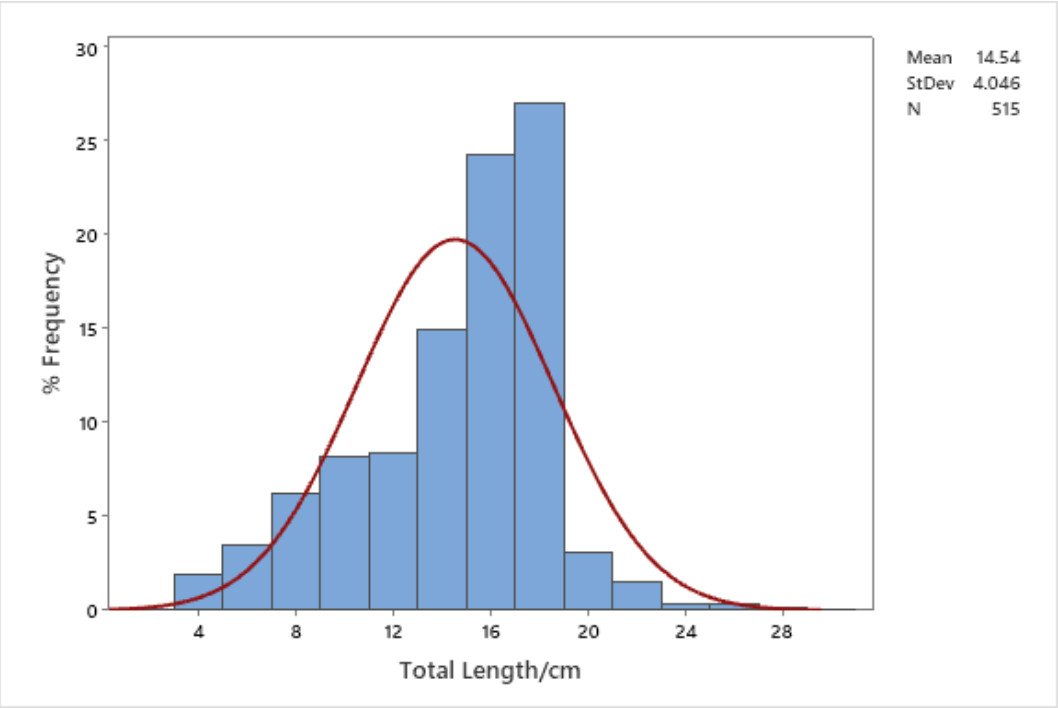

Figure 2. Length distribution of *D. africana* from the coast of Ghana

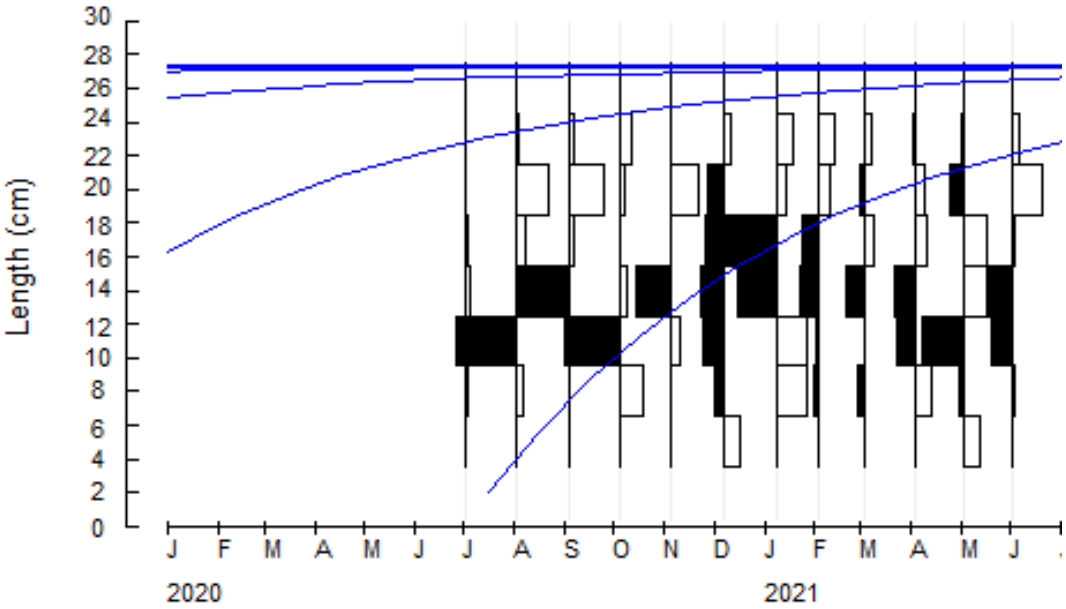

Figure 3. Length-frequency distribution data and growth curves estimated using the ELEFAN method for *D. africana*

