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Aims and Scope

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SHORT COMMUNICATION

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Status of benthic cover in Carbin Reef, Sagay Marine Reserve, Western Visayas, the Philippines

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ABSTRACT

This study determined the status of Carbin Reef benthic cover, Sagay Marine Reserve, Negros Occidental. The underwater benthic assessment was conducted on March 2019 by laying eight 50-meter transect lines at the depths of 6 meters (crest) and 12 meters (slope). Photo-quadrat method was used in obtaining still benthic images for every 1-m across eight 50-meter transect lines. A total of 400 still images were acquired and analyzed using Coral Point Count with Excel Extension (CPCe). The sedimentation rate was also determined by deploying sediment collectors. Results showed that Carbin Reef is characterized by 26.09% live coral cover and 43.25% abiotic structure such as rocks, sand, and coral rubbles. Fifty-five (55) coral species belonging to thirty-three (33) genera were identified, dominated by *Porites*, *Fungia*, and *Goniastrea*. Throughout the area, *Porites lobata* was the most dominant coral species. The overall hard coral cover seemed to have decreased since 2014. Further analysis showed the prevalence of bleached corals and diseases such as white syndrome, and white band disease. Similarly, two signs of compromised health including sediment damage and competition overgrowth were also present. The sedimentation rate per day was below the maximum tolerable levels by most corals during dry season. Overall, this study presents the first extensive study of coral diversity in Carbin Reef–characterized by a moderately diverse area and considered as a moderately stressed habitat.

Keywords: Live coral cover, CPCe, Transect images, Species diversity, Sedimentation

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Introduction

The Philippines, lying at the apex of Coral Triangle, has an estimated coral reef area of 26,000 km^2 and considered as the second largest in Southeast Asia (Spalding, 2001; Burke et al., 2002; Burke et al., 2011; Carpenter et al., 2011). There are approximately 500 species of scleractinian or stony corals in the country, with 12 of them considered as endemic (ADB 2014). However, an average of 15% decline on coral reefs globally happens caused by climate change, typhoons, diseases, pollution, overfishing, etc. (Hughes et al., 2017). Philippine coral reefs suffer the same, which is in a declining state. Based from 1976-1981 nationwide coral reefs assessment, only 5.5% of the Philippine coral reefs are in excellent condition, while 70.1% are considered fair to poor (Gomez et. al., 1981). Subsequent studies were conducted between 1987-1994 and revealed a decline in excellent live coral cover from 5.5% to 2.4%, with 75.3% categorized as poor or fair (Gomez et. al., 1994). Similarly, a nationwide survey from 1990 to 1999 found that 4.3% of the coral reef area is in excellent condition (Licuanan and Gomez, 2000). With the declining state of the Philippine coral reefs and increasing environmental pressures, continuous monitoring of their conditions is important for proper management and protection.

Due to increased coral reef declines, the Philippine Coral Reef Information Network (PhilReefs) produced a series of publications serving a venue to monitor the status of Philippines coral reefs. These publications were cascaded from national to local levels (from province to barangay) providing specific information on coral reef status (Aliño et. al., 2002; PHILREEFS, 2010). The country's coral reef monitoring sites were divided into six biogeographic regions: the West Philippine Sea, North Philippine Sea, South Philippine Sea, Sulu Sea, Celebes Sea, and Visayan Sea (Aliño and Gomez, 1994) represented with 61 municipalities/cities (PHILREEFS, 2010). In the Visayan region, particularly in the province of Negros Occidental, three municipalities/cities (6 stations) were included in the survey between January 2015 and January 2017 (Licuanan et al., 2017). The results revealed 33% hard coral cover (HCC) present in Northern Negros, ranking as the 6th highest percentage of HCC in the country. While majority of the reefs surveyed in Visayas are categorized as poor, excellent conditions are found in protected areas reiterating the importance of marine reserves in coral reef protection.

Negros Occidental is known for its largest marine reserve in the Philippines the Sagay Marine Reserve (SMR). Being the largest marine reserve in the country, SMR is an important management initiative for national and international sustainability and food security. It is also known for its rich marine ecosystem diversity (Maliao et al., 2004; Manejar et al., 2019; Albarico et al., 2020). SMR was established in 1983 and was previously known as Sagay Marine Sanctuary. The Department of Environment and Natural Resources (DENR), recognized the potential of Sagay's coast to contribute to the preservation of Philippine biodiversity and advocated its inclusion in the National Integrated Protected Areas System (NIPAS) (Surtida, 2001; NIPAS 1992). The Presidential Proclamation 592 declared the 32,000 ha Sagay's coastal waters on June 10, 1995 as protected seascape (NIPAS, 1992). The Sagay Marine Reserve Law was then strengthened through Republic Act No. 9106, enacted on April 1, 2001 (Proclamation No. 592) to include the islands of Molocaboc Daku, Molocaboc Diut, Matabas, and Suyac, as well as their surrounding reefs such as Carbin, Molocaboc, and Panal, and the coastal waters of Himo-gaan Baybay, Old Sagay, Taba-ao, Bulanon, Molocaboc, and Vito in the 32,000 ha SMR (NIPAS 1992; Proc. 592; R.A 9106).

Carbin Reef is one of the reef components inside SMR, and the nearest to the mainland (about 5.4 km), and an estimated area of 200 hectares. This was first proclaimed through Municipal Ordinance No. 2 as a marine sanctuary in 1983, hence, it became an important biodiversity conservation model locally and internationally (Alcala, 1988; Webb et al., 2004). A previous study recorded twenty-eight (28) genera of scleractinian corals dominated with massive species *Porites* spp., and sub-massive types (*Favia stelligera*) in Carbin Reef (Maliao et al. 2004). The area was assessed to evaluate its effectiveness on the restoration and conservation of donkey's ear abalone stocks considering the reef's protected status. While the reserve is monitored by SMR Office, scientific reports and published diversity studies are important to provide scientifically sound literatures. Currently, there is a lack of up-to-date information on the status of Carbin Reef. Species-level data is also lacking. Therefore, the aim of this study was to determine the benthic cover, coral diversity, and health status of corals in Carbin Reef in order to establish an in-depth diversity background and to strengthen its management.

Material and Methods

Sampling

Sampling was done in March 2019 in Carbin Reef, Sagay City, Negros Occidental (Figure 1); one of the major reefs within the 32,000 hectares' SMR. Carbin reef covers about 200 hectares and is characterized as a sand cay at the southern end. The most common substrate are dead corals, found in the north, east, and west of the cay. A sandy bottom characterizes the area in the south. On the northern extremity of the reef, a dense growth of *Sargassum* spp. are found. In 1983, a reef watch tower was built in Carbin reef and is still in use by the local government unit's *bantay dagat* or local deputized fisheries enforcers (Maliao et al., 2004). The Philippines' *bantay dagat* or sea guardian program is a community-based law enforcement that uses volunteers from coastal villages or barangays to help detect and apprehend illegal fishing in municipal waters (Maderazo and Advisors, 2016).

Carbin reef is within the Strict Protection Zone of SMR wherein fishing activities are prohibited. However, some portion of the area is intended for recreational activities (i.e. diving, snorkeling, swimming). Extending approximately 500 meters from the edge of Carbin reef is considered as buffer zone, allowing migration of species with relatively limited human disturbance.

An underwater survey was conducted between the depths of 6–12 meters at eight sampling points (Table 1) using Self-Contained Breathing Apparatus (SCUBA) diving equipment. Transects were laid and the photo-quadrat method was used to gather benthic images (van Woesik et al., 2009). After the photo-quadrat, individual coral species on the same transect were photographed to attain closed-up photos for species verification. Table 1 listed the transect coordinates and depth profiles.

Benthic photographs were obtained using an underwater camera (GoPro Hero 5 with underwater casing) mounted on a 0.75-meter-high improvised PVC tetrapod (1 x 1 m base frame/ quadrat) (Figure 2a,b) with a constant vertical distance between the camera and the transect line, starting from the zero mark of the transect. Benthic still images were captured for every 1 m, across the 50 m transect line (DENR-BMB Technical Bulletin No. 05, s. 2017). As a result, each transect acquired fifty (50) photographs, for a total of 400 images for all transects. Photographs were processed and analyzed using the CPCe software by Kohler and Gill (2006). Closed-up individual coral photos were taken in the same transect for species identification. Corals were identified using published literatures (Aliño et al., 2002; Richards, 2018), and verified in the Corals of the World website (Veron et al., 2019). Coral diversity was calculated using Shannon-Weaver and Simpson's diversity indices (Arias-González et al., 2012; Ismail et al., 2022).

Table 1. Location and depth profile of the sampling points.

Transect No.	**Depth (meter)**	**Coordinate**	
		Latitude	Longitude
Transect 1	6	10°58'55.0''	123°27'13.0''
Transect 2	12	10°59'00.0''	123°27'08.4''
Transect 3	6	10°58'51.5''	123°28'05.0''
Transect 4	12	10°58'52.2''	123°28'06.2''
Transect 5	6	10°59'22.4''	123°27'54.7''
Transect 6	12	10°59'22.5''	123°27'51.7''
Transect 7	6	10°58'43.0''	123°27'52.0''
Transect 8	12	10°58'42.6''	123°27'53.8''

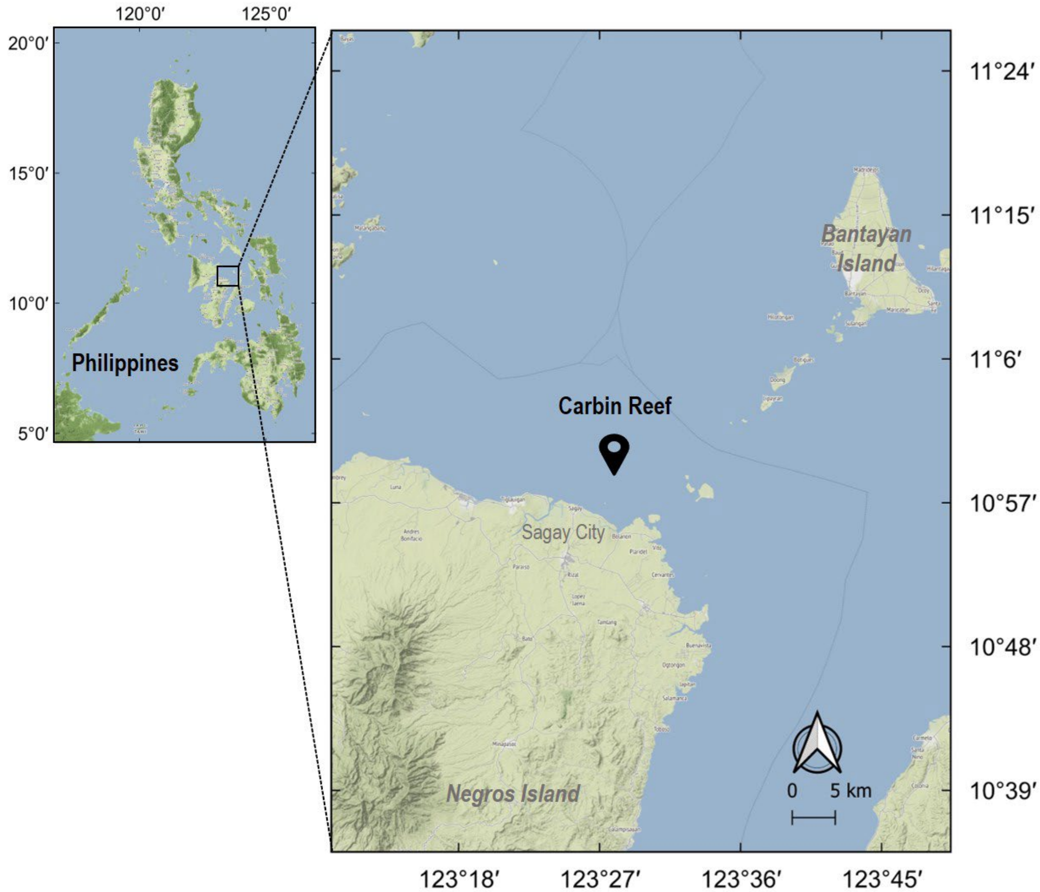

Figure 1. Study site indicating the location of Carbin Reef, Sagay Marine Reserve.

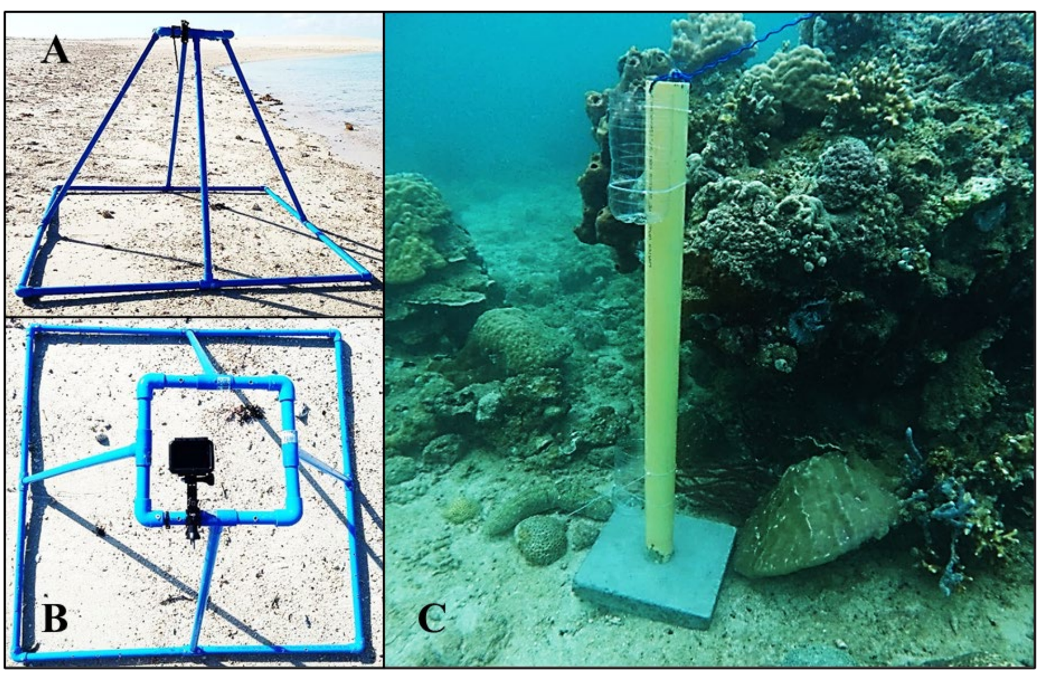

Figure 2. PVC tetrapod with 0.75-meter-height from the substrate with a 1 x 1 m base frame/quadrat (a,b); and sediment collector installed at 6-meter depth (c)

Coral diseases were also noted to provide additional background on coral health status. Prevalence of diseases and signs of compromised health were morphologically identified through the acquired photographs. Closed-up photos were taken to further verify disease prevalence. Disease identification was based from the Coral Disease Handbook of Raymundo et al. (2008).

Sediment Collection

The sedimentation rate per day was determined to gain insights on potential impacts of weather events and anthropogenic run-offs on the reef (Rogers, 1990). Four sediment collectors (Figure 2c) were deployed in each of the 6 m deep transect sites, vertically mounted to the reef. These sediment collectors were made of PVC pipe (which serves as a post) and a cement platform to stabilize the post. At 70 and 20 cm, respectively above the substrate, plastic bottles (about 15 cm high and 8 cm diameter) were affixed to a reference pvc pipe/post. The plastic bottles containing sediment were removed from the post after 7 days and taken to the laboratory for processing. Small organisms were removed from the plastic jar with tweezers. The sediments were filtered through a filter paper (Whatman Filter No. 1) and gently washed multiple times with distilled water to remove salts. The filtered sediment were oven-dried at 70°C until a constant weight was attained. Sedimentation rate was determined in milligrams of sediment per square centimeter (cm^2) per day. Sedimentation rate was calculated as: Sedimentation rate = (sediment weight) ÷ (number of days at the site × r^2). The sediment weight is the total weight minus the filter weight, and the area of the jar opening is πr^2 (r= radius in cm) (Rogers et al., 1994).

Results and Discussion

Benthic Cover

Coral ecosystem of Carbin reef was characterized as a fringing reef. Carbin reef had 26.09% live hard coral cover (HCC), while abiotics such as sand, rock, and rubble had a greater average proportion of 43.25% (Figure 3). The coral cover of Carbin reef was in fair condition, according to the criteria (23–33%) outlined in DENR-BMB (2017). Dead corals, on the other hand, had a greater percentage cover of 22.36, which was closer to the overall mean live HCC. This has caused a decline in the hard coral cover within Sagay Marine Reserve from the 33% HCC estimated by Licuanan et al. (2014). This suggests that ecological processes may be present that are causing the coral reef community to deteriorate (Tabugo, 2016). Anthropogenic influences such as fishing, underwater leisure activities (i.e., diving, snorkeling) (Gomez, 2004) and natural disturbances such as intense ocean currents associated with monsoons and typhoons (Hughes and Connell, 1999) were identified as possible reasons of significant rubble cover and coral death in the area. Conversely, the active patrol by the local fisheries enforcers and the absence of apprehensions does not connect the deterioration of the reef to illegal fishing activities. The most significant damaged could have happened during Typhoon Haiyan which traversed across the Sagay Marine Reserve's four bordering reefs, including Carbin Reef, in November 2013, resulting to the loss in coral reef cover (Bantigue, 2016). Dead corals with algae showed 22.36% prevalence (Fig. 3) which is comparable to less impacted areas of Eastern Samar. Being one of the directly hit areas, dead coral cover in Eastern Samar after the onslaught of Typhoon Haiyan ranged from 20-60% (Anticamara and Go, 2017).

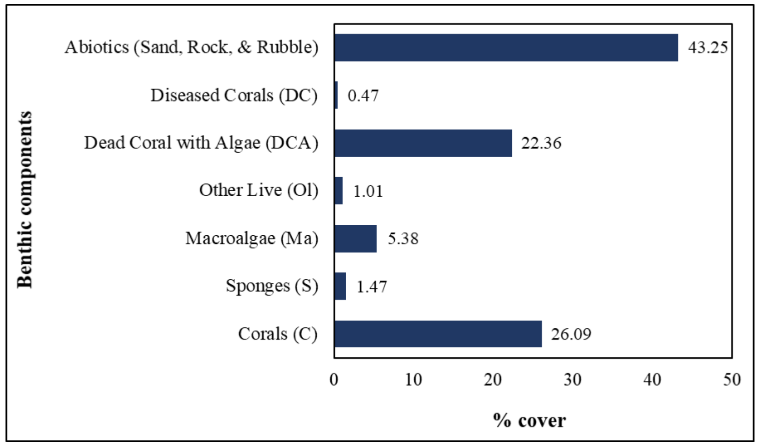

Figure 3. Percentage of benthic cover in Carbin Reef, Sagay Marine Reserve

Hard Coral Species

Using the CPCe software, this study identified a total of 55 hard coral species belonging to 33 genera and 12 families (Figure 4). Carbin reef is dominated by the three genera *Porites*, *Fungia*, and *Goniastrea*. The genus *Porites* included *P. asteroides, P. cylindrica,* and *P. lobata.* The genus *Fungia* included *F. concinna*, while genus *Goniastrea* was composed of *G. deformis*, and *G. edwardsi*. *Porites lobata* was the most dominant species having a mean of 3.32%, followed by *F. concinna* (1.99%), and *G. edwardsi* (1.81%) (Figure 4). The dominant species, *P. lobata*, is a member of the genus *Porites* and is one of the most prolific reef-building coral genera in the tropical Pacific Ocean, with a massive growth form (Lough and Cooper, 2011). Both massive and sub-massive corals are resistant to strong water currents and can be found in shallow and mid-depth environments (Roberts, 2018) indicating a good reef characteristic for Carbin Reef.

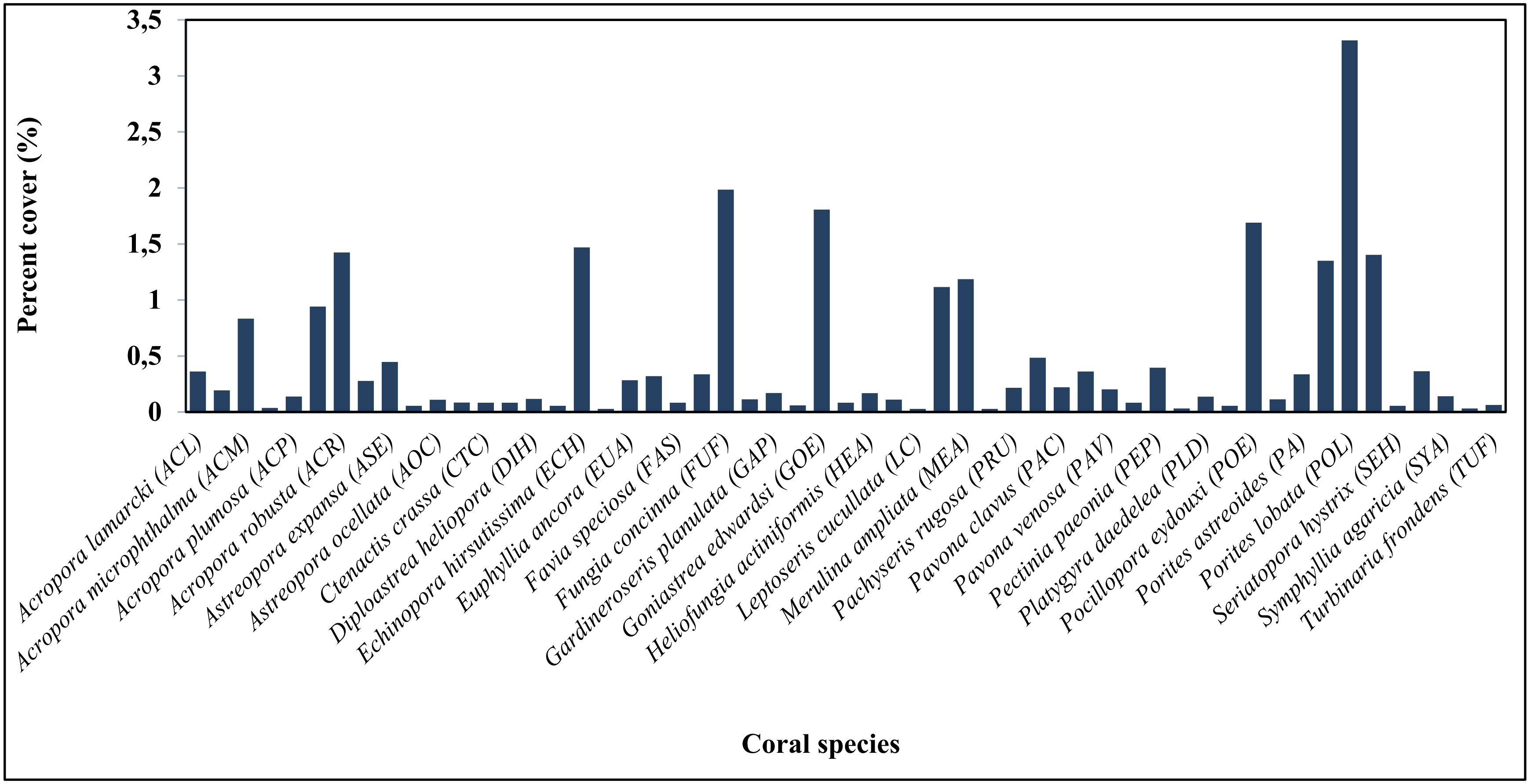

Figure 4. Species composition and percent cover of live hard corals in Carbin Reef, Sagay Marine Reserve

Considering the dearth of diversity data in Carbin reef, the total number of species identified in this study expand the previous report of Maliao et al. (2004) having 28 coral genera. Unfortunately, previous studies only give an overall number and do not detail which corals were found, therefore it is not possible to compare our results with previous reports in Sagay Marine Reserve (Maliao et al., 2004; Licuanan et al., 2017). While Maliao et al., 2004 noted *Porites* spp. dominance comparably observed in this study (*Porites lobata*), *Favia stelligera* mentioned by the latter authors were not found in this study. This could either be due to their population decline or with the differences on surveyed area. Thus, we could assume that this is the first comprehensive assessment of coral diversity in Carbin Reef which could be extended in other major reefs of Sagay Marine Reserve.

Coral Diversity

Table 2 shows the coral diversity in Carbin reef using Shannon-Weaver and Simpson's Indices for each transect. These indices were used to characterize the numerical structure of a coral community and quantify its diversity. In terms of species diversity, Transect 6 had the highest value of H = 2.87, while Transect 3 had the lowest diversity of H = 2.45. Transects 1, 2, 4, 5, and 6 fell into the moderate category, whereas Transect 3 fell into the low species diversity category, according to definition of diversity value and its qualitative equivalence (Fernando, 1998). Transects 1, 2, 4, 5, and 6 may be significantly stressed based on these findings. Gonçalves and Menezes (2011) noted that values greater than 3.0 suggest a stable ecosystem, while values less than 1.0 indicate pollution and habitat destruction.

Table 2. Diversity indices of hard coral cover found in different transect locations

Transect No.	Depth (m)	Shannon-Weaver Index (H)	Simpson's Index (1-D)
1	6	2.66	0.90
2	12	2.75	0.92
3	6	2.45	0.87
4	12	2.63	0.91
5	6	2.69	0.90
6	12	2.87	0.90
Overall Mean		**2.68**	**0.90**

Coral Diseases

The overall prevalence of coral diseases, including symptoms of reduced health was 0.47% in the Carbin Reef. White band illness, bleached coral point, and white syndrome were examples of coral diseases observed. These symptoms of deterioration could be due to various natural stressors and competitive overgrowth—notably of sponges, and red and brown algae, observed in four transects (2, 4, 5, and 6). Another sign of compromised health is sediment damage where corals covered with silt/organic particulates exhibited discoloration and minimal coral die-offs along the sediment-covered area. However, even the sedimentation observed in these transects were relatively high, this could not be attested due to the loss of sedimentation traps, which requires further studies. Transect 2 particularly exhibited a greater percentage of white syndrome (1.2) (Figure 5). White syndrome can be caused by a variety of infections, including *Vibrio harveyi* (Luna et al., 2010), as well as stresses in the environment (Hughes and Conell, 1999). *Porites*, *Montipora*, *Echinopora*, and *Heliopora* were the four genera most usually affected by white syndrome. Some authors have concluded that *Vibrio* infections in corals are opportunistic in nature (Bourne and Munn, 2005; Bourne et al., 2007) as *Vibrio* spp. were both observed in healthy and ill corals. Multiple factors, such as host density (Bruno et al., 2007) and temperature (Colwell, 1996) have been shown to influence the chance of successful infections in models of illness happening in environmental context.

Sedimentation Rate

In this study, force majeure did not permit the researchers to acquire enough data for sedimentation rate. Four sediment collectors were installed within the 6-meter transect depth. However, only one sediment collector was recovered and retrieved after 7 days. The sediment collector either tipped over due to strong wave currents and loss due to human disturbances. Nonetheless, one sediment collector installed in Transect 3 revealed a daily sedimentation rate of 1.62 mg/cm^2. This number is much lower than Pastorok and Bilyard's (1985) predicted sedimentation rates of >50 mg/cm^2/day, which are considered catastrophic for some coral communities. Varied corals can endure different sedimentation rates, ranging from 10 mg/cm^2/d to >400 mg/cm^2/d (Patorok and Bilyard, 1985). While this provide a positive outlook on potential sedimentation impacts in Carbin reef, effective monitoring techniques is further required. This study was done in summer, when total rainfall was at its lowest. The results could have been different during the rainy season. In the same way, spatial variability may occur when water current changes and the effects of adjacent Himoga-an River run-offs potentially reach the reef area.

