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Aquat Res 6(1), 1-10 (2023) • https://doi.org/10.3153/AR23001 *Research Article*

Effects of the fresh and dried housefly (*Musca domestica*) larvae in the diets of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758): growth, feed utilization efficiency, body composition and biological indices

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ABSTRACT

A 56-day feeding trial was undertaken to assess the effects of housefly maggots (HM) forms (dried and fresh) as protein sources on growth, feed efficiency, and body indices of *Oreochromis niloticus* fingerlings. A control diet (T0) contained 300 g/kg of fishmeal (without HM). Two practical diets with the same formula were prepared with dried HM (T1) and fresh HM (T2) where 66 % of the fishmeal was replaced. Diets were fed to triplicate groups of tilapia (mean initial weight: 10.26 ±0.12 g). There was no difference in survival, condition factor, feed conversion ratio, and protein efficiency ratio. Fish fed diets T0 and T1 had significantly increased ($P < 0.05$) mean final weight (50.25 ±1.39 - 52.24 ±1.03 g), specific growth rate (2.84 ±0.03 - 2.88 ±0.03 %/day) and weight gain (389.70 ±7.63 - 402.78 ±8.16 %) compared to T2 diet ones (46.30 ±2.03 g; 2.67 ±0.07 %/day and 356.70 ±7.76 % respectively). Viscerosomatic and hepatosomatic index in fish fed T1 and T2 diets were significantly higher than those fed T0. The present findings indicate that the dried form of housefly maggot has given the best results in terms of growth compared to the live form. However, housefly forms did not affect feed efficiency parameters.

Keywords: Maggot meal, Fresh maggot, Forms, Carcass composition, Tilapia

Introduction

The continued decline in capture fisheries and the increasing demands for livestock and aquaculture have ensued in a quick decrease in the supply of fish meal (FM) coupled with its price increasing (Henry *et al.,* 2015). The current global human consumption of fish is estimated at 20 kg of fish per year per capita, nearly half of which is provided by aquaculture (FAO, 2018). To satisfy the increasing demand for fish facing a growing number of population, there is an urgent need to improve the efficiency of aquaculture production. The necessity for more ecologically and durable aquafeed materials has previously been examined for fish farming to replace a pricey and unsustainable fish meal (Gasco *et al.,* 2018). Among alternative sources of protein for the aquafeed formulation, insects are greatly promising as sustainable ingredients for future commercial eco-friendly aquafeed production (Henry *et al.,* 2015; Tran *et al.,* 2015).

Generally, the amino acids profiles of insects are taxon-dependent, with Diptera profiles thought to be close to those of fish meal (Henry *et al.,* 2015). Insects larvae are very good for its high protein content and easy digestibility and amino acid profile comparable to fish meal (Fitches *et al.,* 2018). Moreover, their production can be examined as environmental-friendly animal farming and they do not rival along with human nutrition (van Huis *et al.,* 2013). One species that has saved more attention than others is the housefly *Musca domestica* (Diptera: Muscidae), whose larvae are recognized to develop on a various types of substrates, such as poultry droppings (Ogunji *et al.,* 2008), chicken manure (Fitches *et al.* 2018), chicken viscera (Djissou *et al.,* 2016; Alofa *et al.,* 2020; Alofa & Abou, 2020) and wheat bran wet (Wang *et al.,* 2017). The successful use of housefly larvae as a fish meal substitution has been documented in these different investigations.

Tilapias are the most commonly aquacultured fish in the world, as classified second only to carp in world fish production (Caï *et al.,* 2018). Nile tilapia *Oreochromis niloticus* is one of both major species, most reared in Benin (Rurangwa *et al.,* 2014). This species grows fast, tolerates a large range of environmental factors, is resistant to stress and diseases, has a low trophic level and accepts dry feeds just after absorption of the yolk sac, and is highly appreciated for its tasty flesh (El-Sayed, 2006; Bhujel, 2014). The potential of housefly maggot as a fish meal replacement has been assessed in several species, such as *O. niloticus* (Alofa *et al.,* 2020; Alofa and Abou, 2020; Wang *et al.,* 2017), *Carassius gibelio* (Dong *et al.,* 2013), *Clarias gariepinus* (Aniebo *et al.,* 2011; Oyelese, 2007; Fasakin *et al.,* 2003) and *Heterobranchus longifilis* (Ossey *et al.,* 2012). The feasibility of incorporating housefly larvae in the diet of tilapia has been successfully tested in several previous investigations. Feeding Nile tilapia with 27 % of maggots meal was also shown not to affect the growth compared to a commercial feed (Wang *et al.,* 2017). Partial replacement of FM with maggot meal may be more effective. This was verified in the aquaculture recirculation system in our previous study, where *O. niloticus* was fed successfully with live housefly larvae (25 % of maggots) (Alofa *et al.,* 2020). We have demonstrated that *O. niloticus* fed live maggots has a higher growth performance and feed efficiency than those fed spirulina waste, probably because the diet containing maggots was more balanced and matched to the requirements of this species. In addition, the housefly maggot meal has been used in combination with chicken viscera to substitute substantially the fish meal in the diet of the same species (Alofa and Abou, 2021).

On the other hand, the use of fresh maggots directly in the diet of fish would be very beneficial for them. Thus, feeding *C. gariepinus* with live maggots mixed with an artificial diet (3.5 % FM) resulted in higher growth performances than an artificial feed alone (Oyelese, 2007). The development of good quality and less expensive feeds for aquaculture is very important in underdeveloped countries such as Benin. In these countries, the efficient use of simple materials in the production of diets can define the success of aquaculture exploitation (Ekpo and Bender, 1989). The use of fresh maggots directly in fish feeds could allow not only a better valorization of the nutritional elements by fish but also a reduction of the energetic costs linked to its processing. Indeed, the direct use of fly larvae in fresh form in fish diets is the easiest method but it could generate the presence of pathogens if the treatment is not correctly carried out. Oven drying, however, could generate supplementary expenses in fish production. However, research on the different forms in which maggots can be successfully used in the diet is still limited and no investigation has not been reported in Nile tilapia. Moreover, there are no investigations assessing the efficiency of the use of a different form of housefly maggots for tilapia aquaculture in single-growth experiments. Therefore, the current study could contribute to filling this gap of information by examining for the first time, the effects of the use of fresh and dried *Musca domestica* larvae on biological indices of *Oreochromis niloticus*. In this investigation, we examined the effects of using a dried and fresh HM to replace 66.6 % of the fishmeal in a diet for Nile tilapia juveniles. Specifically, we analyze feed utilization, growth performance, and carcass quality and identify the most appropriate form of HM for diet formulation in this species.

Material and Methods

Experimental Design

The experiment was conducted in a Recirculating Aquaculture System at the Aquaculture Research Center (ARC) of the Laboratory of Ecology and Aquatic Ecosystem Management, University of Abomey-Calavi, Benin (06°24'49.4''N Latitude and 002°20'17.1''E Longitude). Nine circular cement tanks (diameter: 1.2 m with a volume of 1 m^3) were used. All tanks were connected to a mechanical and biological filter and water pump to maintain optimal water quality. The water was continuously aerated using a compressed air pump (Axair 300). The water flow rate was 4 L/min per tank. During the experiment, the photoperiod was 12 hours dark and 12 hours light (7:30 - 19:30 h) cycle, and tanks were covered two-thirds of their surface by racks to prevent algal development. The feeding trial lasted 8 weeks.

Fish and Feeding Management

A total of four hundred and fifty *O. niloticus* juveniles were purchased from the fish farm ''Dieu Exauce'' situated in Tori Avamey (Benin), and transported to ARC in closed oxygenated plastic bags. All male Nile tilapia with an average weight of 10.26 ±0.12 g (n = 30) was used in this trial. They were distributed randomly into tanks with a density of 50 fish per tank. Prior to the start of the experiment, all fish were fed with an equal mixture of the test diet (1:1:1) at a rate of 3 % of biomass twice daily for one week to acclimate with the experimental condition. Fish were weighed using a digital scale every 2 weeks to determine gain in weight and each tank was cleaned.

Fish were fed manually to apparent visual satiation thrice daily (at 09:00 h, 13:00, and 17:00 h) for a period of 56 days. Test diets were randomly distributed over nine tanks, with three replicates per treatment. Feed distributed each days was noted, and the uneaten diet was collected one hour after distribution. The collected feed has been dried and weighed to evaluate the real feed consumption.

Ingredients, Experimental Diets, and Preparation

Housefly (*Musca domestica*) maggots and fish meal (sun-dried *Sardinella* sp) were obtained as described by Alofa *et al.* (2020) and blood meal, following the method described by Alofa *et al.* (2016). The other ingredients were purchased from commercial sources.

Table 1. Biochemical composition (as % dry matter) of feeds ingredients used in the experimental diets

Ingredients	Dry matter	Crude protein	Crude lipid	Ash
Fish meal	92.2	66.7	6.8	14.7
Housefly maggot	92.7	48.8	21.0	6.3
Soybean meal	93.3	38.0	12.7	4.2
Cottonseed meal	91.1	39.8	8.2	8.5
Blood meal	91.2	72.2	1.5	6.7

All values are mean of triplicate samples

Table 2. Ingredients and proximate composition of the experimental diets: T0 (control diet), T1 (dried housefly maggots diet) and T2 (fresh housefly maggots diet)

	Experimental diets		
Ingredients (g/kg)	T0	T1	T2
Fish meal	300	100	100
Maggot	–	250	X[a]
Blood meal	70	70	70
Corn bran	340	265	265
Soybean meal	160	170	170
Cottonseed meal	100	105	105
Palm oil	20	20	20
Vitamins premix[b]	10	10	10
Minerals premix[c]	10	10	10
Total	1000	1000	750 + X[a]
Chemical composition			
Dry matter (%)	90.16	90.54	90.54
Crude protein (%)	35.36	35.13	35.13
Crude lipid (%)	8.19	11.88	11.88
NFE[d] (%)	36.48	31.35	31.35
Ash (%)	7.98	6.45	6.45
Gross energy[e] (kJ/g)	17.92	18.58	18.58

a. X represents the quantity of fresh maggots. Based on the dry matter, inclusion level, and used as a complement in the diet. Thus, 25 % of fresh maggots correspond to 935 g/kg basis live weight, because the water content of fresh maggots is 77% (Alofa *et al.,* 2020).
b. Vitamin premix contains (mg or IU/kg diet) : Vitamin B1, 15 mg; Vitamin B2, 15 mg; Vitamin B3, 30 mg; Vitamin B5, 35 mg; Vitamin B6, 6 mg; Vitamin B12, 0.03 mg; Vitamin C, 200 mg; Vitamin D3, 2.000 IU; Vitamin E, 50 mg; Vitamin K3, 5 mg; Inositol, 200 mg; Folic acid, 3 mg; Biotin, 0.2 mg.
c. Mineral premix contains (mg/kg diet): I, 0.4 mg; Co, 0.1 mg; Cu, 4 mg; Fe, 150 mg; Zn,80 mg ; Mn, 20 mg; Se, 0.1 mg; Mg, 100 mg.
d. Nitrogen-Free-Extract (NFE) = 100 - (moisture + crude protein + crude fat + ash + crude fibre).
e. Gross energy was estimated using the following values: 23.7 kJ/g for protein, 39.5 kJ/g for fat, and 17.2 kJ/g for carbohydrates (Guillaume *et al.,* 1999).

Three experimental isoproteic (35 % crude protein) and isocaloric (18 kJ/g gross energy) diets were formulated to fill the nutrient requirements of *O. niloticus* fingerlings (FAO, 2020). Before the formulation, the proximate composition of the experimental ingredients was analyzed (Table 1). Ingredients and formulation of the practical diets are shown in Table 2. A control diet (T0) contains a high level of fishmeal (300 g/kg). The other diets were formulated by replacing 200 g/kg with dried and fresh maggots designed as T1 and T2 respectively.

The ingredients were finely ground and blend in a food mixer. During mixing, vitamins and minerals mixtures were added. Then, oil was added and mixed for another 5 minutes. For the preparation of control and dried maggot diets, warm water (60 °C) was added (40 % of the dry ingredients) (Wang *et al.,* 2017). The fresh maggot diet was processed following the procedures described by Alofa *et al.* (2020). Then, the dough was passed through a kitchen meat grinder (Bosh MFW). The diets had a diameter of 3.0 mm, and were oven-dried for 24 hours at 60°C and stored at -4°C until use. Tables 1 and 2 summarize the formulation and composition of ingredients and the test diets.

Somatic Indices and Body Composition

At the end of the trial, the fish were fasted for 24 h to drain the stomach contents. All fish were weighed individually and counted to assess the survival rate and mean weight. Fish from each treatment (six fish per treatment) were randomly selected and dissected to assess the viscero somatic index (VSI, %). The liver was separated from the viscera and weighed to calculate the hepato somatic index (HSI, %).

To determine the whole-body composition, six other fish (two specimens per tank) were randomly selected by treatment and put in a freezer at -20°C.

- VSI = (viscera weight [g] / fish weight [g]) × 100 (Guroy and Karadal, 2019)
- HSI = (liver weight / body weight) × 100 (Guroy and Karadal, 2019)
- Fulton's Condition factor (CF) = [body weight (g)/length^3 (cm)] × 100 (Nash *et al.,* 2006)

Sample Collection and Analyses

The proximate composition of ingredients, diets, and fish from each treatment were determined following procedures of the Association of Official Analytical Chemists Statistical analysis (AOAC, 2005). Before chemical analysis, samples were finely cut, homogenized by grinding in a mincing machine. Dry matter was determined by oven-drying the samples at 105°C for 24 h. Ash content was measured by incinerating the sample at 550°C for 12 h in furnace. Crude proteins was determined using the Kjeldahl method. Crude fat was extracted according to Folch *et al.* (1957) method.

Water Quality

The water temperature, pH, dissolved oxygen and conductivity were measured *in situ* with a multiparameter (HANNA HI-9828). The weekly water samples were collected at 10 cm depth from tanks to determine nitrate and nitrite. These parameters were analyzed by cadmium reduction and phenate methods respectively using a spectrophotometer (Hach Lange DR6000). During the feeding trials, nitrite (NO_2–N, mg/L) concentration was lower than 0.5 mg/L.

Calculations

Growth parameters and feed utilization indices were calculated as followed :

- Survival rate (SR, %) = (Nf/Ni) × 100], where Nf and Ni refer to the final number of fish and the initial number of fish respectively.
- Specific growth rate (SGR, %/day) = 100 × [ln(Final body weight) − ln(Initial body weight)]/days.
- Daily weight gain (DWG) = (Wf - Wi)/T), where Wf is the mean final body weight and Wi, the mean initial body weight, and T is the duration in days.
- Percent weight gain (PWG) = (Final body weight - Initial body weight/Initial body weight × 100
- Total feed intake per fish (FI) = [total feed consumed (g)/number of fish].
- Yield = [final biomass per tank (kg)−initial biomass per tank (kg)]/ Volume (m^3)
- Production (P) = Yield × 365 days/ rearing period (days)
- Feed conversion ratio (FCR) = dry feed consumed/weight gain.
- Protein efficiency ratio = weight gain/crude protein consumed.

Statistical Analysis

All data are expressed as means ± standard deviation. Data were analyzed for homogeneity of variance by Levene's test. Differences were regarded as significant when $P<0.05$. Data were analyzed using a one-way analysis of variance (ANOVA I) and Tukey test. All statistical analyses were executed using the SPSS v20.0 software and graphs were executed with Microsoft Excel 2016.

Results and Discussion

During the study, the levels of pH, dissolved oxygen, temperature and nitrite did not differ significantly by treatment (Table 3). Values of physicochemical parameters of water measured in the tanks during the test are as follows: pH ranged between 7.14 to 7.19, temperature from 29.88 to 30.08 °C, dissolved oxygen from 5.34 to 5.47 mg.L^{-1}, nitrate from 0.24 to 0.26 mg.L^{-1} and nitrite from 3.2 to 3.31 mg.L^{-1}. These physical and chemical water parameters were preserved within the tolerable range for tropical fish (Delong *et al.,* 2009; Bhujel, 2014), signifying that the environmental conditions of *O. niloticus* during the trial were appropriate. This result is in agreement with the study of Alofa *et al.* (2020) with the same species reared in the tank.

Table 3. Water quality parameters measured during the feeding trial

Parameters	T0	T1	T2	$F_{\text{-value}}$	$p_{\text{-value}}$
pH	7.14 ±0.22	7.19 ±0.10	7.17 ±0.11	1.2	0.303
Temperature (°C)	29.88 ±0.68	30.08 ±0.72	29.88 ±0.78	1.25	0.29
Dissolved oxygen (mg/L)	5.47 ±0.43	5.39 ±0.39	5.34 ±0.65	0.81	0.447
Nitrite (mg/L)	0.24 ±0.09	0.26 ±0.07	0.25 ±0.06	1.7	0.186
Nitrate (mg/L)	3.20 ±0.55	3.30 ±0.32	3.31 ±0.28	1.13	0.325

Values represent the mean and standard deviation. T0: a control diet ; T1: a diet containing dried maggot and T2: a diet containing fresh maggot.

No lesions were observed with any treatment and no dead fish were recorded during the trial, suggested that the rearing facilities and quality of experimental ingredients were in adequate conditions. Growth performance after the 8-week feeding trial is given in Table 4. The initial weight of the *O. niloticus* ranged from 10.26-10.39 g and did not significantly (P>0.05) between all treatments. However, the mean final weight (FW) for some of the groups was significantly different (P < 0.05). The mean FW, weight gain, SGR, and DWG of *O. niloticus* of T1 groups did not significantly differ from T0 group. However, all these growth parameters significantly decreased in T2 (P < 0.05). Changes in weight during 56 days of rearing are shown in Fig 1. In the first 4 weeks, weights did not vary significantly between the T1 and T2 groups. On weeks 4-8, T0 and T1 individuals weighed more than T2 individuals. Thus, body weight was significantly greater in the fish fed T0 and T1 than in fish fed T2 (P < 0.05).

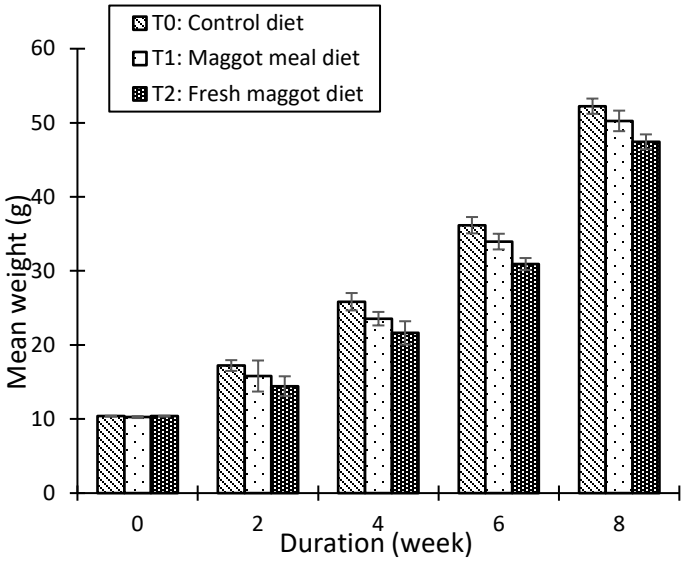

Figure 1. Average weight (g) of *Oreochromis niloticus* during 56-day experimental period.

Table 4. Growth parameters of *Oreochromis niloticus* fed with each practical diet

	Experimental diets			ANOVA	
Parameters	T0	T1	T2	p-value	F-value
Mean initial weight (g)	10.39 ±0.06	10.26 ±0.12	10.38 ±0.06	0.192	2.2
Mean final weight (g)	52.24 ±1.03^{a}	50.25 ±1.39^{a}	46.30 ±2.03^{b}	0.009	11.59
PWG (%)	402.78 ±8.16^{a}	389.70 ±7.63^{a}	356.70 ±7.76^{b}	0.001	27.43
SGR (%.day^{-1})	2.88 ±0.03^{a}	2.84 ±0.03^{a}	2.67 ±0.07^{b}	0.003	18.25
DWG (g.day^{-1})	0.75 ±0.02^{a}	0.71 ±0.02^{a}	0.64 ±0.04^{b}	0.007	12.79
Yield (kg.m^{-3})	1.94 ±0.08^{a}	1.82 ±0.08^{ab}	1.63 ±0.09^{b}	0.009	11.42
P (kg.m^{-3}.year^{-1})	12.62 ±0.50^{a}	11.83 ±0.50^{ab}	10.94 ±0.23^{b}	0.009	11.4

PWG: Percent weight gain, **SGR:** Specific Growth Rate, **DWG:** Daily Weight Gain, **T0:** a control diet; **T1:** a diet containing dried maggot and **T2:** a diet containing fresh maggot. Values represent the mean and standard deviation. In each line, different superscript letters indicate the significant differences between the treatments (P<0.05).

The present study provides important knowledge on the different forms of housefly larvae that can be used in the Nile tilapia diet. Housefly larvae have been found as a durable alternative source of protein for fish feeds (Henry *et al.,* 2015; Makkar *et al.,* 2014). It's been shown that maggots efficiency in the fish diet depends on the species and/or stage, the feed substrate and the process (Fasakin *et al.,* 2003). Diets containing maggots were easily acceptable by the *O. niloticus* used in this experiment, suggesting that this ingredient could contain free amino acids, which could improve feed intake. The specific growth rate of Nile tilapia (weighing 10.23 g) fed with the fishmeal based-diet (T0) was around 2.88 %/day, which is higher than those (1.12-2.26 %/day) recorded for the same species (weighing 25-68.89 g) (Wang *et al.,* 2017; Obirikorang *et al.,* 2015), probably because of the size of the fishes. Generally, the smaller fish get a higher growth rate than the bigger fish (Dong *et al.,* 2013). This experiment shows that the ingredient form of housefly maggots affects significantly the growth performance ($P<0.05$). Fish fed the diets T0 and T1 performed significantly better ($P<0.05$) than that fed fresh based diet. This is consistent with the results of Awom and Eyo (2016), where the weight gain, PER, and specific growth rate for *Clarias gariepinus* after 70 days were lower in fish fed 100% of live maggots. But, on the other hand, maggots were used alone in their investigation. The highest Specific Growth Rate (2.84 ±0.03 %/days) and final body weight (50.25 ±1.39 g) are obtained with the dried form of maggots diet (T1), indicating that the omnivorous Nile tilapia can well utilize dried maggots based diet. For these parameters, the values (2.67 ±0.07 %/days and 46.30 ±2.03 g respectively) recorded with fish fed with fresh form are lower. The findings of this research suggest that the cheaper dried housefly maggots can partially substitute fish meal as a dietary protein source in *O. niloticus* diets. This is in accordance with other investigations where fish meal was replaced by maggots, producing good growth performance in Nile tilapia. (Ogunji *et al.,* 2008; Wang *et al.,* 2017). Nile tilapia fed 25 % of live maggots showed a better growth performance, and specific growth rate, than fish fed spirulina waste (Alofa *et al.,* 2020), probably because the diets were better adapted to the needs of the fish and palatable. The growth reduction observed by Awom and Eyo (2016) in African catfish fed live maggots may be due to the fact that it does not provide all the nutrients necessary for optimal growth of this fish. In their study, maggots were used solely.

Table 4 shows that there was no significant difference in body weights at the beginning of the experiment and that the final body weight of fish fed the fresh maggot diet was significantly lower than that of the other diets throughout the experiment ($P< 0.05$). The poor growth of fish fed the diets containing fresh maggots could be due to the presence of chitin in the exoskeleton of housefly (Cummins Jr *et al.,* 2017), imbalance of fatty acid profile (Lin and Mui, 2017), and lower lipid digestibility (Shiau and Yu, 1999; Diener *et al.,* 2009). These factors could decrease palatability and nutrient absorption resulting in reduced growth. Furthermore, the processing (different forms) of maggots are worthy to consider. Therefore, the decrease in growth performance observed in the fish fed diet containing fresh maggots could be associated with the processing conditions of the maggots used.

The effects of different forms of maggots on the feed utilization parameters of *O. niloticus* are shown in Figure 2. Palatability is an important factor in fish nutrition that influences feed consumption and digestibility by returning the diet to a greater or lesser extent acceptable (Bowker, 2013). Feed conversion ratio (FCR) and protein efficiency ratio (PER) did not differ between the three groups ($P > 0.05$). In this study, the highest feed intake (FI) was observed in fish fed with T0, while the lowest was observed in fish fed with T2 (Fig. 2.c). However, feed intake did not differ significantly in fish fed maggot-based-diets. A similar finding was found by substituting the maggot meal for fish meal in the *O. niloticus* diet (Wang *et al.,* 2017). These authors reported that this ingredient was widely palatable and attractive for Nile tilapia.

The findings of biological indices and whole body composition are presented in Table 5. Hepatosomatic index (HSI) and viscerosomatic index (VSI) were significantly increased in fish fed maggots ($P < 0.05$) but condition factor (CF) did not significantly differ between treatments. VSI of fish fed with T0 was significantly decreased than that of fish fed with the other experimental diets. This could be explained by the increased lipid content of the maggot diet. Studies have revealed a positive relationship between HIS and liver metabolism and usually, increased lipids content generates higher HSI (Liu *et al.,* 2010). This is in accordance with the research on Japanese sea bass *Lateolabrax japonicus*, which showed hepatic steatosis and higher HSI and liver lipid values when high levels of animal protein blend were utilized as a substitute for fish meal (Hu *et al.,* 2013). Nevertheless, the effects of dietary incorporation of animal protein and high-fat sources on the liver forms of fish are contrasted. For example, the histopathology of the liver of Atlantic cod and sea bream showed no changes when these fish are fed high-fat diets (Caballero *et al.,* 1999; Hansen *et al.,* 2006). However, no significant difference was observed for the hepatosomatic index between experimental treatments, indicating that the presentation form of maggots has no adverse effect on the hepatopancreas.

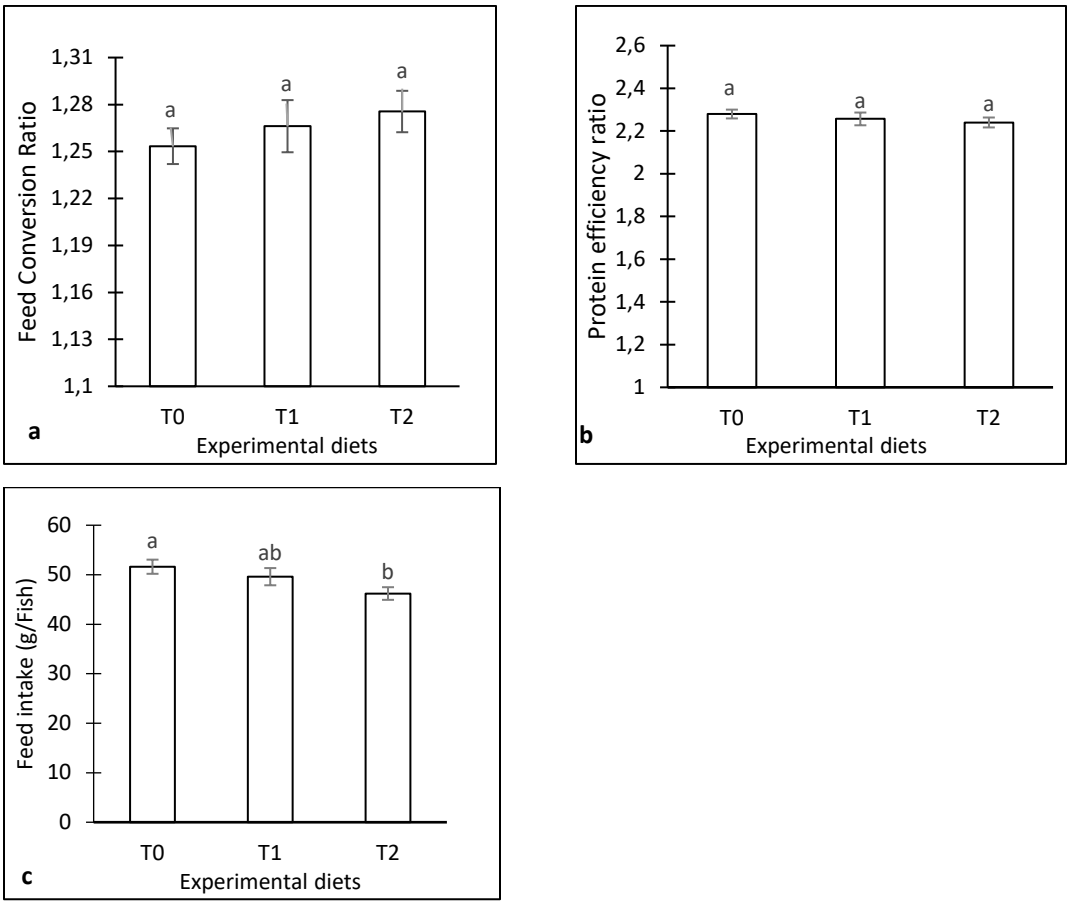

Figure 2. Histogram of the mean of food conversion ratio (a), protein efficiency ratio (b), feed intake (c) with standard deviations of Nile tilapia fed experimental diets (T0: a control diet ; T1: a diet containing dried maggot and T2: a diet containing fresh maggot). Different letters indicate a significant difference among treatments ($P < 0.05$).

Table 5. Morphological and organosomatic indices and carcass composition of Nile tilapia fed the test diets

Parameters		T0	T1	T2	*p-value*
Biological indices (%)					
VI		8.88 ±0.54[b]	10.61 ±0.38[a]	10.41 ±0.79[a]	0.005
HI		3.07 ±0.36[b]	4.95 ±0.21[a]	4.71 ±0.14[a]	0.000
CF		1.90 ±0.02[a]	1.89 ±0.02[a]	1.88 ±0.02[a]	0.83
Proximate composition (%)					
	Initial body				
Dry matter	89.92 ±0.16	90.06 ±1.02	89.95 ±1.24	90.64 ±1.23	0.68
Crude protein	62.11 ±0.21	61.30 ±0.61	60.97 ±0.33	60.68 ±1.00	0.493
Crude lipid	12.76 ±0.59	19.38 ±0.74[b]	26.36 ±0.60[a]	26.45 ±0.49[a]	0.000
Ash	16.22 ±0.39	15.53 ±0.78[a]	13.80 ±0.51[b]	13.84 ±0.29[b]	0.003

Values are means ± standard deviation and values within the same row with different superscript letters are significantly different ($P < 0.05$). **HI** (Hepatosomatic index), **VI** (Viscerosomatic index), **CF** (Condition factor). **T0:** control diet; **T1:** diet containing dried maggot and **T2:** diet containing fresh maggot.

Dietary housefly maggots replacement did not modify body proximate composition, which was also observed in studies with maggots substitution in fish feeds (Ogunji *et al.,* 2008; Wang *et al.,* 2017). Nevertheless, in the present investigation, the fat content of the whole-body increased by maggots supplementation, indicating that the lipid metabolism in Nile tilapia may be affected by the inclusion of insects larvae, which was consistent with our previous findings (Alofa *et al.,* 2020). The dry matter and crude protein contents of the whole body were not affected by any treatment. However, the ash content decreased significantly with the incorporation of maggots (Table 5). This might be associated with the increase of VSI in T1 and T2 groups and the effect of the high-fat content of MM used in the diet formulation, resulting in increased fat content in a substrate (chicken viscera) used for their production.

Conclusion

Findings from this investigation showed that better growth performance in Nile tilapia, *O. niloticus* could be achieved by the using of dried maggot meal in the formulated fish diet compared to live maggots. The beneficial effects of dried maggots supplementation for FM substitution were demonstrated at growth performance and feed efficiency. However, the use of the fresh maggot, significantly decreased growth performance. Nutrient utilization efficiencies were not affected by the inclusion of both forms of maggots.