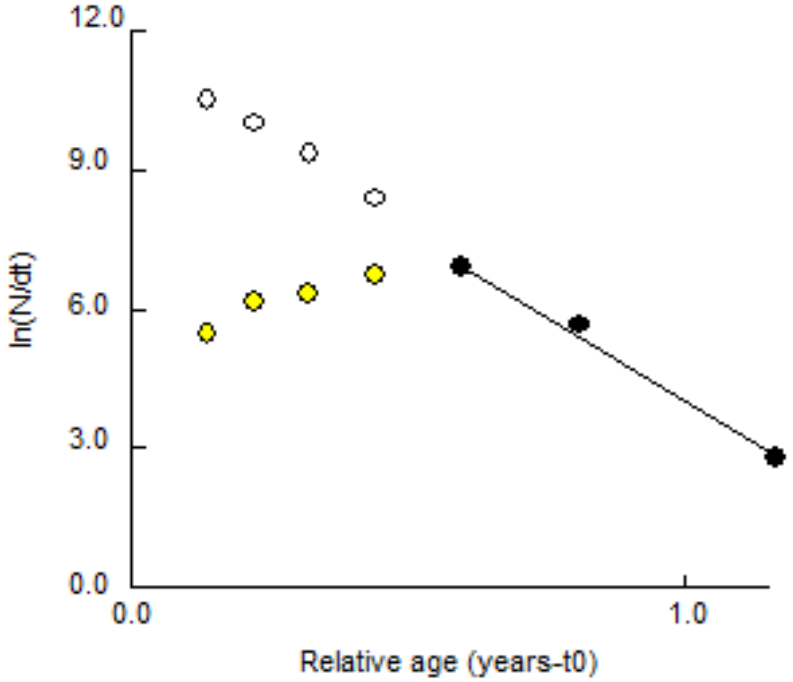

Figure 4. Estimation of 'Z' by length converted catch curve method for *D. africana*