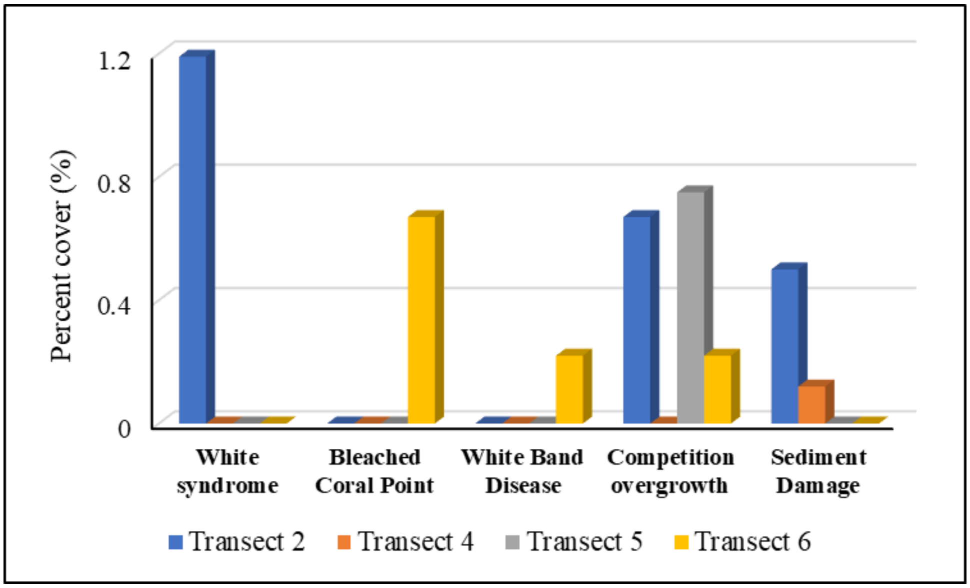

Figure 5. Diseases and signs of compromised health prevalent in corals found in four transects

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Conclusion

This study identified a total of 55 coral species throughout Carbin Reef, providing the first comprehensive species list for Sagay Marine Reserve. Overall, benthic cover suggests that the reef is considered in fair condition but might potentially be in decline, based on the established criteria by the Department of Environment and Natural Resources of the Philippines. Thus, this study showed that Carbin Reef has a moderately diverse reef ecosystem, but experience moderate stress considering the presence of various coral diseases. The two signs of compromised health include sediment damage and competition overgrowth. Likewise, natural disturbances like intense wave current and occurrence of Typhoon Haiyan in year 2013 could have attributed to the deterioration of the reef, causing the presence of massive coral rubble and dead corals with algae. Although, sedimentation rate per day was below the maximum sedimentation that can be tolerated by most corals, this only represented the dry season. Future year-round studies with considerations of water current and pollution sources are needed.

Compliance with Ethical Standard

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

Ethics committee approval: Ethics committee approval is not required.

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Disclosure: -

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Aggregation of Nematode, *Hysterothylacium aduncum* in whiting, *Merlangius merlangus*

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ABSTRACT

Although aggregation of macroparasites characterized by unequal distribution of parasites on the host is a known axiom in marine fishes, aggregation of nematode, *Hysterothylacium aduncum* was not previously studied in whiting, *Merlangius merlangus*. Here, we investigated the host-related (fish condition factor) and parasite-related factors (parasite load) as well as the distribution pattern of *H. aduncum* in whiting to determine whether aggregation existed or not. The distribution of *H. aduncum* (third larval stage) in whiting has been shown to be aggregated. Aggregation of *H. aduncum* was assessed by Weibull distribution. The aggregation degree of nematode, *H. aduncum* in whiting was changed by the individual fish. The observed pattern of parasite distribution by the individual fish enabled the recognition of aggregation for the first time in whiting. The prediction of the intensity of *H. aduncum* in whiting improved the understanding of the host-parasite system, particularly for the dynamics of the parasite.

Keywords: Aggregation, *Hysterothylacium aduncum*, nematode, whiting, *Merlangius merlangus*, Weibull

Introduction

Parasites are important elements in fish ecology. Fish morphology, behavior, physiology, and reproduction can be affected by parasites (Timi and Poulin, 2020). The macroparasite, *Hysterothylacium aduncum* (Nematoda: Raphidascarididae) has been categorized as the generalist nematode because it has been registered for about 30 various fish species in marine teleost (Özer et al., 2016). The occurrence of *H. aduncum* has been reported for whiting (*Merlangius merlangus*) in the Black Sea previously (Ismen and Bingel, 1999). The third larval stage of *H. aduncum* is the infective period for fish. Previous stages are found in one or more crustacea as intermediate hosts (Navone et al., 1998). Quantitative descriptors of *H. aduncum* and host-parasite interaction in whiting have been studied in detail (Özer et al., 2016). The molecular description of *H. aduncum* in whiting has been published in recent studies by Pekmezci et al. (2013) and Pekmezci (2019) as well as in some other marine fish species (*Sparus aurata*, *Diplodus vulgaris* and *Solea solea*) by Keskin et al. (2015). The studies on the characteristics of nematode, *H. aduncum* in the whiting revealed its occurrence throughout the year and the increase in infection by the increase in age and length (Ismen and Bingel, 1999; Özer et al., 2016).

Macroparasites are typically aggregated on their host populations (Gaba et al., 2005; McVinish and Lester, 2020). Aggregation defines the uneven distribution of parasites within hosts. It is particularly common for the parasites hence some individuals have lots of parasites while most have very few. Aggregation is of importance in aquatic ecology concerning the factors such as reducing the size of parasite population and parasite fecundity (Wilson et al., 1996; McVinish and Lester, 2020). Fish mortality due to excessive parasite load as reflected by aggregation is also significant (McVinish and Lester, 2020). Macroparasites can be aggregated over their host (fish) populations; some fish have high parasite burdens with the uncountable number of parasites while others have one or a countable number. The distribution pattern of parasites across their host is not quantitatively identical. The aggregated distribution of parasites has been known as a law of parasite ecology (Poulin, 2011). The aggregation degree can affect the balance between host (fish) and parasite (Wilson et al., 1996). The heterogeneity in the counts of parasites per host (fish) is so conspicuous that the mean number of parasites per fish is generally a pointless characteristic for the parasitic infection level (Rózsa et al., 2000). Parasites have a tendency towards aggregation and log-normal distribution does not provide a good fit to parasite data, that's why the classical linear regression analysis can be considered irrelevant. Although there is no universally recognized standard method or mathematical model to measure aggregation (McVinish and Lester, 2020) the Weibull distribution was obviously more convenient for various degrees of aggregation (Gaba et al., 2005; Balard et al., 2020). Weibull distribution has the advantages of fitting the heavily invaded host in relation to its flexibility and low sensitivity to small sample size (Gaba et al., 2005).

The purpose of the present study was to analyze the distribution of the macroparasite, *H. aduncum* in whiting, *M. merlangus* to determine if the aggregation was prevailing form or not. The estimation of parasite intensity and aggregation pattern of *H. aduncum* in whiting described for the first time in this study contribute to our knowledge of the characteristic of parasitic systems and disease ecology.

Material and Methods

Data Sources

The whiting, *M. merlangus* (N=70) were obtained in January of 2019 from the fish market in Ankara (Turkey). The origin of the fish was the city of Sinop in the Black Sea. Fish and their parasites were examined at the Laboratory of Fish Health, the Department of Fisheries and Aquaculture, Ankara University. Fish weight and length were measured. The number of parasites (third larval stage) in the digestive system of whiting, *M. merlangus* were examined and recorded (Figure 1).

Figure 1. Nematode, *Hysterothylacium aduncum* in the digestive system of whiting

The prevalence was determined by Bush et al. (1997). The condition factor (K) was calculated from whiting samples by the equation

K=W/L3 (W: body weight (g), L: total length (cm))

Molecular Identification

Taxonomic identification of the nematode was done by adequate molecular analyses by Keskin et al. (2015). Briefly, nematode samples were preserved in absolute alcohol. DNA was extracted using QIAGEN QIAquick Extraction kit (Germany) following the manufacturer's protocol. Sequencing was carried out on an ABI Prism 310 genetic analyzer. Sequences have been archived in GenBank and accession numbers are OM884008 and OM884009.

Statistical Analysis

The relationship between the condition factor of fish and the parasite counts was tested using regression analysis.

Weibull distribution is adequate for macroparasite distributions (Gaba et al., 2005). For aggregation assessment, Weibull analyses were applied to the parasite counts using SigmaExcel. The Weibull distribution is expressed by its shape and scale parameters. The maximum likelihood method is suitable for the estimation of these parameters in the Weibull distribution (Yang et al., 2019). The parameters related to aggregation are the scale and shape in Weibull analyses (Balard et al., 2020).

Results and Discussion

Weibull distribution displayed the aggregation of the nematode. *H. aduncum* in whiting. The variance in parasite numbers was higher than the mean of *H. aduncum* counts. The model was fit using likelihood maximization (Table 1). The predicted *H. aduncum* intensity (in parasite number) can be compared to the recorded parasite distribution (Figure 2).

The aggregation expresses the case of variance higher than the mean number of parasites. The aggregation of macroparasites has been described in marine fish (Lester, 2012). Aggregation of parasites is known to be related to fish characteristics such as feeding behavior, sex, age, and resistance (Timi and Poulin, 2020). Aggregation provides a better indication for parasite distribution than the mean number of parasite load. In this study, the distribution of nematode (*H. aduncum*) in whiting (*M. merlangus*) showed aggregated pattern and the aggregation degree varied by the individual fish. The primer outcome of the present study is that the actual differences of parasite distribution among the fish is associated with generating an aggregated distribution of nematod in whiting.

Weibull distribution showed the aggregation type of nematode in fish, giving predictions, particularly for the probability of higher parasite intensity in our study. Heterogeneity in parasite loads is well represented in maximum likelihood models as with Weibull distribution (Wilson et al., 1996; Gaba et al., 2005). The variance of the parasite numbers (26.52) is higher than the mean (5.1), showing the aggregated distribution of nematodes in whiting. The shape parameter of Weibull analysis is a valuable descriptor and inversely proportional to aggregation (Gaba et al., 2005). In our study, the low level of shape parameter (1.208) showed the aggregated distribution of the nematode. Scale parameter correlated to aggregation was higher than 1, evidencing aggregation modulated by the mean number of nematode, *H. aduncum.* Weibull distribution fitted the heavily infected fish with *H. aduncum* in relation to the flexibility of the Weibull. Thus, the parasite frequency ranged from 1 to 33 in heavily infected fish. In the evaluation of aggregation degree for our study, it can be concluded that the major determinant of aggregation may be the number of infected intermediate hosts ingested by fish. The life cycle strategy of nematod may play a critical role in this type of aggregation. The findings of Klimpel and Rückert (2005) supported our interpretation of the link between parasite aggregation and fish feeding on crustacea, reporting that Hyperiids (Crustacean) identified as the obligatory intermediate host of *H. aduncum* beared nematode larvae in their haemocoel in high numbers. The resistance of fish in the context of fish immunity can be another factor in the aggregation of nematodes in fish as stated by Lester (2012). However, we have no data on the immune response of fish to support this phenomenon.

The parasite prevalence was 85% (60/70). The median values for the nematode and condition factor were 4 (min 1 and max 33) and 0.73 (min 0.013 and max 1.38), respectively. The relation between the parasite number and the condition factor of fish was not significant ($p>0.01$) as assessed by linear regression analysis (Table 2). The multiple R was 0.46, indicating a fairly weak linear relationship between the condition factor and parasite counts.

Concerning quantitative characteristics of *H. aduncum*, we found similar prevalence values in the whiting obtained from Sinop (South Black Sea), however, higher than the Balaklava Bay (North Black Sea) samples (Özer et al., 2016). In general, increasing parasite aggregation has been considered to be linked to the host's age or body size and higher parasitism has been expected in larger fish (Wilber et al., 2017). Nematode (*H. aduncum*) load has been reported to be more in the larger or longer whiting (Ismen and Bingel, 1999; Özer et al., 2016). Conversely, in the present study, the relation between the condition factor and mean parasite load was not significant, showing the condition factor as a measure of fish length and fish weight is not important in host-parasite distribution. The possible explanation can be related to the life cycle of *H. aduncum* because fish can feed an unknown number of infected intermediate hosts. This approach in relation to the feeding intake of fish was also reported to explain the seasonal infection pattern of *H. aduncum* in whiting stocks in the Black Sea, interpreting the seasonally changing amount of infected intermediate host (Özer et al., 2016). We also elucidated the aggregation of *H. aduncum* with a similar argument as explained above. Consequently, the parasite aggregation can be interconnected to the heterogeneity in the process of fish-infected intermediate host encounters, resulting in varying aggregation degrees by the individual fish. In this respect, the infected intermediate host (fed by fish) might be essential in considering interrelations between the aggregations and *H. aduncum* infections.

Table 1. Weibull values based on maximum likelihood estimates (Goodness-of-Fit: Log-likelihood -155.210, confidence level 95%, Anderson-Darling 1.548, AD *P*-value < 0.01).

Parameter	Estimate	SE Estimate	Lower 95.0% CI	Upper 95.0% CI
Shape	1.208	0.10868337	1.01281112	1.441
Scale	5.432	0.616627	4.348	6.785
Mean (MTTF)	5.101	0.550829	4.128	6.303
Standard Deviation	4.241	0.542502	3.301	5.450
Variance	26.522			
Variance/mean	5.2			

Percentage	Percentile (Time)	SE Percentile	Lower 95.0% CI	Upper 95.0% CI
0.1	0.01786015	0.010053541	0.005925715	0.053830629
0.135	0.022899764	0.012388002	0.007931579	0.066115365
0.5	0.067791268	0.030212635	0.028302024	0.162379
1	0.120574	0.04769606	0.055531545	0.261799
5	0.464726	0.130418	0.268114	0.805515
10	0.843264	0.195612	0.535193	1.329
25	1.937	0.327546	1.390	2.698
50	4.010	0.502877	3.136	5.128
75	7.118	0.767136	5.763	8.792
90	10.833	1.186	8.741	13.426
95	13.470	1.554	10.744	16.886
99	19.228	2.515	14.880	24.846
99.5	21.594	2.960	16.507	28.250
99.865	25.925	3.835	19.400	34.645
99.9	26.896	4.041	20.036	36.105

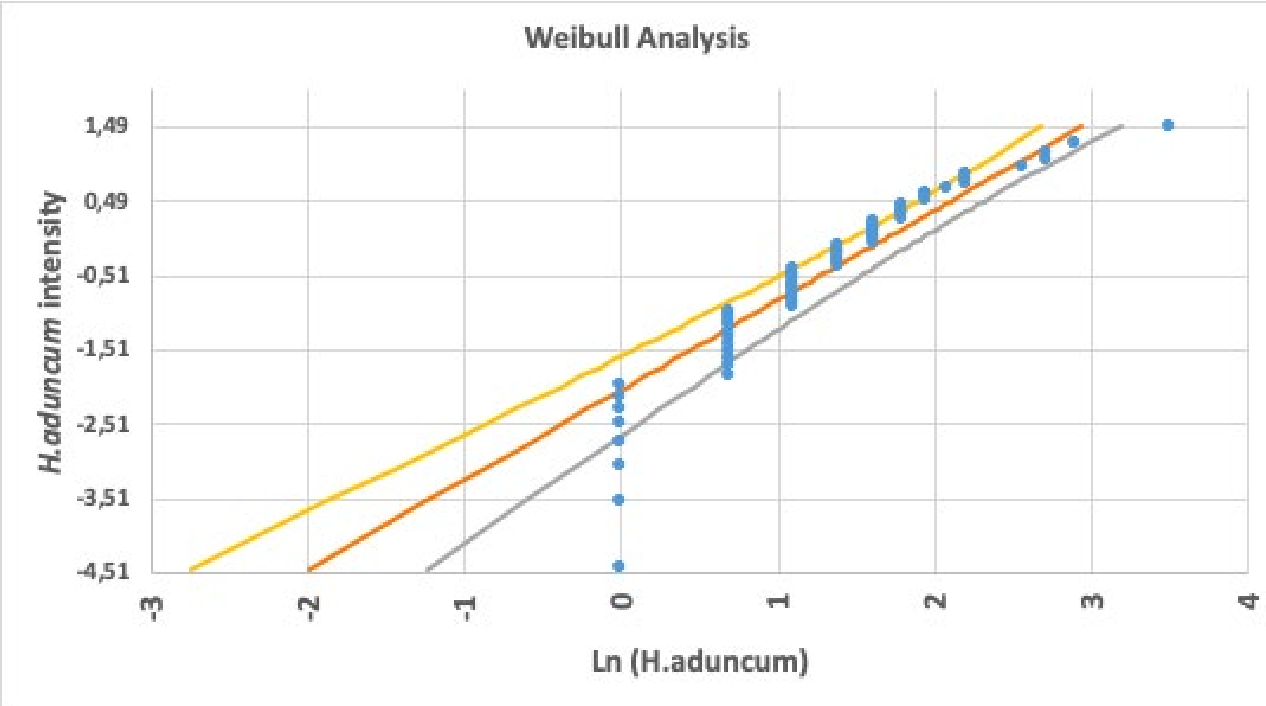

Figure 2. Weibull probability plots of *H. aduncum* intensity with 95% two-sided confidence bounds (The optimized fit is shown by a red line, the 95% CI of the fit as all parameters varying in their 95%CI, is plotted as a yellow and gray line).

Table 2. Parameters of linear regressions concerning nematode, *H. aduncum* counts and condition factor (K) of whiting

Regression statistics		ANOVA					
Multiple R	0.04696849		*df*	*SS*	*MS*	*F*	*Significance F (p)*
R Square	0.00220604	Regression	1	0.00813924	0.00813924	0.12823318	0.72157104
Adjusted R Square	-0.0149973	Residual	58	3.68138694	0.06347219		
Standard Error	0.25193687	Total	59	3.68952618			
Observations	60						

Conclusion

The aggregation of nematode, *H. aduncum* occurs in whiting and its degree varies by the individual fish, showing clear heterogeneity. The Weibull distribution gave suitable fits for the probability of *H. aduncum*. The aggregation and predicted intensity results may provide evidence for the potential for the alteration in the size of the nematode or fish population from the ecological perspective. The aggregation of *H. aduncum* in whiting is described for the first time in this study, adding to current knowledge of the interaction of marine fish and nematode in host-parasite systems.

Compliance with Ethical Standard

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

Ethics committee approval: The research was not subjected to animal ethical permission because fish were dead and obtained from the fish market.

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Disclosure: -

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Antibacterial potential of different red seaweed (Rhodophyta) extracts against ornamental fish pathogen *Salmonella arizonae*

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ABSTRACT

This study evaluated the antibacterial effects of different red seaweed (*Kappaphycus striatus*, *Eucheuma denticulatum*, *Hydropuntia edulis*) against *Salmonella arizonae* that caused disease in goldfish *Carassius auratus*. *In vitro* antibacterial susceptibility was determined using a standard disc diffusion assay. Further *in vivo* experiments were conducted on seaweeds with the highest zone of inhibition. Results showed that *K. striatus* had the highest zone of inhibition with 30.9 ±0.62 mm followed by *H. edulis* (29.6 ±1.61 mm), and *E. denticulatum* (27.6 ±0.51 mm). Promisingly, the antibacterial activity of seaweeds tested was comparable with that of cefixime, trimethoprim, and novobiocin and was significantly higher than the other seven antibiotics tested in this study. Moreover, the *in vivo* treatment of *K. striatus* to *S. arizonae* challenged *C. auratus* significantly decreased the mortality; the positive control group attained 100% mortality while the treated group had 40% mortality after 10 days of post-infection. This study showed the potential use of *K. striatus* to control *S. arizonae* infection in aquarium fishes.

Keywords: Antibacterial, Bioassay, Seaweeds, Goldfish, *Salmonella arizonae*

Introduction

The emergence of multiple drug-resistant (MDR) pathogens has created a worldwide public health problem in the recent past. The concern on MDR has shifted the research priority of epidemiologists and allied researchers into the discovery of alternative sources of antimicrobial agents, wherein the zoonotic pathogen is of top concern. The development of MDR, moreover, is one of the major drivers of research to explore alternative natural antimicrobial agents that are locally available, cost-effective, and have minimal toxicity, but have lesser health impacts than commercial antibiotics (Cheung et al., 2014; Pérez et al., 2016; Cotas et al., 2020).

Seaweeds are marine plants without true leaves, stems, and roots and are mostly found on rocky shorelines (Cordero, 2009). Among the groups of seaweeds, red algae (Rhodophyta) have received much attention for it contains high amounts of polyunsaturated fatty acids, sterols, terpenes, mycosporine-like amino acids, essential amino acids, phycobiliproteins and carotenoids, and phenolic compounds (Torres et al., 2019; Cotas et al., 2020; Lopez-Santamarina et al., 2020). `In aquaculture, seaweed extracts have been used extensively for the prevention and treatment of viral and bacterial diseases (Noorjahan et al., 2022). Recently, the use of seaweed extract has received attention in pharmaceutical industries after research has revealed its inhibitory potential against the antibiotic-resistant pathogen (Cabral et al., 2021; Cotas et al., 2020; Klimjit et al., 2021; Lu et al., 2021)

Salmonella spp. is traditionally treated with antibiotics; however, recent reports on its antibiotic resistance have alarmingly increased (Wang et al., 2017; Cameron-Veas et al., 2018; Khademi et al., 2020). In the meta-analysis of Shen et al. (2022), it was revealed that *Salmonella* spp. isolates were highly resistant to tetracycline, sulfisoxazole, ampicillin, streptomycin, and sulfamethoxazole. This issue has contributed to the economic burden of most developing countries, where added costs are devoted to the prevention and treatment of persistent diseases caused by drug-resistance bacteria (Dodgostar, 2019). To date, research efforts and a vast literature has been published on the antibacterial activity of medicinal plant extracts against *Salmonella* spp., however, the focus was on common foodborne pathogens such as *S. typhi*, *S. enterica*, and *S. typhimurium* (Dayuti, 2017; Dhas et al., 2020; Martelli et al., 2020; Silva et al., 2020; Nozohour and Jalilzadeh, 2021; Gavriil et al., 2021; Wang et al., 2021; Naz et al., 2022). Meanwhile, studies on equally important health concerns and uncommon species and subspecies have remained elusive.

Salmonella enterica subsp. *arizonae* (Caldwell and Ryerson, 1939) is an uncommon pathogen initially described as a pathogen of cold-blooded animals, especially snakes until infection on humans, poultry, and fish have been published (Caldwell and Ryerson, 1939; Seligmann et al., 1944; Jortner and Larsen, 1984; Kodama et al., 1987; Hoag and Sessler, 2005; dos Santos et al., 2019; Limbago et al., 2021). By then, *S. arizonae* was considered zoonotic, mediating diseases in a wide array of animal species. In 2017, Nishioka et al. (2017) reported that *S. arizonae* developed resistance to the acceptable antibiotic, where there is recurring pyelonephritis secondary to *S. arizonae* infection even after cephalosporin treatments.

In 2018, a concerning record of *S. arizonae* infection was reported in *Carassius auratus* (Linnaeus, 1758) in the Philippines (Limbago et al., 2021). Mass mortality of *C. auratus* from a nearby fish pet shop in Barotac Nuevo, Iloilo, Philippines has been noticed, and *S. arizonae* is inferred to be one of the causes after performing Koch's postulate. Despite the study of Gut et al. (2022) on the antibacterial potential of traditional kefir against *S. arizonae*, the utilization of this dairy product in aquaculture might be expensive. Furthermore, the use of commercial antibiotics in aquaculture is currently discouraged since antibiotic residue may lead to the development of bacterial drug resistance (Santos and Ramos, 2018; Albarico and Pador, 2019). It is imperative, therefore, to screen locally available materials as a possible source of antimicrobials against *S. arizonae*, which motivated this study. In this study, the antibacterial activity of three red macroalgae species ethanol extracts was screened against a zoonotic pathogen, *S. arizonae*. This study aimed to contribute and answer the lack of research on uncommon *Salmonella* serotypes. Moreover, the results will be beneficial to aquaculturists and hobbyists should *S. arizonae* infection occur.

Material and Methods

Seaweed Sample Collection

Seaweed samples were collected from the coastal areas of Estancia, Northern Iloilo, Philippines. Collected samples were identified with the aid of the Field Guide and Atlas of the Seaweed Resources of the Philippines (Trono, 1997). Identified seaweed species were *Kappaphycus striatus* (F. Schmitz) Doty ex P. C. Silva (1996), *Hydropuntia edulis* (S.G. Gmelin) Gurgel and Fredericq, (2004), and *Eucheuma denticulatum* (N.L. Burman) Collins and Hervey, (1917). Seaweeds were then washed with distilled water to remove the adherent soils and salts. Cleaned samples were oven-dried at 60°C for 72 h and were cut into pieces using sterile scissors.

Ethanolic Extract Preparation

Samples were soaked into 500 mL 80% ethyl alcohol in a 1 L capacity Erlenmeyer flasks. The flasks were covered with carbon paper and stored in a dark cabinet at ambient temperature for 72 h. Each extract obtained was separately filtered using a Buchner funnel lined with Whatman No. 1 filter paper (Manilal et al., 2009; Lavanya and Veerapan, 2011; Salem et al., 2011). Then, a 100 mL filtered extract was concentrated to about 20 mL using a thermostatic waterbath (Lavanya and Veerappan, 2011). The remaining pure extracts were stored in a dark cabinet and were used in subsequent analysis.

Preparation of McFarland Standards

A 15×10^8 CFU/mL MacFarland standard was prepared by mixing 5 mL of 1.175% Barium Chloride dihydrate ($BaCl_2.2H_2O$) with 95 mL of 1% sulfuric acid (H_2SO_4). The mixture was then vortex for 30 secs. On the other hand, 30×10^8 CFU/mL MacFarland standard was prepared by mixing 10 mL 1.175% $BaCl_2.2H_2O$ with 90 mL of 1% H_2SO_4 (Montaño et al., 2022).

Bacterial Isolation and Culture

Pure cultures of *S. arizonae* were isolated from moribund *Carassius auratus* (Linnaeus, 1758) that were brought to the laboratory. Moribund *C. auratus* was brought to the laboratory and was clinically diagnosed with abdominal dropsy, loss of scales, rotten caudal tail, nonintact internal organs, and pale gills and flesh. Pure cultures of *S. arizonae* were maintained in nutrient broth and incubated at 25°C for 48 h. Every 3 days, working cultures were transferred to fresh nutrient broth media. Before subsequent experiments, a loopful *S. arizonae* culture was aseptically transferred to Shigella-Salmonella agar (SSA). Colonies on SSA plates were aseptically transferred into 10 mL tryptic soy broth (TSB) in replicates until bacterial suspension and turbidity reached 15×10^8 CFU/mL (used for *in vitro* test) and 30×10^8 CFU/mL (used for *in vivo* tests) following 0.5 MacFarland Nephelometer Standard (Ruangpan and Tendencia, 2004). Cultures incubated at 25°C for 5 h were used for subsequent study.

Preparation of Impregnated Disc

Sterilized 6 mm Whatman No. 1 filter paper discs were used in this study. In sterilization, discs were placed in a Petri dish and autoclaved at 121°C for 30 minutes under 15 psi pressure (Hossain et al., 2012). Sterilized discs were then oven-dried for 48 h. Subsequently, five compact discs were immersed in either 10 mL antibiotics or algal extract. Discs were dried for 24 h in an oven at 45°C.