Compliance with Ethical Standards

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

Ethics committee approval: This research was conducted in accordance with the framework law N° 2014-19 of August 07, 2014 on fisheries and aquaculture in the Republic of Benin and the Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes

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Disclosure: -

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Investigation of fatty acid profiles in some economically important fish species living in Atatürk Dam Lake, Adıyaman, Türkiye

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ABSTRACT

This study aims to determine the fatty acid profile of 10 fish species (*Carassius gibelio (Bloch, 1782), Chondrostoma regium (Heckel, 1843), Cyprinus carpio (Linnaeus, 1758), Carassius auratus (Linnaeus, 1758), Alburnus sellal (Heckel, 1843), Planiliza abu (Heckel, 1843), Capoeta trutta (Heckel, 1843), Arabibarbus grypus (Heckel, 1843), Mastacembelus mastacembelus (Banks & Solander 1794)*) caught from Atatürk Dam Lake, sold by fishermen and consumed by local people. Accordingly, the fish species identification was made and the individuals belonging to the determined species were randomly selected and muscle tissue was taken from each sample for fatty acid analysis and put into tubes. Extraction of fats from muscle tissue was carried out using a chloroform-methanol solvent mixture. The methylation process of fatty acids was carried out using the TS EN ISO 12966:2 method. Analyzes were performed with Thermo brand Trace GC model GC with FID (Flame Ion Detector) detector. A 60 m HP-88 column was used in the analysis. It has been determined that 10 fish species contain a total of 26 different fatty acids. Palmitic acid is the major fatty acid among saturated fatty acids (SFA). Palmitic acid values vary between 18.31 % and 25.51 %. It was determined the most in *C. carpio* and the lowest in *C. auratus*. Among the mono unsaturated fatty acids (MUFA), oleic acid is the major fatty acid and was found between 12.70 % and 29.77 %. It was detected at most in *A. sellal* with a rate of 29.77 % and in *P. abu* with a minimum rate of 12.70 %. Of the polyunsaturated fatty acids (PUFA), docosahexaenoic acid is seen as the major fatty acid. It was detected at most in *C. trutta* with a rate of 14.24 %, and at a minimum in *C. carpio* with a rate of 0.62 %.

Keywords: Atatürk Dam Lake, Economic fish species, Fatty acid, Fish meat, Nutrition

Introduction

Fisheries are very important source of natural life, as it contributes to the economy of countries with a certain investment and labor, as well as human nutrition. A healthy and balanced diet can be provided by making high use of marine and freshwater fish (Doğan, 2007). Atatürk Dam Lake, built on the Fırat (Euphrates) River, is Türkiye's largest dam lake, and besides electricity generation, and agricultural irrigation, it also constitutes a significant source of aquaculture for the local people (Alhas et al., 2009; Fırat et al., 2018).

About 28 fish species from eight families live in the Fırat (Euphrates) River. Some of them are caught by the fishermen of the region due to their economic value. (Oymak, 1998). These caught fish are important both in terms of providing the food needs of the local people and in terms of creating employment opportunities. The meat of fish is an important food due to its chemical properties, low cholesterol content, being rich in minerals and vitamins, as well as having essential amino acids (Kaçar et al., 2016). The interest in the fatty acid profiles of fish has increased day by day with the understanding that the omega-3 group fatty acids contained in fish oil are also good for hypertension, cardiovascular diseases, depression, cancer and similar diseases (Kaçar, 2010).

Lipids in fish, which are considered a great source of essential fatty acids recommended in human nutrition, are important biochemical components that perform energy and structural functions, and the lipid component of muscle tissue is largely represented as the composition of the total lipid content. It is important to determine the fatty acid profiles of fish species consumed by the public (Kaçar et al., 2018).

The aim of this study is to determine the fatty acid profile of 10 fish species that are sold by fishermen from Atatürk Dam Lake and consumed by local people. Accordingly, the types and amounts of fatty acids belonging to the consumed species will be revealed and the chance to be preferred for diet will be obtained. In this way, it will be possible to compare the fatty acid profiles of fish caught and sold at the same time by fishermen at the species level. Seasonal changes of fatty acid amounts of some fish species related to fatty acid analysis were studied (Satar et al., 2012; Kaçar et al., 2016; Yılmaz et al., 2016; Kaçar et al., 2018; Dağtekin et al., 2018). However, the amount of fatty acid in the fish consumed by the public and sold at the same time has not been determined. In this study, these analyzes were made and the data of the species were compared.

Material and Methods

The fishes used in the study are the samples caught from the Atatürk Dam Lake fed by the Euphrates (Euphrates) River in October 2020 and were purchased from fishermen in Adıyaman. Fish species that are especially consumed by people were selected for the study. Individuals belonging to the species identified by species identification were randomly selected and muscle tissues was taken from each sample for fatty acid analysis and put into tubes.

Extraction of oils from fish muscles was carried out using a solvent mixture of chloroform and methanol (2:1) according to the method specified by Folch et al (1957). The methylation process of fatty acids was carried out according to the TS EN ISO 12966:2 method. For this, 100 mg of oil sample was weighed in a 10 ml capped test tube, 2 ml of isooctane and 100 µl of 0.2 M methanolic potassium hydroxide solution were added. The cap of the test tube was closed and mixed in vortex for 1 minute. 2 ml of 40% NaCl solution was added into the tube and shaken again. The isooctane phase was transferred to a vial and mixed by adding about 1 g of sodium hydrogen sulfate. After waiting for about 30 minutes, 1 µL of the upper phase was taken and injected into the device.

Analyzes were carried out with GC with Thermo brand {Trace GC model with FID (Flame Ion Detector)} detector. A 60 m HP-88 column was used for the analyses. Detector and injection block temperatures were set at 280 °C and 250 °C, respectively. A temperature program was applied to the column: After it was kept at 50 ºC for 2 minutes, it was increased to 180 ºC with an increase of 20 ºC / min, and from this temperature to 230 ºC with an increase of 5 ºC / min, and waited at this temperature for 5.5 minutes. The split ratio was set at 1/50 and the injection amount was 1 µL.

Atherogenic and Thrombogenic Health Lipids Indices

The atherogenicity (AI) index indicates the risk of developing diseases such as the accumulation of fat on the artery walls. Thrombogenicity (TI) describes the likelihood of a blood clot forming. Fatty acid data of fish oils were used to calculate (AI) and (TI) indices (Garaffo et al., 2011). AI and TI indices were calculated according to the following formulas:

$$AI=(4\times C14:0+C16:0)/(\Sigma MUFA+\Sigma PUFA);$$

$$TI=[(C14:0+C16:0+C18:0)/(0.5\times\Sigma MUFA+0.5\times\Sigma n6\ PUFA+3\times\Sigma n3\ PUFA+(n3/n6))].$$

Statistical Analyzes

Statistical analyzes of the obtained data were performed with the SPSS (22.0) computer program. For all samples, the analysis was performed in triplicate. Results were reported as mean value and standard deviation (mean±SD). Fatty acids were analyzed by analysis of variance (ANOVA) and comparisons between means were made with the Tukey test. The differences between the means were considered statistically significant at the $p<0.05$ level.

Results and Discussion

A total of 26 different fatty acids were determined in 10 fish species collected from Adıyaman Province, sold by fishermen and consumed by the public. Fatty acid compositions of fish species are given in Table 1. When Table 1 and Figure 1 are examined, it is seen that palmitic acid is the major fatty acid among saturated fatty acids (SFA). Palmitic acid values vary between 18.31 % and 25.51 %. It was determined the most in *C. carpio* and the lowest in *C. auratus* ($p<0.05$). Among the mono unsaturated fatty acids (MUFA), oleic acid is the major fatty acid and was found between 12.70 % and 29.77 %. It was detected at most in *A. sellal* with a rate of 29.77 % and in *P. abu* with a minimum rate of 12.70 % ($p<0.05$) (Table 1 and Figure 1). Of the polyunsaturated fatty acids (PUFA), docosahexaenoic acid is seen as the major fatty acid. It was detected at most in *C. trutta* with a rate of 14.24 %, and at a minimum in *C. carpio* with a rate of 0.62 % ($p<0.05$) (Table 1 and Figure 1).

Total (SFA) were highest in *C. carpio* (41.60%), least in *A. sellal* species (27.83 %) ($p<0.05$), total (MUFA) were highest in *A. sellal* species (44.34 %), at least in *C. trutta* (27.91 %) ($p<0.05$), total (PUFA) were the most in *C. auratus* (35.18 %), the least in *C. carpio* type (18.92 %) ($p<0.05$) (Table 1 and Figure 1). ω-3 fatty acids are highly beneficial on diseases such as cardiovascular diseases, diabetes, cancer, depression, various mental illnesses, age-related cognitive decline, rheumatoid arthritis, and periodontal disease (Shahidi and Ambigaipalan, 2018). Although ω-3 fatty acids, which are very important for health, are observed to be the most in *C. trutta* species, it has been determined that *P. abu, C. gibelio, C. auratus,* and *C. regium* species also contain high levels of ω-3 fatty acids. The poorest species in terms of ω-3 was *C. carpio* with 6.33 % (Table 1 and Figure 1).

The ratio ω-3/ω-6 is used to compare the relative nutritional values of fish oils. It has been reported that this ratio is between 1-5 (Inhamuns, 2008; Fallah ve ark., 2011; Gökçe ve ark., 2011; Linhartova, 2018; Bayar ve ark., 2021). A balanced ω-3/ω-6 diet is essential for normal growth and development and plays an important role in the prevention of coronary artery disease, hypertension, cancer, and rheumatoid arthritis (Cengiz et al., 2010; Kaçar and Başhan, 2016). In this study, this value was determined as 0.69 in *C. carpio* species, while it was determined as 3.53 in *C. trutta* species (Table 1 and Figure 1). Bulut et al., (2006) determined the fatty acid composition of *C. carpio* species caught in Apa Dam Lake in Konya. In the study in which male and female fish were evaluated separately, they found the average SFA as 33.18 %, the average MUFA as 42.19 %, and the average PUFA as 7.86 %. They determined the total amount of ω-3 as 1.92 % and the amount of ω-3/ω-6 as 0.66. While the amount of ω-3/ω-6 they detected was similar to this study, there were differences in other values. Kaçar and Başhan (2016) carried out a study to determine the fatty acid composition of 12 different fish species grown in Atatürk Dam Lake. 6 of these species are similar to the species we used in this study. When we compared the fatty acid profiles of 6 similar species, in this study, the highest SFA, ω-3 and ω-3/ω-6 values were found in *C. trutta*, the highest MUFA in *C. luteus* and the highest PUFA in *C. auratus*. In the study of Kaçar and Başhan, 2016), the highest SFA and ω-3 values were found in *C. regium*, the highest MUFA and ω-3/ω-6 ratios in *C. trutta*, and the highest PUFA in *C. auratus*, similar to this study. Bayar et al. (2021) determined the highest SFA in S. erytroptholmus species, and the highest MUFA, PUFA, and ω-3 values in *A. anguilla* species in 2 species of freshwater fish they caught in Büyük Menderes River (Aydın/Türkiye). Öksüz et al., (2019) found the most SFA and MUFA in carp, and the highest PUFA and ω-3 values in Sudak in their study on Carp and Sudak species they obtained from Beyşehir Lake. Başhan and Kaçar (2017) determined the fatty acid composition of *C. trutta* species in Atatürk Dam Lake in their study. Compared to this study, they found lower SFA and PUFA values, and higher MUFA, ω-3, and ω-3/ω-6 values. Özyılmaz (2014) determined the fatty acids in 3 different freshwater fish in Atatürk Dam Lake. They found that the highest SFA, MUFA, and ω-3/ω-6 values were in *C. trutta* and the highest PUFA and ω-3 values were in *C. gibelio*. On the other hand, Taşbozan et al. (2013) found SFA, MUFA, and ω-3 values as 33.87 %, 39.96 %, and 13.96 %, respectively, in *M. mastacembelus* species they caught from Atatürk Dam Lake. These values are similar to the values in this study. They found a higher PUFA value and a lower ω-3/ω-6 value compared to this study.

Table 1. Fatty acid compositions of 10 fish species used in the study

Fatty acids	*C. carpio*	*P. abu*	*A. grypus*	*M. mastacembelus*	*A. sellal*	*C. gibelio*	*C. auratus*	*C. regium*	*C. luteus*	*C. trutta*
(C14:0) MyristicAcid	1.95±0.15^{b}	5.19±0.36^{e}	4.45±0.29^{d}	1.67±0.12^{a}	2.57±0.08^{c}	1.92±0.20^{b}	2.08±0.21^{b}	2.69±0.20^{c}	1.97±0.13^{b}	2.97±0.19^{c}
(C14:1) MyristoleicAcid	0.17±0.03^{ab}	0.25±0.03^{bc}	0.53±0.07^{e}	0.23±0.06^{bc}	0.43±0.06^{d}	0.29±0.04^{c}	0.19±0.03^{abc}	0.14±0.01^{ab}	0.12±0.02^{a}	0.18±0.01^{ab}
(C15:0) PentadecanoicAcid	0.81±0.09^{c}	2.25±0.14^{e}	0.70±0.05^{bc}	0.50±0.10^{ab}	0.43±0.06^{a}	0.67±0.07^{bc}	0.79±0.08^{c}	0.56±0.06^{ab}	0.67±0.05^{bc}	1.73±0.09^{d}
(C15:1)-PentadecenoicAcid	0.61±0.06^{d}	0.35±0.03^{b}	0.36±0.04^{b}	0.18±0.03^{a}	0.21±0.02^{a}	0.59±0.07^{d}	0.40±0.04^{bc}	0.31±0.03^{ab}	0.52±0.09^{cd}	0.37±0.07^{b}
(C16:0) PalmiticAcid	25.51±0.79^{e}	21.79±0.28^{c}	20.38±0.24^{b}	19.05±0.36^{a}	18.75±0.21^{a}	22.47±0.23^{c}	18.31±0.32^{a}	20.47±0.29^{b}	23.82±0.19^{d}	21.96±0.27^{c}
(C16:1) PalmitoleicAcid	6.48±0.18^{c}	15.41±0.37^{g}	9.97±0.17^{e}	5.22±0.17^{a}	10.13±0.17^{e}	6.54±0.32^{c}	7.06±0.19^{d}	11.94±0.22^{f}	7.20±0.22^{d}	5.76±0.14^{b}
(C17:0) HeptadecanoicAcid	0.92±0.08^{b}	0.66±0.07^{a}	0.59±0.04^{a}	0.62±0.04^{a}	0.55±0.06^{a}	0.93±0.11^{b}	0.67±0.08^{a}	1.01±0.14^{b}	0.57±0.08^{a}	0.62±0.04^{a}
(C17:1)-HeptadecenoicAcid	0.39±0.05^{b}	2.97±0.10^{e}	0.54±0.05^{bc}	0.52±0.04^{bc}	0.64±0.03^{c}	0.54±0.06^{bc}	0.57±0.04^{c}	1.26±0.11^{d}	0.49±0.05^{bc}	0.20±0.02^{a}
(C18:0) StearicAcid	12.13±0.32^{g}	3.50±0.22^{a}	4.84±0.12^{b}	11.41±0.27^{f}	5.35±0.22^{b}	10.25±0.27^{e}	9.17±0.23^{d}	7.45±0.26^{c}	9.63±0.10^{d}	10.23±0.36^{e}
(C18:1n9c) OleicAcid	19.64±0.21^{c}	12.70±0.26^{a}	25.04±0.28^{g}	23.39±0.47^{f}	29.77±0.38^{i}	21.48±0.12^{d}	21.63±0.13^{d}	22.51±0.16^{e}	27.92±0.25^{h}	17.35±0.27^{b}
(C18:2n6c) LinoleicAcid	4.65±0.17^{c}	2.56±0.20^{a}	6.02±0.13^{e}	5.30±0.30^{d}	3.65±0.26^{b}	4.40±0.19^{c}	9.17±0.26^{f}	3.72±0.17^{b}	2.23±0.12^{a}	2.29±0.08^{a}
(C18:3n6) G-LinolenicAcid	0.50±0.08^{d}	0.80±0.08^{e}	0.26±0.05^{c}	0.26±0.04^{c}	0.07±0.02^{a}	0.24±0.06^{bc}	0.22±0.04^{bc}	0.12±0.01^{ab}	0.28±0.03^{c}	0.20±0.03^{bc}
(C18:3n3) A-LinolenicAcid	1.54±0.10^{b}	1.95±0.06^{c}	3.03±0.16^{d}	1.98±0.12^{c}	3.47±0.21^{e}	2.01±0.10^{c}	2.00±0.11^{c}	3.59±0.18^{e}	1.53±0.13^{b}	1.16±0.06^{a}
CLA c9-t11	0.08±0.01^{abc}	0.06±0.01^{ab}	0.07±0.01^{ab}	0.20±0.02^{e}	0.07±0.01^{ab}	0.14±0.01^{d}	0.07±0.02^{ab}	0.05±0.01^{a}	0.09±0.01^{bc}	0.11±0.02^{c}
CLA t10-c12	0.61±0.07^{a}	3.16±0.18^{e}	1.23±0.07^{c}	0.41±0.07^{a}	1.25±0.14^{c}	0.66±0.09^{a}	0.93±0.06^{b}	1.46±0.09^{c}	0.45±0.07^{a}	2.77±0.12^{d}
(C20:0)- ArachidicAcid	0.29±0.04^{e}	0.17±0.03^{bc}	0.11±0.03^{a}	0.27±0.02^{e}	0.19±0.03^{c}	0.25±0.05^{e}	0.28±0.04^{e}	0.22±0.02^{d}	0.15±0.01^{b}	0.26±0.04^{e}
(C20:1n9)-EicosenoicAcid	1.00±0.12^{de}	0.26±0.04^{a}	0.34±0.05^{b}	1.41±0.10^{f}	0.85±0.14^{d}	0.79±0.09^{d}	1.27±0.08^{e}	0.58±0.07^{c}	3.11±0.11^{g}	0.94±0.13^{de}
(C20:2)-EicosadienoicAcid	0.49±0.09^{d}	0.20±0.02^{ab}	0.37±0.06^{c}	0.90±0.06^{f}	0.35±0.04^{c}	0.28±0.03^{bc}	0.67±0.04^{e}	0.38±0.03^{c}	0.13±0.02^{a}	0.13±0.01^{a}
(C20:3n6)- EicosatrienoicAcid	0.30±0.03^{cd}	0.23±0.03^{abc}	0.26±0.03^{bcd}	0.58±0.04^{f}	0.35±0.03^{d}	0.28±0.05^{cd}	0.52±0.05^{ef}	0.44±0.06^{e}	0.17±0.03^{ab}	0.15±0.03^{a}
(C20:4n6)- ArachidonicAcid	3.83±0.14^{c}	3.18±0.15^{b}	4.68±0.27^{d}	5.65±0.12^{e}	4.49±0.26^{d}	4.03±0.26^{c}	3.89±0.22^{c}	2.65±0.12^{a}	3.93±0.15^{c}	3.98±0.10^{c}
(C20:3n3)-EicosatrienoicAcid	0.39±0.04f^{g}	0.16±0.01^{abc}	0.24±0.03^{cd}	0.47±0.06^{g}	0.41±0.03^{g}	0.27±0.04^{de}	0.22±0.03^{bcd}	0.32±0.02^{ef}	0.13±0.02^{a}	0.14±0.03^{ab}
(C20:5n3)-EicosatrienoicAcid	3.78±0.07^{b}	10.81±0.38^{f}	4.94±0.31^{c}	0.97±0.09^{a}	7.93±0.10^{d}	5.30±0.18^{c}	7.71±0.27^{d}	9.38±0.33^{e}	3.43±0.17^{b}	7.81±0.17^{d}
(C22:1n9)-ErucicAcid	0.27±0.02^{cde}	0.53±0.05^{f}	0.31±0.03^{e}	0.29±0.04^{de}	0.14±0.01^{ab}	0.28±0.03^{cde}	0.21±0.04^{bc}	0.10±0.02^{a}	0.15±0.01^{ab}	0.22±0.03^{bcd}
(C22:2)-DocosadienoicAcid	2.14±0.09^{g}	0.47±0.03^{b}	0.60±0.08^{b}	2.22±0.13^{g}	0.18±0.03^{a}	1.79±0.18^{f}	0.84±0.09^{c}	0.85±0.08^{c}	0.12±0.02^{a}	1.12±0.09^{d}
(C24:1n9) Nervonic Acid	9.34±0.40^{e}	3.21±0.35^{c}	2.87±0.19^{bc}	4.49±0.24^{d}	2.17±0.15^{a}	1.84±0.12^{a}	2.01±0.33^{a}	2.43±0.29^{ab}	1.94±0.17^{a}	2.89±0.14^{bc}
(C22:6n3) Docosahexaenoic Acid	0.62±0.07^{a}	6.81±0.31^{c}	7.16±0.27^{c}	10.54±0.22^{e}	5.37±0.44^{b}	11.45±0.24^{f}	8.93±0.24^{d}	4.93±0.33^{b}	9.22±0.53^{d}	14.24±0.31^{g}
SFA	41.60±0.97^{e}	33.57±0.69^{c}	31.07±0.70^{b}	33.52±0.28^{c}	27.83±0.20^{a}	36.48±0.64^{d}	31.30±0.37^{b}	32.39±0.41^{bc}	36.81±0.28^{d}	37.77±0.37^{d}
MUFA	37.90±0.50^{d}	35.98±0.52^{c}	39.96±0.18^{e}	35.73±0.56^{c}	44.34±0.18^{f}	32.35±0.22^{b}	33.35±0.29^{b}	39.27±0.19^{e}	41.43±0.44^{f}	27.91±0.23^{a}
PUFA	18.92±0.52^{a}	30.40±0.21^{e}	28.85±0.68^{cd}	29.48±0.59^{de}	27.59±0.35^{c}	30.86±0.74^{e}	35.18±0.68^{f}	27.88±0.49^{c}	21.72±0.21^{b}	34.09±0.40^{f}
ω-3	6.33±0.08^{a}	19.73±0.38^{f}	15.37±0.43^{c}	13.96±0.22^{b}	17.19±0.71^{d}	19.03±0.29^{ef}	18.86±0.22^{ef}	18.21±0.20^{de}	14.31±0.24^{b}	23.35±0.38^{g}
ω-6	9.28±0.19^{b}	6.77±0.09^{a}	11.23±0.13^{c}	11.79±0.36^{c}	8.55±0.28^{b}	8.96±0.37^{b}	13.81±0.48^{d}	6.93±0.29^{a}	6.61±0.02^{a}	6.62±0.04^{a}
ω-3/ ω-6	0.69±0.00^{a}	2.91±0.05^{e}	1.37±0.03^{b}	1.18±0.02^{b}	2.01±0.14^{c}	2.13±0.08^{c}	1.37±0.04^{b}	2.63±0.11^{d}	2.16±0.03^{c}	3.53±0.08^{f}
AI	0.85±0.02^{g}	0.74±0.03^{f}	0.64±0.03^{cde}	0.60±0.01^{cd}	0.49±0.00^{a}	0.67±0.03^{e}	0.54±0.01^{ab}	0.60±0.01^{bc}	0.66±0.01^{de}	0.76±0.02^{f}
TI	0.92±0.03^{f}	0.37±0.01^{ab}	0.41±0.01^{cd}	0.48±0.00^{e}	0.33±0.01^{a}	0.44±0.01^{d}	0.36±0.00^{ab}	0.38±0.00^{bc}	0.51±0.00^{e}	0.39±0.01^{bg}

*: Values shown with different letters on the same line are statistically different from each other ($p<0.05$).

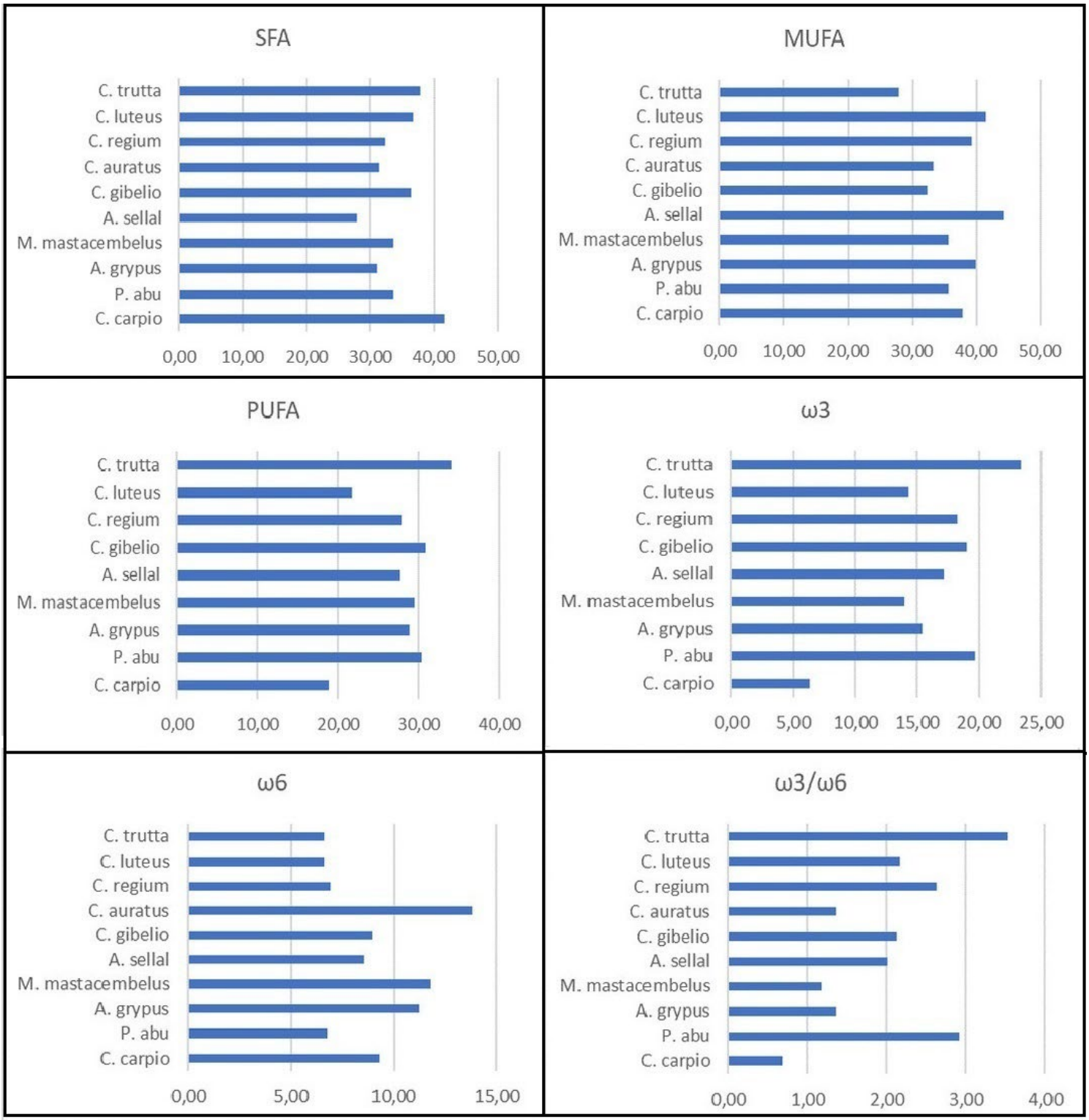

Figure 1. Some fatty acid values of the studied species (%)

The AI and TI indices are indicators of lipid quality that depend on the relative content of particular fatty acid groups, showing the potential effects of lipids on diet quality and the development of coronary disease (Ulbricht and Southgate, 1991; Cengiz et al., 2010; Kaçar and Başhan, 2016). AI and TI are desired to have low values, as they are indices of coronary diseases. It is recommended that AI and TI values in the diet should be less than 1.0 and 0.5, respectively (Woloszyn et al., 2020). In this study, the lowest AI and TI values were determined in the A. sellal species (0.49 and 0.33). The highest values were determined in C. carpio species (0.85 and 0.92). Kaya and Turan (2008) determined the AI and TI values of anchovy as 1.52 and 0.36 (January). Fallah et al. (2011) found it to be 0.45 and 0.33 in wild rainbow trout, respectively. Linhortova et al. (2018) found AI and TI values lower than the stated values in 13 different freshwater fish. They found a high TI value (0.61) only in Nile Tilapia.

Conclusion

In this study, 26 different fatty acid compositions detected in 10 different species living in Atatürk Dam Lake were compared. The ratio of ω3/ω6, which is used to compare the relative nutritional values of fish oils and which should be in balance for normal growth and development, was determined as 0.69 in *C. carpio* species and 3.53 in *C. trutta* species. When the species are examined in terms of ω-3 fatty acids content, which are important for health, it can be said that *C. trutta, P. abu, C. gibelio, C. auratus, C. regium, A. sellal* species are good sources. However, *C. carpio* was the poorest species in terms of ω3. Considering the AI and TI indices, it can be suggested that it would be more suitable for *A. sellal* .

Compliance with Ethical Standards

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

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

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Mitochondrial DNA sequence analysis of *Arabibarbus grypus* (Heckel, 1843) living in Great Zab (Erbil, Iraq)

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ABSTRACT

Arabibarbus grypus (Heckel, 1843), which naturally lives in the Euphrates and Tigris River systems and is endemic, is an economically important fish species consumed by humans. Since the population of this species, which is hunted by both fishermen and local people, is decreasing day by day, its genetic characteristics need to be determined. This study aims to determine the genetic characteristics of *A. grypus* samples living in Great Zab based on sequence analysis of mtDNA *cyt b* and mtDNA *d loop* gene regions. Total DNA isolation was performed from muscle tissue using the kit. Then, Polymerase Chain Reaction was applied through specific primers to mtDNA *cyt b* and mtDNA *d loop* gene regions, and the target regions were amplified. The products with the target length were sent to the commercial firm and sequence analysis was performed. Regarding these specimens living in Great Zab, two haplotypes were determined for the mtDNA *cyt b* gene region and five haplotypes for the mtDNA *d loop* gene region. These haplotypes were compared with the haplotypes in the gene bank and the results were evaluated. Some important data has been obtained regarding the conservation and management of this fish species. Since three new haplotypes were detected for the *d loop* region in this studied locality, it is important to include the samples in this locality in conservation studies.

Keywords: *Arabibarbus grypus*, mtDNA, Euphrates River, Tigris River

Introduction

Arabibarbus grypus (Heckel, 1843) is a member of the Cyprinidae family and is one of the most valuable fish in the world in terms of meat quality (Olgunoglu et al., 2011; Moradkhani et al., 2020). This fish, popularly known as Şabut (Shabout) fish, is an endemic species living in the Euphrates and Tigris river systems in Türkiye, Iraq, Iran, and Syria (Nikpey, 1996; Abdoli, 2000; Göçer, 2022). There are many studies on *A. grypus* living in this basin, some of which are as follows; growth, reproduction and heavy metal characteristics (Oymak et al., 2008; 2009); spermatological and hematological features (Dogu et al., 2014; Khodadadi et al., 2016), genetic features (Parmaksız et al., 2017; 2018). The delicious meat of the *A. grypus* species causes it to be preferred by the local people and increases its economic importance. Therefore, there is excessive hunting pressure on the populations of this species and it is known that its stocks are decreasing day by day (Parmaksız and Şeker, 2018). This species is sensitive in the "VU" (Vulnerable/Sensitive) category according to the 2020 IUCN criteria (Bayhan, 2021). Both the protection of these diminishing resources and the cultivation of this species have gained importance today. However, no measures have yet been taken to protect this species. To be evaluated, especially in terms of production, the individuals to be raised must adapt to the environmental conditions, be resistant to diseases, and the meat quality must be at an optimum level. Populations that will provide these characteristics must also have high genetic diversity. Therefore, first of all, populations in different locations should be evaluated genetically, and the similarities, differences and genetic diversity levels of the populations should be revealed. There are many genetic markers used in genetic studies, but in recent years, mtDNA markers have become popular with the developments in sequence analysis (Liu and Zhou, 2016). Therefore, mtDNA is used for population genetics in different species and can provide information about genetic structure.

In this study, it was aimed to establish genetic data by sequence analysis of mtDNA *cyt b* and mtDNA *d loop* gene regions in *A. grypus* individuals living in Great Zab, near Erbil province in Northern Iraq, and to compare them with the data in the gene bank to determine similarities and differences. Thus, an important data set will be created in terms of providing some of the preliminary information necessary for management and conservation studies.

Material and Methods

The material for this study was created by purchasing nine randomly selected fish from the fish caught by the fishermen living in the Great Zab close to the Erbil region. From these samples, 1 g of the muscle tissue at the base of the dorsal fins was taken and placed in microcentrifuge tubes containing 90% ethyl alcohol and stored in the refrigerator at +4°C until DNA isolation.

In this study, total DNA isolation was performed from muscle tissue using the GeneJET Genomic DNA Purification Kit (Thermo Scientific). Total DNA was obtained by applying the protocol in this kit. To control the presence of DNA, 2 µl of DNA samples from each individual were taken, and 2 µl of dye (2x loading dye) was added to 1% agarose gel added to SYBR Green, and placed in a tank containing 0.5x TBE (Tris/Boric acid/EDTA Buffer) solution. It was run at 120 V electrophoresis for 30 minutes and visualized in the device emitting ultraviolet (UV) light (Figure 1a).