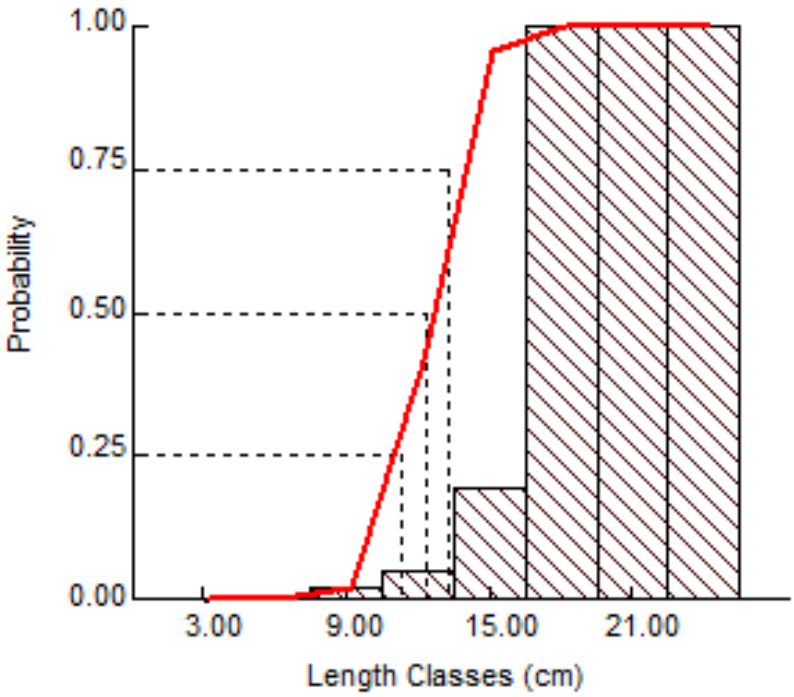

Figure 5. Probability of capture of *D. africana*

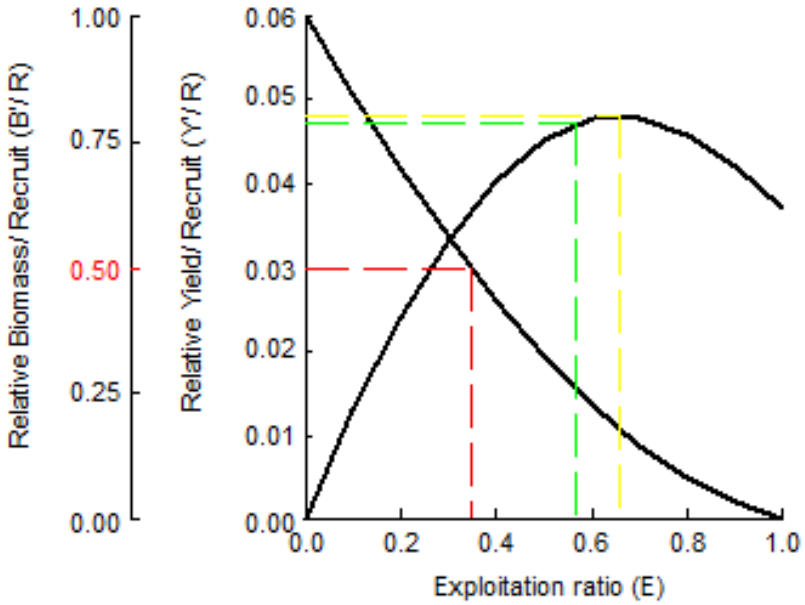

Figure 7. Yield per recruit analysis of *D. africana* in the present study

This is the first study on the population dynamics of *D. africana* from the coast of Ghana. Hence, little information exists for effective comparison. As such, the information gained will serve as preliminary scientific resources for managing this species from the coast of Ghana. Growth parameters are essential to estimate the stock size, recruitment, and mortality of fish population (Shojaen et al., 2007). The asymptotic length recorded from the study was lower than the estimate that Thiam (1988) recorded from the waters of Senegal (51.4 cm). Nonetheless, the growth rate of 1.80 per year in the present study was higher than that of Thiam (1988), who reported a growth rate of 0.15 per year. The variation in asymptotic length and growth rate reveal the presence of small-sized individuals of stock within the coast of Ghana than in other regions, potentially due to the high, unsustainable fishing pressure exerted by fishermen along the coast of Ghana (Amponsah et al., 2019; Arizi et al., 2022). According to Pinsky and Byler (2015), fishes with a fast growth rate are three times more likely to experience a population collapse. This implies that the individuals of the sampled fish species are highly vulnerable to collapse, especially in absence of proper management measures.

The length at first capture (Lc) from the current study was lower than estimate from Thiam (1988), who reported 19.0 cm as the length at first capture. This comparison confirms that individuals of *D. africana* landed along the coast of Ghana are largely of a small size. This is also characterised by the length at capture to asymptotic length ratio being lower than 0.5 (Pauly & Soriano, 1986). This potentially reflects the existence of growth overfishing within the fishery of species. According to Ben-Hasan et al. (2021), poor management of fish species occasions the presence of small-sized individual fish species fishes.

The length at first catch (Lc) from the current study was less than length at first maturity (Lm), suggesting that the species becomes vulnerable to capture before reaching sexual maturity. The presence of immature small-sized individuals from the study may be attributed to artisanal fishermen using small mesh-sized fishing gear (Zhai et al., 2019). Furthermore, high fishing pressure will result in a high likelihood of decline in spawning biomass to the point where recruitment is impaired (i.e. recruitment overfishing) when exploitation is unsustainably high (Ben-Hasan et al., 2021).

From the present study, the fishing mortality rate was higher than the natural mortality rate, indicating that the decline in the population of the fish species is hugely accounted for by fishing-related activities (Aheto et al., 2019). Furthermore, the fishing mortality rate from the study was higher than the value (F = 0.73 per year) Thiam (1988) reported. This suggests that the sampled fish species is experiencing high fishing pressure, evinced by the significant drop in landings (FAO, 2019).

According to Gulland (1971), an exploitation ratio (E) at 0.5 reveals a sustainable level of fishing, while a value above 0.5 signals that the species is in an over-exploited state. This shows that with an exploitation rate of 0.63, the species from

the coast of Ghana is over-exploited. Nonetheless, the exploitation rate from the current study favoured the finding by Thiam (1988), who documented an exploitation rate of 0.63. Compared to the exploitation at maximum sustainable yield (Emax), the current exploitation rate (E) was slightly lower than the Emax, a condition that may result in a reduction of the stock from the coast of Ghana.

Conclusion

The current study sheds light on the population dynamics of the *D. africana* from the coast of Ghana. According to the study, *D. africana* exhibited signs of fast growth, with individuals becoming susceptible to capture before they reach maturity. The stock was overexploited and marginally below the maximum exploitation rate, placing it at risk of future depletion. Revision of mesh size regulation and lowering fishing capacity are some of the measures needed to protect the resources from further depletion.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: Not applicable

Data availability: Data will be made available on request.