In Vitro Antibacterial Test

In vitro antimicrobial activity of three seaweeds and ten commercial antibiotics (as positive controls) against *S. arizonae* were conducted using the standard disc diffusion method (Ruangpan and Tendencia, 2004). Briefly, the pure culture of *S. arizonae* was lawn on Muller Hinton agar (MHA) plates and then was dried for 10 minutes. Prepared impregnated discs were then placed on the MHA surface using sterile forceps. Samples were incubated for 72 h at 25°C, and plates were kept in an inverted position.

Antibiotics used as positive controls were (1) 10 g Gentamycin, (2) 5g Ciprofloxacin, (3) 30g Vancomycin, (4) 10g Streptomycin, (5) 30g Chloramphenicol, (6) G 10 units Penicillin, (7) 5g Cefixime, (8) 2.5g Trimethoprim, (9) 25g Amoxycillin, and (10) 30µg Novobiocin.

Bioassay Test

Twenty-five healthy *C. auratus* with an average weight of 15 g were brought to the laboratory and were acclimatized for five days before the experiment. Fish were subdivided into five treatments comprising five fish per 20 L capacity aquarium. Samples were exposed to different concentrations of seaweed extract to determine the maximum allowable concentration (MAC). The treatment I was exposed to 50 ppm; Treatment II (100 ppm); Treatment III (200 ppm); Treatment IV (300 ppm); and Treatment V (500 ppm). Concentrations in bioassay tests were based on Thanigaivel et al. (2015). Daily fish mortality was recorded to determine the toxicity of the extract. The MAC of seaweed extract to the fish was used for the antibactericidal test.

In Vivo Antibacterial Test

Fifteen healthy *C. auratus* were used in the conduct of this experiment. Fish samples were subdivided into three groups. Positive control and treated groups were intraperitoneally injected with 100 µL of *S. arizonae* (30×10^8 CFU/fish) while the negative control group was injected with 100 µL of distilled water. After 72 h of post-infection, groups were exposed to different treatments. The treatment group was exposed to the MAC of *K. striatus* extract while positive control and negative control were not exposed to the extract.

Antibacterial activity was determined by recording the daily fish mortality for 10 days. A second experiment was conducted to validate the results, employing the same methodologies.

Data Analysis

Inhibition zone and mortality rates were determined and statistically analyzed. Data were presented as percentages and means with standard deviation. Statistical differences were computed using One-way ANOVA with Tukey's HSD posthoc test using SPSS (IBM SPSS 22).

Results and Discussion

As shown in Figure 1, *K. striatus*, *H. edulis*, and *E. denticulatum* have a high zone of inhibition against *S. arizonae*. Among these three species, *K. striatus* had the highest antibacterial activity against *S. arizonae* with an average zone of inhibition at 30.9 ±0.62 mm indicating the sensitivity of the pathogen to the marine algae extract. A promising result was also noted in *H. edulis* with an average zone of inhibition of 29.6 ±1.61 mm and *E. denticulatum* with 27.6 ±0.51 mm, respectively.

Furthermore, the zone of inhibition of *K. striatus*, *H. edulis*, and *E. denticulatum* is not significantly different from that of cefixime, trimethoprim, and novobiocin. However, the zone of inhibition of three seaweed ethanolic extracts is statistically higher than that of gentamycin, ciprofloxacin, vancomycin, streptomycin, chloramphenicol, penicillin, and amoxicillin.

Bioassay Test

The bioassay test of *K. striatus* extract in *C. auratus* was carried out at different concentrations. Treatments and concentrations include Treatment I with 50 ppm concentration, Treatment II with 100 ppm, Treatment III with 200 ppm, Treatment IV with 300 ppm, and Treatment V with 500 ppm. The result of this experiment showed that the fish survived with all the treatments after 10 days of monitoring; indicating the non-toxic effect of the marine algal extracts (data not shown). The maximum allowable concentration (500 ppm) was used for the subsequent *in vivo* antibacterial test.

In vivo Antibacterial Test

The effect of *K. striatus* extracts on *S. arizonae*-infected *C. auratus* showed promising results (Figure 2). The positive control group has 20% mortality on the 3rd day after 72 h post-infection. The mortality of the positive control group has further increased to 100% mortality on day seven of post-infection. The treatment group, on the other hand, has a late onset of mortality as compared to the positive control group, starting with 20% mortality on day seven. The mortality in the treatment group has plateaued and was maintained at 40% until the 10th day of the experiment. While negative control group injected with distilled water has 0% mortality throughout the experiment.

Furthermore, treated *K. striatus* treated *C. auratus* showed normal flesh, intact scales, normal caudal fins, intact internal organs, and normal gills (Figure 3A) while the untreated control group showed pale gills, loss of scales, rotted caudal fin, internal organs not intact, and pale gills (Figure 3B).

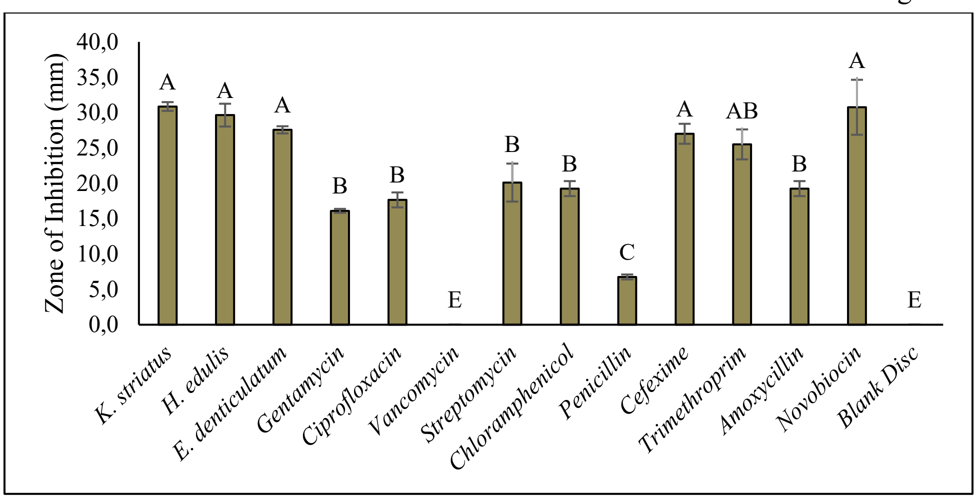

Figure 1. Susceptibility of *S. arizonae* to *K. striatus*, *H. edulis* and *E. denticulatum* ethanolic extract and thirteen commercial antibiotics. Each value is the mean zone of inhibition (mm) ± computed standard deviation from two replicates. Different superscripts mean the significant difference at $p<0.05$ levels.

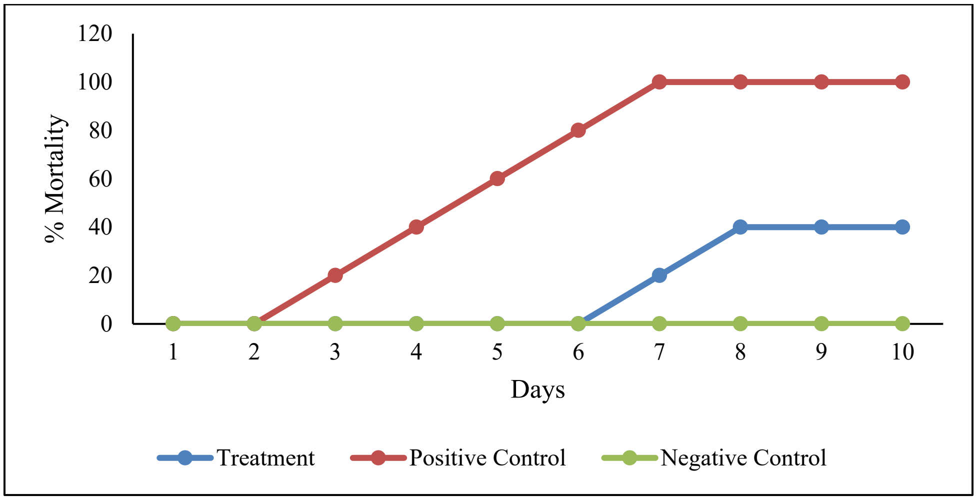

Figure 2. Antibacterial test of *K. striatus* ethanolic extract in *C. auratus* infected with *S. arizonae* for 10 days after 72 h post-infection.

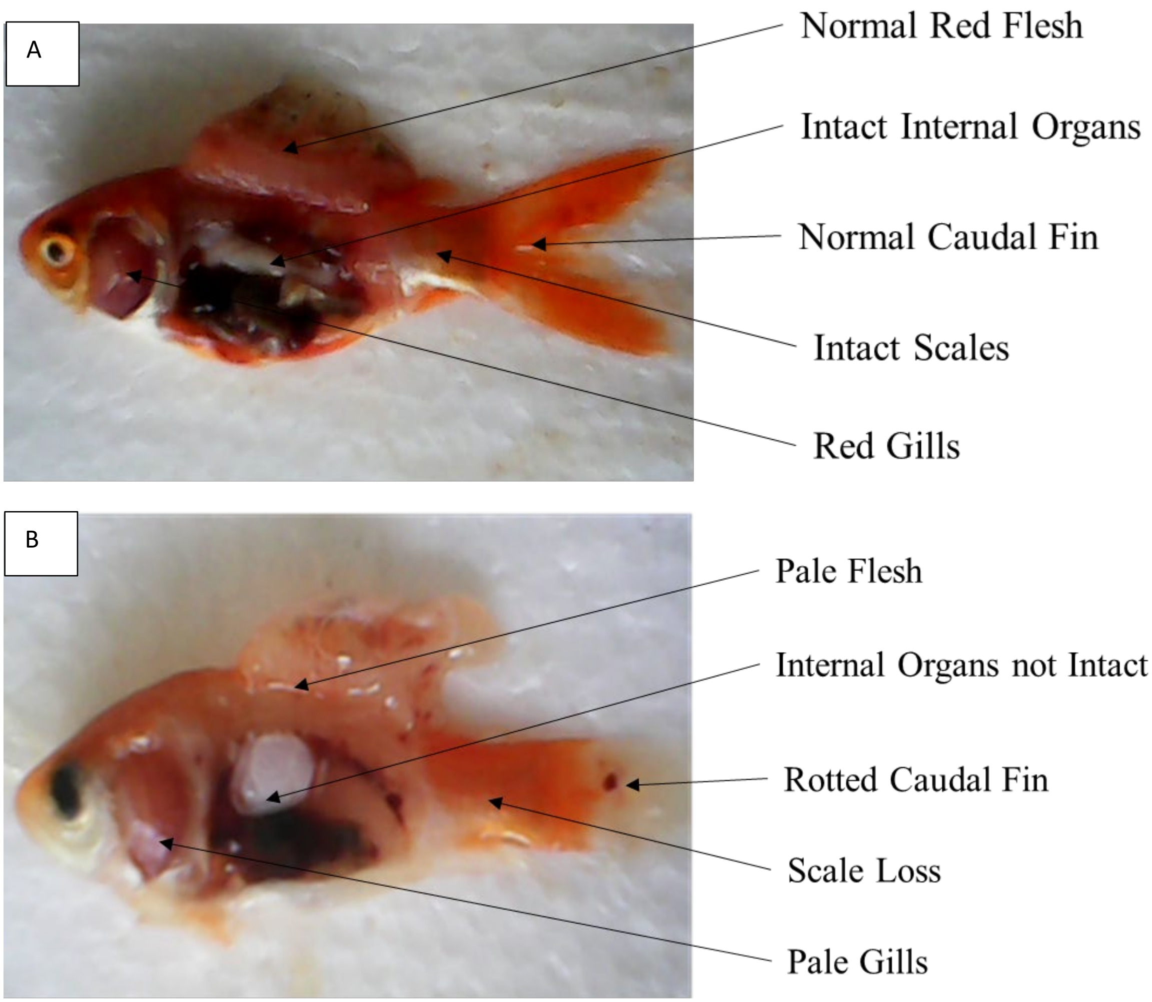

Figure 3. Dissected *C. auratus* infected with *S. arizonae.* (A) Treated group (B) Untreated control group.

In some Eastern countries, like the Philippines, marine autotrophs have been already used for their medicinal purpose. The diversity of plants and their traditional medicinal use has led to vast research to prove their therapeutic activity. Thus, it is worthwhile to conduct studies on life-saving drugs and biologically active substances from this renewable resource.

In the present study, *in vitro* experiments revealed that *K. striatus* has the highest inhibitory activity against *S. arizonae*. Promising antibacterial activity against *S. arizonae* was also observed from *H. edulis* and *E. denticulatum* extracts. This study also revealed that *K. striatus*, *H. edulis*, and *E. denticulatum* antimicrobial activities are comparable with tested commercial antibiotics. Although no studies have been conducted on the antibacterial effect of *K. striatus* on *S. arizonae*, a similar study has been tested on *S. typhi* (Prasad et al., 2013). However, compared to the antimicrobial activity of *K. striatus* in *S. typhi*, *S. arizonae* is highly susceptible to *K. striatus* ethanolic extracts (Prasad et al., 2013). Consequently, the ethanolic extract of *H. edulis* in this study has also a higher zone of inhibition compared to the study of Mahendran et al. (2021) on *H. edulis* against *Salmonella* spp. with only 22 mm. Meanwhile, *E. denticulatum* ethanolic extract in this study has a higher zone of inhibition in contrast with the study of Magallanes et al. (2021) with only a 13.2 mm zone of inhibition against *S. typhi*.

Many factors contribute to the differences in antimicrobial activity of plant extract in a pathogen. Factors include plant species and the solvent-extraction method used in the study (Sameeh et al., 2016). For example, in the study of Chuah et al. (2017) methanol extract of *K. alvarezii* has zero zones of inhibition against *S. enterica* which contradicts the results of this study where the high inhibitory activity of *K. striatus* was observed against *S. arizonae*. In the study of Prasad et al. (2013), results showed that *K. striatus* was slightly more effective than *K. alvarezii*. Moreover, ethanolic *K. striatus* extract showed a higher zone of inhibition than methanol extracts. It could be inferred that methanol and ethanol extraction-method, yielded different amounts of bioactive compounds although it should be noted that authors used different seaweed species. As support, several studies have shown that different extraction method has yielded different amounts of bioactive compounds. For example, the study by Bhuyar et al. (2020) reported that higher polyphenols were detected in the ethanolic extract of *K. alvarezii* than in hot water extract. In the study of Rebecca et al. (2013), it was concluded that ethanolic extraction was the best solvent for maintaining the active compounds in almost species of seaweeds. Ethanolic extracts of *K. alverii* include levoglucosenone, pyridinemethanol, 1,2,5-thiadiazole-3-carboxamide, and 4-[(2-chloroethyl) amino]-N-(2-hydroxyethyl)] (Bhuyar et al., 2020). It should be noted that a higher yield of bioactive compounds such as phytochemicals would likely result in a higher zone of inhibition. Phytochemicals are synthesized in response to microbial infection (Kumar and Pandey, 2013; Mierziak et al., 2014). Phenolic compounds, which are abundant in red seaweeds, have the property to disrupt the cellular membranes of microbes leading to their antimicrobial mechanism (Kumar and Pandey, 2013; Djouossi et al., 2015; Mishra et al., 2017; Cabral et al., 2021). Hence, the abundance of phenolic compounds would likely inhibit the growth of microorganisms including food-related pathogens. However, the exact mechanism of action of phenols is not yet fully understood at the cellular and molecular level (Chibane et al., 2019).

Meanwhile, a bioassay test revealed that 500 ppm of *K. striatus* tested is not toxic to *C. auratus*. It could be attributed to the fact that *K. striatus* is edible, thus, posing no to little toxicity in animals. This result enhances the promising antimicrobial activity of *K. striatus* against *S. arizonae*. Moreover, lower mortality was recorded in treated fish during *in vivo* experiments compared with 100% mortality in the untreated group. Another interesting result is that the treatment of *K. striatus* in infected fish delayed the onset of mortality.

While there is a myriad of studies on the antimicrobial activities of different plant extracts against *Salmonella* spp., the focus has been on the foodborne pathogen, while studies on *S. arizonae* are rather elusive. Previously published studies on the antibacterial activity of plants focused on foodborne pathogens including *S. typhi*, *S. enterica*, and *S. typhimurium* (Dayuti, 2017; Dhas et al., 2020; Sliva et al., 2020; Nozohour and Jalilzadeh, 2021; Gavriil et al., 2021; Wang et al., 2021; Naz et al., 2022). Recent studies on the susceptibility of *S. arizonae* only include the study of Limbago et al. (2021) and Gut et al. (2022) in mangroves leaf extracts and traditional kefir, respectively. While this study contributes to the aforementioned data gap, further research on the isolation of phytochemicals of *K. striatus* and its antimicrobial activity in *S. arizonae* is recommended. It is recommended to maintain the water physio-chemical parameters in the aquarium, and regular water exchange to prevent the occurrence of diseases from opportunistic pathogens like *S. arizonae*.

Conclusion

In conclusion, the present results showed that the ethanolic extract of *K. striatus*, *E. denticulatum*, and *H. edulis* possess antibacterial activity against *S. arizonae*, *in vitro*. Further *in vivo* experiment indicates that *K. striatus* extract can reduce the mortality of *S. arizonae*-infected *C. auratus*. The results of this study project the utilization of red seaweeds in treating *S. arizonae* infection in aquaculture.

Compliance with Ethical Standard

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

Ethics committee approval: The authors declare that all international, national and institutional guidelines for the care and use of laboratory animals have been followed and complied with for this study.

Funding disclosure: -

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Disclosure: -

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Growth parameters of the invasive blue swimming crab *Portunus segnis* (Forskål, 1775) (Crustacea) in the North-Eastern Mediterranean, Türkiye

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ABSTRACT

The blue swimming crab, *Portunus segnis*, is a Lessepsian and the most abundant and economically significant crab species on the Mediterranean Sea coast of Türkiye. However, there are a few studies on the growth of *P. segnis* in Türkiye. Our objective is to determine the allometry, and growth parameters of *P. segnis* in Iskenderun Bay, the Northeastern Mediterranean. Blue swimming crabs were sampled using a bottom trawl net from July 2014 to June 2015. Totally 320 specimens were caught. The carapace width (CW) varied from 38.1 to 163.17 mm (mean: 109.88±27.56 mm) and the total weight (TW) was measured at a minimum of 3.46 and a maximum of 324.36 g. The width (CW)- weight (TW) relationships of the crabs were estimated as log(TW)=2.9028CW-9.0664 (R^2=0.7452) for the females and log(TW)=2.9773CW-9.3842 (R^2=0.8433) for the males. The carapace width-weight relationships of both sexes indicated that the growth pattern is allometric ($p<0.05$). The von Bertalanffy growth parameters were computed as CW_∞=166.00 mm, K=1.2 year^{-1}, t_0=-1.62 years, C=0, WP=0.20, and Φ′=4.519 for all crabs. The von Bertalanffy growth parameters of *P. segnis* were determined on the coasts of Türkiye, for the first time.

Keywords: Crustacea**,** Decapoda, *Portunus segnis*, von Bertalanffy, Allometry

Introduction

Invasive species, prevalent in marine and estuarine ecosystems cause significant changes in the areas they invade (Havel et al., 2015). In such systems, invading species decrease the number of native species (Brenchley & Carlton, 1983). Lack of predators, parasites, and diseases are the most important reasons for the success of invasive species (Williamson & Fitter, 1996). In detail, a lack of predators gives the invasive species possibility of higher survival, higher growth, and lower mortality (Williamson, 1996). Thus, they cause ecological change and threats to biodiversity (Katsanavakis et al., 2014).

The blue swimming crab, *Portunus segnis* (Forskål, 1775) (Brachyura: Portunidae) is native to the West Indian Ocean and distributed from Pakistan to South Africa and to the Red Sea (Lai et al., 2010). It is one of the most successful invasive species in the Mediterranean (Klaoudatos & Kapiris 2014). The first record of *P. segnis* in the Mediterranean was on the coasts of Egypt, as *Neptunus (Portunus) pelagicus* (Fox, 1924). Lai et al., (2010) revised the Portunus species according to their morphometric, geographic, and genetic differences. Their research showed that in the Mediterranean ecosystem just *P. segnis* species is available. Gruvel (1928) reported the first record of *P. segnis* in Türkiye in Iskenderun Bay. *P. segnis* is distributed in the Sea of Marmara, the Aegean Sea, and the Levantine seacoasts of Türkiye (e.g. Ozcan, 2003; Bakır et al, 2014). In Iskenderun Bay, *P. segnis* is one of the most abundant crab species along with *Callinectes sapidus* and *Charybdis longicollis* (Ozcan et al., 2005). In addition, *P. segnis* is the second most economically significant Decapod Crustacean after *C. sapidus* (Tureli et al., 2000, Doğan et al., 2007). Also, it is a substantial nutrient source for humans with a high protein value and a low lipid ratio (Tureli et al., 2000).

Growth is an important model in understanding the ecology of the species, predicting the recruitment in fisheries, and regulating the stock assessment and management of the species in Crustaceans (Miller & Smith, 2003). Significant aspects of blue swimming crab growth were studied in its native range (e.g. Noori et al., 2015; Giraldes et al., 2016). Although the life history of the blue swimming crab in the endemic range has been investigated extensively, there is limited knowledge about it outside of the natural range (Tureli et al., 2016). In Türkiye, some research were carried out about *P. segnis* concerning meat composition and nutritional quality (e.g. Tureli et al., 2000; Gokoglu & Yerlikaya, 2003), heavy metal content (e.g. Olgunoglu & Olgunoglu, 2016), population biology (e.g. Inandi, 2015) and reproductive biology (Tureli & Yesilyurt, 2017).

This study aimed to estimate the allometry and growth parameters of *P. segnis* in Iskenderun Bay (non-endemic range). Our results were compared with the growth estimations of *P. segnis* within its native region. Thus, it will form an idea about the status of the population of the alien species in Iskenderun Bay. It is thought that the results of this research will contribute to our knowledge about the population and management of non-indigenous species on the Mediterranean coasts of Türkiye.

Material and Methods

Study Area and Sampling Methods

Blue swimming crabs were caught between July 2014 and June 2015 monthly, except for February. Sample collection was done using a small bottom trawl net at 0-50 m depths towed for 45 minutes in Yumurtalik Cove, Iskenderun Bay (Northeastern Mediterranean) (Figure 1). Water quality parameters were quantified by a YSI 6600 multi-parameter probe. Carapace width (CW) (accurate to 0.01 mm) and total weight (TW) (accurate to 0.1 g) of all crabs were measured. The sexes of the crab specimens were recorded.

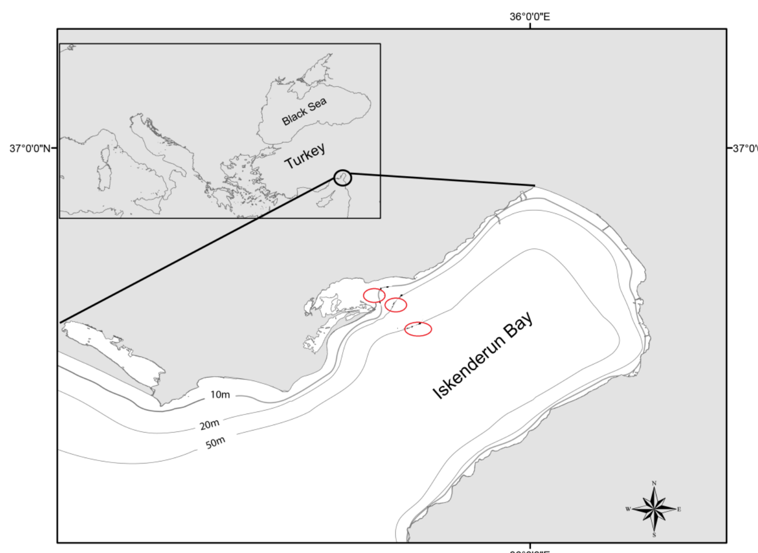

Figure 1. Yumurtalık Cove (Iskenderun Bay, Northeastern Mediterranean-Türkiye)

Data Analysis

To determine the growth was used to allometric $Y=a.x^b$ shaped growth equation. The logarithm of both sides of the allometric growth equation, which is curvilinear, is taken and converted into a linear relation equation in the form of $Log(W)=Log(a)+bLog(CW)$ for females and males. Where W is the total weight (g), CW is the carapace width (mm), a is the allometric constant, and b is the slope of the linear equation (Haefner, 1985).

Growth was estimated using the monthly length-frequency distributions. The crab size distributions were grouped into length classes at 10 mm intervals. Growth parameters were determined with the von Bertalanffy growth function. The FISAT software was used for data analysis (Gayanilo et al., 2005). The seasonal von Bertalanffy growth equation may be defined as:

$$CW(t)=CW_{\infty}[1-\exp\{-K(t-t_0)-(CK/2\pi)(\sin 2\pi(t-t_s)-\sin 2\pi(t_0-t_s))\}]$$

Where CW_{∞} is the asymptotic carapace width, K'= curving parameter of the growth equation (yr^{-1}), t_0= age at the carapace width is zero, t_s= the growth rate that is the highest during the year, C= size of the seasonal variation in growth, which ranges between 0 and 1 (i.e. when C values close to 0 no seasonal variation, when close to 1 the amplitude is maximal). The CW_{∞} and K growth parameters were calculated by ELEFAN, and t_0 was computed for growth fitting with Pauly's equation (1983):

$$\text{Log}(-t_0)=-0.3922-0.2752*\log(CW\infty)-1.038*\log(K)$$

Φ, the growth performance index, was measured as $\Phi'=\log(K)+2\log(CW\infty)$ (Pauly & Munro, 1984).

The winter point (WP) was calculated as $WP=t_{w+}0.5$ shows the period of slowest growth. WP range between 0 and 1.

The von Bertalanffy growth parameters were calculated for all individuals (including females, males, and juveniles altogether) because the females and males numbers were insufficient for calculation separately.

The maximum age was estimated by using the formula: $t_{max}=3/K$. The age-length figure was drawn related to t_{max}.

Results and Discussion

The blue swimming crab *P. segnis* individuals were sampled in all sampling months during the study (except February). A total of 320 specimens (140 females, 110 males, and 70 juveniles) of *P. segnis* were caught. The carapace width and weight ranged from 38.10 to 163.17 mm (mean 109.88 ±27.56 mm) (Table 1) and 3.46 to 324.36 g, respectively for all crabs. The dominant width interval was found between 110 and 120 mm for the females, 130 and 140 mm for the males, and 50 and 60 mm for the juveniles (Figure 2).

The carapace widths (CW) vary between 55.26 mm and 163.17 mm in females, while it varies between 48.77 mm and 154 mm in males (Table 1). Our results indicated that the carapace width of the females was significantly larger (p=0.00) than that of the males. Figure 3 showed the monthly length frequency of the females and males.

The CW-TW relationships of the specimens were Log TW=2.9028CW-9.0664 (R^2=0.7452) for the females and Log TW=2.9773CW-9.3842 (R^2 =0.8433) for the males (Figures 4 and 5). The CW and TW relationships showed that, in both sexes, growth is allometric ($p<0.05$).

The seasonal von Bertalanffy growth function was measured using the length-frequency distribution analysis, for all samples, including juveniles (Table 2). All individuals had at least two cohorts during the studied period (Figure 6). Seasonality was not determined (C=0). The growth performance index (Φ´) was 4.519 for all specimens (Table 2).

Figure 7 showed that, in the first phase of life, the blue swimming crab had fast growth rates. In the second phase, the growth began to decline, but it did not become too slow. In the third phase of its life, the crab's growth was slow and stagnant until it reached its maximum size.