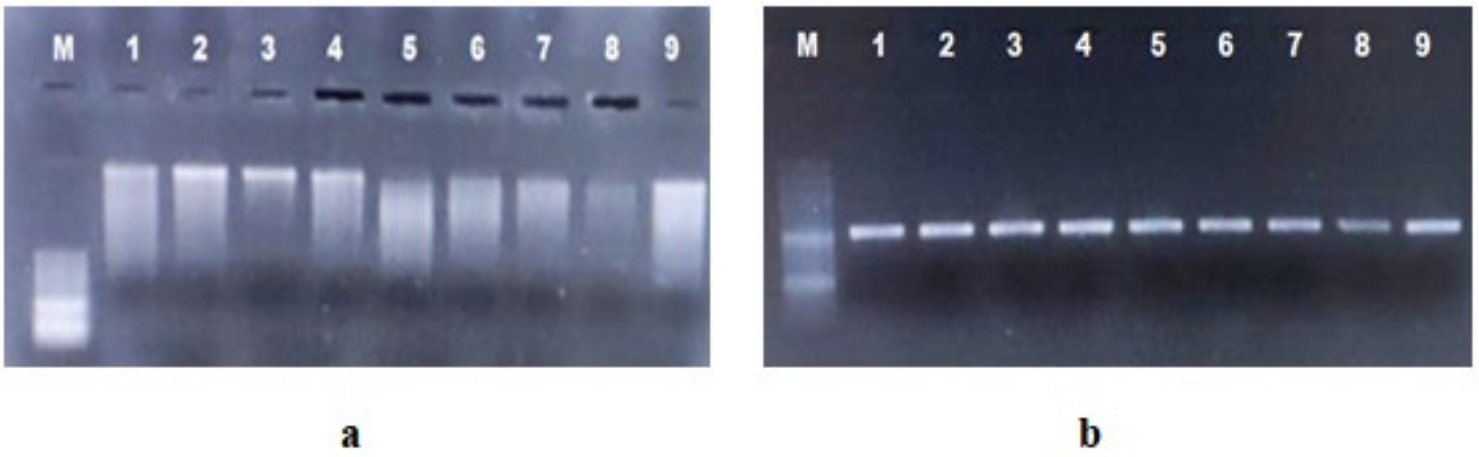

Figure 1. Individuals of the *A. grypus* species: a) Total DNA, b) Agarose gel image of mtDNA *cyt b* PCR products (M: Marker)

In this study, the Polymerase Chain Reaction (PCR) process was carried out in a thermal cycler (BIO-RAD T100™) device. The primer sequence used for the mtDNA *cyt b* gene region was taken from Briolay et al. (1998), and all PCR chemicals and conditions were made according to the criteria in the Parmaksız and Şeker (2018) study. The primer sequence used for amplification of the mtDNA *d loop* region is Iguchi et al. (1997), taken from Inoue et al. (2000) studies, and all PCR chemicals and conditions were adapted according to Oymak and Parmaksız (2018) study. PCR conditions; 3 minutes initial denaturation at 95 °C, and 35 cycles of 30 seconds at 95°C for denaturation, 30 seconds at 58°C for *cyt b* and 51°C for *d loop* annealing and 45 seconds at 72 °C for extension, and a final extension at 72 °C for 10 minutes. PCR mixture used to amplify *cyt b* and *d loop* regions are as follows; a total volume of 25 µL containing 1x PCR buffer, 1 unit Taq polymerase, 2.5mM MgCl2, 0.5 mM of each primer, 0.2 mM of each dNTP, and approximately 50 ng of template DNA. A 1.5% agarose gel was used to control the products formed after the PCR process. The agarose gel added to SYBR Green was placed in the tank containing 0.5x TAE solution and loaded into the wells with 5 µl of PCR products and 5 µl of dye, then run at 120 V electric current for 30 minutes and displayed on a UV light-emitting imaging device (Figure 1b). Target-length PCR products were sent to a commercial firm and sequence analysis was performed on the 3500 XL Genetic Analyzer (Thermo Fisher Scientific).

Data Analysis

Raw data of mtDNA *cyt b* and mtDNA *d loop* sequences were evaluated using the FinchTV 1.4 program, and sequences of all individuals were aligned using BioEdit software version 7.2.5. Those with the highest similarity to the sequences of the *cyt b* and *d loop* regions found in the NCBI Genbank were included in this study. Relationships between haplotypes and Neighbor-joining tree phylogenetic analyzes were performed in the MEGA X program according to the K2 parameter model and the phylogenetic tree was created (Kumar et al., 2018). A bootstrap test (1000 replicates) was used to test the reliability of tree branches (Nodes).

Results and Discussion

Sequences of the mtDNA *cyt b* region with an average length of 580 bp were obtained at the end of this study and the same species in the NCBI gene bank were evaluated together and are shown in Table 1.

As a result of the analysis of the sequences obtained from the *cyt b* gene region, it was seen that the samples taken from the Erbil locality consisted of individuals with H1 and H2 haplotypes. As a result of the analyzes of all individuals, a total of five variable regions and five haplotypes were determined for the cyt b gene region. The H1 haplotype has been seen in all countries and all localities, including Türkiye, Iraq, Iran, and Syria, and is the ancestral haplotype. The phylogenetic tree of haplotypes is shown in Figure 2.

In the phylogenetic tree created according to the analyzed *A. grypus cyt b* haplotypes, kinship relationships for a total of five haplotypes emerged. Accordingly, it is seen that the haplotypes H4 - H5 and H2 - H3 are located closer to each other.

The analysis of the sequences obtained from the *d loop* gene region, it was seen that the samples taken from the Erbil locality consisted of individuals with H1, H2, H3, H4 and H5 haplotypes. As a result of the analyzes of all studies, a total of twelve variable regions and seven haplotypes were determined for the *d loop* gene region (Table 2). The H4 haplotype was seen in all localities, including Türkiye and Iraq, and it can be said to be the ancestral haplotype. The phylogenetic tree of haplotypes is shown in Figure 3.

Table 1. Haplotypes and information about the mtDNA *cyt b* region of *A. grypus* species

Haplotype	Accesion Number	Observed Locations	Reference
H1	ON921337	Erbil, Siverek, Bozova, Çermik, Dicle; Iraq, Türkiye	Parmaksız and Şeker, 2018
	KF876028	Dayr az Zawr; Syria	Borkenhagen, 2014
	KF876025	Rūd-e Mand; Iran	Borkenhagen, 2014
	AF145945	Adıyaman, Diyarbakır; Türkiye	Durand et. al., 2002
	KF876027	Rūdkhāneh-ye Kheyrābād; Iran	Borkenhagen, 2014
H2	ON921338	Erbil, Dicle; Iraq, Türkiye	Parmaksız and Şeker, 2018
H3	ON921339	Bozova; Türkiye	Parmaksız and Şeker, 2018,
	KF876026	Rūdkhāneh-ye Kheyrābād; Iran	Borkenhagen, 2014
H4	ON921340	Çermik, Dicle; Türkiye	Parmaksız and Şeker, 2018
H5	ON921341	Dicle; Türkiye	Parmaksız and Şeker, 2018

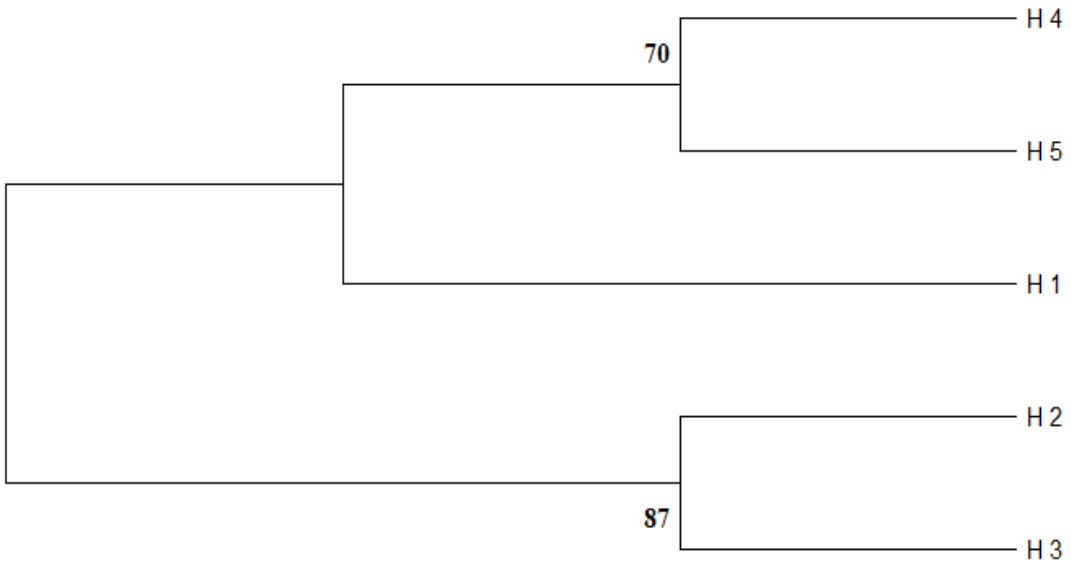

Figure 2. A neighbor-joining tree of *A. grypus* based on observed haplotypes of *cyt b* gene sequences

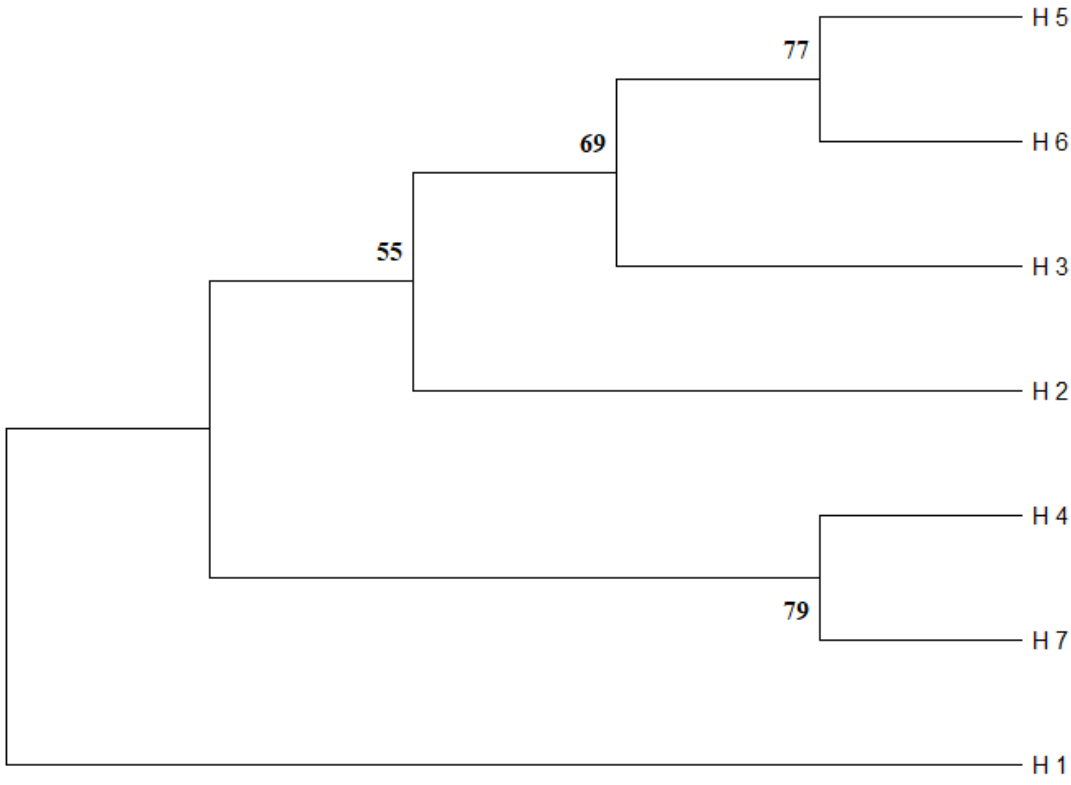

Figure 3. A neighbor-joining tree of *A. grypus* based on observed haplotypes of *d loop* gene sequences

Table 2. The haplotypes and information from the mtDNA *d loop* region of the *A. grypus* species

Haplotype	Accesion Number	Observed Locations	Reference
H1	OP234423	Erbil, Dicle; Iraq, Türkiye	This study, Oymak and Parmaksız, 2018
H2	OP234424	Erbil; Iraq	This study
H3	OP234425	Erbil; Iraq	This study
H4	OP234426	Erbil, Siverek, Bozova, Çermik, Dicle; Iraq, Türkiye	This study, Oymak and Parmaksız, 2018
H5	OP234427	Erbil; Iraq	This study
H6	OP234428	Bozova; Türkiye	Oymak and Parmaksız, 2018
H7	OP234429	Dicle; Türkiye	Oymak and Parmaksız, 2018

In the phylogenetic tree created according to the analyzed *A. grypus d loop* haplotypes, a total of seven haplotypes were related to each other. Accordingly, it is seen that haplotypes H5-H6 and H4 -H7 are located closer to each other.

The Great Zab locality, which was taken as an example in this study, is close to the city of Erbil and this fish species is caught by fishermen and local people. The local people consume the fish caught. Since the populations of these endemic fish species are decreasing day by day, breeding has gained great economic importance. To maintain the stocks of this fish species, which is, considered an alternative to carp or trout in inland fish farming (Gökçınar, 2010), its genetic diversity should be known very well to obtain high yields from these stocks. Because the degree of genetic diversity can be an indicator of the continuity of the population. For the future of this species, the unique genetic heritage found in different localities must be protected by taking necessary precautions. In addition, in the case of breeding this fish species, it is of great importance to take broodstocks from all localities and to create a population that will accommodate each haplotype. Although there is a no different genetic structure in terms of *cyt b* haplotype in the samples taken from the Erbil locality, it is noteworthy that the H2, H3 and H5 haplotypes for the *d loop* region are only in this region. Since phylogenetic analyzes were generally performed on this species, it was possible to find sequences related to *cyt b* in the gene bank. Since the *d loop* region is generally used in studies on population genetics and population genetics is limited for this species, it is expected that the *d loop* data in the gene bank will be low. For the *d loop* region detected only in Erbil, there is a strong possibility that some haplotypes will be found in similar localities if other localities are also studied.

Conclusion

The destruction or change of habitats as a result of anthropogenic effects, especially in localities close to city centers, may cause both populations and species diversity to decrease and even some species to disappear (Parmaksız et al., 2022). The decline of individuals in their natural populations can cause the disappearance of unique genotypes found nowhere else, and when this genetic information is lost, it is almost impossible to recover it (Parmaksız, 2020; 2021). Therefore, urgent measures should be taken for all factors that reduce the genetic diversity of susceptible species. Because genetic diversity directly reflects the ability of species or populations to adapt to environmental factors in alien environments (Frankham et al., 2002; Spielman et al., 2004). Therefore, it will be more beneficial for future studies to determine the genetic diversity of all populations of this fish, especially by using *d loop* and microsatellite markers, and to carry out detailed conservation and brodstock studies based on this data.

Compliance with Ethical Standards

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

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Aquat Res 6(1), 26-42 (2023) • https://doi.org/10.3153/AR23004 *Research Article*

Production and marketing practices of mangrove crab industry towards sustainability in Bataan, Philippines

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ABSTRACT

This showed a comprehensive study on the mangrove crab industry in Bataan, Philippines. Mangrove crab growers (35%) were between 51-60 years old, mostly married with 5-7 family members. Farming was highest (30.77%) between 6-10 years while some were farming for 40 years (1.29%). Monthly income has strong positive correlation (r=676) with production area. *Scylla serrata*, *S. olivacea,* and *S. tranquebarica* were identified using traditional ecological knowledge (TEK). Results revealed 69.57% of the pond used for farming was 1-10 ha employing the polyculture method. About 52.17% used coin-sized crablets while 39.13% fly-sized. Annual production was less than a ton for 46.38% growers while 1.45% have >5 tons. Annual income showed a strong positive correlation (r=0.886 and r=0.815) with production area and annual production, showing a significant difference (p<001). Harvested crabs were marketed live in Orani Port. Grading and pricing were due to sex, weight, and characteristics. Problems include the implementation of FAO 264 s. 2020, weather, market practice, pandemic, and high rejections. Men and women play significant roles where laborious were perceived to be men's sphere while less laborious and financial aspects were for women.

Keywords: Mangrove crab industry, Sustainability, Marketing practices, Crab production, Gender role

Introduction

Mangrove crab (*Scylla spp*.) are widely distributed in the Indo-Pacific Region from Hawaii, Southern Japan, Taiwan, Australia to the Philippines ((Bhuiyan et al., 2021) especially those inhabiting marine or brackishwater areas. In the Philippines, these are considered high value resources abundant in the area (Vince Cruz et al. 2015) thus, many fishers depend on crabs as their livelihood (Castrence-Gonzales et al., 2018). Farming of mangrove crab (formerly known as mud crab) species has long been established in the Philippines including *Scylla serrata*, *S. tranquebarica* and *S. olivacea,* although the preferred species for growing is *S. serrata* (Quinitio and Parado-Estepa, 2017; Orario et al. 2021*).* Morphologically, *S. serrata* has a pointed dactyl prominence same with *S. tranquebarica* while *S. olivacea* is blunted as described by Orario et al. (2021). In terms of the inner carpus spine, it is present in the first two species while absent in the former. These are locally known as "alimango" fetching high value for local and export markets (Orario et al., 2021) making it a great livelihood opportunity making it prone to overfishing as an evident of decreasing harvest over the years (*Castrence-Gonzales et al., 2018)*. The main source of stocks for mangrove crabs are still from the wild-sourced crablets due to its limited supply from the hatcheries (Vince-Cruz-Abeledo et al. 2020).

Mangrove crabs signifies a major economic resource in the country (Vince Cruz et al. 2015) with Northern Mindanao and Central Luzon as major producers (Philippine Statistics Authority 2020). In fact, Central Luzon contributed a mangrove crab production of 24.06%, 20.89% and 22.25% in the national production in the years 2017, 2018 and 2019, respectively earning Php 2,109,462.96 in 2019. In Central Luzon, Bataan is known for bulk production in the region (Philippine Statistics Authority 2020) as the province is known to yearly celebrate "*Alimango Festival*" commanding high prices due to the quality of its meat (Vince-Cruz et al. 2015) and aquaculture qualities. In fact, Bataan ranked 2nd highest-producing province next to the province of Pampanga (Philippine Statistics Authority 2020).

Mangrove crab industry in the province of Bataan starts from either crab gatherers (from the open sea or mangrove areas) or aquaculture which are performed in brackishwater ponds primarily dependent to trash fish and/or shellfish. While considered as trivial product in shrimp in Southeast Asian countries (Sultana et al. 2019), mangrove crab industry has now emerged as an alternative and profitable livelihood when cultured with other aquaculture species. Mangrove crab are good aquaculture candidate as these are less susceptible to disease, easier to culture, more resistant to adverse environmental conditions, and has a high commercial value and market price both locally and internationally thus, provides a source of income (Salam et al. 2012) for coastal fisher folk of Bataan province. Marketing of harvested or collected mangrove crab in the province is a complex system including various middlemen which is both beneficial and disadvantageous, especially to the producers.

Production, marketing and actors in the mangrove crab industry all play integral part in the mangrove crab industry. This is composed of series of intermediaries with their own unique roles in the chain. While studies on mangrove crab have been conducted in the country, comprehensive study on the production and marketing of mangrove crab in the province of Bataan is still in paucity. In spite of its increasing demand and great export potential, issues on different activities in the industry are determined for a sustainable production and food security in the province.

This study was conducted to assess the present status of mangrove crab industry which shall cover the socio-demographic profile of farmers and aquaculture practices. This also showed the marketing network extent in the province and the challenges encountered in the process. In addition, this showed marketing system, actors and their roles in the chain, constraints and challenges encountered in the industry and the role of men and women in the province's mangrove crab industry.

Material and Methods

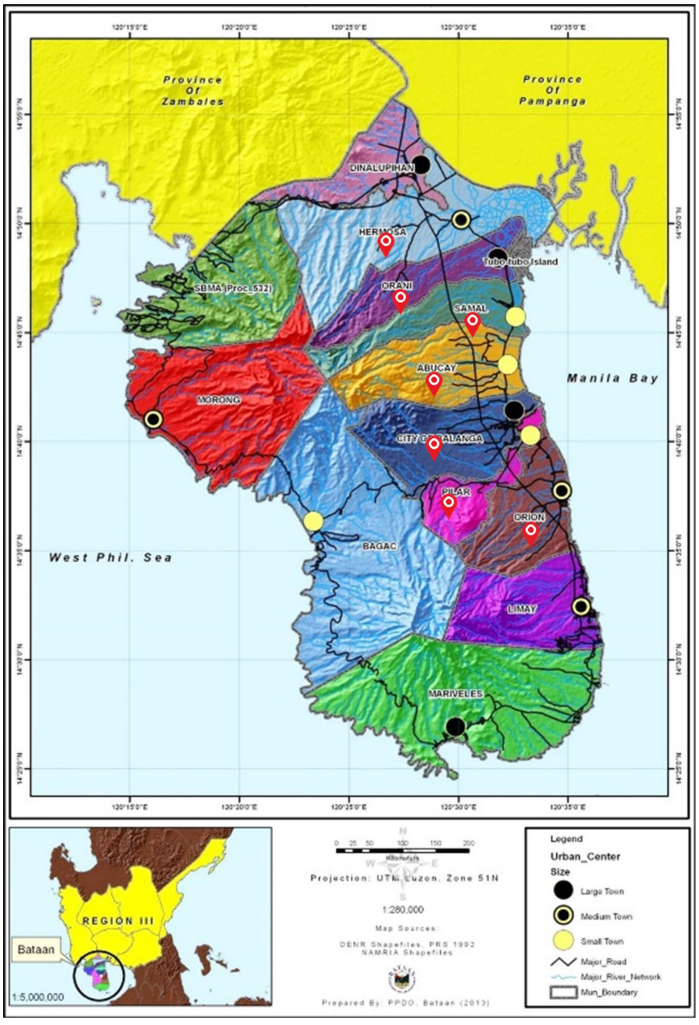
Study Area

The study was conducted from January 2021 to June 2021 in the province of Bataan. The province is a peninsula facing South China Sea to the west and Subic Bay to the northwest, and encloses Manila Bay to the east. It is located 14°41′06″N 120°25′55″E. Study areas were Hermosa, Orani, Samal, Abucay, Pilar, Orion and City of Balanga Identified locations (red locators) were of the major producers of mangrove crabs in the province.

Data Collection

Structured and semi-structured questionnaire were used to primarily collect data from all actors in the mangrove crab industry following and modifying models from SEARCA (2017), Sulatana et al. (2019) and Bhuiyan et al. (2021). Respondents included three (3) input suppliers, nine (9) crab gatherer colleting marketable mangrove crab from the open

sea or mangrove areas, 69 crab farmers, 50 intermediaries and 20 consumers. Key Informant Interview (KII) and Focus Discussion Group (FGD) were applied to collect information from a wide range of actors. All data were recorded. Direct observation during culture and actual marketing activities were also done to observe production and marketing extents in mangrove crab industries. Marketing dynamics were observed and noted including grading, pricing and mode of payments. Secondary data have been collected from published articles, Philippine Statistic Authority (PSA) and from Local Government Units (LGUs). These were used in describing the actors, their roles and interrelationships among other actors.



Source: Bataan provincial planning office

Figure 1. Location of the study

Data Analysis

Both qualitative and quantitative analyses were used in treating the collected data for this study. Qualitative data were encoded and analyzed using Microsoft Excel. Qualitative data were prepared, organized, reduced to themes and were represented in tables, charts and graphs as part of the discussion. For quantitative analysis, data were encoded and analyzed using Microsoft Excel and SPSS 16.0. Data were collected, organized, encoded, treated and interpreted. Results were represented in graphical and tabular form.

Results and Discussion

Mangrove Crab Respondent's Socio-Demographic Profile

Mangrove crab production in the province is primarily comes from aquaculture. Data showed that most of the mangrove crab growers and collectors are between 51-60 years old with 35% of the total population while only 3.84% are between 20-30 years old (Table 1). There are about 92.31% of the respondents were married with a family size of mostly between 5-7 members with 57.69% of the total population (Table 1). Respondents engagement in the mangrove industry were fish farmers (caretakers) at about 67.95% while owners (also fish farmers) were 20.51% and 11.54% were gatherer from the open sea and mangrove areas. The results showed that about 30.77% of the respondents were engaged in mangrove crab industry for 6-10 years, 25.64% were engaged between 21-20 years, 19.23% were engaged for about 11-20 year while there were 10.26% of about 31-40 years of engagement and only 1.28% has above 40 years of engagement, respectively.

The income of farmers and collectors vary from activities, years of engagement and other internal benefits agreed upon. The results showed that 53.85% of the respondents earned Php 5,000-Php 10,000 per month, 32.05% earned Php 10,000-Php 20,000, 10.26% earned below Php 5,000 and 3.85% earned Php 21,000-Php 30,000 (Table 1) with 69.57% respondents with farming a 1-10 ha production area.

Monthly income of the mangrove crab growers, specifically the caretakers (n=53), was correlated with the years of their engagement in farming and the area of production. Results showed that the monthly income of farmers had a very weak correlation (r=0.110) with the years of engagement with no significant difference ($p>0.05$) showing that years of their experience has no bearing when with their monthly wages from their employers. On the other hand, correlation of the monthly income and production area showed a strong correlation (r=676) and a significant difference ($p<0.05$) showing

that as the production area increases, the monthly income of mangrove crab growers also increases (Figure 2).

Most frequent number of mangrove crabs and collectors of 51-60 years of age and showing decades of farming proves that mangrove farming, aquaculture in general, has been widely practiced in the province serving as one of the most important livelihoods in the coastal areas. This supports the same findings described by Quinitio (2015) and Quinitio and Parado-Estepa (2017) suggesting that wide and long farming of wild crabs have been overexploited in the country.

Monthly income of crab growers (caretakers) were found to be significantly correlated with the production area. Caretakers are usually paid on monthly salary, commission basis or both. Study of Torres and Ventura (1983) suggested the same salary scheme observed in Central Luzon. In this study, most of the caretakers have a fixed salary monthly and an additional commission upon a good harvest. There were some who worked for a commission basis, as according to employers, this set-up shall ensure the engagement and dedication of the caretakers in performing well. Monthly salary depends on the pond size as it requires more stocks to be cultured and monitored as well as larger size to be handled and managed. This suggests that monthly salary of caretakers was directly dependent with pond size.

Table 1. Socio-demographic profile of mangrove crab producers and collectors

Characteristics	Categories	No. of Respondents	Percentage (%)
Age Group	20-30	3	3.84
	31-40	15	19.23
	41-50	18	23.08
	51-60	28	35.9
	Above 60	14	17.95
Family size	2-4	25	32.05
	5-7	45	57.69
	8-10	7	8.97
	11-13	1	1.28
Civil status	Married	72	92.31
	Single	6	7.69
Engagement	Caretaker	53	67.95
	Pond owner	16	20.51
	Collector	9	11.54
Engagement in mangrove crab industry (Years)	<5	10	12.82
	6-10	24	30.77
	11-20	15	19.23
	21-30	20	25.64
	31-40	8	10.26
	Above 40	1	1.28
Monthly income (Php)	<5,000	8	10.26
	5,000-10,000	42	53.85
	10,000-20,000	25	32.05
	21,000-30,000	3	3.85
Production area (ha)	<1	1	1.45
	1-10	48	69.57
	11-20	5	7.25
	21-30	9	13.04
	31-40	1	1.45
	Above 40	5	7.25

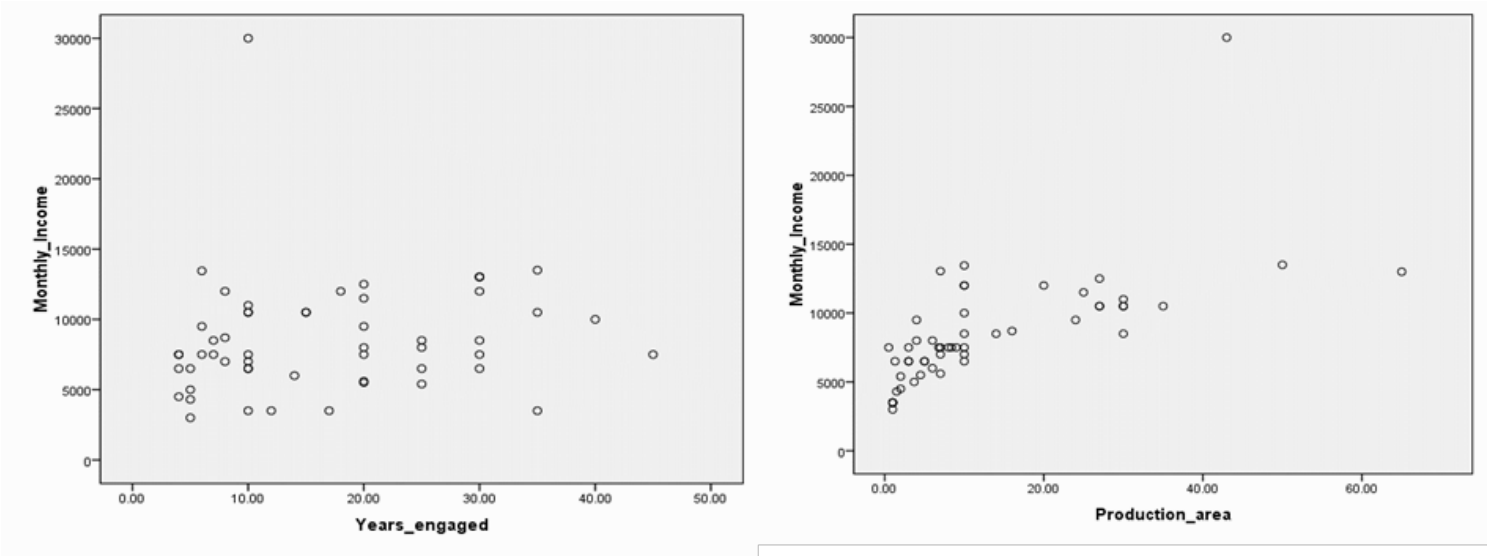


Figure 2. Correlation (r) of monthly income with years of engagement and production area

Production of Scylla spp.

Mangrove Crab Species Cultured in the Province

There were three species produced and collected in the province of Bataan namely Scylla serrata (bulik), S. olivacea (pulahan) and S. tranquebarica (native) (Figure 3). Identification of the species was through observation of the carapace and chelae color. Farmers and collectors identified S. serrata with the one with dark-green color, S. tranquebarica, also called as native by the respondents, was associated with the purplish color and S. olivacea with the red to almost orange in color. This method applied in the identification of species was described by Nakashima et al. (2012) as the traditional ecological knowledge or TEK as cited by Orario et al. (2021). The TEK method was described in the study of Orario et al. (2021) for identifying Scylla juveniles through the color of its ventral carapace and chellae. Apart from it, Bataan farmers also pointed out that same species of the same age, S. serrata was of the largest (Figure 2) among the three.

Of the four of four species in the genus Scylla occurring in the country (Orario et al. 2021), there were three existing in Bataan province namely S. serrata, S. olivacea and S. tranquebarica. Of the three, S. serrata "bulik" was the most preferred species for culture as experience showed that this was the fastest growing species. Despite the preference of S. serrata, results showed wild caught crablets were combination of the three species. This was also revealed by Walton et al. (2006; 2006b) as these species have overlapping ranges.

Left to right: a) Bulik (Scylla serrata); b) Native (S. tranquebarica); c) Pulahan (S. olivacea)

Figure 3. Three species identified in the ponds of Bataan province

Aquaculture Engagement

Results showed that about 69.57% of the mangrove crab growers are faming ponds with 1-10 hectares in size while 13.04% were farming 21-30 hectares. On the other hand, big ponds of 31-40 and above 40 hectares are farmed by 1.45% and 7.25% of mangrove crab growers, respectively. Production cycles are usually done thrice a year with 60.87% while some grow crabs twice a year with 17.39% and four times a year with 21.74%, respectively.

Mangrove crab species grown in the province showed a mixed or combination of species (bulik, pulahan, purplish-Figure 3) grown in ponds with 59.42% while 39.13% disclosed that they have grown a pure "bulik", S. serrata in their ponds. Growers have a low preference with purplish crab with 1.45% and no growers have grown pulahan or S. olivacea solely. Culture method employed in the province was a 100% polyculture i.e. milkfish-crab, milkfish-crab-shrimp, milkfish-crab-prawn.

Results showed that 52.17% of the growers used coin-sized or single-an as seed stocks while 39.13% cultured fly size or langaw langaw. Only 8.70% of the growers revealed using both sizes due to availability and growth performance. Despite the prohibition of Fisheries Administrative Order (FAO) 264 series of 2020 to collect and transport of fly-sized or langaw langaw juvenile, records showed that farmers still opt to use the langaw langaw.

While results showed that majority of the growers (52.17%) used coin-sized as seed stocks, the use of fly-sized crablets was still evident. Fly-sized crablets were also used for grow-out in different provinces including Pampanga, Bulacan and Roxas (Ballad and Bañares 2019). Growers claimed that fly-sized were used due to its lower price compared to coin-sized resulting to same or more harvest. No comparative study has done to verify this claim.

Further, volume of production showed that about 46.38% of the growers are producing less than a ton annually. There were 40.58% harvesting between one to three tons, 10.14% between 3.01 to 5.0 tons while both 1.45% were recorded harvesting 5.01-7 tons and above 7 tons.