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Disclosure: -

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Growth variability of selected *Vibrio parahaemolyticus* strains isolated from seafood

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ABSTRACT

The aim of this study was to quantify the growth and assess the variability of *V. parahaemolyticus* strains isolated from seafood. A total of 35 *V. parahaemolyticus* strains were assessed, and their maximum specific growth rate (μ_{max}) was estimated by the Time-to-Detection Method by regression analysis using the Generalized Reduced Gradient algorithm. The highest μ_{max} (h^{-1}) value was 2.33 for *V. parahaemolyticus* isolated from Atlantic salmon, followed by 2.30 for Mediterranean horse mackerel and European seabass, 2.26 for Mediterranean mussels, 2.20 for veined rapa whelk, 1.88 for the pandemic strain O3:K6, 1.57 for oysters, 1.43 for bluefish, and 1.29 for Gilthead bream. This study provides useful information for the quantitative characterisation of *V. parahaemolyticus* growth, which can be a main input for microbial exposure assessments.

Keywords: *Vibrio parahaemolyticus*, Seafood, Specific growth rate

Introduction

Vibrio parahaemolyticus is a Gram-negative and halophilic bacterium which causes seafood-borne gastroenteritis worldwide (Narayanan et al., 2020). It is a normal habitant in marine and estuary environments. Hence, *V. parahaemolyticus* dwells freely in the water body, attached to the surface or parasite in the gastrointestinal tract of hydrobionts (Tan et al., 2020). The prevalence of *V. parahaemolyticus* varies significantly between geographical regions or different climatic conditions (Ma et al., 2023). However, this pathogen is usually higher in warmer months (Ndraha & Hsiao, 2021). *V. parahaemolyticus* can be more prevalent in fish, shrimps, oysters, mussels, clams, scallops, and squid (Vu et al., 2022; Wang et al., 2022). Seafood is contaminated with *V. parahaemolyticus* because of improper handling, lack of hygiene and refrigeration, and cross-contamination (Stratev et al., 2023). The pathogen can accumulate in hydrobionts, but it could be at higher levels in shellfish because of their filter-feeding behavior. The main pathogenic factors of *V. parahaemolyticus* are thermostable direct hemolysin (tdh) and thermostable direct-related hemolysin genes (trh) (Flynn et al., 2019). *V. parahaemolyticus*-associated gastroenteritis is due to ingesting raw or undercooked seafood. They are seasonally dependent because 67% of the gastroenteritis appear in August and September (Mok et al., 2021). The main clinical symptoms are diarrhoea, abdominal cramps, nausea, vomiting, and fever (Mai et al., 2022). The first outbreak of *V. parahaemolyticus* gastroenteritis was reported in Japan in 1950 after consuming contaminated fish. More outbreaks of contaminated seafood consumption have been reported in the United States, China, Taiwan, Spain, Italy, Chile, and Peru (Odeyemi, 2016).

Maximum specific growth rate (μ_{max}) is considered a universal indicator, relating kinetic information to food-borne pathogens' proliferation. Mathematical models based on μ_{max} allow predicting the behaviour of bacteria in different conditions while having a quantitative assessment at a population level. The maximum specific growth rate is a crucial parameter for developing predictive models which show the practical meaning of strain variability and provide key information for quantitative risk assessment (McMeekin, 1997).

Considering the scarce information and importance of μ_{max} for microbial exposure assessments of *V. parahaemolyticus*, we designed this study to fill these gaps and provide deeper knowledge.

Materials and Methods

Strains Used

In total, 35 *V. parahaemolyticus* strains previously isolated from Mediterranean mussel (*Mytilus galloprovincialis*) (M) (n=12), veined rapa whelk (*Rapana venosa*) (R) (n=7), Mediterranean horse mackerel (*Trachurus mediterraneus*) (SF) (n=5), oysters (*Ostreidae*) (OST) (n=3), Gilthead bream (*Sparus aurata*) (CP) (n=3), Atlantic salmon (*Salmo salar*) (SAL) (n=2), bluefish (*Pomatomus saltatrix*) (CH) (n=1), and European seabass (*Dicentrarchus labrax*) (LAV) (n=1) were used in this study (Stratev et al., 2023). The pandemic strain *V. parahaemolyticus* O3:K6 provided by the National Bank for Industrial Microorganisms and Cell Cultures (Sofia, Bulgaria) was also used as a reference strain.

Preparation of Inoculum

All strains were kept in CASO broth (HiMedia, India) supplemented with glycerin in a fridge at –20°C. After defrosting, each strain was streaked onto Zobell Marine Agar (HiMedia, India) and incubated overnight at 37°C. After that, a single colony was inoculated in alkaline saline peptone water (HiMedia, India) with 2% NaCl and pH 8.6 and incubated at 37°C for 24h to achieve an enriched broth culture of at least log 7 CFU/mL. The enriched broth was centrifuged at 6450 rcf for 5 min. Moreover, decanted, the cell pellet was washed twice, and the bacterial suspension was recovered in alkaline saline peptone water (HiMedia, India).