Table 1. The mean carapace width (mm), range in width for blue swimming crabs from Yumurtalik Cove sampled between July 2014-June 2015

Sex	n	Mean ±SD	Minimum CW	Maximum CW
Females	140	125.52 ±16.80	55.26	163.17
Males	110	111.65 ±23.11	48.77	154.00
Juvenile	70	75.82 ±20.54	38.10	117.45
All	320	109.88 ±27.56	38.10	163.17

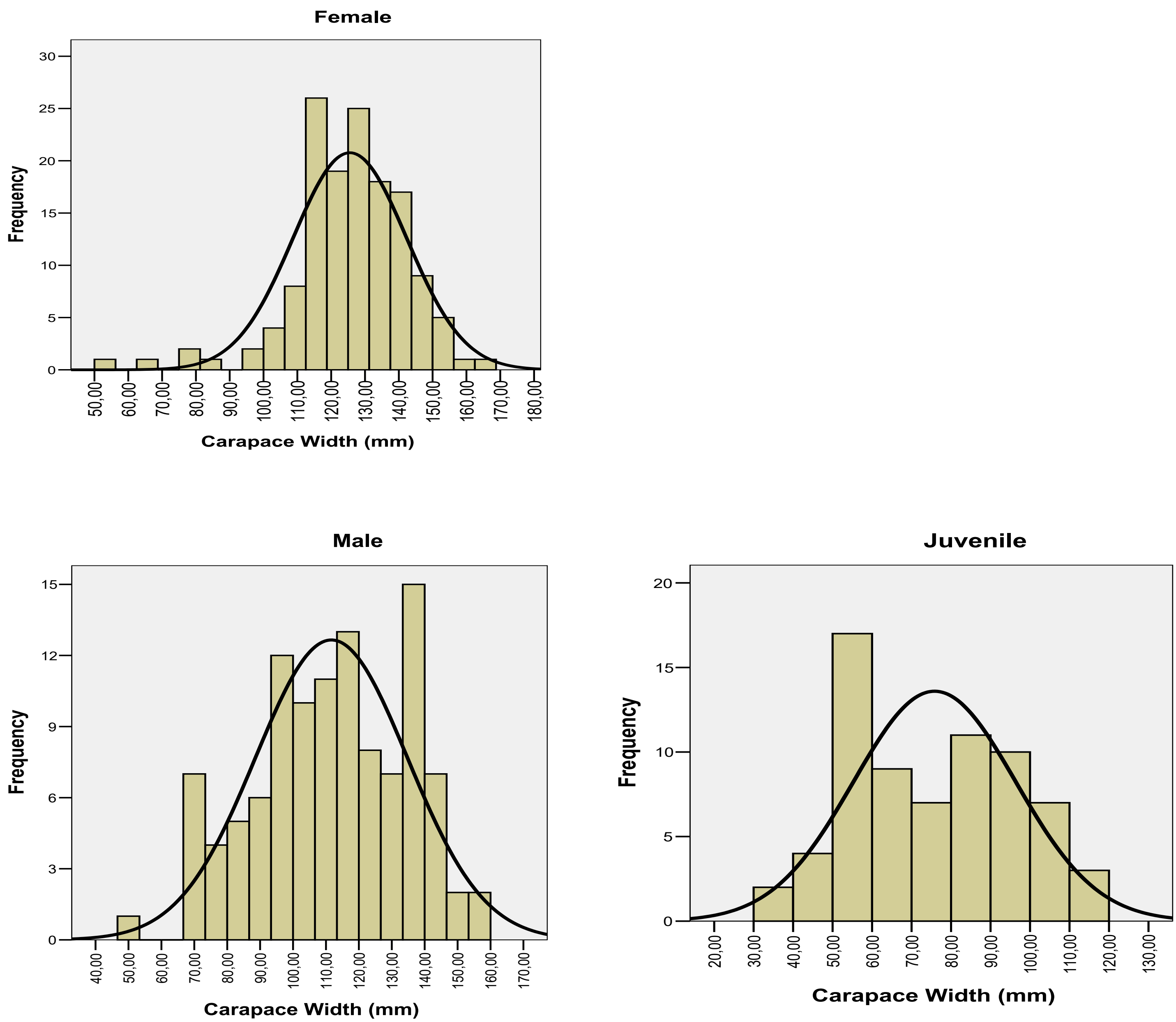

Figure 2. Length-frequency distribution of females, males and juvenile of blue swimming crab between July 2014-June 2015 in Yumurtalik Cove

Figure 3. Monthly length- frequency distribution of female, male and all blue swimming crabs

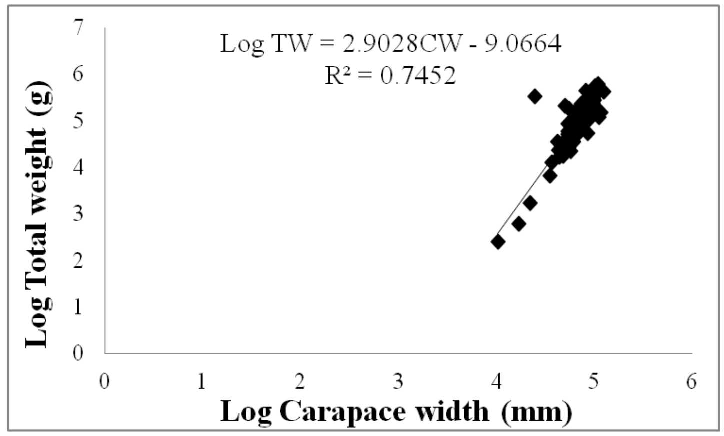

Figure 4. Carapace width- total weight relationship of *P. segnis* females

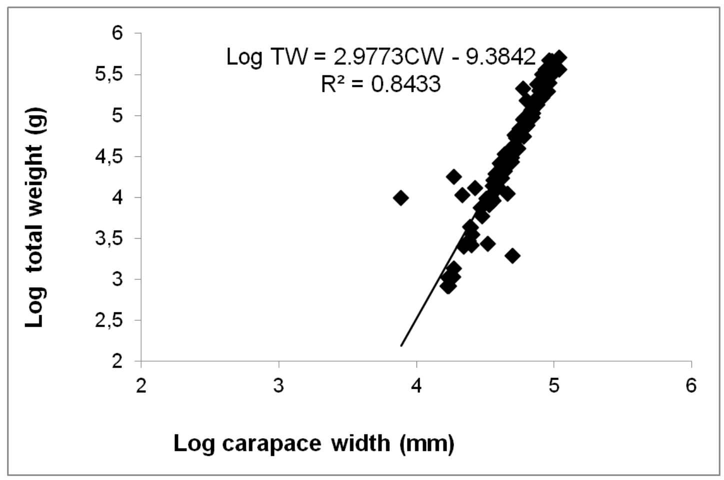

Figure 5. Carapace width- total weight relationship of *P. segnis* males

Table 2. von Bertalanffy growth parameters for *P. segnis*

CW∞ (mm)	K (year-1)	t_0	C	WP	Φ’	Rn
166.00	1.2	-1.62	0	0.20	4.519	0.270

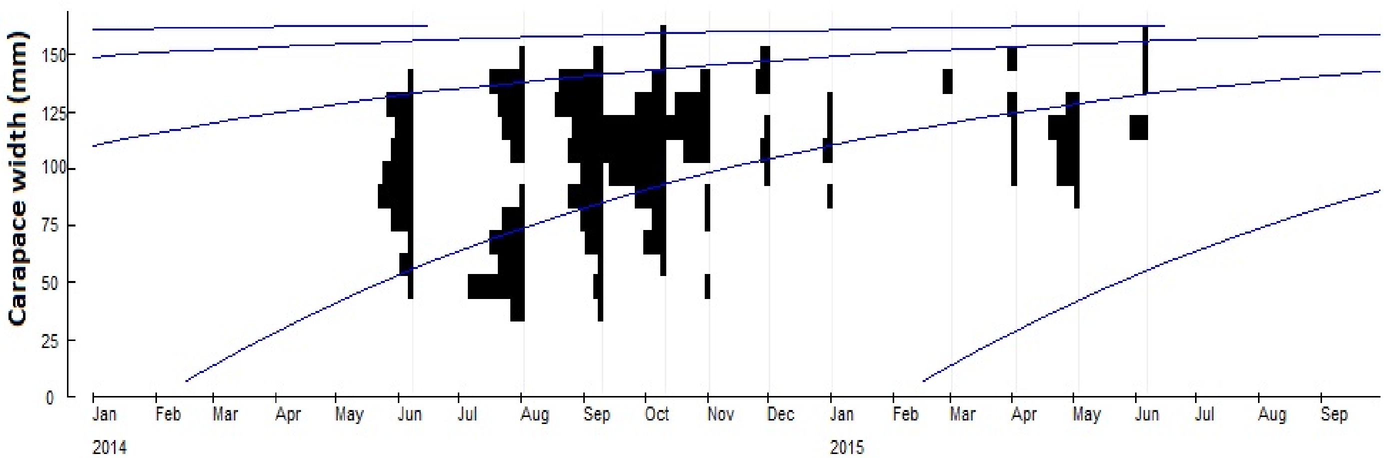

Figure 6. Length-Frequency distribution with seasonal von Bertalanffy growth curves for both sexes of *P. segnis*

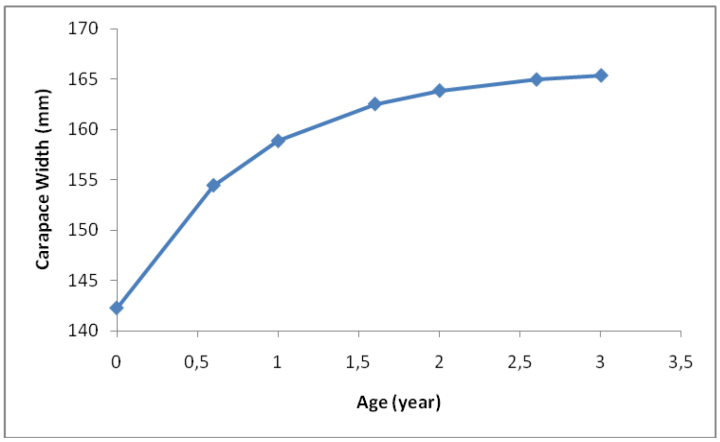

Figure 7. Growth curve of blue swimming crab, *P. segnis*

The growth parameters for blue swimming crabs were estimated in the Eastern Mediterranean, in its non-native range. In this study, CW was measured with a minimum of 55 and a maximum of 163 mm in females and a minimum of 48 and a maximum of 154 mm in males (Table 1). In Iskenderun Bay, the largest male and female sizes were determined as 170 mm CW and 171.5 mm CW, respectively, by Inandi (2015). The smallest male and female sizes were measured as 47.2 mm CW and 51.3 mm CW, respectively. Likewise, Ozcan & Akyurt (2006) proved that the CW frequency of males ranged between 40 and 169.9 mm, and the CW frequency of females ranged between 40 and 182.2 mm in Iskenderun Bay. As seen, our results on the CW range were found lower than those notified in earlier research in the same area. In the Gulf of Gabes, the minimum CW was lower than that in our study for males and females but in the Persian Gulf, the minimum CW was larger than that in our study for males and females (Hosseini et al., 2014a; b; Noori et al., 2015). In some of these previous studies, the maximum CW was larger than that in our study for males and females (Hosseini et al., 2014b), while in others, it was similar to those of females in our study (Hosseini et al., 2014a; Noori et al., 2015).

Our results indicated the carapace width of the females was larger than that of the males, and the difference was found to be statistically significant ($p=0.00$). Moreover, in Iskenderun Bay, Inandi (2015) reported similar data, but the difference was not statistically significant. In another study in the Gulf of Gabes, in the species' nonnative area, Hajjej et al., (2016) found that the mean carapace width of males was significantly bigger than that of females ($p<0.05$), same as those in the Persian Gulf, in its native area (Hosseini et al., 2014a; b).

On the Mediterranean Sea coast of Türkiye, a negative allometric growth pattern was found between the carapace width and weight in both males and females (Pauly's t-test $P<0.05$) (Figures 3 and 4, Table 3). In accordance with the morphology of the species, it can be said that the increase in weight is slower than the increase in carapace width. In Türkiye, there had been no data on the carapace width-total weight relationship of *P. segnis*. So, the results of this study were compared to those of earlier research from the native and other non-native ranges of *P. segnis*. Some other studies in native regions also provided similar results (Kamrani et al., 2010; Hosseini et al., 2014a; Giraldes et al., 2016), but Safaie et al., (2013) and Noori et al., (2015) found that growth is a positive allometric in both sexes and in males, respectively. In a non-native area of the species, the Gulf of Gabes (Tunisia), one study showed similar results (Hajjej et al., 2016). On the other hand, Ben Abdallah-Ben Hadj Hamida et al., (2019) indicated different results (Table 3).

In this study, for the first time, the von Bertalanffy growth parameters of *P. segnis* were determined on the coasts of Türkiye. The data of growth studies of blue swimming crab in its native range were summarized in Table 4. Findings of these researches in its native region showed a difference from our results. The value of CW_∞ of the males and females was found to be lower than those reported from the Persian Gulf (Kamrani et al., 2010; Safaie et al., 2013). The value of K for both sexes for the reported estimates in the native region (in the Persian Gulf) (Kamrani et al., 2010) was 0.98 yr^{-1}, whereas, for the North-Eastern Mediterranean, it was 1.2 yr^{-1}. The seawater temperature is high throughout the year in İskenderun Bay (between 16-28°C). At higher temperatures, growth rates increase. So our findings were normal. It can be said that results compatible with the high growth rate of invasive species are obtained. (Table 4). The growth performance index Φ′ calculated from our research was smaller than those reported from the Gulf of Oman, Iran (male: 11.04, female: 10.91) (Safaie et al., 2013). But, in the Gulf of Gabes (Tunisia), in a non-native area, similar results were reported (Hajjej et al. 2016; Ben Abdallah-Ben Hadj Hamida et al., 2019).

Tablo 3. The carapace width (CW)- weight (TW) relationship parameters for *P. segnis* in other studies

Sex	Sample number	CW (mm)	TW-CW relationship	R^2	Location	Distribution	Researcher
M	424	-	$TW = 0.0002CW^{2.757}$	0.93	Northern Persian Gulf	Native	Kamrani et al. 2010
F	348	-	$TW = 0.0002CW^{2.748}$	0.88			
T	772	23-173	$TW = 0.0002CW^{2.762}$	0.91			
M	1839	-	$TW = 0.00003CW^{3.214}$	0.96	Persian Gulf and Gulf of Oman	Native	Safaie et al. 2013
F	1769	-	$TW = 0.00001CW^{3.299}$	0.84			
T		-	$TW = 0.00002CW^{3.232}$	0.89			
M	418	75-175	Log TW = −16.532 + 2.334 log CW		Persian Gulf	Native	Hosseini et al. 2014a
F	448	70-165	Log TW = −15.278 + 2.554 log CW				
M	148	80.75-148.96	Ln TW = 3.45 LnCW-11.64	0.96	Persian Gulf	Native	Noori et al. 2015
F	154	84.54-163.42	Ln TW = 3.03 LnCW-9.72	0.93			
M	40	-	$TW=0.36*CW^{2.567}$	0.95	Western Arabian Gulf	Native	Giraldes et al. 2016
F	27	-	$TW=0.26*CW^{2.665}$	0.93			
M	335	39.26-155.5	Log TW = 3.1444CW-10.181	0.96	Gulf of Gabes	Non-native	Hajjej et al. 2016
F	299	34.27-148.5	Log TW = 2.7433CW-8.4617	0.93			
T			Log TW = 2.9796CW-9.47	0.94			
M	1552	45-168	Log TW=-10.287+3.1870Log CW	0.97	Gulf of Gabes	Non-native	Ben Abdallah-Ben Hadj Hamida et al. 2019
F	1392	50-159	Log TW=-9.1512+2.9198Log CW	0.96			
T	2944	-	Log TW=-9.8910+3.0931Log CW	0.96			
M	110	48.77-154	Log TW = 2.9773CW-9.3842	0.84	Mediterranean of Türkiye	Non-native	This study
F	140	55.26-163.17	Log TW = 2.9028CW-9.0664	0.74			

Table 4. Published von Bertalanffy growth coefficients for *P. segnis*

Location	k (yr^{-1})	CW_{∞} (mm)	t_0 (yr)	Source
Northern Persian Gulf	♂ 1.2	♂ 168	-	Kamrani et al., 2010
	♀ 1.1	♀ 177.9	-	
	B: 0.98	B: 172.5	-	
Persian Gulf and Gulf of Oman	♂ 1.7	♂ 191	♂ -0.055	Safaie et al., 2013
	♀ 1.6	♀ 185	♀ -0.059	
Gulf of Gabes	♂ 1.34	♂ 206.48	♂ -0.130	Ben Abdallah-Ben Hadj Hamida et al., 2019
	♀ 1.42	♀ 183.89	♀ -0.127	
	B: 1.02	B: 190.60	B: -0.177	
Mediterranean of Türkiye	B: 1.2	B: 166	B: -1.62	Present study

B: both

Growth was observed to be relatively constant throughout the year. As for the water temperature, the lowest mean value was measured as 16.60 ±0.24°C, and the highest mean value was measured as 28.55 ±0.52°C. The salinity change during the study was between 37.40 ±0.65‰ and 38.61 ±0.78‰ in Iskenderun Bay. This supported our interpretation of the data regarding the continuity of growth throughout the year.

Conclusion

The present research findings provided new information which is morphometric characters, growth patterns, and population status of *P. segnis* on Turkish coasts. Blue swimming crab is commercially significant at local restaurants in Mersin and Iskenderun Bay. Also, it will be formed the basis for future research which is fisheries' biology and management, *P. segnis* population dynamics, stocks assessment, and impact on ecological systems of the Northeastern Mediterranean, Türkiye.

Compliance with Ethical Standard

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

Ethics committee approval: Ethics committee approval is not required for this study.

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Research Article

Trichodinid ectoparasites (Ciliophora: Peritrichida) from gills of some marine fishes of Sinop Coasts of the Black Sea, with the first report of *Trichodina rectuncinata*

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ABSTRACT

Four species of marine fishes, turbot *Scophthalmus maeoticus*, common sole *Solea solea*, shore rockling *Gaidropsarus mediterraneus*, and rusty blenny *Parablennius sanguinolentus* from Sinop coasts of the Black Sea were examined for ectoparasitic trichodinids. A total of four trichodinid species, *Trichodina rectuncinata, T. ovonucleata, T. jadranica,* and *T. domerguei* were described using the silver nitrate impregnation technique and morphologically studied. All morphometric data and photomicrographs of these trichodinids were presented along with details of their host preferences, prevalence and intensity of infestation. This study is the first report on the trichodinid ectoparasites on *Scophthalmus maeoticus*, *Solea solea*, *Gaidropsarus mediterraneus*, and *Parablennius sanguinolentus* in Türkiye. Moreover *Trichodina rectuncinata* is as a new record for Turkish fish parasite fauna.

Keywords: Ectoparasite, *Trichodina rectuncinata, T. ovonucleata*, *T. jadranica, T. domerguei,* Black Sea

Introduction

Trichodinidae is a family of ciliates of the order Mobilida, and is consist of eleven genera, which are *Dipartiella* Stein, 1961; *Hemitrichodina* Basson and Van As, 1989; *Heterobladetrichodina* Hu, 2011; *Pallitrichodina* Van As and Basson, 1993; *Paratrichodina* Lom, 1963; *Semitrichodina* Kazubski, 1958; *Trichodina* Ehrenberg, 1830; *Trichodinella* Raabe, 1950; *Trichodoxa* Sirgel, 1983; *Tripartiella* Lom, 1959; and *Vauchomia* Mueller, 1938 (Van As and Basson, 1989; Hu, 2011, Kibria and Asmat, 2019). The largest group within this family is the genus *Trichodina* Ehrenberg, 1838 that consists of more than 300 described species to date (de Jager and Basson, 2019). Trichodinids are well known as the ectoparasites of numerous aquatic invertebrate and vertebrate hosts, especially both cultured and wild fish (Van As and Basson, 1989).

Trichodinid species have some specific characteristic structures called the adhesive disc and the denticle. The taxonomy and identification of trichodinid species are based on the appearance and the size of these structures. Klein's silver impregnation technique is the only method used to reveal these structures of trichodinids (Lom, 1958).

The first report on trichodinid species in Türkiye was presented by Özer and Erdem (1998), up to present a total of 21 trichodinid species have been recorded at the species level (excluding genus level) in various fish species living in the freshwater, lagoon, and marine environments.

Most of the trichodinid species reported in our country have been identified from freshwater fish, but the trichodinids of marine fish have not been adequately studied (see Table 1 for details). *T. claviformis*, *T. gobii*, T. *ovonucleata* and *Paratricodina obliqua* have so far been reported only from the marine environment, while *T. domerguie* and *T. puytoraci* have also been reported from marine fish as well as freshwater and lagoon environments.

The objective of this study is to investigate the trichodinid species of four marine fish collected from the Sinop region of the Black Sea, and to present information about the distribution of the trichodinid species in Türkiye.

Material and Methods

Study area and Sampling

The study was conducted between May 2015 and April 2017 in in the Sinop coast of the Black Sea (Figure 1). The host fish were caught by gill net from local fishermen. All the fish specimens were maintained in Faculty of Fisheries and Aquatic Sciences at Sinop University for examination. A total of 223 fish specimens, turbot *Scophthalmus maeoticus* (8), common sole *Solea solea* (140), shore rockling *Gaidropsarus mediterraneus* (70), and rusty blenny *Parablennius sanguinolentus* (5) were examined for trichodinids.

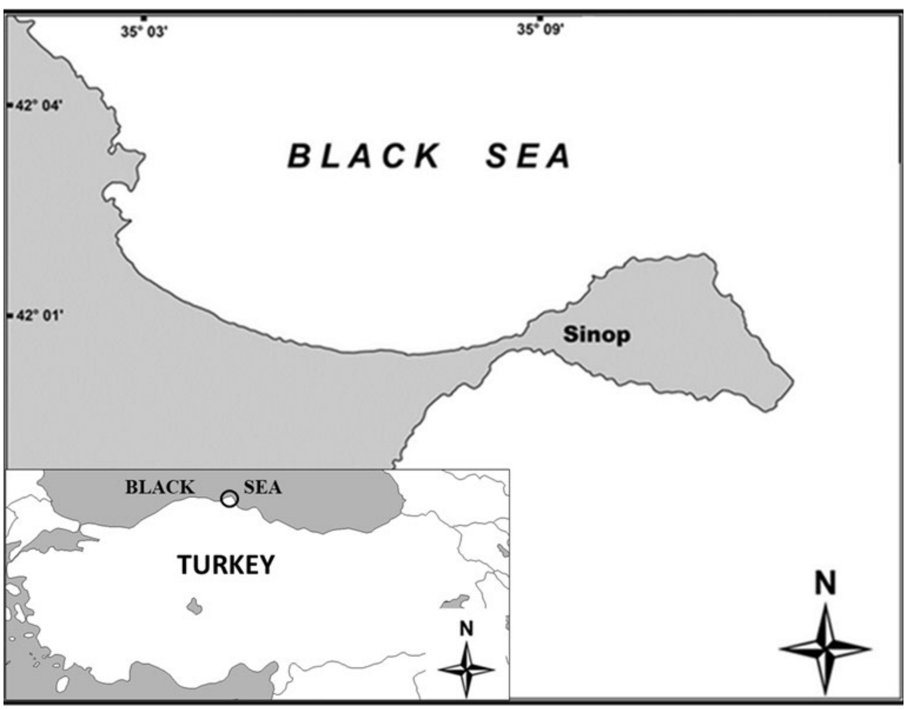

Figure 1. Map of sampling area

Searching for Trichodinids and Identification

At necropsy, the gills of the hosts were scraped and wet smears were prepared on several slides. These slides were examined using a microscope for the presence of trichodinids, later slides with trichodinids were air-dried and impregnated with silver nitrate technique to examine the details of the adhesive disc (Lom and Dykova, 1992). Examinations of prepared and stained slides and morphological measurements of trichodinids were made under a light microscope (Model Olympus BX53) with an oil immersion lens (100X) and their photographs were taken with a digital camera (Model Olympus DP25). The description of denticle structure followed the format recommended by Van As and Basson (1989) and Lom and Dykova (1992). All measurements were presented in micrometers (µm). Measurement values of trichodinids are given as minimum and maximum, followed by the arithmetic mean and standard error in parentheses. Radial pins and thread counts were presented as modes instead of arithmetic mean.

The prevalence of infestation value was determined according to Bush et al. (1997), while the density of infestation was evaluated as low (<10 individuals/slide), moderate (10–100 individuals/slide), high (>100 individuals/slide) and very high (>1000 individuals/slide).

The prevalence and intensity of infestation in host fish infested with more than one trichodinid species were presented as pooled data rather than for each trichodinid species.

Results and Discussion

In the present study, four known species of trichodinids were identified from the gills of four marine fishes from the Sinop coast of the Black Sea. These are *Trichodina rectuncinata, T. ovonucleata, T. jadranica,* and *T. domerguei.* Trichodinids list, their hosts and presence in their hosts are presented in Table 2 and representatives of the trichodinid species are illustrated in Figure 2 to Figure 5.

***Trichodina rectuncinata* Raabe, 1958**
(Figure 2, 5A, Table 3)

Host: shore rockling, *Gaidropsarus mediterraneus,* and rusty blenny, *Parablennius sanguinolentus*

It is a medium-sized trichodinid and body is disc-shape. The centre of adhesive dics is dark-stained (Figure 2A). The blade of the denticle is triangular with straight edges and a triangular or pyriform cavity in the center of each blade (Figure 5A). The distal blade surface is prominently rounded. The blade's distal margin touches the border membrane. The apex is rounded and the tangent point is not clear. The posterior blade surface extends straight and parallels to y axes, touching y axes almost along its full length in some blades. The anterior margin of the blade is straight in a population of *P. sanguinolentus*, and slightly curved in a population of *G. mediterraneus* (Figure 2). The posterior blade margin is straight and parallel to the anterior blade margin. Blade apophysis is not visible. The blade connection is not thin. The central part of the denticle is narrow, with a round point fitting tightly into the preceding denticle and extending three-fourths to the y-1 axis. Rays are very variable in shape, direction, and thickness. They are relatively short and curved backward, tapering slightly pointed (Figure 5A). Ray apophysis is not visible. The morphometrical data are presented in Table 3.

Previous comparative studies based on morphometric data have noted significant morphological variations among *T. rectuncinata* populations from different hosts (Loubser et al., 1995). In this study, it was determined that the populations from *G. mediterranneus* and *P. sanguinolentus* were almost similar to each other, with no obvious differences.

Trichodina rectuncinata is one of the trichodinids most widely distributed in marine fish. It has been recorded from more than 20 host species belonging to 17 fish families worldwide. To date, it has been frequently reported in Balistidae, Cottidae, Lotidae, Sygnathidae, Blennidae, Moridae, Gadidae, Hexagrammidae, Gobiesocidae, Scorpaenidae, Labridae, Gobiidae, Serranidae, Lateolabracidae, Mullidae, Scianidae, and Tripterygiidae (Lom and Dykova, 1992; Xu et al. 2001; Aguilar-Aguilar and Islas-Ortega, 2015; Islas-Ortega et al. 2020). In the Black Sea, it has been reported from fish belonging to 10 families, predominantly Bleniidae and Labridae (Zaika, 1968; Grupcheva et al. 1989; Gaevskaya and Korniychuk, 2003). This trichodinid, in addition to having a broad host spectrum, has been reported mostly in demersal or benthic fish.