There were various pond sizes used in mangrove crab farming in the province used for polyculture of milkfish, crab and/or shrimp. The growers did not recommend monoculture of crab. Crabs were considered as the 'jackpot' species increasing income however, culturing it alone will cost high inputs and the risk of low survival will end up farmers to empty pocket. Polyculture tends to decrease input cost as this could be divided to the species farmed. Khor et al. (2022) revealed that polyculture ponds produce higher aquaculture revenue and profits than monoculture ponds.

Annual income covering the polyculture of aquaculture species showed that 37.68% were earning Php 100,000-Php 200,000 while 24.64% obtained below Php 100,000. Results also showed that 15.94% had earned Php 200,001-Php 300,000, 15.94% had a whooping above Php 500,000 annual income while there were 4.35% who earned Php 400,001-Php 500,000.

Estimated annual income was correlated to production area (ha), annual production and number of cycle per year (Figure 4). Results showed that annual income had a very strong positive correlation with production area (r=0.886) and annual production (r=0.815) showing significance at 0.01 level.

On the other hand, number of cycle per year showed a weak to no correlation to estimated annual income, production area and annual production (Figure 4). Results revealed that number of cropping per year do not necessarily affect the changes in annual income and annual production.

Correlation of estimated income, production area (ha) and annual production suggests that as the production area increases, annual production and estimated annual income also increase for brackishwater ponds. Study suggested that brackishwater ponds could generate a higher profit, but this was largely due to farm size (Irz and Mckenzie 2003), thus the potential to increase stocking density which could lead to higher production.

Marketing

Market Trends

Data collected showed the market trend of estimated mangrove crab supplied in Orani Fish Port for the last three years. Highest recorded supplied mangrove crab was in 2019 with 999,442.50+24,074.63 kgs (Table 3). There were 561,102.50+15,731.46 kgs and lowest observed supplied crab was recorded at 553,437.50+18,026.48 kgs.

Mangrove crabs are mainly marketed in the whole live form. Presently, the major market of mangrove crab and seafood products is Orani Fish Port (Consignacion). Collectors used to sell their product directly to the buyers or at the municipal market at small volume. Larger volume of mangrove crabs have been transported and marketed either at Balanga City public market or Orani Consignment. On the other hand, most of the mangrove crab growers brought their product in Balanga City and Orani Consignment. Nearby province, Pampanga became an option to some municipalities such as Hermosa and Orani when the current price in Bataan was relatively low. While Bulacan and Manila were considered as existing markets for traders, these were seen by most of the growers to be part of their options in the coming cycles.

Crabs are marketed live in the area and sold per piece commanding a higher price than selling it in kilo. Wholesalers supplying exporters and retailers (restaurants and nearby provinces) were directly negotiating in Orani Fish Port.

Table 2. Production engagement of mangrove crab farmers in Bataan province

Characteristics	Categories	Number of Farmers n=69	Percentage (%)
Production area (ha)	<1	1	1.45
	1-10	48	69.57
	11-20	5	7.25
	21-30	9	13.04
	31-40	1	1.45
	Above 40	5	7.25
Number of Cycle Per year	1	0	-
	2	12	17.39
	3	42	60.87
	4	15	21.74
Species cultured	Bulik (*S. serrata*)	27	39.13
	Pulahan (*S. olivacea*)	0	-
	Purplish (*S. tranquebarica*)	1	1.45
	Mixed	41	59.42
Type of culture	Monoculture	0	-
	Polyculture	69	100
Seed size	Fly size	27	39.13
	Coin size	36	52.17
	Mixed	6	8.70
Annual Production (Ton)	Below 1	32	46.38
	1.00-3.00	28	40.58
	3.01-5.00	7	10.14
	5.01-7.00	1	1.45
	Above 7.00	1	1.45
Estimated Annual income (Php)	Below 100,000	17	24.64
	100,000-200,000	26	37.68
	200,001-300,000	11	15.94
	300,001-400,000	1	1.45
	400,001-500,000	3	4.35
	Above 500,000	11	15.94

Adopted and modified from Bhuiyan et al. (2021)

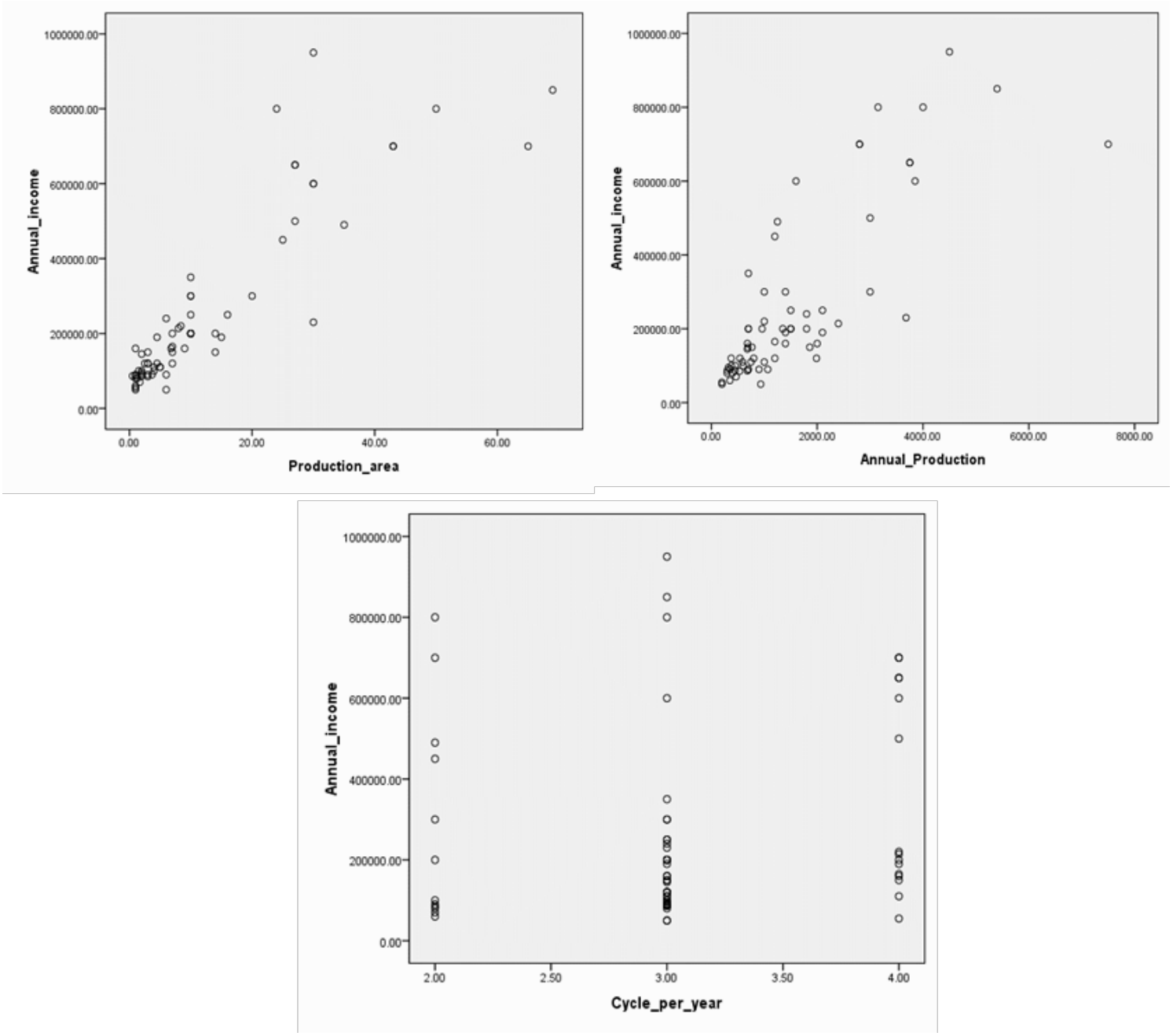


Figure 4. Annual income (Php) in correlation with production area (ha), annual production and cycle per year

Table 3. Mangrove crab quantity supplied, type and markets

Quantity supplied (Kilo)			Product type	Existing market (from the producers)	Potential market
2019	2020	2021			
999,442.50 SD=24,074.63	561,102.50 SD=15,731.46	553,437.50 SD=18,026.48	Live	Local market, Bataan, Pampanga	Bulacan, Manila

Adopted and modified from SEARCA (2017)

Grading and Pricing System

Crabs could be marketed according to grades, weight, meat characteristics and shell condition. Results showed that there were three (3) classifications of mangrove crab for marketing in the province. Classifications were male, female and the immature or locally termed as bakla. Highest grade for males was XL having > 500 g in weight fetching P 1,025.00+230.12 each crab while lowest grade was the reject fetching a price of P179.58+146.79. Rejected crabs were those of different sizes with physical damage, no claws, under sized and with soft shell (Table 4).

Grading of female crabs included primera (F1) for highest grade followed by segunda (F2), tresera (F3) and reject as the lowest grade. Highest grade was usually observed at > 500 g marketed at Php 920+313.89 while reject has the same characteristics as those of the males fetching Php 252.50+106.10 (Table 4).

Immature, locally termed as bakla, are the crabs, which were not fully developed into females. These were graded as primera (F1) and segunda (F2) of weight at about 350 g and 250 g. These were sold at Php 305.25+212.74 and 223.64+100.13, respectively (Table 4).

Grading of crabs vary due to sex, weight, characteristics and markets (exporters and retailers). Results an almost similar grading system of crabs in other countries which vary due to sex, weight and domestic and international market (Mahmud and Mamun 2013; Ferdoushi et al. 2010; and Huq 2010). In this study, exporters decide on what crab sex and weight is demanded for the day. Size grading is almost similar to the Philippine National Standards (PNS) for the male while female was categorized in PNS as XL with >400 g weight. Males were marketed for its full meat and hard shell condition. Females were marketed for its full hard gonad characteristics. These are marketed as due to its partial development of gonad and meat in one.

Market Channels in the Mangrove Crab Industry

Figures 5 and 6 presents the marketing channel of a mangrove crab collector and farmer. The market channel from collecting crabs showed that from crab collector, crab are sold in different ways (Figure 4). Crabs are sold directly to the consumers, supplied to the retailers or brought to the fish port through consignment. Shortest chain was observed in the first channel while longest was recorded in the last chain.

Table 4. Grading and classification of mangrove crab (Scylla spp.)

CLASSIFICATION	GRADE	WEIGHT (G)/PIECE	CHARATERISTIC	SHELL CONDITION	PRICE (Php/kilo)
MALE	XL	≥500	Full meat	Hard	1,025.00±230.12
	L	≥400	Full meat	Hard	820.83±133.92
	M	≥300	Full meat	Hard	662.50±149.43
	S	≥200	Full meat	Hard	288.33±158.74
	REJECT	Variety	No claws, soft shelled, under sized	Hard/Soft	179.58±146.79
FEMALE	Primera (F1)	≥500	Full hard gonad	Hard	920±313.89
	Segunda (F2)	300-400	Full hard gonad	Hard	807.08±260.35
	Tresera (F3)	≥200	Full hard gonad	Hard	681.25±235.76
	REJECT	Variety	No claws, soft shelled, under sized	Hard/Soft	252.50+106.10
IMMATURE (*Bakla*)	Primera (F1)	350	Partial development	Hard	305.25±212.74
	Segunda (F2)	250	Partial development	Hard	223.64±100.13

Adopted and modified from Bhuiyan et al. (2021) and Sultana et al. (2019)

Channels in farming crabs include the input supplier, grower, fish port (consignment, wholesaler, exporter or retailer and consumer (Figure 5). Input supplier served as the link between the crab catchers (usually from Bicol Region) in the chain. All harvested crabs were directly brought in the fish port (Orani, Balanga City or Pampanga) due to its bulk. The first channel was input supplier to grower to fish port to wholesaler to exporter to consumer. The second channel involved input supplier, grower, fish port, wholesaler, retailer and consumer. Exporter in the former involves processors located at Paranaque while retailers in the latter referred to restaurants and local markets of adjacent and nearby provinces.

The present market channels of mangrove crab depend from the origin of the production. Mangrove crab collectors revealed a simple channel directly supplying to the consumer. A more complex channel involving few intermediaries was also found in this study when the volume of catch was high. For mangrove crab growers, channel starts at input supplier passing different intermediaries from consignment, wholesalers to retailers and exporters. Ballad and Bañarez (2019) reported that collection and trading systems of crablets in Cagayan involved series of intermediaries, from gatherers to consolidators to grow-out operators including Bataan province.

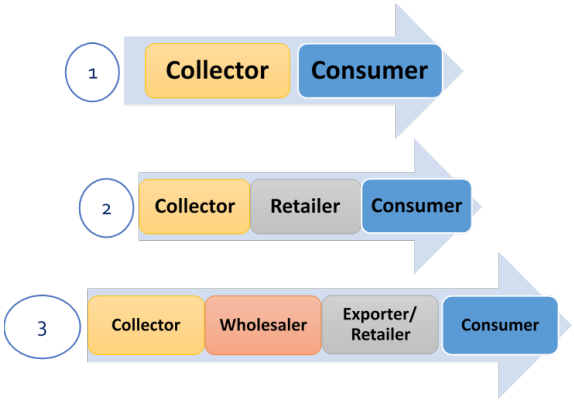


Figure 5. Mangrove crab collector's market channel

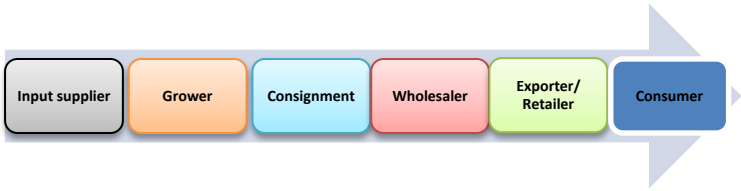


Figure 6. Mangrove crab farmer's market channel

Mode of Payment and Transportation

The payment activity was found to be dependent on the mutual understanding between the parties (Table 5). Payment was done through cash, check or credit in the study area. Input suppliers were found to demand cash-basis payment from the mangrove growers. Study revealed that mangrove crab growers were paid either cash, check or credit. Cash were handed immediately upon completion of the transaction provided that the consignee owner has available cash-on-hand while check was issued in the absence of cash. Credit was practiced between consignment and mangrove crab growers where payment was done between 2-3 days after transaction. Consignment was found to allow credit to wholesalers who travel their products in Manila and practiced payment 5-7 days upon transaction. Study showed that wholesalers demand cash and check basis from exporters while cash and credit basis for retailers. Retailers were allowed to pay the wholesaler 2-3 days upon transaction. Retailers practiced cash-basis from consumers. Credit was practiced through mutual and verbal agreement between parties.

Live mangrove crabs were generally transported to the Fish Ports. Crabs were put in trays with open holes (side and bottom). Some growers were found to be transporting the harvest using boats while others rent vehicles. Areas adjacent to the fish port spent little transportation cost than those who were of a relative distance.

Table 5. Payment scheme in mangrove crab industry

Actor	Mode of payment	Payment scheme
Input supplier to Source	Cash basis	Immediately after transaction
Mangrove crab to Input supplier	Cash basis	Immediately after transaction
Consignment owner to Grower	Cash basis, check or credit	Immediately; 2-3 days
Wholesaler to Consignment owner	Credit basis	5-7 days
Retailer to Wholesaler	Cash; credit	Immediately; 2-3 days
Exporter to wholesaler	Cash	Immediately after transaction

Actors, Roles and Interrelationship

Data were collected through direct interview, FGD and KII. The study revealed that there are two sources of mangrove crab production in the province: collection and aquaculture. The former was considered as minimal to low production contribution while the latter was considered as the major. The marketing channel for aquaculture in the province was found to start from the input supplier while collectors directly gather crabs from the sea. Different actors and intermediaries were discussed in the Table 6.

Actors Roles and Interrelationships

Input supplier The input suppliers are the main source of crablets in the province, thus playing a vital role in the industry. They serve as the link between mangrove crab growers and the crab catchers. Since crab catchers were mostly from Bicol Region, they are the responsible link to order and negotiate desired number from the growers. Growers and input suppliers agree to a certain price to close the deal. Verbal understanding serves as their main agreement. Hundred percent (100%) of the input suppliers were commission basis. Their main inputs were mouth and cellphone communication. They have the capable of controlling the price of crablets. Mangrove crab growers were bound to agree to be able to avail crablets for their pond operation. Payments were made directly to the input suppliers.

Mangrove Grower

Growers are the main producers and key actors who buy crablets of either fly size or coin size. Culture for fly size usually takes 5-6 months while coin size farming takes 3-4 months. Fly size are bought at P5.00/each while coin size were commonly bought at P32.00/each. There were 52.17% growers who used coin size as seed stocks while 39.13% still use fly size and 8.70% of the growers used either of the two despite the Fisheries Administrative Order (FAO) 264 series of 2020 prohibiting the collection and transport of fly-sized or langaw langaw juvenile, records show that farmers still opt to use the langaw langaw size due to its lower price compared to the coin-sized.

Collector

Crab collectors are those collecting crabs seasonally of which most abundant were during the –ber months. Collector collects crabs from the open sea 3-7 times a week with > 3 kgs catch per day. Low volume collected crabs are usually marketed directly to consumers who are waiting in the port or sold directly by house to house. Given a great number of catch, collectors are saving the catch and bring to fish port (consignment) where price is higher.

Fish Port (Consignment Owner)

Consignment, some termed it depot, are the main market of mangrove crab and other sea foods in the province. Orani, Bataan was the most known Consignment. Consignment owners and producers (growers or collectors) commonly have verbal agreement in the marketing of the products. Consignment will sell the harvested mangrove crab at the highest possible price since its income depends on it. Consignment trades the crabs and get 6-7% as commission. In addition, a cha-cha as they termed was also implemented in the consignment on which, every 40-50 pcs of crabs there will be 1 crab deduction which according to the consignment are for the staff. Same goes with shrimp where an actual 15 kgs will be deducted with ½-1 kg as cha-cha. Much as the producers felt aggrieved with cha-cha, they have to agree for them to sell their harvest.

Wholesaler

Wholesalers were the actors who acquire crabs from the consignment. They are the bulk buyers, sometimes called as Viajero (Byahero) bringing the crabs in either exporter or retailer (restaurants) or marketing them in adjacent and nearby provinces. They are sometimes retailers, too. They can control the price of the crabs as they are the direct contacts of the exporters or restaurant owners. If the customers tell that they are in need of this certain crab species and sex at a given amount, the wholesalers negotiate with each other and make an agreement on the ceiling price of the goods. In addition, they also decide if the product could fetch a low or high price. They acquire the products from the consignment in a credit-basis, where they pay the amount due in a week or two. No document serves as binding agreement but verbal negotiation.

Retailer

Retailers are actors who usually acquire crabs from the wholesaler. They could be restaurants or a stall owner in the local wet market selling the goods per piece or per kilogram. Retailers (restaurants) usually change the form of the crabs adding value such as ingredient of soup or added with spices.

Exporter

Exporters are the part of the channel playing significant role in the uplifting of the price. Crabs are bought from wholesalers and the crabs are processed for export. There are about 10 exporters supplied by the wholesalers.

Table 6. Actors and their roles in the mangrove crab industry

Actors	Roles and interrelationships
Input supplier	The input suppliers are the main source of crablets in the province, thus playing a vital role in the industry. They serve as the link between mangrove crab growers and the crab catchers. Since crab catchers were mostly from Bicol Region, they are the responsible link to order and negotiate desired number from the growers. Growers and input suppliers agree to a certain price to close the deal. Verbal understanding serves as their main agreement. Hundred percent (100%) of the input suppliers were commission basis. Their main inputs were mouth and cellphone communication. They have the capable of controlling the price of crablets. Mangrove crab growers were bound to agree to be able to avail crablets for their pond operation. Payments were made directly to the input suppliers.
Mangrove grower	Growers are the main producers and key actors who buy crablets of either fly size or coin size. Culture for fly size usually takes 5-6 months while coin size farming takes 3-4 months. Fly size are bought at P5.00/each while coin size were commonly bought at P32.00/each. There were 52.17% growers who used coin size as seed stocks while 39.13% still use fly size and 8.70% of the growers used either of the two despite the Fisheries Administrative Order (FAO) 264 series of 2020 prohibiting the collection and transport of fly-sized or *langaw langaw* juvenile, records show that farmers still opt to use the *langaw langaw* size due to its lower price compared to the coin-sized.
Collector	Crab collectors are those collecting crabs seasonally of which most abundant were during the –ber months. Collector collects crabs from the open sea 3-7 times a week with $\geq$ 3 kgs catch per day. Low volume collected crabs are usually marketed directly to consumers who are waiting in the port or sold directly by house to house. Given a great number of catch, collectors are saving the catch and bring to fish port (consignment) where price is higher.
Fish Port (Consignment Owner)	Consignment, some termed it depot, are the main market of mangrove crab and other sea foods in the province. Orani, Bataan was the most known Consignment. Consignment owners and producers (growers or collectors) commonly have verbal agreement in the marketing of the products. Consignment will sell the harvested mangrove crab at the highest possible price since its income depends on it. Consignment trades the crabs and get 6-7% as commission. In addition, a *cha-cha* as they termed was also implemented in the consignment on which, every 40-50 pcs of crabs there will be 1 crab deduction which according to the consignment are for the staff. Same goes with shrimp where an actual 15 kgs will be deducted with ½-1 kg as *cha-cha*. Much as the producers felt aggrieved with *cha-cha*, they have to agree for them to sell their harvest.
Wholesaler	Wholesalers were the actors who acquire crabs from the consignment. They are the bulk buyers, sometimes called as *Viajero* (*Byahero)* bringing the crabs in either exporter or retailer (restaurants) or marketing them in adjacent and nearby provinces. They are sometimes retailers, too. They can control the price of the crabs as they are the direct contacts of the exporters or restaurant owners. If the customers tell that they are in need of this certain crab species and sex at a given amount, the wholesalers negotiate with each other and make an agreement on the ceiling price of the goods. In addition, they also decide if the product could fetch a low or high price. They acquire the products from the consignment in a credit-basis, where they pay the amount due in a week or two. No document serves as binding agreement but verbal negotiation.
Retailer	Retailers are actors who usually acquire crabs from the wholesaler. They could be restaurants or a stall owner in the local wet market selling the goods per piece or per kilogram. Retailers (restaurants) usually change the form of the crabs adding value such as ingredient of soup or added with spices.
Exporter	Exporters are the part of the channel playing significant role in the uplifting of the price. Crabs are bought from wholesalers and the crabs are processed for export. There are about 10 exporters supplied by the wholesalers.

Challenges in the Mangrove Crab Industry

Various factors affecting the crab industry have been identified through direct interview and Focus Group Discussion (FGD). Respondents were allowed to choose as many challenges as they can but encouraged them to pick the top concerns. Varying factors have been identified in each actor (Table 7). Seeds and transportation have been marked with 100% as main constraints for the input suppliers. Low to no collection and weather at 100% were the challenges for the collectors. Mangrove crab growersrevealed that weather has the greatest impact in their farming with 79.71% and the price of crablets with 50.72%. Among other challenges faced by the growers include water quality (37.68%), the occurrence of the pandemic (36.23%), marketing practices (21.74%) which includes the deceiving of growers in grading and pricing system and the system of cha-cha.

Traders identified their constraints including the high rejection of the crabs (100%) and occurrence of pandemic (100%). Season of the year which can also be associated with the supply and demand was also identified with 56% response. Consumers on the other hand, all (100%) agreed to have been cheated with the quality of the crabs and buying too expensive crabs. Crabs for the consumers were usually served during meetings, conferences, family occasions and holidays.

Table 7. Challenges in the mangrove crab industry

Actor	Problems	Response	Response (%)
Input Supplier (n=3)	Seeds	3	100.00
	Transportation (Inspection)	3	100.00
Collectors (n=9)	Low to no collection	9	100.00
	Weather	9	100.00
	Water quality	3	33.33
	Garbages	3	33.33
Mangrove crab growers(n=69)	High crablets price	35	50.72
	Weather	55	79.71
	Water quality	26	37.68
	Marketing practices	15	21.74
	Pandemic	25	36.23
	Supply/Demand	14	20.29
Traders (n=50)	High rejection	50	100.00
	Pandemic	50	100.00
	Season (Supply/Demand)	28	56.00
	High Transportation Cost	18	36.00
Consumer (n=20)	Cheated in quality	20	100.00
	High price	20	100.00

Several challenges were observed in every step of the of mangrove crab industry. Input supplier revealed that source and transportation of seeds, specifically the fly-sized, due to the implementation of FAO 264, s. 2020. In has become worse when the pandemic hits the country where mandatory checkpoints were implemented in every boundary. Mangrove crab growersrevealed that most challenging part in aquaculture is having no to little harvest which could be a result of typhoon, mortality and water quality or the combination thereof. Pandemic has also affected the production sector when movements were restricted which caused price increase in aquaculture inputs. Traders disclosed that high rejection rates and pandemic affected their livelihood the most. High rejection was attributed to the quality loss of the product thus, goods were sold at very low price if not thrown away. The pandemic stopped all activities resulting to low income. Both led to economic loss for the traders.

Role of Men and Women in the Mangrove Crab Industry, Fisheries Sector in General

Activities in the mangrove crab industries have been identified (Table 8). Respondents were asked to identify their gender based on the social norms, behaviors and roles associated with woman, man, girl or boy. Respondents were asked to describe how gender plays role in the mangrove crab industry. Trainings were determined from an agency conducting fisheries trainings through KII. Activities were categorized into two: laborious and less-laborious as how the respondents perceived it.

Crab gathering (100%), pond operating/owning (81.25%), pond constructing (100%), pond preparing (94.34%), feeding and monitoring (84.91%), harvesting (100%) and aquaculture training (93.75%) were revealed to be men dominated activities while marketing and trading (74%) and fish processing training (85%) showed a women dominated activities (Figure7). It however revealed that both genders are performing feeding and monitoring (15.09%) and pond preparing (5.66%).

Chi-square test revealed a significant (f=0.000) association between activities and gender. Activities are not independent from gender but the other way around.

The study revealed that men and women both played important roles in the mangrove industry. Activities were categorized as laborious and non-laborious. Laborious activities were those which productive works requiring physical strength, spending more time to do works and decision-making. On the other hand, less laborious activities were defined as lesser physical strength involved, short to limited time of involvement and focus more on financial management. Results revealed that laborious activities were men's sphere while less laborious were for women. Involvement of women in pond preparing and feeding and monitoring (8%) were more on supportive role like handing over supplies and materials. Nabayunga et al. (2021) found out that women could play a supporting role in aquaculture activities.

Table 8. Gender roles in the mangrove crab industry

Category	Activity Indicator	Men	Women	Both	Laborious	Less Laborious
Collection	Crab gathering (n=9)	9	0	0	9	0
Pond owners	Pond operating/owning (n=16)	13	3	0	16	0
	Pond constructing (n=53)	53	0	0	53	0
	Pond preparing (n=53)	50	0	3	53	0
Aquaculture	Feeding & monitoring (n=53)	45	0	8	45	8
	Harvesting (n=53)	53	0	0	53	0
Marketing & Trading	Marketing (n=50)	13	37	0	18	32
	Aquaculture training (n=48)	45	3	0	45	0
Training & education	Fish Processing training (n=140)	21	119	0	140	0

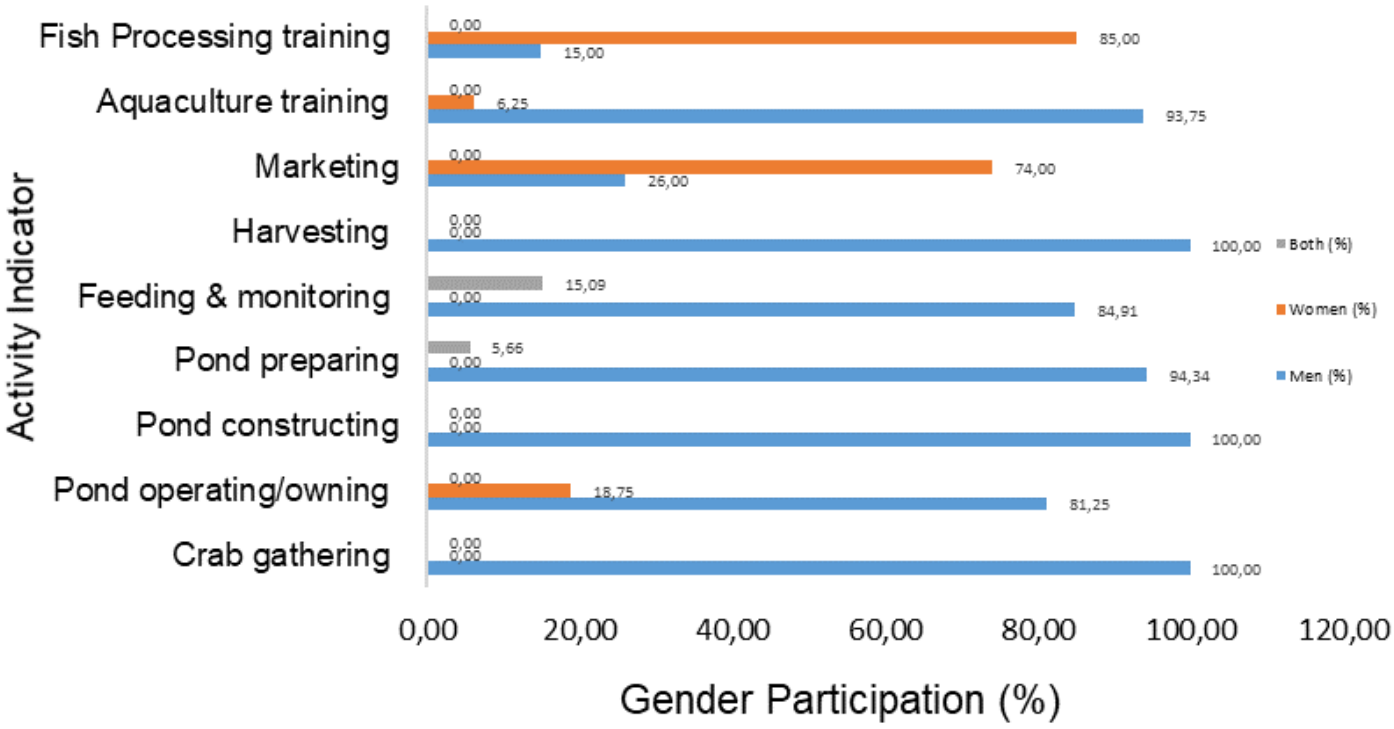


Figure 7. Role of Men and Women in mangrove crab industry

Women, according to men respondents, still need to attend their children at home thus, marketing is suited for them as it only takes few hours. In addition, most of the respondents suggested that women are more particular on finances rather than men. Iguban et al. (2017) reported that the shrimp industry was a men's sphere, where more men were identified to be actively engaged as owners or members of the shrimp catching operation crew. Thus, supporting claims that in fishing communities, women are less engaged in productive work but generally for housework (Iguban et al. 2017)

This study revealed that men acknowledged the roles of women in the mangrove crabs however, men perceived that letting women work on laborious activities was likely to be ungentlemanly. In addition, it would take more time for women to do a man's day job.

Conclusion

Mangrove crabs are valuable seafoods with high demand both for local and export purposes. Crab collection and farming, fisheries in general, has long been a source of livelihood in the province. Majority of the mangrove crab growersworked as caretakers depending on their salary as their main income. Monthly salary was found to be directly dependent on the size of the pond. A 5-10% commission was usually granted upon good harvest. *Scylla serrata* was the most preferred species for culture however, results showed overlapping of three species cultured in brackishwater ponds. Despite the implementation of FAO 264, s. 2020, growers were found to be using fly-sized crablets as seedstocks. Pond sizes vary in the province preferably using polyculture as this shows higher profitability than monoculture.

Intermediaries play significant roles in the crab industry. Input players serve as the main link between crablet catchers while consignment serves as the main link between growers and the market. Highest gross profits and markups was attained by wholesaler as he/she can has the sole control on how to price the crabs from the consignment to the exporters and retailers. Of all actors, crab growers were the most affected as they cannot complain from the price set by the input supplier as they depend their stocks from them. *Cha-cha* implemented by the consignment was found to be not beneficial to growers.

Several problems in the mangrove industry include the implementation of FAO 264, s. 2020 for input suppliers, weather, market practice and crablets price in aquaculture sector while pandemic and high rejections for market traders.

Men and women both play significant roles in the fisheries sector. Laborious/productive activities were perceived to be men's sphere while less laborious and financial aspects were for women. Results of this study is anticipated to be beneficial to different stakeholders to ensure sustainability of mangrove crab and fisheries industry in general.