Determination of Maximum Specific Growth Rate (μ_{max})

A standard 96-well flat-bottom microplates were inoculated with 2-fold serial diluted bacterial cultures, and the optical density was measured every 30 min for 10 hours at 630 nm (Microplate Reader Rayto RT-2100C, China). The method of Cuppers & Smelt (1993) and Membre et al. (2002) was applied for computing the μ_{max} by regression analysis using the Generalized Reduced Gradient algorithm (Excel solver). Each isolate was assessed in triplicate, and the mean values of μ_{max} were calculated using the following basic formula:

$$Mean\ value = \frac{a + b + c}{3}$$

where **a** is the value of μ_{max} from the first assessment, **b** is the value of μ_{max} from the second assessment, and **c** is the value of μ_{max} from the third assessment.

Statistical Analysis

GraphPad Prism (ver. 8.0.1) was used for statistical data processing. Two-way ANOVA with Tukey's multiple comparisons test was performed to show significant differences in the specific growth rate between the investigated strains. The results are presented as mean values. The statistical significance was determined at $p<0.05$.

Results and Discussion

The mean μ_{max} (h^{-1}) of *V. parahaemolyticus* ranged from 0.73 to 2.26 for Mediterranean mussels, 1.63 to 2.20 for veined rapa whelk, 1.67 to 2.30 for Mediterranean horse mackerel, 1.19 to 1.57 for oysters, 0.99 to 1.29 for Gilthead bream, 2.01 to 2.33 for Atlantic salmon, while it was 1.43 for bluefish, 2.30 for European seabass, and 1.88 for the pandemic strain O3:K6. The strain with the highest growth was isolated from Atlantic salmon, i.e. SAL9 – 2.33, and the slowest grower was isolated from Mediterranean mussels, i.e. M5 – 0.73. There was a significant difference ($p<0.05$) in the growth characteristics between the investigated strains from Mediterranean mussels (Figure 1), Mediterranean horse mackerel (Figure 2), Gilthead bream (Figure 3), and between the strains isolated from oysters (Figure 4). No significant difference ($p>0.05$) in the growth characteristics between the strains from veined rapa whelk was found.

V. parahaemolyticus has been reported to be a major seafood-borne pathogen in Asia and the USA responsible for severe infections (Wang et al., 2020a). In China, 322 *V. parahaemolyticus*-associated gastroenteritis outbreaks were recorded, resulting in 9041 illnesses and 3948 hospitalisations between 2003 and 2008 (Wu et al., 2014), while vibrions cause 80000 illnesses and 100 deaths in the United States each year (Hanna et al., 2022). From the above, it is evident that *V. parahaemolyticus* is the most common pathogen in seafood, and the development of a predictive model has market importance for providing safe aquatic products (Wang et al., 2020b). Quantitative risk assessment can be applied to develop effective and efficient risk-based food safety programs. It comprises hazard identification, dose-response assessment, exposure assessment, and risk characterisation (Potter & Brudney, 1994). The exposure assessment step includes determining a few indicators, including the maximum specific growth rate or briefly μ_{max} (Hu et al., 2017). In this study, we determined the μ_{max} of 35 *V. parahaemolyticus* strains using a turbidimetric assay. This method is reliable for estimating bacterial growth under various conditions (Cuppers & Smelt, 1993). It is also rapid, non-destructive, inexpensive, and easily automated (Dalgaard & Koutsoumanis, 2001). Lianou & Koutsoumanis (2011) found higher intra-specific variability of μ_{max} among *S. enterica* strains compared to that observed among the different replicates of one strain. Our results align with this finding as we computed a high range of μ_{max} values, between 0.73 and 2.33, in the investigated strains. Whiting and Golden (2002) stated that this point is important for properly interpreting experimental results because some food microbiologists incorrectly assume that strain-to-strain variation is equal. It is not necessary to be estimated. Moreover, research data generated in this study should be useful in strain selection for food safety challenge tests, assessing the effect of hurdles, and the development of quantitative risk assessment models (Lianou & Koutsoumanis, 2013). Shi et al. (2021) calculated μ_{max} of 18 *V. parahaemolyticus* strains isolated from shrimps by the modified Gompertz model and found values ranging from 0.16 to 0.64 in 2-fold dilution broth culture. Similarly, Wang et al. (2020b) also applied the modified Gompertz model for the μ_{max} calculation of 27 *V. parahaemolyticus* strains isolated from shrimps, and the values ranged from 0.45 to 1.00. At 37°C, Liu et al. (2016) found that μ_{max} ranged from 0.03 to 0.24 at 0.5% NaCl, from 0.02 to 0.44 at 3% NaCl, from 0.01 to 0.26 at 5% NaCl, from 0 to 0.15 at 7% NaCl, and from 0 to 0.12 at 9% NaCl among 50 *V. parahaemolyticus* strains isolated from shrimps. When these results were compared with those of our strains, the higher μ_{max} estimates were evident.