Although *T. rectuncinata* has been previously reported from marine fish in many regions of the world, this is the first record of this species in Türkiye.

Table 1. Records of Trichodinid parasites in Turkish waters

Trichodinid species	Fish host(s)	Environment/Locality	Reference(s)
Trichodina acuta Lom, 1961	*Cyprinus carpio*	Freshwater / BS	Özer and Erdem (1998), Özer and Öztürk (2015)
Trichodina claviformis Dobberstein & Palm, 2000	*Merlangius merlangus*	**Marine / BS**	Öğüt and Palm (2005)
Trichodina cobitis Lom, 1960	*Cobitis taenia*	Freshwater / BS	Özer and Öztürk (2015)
Trichodina domerguei Wallengren, 1897	*Merlangius merlangus*	**Marine / (BS)**	Özer et al. (2012)
	Mullus barbatus ponticus	**Marine / (BS)**	Öztürk and Yeşil (2019)
	Platichthyes flesus	Freshwater / BS	Öztürk and Özer (2010)
	Aphanius danfordii	Freshwater / BS	Öztürk and Özer (2007)
	Gasterosteus aculeatus	Freshwater / BS	Özer (2003a)
	Neogobius melanostomus	Freshwater / BS	Özer (2003b)
	Sparus aurata	Lagoon / MS	Canlı (2010)
	Dicentrarchus labrax	Lagoon / MS	Canlı (2010)
	Gambusia holbrooki	Freshwater / BS	Özer and Öztürk (2015)
	Neogobius fluviatilis	Lagoon / BS	Öztürk and Çam (2013)
	Pomatoschistus marmoratus	Lagoon / BS	Öztürk and Çam (2013)
Trichodina fultoni Dawis 1947	*Oncorhynchus mykiss*	Freshwater / (AS)	Şimşek and Aldemir (2020)
Trichodina gobii Raabe, 1959	*Merlangius merlangus*	**Marine / (BS)**	Özer et al. (2015)
Trichodina heterodentata Duncan, 1977	*Neogobius fluviatilis*	Lagoon / BS	Öztürk and Çam (2013)
	Pomatoschistus marmoratus	Lagoon / BS	Öztürk and Çam (2013)
	Proterorhinus marmoratus	Freshwater / BS	Öztürk and Çam (2013)
	Aphanius danfordii	Freshwater / BS	Özer and Öztürk (2015)
	Sander lucioperca	Freshwater / BS	Özer and Öztürk (2015)
	Sciaenochromis fryeri	Freshwater / (aquarium)	Çelik and Korun (2018)
	Poecilia sphenops	Freshwater / (aquarium)	Çelik and Korun (2018)
Trichodina jadranica Raabe, 1958	*Platichthyes flesus*	Freshwater / (BS)	Öztürk and Özer (2010)
Trichodina lepsii Lom, 1962	*Mugil cephalus*	Lagoon (BS)	Özer and Öztürk (2004)
	Liza aurata	Lagoon (BS)	Özer and Öztürk (2004)

Table 1. (contiuned)

Trichodinid species	Fish host(s)	Environment/Locality	Reference(s)
*Trichodina lucioperca*e Lom, 197	*Sander lucioperca*	Freshwater / BS	Özer and Öztürk (2015)
Trichodina modesta Lom, 1970	*Aphanius danfordii*	Freshwater / BS	Öztürk and Özer (2007), Özer and Öztürk (2015)
Trichodina mutabilis Kazubski & Migala, 1968	*Cyprinus carpio*	Freshwater / BS	Özer and Erdem (1998)
Trichodina nigra Lom, 1960	*Cyprinus carpio*	Freshwater / BS	Özer and Erdem (1998)
	Cyprinus carpio	Freshwater / MS	Cengizler et al. (2001), Kılınçaslan and Cengizler (2008)
	Silurus glanis	Freshwater / MS	Kılınçaslan and Cengizler (2008)
	Leuciscus cephalus	Freshwater / MS	Kılınçaslan and Cengizler (2008)
Trichodina ovonucleata Raabei, 1958	*Mullus barbatus ponticus*	**Marine / (BS)**	Öztürk and Yeşil (2019)
Trichodina pediculus	*Cyprinus carpio*	Freshwater / MS	Çelik and Korun (2018)
	Labidochromis caeruleus	Freshwater / (aquarium)	Çelik and Korun (2018)
	Pseudotropheus socolofi	Freshwater / (aquarium)	Çelik and Korun (2018)
	Poecilia velifera	Freshwater / (aquarium)	Çelik and Korun (2018)
	Poecilia sphenops	Freshwater / (aquarium)	Çelik and Korun (2018)
Trichodina puytoraci Lom, 1962	*Liza aurata*	Freshwater / BS	Özer and Öztürk (2004), Öztürk (2013)
	Mugil cephalus	Freshwater / BS	Özer and Öztürk (2004)
	Merlangius merlangus	**Marine (BS)**	Öğüt and Palm (2005)
Trichodina tenuidens Faure-Fremiet, 1944	*Gasterosteus aculeatus*	Freshwater / BS	Özer (2003a), Özer and Öztürk (2015)
	Gasterosteus aculeatus	Lagoon / (BS)	Özer and Öztürk (2015)
Tripartiella macrosoma Basson & Van As, 1987	*Aphanius danfordii*	Freshwater (BS)	Öztürk and Özer (2007), Özer and Öztürk (2015)
Trichodinella subtilis Lom, 1959	*Cyprinus carpio*	Freshwater / BS	Özer and Erdem (1998)
Paratrichodina corlissi Lom & Haldar, 1977	*Neogobius fluviatilis*	Lagoon / BS	Öztürk and Çam (2013)
	Pomatoschistus marmoratus	Lagoon / BS	Öztürk and Çam (2013)
Paratrichodina obliqua Lom 1963	*Mullus barbatus ponticus*	**Marine** (BS)	Öztürk and Yeşil (2019)

BS: Black Sea, MS: Mediterranean Sea, AS: Aegean Sea, MS: Marmara Sea, CA: Central Anatolia Region

Table 2. List of identified trichodinid species at study and presence in their hosts

Host	P (%) (Nin/Nex)	Trichodinid species	Intensity of infestation
Gaidropsarus mediterraneus	11.4 8/70	*Trichodina rectuncinata* *T. ovonucleata*	high moderate
Parablennius sanguinolentus	80.0 (4/5)	*T. rectuncinata* *T. ovonucleata*	high moderate
Scophthalmus maeoticus	100 (8/8)	*T. jadranica* *T. ovonucleata*	high moderate
Solea solea	7.9 (11/140)	*T. jadranica* *T. domerguei*	very high low

Nin, Number of fishes infested. Nex, Number of fishes examined

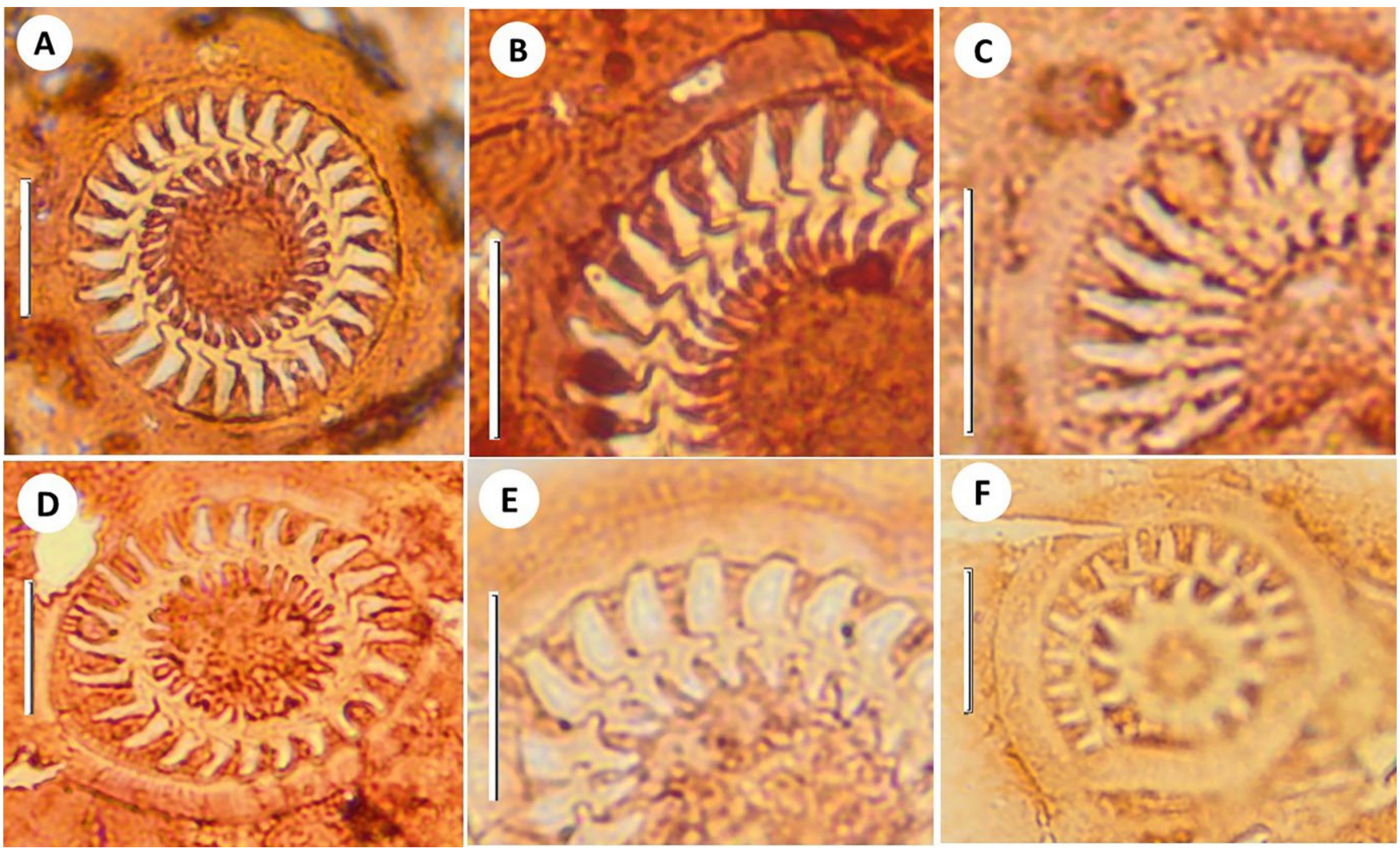

Figure 2. *Trichodina rectuncinata* Raabe, 1958. A-C: *T. rectuncinata* from *P. sanguinolentus,* D-E: *T. rectuncinata* from *G. mediterraneus*. F. An adhesive disc of *T. rectuncinata* during binary fission. Specimens stained with silver-nitrate. Scale bar 10 µm.

Table 3. Morphometric (in micrometers) data on populations of *Trichodina rectuncinata*, *T. ovonucleata* and *T. jadranica*

Trichodinidid species	*Trichodina rectuncinata*		*Trichodina ovonucleata*			*Trichodina jadranica*	
Hosts	*G. mediterraneus*	*P. sanguinolentus*	*G. mediterraneus*	*P. sanguinolentus*	*S. maeoticus*	*S. maeoticus*	*S. solea*
Body diameter	34.5-39.1 (36.1±1.6)	29.3 – 37.9 (34.3 ±1.8)	26.2 - 28.6 (26.9 ±0.4)	25.4 – 27.7 (26.3 ±0.3)	26.7 – 29.1 (28.1 ±0.4)	27.7 -34.6 (31.0 ±1.2)	25.4 – 28.3 (26.4 ±0.4)
Adhesive disc diameter	22.4 – 24.2 (23.4 ±0.5)	22.8 - 30.3 (27.5 ±1.7)	18.8 – 21.9 (20.8 ±0.5)	20.5 – 22.8 (21.6 ±0.4)	20.2 – 24.3 (21.6 ±0.7)	22.8 – 30.3 (26.0 ±1.3)	18.4 – 22.8 (20.5 ±0.6)
Border membrane width	2.9 – 3.0 (3.0 ±0.05)	2.1 – 3.2 (2.6 ±0.2)	2.4 – 2.8 (2.6 ±0.08)	2.1 – 2.6 (2.5 ±0.07)	2.3 – 2.9 (2.6 ±0.09)	2.0 – 2.8 (2.5 ±0.1)	2.4 – 2.8 (2.6 ±0.06)
Denticle ring diameter	13.5 – 15.8 (14.3 ±0.8)	13.6 – 19.3 (16.6 ±1.4)	11.9 – 15.6 (14.1 ±0.7)	11.5 – 14.3 (13.4 ±0.5)	13.3 – 15.5 (14.5 ±0.4)	13.4 – 16.9 (15.9 ±0.5)	12.3 – 13.3 (12.3 ±0.4)
Denticle span	3.1 – 3.8 (3.3 ±0.2)	2.1 – 3.4 (2.8 ±0.4)	2.8 – 3.6 (3.3 ±0.1)	2.6 – 3.9 (3.4 ±0.3)	3.1 – 3.7 (3.5 ±0.1)	3.7 – 4.6 (4.2 ±0.2)	3.4 – 4.0 (3.7 ±0.09)
Denticle length	7.1 -7.7 (7.4 ±0.2)	7.2 – 9.3 (8.7 ±0.6)	4.2 – 6.3 (5.5 ±0.4)	5.9 – 6.9 (6.5 ±0.2)	5.2 – 6.0 (5.8 ±0.1)	6.0 -6.6 (6.3 ±0.08)	5.3 – 6.0 (5.6 ±0.2)
Blade length	3.5 - 3.7 (3.3 ±0.08)	3.1 – 4.5 (3.9 ±0.3)	1.7 – 2.2 (2.0 ±0.07)	2.1 – 2.6 (2.3 ±0.1)	1.5 – 2.0 (1.8 ± 0.08)	2.2 – 2.8 (2.5 ± 0.08)	2.4 – 2.6 (2.6 ±0.02)
Central part width	1.3 -2.2 (1.6 ±0.3)	1.7 – 2.3 (1.9 ±0.2)	0.7 – 1.5 (1.2 ±0.2)	0.9 – 2.1 (1.3 ±0.2)	1.4 – 2.0 (1.7 ± 0.1)	1.2 -2.0 (1.6 ±0.1)	1.0 – 1.7 (1.5 ±0.1)
Ray length	2.1 – 2.4 2.2 ±0.1)	2.3 – 2.8 (2.6 ±0.1)	2.1 – 2.8 (2.4 ±0.1)	2.6 – 3.0 (2.8 ±0.07)	2.1 – 2.6 (2.4 ± 0.07)	1.7 – 2.8 (2.2 ±0.2)	1.7 – 1.9 (1.8 ±0.1)
Denticle number	24 - 27	26 - 29	20-22	20-23	22-24	21 - 23	19 - 21
Radial pins per denticle	6	5-6	6	6	6-8	6-8	6
n	10	10	10	10	10	10	10

n: number of specimens measured

Trichodina ovonucleata Raabe, 1958

(Figure 3, 5B, Table 3)

Host: Shore Rockling, *Gaidropsarus mediterraneus,* rusty blenny, *Parablennius sanguinolentus* and Turbot, *Scophthalmus maeoticus*

It is a small to medium-sized trichodinid with body disc-shape. The centre of the adhesive dics is dark-stained (Figure 3). The blade of denticle is sickle-shaped and fill most of space between Y-axes. The anterior margin of blade is curved than posterior blade surface. Blade distal margin touches the border membrane in some. Blade apophysis is not visible. Blade connection is evident. The central part of denticle is narrow, rounded end and fitted loosely into preceding denticle, not extending to y-1 axis, shapes of the central part above and below the x-axis similar (Figure 5B). Ray is longer than the blade. Ray is straight and pointed. There is no ray apophysis. Rays are extends straight and parallel to Y-axes, touching Y-axes almost along its full length in some. The morphometrical data are presented in Table 3.

Trichodina ovonucleata was originally described by Raabe (1958) from Adriatic blennies. Since then, it shows a broad host preference and has been reported by a number of authors from various marine fish in worldwide, including the Pacific and the Atlantic Oceans, the Mediterranean and the Black Seas (Zaika, 1968; Stein, 1979; Grupcheva et al. 1989; Xu et al. 2002). In the Black Sea, *T. ovonucleata* has so far been reported in *Parablennius sanguinolentus*, *P. tentacularis*, *Gaidropsarus mediterraneus*, *Merlangius merlangus euxinus*, *Lisa saliens*, *Trachurus mediterraneus ponticus, Spicara smaris, Diplodus annularis, Gobius cobitis, G. niger, Mullus barbatus ponticus, Symphodus cinereus, S. tinca, S. roissali, Ophidion rochei, Psetta maxima maeotica, Sciaena umbra, Serranus scriba, Scorpaena porcus, Syngnathus typhle, S. abaster, Solea nasuta* (Lom, 1970; Zaika, 1968; Grupcheva et al. 1989; Gaevskaya and Korniychuk, 2003). As can be seen from Table 1, *T. ovonucleata* was reported from the Black Sea coast of Türkiye, only in *M. barbatus ponticus*.

T. ovonucleata, which was previously recorded in *G. mediterraneus*, *P. sanguinolentus* and S. *maeoticus* in the Black Sea, had not been previously recorded from these hosts in Turkish coasts of the Black Sea. There fore, this study is first report on trichodinids of *G. mediterraneus*, *P. sanguinolentus* and *S. maeoticus* in Turkish coasts.

***Trichodina jadranica* Raabe, 1958**

(Figure 4A-B, 5C, Table 3)

Host: Turbot *Scophthalmus maeoticus*, Common Sole *Solea solea*

It is a medium-sized trichodinid and body is disc-shaped. The centre of the adhesive dics is an unstained, clear circle with several dark granules close to each other (Figure 4A-B). The blade of denticle is broad, sickle-shaped, filling most of space between Y-axes, even, extending beyond Y-axes (Fig. 5C). Blade apophysis is present, but is not clearly visible. The central part of denticle is ovoid end and fitted firmly into preceding denticle. Ray is short and rounded end. Ray apophysis is present. The morphometrical data are presented in Table 3.

Trichodina jadranica has a wide geographical distribution including the Adriatic, Baltic, Black, and Azov seas as well as the Atlantic and Pacific Oceans. To date, have been reported from a large number of fish species living marine, brackish and freshwater environments worldwide (Arthur and Lom, 1984; Grupcheva et al. 1989; Loubster et al. 1995; Su and White, 1995; Dobberstein and Palm, 2000; Madsen et al. 2000; Xu, 2007; Öztürk and Özer, 2010; Islas-Ortega et al. 2020). Although this trichodinid has been reported in Perciformes, Pleuronectiformes, Scorpaeniformes, Tetraodontiformes and Anguilliformes to date, it is noteworthy that it has been reported more frequently, especially in flatfish. Similarly, *T. jadranica* has previously been reported from a flatfish, *P. flesus* in the Black Sea coast of Türkiye (Table 1). In this study, *Trichodina jadranica* was also detected from two flatfish, *S. maeoticus* and *S. solea*. Besides, the current study is the first report on trichodinids of turbot and common sole fish captured from Turkish coasts of the Black Sea.

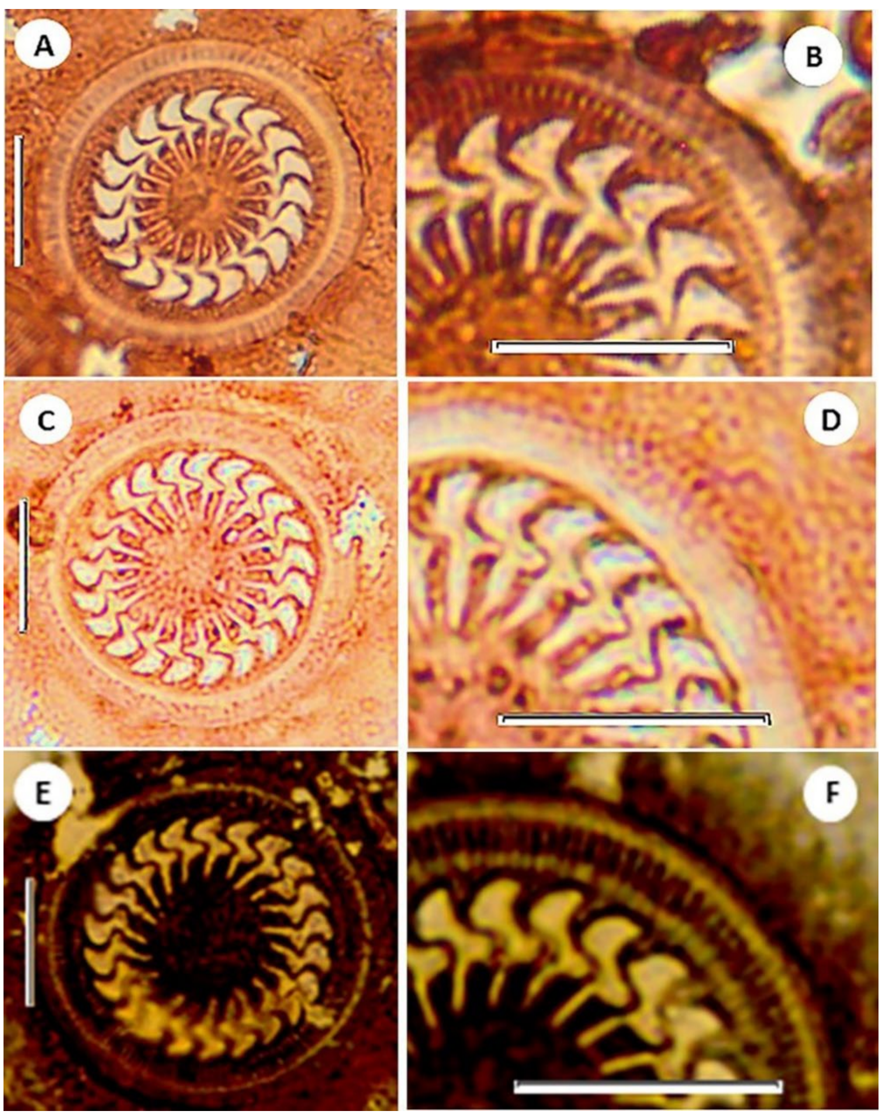

Figure 3. *Trichodina ovonucleata* Raabei, 1958. A-B: *T. ovonucleata* from *P. sanguinolentus,* C-D: *T. ovonucleata* from *G. mediterraneus,* E-F: *T. ovonucleata* from *S. maeoticus.* Specimens stained with silver-nitrate. Scale bar 10 µm.

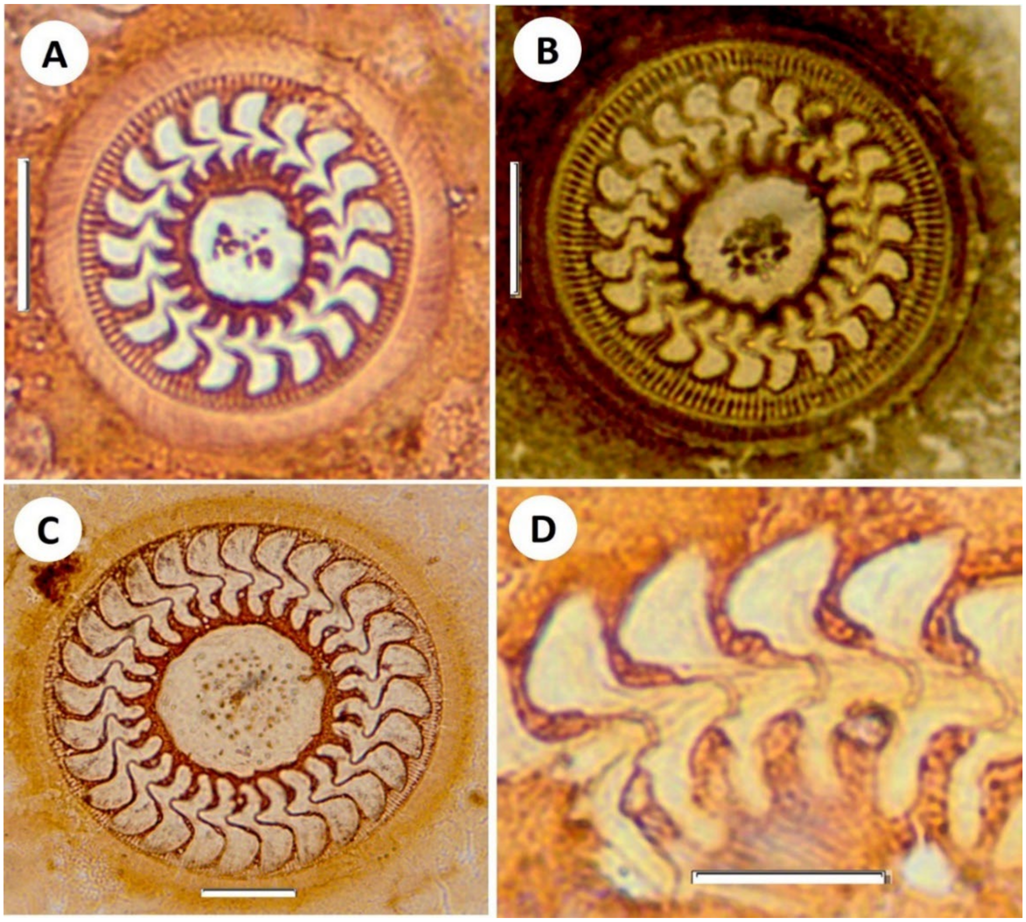

Figure 4. *Trichodina jadranica* Raabe, 1958 and *Trichodina domerguei* Wallengren, 1897. A: *T. jadranica* from *S. solea*, B. *T. jadranica* from *S. maeoticus,* C: *T. domerguei*, D: the denticles of *T. domerguei*. Specimens stained with silver-nitrate. Scale bar 10 µm.

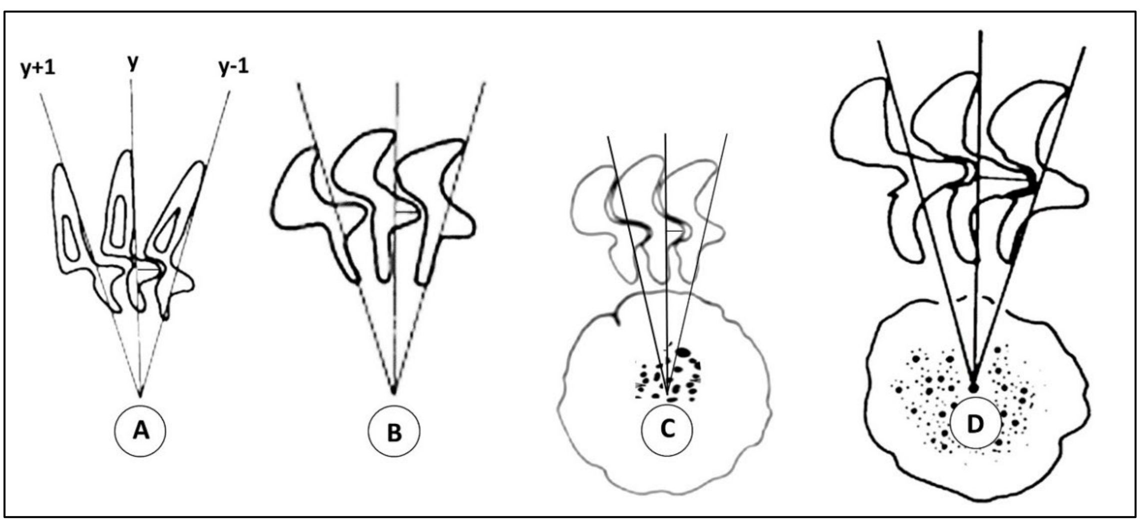

Figure 5. Diagrammatic drawings of the denticles of trichodinids in the present study. A. *Trichodina rectuncinata* Raabe, 1958, *Trichodina ovonucleata* Raabei, 1958, *Trichodina jadranica* Raabe, 1958, *Trichodina domerguei* Wallengren, 1897 (y+1, y, y-1. Y axes).