Compliance with Ethical Standards

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

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

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Investigation of different lighting (LED, HPS and FLO) in aquaponics systems for joint production of different plants (Lettuce, Parsley and Cress) and koi carp

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ABSTRACT

We investigated the effects of growth performance of three plant species parsley (*Petroselinum crispum*), lettuce (*Lactuca sativa*) and cress (*Lepidium sativum*) under the three different lighting sources, Light-Emitting Diode lamp (LED; 200w), High-Pressure Sodium lamp (HPS; 200w) and Fluorescent lamp (FLO; 200w) in an aquaponic system. A total number of 43 koi fish (*Cyprinus carpio* var. *koi*) with 3628 g total biomass (84.4 g per individual) were used. The fish used in the experiment recorded 36% growth and reached an average individual weight of 132.7 g at the end of the experiment. The parsley plant was measured as 8.76 ±7.32 g; 7.45 ±4.13 g; 2.04 ±1.96 g weight after 45 days, the lettuce plant was 54.09 ± 25.60 g; 60.83 ±19.39 g; 17.81 ±6.40 g weight after 54 days, cress plant was 1.03 ±0.58 g; 1.15 ±0.46 g; 1.31 ±0.58 g weight after 42 days, under the HPS, LED, and FLO light sources, respectively. HPS and LED light sources in lettuce and parsley showed better plant development than the FLO, while no significant difference occurred in cress plants under three light conditions. We conclude that using HPS or LED lights in indoor aquaponics has the potential to produce good quality and adequate amounts of plants.

Keywords: Aquaponics, Koi, Light sources, *Petroselinum crispum, Lactuca sativa, Lepidium sativum*

Introduction

Aquaponics combines fish production in a recirculating aquaculture system (RAS) and plant production in a hydroponic unit (Rakocy, 2012; Goddek et al., 2015; Yavuzcan Yildiz, 2017). Considering global climate change, loss of soil productivity and biodiversity, and lack of sources and drinking water, the practice of aquaponics in both commercial and academic fields has dramatically expanded in recent years and has the potential to play an essential role in food production in the future (Goddek et al., 2016; Junge et al., 2017). In addition, aquaponics has the potential to move food production to regions with severe water restrictions. The location of the aquaponics facility close to the town center can reduce the costs associated with the transportation, storage, and processing of vegetable crops (Savidov and Brooks, 2004).

Aquaponic systems are scalable and flexible, featuring systems; therefore, they could be done from small systems to extensive capacity facilities and installed in a broad environment from the basement to the roof, even to the desert. In addition, aquaponics is a suitable food production technique for indoor culture (Yanes et al., 2020). However, artificial lighting must be installed in places where the sun does not reach, such as basements in the city, and to increase efficiency in winter when the sunlight is limited (Hernández and Kubota, 2015). Indoor lighting can be carried out with different lighting types such as fluorescent (FLO), high-pressure sodium (HPS), induction, and light-emitting diode (LED). The source of artificial lighting may dramatically effect on plant anatomy and morphology, food intake, and pathogen development (Massa et al., 2008). Light is a source of energy for photosynthesis and has a signal feature that affects plant growth, flowering timing, and morphogenetic features such as plant height and shape (Xu, 2019). Thus, it is also possible to increase production efficiency by expanding the photoperiod in the seasons when the natural daylight is short and insufficient (Nelson and Bugbee, 2014). Each light source has different electricity consumption, which is another crucial point of view of aquaponics sustainability. Considering that electricity consumption causes the highest cost in aquaponic systems (Forchino et al., 2017), the correct light source selection becomes even more important. In addition, it may be possible to change the light spectrum to suit the welfare of the fish cultured in indoor RAS (Karakatsouli et al., 2010). Light systems impact aquaculture systems' productivity, particularly regarding animal health, growth, and product quality (Tielmann et al., 2017; Bögner et al., 2018). Although research on indoor lighting has been going on for two decades, more information should be learned about the effects of LEDs on a variety of vegetables for larger-scale industrial applications (Olle and Viršilė, 2013). While light-emitting diodes (LEDs) have technical advantages over conventional lighting sources, they have recently been tested only for horticultural applications (Mitchell et al., 2012).

This study aimed to investigate LED, HPS, and FLO light sources in aquaponics systems for joint production of different plants (lettuce (*Lactuca sativa*), parsley (*Petroselinum crispum*) and cress (*Lepidium sativum*)) and koi fish (*Cyprinus carpio* var. koi*).*

Material and Methods

Experimental Design

This research was carried out at Sapanca Inland Fisheries Production Research and Application Unit, Faculty of Aquatic Sciences, Istanbul University. The aquaponics system in which the experiment was conducted consisted of three plant grow beds (220 x 50 x 25 cm) with a volume of 200 L, a circular fish tank with a volume of 750 L, and a sump with a volume of 330 L. Fish reared in a circular fiberglass growing tank which has 750 L water volume. Discharge of nutrient-rich water flows by gravitation from the fish tank into the sump. The mechanical filtration unit removed organic material from the fish tank and let the clean water pass into the biological filter. After the microbial process by which autotrophic bacteria oxidize ammonium to nitrite and then to nitrate, water is pumped to the plant-growing beds by a submersible water circulation pump. Nutrients from the plants and effluent water turn again into the fish tank (Figure 1).

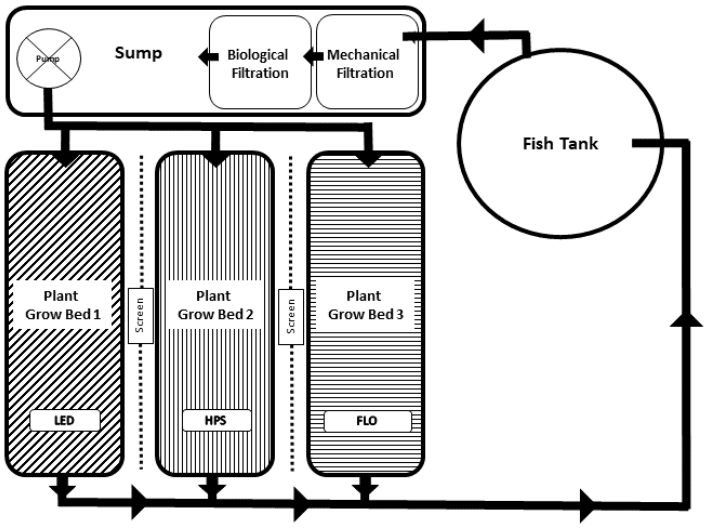

Figure 1. Schematic design of experimental aquaponics system

Light Sources

LED (light emitting diode lamp 200 w, 20000 lumens), HPS high-pressure sodium lamp 200w, 20000 lumens), and Fluorescent (FLO) (200w, 20100 lumens) lamps are used on plant grow beds in the trials. The light sources were adjusted to close standards according to the number of LUX values falling on the leaves and monitored with a digital light meter (MASTECH MS6610, Pittsburgh) (Figure 2).

Figure 2. The plant grow beds under LED (upper left), HPS (bottom middle), and FLO (upper right) lights in the experimental aquaponics system

Fish and Growth Performance

The total biomass of 43 koi fish (*Cyprinus carpio* var. *koi*) used in the experiment was 3628 g (average fish weight 84.37 g). Fish weight and height were measured at two-week intervals. The mean weight of fish was calculated total biomass of fish x number of fish^{-1}. According to Bhaskar et al. (2015), to determine the growth performance of koi fish in the aquaponics system;

Fish Weight Gain (FWG) is calculated as the final weight of fish (g) – the initial weight of fish (g). Fish Growth Rate (FGR) is calculated as (W_2(g) – W_1(g) x W_1^{-1}(g)) x 100. Specific Growth Rate (SGR) was calculated as ($ln W_2 - ln W_1$) x t^{-1} x 100, where W_1 and W_2 were fish weights (g) at the beginning and end of the experiment, and t was the length (d) of the experiment. Feed Conversion Ratio (FCR) was calculated as feed intake (g) x biomass gain^{-1}.

Plant and Growth Performance

In each plant experiment, a total of 84 plant seedlings were used, a total of 28 each under the three different lights on the plant beds. Since three different plants (parsleys, lettuces, and cress) were used, a total of 252 plants were cultivated during all experiments. Measurement of the plant height (cm), root length (cm), and total plant weight (g) of the parsley, lettuce and cress plants were made at the beginning and end of the experiment in each group using scales with a precision of ±0.01 (Radwag, Poland), and the total number of leaves (pieces) were counted (Figure 3).

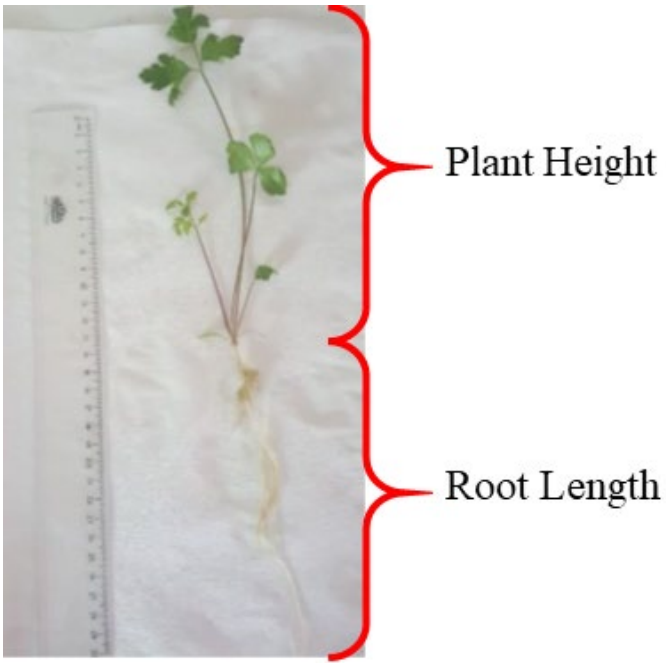

Figure 3. Measurements of plant height and root length

When all the measurements were done, seedlings were transplanted onto the rockwool cubes (cube size: 4.0 cm long x 4.0 cm wide x 4.0 cm high) onto plant growth beds of the aquaponics system, which sets subjected to the different lighting treatments. In the initial plant measurement data, the difference of variation between individuals was kept to a minimum in terms of not creating an effect between groups. The initial values for the plants are summarized in Table 1.

Table 1. Initial plant growth parameter values for plants (parsley, lettuce, and cress)

Parameters	Parsley	Lettuce	Cress
Individual biomass (g)	0.81±0.01	1.50±0.21	0.19±0.05
Plant Height (cm)	5.33±1.67	10.22±0.98	8.24±0.99
Leaf number	1.00±0.01	3.00±0.01	5.14±0.42
Root Length (cm)	3.94±0.63	6.08±0.79	3.63±0.82

In this study, harvest periods were reported as 45 days for parsleys, 44 days for lettuces, and 42 days for cress.

Water Quality Parameters in Aquaponics System

Dissolved oxygen (DO), temperature (°C), electrical conductivity (EC), and pH were checked before fish were fed with extruded pellet feed in the morning daily. Temperature and pH were measured using a 3110 pH meter with SenTix® 41 Epoxy Electrode (WTW, Germany), DO was measured with an oxygen meter (OxyGuard Handy Polaris probe, Birkerød, Denmark), and EC using a digital pen-type portable TDS meter (Az Instrument, Taiwan).

Total ammonia-nitrogen (TAN), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), and iron were measured according to APHA (2005) once a week using by spectrophotometer (Shimadzu, Kyoto, Japan).

Statistical Analysis

The obtained data were analyzed by using statistical software (IBM SPSS v.21, USA) in which one-way ANOVA and the post hoc Tukey's test were performed at a significance level of ($P<0.05$) at 95% confidence limits to know the significant difference between the treatments means for different parameters.

Results and Discussion

The water quality of the aquaponics system was monitored as the mean of daily temperature (°C), dissolved oxygen (mg/L), pH, and electrical conductivity (µS/cm) parameters were presented in Table 2 during each plant growing period.

Table 2. Temperature (°C), dissolved oxygen (mg/L), pH and electrical conductivity (µS/cm) parameter values of system water which measured daily during each plant growing period.

Groups	Temperature (°C)	Oxygen (mg/L)	pH	EC (µS/cm)
Parsley	21.23±0.98	6.99±0.34	7.08±0.41	503.43±49.58
Lettuce	17.82±1.40	6.31±0.56	7.22±0.13	536.11±30.79
Cress	16.58±1.57	5.43±0.54	7.26±0.09	442.88±32.14

Mean water temperature has been measured lower than other species during the trial of the cress plant due to the ambient temperature in winter. In addition, when the electrical conductivity values were examined, it was seen that it was lower than lettuce and parsley during the growth of the cress plant. However, during the study, each plant's growing conditions were kept suitable for aquaponic systems for each species according to Somerville et al. (2014). At the end of the experiments, the mean measurements such as phosphate, total phosphorus, nitrite, nitrate, and iron, are shown in Table 3.

It has been determined that lettuce was more successful in using nitrate in water than parsley and cress plants. Looking at the values in Table 3, it is understood that as time progress, the nitrate in the water in the lettuce plant decreases continuously, while it remains relatively constant in the cress, and it increases in the parsley. According to Liu et al. (2016), the lettuce plant absorbed more nitrate under LED light than fluorescent light and HPS light. This study showed why lettuce plant creates larger biomass in LED light, and the results confirm our work.

Harvest results of plants, such as individual biomass (g), plant height (cm), leaf number, and root length (cm) parameters, were summarized in Table 4.

Table 3. Water quality parameter values such as Phosphate (PO_4), total phosphorus (TP), nitrite (NO_2), nitrate (NO_3), and iron (Fe) during the Parsley, Lettuce, and Cress production periods.

Parameters (mg/L)	Species	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	(Mean ±SD)
PO_4	Parsley	0.63	1.26	2.05	2.74	3.19	3.61	3.62	2.44±1.08
	Lettuce	0.81	5.78	5.2	5.25	5.78	4.7	4.71	4.60±1.60
	Cress	4.71	1.86	2.86	2.91	3.45	2.45	2.13	2.91±0.88
TP	Parsley	0.96	1.89	2.4	3.08	3.88	4.51	4.15	2.98±1.20
	Lettuce	1.23	6.08	6.03	5.96	5.91	5.44	5.27	5.13±1.62
	Cress	5.27	2.39	3.58	3.66	4.19	2.79	2.3	3.45±0.99
NO_2	Parsley	0.07	0.03	0.14	0.08	0.12	0.14	0.02	0.09±0.05
	Lettuce	0.04	0.73	0.42	0.34	0.27	0.39	0.09	0.33±0.21
	Cress	0.09	0.15	0.09	0.07	0.12	0.44	0.13	0.16±0.12
NO_3	Parsley	9.05	8.42	13.24	19.16	21.92	28	29.84	18.52±8.01
	Lettuce	29.08	26.07	23.75	15.31	13.54	9.98	2.38	17.16±8.88
	Cress	2.38	0.35	0.3	1.24	0.37	1.11	1.04	0.97±0.68
Fe	Parsley	0.02	0.03	0.05	0.04	0.04	0.05	0.04	0.04±0.01
	Lettuce	0.03	0.09	0.07	0.09	0.1	0.1	0.16	0.09±0.04
	Cress	0.16	0.24	0.4	0.34	0.25	0.22	0.15	0.25±0.08

Table 4. Effect of lighting with HPS, LED, and FLO lamps on the individual biomass (g), plant height (cm), leaf number, and root length (cm) of parsley, lettuce, and cress end of the experiments.

Plant variable	Species	HPS	LED	FLO
Individual biomass (g)	Parsley	8.76±7.32 ^{a}	7.45±4.13 ^{a}	2.04±1.96 ^{b}
	Lettuce	54.09±25.60 ^{a}	60.83±19.39 ^{a}	17.81±6.40 ^{b}
	Cress	1.03±0.58 ^{a}	1.15±0.46 ^{a}	1.31±0.58 ^{a}
Plant Height (cm)	Parsley	22.04±5.32 ^{a}	15.14±3.71 ^{b}	12.45±3.57 ^{b}
	Lettuce	55.25±14.85 ^{a}	54.96±15.22 ^{a}	29.79±12.40 ^{b}
	Cress	10.37±2.62 ^{a}	9.35±1.73 ^{a}	12.70±2.66 ^{b}
Leaf number	Parsley	7.54±1.07 ^{a}	7.96±1.40 ^{a}	5.61±0.99 ^{b}
	Lettuce	28.25±4.03 ^{a}	24.54±3.31 ^{b}	20.18±1.59 ^{c}
	Cress	12.96±2.81 ^{a}	12.61±1.77 ^{a}	15.04±2.06 ^{b}
Root length (cm)	Parsley	22.54±12.67 ^{a}	18.00±7.29 ^{a}	9.25±5.17 ^{b}
	Lettuce	37.30±12.57 ^{a}	43.54±11.58 ^{a}	18.59±11.80 ^{b}
	Cress	6.22±1.23 ^{a}	7.06±1.83 ^{b}	6.20±0.61 ^{a}

Data were subjected to one-way ANOVA (n = 29). Means within a line followed by different letters are significantly different at $p < 0.05$ according to the posthoc Tukey test.

There was no significant difference between groups for parsley and cress on individual biomass (g) values. However, in the lettuce plant, the individual biomass (g) of the fluorescent light group (17.81 ±6.40 g) was three times lower compared to HPS (54.09 ±25.60 g) and LED (60.83 ±19.39 g) light.

The plant height of lettuce and cress plants was statistically the same between HPS (55.25 ±14.85 cm, 10.37 ±2.62cm respectively) and LED (54.96 ±15.22 cm, 9.35 ±1.73 cm respectively) but low in fluorescent light (29.79 ±12.40 cm, 12.70 ±2.66 cm respectively) have been found. The highest results in the height of the parsley plant were observed in HPS (22.04 ±5.32 cm) light, and the difference between LED (15.14 ±3.71 cm) and FLO (12.45 ±3.57 cm) lights were found to be insignificant.

In the number of leaves of parsley and cress plants, between HPS (7.54 ±1.07 and 12.96 ±2.81, respectively) and LED (7.96 ±1.40 and 12.61 ±1.77, respectively) lights, there was no significant difference. The number of leaves in plants under the FLO light (5.61 ±0.99 and 15.04 ±2.06, respectively) was significantly different and lower than in other light sources. In the lettuce plant, we found that the HPS group had the highest leaf number (28.25 ±4.03), followed by LED (24.54 ±3.31) and the lowest FLO (20.18 ±1.59).

The harvest period, after the seedlings are planted in the system, varied widely among the species. Martineau (2012) harvested the lettuce plants in 28 days, Roosta (2014) reached the harvest time in 45 days in his study with parsley, and Buzby et al. (2016) harvested the cress plant in 36 days. These harvest times are obtained as similar in other scientific studies.

Although there was no significant difference between the groups in the cress plant, the performance of HPS and LED lights in parsley and lettuce plants was found to be higher than the FLO light source. As reported by Martineau (2012), HPS, LED, and regular light applications achieved 114.3 ±54.2 g, 94.3 ±46.5 g, and 102.5 ±28.7 g fresh lettuce mass values, respectively. Lettuce plants not exposed to any additional artificial light created 82.3 ±38.2 g of fresh biomass in the same study. Both LED and HPS light applications were significantly similar in the production of fresh and dry biomass for lettuce; the HPS light is reported to be slightly larger in fresh biomass compared to LED light, but the difference is not significant. This study confirms the results of our study.

There is a strong relationship between biomass gain and feed intake of the fish in aquaponics. The mean initial biomass of fish was 3628 g at the start of the experiment, and it reached 5442 g at the end of the study with the feed intake of fish. At the end of the experiment, it was determined that among the 3 groups, the fish in the lettuce group showed the highest growth rate of 17.86% (Tables 5 and 6).

Table 5. Total Initial Fish Weight, Individual Initial Fish Weight, Total Final Fish Weight, and Individual Final Fish Weight values in the experimental groups.

Groups	Total Initial Fish Weight (g)	Individual Initial Fish Weight (g)	Total Final Fish Weight (g)	Individual Final Fİsh Weight (g)
Parsley	3628	84.4	4193	99.8
Lettuce	4193	99.8	4942	117.7
Cress	4942	117.7	5442	132.7

Table 6. Fish weight gain (g), Fish growth rate (%), Specific growth rate (%), and Feed conversion ratio values for each plant growth period.

Periods	FWG (g)	FGR (%)	SGR (%)	FCR (%)
Parsley	565	15.57	0.33	2.38
Lettuce	749	17.86	0.35	3.74
Cress	500	10.12	0.23	3.13

FWG: Fish Weight Gain calculated as the final weight of fish (g) – the initial weight of fish (g)
FGR: Fish Growth Rate calculated as (W_2 (g) – W_1 (g) x W_1^{-1} (g)) x 100
SGR: Specific Growth Rate calculated as ($ln W_2 - ln W_1$) x t^{-1} x 100, where W_1 and W_2 were fish weights (g) at the beginning and end of the experiment, and t was the length (d) of the experiment.
FCR: Feed conversion ratio was calculated as feed intake (g) x biomass $gain^{-1}$

The water quality parameters of the system were determined to be of good quality for the plant growing conditions. In this study, in which three different plant and light trials were used, it was observed that the koi fish were healthy during the experiment in the aquaponics system.

The FCR value ranged from 1.95 to 6.49 and the SGR value ranged from 0.29 to 0.84 in a study by Hussain et al. (2014) with juvenile koi fish, which they raised from 4.22 g initial weight to 6.81 g final weight at a water temperature of 24.03°C (Hussain et al., 2014). In a study by Hussain et al. (2015) using juvenile koi fish that they reared from 5.97 grams initial weight to 8.60 g final weight at a water temperature of 25°C, the FCR value varied from 2.28 to 2.34, and the SGR value was varied from 0.80 to 0.83 (Hussain et al., 2015). In a study conducted by Nuwanski et al. (2016) with juvenile koi fish, which they brought from 2.45 grams initial weight to 3.36 grams final weight, at 25.56°C water temperature, the FCR value was 5.6, and the SGR value was 0.7 (Nuwanski et al., 2016). Nuwanski et al., (2017) used koi fish that had an initial fish weight of 0.30 and final fish weight of 2.24, and they found SGR 3.32 ±0.03, FCR 1.32 ±0.03. In an aquaponic study, which was carried out at 22.5-27.4°C water temperature and 2% feeding rate, in 60 days when juvenile koi weighing 4.04 g were brought up to 6.99 g, FCR values

of 4.13-5.29 and SGR values of 0.73-0.94 has been reported (Nuwanski et al., 2019). According to Nuwansi et al. (2020), which is also an aquaponic study, while koi fish were increased from 6.94 to 12.66 g at high temperatures (27.8-28.3°C), FCR values of 3.31-3.41 and SGR values of 1-1.04 were obtained. In this study, the water temperature was kept at about 17-21°C in the system, which is a relatively low water temperature compared to mentioned studies. In addition, the initial fish weights used in our study were relatively higher than in the mentioned studies. These could explain FCR, and SGR differentiation from the mentioned koi fish reared in aquaponics studies.

Although indoor lighting is performed with different types of illumination sources such as fluorescent (FLO), high-pressure sodium (HPS), induction, and light-emitting diode (LED), it is essential to know which source is the most suitable for the particular plant. It is known that artificial lighting can have dramatic effects on the source, plant anatomy and morphology, food intake, and pathogen development (Massa et al., 2008). In addition, light is both an energy source for photosynthesis and a signal property that affects plant growth, flowering timing, and morphogenetic characteristics such as plant height and shape (Xu, 2019).

Each light source needs its climate set points for the optimum growth performance of the plant (Dueck et al., 2011). It has been reported that different light sources can alter the metabolite status in plant bodies (Fukuda, 2019). LED lights are known to have some advantages such as adjustability of the light spectrum, small size, long-lasting, low heat effects to the ambient for plants (Lin et al., 2013; Oliver et al., 2018). At the same time, it has been shown in studies that LED lights can achieve the same efficiency by consuming 75% less energy compared to light sources such as Metal Halide (Singh et al., 2015).

Conclusion

In conclusion, the experiment realized in the aquaponics system showed that using HPS or LED lights has the potential to produce adequate amounts of parsley and lettuce. It has been found that HPS and LED light sources provide a similar increase in plant biomass in all species, but fluorescent light is insufficient against these two light sources. Despite the high initial cost, LEDs stand out as the right choice for installation for their narrow bandwidths and easy adjustment, allowing their arbitrary combinations to suit any plant at any stage. Therefore, LEDs can be considered the most promising source for plant lighting. In the future, it seems possible that other artificial lighting sources will be gradually replaced by LEDs by providing technological developments and price reductions. Further experimentation for different plants under various growing conditions is recommended to obtain the necessary data on improving the artificial lighting performance of the aquaponics system.

Compliance with Ethical Standards

Conflict of interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Ethics committee approval: All experiments in this study were carried out in accordance with the "Regulation on the Welfare and Protection of Aquatic Vertebrates Used for Scientific Purpose" from the Republic of Turkey Ministry of Agriculture and Forestry (30751).

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Deniz MERCAN

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ÖZ

Porsuk Çayı'nda bazı çevresel parametrelerin zoobentik komünite yapısı üzerine etkilerinin incelenmesi amacıyla 2020-2021 yılları arasında 6 istasyondan mevsimsel olarak zoobentik örnekler toplanmış ve aynı zamanda bazı fizikokimyasal parametreler de ölçülmüştür. Çalışma kapsamında Gastropoda sınıfından 4, Bivalvia sınıfından 1, Clitellata sınıfından 3, Insecta sınıfından 22 familya olmak üzere toplam 30 familya tespit edilmiştir. Tubificidae, Erpobdellidae ve Chironomidae familyaları her istasyonda tespit edilmiştir. Porsuk Çayı zoobentozunda baskın familyaların; Tubificidae (%41.90), Chironomidae (%16.68), Gammaridae (7.84), Valvatidae (%6.16), Naididae (%5.30), Asellidae (%3.73), Physidae (%3.48), Dreissenidae (%3.16), Baetidae (%3.07), Simuliidae (%1.70), Erpobdellidae (%1.69) ve Lymnaeidae (%1.32) şeklinde sıralandığı görülmektedir. En yüksek çözünmüş oksijen değeri ortalama 11.3 mg/L ile 1. istasyon olan Sobran Deresi'nde ölçülmüştür. Bu istasyonda kirliliğe toleransı düşük gruplardan Ephemeroptera takımından Caenidae ve Trichoptera takımından Hydropsychidae familyalarının tespit edilmesi ve Kanonik Uyum Analizi sonuçları havzada belirlenen taksonların dağılışına çözünmüş oksijen konsantrasyonun etkili olduğunu göstermektedir. Aynı zamanda bu istasyon en yüksek BMWP (64) ve Shannon (2.22) değerlerine de sahiptir.

Anahtar Kelimeler: Zoobentik komünite, Su kalitesi, Porsuk Çayı, Eskişehir

ABSTRACT

Investigation of the effects of some environmental variables on the zoobenthic community structure: The case of Porsuk Stream (Sakarya River, Türkiye)

In order to examine the effects of some physicochemical parameters on the zoobenthic community structure in Porsuk Stream, seasonal zoobenthic samples were collected from 6 stations between 2020-2021, and some physicochemical parameters were also measured. Within the scope of the study, a total of 30 families, 4 from the Gastropoda, 1 from the Bivalvia, 3 from the Clitellata, and 22 from the Insecta, were identified. Families Tubificidae, Erpobdellidae, and Chironomidae were identified at each station. Dominant groups in Porsuk Stream zoobenthos; Tubificidae (41.90 %), Chironomidae (16.68 %), Gammaridae (7.84), Valvatidae (6.16 %), Naididae (5.30 %), Asellidae (3.73 %), Physidae (% 3.48 %), Dreissenidae (3.16 %), Baetidae (3.07 %), Simuliidae (1.70 %), Erpobdellidae (1.69%) and Lymnaeidae (1.32 %), respectively. The highest dissolved oxygen value was measured in the Sobran Stream with an average of 11.3 mg/L. Detection of Caenidae from Ephemeroptera and Hydropsychidae from Trichoptera with low tolerance to pollution and results of the Canonical Correspondence Analysis in this station shows that dissolved oxygen concentration is effective on the distribution of taxa determined in the basin. At the same time, this station has the highest BMWP (64) and Shannon (2.22) values.

Keywords: Zoobenthic community, Water quality, Porsuk Stream, Eskişehir

Giriş

Doğal kaynakların ve biyolojik çeşitliliğin korunması ve ayrıca sürdürülebilir yönetimi, dünya gündeminde önceliği giderek artan bir konudur. Genetik çeşitliliğin, ekosistem, tür ve proses çeşitliliği ile birlikte devamlılığının sağlanması ve korunması, gelecek nesiller için oldukça önemli bir konudur (Eken vd., 2006). Bir ekosistemin işlevini yerine getirebilmesi ve sistemdeki tüm anahtar birimlerin çalışabilmesi, biyoçeşitliliğin orta veya yüksek seviyelerde sürdürülmesi ile sağlanmaktadır. Ayrıca çeşitlilik, ekosistemin kendi kendini yenileyebilmesinin ve baskılara karşı koyabilmesinin de bir güvencesidir (Odum ve Barrett, 2008). Türkiye, Palearktik bölge içerisinde biyoçeşitliliği yüksek olan ülkeler arasındadır. Türkiye, eski dünya kıtaları arasında köprü görevi görmüş ve son 2 milyon yıl içinde meydana gelen buzul çağlarında birçok canlı tarafından sığınak olarak kullanılmış ve şimdiki biyoçeşitliliğine kavuşmuştur (Şekercioğlu vd., 2011). Türkiye'nin topografik yapısı farklı olduğu ve özellikle kısa mesafelerde ekolojik faktörleri çok farklı ortamlar içerdiği için, çok farklı canlı gruplarını barındırma özelliğine sahiptir (Demirsoy, 1996). Türkiye sucul ekosistemler bakımından oldukça zengin bir ülke olmakla birlikte 25 su havzasına sahiptir (Çevre ve Orman Bakanlığı, 2010).

Sucul sistemlerde yaşayan bentik makroomurgasız grupları, biyolojik zenginliğimizin büyük kısmını oluşturmaktadır. Ancak sucul ekosistemler ciddi şekilde antropojenik kaynaklı kirliliğe maruz kalmakta ve birçok bentik makroomurgasız grubu daha varlığı dahi tespit edilemeden yok olup gitmektedir. Ekosistem değerlendirmelerinde kullanılan kirlilik ve çeşitlilik indeksleri, biyolojik değişken olarak kullanımlarının kolay olması nedeniyle çoğunlukla 5 biyolojik bileşen (diatom, plankton, makrofit, makroomurgasız ve balık) ile yapılmaktadır (Kazancı vd., 1997). Makroomurgasızlar, bu bileşenlerin içinde en kalabalık ve heterojen gruptur. Gerek birey sayısı gerekse tür çeşitliliği diğer bileşenlerden çok daha yüksektir. Ayrıca zoobentik komünite üyelerinin tür bazında ekolojik istek ve değişen çevre koşullarına karşı tolerans sınırları farklı olduğundan bulundukları ekosistemin canlı hafızaları olarak işlev görürler. Bu yüzden yüzey suları izleme ve kalite değerlendirmelerinde biyoindikatör olarak kullanılırlar (Demir, 2020).

Porsuk Çayı, Türkiye'nin büyük nehirlerinden biri olan Sakarya Nehri'ni besleyen en önemli koludur. Porsuk Çayı bazı önemli yerleşim (Eskişehir ve Kütahya gibi) yerlerinden geçerken gerek endüstriyel gerekse evsel atıklarla kirlenebilmektedir. Bu kirliliğin en önemli etkisi, başta sucul ortamda yaşayan ve balıkların besinini oluşturan zoobentik grupların yaşam ve dağılım alanları kısıtlaması şeklinde gözlemlenebilmektedir. Bu ise henüz varlığından dahi haberdar olamadığımız bazı taksonomik grupların yok olması anlamına gelmektedir. Belirli bir bölgede bazı canlı grupların bulunması veya bulunmaması bize su kirliliği ve boyutları hakkında ekolojik bilgiler verebilir. Çünkü yüzey sularının fizikokimyasal parametreleri (sıcaklık, pH, bulanıklık, çözünmüş oksijen, nitrat, nitrit ve amonyum azotu, fosfor vb.) uzun dönemler boyunca günlük ölçülmedikçe, sudaki kalıcı değişiklikleri yansıtmaz, anlık değişimleri verir. Hâlbuki aynı suda yaşayan zoobentik komünite üyelerinin varlığı veya yokluğu (özellikle de daha önceden varlığı bilinip de güncel olarak aynı bölgede tespit edilemeyen) ise bize uzun süreli değişimleri gösterir.