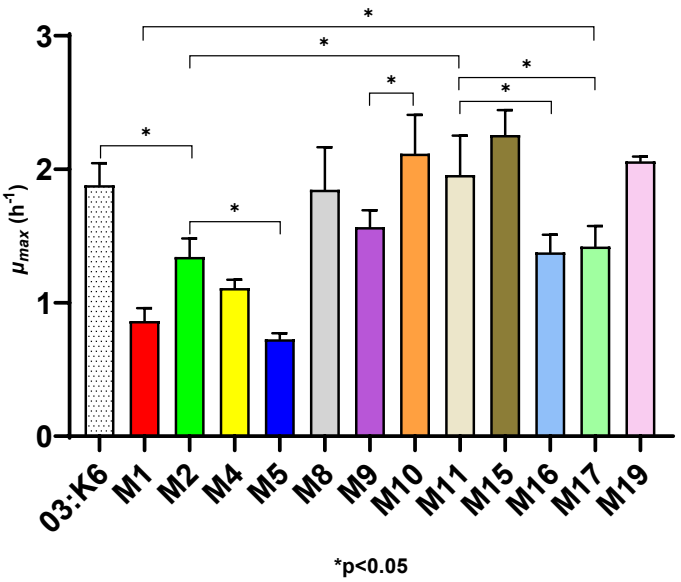

Figure 1. Significant differences between *V. parahaemolyticus* strains from Mediterranean mussels

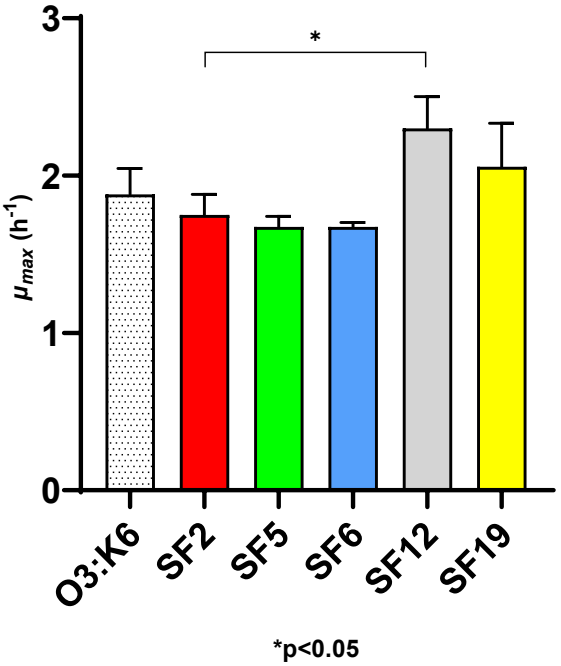

Figure 2. Significant differences between *V. parahaemolyticus* strains from Mediterranean horse mackerel

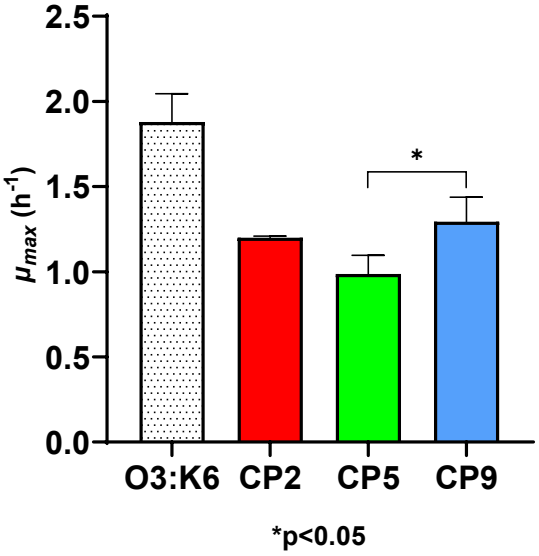

Figure 3. Significant differences between *V. parahaemolyticus* strains from Gilthead bream