***Trichodina domerguei* Wallengren, 1897**

(Figure 4C-D, 5D)

Host: Common Sole, *Solea solea*

Only three *T. domerguei* specimens were found in stained slides. All morphological measurement were carried out of three specimens.

Trichodina domerguei (Figure 4C-D) is a large-sized species with body diameter 65–75 (70.6) µm. The adhesive disc 51–65 (58.6) µm in diameter and the centre of the adhesive disc of the specimens impregnated with silver nitrate is clear with numerous dark granules (Figure 4C). Adhesive disc surrounded by a border membrane of 4.0–5.0 (4.5) in width. Diameter of denticulate ring 35–45 (39.3) µm, number of denticles 23-29 and number of radial pins per denticle 9-10. Span of denticle 12–16 (15), length of denticle 9–11 (10.5) µm. Length of blade 7–9 (8) µm and it is broad and sickle-shaped, filling a large area between the y axes (Figure 5D). The distal margin of the blade is close to the border membrane (Figure 4C). Posterior blade margin is fairly curved. Blade apophysis is present (Figure 4D). The central part of denticle is well developed, but thin and long tapering to rounded point fitting tigthly into preceding denticle (Figure 4D). Rays is short, length of its 4-5 (4.7) µm and curved in posterior direction with tips extending beyond y axes (Figure 5D).

Trichodina domerguei is a cosmopolitan species and it has been reported to be one of the most widest distrubition with low specifitiy, able to infest variety of fish hosts living in freshwater, brackish and marine habitats (Lom 1970; Xu et al. 1999; Özer, 2003a; 2003b; Öztürk and Özer 2007; 2010). As can be seen from Table 1, it is one of the most frequently reported trichodinid species in our country. *Trichodina domerguei* has so far been reported from *Mullus barbatus ponticus* and *Merlangius merlangus* living marine habitat excluding hosts found in freshwater and brackishwater habitats (Table 1). This is the first report of the presence of *T. domerguei* on *Solea solea*.

Conclusion

The trichodinid ectoparasites of *S. maeoticus*, *S. solea*, *G. mediterraneus*, and *P. sanguinolentus* in Turkish Black Sea coasts firstly reported with this study. *Trichodina rectuncinata* is new record for Turkish parasite fauna, while *T. jadranica* and *T. domerguei* are parasite records for *Solea solea.* This paper is the first report on present of *T. ovonucleata* in *G. mediterraneus*, *P. sanguinolentus* and *S. maeoticus* hosts in Türkiye.

Compliance with Ethical Standard

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

Ethics committee approval: Ethics committee approval is not required for this study.

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Disclosure: -

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Aras Nehri havzasındaki (Kuzeydoğu Türkiye) üç gölün (Çıldır, Aktaş ve Aygır) makrozoobentik fauna yapısının ilk değerlendirmesi

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ÖZ

Çıldır, Aktaş ve Aygır göllerinin makrozoobentik fauna yapısının ortaya konması amacıyla 2013, 2017 ve 2019 yıllarında farklı dönemlerde göllerde yapılan örneklemeler sonucunda 47 takson tespit edilmiştir. Tür düzeyinde teşhisi yapılan 43 taksondan 41 tanesi araştırma yapılan göller için yeni kayıt niteliğindedir. Çıldır Gölü'nün dominant türleri *Gyraulus (Armiger) crista* (% 4.49), *Paratanytarsus lauterborni* (% 3.90) ve *Virgotanytarsus arduensis* (% 3.80) olarak tespit dilmiştir. Aktaş Gölü zoobentozunda gammarid populasyonunun çok baskın olduğu görülmektedir. Gölde *Gammarus pseudosyriacus* %15.24 baskınlık oranı ile dominant tür iken bunu % 7.53 oran ile *Cricotopus (Isocladius) reversus* ve % 7.29 oran ile *Gyraulus albus* takip etmektedir. Aygır Gölü'nde de gammarid populasyonu yüksektir ancak Aktaş Gölü'nden farklı olarak bu gruptan *Gammarus balcanicus* % 6.28'lik baskınlık oranı ile; *Gyraulus albus* (% 8), *Virgotanytarsus arduensis* (% 6.92) ve *Cricotopus (Isocladius) tricinctus*'tan (% 6.64) sonra dördüncü sırada yer almaktadır. Makrozoobentik fauna çeşitliliği açısından en yüksek Shannon ve Margalef İndeks değerleri Çıldır Gölü'nde tespit edilmiş olup (Shannon İndeks H': 1.73-2.23 arasında (ortalama 2.0), Margalef İndeks: 1.89-2.06 arsında (ortalama 2.03)) Aktaş ve Aygır göllerinde ise daha düşüktür. Her üç göl genelinde de tespit edilen taksonlar α ve β mesosaprob su kalitesi sınıfına ait türler olup, populasyon yoğunluklarının fazla olması özellikle Çıldır Gölü için oligotrofik düzeyden mesotrofik düzeye geçişin bir işareti olarak değerlendirilebilir.

Anahtar Kelimeler: Çıldır Gölü, Aktaş Gölü, Aygır Gölü, Makrozoobentoz

ABSTRACT

First evaluation of three lakes' (Çıldır, Aktaş and Aygır) macrozoobentic community structure in Aras River basin (North Eastern Türkiye)

In order to evaluate the macrozoobenthic fauna of Çıldır, Aktaş and Aygır lakes, 47 taxa were identified as a result of the samplings carried out in different periods in 2013, 2017 and 2019. 41 of 43 taxa identified at species level were new records for the study area. The dominant species of Lake Çıldır were *Gyraulus (Armiger) crista* (4.49%), *Paratanytarsus lauterborni* (3.90%) and *Virgotanytarsus arduensis* (3.80%). It was seen that the gammarid population was determined dominant among zoobenthos of Aktaş Lake. *Gammarus pseudosyriacus* was the dominant species with 15.24% dominance rate in Aktaş Lake, followed by *Cricotopus (Isocladius) reversus* with 7.53% and *Gyraulus albus* with 7.29%. Gammarid population was also high in Aygır Lake, but unlike Aktaş Lake, *Gammarus balcanicus* from this group with a dominance rate of 6.28%; it took the fourth place after *Gyraulus albus* (8%), *Virgotanytarsus arduensis* (6.92%) and *Cricotopus (Isocladius) tricinctus* (6.64%). In terms of macrozoobenthic fauna diversity, the highest Shannon and Margalef Index values were found in Lake Çıldır (Shannon Index H': between 1.73-2.23 (average 2.0), Margalef Index: between 1.89-2.06 (average 2.03)) while diversity indices were lower in Aktaş and Aygır lakes. The taxa detected in all three lakes are α and β mesotrophic species, and the high population density can be considered as a sign of the transition from the oligotrophic level to the mesotrophic level, especially for Lake Çıldır.

Keywords: Lake Çıldır, Aktaş Lake, Lake Aygır, Macrozoobenthos

Giriş

Türkiye, lentik ve lotik ekosistemleri ile biyolojik çeşitlilik açısından önemli yüzey suyu kaynaklarına sahiptir. Türkiye, 5 tanesi sınır aşan nehir havzası (Meriç Nehri Havzası, Çoruh Nehri Havzası, Kura-Aras Nehri Havzası, Asi Nehri Havzası ve Fırat-Dicle Nehirleri) olmak üzere 25 nehir havzasına sahiptir; sınır aşan nehir havzaları, ülkenin üçte birini oluşturan yaklaşık 256000 km^2'lik bir alanı kaplamaktadır. Türkiye'nin yıllık ortalama 143 milyar metreküplük ortalama su potansiyelinin % 40'ının bu nehir havzalarından kaynaklanması nedeniyle sınır aşan sular Türkiye için büyük önem taşımaktadır. Türkiye, sınır aşan nehirlerimizden biri olan Kura-Aras nehrini Gürcistan, Ermenistan, Azerbaycan ve İran ile paylaşmaktadır (Bilen, 2001). Kafkasya'nın en büyük akarsu sistemlerinden biri olan Kura-Aras Nehri Havzasının toplam alanı yaklaşık 27548 km^2 olup, Aras ve Kura nehirleri birleşerek Hazar Denizi'ne dökülmektedir. Aras Nehir Havzası 7 doğal göl içermekte olup, bunlardan üçü makroomurgasız fauna yapısı araştırılan Çıldır, Aktaş ve Aygır gölleridir. Her üç gölde de bugüne kadar su kalitesi (Kükrer vd., 2021), balık (Kırpık, 2018), fitoplankton (Çelekli vd., 2020), zooplankton (Apaydın Yağcı, 2013) ve diatom (Akbulut ve Yıldız, 2002) çalışmaları yapılmış olmasına rağmen, göllerde yer alan istilacı ve ekonomik öneme sahip bivalv türleri dışında (Başçınar ve Düzgüneş, 2009; Başçınar vd., 2009; Berber vd., 2018) makroomurgasız komunite yapısını bir bütün olarak ortaya koyan detaylı bir çalışma bulunmamaktadır. Bu çalışmaların arasında, Tarım ve Orman Bakanlığı, Doğa Koruma ve Milli Parklar Genel Müdürlüğü'nce 2017 yılında tamamlanan Aktaş Gölü Yönetim Planı Projesi kapsamında yapılan çalışmada Kırpık vd. (2019) tarafından Aktaş Gölü ile ilgili yapılan çalışmalar ve literatür verileri birlikte değerlendirilerek sunulmuş ve Aktaş (Ardahan) Gölü sucul omurgasız faunası tür adı belirtilmeden 14 Coleoptera, 1 ekzotik Decapoda türü ve istilacı Decapoda türü (*Astacus leptodactylus*) olarak verilmiştir. Gündüz (1996) tarafından Çıldır Gölü'nde yeni bir cladocer türü tanımlanmıştır.

Başta Çıldır Gölü olmak üzere her üç göl de yüksek oranda çeşitlilik barındıran ülkemizin önemli sucul ekosistemlerindendir. Çıldır Gölü hem önemli bitki alanı (ÖBA) hem de önemli kuş alanı (ÖKA) olarak değerlendirilmekte olup koruma statüsünde değildir. Floristik açıdan Küresel ve Avrupa ölçeğinde tehlike altındaki türleri içermemekle birlikte ulusal ölçekte nadir ve diğer türler açısından 10 bitki taksonu içermekte, angıt ve Van Gölü martısı populasyonları bakımından da (ÖKA No. 88) ÖKA olarak kabul edilmektedir (Magnin ve Yarar, 1997). Türkiye ile Gürcistan sınırında yer alan Aktaş Gölü ise iki farklı ülkeye ait olmak gibi ilginç, aynı zamanda hassas bir jeopolitik konumdadır. Bu çalışmada daha öncede belirtildiği gibi i:Çıldır, Aktaş ve Aygır göllerinin ilk defa makroomurgasız komunite yapısının mümkün olduğunca tür düzeyinde ortaya konulması, ii: ülkemiz sucul ekosistem omurgasız türleri biyoçeşitliliğine katkıda bulunulması, iii: bundan sonra aynı bölgede yapılacak olan çalışmalara ışık tutması amaçlanmıştır.

Materyal ve Metot

Çalışma Alanı

Yukarı Kura Havzasının en büyük gölü olan Çıldır Gölü, Ardahan ili Çıldır ilçe merkezine 2 km. uzaklıkta bulunmaktadır. Gölün %60'a yakın kısmı Ardahan il sınırları içerisinde olup kalan kısmı Kars il sınırları içerisindedir. Deniz seviyesinden 1959 m. yüksekliğinde, 124 km^2 yüzölçümünde, 100 m. den fazla derinliktedir. Tatlı su gölü olan Çıldır Gölü, Gölebakan ve Gülyüzü, Doğruyol derelerinden ve kar sularından beslenmektedir. Gölden enerji üretimi ve sulama suyu olarak faydalanılmaktadır. Çıldır I Hidro Elektrik Santrali, aktif olarak işletilmektedir (Ardahan il çevre ve orman müdürlüğü Çıldır Gölü kısa rapor). Hazapin Gölü olarak da bilinen Aktaş Gölü, Van Gölü'nden sonra Doğu Anadolu Bölgesi'nin ikinci büyük sodalı gölü olup tektonik kökenli bir göldür. Aktaş gölü, Türkiye-Gürcistan sınırı boyunca 2700 ha yüzey alanına ve 3 m ortalama derinliğe (maksimum derinlik yaklaşık 10 m) sahip sınır aşan bir göldür (Çelekli vd,. 2020). Gölün 11.7 km^2 'si Türkiye, 13.8 km^2 'si Gürcistan sınırları içerisindedir. Göl, Kenarbeli köyü yakınlarındaki kısa boylu mevsimlik dereler ile güney kıyılardaki Ema, Ortaklı ve Güngörmez pınarlarından beslenmektedir (Doğanay ve Zaman, 2006). 2216 m. rakımda yer alan Aygır Gölü, 400 ha yüzey alanına ve 22 m derinliğe sahip volkanik bir dolgu gölü olup, gölün suları göl havzasındaki kar suları ve kaynaklardan gelmektedir (Çelekli vd,. 2020).

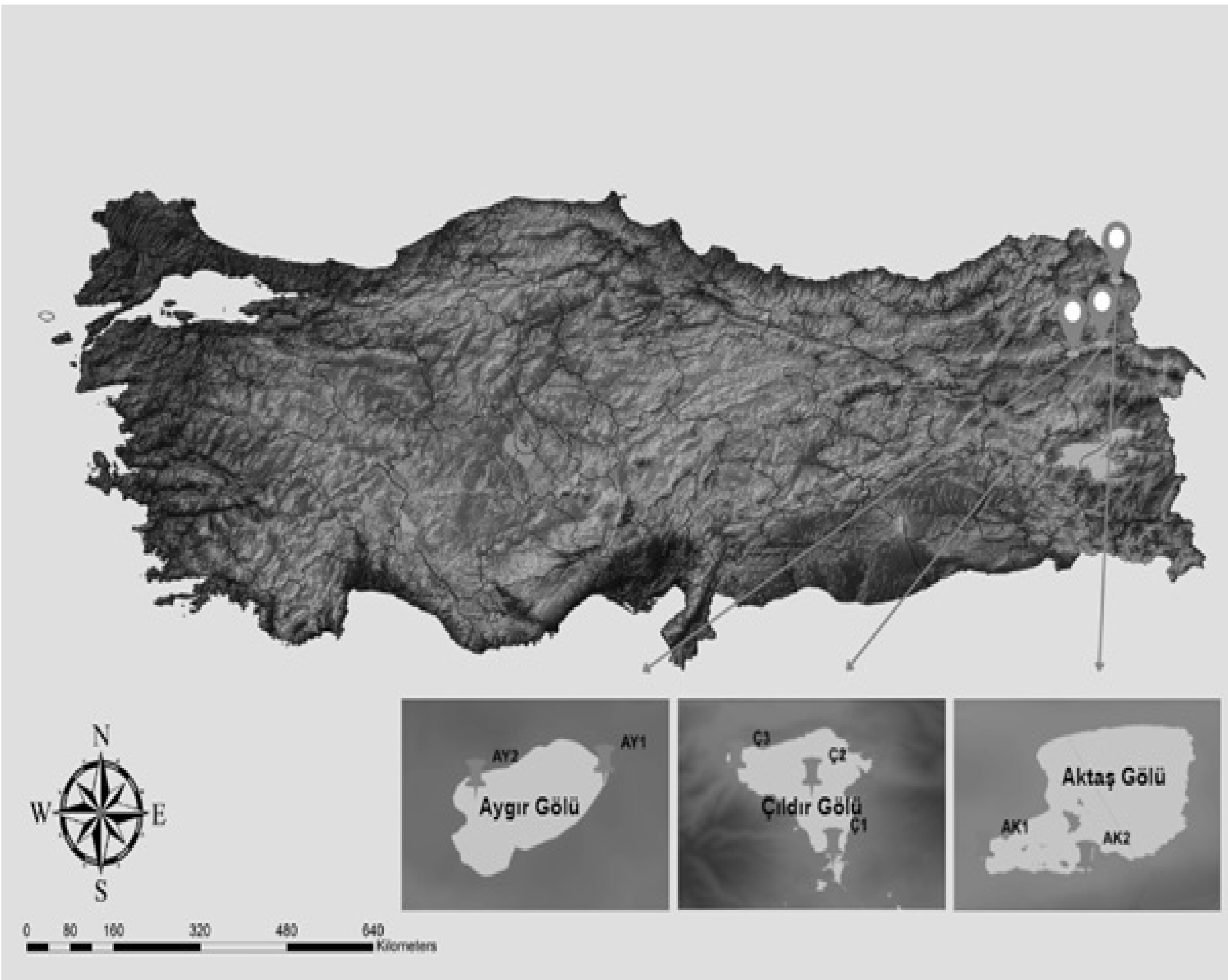
Örneklerin Toplanması ve Teşhisi

Çıldır, Aktaş ve Aygır Gölleri'nin makroozoobentik faunasının belirlenmesi amacıyla 2013, 2017 ve 2019 tarihlerinde farklı projeler kapsamında (ESOGU BAP 201619A224-Tübitak 117Y347 no'lu projeler) Çıldır Gölü'nden biri litoral olmak üzere 3, Aygır ve Aktaş göllerinden 2'şer litoral örnekleme yapılmıştır (Şekil 1). Örneklerin toplanmasında el kepçesi ve yüzey alanı 225 cm^2 alan ekman grab kullanılmıştır. Elde edilen örnekler 5'li elek sistemi ile elenerek % 70 lik etil alkol ile *in situ* olarak fikse edilmiş ve Eskişehir Osmangazi Üniversitesi, Biyoloji Bölümü, Hidrobiyoloji Laboratuvarı'na getirilmiştir. Ayıklanan örneklerin teşhisleri mümkün olan en alt taksonomik seviyeye kadar gerçekleştirilmiştir.

Zoobentoz örneklerinin teşhisinde Schütt (1965), Zhadin (1965), Müller-Liebenau (1969), Bilgin (1980), Şahin (1984), Eliot vd. (1988), Harker (1989), Sauter (1992), Epler (1995), Cranston (1995), Nilsson (1996), Nilsson (1997), Glöer ve Meier-Brook (1998), Glöer (2002), Bouchard (2004), Eiseier (2005), Bauernfeind (2012) teşhis anahtarları kullanılmıştır.

Verilerin Hesaplanması

Makrozoobentik verileri PAST 1.75b programı kullanılarak analiz edilmiştir (Hammer vd., 2001). Dominansi değerleri Bellan-Santini'nin (1969) nicel baskınlık indeksi ile ($Di = Ni/Nt \times 100$; N_i = i türünün birey sayısı; N_t = makrozoobentik örneklerin toplam sayısı) hesaplanmıştır. UPGMA algoritmasına dayalı Bray-Curtis benzerlik analizleri de PAST 1.75b kullanılarak değerlendirilmiştir (Hammer vd. 2001).



Şekil 1. Örnekleme yapılan istasyonları gösterir harita

Figure 1. Map of sampling stations

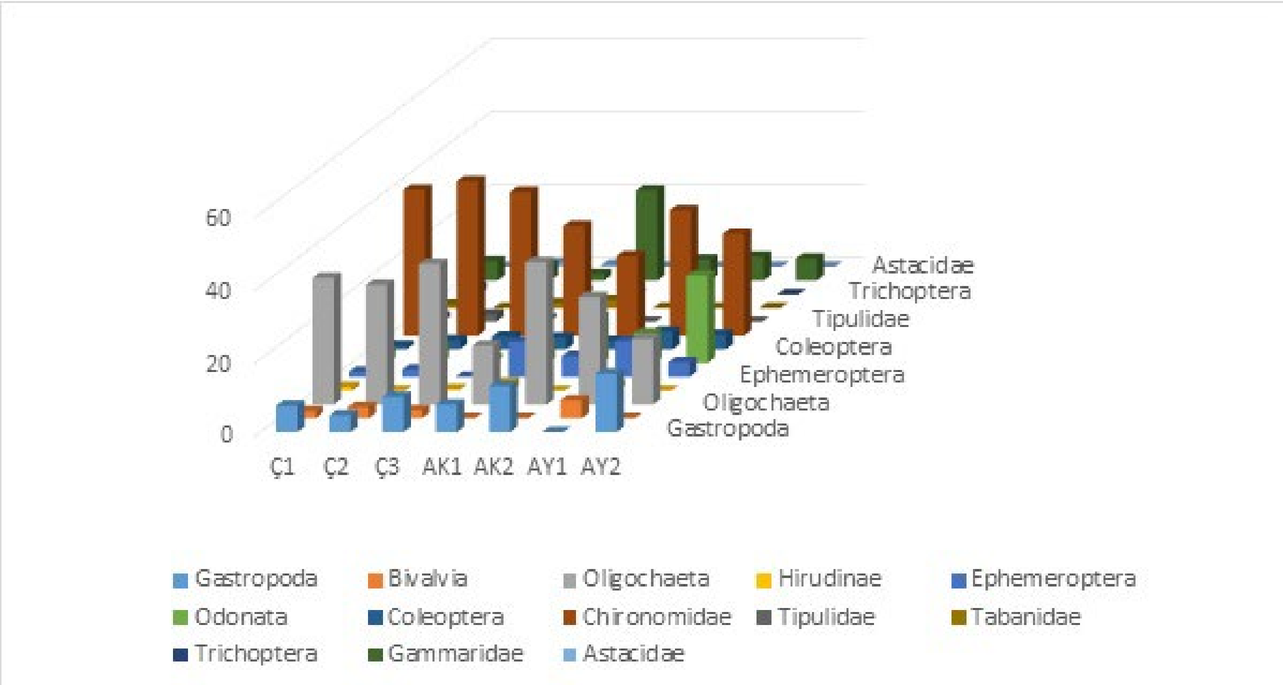
Bulgular ve Tartışma

Çıldır, Aktaş, Aygır göllerinin makrozoobentik fauna yapısının ortaya konması için yapılan çalışmada toplam 47 takson tespit edilmiştir (Tablo 1, Şekil 2). Tür düzeyinde teşhisi yapılan 43 taksondan *Anodonta cygnea* ve *Astacus leptodactylus* hariç bu çalışmada tanımlanan türlerin tamamı araştırma yapılan göller için yeni kayıt niteliğindedir. Her üç gölünde makrozoobentik faunasını yüzey sularında en sık rastlanılan ve genellikle toleranslı bireyleri içeren Oligochaeta ve Chironomidae türleri oluşturmaktadır. Çıldır Gölü'nün dominant grubu % 40.66 baskınlık oranı ile Chironomidae olup bunu % 35.40 baskınlık oranı ile Oligochaeta takip etmektedir. Göldeki baskın diğer taksonlar ise Gastropoda (% 7.15) ve Gammaridae'dir (% 3.70). Aktaş ve Aygır göllerinde de baskın ilk iki takson aynı olup, Aktaş Gölü'nde % 15.24 oranı ile üçüncü sırada yer alan Gammaridae bireylerinin yoğunluğu dikkat çekicidir. Her iki gölde de Çıldır Gölü'nden farklı olarak Ephemeroptera bireylerinin populasyon yoğunluklarının daha fazla olduğu görülmektedir. Makrozoobentik fauna çeşitliliği açısından en yüksek Shannon ve Margalef İndeks değerleri Çıldır Gölü'nde olup (Shannon İndeks H': 1.73-2.23 arasında (ortalama 2.0), Margalef İndeks: 1,89-2,06 arsında (ortalama 2.03)) Aktaş ve Aygır göllerinde ise daha düşüktür (Tablo 1).

Tablo 1. Çıldır, Aktaş, Aygır Göllerinde tespit edilen makrozoobentik taksonlar, % dominansi oranlarının istasyonlara göre dağılımı ve indeks değerleri (Ç: Çıldır Gölü, AK: Aktaş Gölü, AY: Aygır Gölü; OD: Ortalama dominansi; O: Ortalama)

Table 1. Macrozoobenthic taxa detected in Lakes Çıldır, Aktaş and Aygır, distribution of % dominance ratios according to stations and index values (Ç: Çıldır Lake, AK: Aktaş Lake, AY: Aygır Lake; OD: Average dominance; M: Average)

	Çıldır Gölü				Aktaş Gölü			Aygır Gölü		
Takson/İstasyon	**Ç1**	**Ç2**	**Ç3**	**OD**	**AK1**	**AK2**	**OD**	**AY1**	**AY2**	**OD**
Gastropoda	7.25	4.55	9.65	7.15	7.53	12.64	10.09	0.00	16	8
Bivalvia	1.93	3.03	2.19	2.39	0.00	0.00	0.00	4.92	0.00	2.46
Oligochaeta	34.78	32.83	38.60	35.40	16.13	39.08	27.60	29.51	18	23.75
Hirudinae	0.97	0.00	0.44	0.47	2.15	0.00	1.08	1.64	0.00	0.82
Ephemeroptera	1.45	2.02	0.00	1.16	9.68	5.75	7.71	9.84	4.00	6.92
Odonata	2.90	4.55	1.32	2.92	4.30	12.64	8.47	8.20	24	16.10
Coleoptera	0.97	2.53	3.51	2.33	3.23	2.30	2.76	4.92	4.00	4.46
Chironomidae	40.10	42.42	39.47	40.66	30.11	21.84	25.97	34.43	28.00	31.21
Tipulidae	1.45	2.02	0.88	1.45	0.00	0.00	0.00	0.00	0.00	0.00
Tabanidae	0.97	0.00	1.32	0.76	2.15	0.00	1.08	0.00	0.00	0.00
Trichoptera	1.93	1.01	0.44	1.13	0.00	0.00	0.00	0.00	0.00	0.00
Gammaridae	5.31	4.04	1.75	3.70	24.73	5.75	15.24	6.56	6.00	6.28
Astacidae	0.00	1.01	0.44	0.48	0.00	0.00	0.00	0.00	0.00	0.00
İndeksler										
	Ç1	**Ç2**	**Ç3**	**O**	**AK1**	**AK2**	**O**	**AY1**	**AY2**	**O**
Takson sayısı	12	11	12	12	9	7	8	8	7	8
Birey sayısı	207	198	228	211	93	87	90	61	50	56
Çeşitlilik (Shannon İn. H')	2.23	1.73	1.96	2.0	1.59	1.64	1.6	1.70	1.59	1.6
Çeşitlilik (Simpson_1-D)	0.71	0.70	0.68	0.7	0.80	0.76	0.8	0.77	0.80	0.8
Çeşitlilik (Margalef İn.)	2.06	1.89	2.03	2.0	1.77	1.34	1.6	1.70	1.53	1.6
Çeşitlilik (Evenness)	0.41	0.45	0.36	0.4	0.69	0.73	0.7	0.69	0.80	0.7



Şekil 2. Çıldır, Aktaş ve Aygır Gölleri'nde tespit edilen makrozoobentik taksonların göller ve istasyonlara göre dağılımı

Figure 2. Diagrapham of macrozoobenthic taxa detected in Lakes Çıldır, Aktaş and Aygır according to lakes and stations.