Bu bakış açısı ile bu araştırmada, daha önce yapılan bazı çalışmalar (Arslan ve İlhan, 2010; Kırkağaç vd., 2011; Köse vd., 2015; Köse vd. 2016; Arslan ve Mercan, 2020) ile bilinen ve var olan Porsuk Çayı'nın kirliliğinin, havzadaki euryo-stenök taksonların dağılışı üzerine olası etkileri araştırılmıştır. Böylece, kirliliğin yaşayan doğal sistemler olarak bilinen tatlı su zoobentik canlıları üzerine olası etkilerinin belirlenmesi amaçlanmıştır.

Materyal ve Metot

Çalışma Alanı

Porsuk Çayı, Sakarya Havzası Porsuk Alt Havzası'nda yer almaktadır. Sakarya Havzası Türkiye yüzölçümünün 1/8'ini oluşturmaktadır. Porsuk Alt Havzası da 1.082.500 ha alanı kaplamaktadır. Porsuk Alt Havzası, kuzeybatı Anadolu'da 38°44'-39°99' kuzey enlemleri ile 29°38'-31°59' doğu boylamları arasında yer almaktadır. Porsuk Çayı 448 km uzunluğu ve 1.082.519 ha'lik drenaj alanı büyüklüğü ile Sakarya Nehri'nin en uzun koludur (Tarım ve Orman Bakanlığı, 2022). Sakarya Nehri 810 km uzunluğu ile Türkiye'nin en uzun üçüncü nehridir. Nehir Eskişehir ili Çifteler ilçesinde Sakarbaşı bölgesinden doğar ve birçok dere ile beslenerek kuzey yönüne akıp Karasu Bölgesi'nde denize dökülmektedir (Işık vd., 2008).

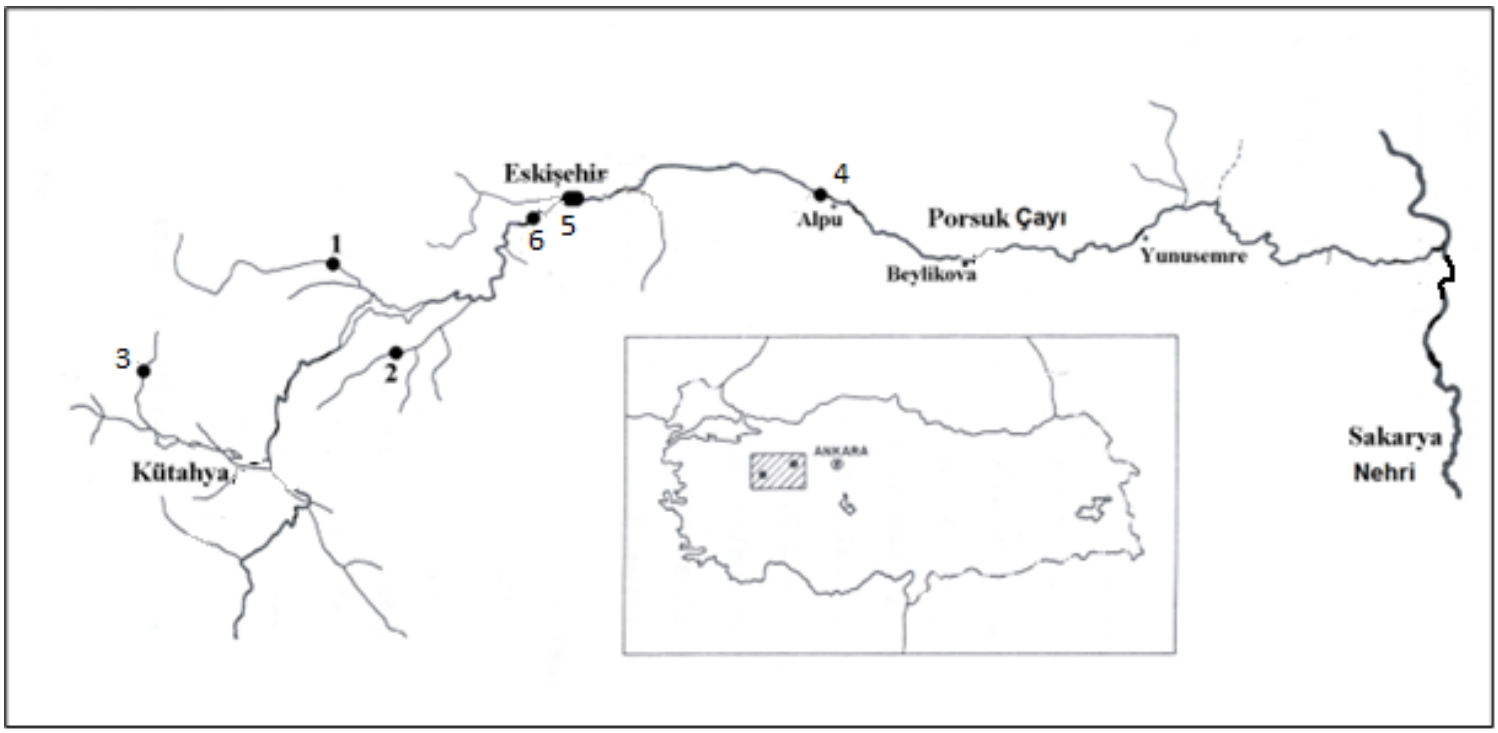
Örneklerin Toplanması ve Teşhisi

Porsuk Çayı'nda 2020-2021 tarihleri arasında 6 istasyondan zoobentik örnekler dört mevsimi yansıtacak şekilde el kepçesi kullanılarak toplanmıştır (Şekil 1). Toplanan materyal elenerek %70'lik alkol ile fikse edilmiş ve laboratuvara getirilmiştir. Örnek alımı esnasında suyun bazı parametreleri de (sıcaklık (^{o}C), çözünmüş oksijen (mg/L), pH) arazide Hach Lange marka cihaz ile *in situ* olarak ölçülmüş, Biyolojik Ok-

sijen İhtiyacı (BOI5) ise laboratuvara getirilen su örneklerinden Enotek marka cihaz ile analiz edilmiştir. Laboratuvara getirilen örnekler Zeiss marka stereomikroskop altında ayıklanmış, her bir takson familya düzeyine kadar teşhis edilmiş, daha sonra sayılarak her taksonomik grup ayrı ayrı %70'lik alkol içeren flakonlarda etiketlenerek saklanmıştır. Zoobentik örneklerin teşhisinde Macan (1965; 1977; 1979), Kruse ve Pritchard (1982), Brinkhust (1986), Nilsson (1996), Mandaville (2002), ve Boucherd (2004) teşhis anahtarları kullanılmıştır. Teşhis edilen örnekler Eskişehir Osmangazi Üniversitesi Fen Fakültesi Biyoloji Bölümü Hidrobiyoloji Laboratuvarı'nda muhafaza altına alınmıştır.

Verilerin Hesaplanması

Familyaların istasyonlardaki baskınlık değerlerini hesaplamak için; D = n / N x 100 (n=bir türün istasyondaki birey sayısı; N= istasyondaki toplam birey sayısı) formülü kullanılmıştır (Bellan-Santini, 1969). Biyotik indekslerden BMWP (İspanyol versiyonu) ve ASPT indeksleri ve çeşitlilik indekslerinden Shannon-Wiener, Margalef, Simpson ve Evenness indeksleri su kalitesi parametrelerinin zoobentik gruplar üzerine etkisini belirlemek amacıyla kullanılmıştır. İndeksler ASTERICS 3.1 programı kullanılarak hesaplanmıştır (AQEM Consortium 2002). Zoobentik komünite ile çevresel parametreler arasındaki ilişkiyi değerlendirmek için Past programı kullanılarak Kanonik Uyum Analizi (CCA) yapılmıştır (Hammer vd., 2001).



(1. ist: Sobran Deresi; 2. ist: Sabuncupınar Deresi; 3. ist: Enne Çayı; 4. ist: Porsuk Çayı; 5. ist: Regülatör; 6. ist: Fidanlık)

Şekil 1. Porsuk Çayı'nda örnekleme yapılan istasyonlar (Çabuk vd., 2004'ten revize edilmiştir)

Figure 1. Map of sampling stations in Porsuk Stream (revised from Çabuk et al., 2004)

Bulgular ve Tartışma

Porsuk Çayı'nın zoobentik faunasına ait; Gastropoda sınıfından 4, Bivalvia sınıfından 1, Clitellata sınıfından 3, Insecta sınıfından 22 familya olmak üzere toplam 30 familya tespit edilmiştir (Tablo 1). Porsuk Çayı'nda tespit edilen zoobentik taksonların dominansi değerleri ve suda ölçülen bazı parametrelerin minimum-maksimum ve ortalama değerleri Tablo 1'de verilmiştir. Porsuk Çayı'nda 6 istasyonda gerçekleştirilen çalışmada yüzey sularında oldukça sık rastlanılan ve toleranslı bireyleri içeren Chironomidae ve Oligochaeta bireylerinin baskın olduğu görülmektedir.

Tablo 1. Porsuk Çayı'nda ölçülen bazı su parametrelerine ve tespit edilen zoobentik taksonların dominansi değerlerine göre dört mevsim genelinde minimum-maksimum ve ortalama değerleri

Tablo 1. The minimum-maximum and average values according to the some water parameters measured in Porsuk Stream and the dominance values of the determined zoobenthic taxa.

	Sobran Deresi	**Sabuncupınar Deresi**	**Enne Çayı**	**Porsuk Çayı**	**Regülatör**	**Fidanlık**
Parametre	**min-mak (ort)**	**min-mak (ort)**	**min-mak (ort)**	**min-mak (ort)**	**min-mak (ort)**	**min-mak (ort)**
Sıcaklık (ºC)	5.9-13.6 (9.6)	8.5-16.5 (13.0)	11.9-19.0 (15.7)	8.4-12.9 (10.8)	5.7-11.6 (9.6)	5.5-13.1 (9.7)
pH	8.2-8.5 (8.3)	7.5-7.7 (7.6)	7.4-7.8 (7.6)	7.2-7.5 (7.4)	7.9-8.2 (8.1)	8.1-8.5 (8.3)
Su Kalite Sınıfı	I-I (I)	I-I (I)	I-I (I)	I-I (I)	I-I (I)	I-I (I)
Çözünmüş Oksijen (mg/L)	8.7-13.4 (11.3)	4.6-8.2 (7.1)	5.0-8.9 (7.3)	4.7-5.6 (5.0)	3.0-7.5 (5.1)	4.7-6.4 (5.3)
Su Kalite Sınıfı	I-I (I)	III-I (II)	III-I (II)	III-III (III)	III-II (III)	III-II (III)
Biyolojik Oksijen İhtiyacı (mg/L)	1.3-3.0 (1.8)	2.9-5.0 (3.9)	3.0-5.6 (3.8)	2.0-18.0 (9.5)	5.0-11.0 (7.4)	3.0-9.4 (5.1)
Su Kalite Sınıfı	I-I (I)	I-II (I)	I-II (I)	I-III (III)	II-III (II)	I-III (II)
Takson						
Şube: Mollusca						
Sınıf: Gastropoda						
Altsınıf: Pulmonata						
Lymnaeidae	-	1.00-12.42 (7.36)	-	0.59-1.70 (1.27)	-	-
Physidae	0.60-11.83 (5.46)	-	12.83-18.44 (15.21)	-	0.00-1.64 (0.65)	0.00-0.41 (0.16)
Altsınıf: Prosobranchia						
Hydrobiidae	-	1.04-3.94 (2.19)	-	-	-	-
Valvatidae	-	-	15.96-47.91 (34.14)	0.46-2.99 (1.62)	0.00-1.64 (0.65)	-
Sınıf: Bivalvia						
Dreissenidae	0.00-2.37 (0.82)	-	9.22-22.64 (15.20)	0.00-2.74 (1.08)	-	0.00-0.60 (0.25)
Şube: Annelida						
Sınıf: Clitellata						
Oligochaeta						
Naididae	3.01-7.34 (4.64)	13.93-20.69 (16.73)	2.09-4.52 (3.22)	0.94-13.29 (5.08)	-	1.62-8.33 (5.07)
Tubificidae	18.06-31.65 (25.40)	16.26-40.48 (27.24)	7.72-29.48 (17.67)	32.56-57.51 (49.09)	60.53-92.58 (74.31)	25.10-81.14 (50.06)
Altsınıf: Hirudinea						
Erpobdellidae	0.00-0.69 (0.29)	0.00-8.87 (2.60)	2.13-9.39 (4.37)	0.86-2.74 (1.49)	0.30-0.82 (0.60)	0.00-0.60 (0.25)
Sınıf: Insecta						
Takım: Ephemeroptera						

Baetidae	3.61-9.03 (6.43)	2.77-5.23 (3.98)	0.00-3.83 (2.16)	-	0.59-2.91 (1.99)	0.41-13.79 (5.30)
Caenidae	1.20-3.47 (2.33)	-	-	-	-	-
Ephemerellidae	-	0.00-0.65 (0.29)	-	0.00-0.57 (0.18)	-	-
Ephemeridae	-	-	0.00-0.17 (0.08)	-	-	-
Takım: Odonata						
Calopterygidae	0.00-3.67 (1.07)	-	-	-	-	-
Libellulidae	-	-	-	0.00-1.15 (0.37)	-	-
Coenagrionidae	-	-	-	-	-	0.00-2.38 (1.10)
Takım: Hemiptera						
Gerridae	0.00-0.69 (0.29)	-	-	-	-	-
Takım: Coleoptera						
Empididae	0.00-0.69 (0.29)	-	-	-	-	-
Haliplidae	-	-	-	0.00-2.29 (0.61)	-	-
Dytiscidae	0.00-13.61 (4.16)	-	0.00-0.17 (0.08)	-	-	0.00-4.76 (1.19)
Dryopidae	0.00-0.69 (0.29)	-	-	-	0.00-0.59 (0.27)	0.00-1.19 (0.38)
Psephenidae	-	-	-	-	-	0.00-0.41 (0.10)
Takım: Diptera						
Chironomidae	15.97-27.11 (22.21)	1.49-28.10 (16.14)	0.35-13.48 (7.17)	26.65-41.86 (35.29)	3.86-9.43 (6.19)	4.73-33.14 (14.17)
Simuliidae	0.00-25.69 (9.28)	-	0.00-0.17 (0.08)	-	0.00-1.12 (0.28)	0.00-4.76 (2.34)
Ceratopogonidae	0.00-0.60 (0.30)	0.00-0.50 (0.25)	-	-	0.00-4.92 (1.80)	0.00-0.60 (0.20)
Tabanidae	0.00-0.69 (0.29)	0.00-1.49 (0.37)	-	-	0.00-0.84 (0.21)	-
Tipulidae	-	-	-	-	0.00-1.23 (0.31)	-
Takım: Trichoptera						
Psychomyiidae	-	-	-	-	0.00-0.82 (0.35)	-
Hydropsychidae	0.60-3.67 (2.23)	0.00-0.50 (0.25)	-	0.00-0.57 (0.18)	-	0.00-2.38 (1.02)
Altşube: Crustacea						
Sınıf: Malacostraca						
Takım: Isopoda						
Asellidae	-	10.73-47.76 (22.61)	0.15-1.31 (0.54)	-	0.00-0.56 (0.26)	-
Takım: Amphipoda						
Gammaridae	0.59-25.90 (14.23)	-	0.00-0.17 (0.08)	1.56-5.44 (3.74)	1.78-27.36 (12.14)	4.96-40.95 (18.41)

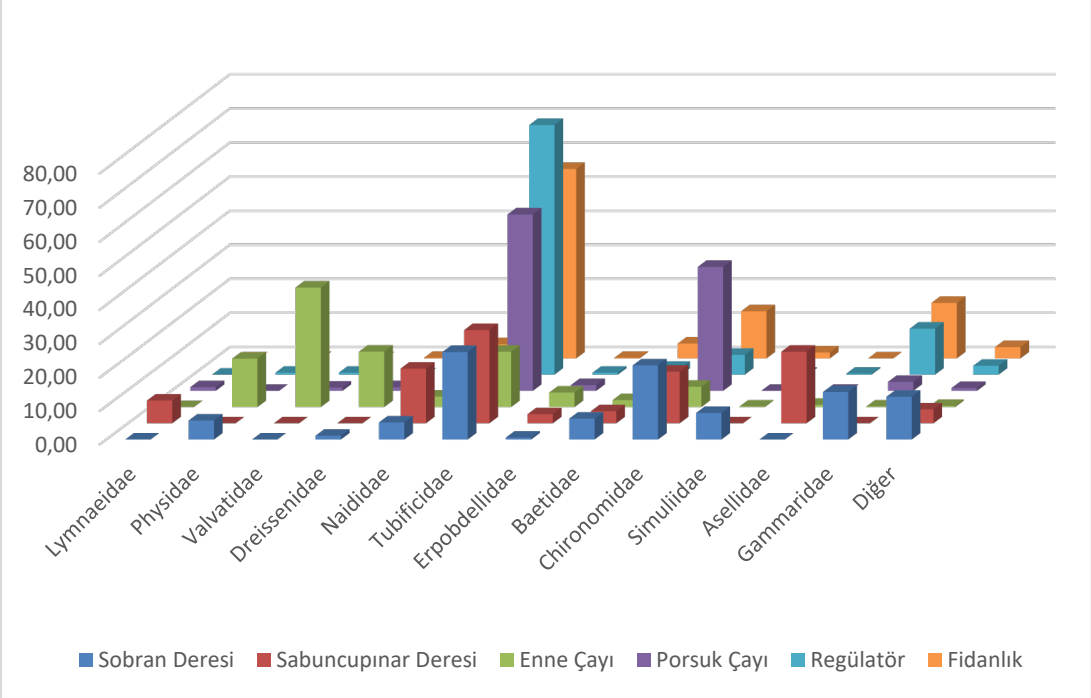
Ortalama bolluk değerlerine göre Porsuk Çayı zoobentozunda baskın gruplar; Tubificidae (%41.90), Chironomidae (%16.68), Gammaridae (7.84), Valvatidae (%6.16), Naididae (%5.30), Asellidae (%3.73), Physidae (%3.48), Dreissenidae (%3.16), Baetidae (%3.07), Simuliidae (%1.70), Erpobdellidae (%1.69) ve Lymnaeidae (%1.32) şeklinde sıralanmaktadır (Şekil 2). Baskınlık değeri %1'in altında olan gruplar ise diğer olarak alınmıştır (Diğer; Hydrobiidae, Caenidae, Ephemerellidae, Ephemeridae, Calopterygidae, Libellulidae, Coenagrionidae, Gerridae, Empididae, Haliplidae, Dytiscidae, Dryopidae, Psephenidae, Ceratopogonidae, Tabanidae, Tipulidae, Psychomyiidae and Hydropsychidae).

Her istasyonda tespit edilen Tubificidae, Erpobdellidae ve Chironomidae familyalarının %100 frekans değerine sahip olduğu tespit edilmiştir (Tablo 1). Clitellata sınıfından Tubificidae ve Erpobdellidae ve Insecta sınıfından Chironomidae bireylerinin (stenök olan az sayıdaki bazı türleri haricinde) oldukça farklı habitatlara uyum sağlayabildikleri, suyun kuruması durumunda bile hayatta kalabildikleri, lağım sularında da yaşayabildikleri bilinmektedir (Brinkhurst, 1986; Şahin, 1991). Kanonik Uyum Analizi, zoobentik komünite ile çevresel parametreler arasındaki ilişkiyi analiz etmek için kullanılmıştır. Analiz sonuçları Şekil 4'te verilmiştir. Çözünmüş oksijen ve pH çevresel parametrelerinin zoobentik komünite

üzerine negatif etkiye sahip olduğu görülmektedir. Çözünmüş oksijen parametresi Naididae, Hydrobiidae, Asellidae, Hydropsychidae, Caenidae ve Physidae familyalarının dağılışı üzerine negatif etkiye sahipken Biyolojik Oksijen İhtiyacı (BOI5) Chironomidae, Tubificidae, Ceratopogonidae, Psephenidae, Coenagrionidae, Psychomyiidae, Tipulidae familyalarının dağılışı üzerine pozitif etkiye sahiptir (Şekil 3).

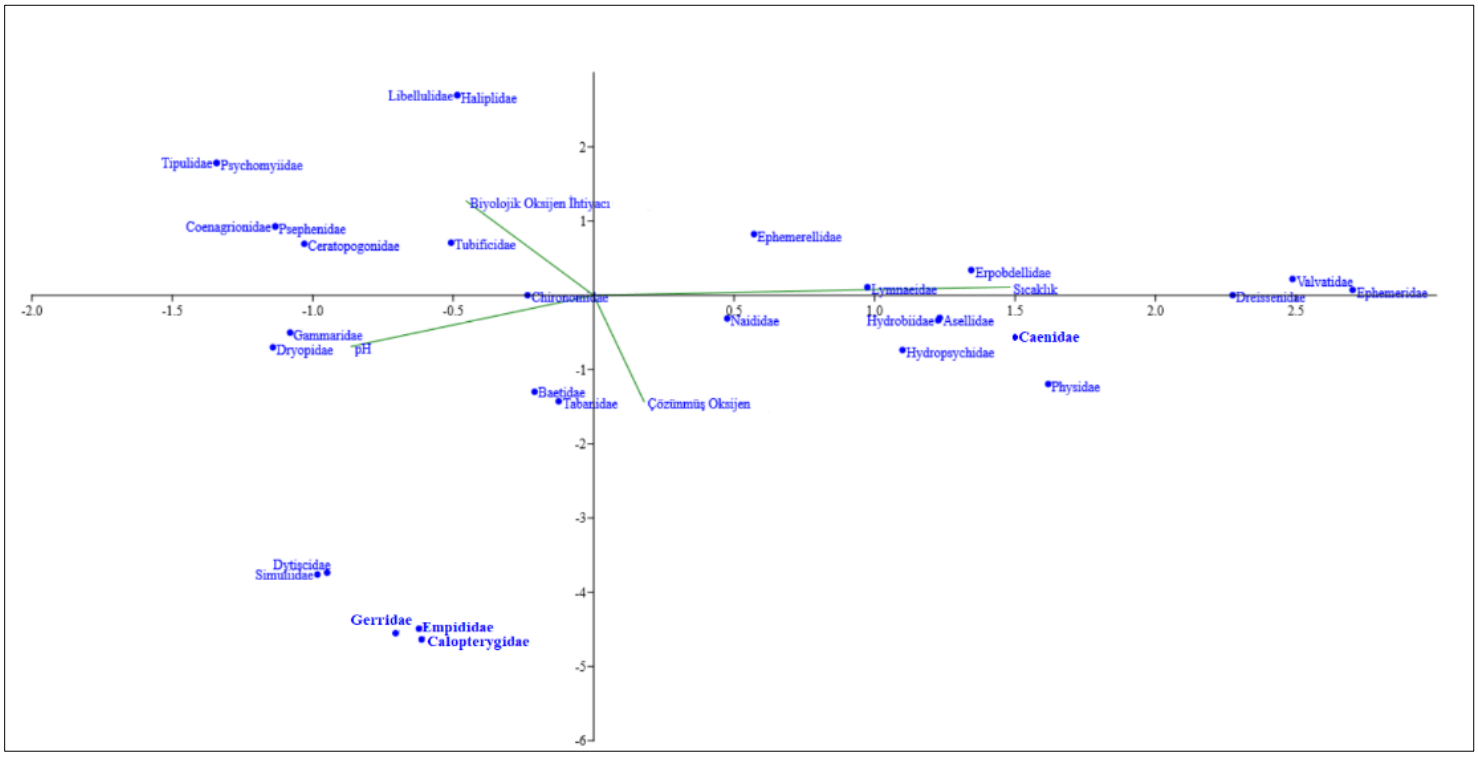
Tablo 1'de de görüldüğü üzere çözünmüş oksijen değerlerinin düşük olduğu Porsuk Çayı, Regülatör ve Fidanlık istasyonlarında Tubificidae bireylerinin yoğunluğu (sırasıyla %49.09, %74.31 ve %50,06) dikkat çekicidir. Benzer durum Porsuk Çayı istasyonunda %36,58 baskınlık oranı ile Chironomidae familyası için geçerlidir. Havzada bu iki grubun bariz bir şekilde baskın olması, havzanın baskı altında olduğunu, çevresel değişikliklerden olumsuz yönde etkilendiğini göstermektedir. Nispeten daha temiz sularda yaşayan türleri içeren Ephemeroptera ve Trichoptera taksonlarına ait familyaların bolluk değerleri az da olsa havzada bulunması, kirliliğe bağlı muhtemel fauna kompozisyonun değiştiği fikrini akla getirmektedir. En yüksek çözünmüş oksijen değeri ortalama 11.3 mg/L değeri ile Sobran Deresi'nde ölçülmüştür. Bu istasyonda Ephemeroptera takımından Caenidae ve Trichoptera takımından Hydropsychidae familyalarının tespit edilmesi, havzada tespit edilen taksonların dağılışına çözünmüş oksijen konsantrasyonun etkili olduğunu göstermektedir (Şekil 3). EPT (Ephemeroptera, Plecoptera ve Trichoptera) grubu taksonlar su kütlesindeki kirleticilere karşı diğer gruplara göre daha hassastırlar ve bu nedenle özellikle bu grubun üyeleri su kalitesini belirlemede önemli biyolojik indikatörlerdir. Çoğu kez akarsu yakınındaki aktif antropojenik aktiviteler EPT grubunun bolluğu ve çeşitliliği üzerinde etkiye sahiptir (Wan Hafezul vd., 2016). EPT içinde Plecoptera taksonları su kalitesi değişimlerine karşı en hassas olan gruptur. EPT türlerinin varlığı, habitattaki parametrelerin türlerin tolerans sınırları içinde olduğunu gösterir. Plecoptera dışında, Trichoptera larvaları ve Ephemeroptera nimfleri de sucul sistemlerin kalitesini ve ekolojik değişimleri belirlemede uygun biyoindikatörlerdir (Karr, 1991; Rosenberg ve Resh, 1993). Çünkü EPT grubunun çoğu cinsi tatlı suların sadece iyi su kalitesine sahip noktalarında yaşayabilirler (Chapman, 1996; Azrina vd., 2006; Suhaila vd., 2014; Suhaila ve Che Salmah, 2014) ve dağılımları bir dizi çevresel faktöre toleransları ile sınırlandırılır (Dudgeon, 1984; Suhaila ve Che Salmah, 2017). Örneklem noktalarında Plecoptera grubu üyelerinin tespit edilmemesi ancak Ephemeroptera ve Trichoptera taksonlarının sınırlı sayıda da olsa tespit edilmesi su kalitesi bakımından bir değişimin olduğunu göstermektedir.

Havzadaki sıcaklık değerleri mevsim normallerine göre seyretmiş, havzada ortalama sıcaklık değerleri 5.5-19 ºC arasında değişmiştir. pH değerleri açısından bakıldığında ise 7.2 ile 8.5 arasında değişmiştir. BOI5 değerleri ise çözünmüş oksijen değerleri ile uyumludur (Şekil 4).



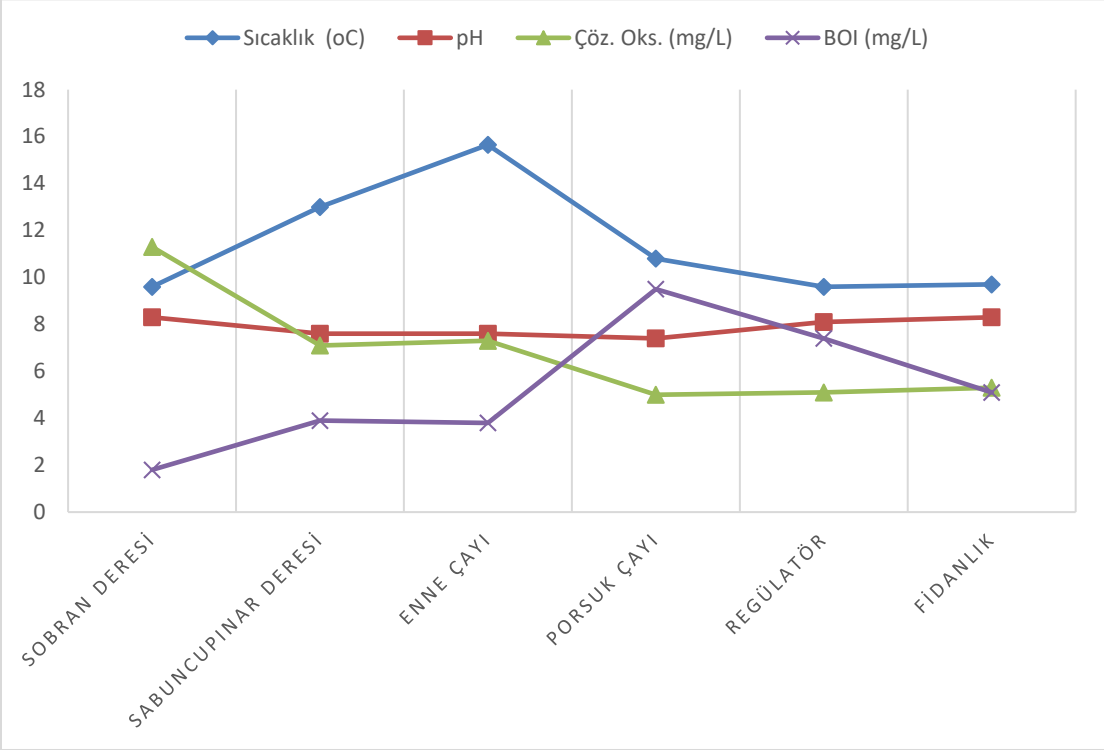
Şekil 2. Porsuk Çayı istasyonlarında tespit edilen zoobentik komünitenin ortalama bolluk değerleri

Figure 2. Avarage abundance values of identified zoobenthic communities in the Porsuk Stream



Şekil 3. Zoobentik komünite ile çevresel parametreler arasındaki ilişkiyi gösterir CCA analizi grafiği

Figure 3. Dendrogram of CCA analysis showing the relationship between of the zoobenthic community and environmental parameters



Şekil 4. Porsuk Çayı'nda istasyonlarda ölçülen bazı parametrelerin ortalama değerleri

Figure 4. Avarage values of the some measured parameters in stations of Porsuk Stream

Tablo 2. Porsuk Çayı'nda araştırılan istasyonların indeks değerleri

Table 2. Indices values of studied stations in Porsuk Stream

Metrikler / İstasyonlar	Sobran Deresi	Sabuncupınar Deresi	Enne Çayı	Porsuk Çayı	Regülatör	Fidanlık
Takson sayısı	18.00	12.00	13.00	12.00	14.00	15.00
Birey Sayısı	180.00	216.00	574.00	812.00	338.00	570.00
BMWP değeri	64.00	39.00	43.00	42.00	56.00	47.00
ASPT değeri	4.00	3.55	3.91	4.20	4.00	3.92
Shannon-Wiener Indeks	2.22	1.87	1.82	1.18	1.00	1.44
Simpson-Indeks	0.86	0.82	0.79	0.60	0.44	0.64
Margalef Indeks	3.27	2.05	1.89	1.64	2.23	2.21
Evenness	0.77	0.75	0.71	0.47	0.38	0.53

Sonuç

Sonuç olarak; Chironomidae ve Oligochaeta, evsel atıkların nehirlere deşarj olduğu polisaprobik ve alfa-mezosaprobik akarsu zonlarında bulunan taksonlardır, ayrıca Chironomidae larvaları ile Oligochaeta bireyleri sınıf IV ve sınıf III akarsu zonlarının indikatörü olarak görülmektedir (Raczynska vd., 2000). Havzada bu iki grubun baskın olması kirletici baskısı ile ilişkilendirilebilir. Özellikle de Oligochaeta üyelerinin euryök formlar olduğu, lağım sularında dahi yaşayabildikleri bilinmektedir (Brinkhurst, 1986). Havzada %47.2 oranında Oligochaeta baskınlığı, kirliliğin boyutlarını göstermektedir. Her iki grupta sahip oldukları hemoglobin ve bazı euryok türlerde görülen solungaç yapısı ve yüzeysel epitel solunumu yapabilmeleri sayesinde düşük çözünmüş oksijen içeren ortamlarda hayatta kalabilmeleri en büyük avantajları oldukları bilinmektedir. Genel olarak havzadaki çözünmüş oksijen değerlerine bakıldığında (Şekil 4) 5.0 mg/L ile 11.3 mg/L arasında değiştiği görülmektedir. Her ne kadar çözünmüş oksijen konsantrasyonu, mevsimlere, suyun sıcaklığına, akış hızına ve rüzgâr durumuna bağlı olarak değişse de havza genelinde düşüktür. Sudaki çözünmüş oksijen bütün aerobik canlıların metabolizması için temel öneme sahiptir. İç sulardaki oksijen çözünürlüğü ve özellikle de oksijen dağılımı, sucul canlıların büyüme, davranış ve dağılımını etkileyen ana faktördür (Wetzel, 2001). Sucul sistemlerdeki oksijen, alglerin ve bitkilerin fotosentezi sonucu üretilirken; bitkilerin, hayvanların ve bakterilerin solunumu, biyolojik oksijen ihtiyacı bozunma süreci, sediment oksijen ihtiyacı ve oksidasyon ile uzaklaştırılır (Radwan vd., 2003; Lin vd., 2006). Yüzey sularında genellikle BOI5 ve çözünmüş oksijen ters orantılı olarak değişmektedir. Bilindiği gibi BOI5 aerob bakteriler tarafından 22 ºC de 5 günde organik materyalin parçalanması için harcanan oksijen miktarıdır. BOI değerinin yüksek olması ortamdaki organik madde miktarındaki artışı işaret etmektedir. En yüksek BOI değerinin ise 4 no'lu istasyon olan Porsuk Çayı'nda tespit edildiği ve çözünmüş oksijen değerinin düşük olduğu görülmektir. Bu istasyon Alpu ilçesi sınırlarında olup küçük ve büyükbaş hayvan besiciliği ve tarım yapılan bölge içindedir. Bu istasyonda Chironomidae ve Tubificidae populasyon yoğunluğu dikkat çekicidir. Ayrıca araştırma alanındaki en düşük BMWP (42) ve Shannon çeşitlilik indeks (1,18) değerlerine sahip istasyonlarından biridir. Bu durum bölge içindeki su kaynaklarında organik materyalin artmasının bir sebebi olarak değerlendirilebilir. İkinci en yüksek BOI5 değeri Eskişehir il sınırları içinde Kütahya yolu üzerinde bulunan Regülatör piknik alanı içinde yer almaktadır. Bu bölgede aynı zamanda bir restoran da bulunmakta, alan il halkı tarafında yaz aylarında piknik alanı olarak yoğun bir şekilde kullanılmaktadır. İl yönetimi tarafından her ne kadar çevre ve su temizliğine özen gösterilerek korunmaya çalışılsa da su analizleri ve zoobentik komünite yapısı ciddi boyutta sinyal vermektedir. Belki de bölgedeki piknik alanı ile ilgili daha yüksek boyutta önlemlerin alınması, mangal yakılmasının önlenmesi bazı faaliyetlerin yasaklanması gerekebilir.