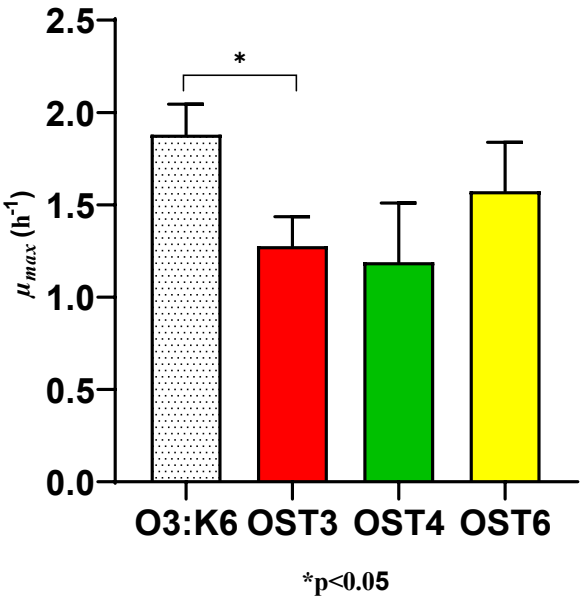

Figure 4. Significant differences between *V. parahaemolyticus* strains from oysters

Conclusion

The results showed that the highest μ_{max} value was for *V. parahaemolyticus* isolated from Atlantic salmon, followed by the values for Mediterranean horse mackerel, European seabass, Mediterranean mussels, veined rapa whelk, oysters, bluefish, and Gilthead bream. This study provides useful information for the quantitative characterisation of *V. parahaemolyticus* growth, which can be a main input for microbial exposure assessments as part of risk analysis of food-borne pathogens.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require an ethics committee or special permission.

Data availability: Data will be made available on request.

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Disclosure: -

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Additional record of devil firefish, *Pterois miles* (Bennett, 1828) (Scorpaenidae) from Izmir Bay (NE Aegean Sea, Türkiye)

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ABSTRACT

This ichthyological note presents a recent observation of a *Pterois miles* within an artificial reef shared with an octopus off Urla in the Bay of Izmir (northern Aegean Sea). On 31 May 2024, a *P. miles* specimen was observed and photographed by a GoPro Hero 9 Black video camera during SCUBA diving at a depth of 9 m. This report provides signs suggesting that a very fast-spreading invasive species may use an artificial reef as a long-term habitat as it moves into the Bay of Izmir.

Keywords: Lionfish, Artificial reef, Octopus, Interaction, Mediterranean Sea

Introduction

The devil firefish (herein lionfish) *Pterois miles* (Bennett, 1828) is a species commonly found in the Indian Ocean and Red Sea (Froese & Pauly, 2024). This species is characterised by a very voracious and high reproductive rate, which renders it a significant threat to Mediterranean species (Albins & Hixon, 2008; Kletou et al., 2016). Consequently, it is considered to be one of the hundred of the world's worst invasive alien species (Lowe et al., 2000). Since its initial observation at Haifa Bay, Israel, in the Mediterranean Sea in 1991 (Golani & Sonin, 1992), the species has exhibited relatively rapid reproduction and spread and further expanded to the coasts of Lebanon (Bariche et al., 2013), the northern Aegean Sea (i.e. Edremit Bay) (Aydın et al., 2022), the southern Aegean Sea (i.e. southern Crete) (Dallianis et al., 2016), Adriatic Sea (Dragičević et al., 2021), Italy, (Azzurro et al., 2017), Tunisia (Dailianis et al., 2016) as well as Libyan Sea (Al Mabruk & Rizgalla, 2019) between 2013 and 2022.

In the Aegean Sea, since the initial observation of *P. miles* on Rhodes Island in 2015 (Crocetta et al., 2015), the fish has continued its northern migration as far north as Kokar Bay (Özgül, 2020), Karaburun, Izmir (Oruç et al., 2022) and Edremit Bay (Aydın et al., 2022; Alkan et al., 2023). Nevertheless, the northernmost record of *P. miles* was reported from Croatia in the Adriatic Sea by Dragičević et al. (2021).

This paper presents a recent observation of a *P. miles* within an artificial reef (AR) shared with an octopus off Urla in the central Bay of Izmir, northern Aegean Sea.

Materials and Methods

On 31 May 2024, a *P. miles* specimen was observed and photographed by a GoPro Hero 9 Black video camera during SCUBA diving in an artificial reef area (AR) off Hekim Island, Urla, Izmir Bay (Figure 1) at a depth of 9 m (Figure 2). The water temperature was 20°C. Additionally, an *Octopus vulgaris* Cuvier, 1797, was sheltering in the same reef. One week later (on 7 June 2024), the same fish was re-examined to ascertain its continued presence, and the fish was still there. However, the octopus was absent.

The AR deployed at two different depths (9 and 18 m) in the coastal area of eastern Hekim Island in 1992 was constructed from reinforced concrete. It consisted of 30 blocks in a cubic form with a hollow 1 m³ volume (Lök et al., 2002).