Tablo 2'de de görüldüğü üzere araştırma alanında 3 Gastropoda, 2 Bivalvia, 6 Ephemeroptera, 25 Chironomidae, 1 Tipulidae, 1 Tabanidae, 1 Trichoptera, 3 Gammaridae ve 1 Astacidae türü tespit edilmiştir. Daha önce makrozoobetik komunite yapısında da bahsedildiği üzere her üç gölün de makrozoobentik faunasının büyük kısmını Oligochaeta ve Chironomidae türleri oluşturmaktadır (bu çalışmada araştırma alanında tespit edilen Oligochaeta grubuna ait türler ve dağılımları Arslan ve Mercan tarafından (2020) 14rd Uluslarası Sucul Oligochaeta Sempozyumu'nda sunulup yayınlandığı için bu listeye dâhil edilmemiştir. Çalışmada 9 Naidid, 11 Tubificin, 1 Lumbriculidae ve 1 Enchytraeid türü rapor edilmiştir.

Çıldır Gölü'nde 1. istasyonun dominant türü % 6,25'lik baskınlık oranı ile *Polypedilum convictum* ve *Cryptotendipes holsatus* olup bunu *Ablabesmyia monilis (*% 5.77), *Gammarus* sp. (% 4.33), *Procladius (Psilotanypus)* sp. (% 4.33) ve *Gyraulus (Armiger) crista* (%4.33) takip etmektedir. 2.istasyonda % 4.55 baskınlık oranı ile *Dicrotendipes tritomus* ve Cricotopus *(Isocladius) tricinctus* dominant iken bunu % 4.04 *Gammarus* sp., % 3.54 *Orthocladius (O.) thienemanni* ve *Dicrotendipes nervosus* takip etmektedir. 3. İstasyonun dominant türü ise % 9.17 baskınlık oranı ile *Paratanytarsus lauterborni* olup bunu *Virgotanytarsus arduensis* (% 7.86), *Gyraulus (Armiger) crista* (% 6.11), *Polypedilum scalaenum* (% 4.80) ve *Cryptochironomus defectus* (% 4.37) takip etmektedir. Çıldır Gölü'nde tespit edilen türlerin baskınlık oranları istasyonlara göre değişmekle beraber göl genelinde ortalama dominansi oranlarına göre baskın türler *Gyraulus (Armiger) crista* (% 4,49)*, Paratanytarsus lauterborni* (% 3.90) ve *Virgotanytarsus arduensis*'dir (% 3.80).

Aktaş Gölü zoobentozunda gammarid populasyonun çok baskın olduğu görülmektedir. Göl genelinde ortalama dominansi değerlerine göre *Gammarus pseudosyriacus* % 15.24 baskınlık oranı ile dominant tür iken bunu % 7.53 oran ile *Cricotopus (Isocladius) reversus* ve % 7.29 oran ile *Gyraulus albus* takip etmektedir.

Aygır Gölü'nde de gammarid populasyonu yüksektir; ancak Aktaş Gölü'nden farklı olarak bu gruptan *Gammarus balcanicus* % 6.28 oran ile *Gyraulus albus* (% 8.00), *Virgotanytar-*

sus arduensis (% 6.92) ve *Cricotopus (Isocladius) tricinctus*'tan (% 6.64) sonra dördüncü baskın tür olarak tespit edilmiştir (Tablo 2).

Tablo 2'de de görüldüğü üzere en yüksek ortalama takson sayısı (26 takson) ve çeşitlilik indeksi (Shannon İndeks ve Margalef İndeks değerleri sırasıyla 2.81ve 5.67) Çıldır Gölü'nde tespit edilmiştir. Her üç gölün tür düzeyindeki makrozoobentik komünite yapısına göre yapılan Bray-Curtis benzerlik analizinde Aktaş Gölü 1. istasyonu ile Çıldır Gölü fauna yapısı benzer bulunmuştur (Şekil 3). İki gölün populasyon yoğunluğu yüksek ortak türleri *Gyraulus albus, Radix labiata, Baetis rhodani, Ephemerella ignita* ve *Cricotopus (Isocladius) reversus*'dur (Tablo 2).

Tablo 2. Çıldır, Aktaş, Aygır Göllerinde tespit edilen makrozoobentik taksonların göller ve istasyonlara göre % baskınlık değerleri (Ç:Çıldır Gölü, AK: Aktaş Gölü, AY: Aygır Gölü; OD: Ortalama dominansi, *: Oligochaeta tür listesi Arslan ve Mercan (2020) tarafından verilmiştir)

Table 2. % dominance values of macrozoobenthic taxa detected in Çıldır, Aktaş, Aygır Lakes according to lakes and stations (Ç:Çıldır Lake, AK: Aktaş Lake, AY: Aygır Lake; OD: Average dominance, *: Oligochaeta species list given by Arslan and Mercan (2020))

	Çıldır Gölü				Aktaş Gölü			Aygır Gölü		
	Ç1	**Ç2**	**Ç3**	**OD**	**AK1**	**AK2**	**OD**	**AY1**	**AY2**	**OD**
Gastropoda										
Gyraulus (Armiger) crista (Linnaeus, 1758)	4.33	3.03	6.11	4.49	-	-	-	-	-	-
Gyraulus albus (O.F.Müller, 1774)	0.96	1.01	2.62	1.53	5.38	9.20	7.29	-	16.00	8.00
Radix labiata (Rossmässler, 1835)	1.92	0.51	0.87	1.10	2.15	3.45	2.80	-	-	-
Bivalvia										
Pisidium subtruncatum Malm, 1855	1.44	1.01	1.31	1.25	-	-	-	4.92	-	2.46
Anodonta cygnea (Linnaeus, 1758)	0.48	2.02	1.31	1.27	-	-	-	-	-	-
Oligochaeta*	34.62	32.83	38.43	35.29	16.13	39.08	27.60	29.51	18.00	23.75
Hirudinae	0.96	-	0.44	0.47	2.15	-	1.08	1.64	-	0.82
Baetis fuscatus (Linnaeus, 1761)	0.48	1.01	-	0.50	-	1.15	0.57	-	-	-
Baetis rhodani (Pictet, 1843)	0.48	1.01	-	0.50	2.15	2.30	2.22	3.28	-	1.64
Ecdyonurus sp.	-	-	-	-	1.08	1.15	1.11	-	-	-
Ephemera vulgata Linnaeus, 1758	-	-	-	-	2.15	1.15	1.65	4.92	4.00	4.46
Potamanthus luteus (Linnaeus, 1767)	-	-	-	-	2.15	-	1.08	-	-	-
Ephemerella ignita Poda, 1761	0.48	-	-	0.16	2.15	-	1.08	1.64	-	0.82
Odonata	2.88	4.55	1.31	2.91	4.30	12.64	8.47	8.20	24.00	16.10
Coleoptera	0.96	2.53	3.49	2.33	3.23	2.30	2.76	4.92	4.00	4.46
Chironomidae										
Ablabesmyia monilis (Linnaeus, 1758)	5.77	-	-	1.92	-	-	-	-	-	-
Procladius (Psilotanypus) sp.	4.33	2.53	-	2.28	-	-	-	-	-	-
Cricotopus (C.) triannulatus (Macquart, 1826)	2.88	-	-	0.96	-	-	-	-	-	-
Cricotopus (C.) fuscus (Kieffer, 1909)	3.85	2.02	-	1.96	-	-	-	6.56	-	3.28
Cricotopus (Isoc) reversus Hirvenoja, 1973	2.88	2.53	-	1.80	15.05	-	7.53	-	-	-
Cricotopus (Isoc) tricinctus (Meigen, 1818)	-	4.55	1.75	2.10	-	-	-	3.28	1-	6.64
Cricotopus (Isoc) suspiciosus Hirvenoja, 1973	-	-	-	-	-	11.49	5.75	6.56	-	3.28
Cricotopus (Isoc) ornatus (Meigen, 1818)	-	-	-	-	11.83	-	5.91	-	-	-
Rheocricotopus sp.	-	-	-	-	-	-	-	6.56	-	3.28

Orthocladius (O.) thienemanni Kieffer & Thienemann, 1906	3.37	3.54	-	2.30	-	-	-	-	-	-
Dicrotendipes nervosus (Staeger, 1839)	-	3.54	0.87	1.47	-	-	-	-	-	-
Dicrotendipes tritomus (Kieffer, 1916)	-	4.55	-	1.52	-	-	-	-	-	-
Cryptochironomus defectus (Kieffer, 1913)	-	2.53	4.37	2.30	-	-	-	-	-	-
Polypedilum scalaenum (Schrank, 1803)	-	3.03	4.80	2.61	-	-	-	-	-	-
Polypedilum convictum (Walker, 1856)	6.25	-	-	2.08	-	-	-	-	-	-
Chironomus viridicollis Wulp, 1859	2.40	-	-	0.80	-	-	-	-	-	-
Chironomus (Campt) tentans Fabricius, 1805	-	-	1.75	0.58	-	-	-	-	-	-
Chironomus anthracinus Zetterstedt, 1860	1.92	-	-	0.64	-	-	-	-	-	-
Cryptochironomus defectus (Kieffer, 1913)	-	-	-	-	3.23	-	1.61	-	-	-
Cryptotendipes holsatus Lenz, 1959	6.25	-	-	2.08	-	-	-	-	-	-
Paratendipes albimanus (Meigen, 1804)	-	2.53	3.06	1.86	-	-	-	-	-	-
Microtendipes pedellus De Geer, 1776	-	3.03	1.75	1.59	-	-	-	1.64	6.00	3.82
Paratanytarsus lauterborni (Kieffer, 1909)	-	2.53	9.17	3.90	-	-	-	-	-	-
Cladotanytarsus mancus (Walker, 1856)	-	2.02	3.93	1.98	-	-	-	-	8.00	4.00
Virgotanytarsus arduensis (Goetghebuer, 1922)	-	3.54	7.86	3.80	-	10.34	5.17	9.84	4.00	6.92
Tipulidae										
Tipula sp.	1.44	2.02	0.87	1.45	-	-	-	-	-	-
Tabanidae										
Tabanus sp.	0.96	-	1.31	0.76	2.15	-	1.08	-	-	-
Trichoptera										
Hydropsyche contubernalis McLachlan, 1865	1.92	1.01	0.44	1.12	-	-	-	-	-	-
Gammaridae										
Gammarus pseudosyriacus Karaman & Pinkster, 1977	-	-	-	-	24.73	5.75	15.24	-	-	-
Gammarus balcanicus Schäferna, 1923	1.44	-	-	0.48	-	-	-	6.56	6.00	6.28
Gammarus sp.	4.33	4.04	1.75	3.37	-	-	-	-	-	-
Astacidae										
Astacus leptodactylus schscholtz, 1823	-	1.01	0.44	0.48	-	-	-	-	-	-

					İndeksler					
İstasyonlar	**Ç1**	**Ç2**	**Ç3**	**O**	**AK1**	**AK2**	**O**	**AY1**	**AY2**	**O**
Takson sayısı	27	28	24	26	16	12	14	15	10	13
Birey sayısı	83	92	87	87	96	96	96	91	100	96
Çeşitlilik (Shannon İn. H’)	2.93	2.92	2.59	2.81	2.51	2.10	2.31	2.37	1.98	2.18
Çeşitlilik (Simpson_1-D)	0.79	0.85	0.77	0.80	0.85	0.78	0.82	0.84	0.86	0.85
Çeşitlilik (Margalef İndeks)	5.88	5.97	5.15	5.67	3.29	2.41	2.85	3.10	1.95	2.53
Çeşitlilik (Evenness)	0.69	0.66	0.56	0.64	0.67	0.60	0.64	0.82	0.82	0.82

Çıldır, Aktaş ve Aygır gölleri bentozunda bu çalışmada tespit edilen 43 taksondan *Anodonta cygnea* ve *Astacus leptodactylus* hariç bu çalışmada tanımlanan türlerin tamamı araştırma yapılan göller için yeni kayıt niteliğindedir.

Her üç gölde de Diptera'dan Chironomidae bireylerinin zoobentozda baskın olduğu görülmektedir. Çıldır Gölü'nde ortalama değerlere göre *Paratanytarsus lauterborni* (% 3.90) ve *Virgotanytarsus arduensis* (% 3.80) en baskın iki chironomid türüdür. Buna ilaveten Çıldır gölünün gölün güney kısmında (1. istasyon) *Cryptotendipes holsatus* (% 6.25) ve *Polypedilum convictum*'un (% 6.25) populasyon oranları çok yüksek olup söz konusu iki tür gölün kuzeybatı (2. istasyon) ve kuzeydoğu (3. istasyon) kesimlerinde tespit edilmediği görülmektedir. *Paratanytarsus lauterborni* ve *Polypedilum convictum* çamurlu-kumlu zeminlerin kazıcı türleri olup ekolojik tolerans değerleri yüksek türlerdir (Grzybkowska ve Witczak, 1990), *Virgotanytarsus arduensis* ve *Cryptotendipes holsatus*'un ise akarsu ve göllerde yumuşak substratları ve sucul bitkilerin olduğu habitatları tercih ettiği bilinmektedir (Epler, 1995). Söz konusu dört chironomid türünün de ekolojik tolerans değerleri yüksek olup temiz sulardan ekolojik kalitesi orta ve düşük sucul sistemler gibi farklı habitatlarda yaşayabilmektedirler. *P. convictum* larvalarının akarsuların üst ve orta kesimlerinde göllere göre daha yaygın oldukları bilinmekle beraber türün kanalizasyon veya zehirli atıklara karşı toleranslı olabileceği de bildirilmiştir (Simpson ve Bode, 1980). *P. convictum* ülkemizde yaygın bir tür olup Özkan (2006) tarafından Trakya Bölgesindeki göl, gölet ve barajların kıyı kesimlerinde, kumlu, çamurlu, kumlu ve detrituslu, habitatlarda, Arslan vd. (2013) tarafından Gölbaşı Gölü'nde (Hatay) çamur substratında yüksek oranda tespit edilmiştir. Çıldır Gölü'nde *Cryptotendipes holsatus* (% 6,25) ve *Polypedilum convictum*'un tespit edildiği tek istasyon olan gölün güney kesimi diğer istasyonlara göre sucul bitkiler bakımından zengin olması türlerin yüksek populasyon oranlarını açıklamaktadır. Aynı zamanda substrat yapısı diğer iki istasyondan farklı olarak çamur, çamur-kum yapısına sahip olması türlerin substrata bağlı olarak da yayılışını göstermektedir. Göl genelinde gastropodlardan *Gyraulus (Armiger) crista* her üç istasyonda da tespit edilmiş olup (ortalama %4,49) dominant türlerden biridir (Tablo 2). Bir Pulmonat türü olan *Gyraulus (Armiger) crista*'nın durgun sularda yaygın olmasına rağmen yavaş akıntılı akarsuların göllenen kesimlerinde de bulanabileceği, düşük pH değerlerine toleranslı bir tür olduğu, m^2 deki populasyon yoğunluklarının 1000'i aşabileceği ve farklı habitat koşullarında konkolojik varyasyonlar gösterebileceği belirtilmiştir (Spyra ve Strzelec, 2013).

Çıldır Gölü, Çelekli vd. (2020) tarafından Carlson's trofik indekse göre oligo-mesotrofik, fitoplanktonik indekslere göre de ekolojik kalite oranının yüksek olarak tespit edilmiştir. Kükrer vd. (2014) tarafından Çıldır Gölü ekolojik durumu bazı metal düzeyleri araştırılarak incelenmiştir. Çalışmada Çıldır Gölü sediment örneklerinde Pb, Cr, Cu, Ni ve Zn düzeyleri doğal düzeylerde bulurken As, Cd, Mn ve Hg düzeylerinin göl geleceği için orta derecede risk oluşturabileceği vurgulanmıştır. Çıldır Gölü zoobentik fauna yapısı yukarıda da açıklandığı gibi genellikle toleranslı türleri yüksek oranda içermektedir. Göl daha önce yapılan su kalitesi değerlendirmesi çalışmalarında her ne kadar oligo-mesotrofik ve ekolojik kalite oranı yüksek olarak değerlendirilse de (Çelekli vd., 2020) omurgasız faunası yapısında EPT (Ephemeroptera-Plecoptera-Trichoptera) olarak değerlendirilen, genellikle oligotrofik ortamları tercih eden indikatör taksonların (Ephemeroptera dışında) bulunmayışı dikkat çekicidir. Gölde EPT grubundan tespit edilen 3 Ephemeroptera türünden ikisi de (*B. rhodani* ve *B. fuscatus)* göllerin kıyı kesimlerinden yapay su kanallarına kadar farklı habitatlarda bulunabilen geniş toleranslı alfa-mezosaprob türlerdir (Bauernfeind vd., 2012). Makrozoobentik canlıların sucul sistemlerdeki varlığı ve(ya) yokluğu o sistem ile ilgili biyolojik anlamda ölçüm anına dayanan fiziko-kimyasal verilerden daha önemli ipuçları vermektedir. Çünkü fauna yapısı içinde hassas türlerin ve ekolojik toleransı geniş türlerin yoğunluğu ve oranı, Su Çerçeve Direktifi uyarınca, su kalitesinin bir göstergesi olarak kullanılan beş biyolojik parametreden biri olarak görülmektedir. Yüksek populasyon yoğunluğuna sahip türlerin varlığı ve sucul sistemlerdeki baskınlığı genellikle orta ve düşük kalite sınıfı olarak tanımlanmaktadır (Langdon vd., 2006). Çıldır Gölü makroomurgasız faunası da yüksek populasyon oranı ile içerdiği toleranslı türlerden dolayı, gölün oligotrof düzeyden üst düzey mesotrofiye geçtiğini işaret etmektedir.

Aktaş Gölü, yüksek pH değerinden dolayı Doğu Anadolu Bölgesi'nin Van Gölü'nden sonra ikinci büyük sodalı gölüdür. Aktaş Gölü zoobentozunda gammarid populasyonunun çok baskın olduğu görülmektedir. Göl genelinde ortalama dominansi değerlerine göre *Gammarus pseudosyriacus* % 15.24 baskınlık oranı ile dominant tür iken bunu % 7.53 oran ile *Cricotopus (Isocladius) reversus* ve % 7.29 oran ile *Gyraulus albus* takip etmektedir. Göldeki makroomurgasız takson sayısı (ortalama 14) ve çeşitliliği (Shannon indeks 0.82, Margalef indeks 2.85) çok yüksek olmamakla beraber sodalı bir göl için oldukça iyidir. Çünkü yüksek pH değerleri pek çok sucul omurgasız için sınırlayıcı bir faktördür (Petrin vd., 2007). Gölün dominant türü olan *Gammarus pseudosyriacus* geniş sıcaklık aralığında (5-21°C) yaşayabilen tolerans değeri yüksek bir türdür (Zamanpoore vd., 2011).

Çelekli vd. (2020) tarafından yapılan çalışmada Aktaş Gölü'nde, Kura-Aras havzasında yer alan göller içinde en yüksek su sıcaklığı (ortalama 20.3 °C) ve toplam fosfor değeri ile yüksek elektriksel iletkenlik değeri (1043 µS/cm) tespit edilmiş, ayrıca havzadaki en düşük su şeffaflığı (0.30 m) yine Aktaş Gölü'nde kaydedildiği bildirilmiştir. Bu durum, yüksek pH değeri ile sodalı bir göl olan suları sıcak Aktaş Gölü'nün zoobentik komünite yapısı içinde diğer makroomurgasızlarla rekabete giren *Gammarus pseudosyriacus*'un yüksek populasyon değerini açıklayabilir. Söz konusu tür ülkemizde de daha önce İzmir, Muş, Artvin, Bitlis ve Van illerindeki akarsu ve göllerden de kaydedilmiştir (Karaman ve Pinkster, 1977). Aktaş Gölü'ndeki ikinci dominant tür olan *Gyraulus albus*, tolerans değeri yüksek olan pulmonat bir gastropodtur. Genellikle göl ve göletlerde yayılış gösteren türün bitkilerce zengin litoral kesimden derin bölgelere kadar yayılış gösterdiği ve değişen ortam koşullarına adaptasyonunu yüksek olduğu bilinmektedir (Anderson, 2016). Göldeki dominansi değerleri yüksek olan diğer taksonlar, Chironomidae grubundan aynı cinsin üç farklı türüdür; *Cricotopus (Isoc.) reversus* (% 7.53), *Cricotopus (Isoc.) tricinctus* (% 5.75) ve *Cricotopus (Isoc.) ornatus* (% 5.91). Her üç *Cricotopus* türü larvaları substrat içindeki debriste iki ucu açık olarak açtıkları tüneller içinde yaşar ve larvalar tünellerde oluşturdukları su akımıyla hem suyun oksijenlenmesini sağlar hem de su içindeki partiküllerle beslenirler (Bland, 1994). Tünel açarak içinde yaşam, pek çok makroomurgasız canlı için değişen ortam koşullarından korunmanın bir yolu olarak değerlendirilebilir.

Aktaş Gölü, Türkiye-Gürcistan sınırında yer alan uluslararası öneme sahip, sınır aşan bir göldür. Gölde daha önce yapılan çalışmalarda 107 kuş türü belirlenmiş, bu kuşlardan ak pelikanların %80'nin Aktaş Gölü'nde ürediği ve kuşların büyük bir bölümünün etçil olduğu vurgulanmıştır (Kırpık vd., 2019). Makroomurgasız canlılar su kuşlarının tercih ettiği önemli besin unsurlarındandır. Aktaş Gölü'nden Kura Nehri'ne akış sağlayan DSI tarafından açıldığı belirtilen bir kanalın olması (Kırpık vd., 2019) göl su seviyesinin düşmesine neden olacağı açıktır. Ayrıca göl kenarında yer alan köylerin hayvansal atık ve hayvan sulama sularının doğrudan göle karışması gölde yüksek oranda belirlenen besleyici maddelerin (Çelekli vd., 2020) artmasına ve ötrofikasyona neden olabilmektedir. Bu durum makroomurgasız fauna yapısı için de bir tehdit unsurudur. Aktaş Gölü Kafkaslar ile Doğu Karadeniz arasında göç eden kuşların dinlenmesi, beslenmesi ve üremesi açısından önemli bir konaklama alanı olduğu için, göldeki mevcut fauna yapısının su kalitesinin kötüleşmesine bağlı olarak oluşacak tehditlere yönelik önlemlerin alınması hem ülkemiz biyolojik çeşitliliği hem de biyolojik miraslarımız açısından önemlidir.

Benzer bir durum Aygır Gölü'nde de gözlenmektedir; ancak dominant türlerin sırası değişmektedir. Aygır Gölü'nde de gammarid populasyonu yüksektir ancak Aktaş Gölü'nden farklı olarak bu gruptan *Gammarus balcanicus* % 6.28'lik baskınlık oranı ile *Gyraulus albus* (% 8), *Virgotanytarsus arduensis* (% 6.92) ve *Cricotopus (Isoc) tricinctus*'tan (% 6.64) sonra dördüncü sırada yer almaktadır (Tablo 2). Aygır Gölü'nde de tespit edilen hem baskın taksonlar hem de diğer zoobetoz üyelerinin büyük bir kısmı yukarıda da açıklandığı gibi toleranslı türlerdir. Ortalama takson sayısı araştırılan üç göl içinde en düşük (13) Aygır Gölü'nde olup, Margalef ve Shannon çeşitlilik indeks değerleri de, en düşük değer (sırayla 2.18 ve 2.53) olarak kaydedilmiştir. Gölün dominant türü olan *Gammarus balcanicus* ekolojik açıdan oldukça toleranslı bir tür olup, Balkanlardan orta doğuya kadar geniş bir dağılım alanına sahip olduğu ve karstik kaynaklardan dere, nehir ve göllere kadar yeterli oksijen ve az tuzluluk içeren tüm yüzey sularında yüksek oranlarda bulunabileceği bildirilmiştir (Karaman ve Pinkster, 1987). Göldeki populasyon yoğunluğunun yüksek olması bu bilgileri desteklemektedir.

Çalışma alanımız olan Çıldır, Aktaş ve Aygır göllerinin de içinde yer aldığı yüksek rakımlı göller özellikle insan baskısına uzun süreli olarak daha az maruz kaldıklarından dolayı, bu tatlı su habitatlarında yaşayan canlıların nispeten düşük sayıda, endemik ya da ekstrem çevre koşullarına uyum sağlamış türler tarafından temsil edildiği ve bu ortamlarda yaşayan canlıların çevresel değişiklikleri incelemede kusursuz modeller olduğu bilinmektedir (Manca vd., 1998; Taşdemir ve Ustaoğlu, 2016). Ülkemizdeki yüksek rakımlı göllerde yapılan bazı bentik çalışmalar incelendiğinde (Taşdemir vd., 2011; Yıldız vd., 2012; Taşdemir ve Ustaoğlu, 2016; Atıcı, 2018; Öztürk vd., 2022) yüksek rakımlı göllerde özellikle Chironomidae bolluğunun düşük verimlilik dolayısıyla çok yüksek değerlere ulaşmadığı, ancak ekstrem koşullara uyum sağlayan türlerin varlığı rapor edilmiştir. Çalışma kapsamında, özellikle Çıldır Gölü'nde tespit edilen bentik makroomurgasız gruplarının kirliliğe toleransı yüksek türlere doğru kaydığı gözlenmektedir. Gölün trofi seviyesinin oligotrofiden üst düzey mesotrofiye geçtiği tespit edilmiştir. Aynı durum yine Aygır Gölü'nde de gözlenmektedir. Bu yüksek rakımlı göllerde artık insan etkisinin arttığı antropojenik faaliyetlerin gölün trofi seviyesini kötü yönde etkilediği açık bir şekilde görülmektedir.

Sonuç

Sonuç olarak, yapılan bu çalışma ile Aras Nehir havzasında yer alan Çıldır, Aktaş ve Aygır göllerinin makroomurgasız faunası belirlenmiş ve tespit edilen 43 taksondan 41'i göller için yeni kayıt niteliği taşıdığı tespit edilmiştir. Her üç gölde ülkemiz Doğu Anadolu Bölgesi'nde yer alan önemli sulak alanlarımızdandır. Sucul sistemler biyotik ve abiyotik öğeleri ile bir bütündür. Bu yüzden barındırdığı canlıların devamlılığı substrat yapısı ve su kalitesi ile yakından ilişkilidir. Her üç göl genelinde tespit edilen taksonlar α ve β mesosaprob türler olup, populasyon yoğunluklarının fazla olması özellikle Çıldır Gölü için oligotrofik düzeyden mesotrofik düzeye geçişin bir işareti olarak değerlendirilebilir. Gerek Çıldır gerekse Aktaş Gölü önemli kuş ve önemli bitki alanı statüsündedir. Bu yüzden sadece bu göllerde değil, ülkemiz genelinde sahip olduğumuz biyolojik zenginliklerin korunabilmesi ve devamlılığının sağlanabilmesi için hem su kalitesi bakımından hem de tür çeşitliliği bakımından izlenmesi, antropojenik etmenlere bağlı baskı unsurları ile oluşan tehditlerin fauna yapısını etkilemesini önleyecek önlemlerin alınması gerekmektedir.

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Short Communication

The recent capture of *Bregmaceros nectabanus* (Bregmacerotidae) from purse-seine fishery in Izmir Bay, NE Aegean Sea

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ABSTRACT

On 25 March 2022, two specimens of *Bregmaceros nectabanus* were captured by a commercial purse-seiner for targeting sardine and anchovy off Kırdeniz, Izmir Bay at a depth of 30 m. The total length of these specimens was between 53 and 63 mm. Thus, the record number has reached at least 37 specimens in the eastern Mediterranean Sea. This paper presents the third confirmed record of *B. nectabanus* from Izmir Bay (Northeastern Aegean Sea). Though the sporadic occurrences of this species in Izmir Bay do not indicate an established population for the time being, it signs that it's getting abundant.