Havzada baskın olan bir diğer grup ise %7,84 baskınlık oranı ile Gammaridae'dir. Gammaridae familyası bireylerinin genellikle alfa-mezosaprobik ve beta-mezosaprobik zonlarda bulunduğu, özellikle de alfa-mezosaprobik zonlarda bol miktarda bulunabildikleri bilinmektedir (Sporka, 2006). Genel olarak değerlendirildiğinde havzadaki su kalite değerlerinin özellikle de çözünmüş oksijen miktarının fauna kompozisyonu üzerine etkili olduğu görülmektedir.

Porsuk Çayı üzerinde 1970 yılından bu yana su kalitesini çevresel parametreler ile belirlemek amacıyla birçok çalışma gerçekleştirilmiş ve Porsuk Çayı'nın yoğun bir şekilde kirlilik baskısı altında olup, evsel ve endüstriyel atıklarla su kalitesinin bozulmaya devam ettiği tespit edilmiştir (Öngel ve Ağaçık, 1970; Ağacık, 1971; 1974; Türkman ve Dirik, 1974; DSİ, 1975; Özbek, 1976; Dirik, 1977; Atıcı, 1997; Özyurt

vd., 2004; Gürel, 2011; Arslan ve İlhan, 2010; Köse vd., 2015; Köse vd. 2016). Bahsi geçen çalışmalar su kalitesini çevresel parametreler ile belirlemeye yöneliktir. Ancak son yıllarda sucul sistemlerin uzun süreli izlenmelerinde makroomurgasız gruplarını temel alan biyotik indeksler kullanılmakta ve daha etkili sonuçlar elde edildiği rapor edilmektedir (Kökmen vd., 2007; Kalyoncu ve Zeybek, 2011; Zeybek vd., 2014; Yorulmaz vd., 2015; Arslan vd., 2016; Kazancı vd., 2016). Bu çalışmada biyotik ve çeşitlilik indeksleri ile çevresel parametreler bir arada kullanılarak Porsuk Çayı'nda belirlenen bazı su kalitesi parametrelerinin zoobentik komünite üzerine etkileri araştırılmıştır. Bu açıdan diğer çalışmalardan farklılık arz etmektedir.

Arslan ve İlhan tarafından 2010 yılında Porsuk Çayı'nda Oligochaeta dağılımları ve çevresel değişkenler incelenmiştir. Çalışmada çözünmüş oksijen bakımından en yüksek istasyon, bu çalışmada da yer alan Sobran Deresi olarak belirlenmiştir. Sobran Deresi'nin Porsuk Çayı'na giriş noktasında olduğu ve bu nedenle endüstriyel kirlilikten etkilenmediği bildirilmiştir. Ayrıca çözünmüş oksijen ve biyolojik oksijen ihtiyacı değerlerine göre de Enne Çayı, Yenibosna ve Sabuncupınar Deresi istasyonlarının aşırı derecede organik kirliliğe maruz kaldığı vurgulanmıştır. Çevresel parametrelerin yanı sırı kirliliğe toleransı yüksek bireyler içeren Oligochaeta taksonları da tür seviyesinde tespit edilmiş ve 26 tür teşhis edilmiştir. Çalışmada, çevresel parametrelerden pH, çözünmüş oksijen, biyolojik oksijen ihtiyacı ve nitrat seviyelerinin Oligochaeta taksonlarının bolluğu ile doğrudan ilişkili olduğu tespit edilmiştir (Arslan ve İlhan, 2010). Bu çalışmada da en yüksek çeşitliliğe ve çözünmüş oksijen değerine Sobran Deresi'nin sahip olduğu ve istasyonda kirliliğe toleransı düşük olan taksonların varlığı tespit edilmiştir. Ayrıca Sabuncupınar istasyonu havzadaki en düşük BMWP değerine sahip istasyondur ve alanda kirliliğe toleransı yüksek Tubificidae, Chironomidae ve Asellidae familyalarının (Henderson ve Christian, 2022) baskınlığı mevcuttur. Bu da daha önce alanda rapor edilen kirliliğin devam ettiğini göstermektedir. Elde edilen veriler ışığında, havzada tespit edilen taksonların dağılışına çözünmüş oksijen konsantrasyonun negatif yönde etkili olduğu da söylenebilir (Şekil 3). Kırkağaç vd. tarafından Porsuk Çayı'nın Eskişehir merkezinde belirlenen 5 istasyonda gerçekleştirilen çalışmada sadece Köprübaşı ve Salhane istasyonlarında makroomurgasız tespit edildiği rapor edilmiş ve genel olarak organik kirliliğe toleranslı Erpobdellidae, Oligochaeta ve Gastropoda takımı bireylerinin varlığından söz edilerek Porsuk Çayı'nın makroomurgasızlar açısından kirli sular (4. sınıf) sınıfına girdiği bildirilmiştir (Kırkağaç vd., 2011). Bu çalışmada bahsi geçen istasyonlar yer almamasına rağmen Porsuk Çayı'nın yerleşim yerlerinden geçen noktalarında antropojenik etkiye bağlı kirlenmelerin olduğu barizdir.

Arslan ve Mercan tarafından Yukarı Sakarya Havzası'nda gerçekleştirilen başka bir çalışmada 1995-2015 yılları arasında makrobentik komünite yapı değişikliği irdelenmiştir (Arslan ve Mercan, 2020). Yukarı Sakarya Havzası'nda çalışılan 13 istasyondan Porsuk Çayı, Enne Çayı ve Regülatör istasyonları bu çalışma ile ortak istasyonlardır. Çalışmada, 1995-2015 yılları arasında Porsuk Çayı ve Enne Çayı'nda Naidine-Tubificine baskınlığının yüksek olduğu ve havza genelinde en düşük su kalitesine sahip olan istasyonlar olduğu rapor edilmiştir. Bu çalışmada da özellikle Porsuk Çayı istasyonunda Chironomidae ve Tubificidae bireylerinin baskınlığı dikkat çekicidir. Ayrıca havzadaki en düşük BMWP (42.00) ve Shannon çeşitlilik indeks (1.18) değerlerine sahip istasyonlarından biridir. Enne Çayı ise bir diğer düşük BMWP değerine (43.00) sahip istasyon olmakla beraber tolerans

Elde edilen veriler ışığında; Porsuk Çayı'nda tespit edilen zoobentik komünite ve ölçülen çevresel parametreler birlikte değerlendirildiğinde taksonomik grupların dağılışında özellikle çözünmüş oksijen parametresinin etkili olduğu görülmektedir. Örneklem istasyonlarından özellikle antropojenik kaynaklı baskıların mevcut olduğu noktalarda (Porsuk Çayı, Regülatör ve Fidanlık) kirliliğe toleransı yüksek Chironomidae ve Tubificidae bireylerinin baskın olduğu görülmektedir. Kirliliğe toleransı düşük olan EPT grubu bireylerinin ise sınırlı sayıda istasyonda düşük dominansilerde tespit edildiği hatta hassas türler içeren Plecoptera grubu üyelerin hiç tespit edilmediği belirlenmiştir. Çalışma alanlarından özellikle yerleşim bölgelerine yakın noktalarda kirliliğin mevcut olduğu ve gerekli önlemler alınmazsa ciddi boyutlara ulaşacağı bariz şekilde görülmektedir.

Etik Standartlar ile Uyumluluk

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

Etik kurul izni: Araştırma niteliği bakımından etik izin gerektirmemektedir.

Finansal destek: -

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New record of the prickly shark *Echinorhinus cookei* (Pietschmann, 1928) and evidence of scavenging by the coyote *Canis latrans* (Say, 1823) in Bahia de Los Angeles, Baja California, Mexico

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ABSTRACT

Echinorhinus cookei is a rare, large, demersal, deep-sea species of shark inhabitant of the Pacific Ocean basin. A sub-adult female with a total length of 262.7 cm was found naturally stranded in 2021 at Bahia de Los Angeles, Baja California, Central Gulf of California. It presents two spineless dorsal fins closer to the pelvic fins, large star-shaped dermal denticles, and distally oriented multi-cusplet cutting-type teeth. Along the shark, a peninsular coyote *Canis latrans peninsulae* was observed scavenging on the shark carcass. This study documents the first record of *E. cookei* in the Bahia de Los Angeles area and the first record of scavenging by the coyote *C. latrans* on any echinorhiniform shark to date.

Keywords: Natural stranding, Dermal denticles, Oceanography, Scavenging

Introduction

The Pacific prickly shark *Echinorhinus cookei* Pietschmann, 1928, (Chondrichthyes: Echinorhiniformes) is a rare species of demersal shark associated with the continental and island slopes of the Pacific Ocean basin (Compagno, 1984), being found up to 1100 m deep (Cox & Francis, 1997). Considered as large sharks (up to 4.5 m in total length) (Ebert et al., 2021), its deep-sea habits make this species little known and little studied; however, it is considered an active and top predator of deep-zone ecosystems (Ebert, 2003). Diagnostic features include the absence of anal fin, the origin of the two spineless dorsal fins closer to the pelvic fins, and large star-shaped dermal denticles grouped in patches along the body, without being fused into plates (Compagno, 1984). For the coasts of California, United States, it is associated with submarine canyons (Dawson & Starr, 2009) however, aggregations of more than 30 individuals have been reported less than 40 m deep for Monterey Bay (Crane & Heine, 1992). There are few confirmed reports of the presence of *E. cookei* along the Mexican Pacific coast: Altata, Sinaloa and Punta Rosa, Nayarit (Chávez-Ramos & Castro-Aguirre, 1974); off the coast of Mazatlán, Sinaloa (Álvarez-León & Castro-Aguirre, 1983); near Isla Cerralvo, Baja California Sur, Southern Gulf of California (Galván-Magaña et al., 1996); Isla San Jose, Baja California Sur, Southern Gulf of California and Isla Socorro at the Revillagigedo archipelago (Mariano & Villavicencio, 1998); off the coast of Boca de Apiza, Michoacan (Aguirre et al., 2002); Vizcaino Bay, Baja California and off the coast of Cabo San Lucas, Baja California Sur (Ruiz-Campos et al*.,* 2010); Isla Guadalupe, off the coast of Magdalena Bay, Baja California Sur and at off the coast of Oaxaca (Del Moral-Flores et al., 2015). For the Gulf of California, none of the reports records the presence of this species further north of the La Paz Bay, Baja California Sur, Southern Gulf of California (Figure 1).

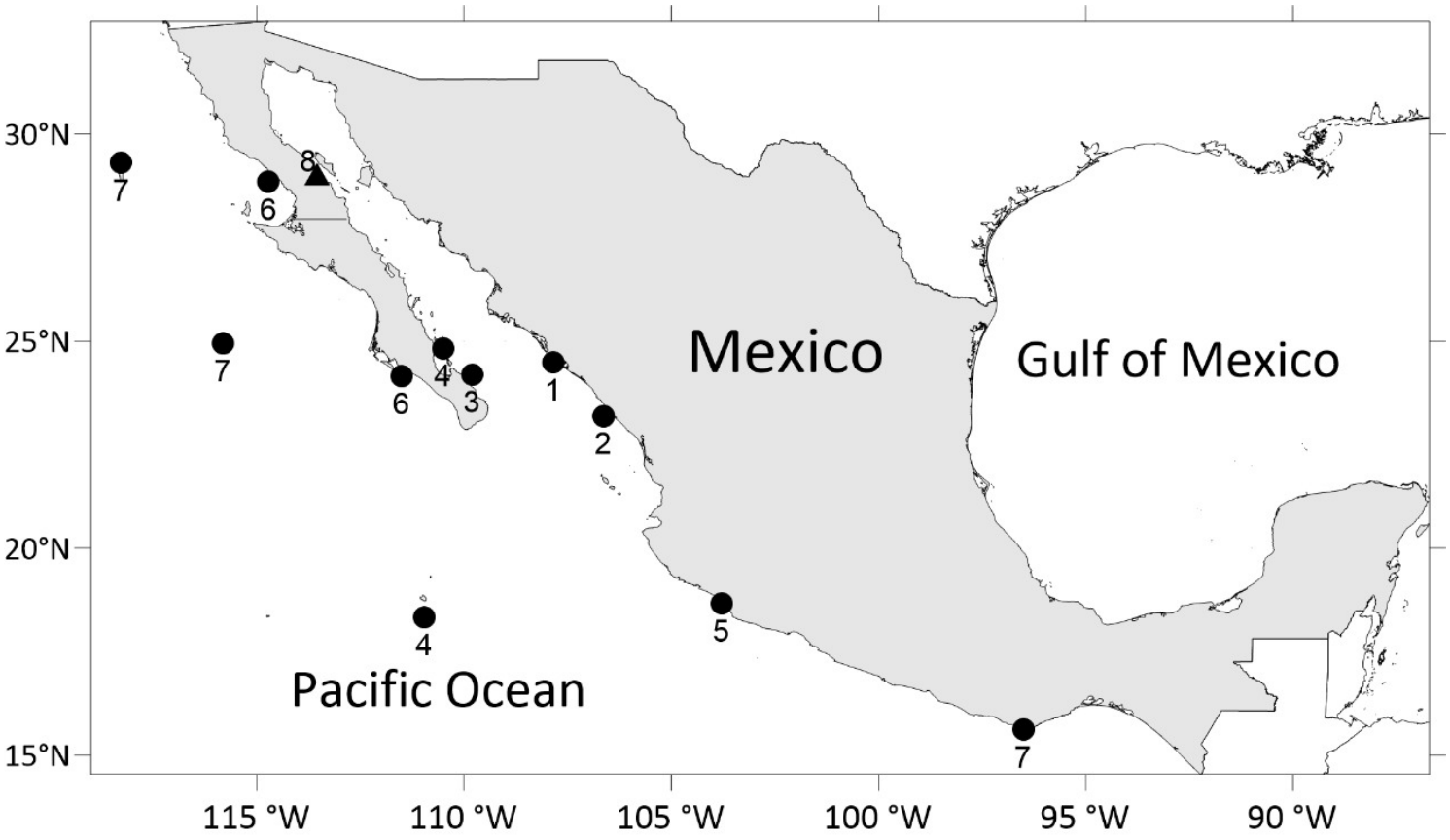

Figure 1. Prickly shark reports from the Mexican Pacific Ocean. 1, Chávez-Ramos & Castro-Aguirre, 1974; 2, Álvarez-León & Castro-Aguirre, 1983; 3, Galván-Magaña et al., 1996; 4, Mariano y Villavicencio, 1998; 5, Aguirre et al., 2002; 6, Ruiz-Campos et al*.,* 2010; 7, Del Moral-Flores et al., 2015; 8, this work (triangle marker)

On the other hand, the coyote *Canis latrans* (Say, 1823) is a species of land mammal of the canid family with wide distribution in North America (Kays, 2018). For the Baja California peninsula, there are two subspecies, the San Pedro Mártir coyote *Canis latrans clepticus* for the northern zone and the peninsular coyote *Canis latrans peninsulae* for the center and south of the peninsula (Nowak, 1979). Considered as a mesopredator (Berger & Conner, 2008), as far as its diet is concerned, it is an omnivore with seasonal variations in their diet covering a wide spectrum of prey. It actively preys on arthropods, crustaceans, small mammals, reptiles, birds, and offspring of large herbivorous mammals, in addition to vegetables (Grajales et al., 2003). The scavenging capacity of C. *latrans* is well-known, even stealing prey hunted by cougars or wolves (Wilmers et al., 2003). For Baja California, it is known that coyote populations related to coastal ecosystems take advantage of the food opportunities that the coastal zone offers, reaching up to 47.8% of their diet (Rose & Polis, 1998). Through scat studies, the consumption of marine organisms such as turtles, marine mammals both pinnipeds and cetaceans, birds, bony and cartilaginous fish has been recorded (Rose & Polis, 1998). Among the reported elasmobranchs are whip and guitar rays (Dasyatidae, Rhinobatidae), and thresher sharks (Alopiidae) (Rose & Polis, 1998).

This work presents the first confirmed record of *E. cookei* for Bahia de Los Angeles, Baja California, as well, a new evidence of the peninsular coyote *C. latrans* scavenging on elasmobranchs.

Material and Methods

At the beginning of April 2021, a local resident reported the presence of a stranded shark carcass of unknown species, in the area known as "La gringa beach", Bahía de Los Angeles (BLA), Baja California, Mexico near to 29.03234 N, 113.53808 W. (Figure 1).

Along with the shark found, the presence of an unsexed coyote *C. latrans* was observed scavenging on the aforementioned carcass (Figure 2).

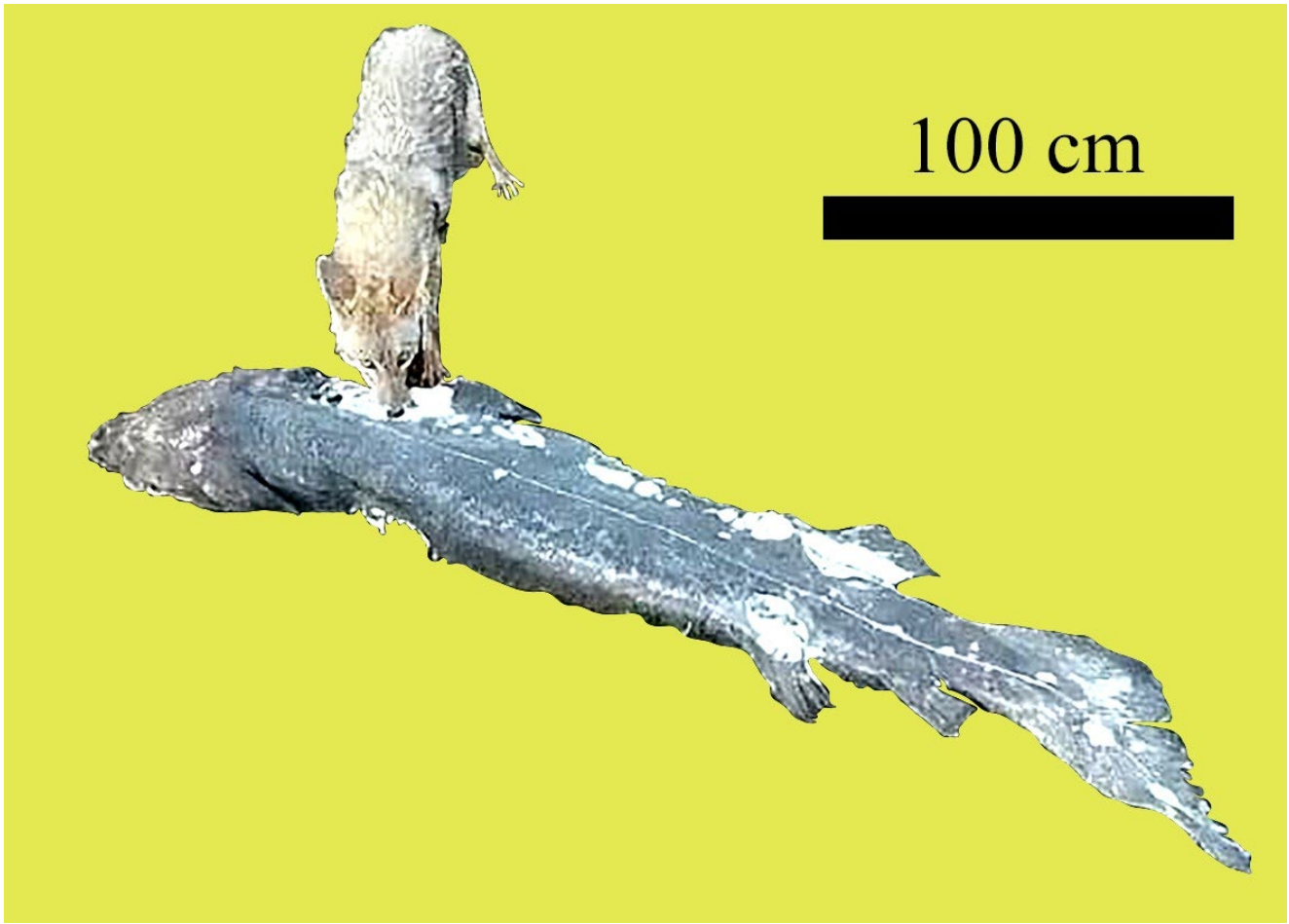

Figure 2. Scavenging by *C. latrans* on the carcass of *E. cookei*. Bite at the right side of the shark's body at the gill slits area. Photograph by Leopoldo M. Amezcua.

In order to know the species of the shark, the local villager kindly shared a photo and video graphics evidence obtained both at ground level and by air (using a drone) with the authors of this document. The photographs taken at the site were used to identify the shark following the identification guide of Compagno (1984) and Ebert et al. (2021). In addition, based on the initial estimate of the total length of the coyote made by the local resident, the precaudal length of the coyote was calculated according to what was established by Young & Jackson (1978), in order to be used as a scale in the photographs. Some of the most important measurements proposed by Compagno (1984) were calculated, as far as possible, for the shark using the ImageMeter® mobile phone application.

Results and Discussion

The initial estimate of the length of the coyote by the local resident was more than one meter in total length (>1 m). According to that observation, it was estimated that the precaudal length of the canid was approximately between 0.7 to 0.8 m. The shark analyzed was a female with an estimated total length (LT) of 262.7 cm and a precaudal length (PCL) of 186.3 cm (Figure 3a). The rest of the measurements obtained are shown in Table 1.

As for the state of decomposition of the shark, there was no evidence that the abdominal cavity was swollen or burst. Likewise, there is no evidence of discoloration or detachment of skin related to advanced decomposition of the body. The local villager said the body expelled a pronounced smell of ammonia. The shark presented superficial marks related to the coyote bite on its right side at the caudal and lateral regions (Figure 3a).

Exposed cartilage was also observed for the pelvic and dorsal fins base regions of the side mentioned above (Figure 3a). The most severe damage was observed in the gill area (Figure 3a) of both sides, in which pieces of detached tissue and exposed muscle was seen. Stands out aerial evidence of the moment in which the coyote was shredding a piece of tissue from the shark's gill area (Figure 2).

Table 1. Diagnostic characteristics and measurements (cm) obtained from the studied specimen based on digital material and comparison with Aguirre et al. (2002) and Álvarez-León & Castro-Aguirre (1983) records for *E. cookei*

Anal fin	Absent					
Number of dorsal fins	2					
Number of gill slits	NA					
	This study	**% TL**	**Aguirre et al. (2002)**	**% TL**	**Álvarez-León & Castro-Aguirre (1983)**	**% TL**
Sex	Female		Male		Female	
Natural total lenght (NTL)	262.7		37.7		350	
Fork lengh (FL)	234.8	89.4	NA		NA	
Precaudal lenght	186.3	70.9	26.1	69.2	246.9	70.5
Pre-first dorsal fin lenght (PD1)	143.9	54.8	21	55.7	179.8	51.4
Pre-second dorsal fin lenght (PD2)	173	65.9	23.8	63.1	220.6	63.0
Prepelvic lenght (PP2)	150.7	57.4	19.2	50.9	167.8	47.9

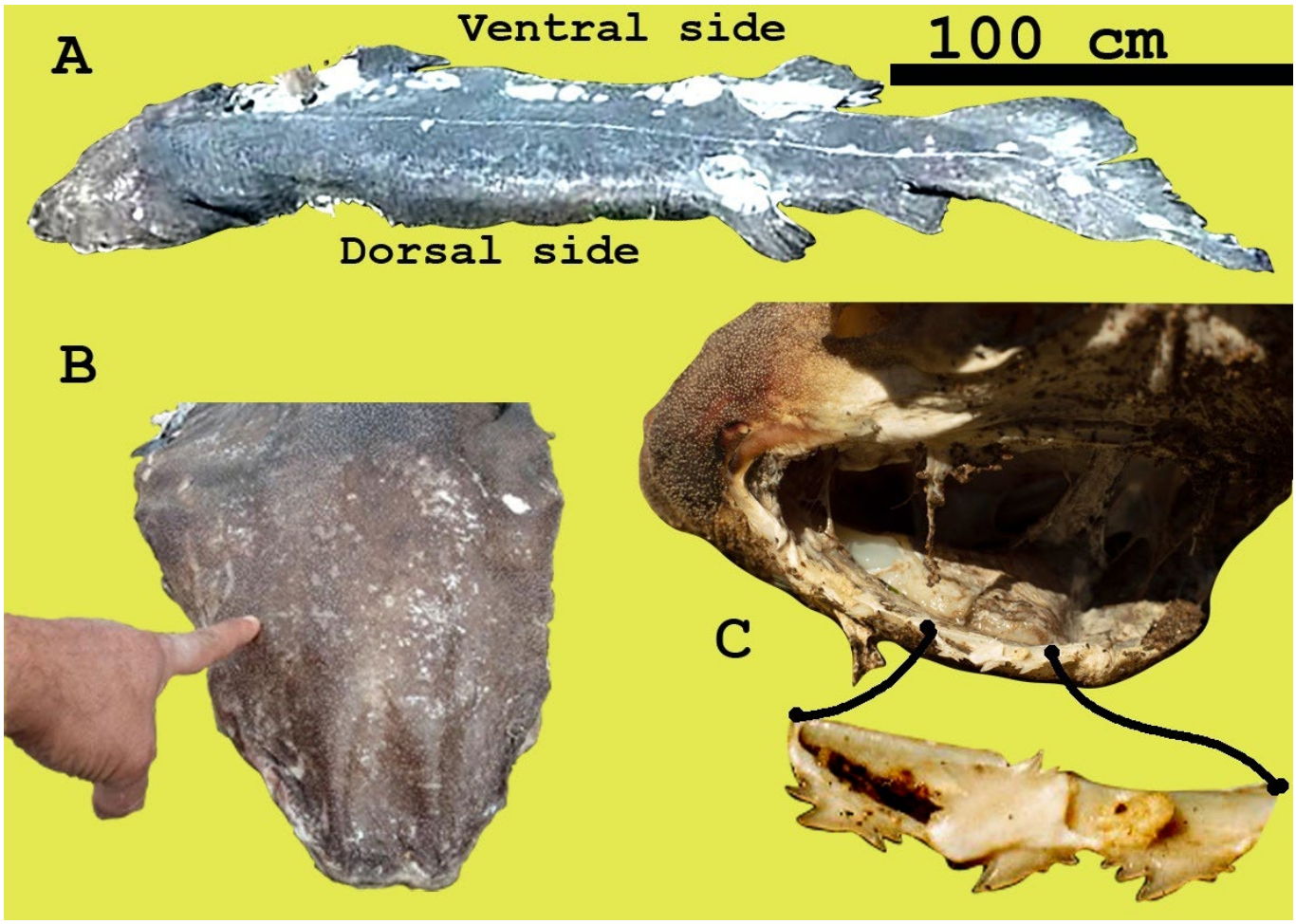

Figure 3. Aerial view of the prickly shark *E. cookei* with scavenging marks made by coyote *C. latrans*. (A). Detail of the large dermal denticles of the head area (B). Close up to the lower multi cusplet teeth of *E. cookei* (C). Photographs A and B by Leopoldo M. Amezcua, C by Meliza Le Alvarado

a) Identification of *Echinorhinus cookei*

In reference to the diagnostic characteristics that can be noticed in the analyzed specimen and following the stipulations of Compagno (1984), the absence of anal fin places it as a Squalomorph shark. Although the gill slit count could not be performed, due to damage caused by coyote scavenging, the presence of two spineless dorsal fins eliminates the possibility that the shark it is a Hexanchiform. The fusiform body, not being dorso-ventrally flattened "ray type" and the short rostrum without the presence of a saw indicates that the analyzed shark belongs to the order of the Squaliformes (Compagno, 1984). However, according to Ebert et al. (2021), the origin of the dorsal fins closer to the pelvic fins, as well as the presence of star-shaped dermal denticles (Figure 3b), appreciated at glance, place the organism in the order Echinorhiniformes. This order have a single family: Echinorhinidae and two species *Echinorhinus cookei* for the Pacific Ocean and *Echinorhinus brucus* for the Atlantic (Ebert et al., 2021). The presence of dermal denticles grouped in patches, unfused, not forming plates and the locality where it was found identified the shark as *E. cookei*. The absence of claspers on the pelvic fins indicates that the specimen was a female. According to Compagno, (1984), the sexual maturity for females of *E. cookei* is reached at 299 cm of total length, the estimated total length of the studied shark (262.7 cm), indicates that it was an immature organism. It is also noteworthy that no evidence of wounds caused by fishing hooks, harpoons or fishing nets were found in the body, so it can be concluded that the observation is the product of a natural stranding, not caused by a

fishing discard. To date, there is no report of any other stranded individual of *E. cookei* for the Pacific Ocean basin, so this is the first record of this type of event for the species. Nakamura et al. (2015) found that *E. cookei* present positive buoyancy and vertical diel migrations, the death of the shark at low deep because of the diel migration behavior, the positive buoyancy of the body and the strong currents at the Bahia de Los Angeles zone could cause the stranding of the shark carcass at the beach. Unfortunately, due to the remoteness of the location and the lack of on-site facilities, no tissue samples or necropsy was made in order to determine the cause of death of the organism.

b) Environmental feasibility of the presence of *E. cookei* in BLA

BLA is located in the central part of the Gulf of California (GC) in the area known as the "midriff islands". This area is characterized by the presence of the two largest islands of the GC (Angel de la Guarda and Tiburon). For the coast of Baja California, the natural channel between the coast and Angel de la Guarda Island is known as the "Ballenas channel" (BC), which has a depth of up to 1600 m and very stable temperatures, around 11° C throughout the year (Álvarez-Borrego, 2007). The oceanography for the BC is considered unique and different from the rest of the GC, with very strong tidal currents that promote an intense mixture resulting in a condition of constant upwelling (Álvarez-Borrego, 2007). This produces the highest surface concentrations of CO_2 and nutrients throughout the GC, which leads to high primary productivity (Álvarez-Borrego, 2007) and the presence of macroplanktophagous organisms such as great whales (*Balaenoptera musculus, B. physalus, B. edeni, Eschrichtius robustus*) or whale-sharks (*Rhincodon typus*), as well as various odontocetes (*Delphinus delphis, Globicephala macrorhynchus, Physeter macrocephalus, Orcinus Orca*) (Heckel et al., 2007). Likewise, BC is characterized by high concentrations of oxygen at great depth (Heckel et al*.,* 2007). These oceanographic conditions are similar to those of Monterey Bay, California (Broenkow https://montereybay.noaa.gov/sitechar/phys2.html), where the presence of *E. cookei* is known. This makes the presence of *E. cookei* feasible for the Ballenas Channel area in the GC. BLA presents small-scale fisheries aimed for geoduck clam, octopus, sharks, bony fish, mullet, squid, crabs, swimming crabs, and seaweed, as well as an industrial sardine fishery (Danemann et al*.,* 2007). With regard specifically to shark fisheries, there is no commercial interest on *E. cookei* and because of that, most of the previous records for Mexican waters are the result of bycatch (Chávez-Ramos & Castro-Aguirre, 1974; Álvarez-León & Castro-Aguirre, 1983; Mariano & Villavicencio, 1998; Ruiz-Campos et al*.,* 2010; Del Moral-Flores et al. 2015) or scientific collect (Galván-Magaña et al. 1996; Aguirre et al. 2002). It is likely that the absence of previous reports of *E. cookei* in BLA is due to fishers not accessing the usual depths (400-1100 m) (Cox & Francis, 1997) inhabited by this species and also, not having any commercial interest on this shark.

c) Coyote scavenging on *E. cookei* carcass

As described by Nowak, (1979) and by the geographical area where the observation was made, the scavenging coyote on the shark's carcass corresponds to the peninsular subspecies *Canis latrans peninsulae*. Coyotes have a maximum length between 1.1 to 1.3 m, being the subspecies of the Baja California peninsula smaller (1.1 m LT max.) than their northern counterparts. Morphometric analysis performed on coyotes indicate that their tails are between 30 and 40 cm long (Nowak, 1979). According to this, the precaudal length of the coyote was 80 cm and was used as a scale in the photograph to make the measurements of the shark. Coyotes are the first know canids that present antibodies to counteract the neurotoxin produced by *Clostridium botulinum*, a deterioration bacterium that occurs in carcasses and is responsible for the disease known as botulism (Ohisi et al., 1979), hence the ability of these mammals to feed on carcasses. It is noteworthy that although it has been previously described that coyotes can feed on elasmobranchs (Rose & Polis, 1998; Morales, 2008) to date there is no record of the scavenging of *C. latrans* or any other canid on *E. cookei* or any other echinorhiniform shark.