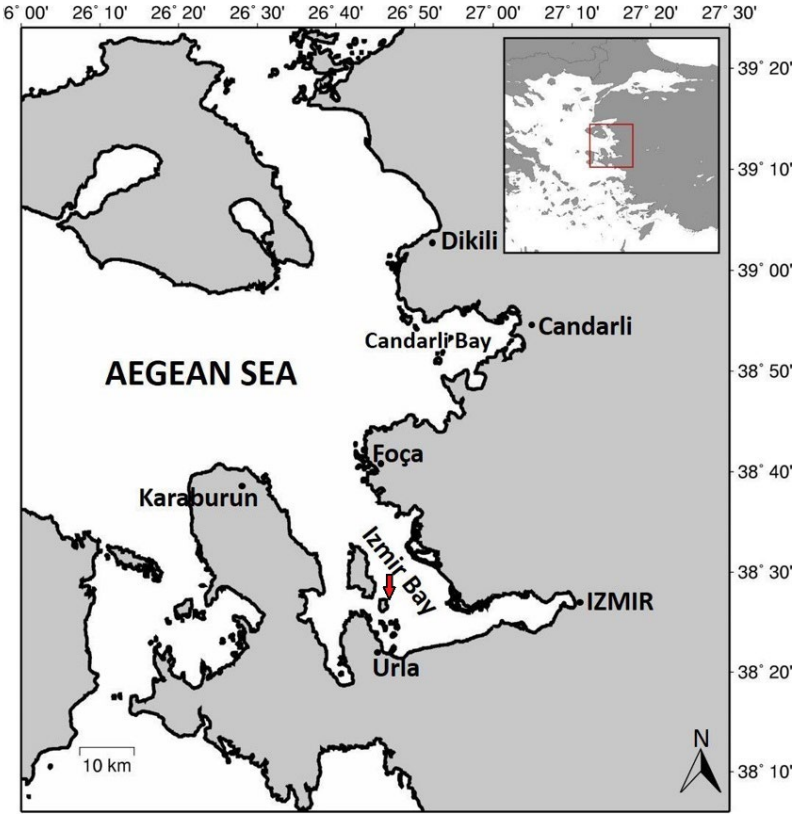

Fig. 1. Artificial reef area where *Pterois miles* were seen (red arrow)

Results and Discussion

The estimated length of *P. miles* was 17–18 cm, and the animal was observed calmly standing near an octopus burrow. Despite the diver's attempts to push *P. miles* away with his hand, the fish initially retreated slightly and then returned to the same location. The *octopus vulgaris* (estimated weight: ~6 kg) sheltered in a hole close to the fish did not leave the area where it laid its eggs and has never been observed interacting with the lionfish. In contrast, the octopus is regarded as one of the few potential predators of *the P. miles* (Crocetta et al., 2021). *P. miles* exhibited a calm behaviour, seemingly aware that the octopus would not harm itself given its protective role in incubating eggs.

Figure 2. *Pterois miles* and *Octopus vulgaris* are sharing a habitat within an artificial reef near Hekim Island, off Urla, Izmir Bay (North-eastern Aegean Sea)

Conclusion

This ichthyological note proves that a highly invasive species may utilise an AR as a long-term habitat as it expands into Izmir Bay. Furthermore, the initial evidence of mutual sharing of habitat with a spawning octopus without any apparent harm to either species may have been identified in this study. In contrast, Crocetta et al. (2021) presented visual material of an octopus catching and eating a lionfish in a similar environment in Famagusta, Cyprus, on 9 February 2021. However, the *P. miles* appeared calm and confident, as though it would not be attacked. We suppose that the octopus is waiting for care of its eggs (it's known that during this time, the female octopus must keep clean eggs and protect them from predators) has played a role in this phenomenon. However, this issue needs to be proven in future studies. Furthermore, the interaction between *P. miles* and the other fishes in ARs should also be examined with further studies. If it settlements in ARs, it is likely to limit the lives of other native fish. The spread of the species in the Bay of Izmir should also be closely monitored.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require an ethics committee or special permission.

Data availability: No funding provided.

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Disclosure: -

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....(Bhujel, 2014).

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(if a DOI number is available)

A book in print

Bhujel, R.C. (2014). A manual for tilapia business. CABI Nosworthy Way Wallingford Oxfordshire OX10 8DE UK, 199 p. ISBN 978-1-78064-136-2. https://doi.org/10.1079/9781780641362.0000
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Craddock, N. (1997). Practical management in the fo od industry A case study. In Food Allergy Issues for th e Food Industry; Lessof, M., Ed.; Leatherhead Food R A: Leatherhead, U.K., pp 25-38. ISBN: 4546465465

A webpage

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