Keywords: Smallscale codlet, Measurement, Occurrence, Eastern Mediterranean

Introduction

Family Bregmacerotidae, commonly known as codlets, comprises 15 valid species (Fricke et al., 2020). The smallscale codlet, *Bregmaceros nectabanus* Whitley, 1941 (misidentified as *B. atlanticus*) is a monotypic species in the Mediterranean Sea and it is one of the three Lessepsian species belonging to the genus *Bregmaceros* (*B. nectabanus* Whitley, 1941, *B. arabicus* D'Ancona and Cavinato, 1965 and *B. mcclellandi* Thompson, 1840) that occurring possibly in the Red Sea (Harold & Golani, 2016). It is native to the Indo-West Pacific and western Indian Ocean, including the Red Sea (Harold & Golani, 2016).

Only eleven specimens of *B. nectabanus* (most of them reported as *B. atlanticus*) have been documented: Kuşadası Bay (n=1, Filiz *et al*., 2007), Izmir Bay (n=1, Aydın & Akyol, 2013; n=1, Özgül & Akyol, 2017) in the eastern Aegean Sea; and Gulf of Seranikos (n=8, Dogrammatzi & Karachle, 2015) in the western Aegean Sea. Özgül & Akyol (2017) reported 23 specimens, including eleven (above mentioned) throughout the eastern Mediterranean Sea until 2014. Additional records give signs of whether an alien species is established in a region. The paper provides additional records of rare *B. nectabanus* in both the Aegean Sea and the eastern Mediterranean. It is also the first finding of the purse seine catch composition.

Material and Methods

On 25 March 2022, two specimens of *B. nectabanus* (Figure 1) were captured by a commercial purse-seiner for targeting sardine and anchovy off Kırdeniz, Izmir Bay (lat. 38°35'20''N – long. 26°46'21''E) at a depth of 30 m. The specimen was fixed with 5% formaldehyde solution and deposited in the fish collection of the Fisheries Faculty, Ege University (ESFM-PIS/2022-003).

Results and Discussion

Both specimens were measured to the nearest millimeter (Table 1); a brief description of the specimen: body fusiform, elongate, the color is silver-grey on the belly, with dense pigmentation entirely along the dorsum, and a distinctive thin brown dorso-lateral longitudinal stripe below the second dorsal fin. All determined measurements and color patterns are in accordance with the descriptions of Harold & Golani (2016) and Özgül & Akyol (2017).

Although *B. nectabanus* forward to the western part of the Mediterranean Sea since it reached the Adriatic Sea in 2019 (see, Palermino et al., 2022), especially its population tends to rise in the eastern Mediterranean, and successive records of *B. nectabanus* are shown in Table 2. Thus, the record number has reached at least 37 specimens in the eastern Mediterranean Sea. Interestingly, some of *B. nectabanus* have been found in the stomach content of *Saurida lessepsianus* (Yılmaz et al., 2004) and *Trachurus trachurus* (Filiz et al., 2007; Othman & Galiya, 2018).

A recent study proved that *B. nectabanus* is a Lessepsian migrant and this fish was previously misidentified as *B. atlanticus* (Harold & Golani, 2016). However, Özgül & Akyol (2017) expressed that *B. nectabanus* was also likely introduced in the eastern Mediterranean via ship ballast water in terms of occurring near large ship harbours during the last decade, and secondarily, it has been proved as a Lessepsian migrant. Also, Dulcic et al. (2020) noted that a shipping-related introduction of *B. nectabanus* to the Adriatic Sea, where the area of Bari features a commercial harbour was possible. Vrdoljak et al. (2021) also supported both hypotheses (i.e. Lessepsian migration and ballast water) since two specimens have been found near cargo port Ploče, Crotia.

Figure 1. Two specimens of *Bregmaceros nectabanus,* captured by a purse-seiner in Izmir Bay, NE Aegean Sea (ref. ESFM-PIS/2022-003) (Photo: O. Akyol)

Table 1. Morphometric measurements in mm and as percentage of total length (%TL) and head length (%HL) and counts recorded in *Bregmaceros nectabanus* (ref. ESFM-PIS/2022-003), captured from Izmir Bay, NE Aegean Sea

Specimens	**1**		**2**	
Measurements	Size (mm)	Proportion %	Size (mm)	Proportion %
Total length (TL)	53		63	
Standard length (SL)	48	90.6 TL	56	88.9 TL
Predorsal fin length	20	37.7 TL	22	34.9 TL
Prepectoral fin length	10	18.9 TL	11	17.5 TL
Pre-anal fin length	20	37.7 TL	22	34.9 TL
Head length (HL)	10	18.9 TL	11	17.5 TL
Eye diameter	3.7	37.0 HL	3.9	35.5 HL
Preorbital length	2	20.0 HL	2.4	21.8 HL
Counts				
Dorsal fin rays	48		48	
Anal fin rays	49		49	
Pectoral fin rays	16		16	
Weight (g)	1.06		1.69	

Table 2. Successive records of *Bregmaceros nectabanus* in the Eastern Mediterranean Sea

Location	Coordinates Lat. N - Lon. E	Depth (m)	Record Date	Number collected	Size (mm)	References
Gulf of Antalya, Türkiye	?	30	12 Oct. 2002	2	30-34 TL	Yılmaz et al. (2004)
Off Palmahim, Israel	31°05’- 34°03’	35	20 Sep. 2004	1	46.5 SL	Goren & Galil (2008)
Kuşadası Bay, Türkiye	?	150	04 Feb. 2005	1	39 TL	Filiz et al. (2007)
Off Palmahim, Israel	31°05’- 34°03’	35	26 May 2006	3	47-62 SL	Goren & Galil (2008)
Off Iskenderun, Türkiye	35°57’- 35°59’	120	15 Dec. 2010	5	70.7-102 TL	Turan et al. (2011)
Izmir Bay, Türkiye	38°28’- 26°47’	50	01 Dec. 2011	1	66 TL	Aydın & Akyol (2013)
Gulf of Seronikos, Greece	37°50’- 23°29’	90	31 July 2014	1	53 TL	Dogrammatzi & Karachle (2015)
Gulf of Seronikos, Greece	37°50’- 23°20’	98	02 Aug. 2014	7	54-64 TL	Dogrammatzi & Karachle (2015)
Rashid, Egyptian Med.	?	29	? Sept. 2014	1	76 TL	Rizkalla & Akel (2015)
Izmir Bay, Türkiye	38°23’- 26°46’	20	09 Sep. 2014	1	95 TL	Özgül & Akyol (2017)
Syrian waters	35.6844°- 35.7837°	100	16 Aug.2018	12	19-53 TL	Othman & Galiya (2019)
Izmir Bay, Türkiye	38°35’- 26°46’	30	25 Mar. 2022	2	53-63 TL	This study

Conclusion

This paper presents the third confirmed record of *B. nectabanus* from the northeastern Aegean Sea. Though the sporadic occurrences of this species in Izmir Bay do not indicate an established population, for the time being, it signs that it's getting abundant. In addition, capturing them by purse-seine shows that *B. nectabanus* might be attracted under the strong light that uses for aggregating clupeid fishes owing to their phototaxy feature.

Compliance with Ethical Standard

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

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Notes on mangrove civil reservation area, Sagay Marine Reserve, the Philippines: A baseline mangrove diversity checklist

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ABSTRACT

Sagay Marine Reserve (SMR) being the largest marine reserve in the Philippines is blessed with great biodiversity. However, studies are very limited and there are no published literature in refereed journals for mangroves. This study photo-documented the true mangroves found at the Mangrove Civil Reservation Area (MCRA), a mangrove forest within SMR. A total of 20 species from 12 families were recorded from a series of biodiversity studies, site visits, and species inventory from 2018 to 2021. The list includes *Ceriops zippeliana*, that has long been mistaken as *C. decandra*. NONESCOST MCRA showed the highest mangrove diversity compared to other parts of Negros Occidental. Overall, 7 species were new records while 11 previously reported species were not found in this study. This is the first comprehensive species inventory of mangroves found at the reservation area, providing baseline mangrove diversity data with photos.

Keywords: Mangrove species, Negros Occidental, New record, Philippines, Species inventory

Introduction

Philippine mangrove diversity is one of the highest in the world with 36 (Primavera and Dianala, 2009) to 42 species (ADB, 2014) found in the country. However, despite many researches in the Philippines, there are limited published studies on mangrove species found in Negros Occidental Province. Fourteen (14) species were reported in Danjugan Island, Southwestern Negros (King et al., 2002), while 10 were found in Binalbagan, Central Negros (Malo and Cari-an, 2012). Apparently, there were no published surveys conducted in Northern Negros, particularly in Sagay Marine Reserve (SMR); even it bears the title of being one of the largest marine reserves in the Philippines (Bocario et al., 2019).

It is within SMR where the Northern Negros State College of Science and Technology (NONESCOST) Mangrove Civil Reservation Area (MCRA) is located. NONECOST MCRA is a 19.84 ha mangrove forest managed by the Northern Negros State College of Science and Technology (former Iloilo State College of Fisheries–Sagay Branch). The stewardship of the area was awarded to the College through a Presidential Proclamation signed by the late President Corazon C. Aquino in March 9, 1987 (Proclamation No. 83 1987). Since then, mangrove conservation and rehabilitation initiatives were implemented.

The reservation has become a research and practicum ground for the faculty and students in Negros Island but no studies has been published in refereed journals on its biodiversity. Thus, references on research undertakings in the area are only grey literatures. This includes the sole reference on mangroves found at NONESCOST MCRA by Bantigue (2010) with 22 recorded species. Although Bantigue (2010) reported a significant number of species, there were no means to verify the identification due to a lack of specimens and photo-documentations. Therefore, to verify and update the species checklist at NONESCOST MCRA, this recent study is presented with photo-documentations.

Material and Methods

This study was conducted at the NONESCOST MCRA (10°56'43" N, 123°25'30" E; 10°56'54" N 123°24'54" E) located in Old Sagay, Sagay City, Province of Negros Occidental. The reservation area is managed by the College of Fisheries and Allied Sciences of NONESCOST, and now part of its newly established Fisheries and Marine Science Research Station (FaMaRS). The species listing was based on a series of biodiversity studies and site visits conducted between 2018 and 2020. To verify those results, a species inventory was conducted in January 2021 where the entire mangrove reservation area was surveyed to cover the whole area and relatively document all mangrove species (Figure 1). The perimeter was further traced along the residential areas to see other mangrove species commonly found in the dikes e.g., *Acanthus* and *Acrostichum*. The data and photos presented were taken between 2018 and 2021. The species were primarily identified using published identification guides to Philippine mangroves by Primavera et al. (2004) and Primavera and Dianala (2009). Morphological observations on mangrove forms (tree, shrub, palm, etc.), root types (prop, buttress, plank, pneumatophores, cones, knee), bark structures, leaf characteristics, and inflorescence were done for each species. Characteristics were further examined during the fruiting seasons for species that require differentiation based on floral and fruit structures.

Results and Discussion

A total of 20 species were photo-documented at the Northern Negros State College of Science and Technology (NONESCOST) Mangrove Civil Reservation Area (MCRA) as shown in Table 1. Eleven previously reported species were not found in the area, while seven species were new records. Each species was photo documented and presented in the figures below (Figures 2A-E) alphabetically.

Sagay Marine Reserve is being one of the largest marine reserves in the country housing a significant number of species such as mangroves, seagrasses, corals, giant clams, etc. (SMR Office 2012 *cf* Bocario et al., 2019). However, these species are poorly documented in scientific literature; similar to with NONESCOST MCRA, a reservation area managed by the academe within SMR-making diversity data difficult to verify. In this study, a total of 20 mangrove species were photo-documented which is lower than previous records of Bantigue (2010). The new list included one species, *C. zippeliana* that has long been mistaken as *C. decandra* (Sheue et al., 2009). The current species observed consistently agreed with the description of Sheue et al., 2009, having shallow calyx tube, short, but erected calyx lobes, and pointed hypocotyl apex, contrary to the blunt apex of *C. decandra*. The occurrence of *C. zippeliana* in the Philippines have been reported especially in the Mindanao region (Sheue et al., 2010; Middeljans, 2014; Mangaoang and Flores, 2019). However, there is a death of reports in their distribution particularly in the Visayas region where this study was conducted. In total, seven species were new records (Table 1), while 11 previously reported were not found in this study suggesting that a total of 29 mangrove species may have resided the reservation area. This record is far higher than species found in Suyac Island, Sagay City (9 species) but are dominated by centuries-old assemblages of *S. alba*, *A. marina*, and *Rhizophora* spp. (Albarico et al., 2020). In contrast to other studies in Negros Province, King et al. (2002) reported 14 mangrove species in Danjugan Island, while Malo and Carian (2012) reported 10 in Binalbagan. Therefore, northern Negros's area as shown in this study showed the highest mangrove diversity throughout the island-supporting Sagay Marine Reserve's vast marine diversity.

Bantigue (2010) reported a significant number of species over a decade ago for NONESCOST MCRA. However, species reported could not be verified due to a lack of specimens and photo documentation. The latter did not also consider noting key characteristics that is important to verify the species though taxonomy was not the main objective. This can lead to the conclusion that some species either have been lost due to anthropogenic activities or may have been misidentified. Without extensive training/ background in taxonomy, biodiversity reports are easily mislead especially without proper peer review and scrutiny from scientific experts in the field. Ragavan et al. (2014) noted that some mangrove species are difficult to identify like *C. tagal* and *C. zippeliana* due to their almost identical morphology. Hence, whole-year observations are essential to accurately identify some mangroves with distinctions only found in floral and fruit morphology. It is important to note that baseline studies such as that of Bantigue (2010) are essential and should be properly documented with descriptions, vouchered specimens, or photos for appropriate biodiversity assessment and conservation. Thus, this study should be considered as the baseline mangrove diversity data for the Mangrove Reservation Area.

Figure 1. Mangrove Civil Reservation Area, Sagay Marine Reserve. Dashed line represents the rough survey track done during species inventory in January 2021

Table 1. Mangrove species of NONESCOST MCRA, Sagay Marine Reserve. (LC) Least Concern, (NT) Near Threatened, (VU) Vulnerable, (EN) Endangered, (*) not reported by Primavera and Dianala, (+) present, (-) absent

Family	Philippine Mangrove Species (Primavera and Dianala, 2009; Sheue et al., 2009*)	MCRA		IUCN Status	
		Bantigue 2010	This study	Redlist Category	Population Trend
Acanthaceae	*Acanthus ebracteatus*, Vahl 1791	+	-	LC	decreasing
	Acanthus ilicifolius, Linnaeus 1753	+	-	LC	unknown
	Acanthus volubilis, Wallich 1831	+	-	LC	decreasing
	Avicennia alba, Blume 1826	+	-	LC	decreasing
	**Avicennia lanata*, Ridley 1920	+	-	VU	unspecified
	Avicennia marina, (Forsskal) Vierhapper 1907	+	+	LC	decreasing
	Avicennia officinalis, Linnaeus 1753	-	+	LC	decreasing
	Avicennia rumphiana, Hallier fil. 1918	-	+	LC	decreasing
Malvaceae	*Camptostemon philippinensis*, (Vidal) Becc. 1889	-	-	ND	decreasing
Combretaceae	*Lumnitzera littorea*, (Jack) Voigt 1845	-	-	LC	decreasing
	Lumnitzera racemosa, Willdenow1803	+	+	LC	decreasing
Euphorbiaceae	*Excoecaria agallocha*, Linnaeus 1759	+	+	LC	decreasing
Lythraceae	*Pemphis acidula*, Forster & Forster 1775	+	+	LC	decreasing
Malvaceae	*Heritiera littoralis*, Aiton 1789	-	-	LC	decreasing
	Brownlowia tersa, (Linnaeus) Kostermans 1959	-	-	NT	decreasing
Meliaceae	*Xylocarpus granatum*, Köenig 1784	+	+	LC	decreasing
	Xylocarpus moluccensis, (Lamarck) Roemer 1846	+	+	LC	decreasing
Myrtaceae	*Osbornia octodonta*, F. Mueller 1862	-	+	LC	decreasing
Arecaceae	*Nypa fruticans*, Wurmb 1779	+	+	LC	unknown
Primulaceae	*Aegiceras corniculatum*, (Linnaeus) Blanco 1837	-	-	LC	decreasing
	Aegiceras floridum, Roemer & Schultes 1819	-	+	LC	decreasing
Pteridaceae	*Acrostichum aureum*, Linnaeus 1753	-	+	LC	stable
	Acrostichum speciosum, Willdenow 1810	-	+	LC	stable
Rhizophoraceae	*Bruguiera cylindrica*, (Linnaeus) Blume 1828	+	+	LC	decreasing
	Bruguiera gymnorrhiza, (Linnaeus) Lamarck 1798	+	-	LC	decreasing
	Bruguiera parviflora, (Roxburgh) Wight & Arnott ex Griffith 1836	+	-	LC	decreasing
	Bruguiera sexangula, (Lour.) Poiret 1816	+	-	LC	decreasing
	Ceriops decandra, (Griffith) Theob 1883	+	-	NT	decreasing
	**Ceriops zippeliana,* Blume 1849	-	+	LC	decreasing
	Ceriops tagal, (Persoon) Robinson 1908	+	-	LC	decreasing
	Kandelia candel, (Linnaeus) Druce 1913	-	-	LC	decreasing
	Rhizophora apiculata, Blume 1827	+	+	LC	decreasing
	Rhizophora mucronata, Poiret 1804	+	+	LC	decreasing
	Rhizophora stylosa, Griffith 1854	+	+	LC	decreasing
Rubiaceae	*Scyphiphora hydrophyllacea*, Gaertner 1971	+	+	LC	decreasing
Lythraceae	*Sonneratia alba*, Smith 1816	+	+	LC	decreasing
	Sonneratia caseolaris, (Linnaeus) Engler 1897	+	-	LC	decreasing
	Sonneratia ovata, Backer 1920	-	-	NT	decreasing
TOTAL	**36**	22	**20**		

Sagay Marine Reserve is one of the largest marine reserves in the country housing a significant number of species such as mangroves, seagrasses, corals, giant clams, etc. (SMR Office 2012 *cf* Bocario et al., 2019). However, these species are poorly documented in scientific literature; similar with NONESCOST MCRA, a reservation area managed by the academe within SMR-making diversity data difficult to verify. In this study, a total of 20 mangrove species were photo-documented which is lower than previous records of Bantigue (2010). The new list included one species, *C. zippeliana* that has long been mistaken as *C. decandra* (Sheue et al., 2009).

The current species observed consistently agreed with the description of Sheue et al., 2009, having shallow calyx tubes, short, but erected calyx lobes, and pointed hypocotyl apex, contrary to the blunt apex of *C. decandra*. The occurrence of *C. zippeliana* in the Philippines have been reported especially in the Mindanao region (Sheue et al., 2010; Middeljans, 2014; Mangaoang and Flores, 2019). However, there is a dearth of reports in their distribution, particularly in the Visayas region where this study was conducted. In total, seven species were new records (Table 1), while 11 previously reported were not found in this study suggesting that a total of 29 mangrove species may have resided in the reservation area. This record is far higher than species found in Suyac Island, Sagay City (9 species) but is dominated by centuries-old assemblages of *S. alba*, *A. marina*, and *Rhizophora* spp. (Albarico et al., 2020). In contrast to other studies in Negros Province, King et al. (2002) reported 14 mangrove species in Danjugan Island, while Malo and Carian (2012) reported 10 in Binalbagan. Therefore, northern Negros's area in this study showed the highest mangrove diversity throughout the island—supporting Sagay Marine Reserve's vast marine diversity.

Bantigue (2010) reported a significant number of species over a decade ago for NONESCOST MCRA. However, the species reported could not be verified due to a lack of specimens and photo-documentations. The latter did not also consider noting key characteristics that is important to verify the species though taxonomy was not the main objective. This concludes that some species either have been lost due to anthropogenic activities or may have been misidentified. Without extensive training/ background in taxonomy, biodiversity reports are easily mislead especially without proper peer review and scrutiny from scientific experts in the field. Ragavan et al. (2014) noted that some mangrove species are difficult to identify like *C. tagal* and *C. zippeliana* due to their almost identical morphology. Hence, whole-year observations are essential to accurately identify some mangroves with distinctions only found in floral and fruit morphology. It is important to note that baseline studies such as that of Bantigue (2010) are essential and should be properly documented with descriptions, vouchered specimens, or photos for appropriate biodiversity assessment and conservation. Thus, this study should be considered as the accurate baseline mangrove diversity data for the Mangrove Reservation Area.

Figure 2A. Mangroves of NONESCOST MCRA. A) *Acrostichum aureum*, B) *Acrostichum speciosum*, C) *Aegiceras floridum*, D) *Avicennia marina*

Figure 2B. Mangroves of NONESCOST MCRA. E) *Avicennia officinalis,* F) *Avicennia rhumphiana*, G) *Bruguiera cylindrica*, H) *Ceriops zippeliana*

Figure 2C. Mangroves of NONESCOST MCRA. I) *Excoecaria agallocha,* J) *Lumnitzera racemosa*, K) *Nypa fruticans*, L) *Osbornia octodonta*

Figure 2D. Mangroves of NONESCOST MCRA. M) *Pemphis acidula,* N) *Rhizophora apiculata*, O) *Rhizophora mucronata*, P) *Rhizophora stylosa*

Figure 2E. Mangroves of NONESCOST MCRA. Q) *Sciphiphora hydrophyllacea,* R) *Sonneratia alba*, S) *Xylocarpus granatum*, T) *Xylocarpus mollucensis*

However, the absence of *Acanthus* spp. in this study could be attributed by increasing settlements around the mangrove area. *Acanthus* spp. are commonly found in higher elevations such as dikes and in the swash zones. Consequently, increased human settlement around the mangrove forest may have cleared some mangrove shrubs. Having small patches of shrubs makes them difficult to monitor compared to mangrove trees. A small *Acanthus* assemblage (Figure 3) was spotted from a nearby fishpond area approximately 500 m away from the reservation area potentially supporting the results of Bantigue (2010). Newly recorded mangroves such *A. floridum* and *O. octodonta* could be new recruits at NONESCOST MCRA but with some having heights over 3.0 meters suggests older plants. Therefore, these two species while having significant number in the area may either had been misidentified or not included in the previous transect—highlighting the limitation of transect method in mangrove assessment. Unfortunately, the ageing of these two species using height increments was never explored making it difficult to speculate ages without proper tagging.

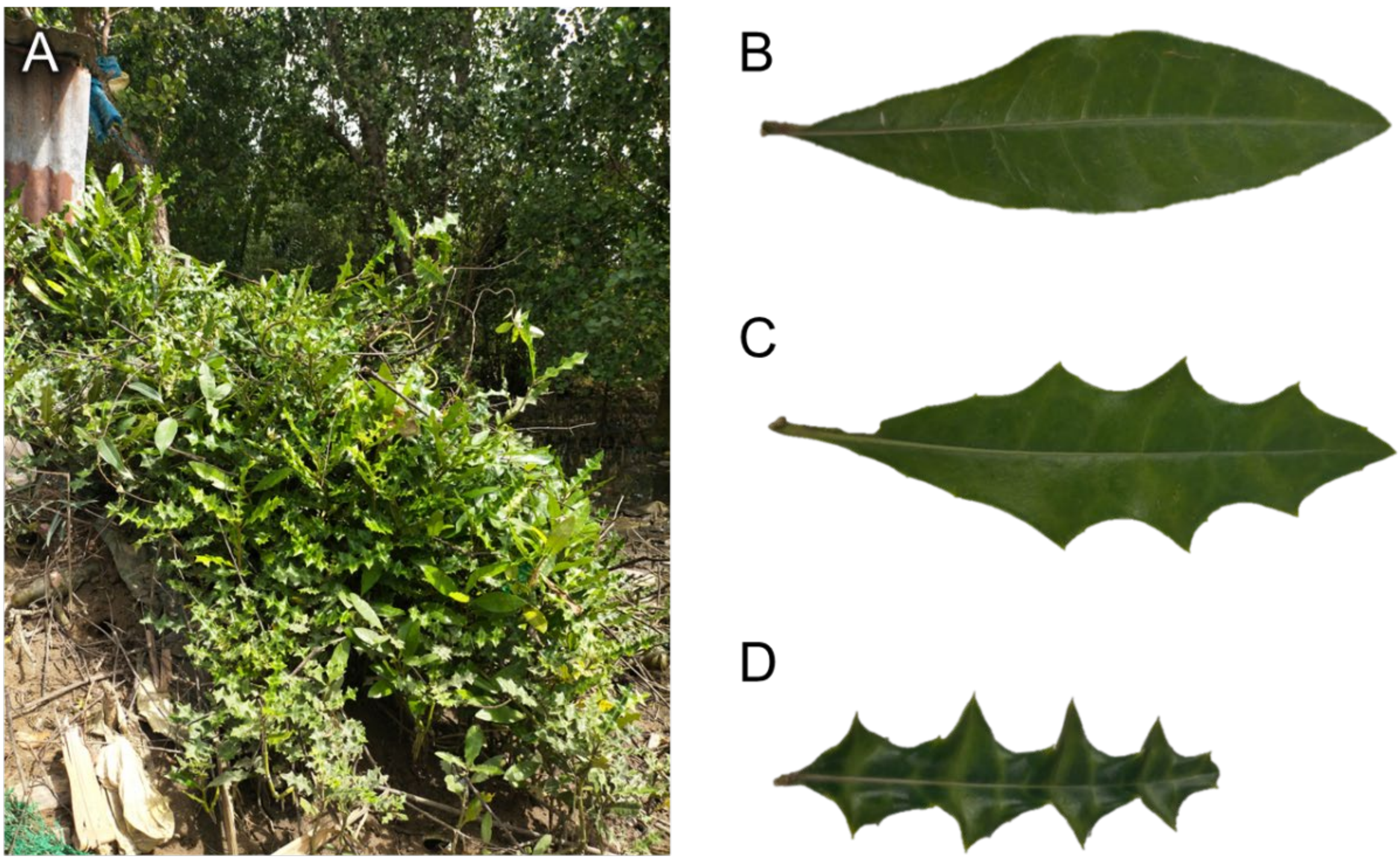

Figure 3. *Acanthus* assemblage in a nearby fishpond (A): B) *Acanthus volubilis* C) *Acanthus ilicifolius*, D) *Acanthus ebracteatus.*

Conclusion

This paper presents an accurate and comprehensive baseline data for mangrove species at Mangrove Civil Reservation Area, Sagay Marine Reserve. This study found seven new mangrove records, with one often mistakenly *C. zippeliana* as *C. decandra*. The series of assessments and a comprehensive species inventory in this study made it possible to hypothetically record all existing mangrove species in the area; and could be used to advance mangrove biodiversity monitoring and management for the reserve, as well as other mangrove forests in Negros Province. Conversely, 11 previously recorded species were not found in the area. The use of the transect method in the previous study have sampled portions of the forest that may lead to species listing discrepancy as far as baseline biodiversity assessment is concerned. This highlights that baseline studies either in large scale or in strict geographical areas should adhere to strict taxonomic accounts with proper documentation, if the vouchered specimen is not possible.

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Disclosure: -

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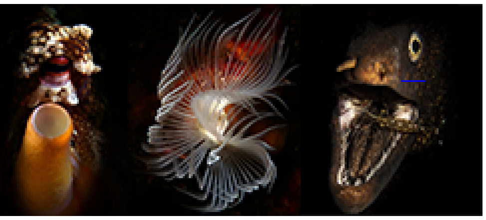

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Photograph (from book, magazine or webpage)	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.2015)
Artwork - from library database	**Clark, L. (c.a. 1960's).** *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
Artwork - from website	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.2015)