Conclusion

The oceanographic characteristics of BLA and BC, similar to other areas (Monterey Bay, CA) with the registered distribution of *E. cookei*, make the presence of this species, not registered for BLA to date, viable. The natural stranding of *E. cookei*, analyzed in this study, confirms the presence of this species for BLA and BC zone and increases the ichthyologic knowledge of the area. Likewise, the scavenging by the coyote *C. latrans* is another evidence of the feeding opportunities that the sea provides to these terrestrial carnivores, being this another record of scavenging feeding by *C. latrans* on elasmobranchs and the first record by any canid over echinorhiniform sharks to date.

Compliance with Ethical Standards

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

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

Funding disclosure: -

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Disclosure: -

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A preliminary life history traits analysis of sharks in the Sea of Marmara (Türkiye), where deoxygenation and habitat deterioration are raising concerns

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ABSTRACT

Life history traits of 17 species of sharks occurring in the Sea of Marmara were analysed based on Gower's distances, Principal Coordinate Analyses, and hierarchical clustering. The analysis shows that the sharks of the Sea of Marmara can ecologically be divided into several clusters. The increasing occurrence of sharks on the Marmara continental shelf, especially in the last few years, suggests that the expected habitat compression due to deoxygenation has begun to take place. This situation, which can be considered as a "habitat trap" for sharks, should be considered as a threat that may lead to shark mortalities due to the intensification of bycatches or even the intentional killing of sharks. Available results are sufficient to predict a disturbing future for sharks of the Sea of Marmara if the factors (deoxygenation, habitat loss, bycatch, etc.) threatening the overall ecosystem do not improve.

Keywords: PCoA, Ecology, Sharks, Habitat compression, Mortality

Introduction

The Sea of Marmara is the center of the hydrographic structure called the Turkish Straits System (Öztürk and Öztürk, 1996). Connected to the Mediterranean Sea via the Dardanelles Strait and the Black Sea via the Bosphorus Strait, the species composition of the fish fauna of the region represents the faunal characteristics of both seas (Eryılmaz and Meriç, 2005). Recent studies showed that the fish fauna of the Sea of Marmara includes 263 species of teleost fishes, representing 78 families (Artüz and Fricke, 2019) and 17 species of sharks, representing 11 families (Kabasakal, 2022a). According to Kabasakal (2022a) presence statuses of 3 out of 17 species of sharks, are currently being considered questionable.

Since it is on the way of one of the most intense maritime trade routes in the world, the Sea of Marmara has been exposed to serious pollution from ships (Erlevent and Kum, 2022). The fact that a significant part of Turkey's population lives in large cities surrounding the Sea of Marmara, as well as the intense industrialization around the region and the drainage waters of agricultural areas carried by the rivers emptying into the marine area, have caused serious marine pollution in the inland sea during the last 40 years (Salihoğlu et al., 2022). Especially in the deep sea trenches of the region, the fact that the oxygen level has decreased below 80 micromolar, which is considered the "hypoxia" limit (Mantıkçı et al., 2022), forces the fauna in these zones to live in extreme conditions. In a recent review, Kabasakal (2022b) pointed out that continued deoxygenation, particularly in the deep-sea region, could lead to "vertical habitat compression", forcing bathydemersal shark species to ascend in the shallows of the continental shelf and inhabit these zones, which could increase bycatch of deep-sea sharks. In the present article, which is a follow-up study of Kabasakal's (2022b) review, the authors aim to provide a preliminary life history traits analysis to a better understanding of the ecological status of the sharks of the Sea of Marmara. The Authors also aim to evaluate the available data regarding the deoxygenation and habitat loss in the Sea of Marmara to project possible consequences of such ecological conditions on the survival of sharks, based on the present analysis of life history traits of species occurring in the region.

Material and Methods

General Characteristics of the Study Area

Based on Kocatas et al. (1993) and Öztürk and Öztürk (1996), the general characteristics of the Sea of Marmara (Figure 1) can be summarized as follows: although it is a very small inland sea with an area of 11,500 km^2 and a volume of 3,378 km^3, the Sea of Marmara occupies an important place in Turkey's fishing economy. Three neighboring deep-sea trenches, (or deep depressions), a narrow continental shelf in the north and a relatively wider continental shelf in the south are the main geomorphological formations that stand out in the bottom structure of the Sea of Marmara (Figure 1). The deep depression zone, which is located in the middle region and reaches 1,335 m in depth, is the deepest place in the Sea of Marmara. A dual current system is dominated the current dynamics in the inland sea, which is connected to the Black Sea via the Bosphorus Strait, and to the Aegean and Mediterranean seas via the Dardanelles Strait. Considering the temperature and salinity stratification, three different water layers, which are surface, bottom, and transitional layers, fill the Marmara basin. The renewal time of the volume of water in the Sea of Marmara, with the oxygen-rich flow from the Mediterranean and Aegean, was estimated at 6 to 7 years (Kocataş et al., 1993).

Data Gathering and Analysis

Life history traits of 17 species of sharks, which are included in the current species list with confirmed or questionable occurrence (Kabasakal, 2022a), were gathered from the relevant literature (Compagno, 1984a,b; Serena, 2005; IUCN, 2007; Ebert and Stehmann, 2013; Froese and Pauly, 2022). In order to test whether the life history traits of the species could help to explain spatial variation in the Sea of Marmara a total of 18 functional traits with subcategories were assigned to each species, and the definitions of these traits (Pimiento et al., 2020) are shown in Table 1. Based on the definition that "any shark species with a total length (TL) >200 cm are classified as a large shark" (Ferretti et al., 2008), maximum sizes of examined species are arbitrarily divisioned into those with TL $\leq$200 cm and those with >200 cm.

To visualize the trait characteristics occupied by the selected shark species we conducted Principal Coordinate Analysis (PCoA) on Gower's distances of the species by trait matrix (Ladds et al., 2018). Convex hulls were created and colour-coded based on vector overlays to show traits that are important for shark species separation in trait space. In order to compute Gower's distances, based on the life history traits of 17 shark species, a "dissimilarity" matrix was computed with "dist.ktab" function of the "ade4" package of R (Pimiento et al., 2020). Since the data matrix is based on binary coding (1, presence; 0, absence), the "dichotomous – D" variable type is selected for computation. PCoA was performed with "pcoa" function of the "ape" package (Pimiento et al., 2020), and results were visualised with the "ggplot" library. Furthermore,

in order to illustrate the clustering of examined shark species, agglomerative cluster analysis, based on Gower's distances, was performed (Akay and Yüksel, 2017). All analyzes were performed in an R environment.

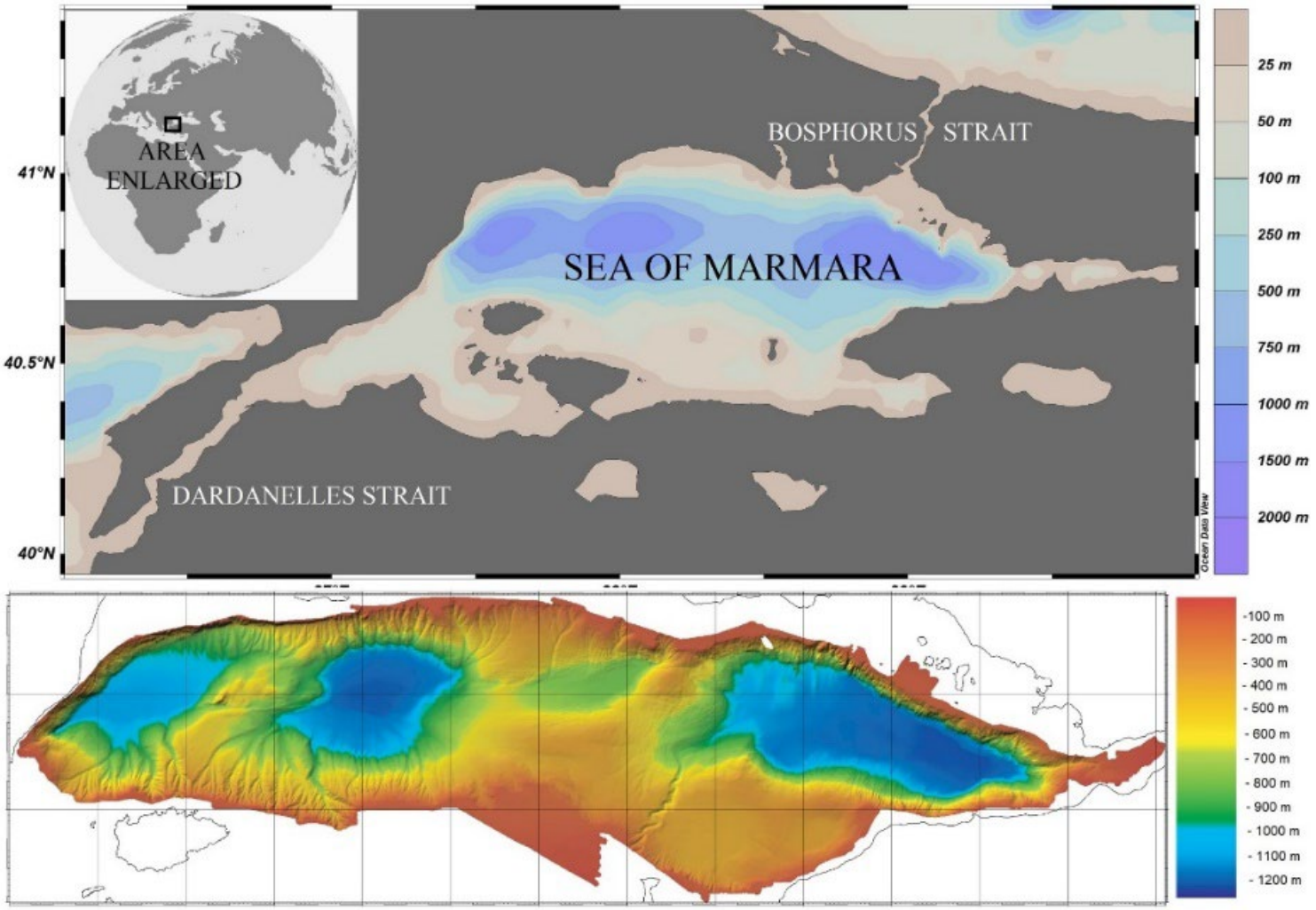

Figure 1. Position of the Sea of Marmara on the globe and the bathymetric map depicting continental shelf and deep-depression zones

Table 1. Definitions of life history traits used in the present analysis

Traits	Definition	Nature	Modality/Unit
Maximum size, TL	Maximum reported total length for each species gathered from relevant literature; TL of examined species arbitrarily categorized as TL ≤200 cm and TL >200 cm, based on definition of large elasmobranchs (Ferretti et al., 2008)	Binary	cm
Thermoregulation	The trait of mesothermy was assigned to two species of thresher sharks (*Alopias superciliosus* and *A. vulpinus*)	Binary	Mesothermy
Terrestriality	A 'land' trait designation was assigned to two angel sharks (*Squatina oculata* and *S. squatina*) and *Mustelus asterias*, since their juveniles or adults may occur in very shallow depths (eg. 5 m), coastal bays, estuaries and brackish water areas	Binary	Coastal bays Shallow water Estuarine Brackish
Habitat zone and vertical position	Habitat was categorized in accordance where organisms occur based on the zone where they occur as adults (i.e., trait designations: continental shelf, slope, offshore, some of these, or all). The vertical position was assigned based on the most frequent part of the water column where they feed (i.e., trait designations: benthic or demersal, pelagic or both). These data were gathered from the relevant literature	Binary	Shelf Slope Coastal Offshore Demersal Pelagic
Migration	Migration was assigned to define the seasonal coastal occurrences of Squatinid angel sharks, and seasonal migrations of Alopiid thresher sharks and *Galeorhinus galeus*, based on relevant literature	Binary	Seasonal
Diet	Diet was categorized based on the information gathered from relevant literature	Binary	Fish Cephalopods Crustaceans Polychates Marine mammals
Reproduction	Since egg-laying oviparous Scyliorhinid sharks depend on bottom structures to fix their egg-cases, mode of reproduction was assigned to each species to identify the location of reproduction; viviparity includes species with or without a yolk-sac placenta	Binary	Oviparous Viviparous

Results and Discussion

Life history traits of the following sharks species were analysed: Hexanchidae, *Hexanchus griseus* (Bonnaterre, 1788); Alopiidae, *Alopias superciliosus* (Lowe, 1841), *A. vulpinus* (Bonnaterre, 1788); Pentanchidae, *Galeus melastomus* Rafinesque, 1810; Scyliorhinidae, *Scyliorhinus canicula* (Linnaeus, 1758), *S. stellaris* (Linnaeus, 1758); Triakidae, *Galeorhinus galeus* (Linnaeus, 1758), *Mustelus asterias* Cloquet, 1819, *M. mustelus* (Linnaeus, 1758); Dalatiidae, *Dalatias licha* (Bonnaterre, 1788); Oxynotidae, *Oxynotus centrina* (Linnaeus, 1758); Centrophoridae, *Centrophorus uyato* (Rafinesque, 1810); Squalidae, *Squalus acanthias* Linnaeus, 1758, *S. blainville* (Risso, 1827); Echinorhinidae, *Echinorhinus brucus* (Bonnaterre, 1788); and Squatinidae, *Squatina oculata* Bonaparte, 1840, *S. squatina* (Linnaeus, 1758).

PCoA analysis and hierarchical cluster analysis, based on Gower's distances showed that 17 species of sharks occurring in the Sea of Marmara were divided into several clusters. Graphical illustrations of PCoA analysis and hierarchical clustering of the examined species are depicted in separate plots (Figures 2 and 3). The first cluster includes *A. superciliosus* and *A. vulpinus*, the last pelagic lamniform sharks still occurring in the Sea of Marmara, due to their pelagic migratory characteristics, and thermoregulation abilities. The second cluster includes predominantly demersal shark species

and redivisioned in several subclusters including *H. griseus*, all of the squaliform sharks, representing in Dalatiidae, Oxynotidae, Centrophoridae, Squalidae and Echinorhinidae families; squatinid angel sharks, and the triakid *G. galeus* (Figure 3). Due to its' specialized feeding modes, *O. centrina*, a specialized polychaete feeder, differs from other species of the second cluster. Although, included in the second cluster, *H. griseus*, the largest demersal shark occurring in the Sea of Marmara, is differentiated from other subclusters due to its large size and extensive depth distribution covering both bathial and shallow shelf waters, as well as the fact that in its stomach contents remains of marine mammals could be found. Angels sharks (*S. oculata* and *S. squatina*) representing terrestriality and form another subcluster. The oviparous (egg-laying) pentanchid (*G. melastomus*) and scyliorhinid sharks (*S. canicula* and *S. stellaris*) were grouped in the third cluster. The fourth cluster is formed by *M. asterias* and *M. mustelus*, and they differed from other demersal species due to their durophagous feeding habits, since they mainly prey on crustaceans.

As Mouillot (2007) emphasizes, biodiversity is being lost rapidly in an increasingly changing world due to human activities. Therefore, we urgently need to identify the factors that control the coexistence of species and thus sustain regional biodiversity and the threats to them. Measurements of species dissimilarities or distances between them, which is a critical step in functional ecology studies, is an important milestone in understanding the factors that make the coexistence of these species possible (Pavoine et al., 2009). Hierarchical clustering methods based on dissimilarities or differences, such as Gower's distances, can help to understand diversity (Pavoine et al., 2009; Ladds et al., 2018). This pioneering study, in which the functional distances or dissimilarities of shark species occurring in the Sea of Marmara were revealed for the first time, showed that 17 species of sharks form distinct clusters that are not far apart. Thus, apart from 7 specialized species, 10 other species (58.8% of total species) share similar niches.

Since sharks are apex predators, they play a fundamental role in maintaining a balanced food chain in the marine ecosystem, and are indicators of healthy oceans (Stevens et al., 2000; Fowler et al., 2005; Motivarash et al., 2020). Despite the vital functional roles they play in the ecosystem, sharks are increasingly exploited in bycatch worldwide (Dulvy et al., 2021), and due to the nature of their K-selective life history, the effects of untargeted removal of these top predators from the marine ecosystem are still not fully understood (Stevens et al., 2000). Bycatch, causing more than half of shark and other cartilaginous fish mortalities worldwide (67.3%; Dulvy et al., 2021), is also the main threat targeting sharks in the Sea of Marmara (see Kabasakal, 2022a for relevant references). Moreover, habitat loss and deterioration as well as pollution are considered as raising threats that seriously affect the survival of sharks (Dulvy et al., 2021).

Since the survival of sharks (and cartilaginous fish in general), which play a decisive role in the health of marine life, depends on perfectly functioning ecosystems (Fowler et al., 2005), studies dealing on the potential effects of habitat loss and deterioration, as well as pollution-related threats on sharks and possible consequences on the ecosystem, are of critical importance. In a recent review, Consales and Marsili (2021) considered habitat loss and environmental contamination as major hazards threatening sharks. However, pollution and the relevant stressors affecting sharks are the least investigated and evaluated threats, as well (Consales and Marsili, 2021; Lipej et al., 2022). Regarding the survival of sharks, critical habitats (e.g. shallow estuaries, bays, gorgonian reefs, deep-sea areas *etc.*) can play crucial roles in certain periods of life cycles (e.g. reproduction and development) of elasmobranchs, and anthropogenic environmental pressures on critical habitats may have devastating consequences (Fowler et al., 2005). Those expected devastating consequences, which threaten the survival of sharks, have begun to occur with increasing frequency in the Sea of Marmara, especially in recent years, as well.

Deoxygenation, mucilage outbreaks and deterioration of benthic habitats in the Sea of Marmara, which has undergone great changes in the last 40 years due to human pressures, severely threaten the entire ecosystem (Topçu and Öztürk, 2015; Özalp, 2021; Çınar et al., 2021; Aksu et al., 2022; Karadurmuş and Sarı, 2022; Mantıkçı et al., 2022; Salihoğlu et al., 2022). Kabasakal (2022b) emphasized that 'vertical habitat compression' may occur in the Sea of Marmara due to deoxygenation and 'dead zone' formation, especially in the deep trenches, and as a result of this situation, deep-sea sharks can be encountered in the shallow continental shelf region more frequently than they used to be in the past. Fishing records of the last 10 years revealed that captures of deep-sea sharks have been increasingly concentrated in the shallower waters of the continental shelf (<100 m depth) (Bayhan et al., 2006; Kabasakal, 2013, 2017;). Mass mortalities of *Raja clavata*, and *Dasyatis pastinaca* were recorded for the first time in the Sea of Marmara during the mucilage outbreak in the summer of 2021 (Karadurmuş and Sarı, 2022). Although not yet observed, mass mortalities of demersal sharks can also be expected, if the current deoxygenation continues.

So, what could be the effects of deterioration of benthic habitats on sharks in the Sea of Marmara? As a result of mucilage aggregations and construction debris being discharged into

the sea, the gorgonian reefs in the Sea of Marmara have been severely damaged in recent years (Topçu and Öztürk, 2015). These reefs are considered to be among the important breeding areas of oviparous scyliorhinid catsharks, and the deterioration of these areas will possibly result in the loss of breeding grounds for *S. canicula* and *S. stellaris*. According to Capapé (2008), *O. centrina* is a specialized suction feeder that preys on worm-like prey such as polychaetes that constitute 60% of its diet, and the deterioration of the benthos can have dramatic consequences on the diet of this species. Crustaceans experienced the most severe mass mortalities during the mucilage outbreak in the Sea of Marmara (Karadurmuş and Sarı, 2022). This situation threatens the diet of crustacean feeders among sharks such as *M. asterias* and *M. mustelus*. Young and adult individuals of *M. asterias* and *S. squatina* inhabit estuarine brackish areas, especially during the breeding and development period (Ebert and Stehmann, 2013). Such areas are inhabited also by some rare species such as *G. galeus* in the Sea of Marmara (Kabasakal and Türetken, 2021). The coastal destruction and human-induced pollution caused the deterioration of the estuarine regions, (Aksan and Ergül, 2022; Tan and Demirtaş, 2022) which subsequently resulted, in habitat loss for the sharks inhabiting these regions.

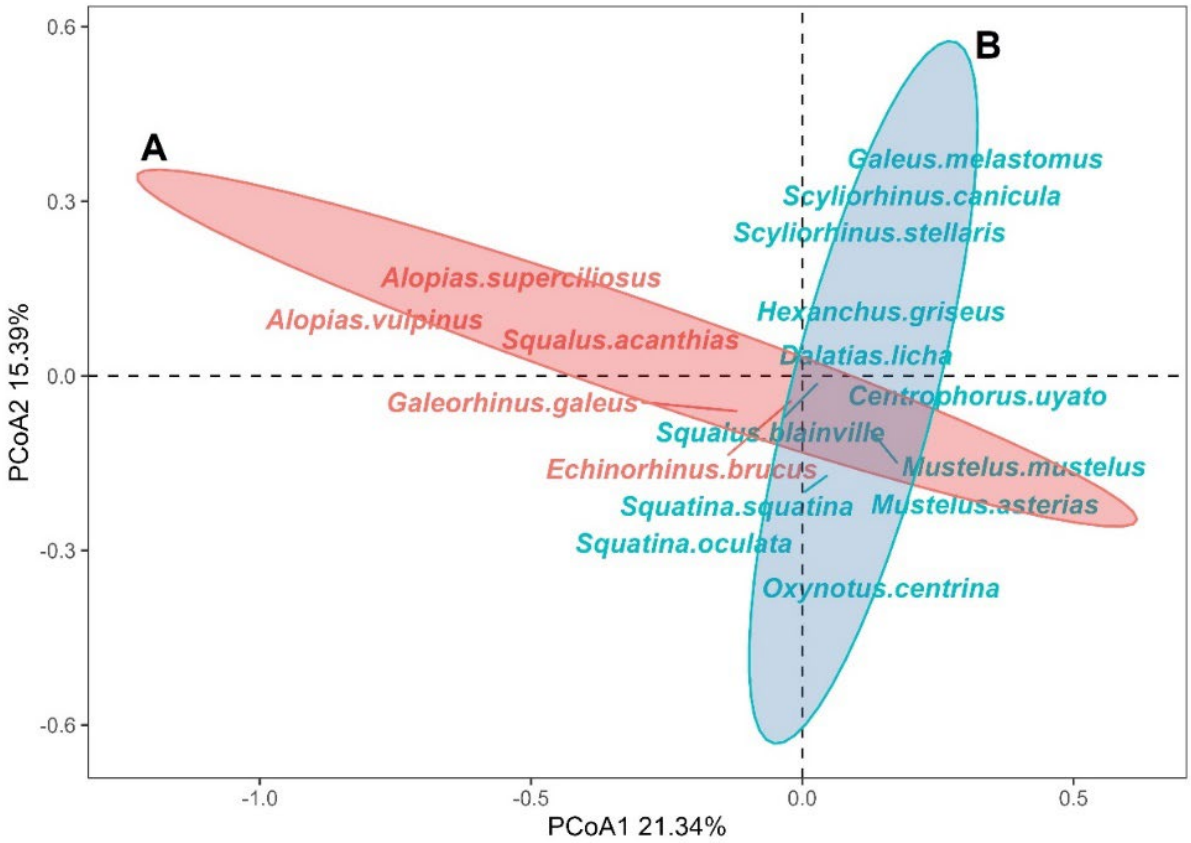

Figure 2. Gower's distances showing differences among shark species of the Sea of Marmara recorded in terms of their species traits. Coloured polygons were determined based on Pincipal Coordinate Analysis

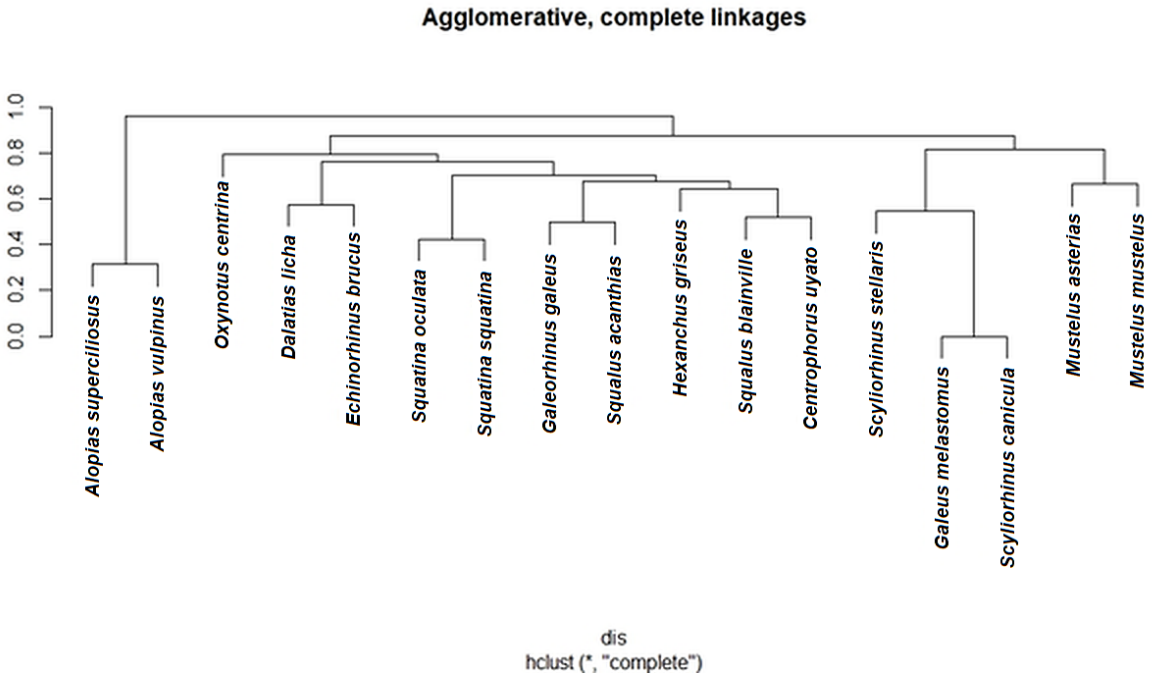

Figure 3. Hierarchical cluster analysis of shark species occurring in the Sea of Marmara, based on Gower's distances

Most pelagic sharks are ram ventilators and can only tolerate moderate hypoxia for a short time (Sims, 2019). These species, which are obligatory swimmers, face a remarkable vertical and horizontal habitat compression in the pelagic realm, as a result of deoxygenation (Vedor et al., 2021). Lamnid sharks, such as *Carcharodon carcharias*, that seasonally migrated into the Sea of Marmara in the past following tuna, *Thunnus thynnus*, have disappeared from the region since the mid-1980s, following the extirpation of tuna populations due to overfishing and marine pollution (Kabasakal, 2016). Although it is currently unpredictable how *A. superciliosus* and *A. vulpinus*, the last remaining pelagic lamniform sharks in the Sea of Marmara, will respond to the increasingly severe deoxygenation, their concentration in habitable oxygenated areas may create a "habitat trap" (Sims, 2019) and can be caused with the increase of pelagic sharks' bycatch.

Mittlebach and Schemske (2015) define the 'mass effect' as the immigration of individuals into a community in a way that influences the recipient community's dynamics. In terms of functional ecology, we do not yet have basic data predicting how the recipient community dynamics in these regions may be affected as a result of the sharks being trapped in the habitable oxygenated areas and shallow continental shelf in the Sea of Marmara. Since the continental shelf of the Sea of Marmara is a region where commercial fishing is intense, as a result of increased predatory pressure on commercial species due to habitat compression, fisher-shark competition may dramatically intensify in the future. For example, *E. brucus*, which normally occurs at depths of 200-1,214 m (Kabasakal et al., 2005; Ebert and Stehmann, 2013), has been observed frequently in the Marmara continental shelf in recent years in areas shallower than 100 m and especially in the fishing grounds of the commercial shrimp species *Parapenaeus longirostris* (Kabasakal *pers. obs*.). The beam-trawl used in *P. longirostris* fishery produces significant amounts of bycatch in the Sea of Marmara (Bayhan et al., 2006; Bök et al., 2011). Although for the moment, most shrimp fishermen are releasing the sharks that have entered the beam trawl (Figure 4), as the economic losses due to predator pressure increase, will fishermen's conservationist perceptions shift towards to intentionally killing of the bycaught sharks over time? Moreover, in the context of the assumption that economic pressures on any ecosystem will eventually turn into a biodiversity problem (Perrings and Walker, 1995), increasing economic losses in shrimp catches (as well as for other commercial species and fishing gears), can trigger a possible fisher-shark competition which could result in the loss of sharks in the Sea of Marmara. This concern, which has just begun to attract attention, seems to intensify if the threats on the Marmara ecosystem continue.

Figure 4. Releasing the bramble shark, *E. brucus*, which was incidentally captured by a commercial beam-trawler catching shrimp, *P. longirostris*, in the shallows (<100 m) of northern shelf of Sea of Marmara. Images were captured from a video footage

Conclusion

On the basis of Gower's distances, Principal Coordinate Analysis and hierarchical clustering, it can be suggested that 17 species of sharks occurring in the Sea of Marmara mostly share very close niches. The increasing occurrence of sharks on the Marmara continental shelf, especially in the last few years, suggests that the expected habitat compression due to deoxygenation has begun to take place. This situation, which can be considered as a "habitat trap" for sharks, should be considered as a threat that may lead to shark mortalities due to the intensification of bycatches or even the intentional killing of sharks. Unfortunately, this situation can only be resolved with an integrated rehabilitation in the Marmara ecosystem. Available results are sufficient to predict a disturbing future for sharks of the Sea of Marmara if the factors (deoxygenation, habitat loss, bycatch etc.) threatening the overall ecosystem do not improve.

Compliance with Ethical Standards

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

Ethics committee approval: Ethics committee approval is not required.

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Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Short Communication" Readers can also present their comments on the published manuscripts in the form of a "Short Communication". The main text should contain **"Introduction", "Materials and Methods", "Results and Discussion", "Conclusion", "Compliance with Ethical Standard**" and **"References"** sections.

Table 1. Limitations for each manuscript type

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

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Tables

Tables should be included in the main document, and presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

Figures and Figure Legends

Figures, graphics, and photographs should be submitted in main document WORD files (in JPEG or PNG format) through the submission system. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in the USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References

Reference System is APA 6th Edition (with minor changes)

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

....(Bhujel, 2014).

....(Mol and Erkan, 2009).

....(Alofa et al., 2023).

....(Mol and Erkan, 2009; Bhujel, 2014; Alofa et al., 2023).

Citations for a Reference Section:

An article

Alofa, C.S., Olodo, I.Y., Chabi Kpéra Orou Nari, M., Abou, Y. (2023). Effects of the fresh and dried housefly (*Musca domestica*) larvae in the diets of Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758): growth, feed utilization efficiency, body composition and biological indices. *Aquatic Research*, 6(1), 1-10. https://doi.org/10.3153/AR23001 **(if DOI number has)**

A book in print

Bhujel, R.C. (2014). A manual for tilapia business. CABI Nosworthy Way Wallingford Oxfordshire OX10 8DE UK, 199 p. ISBN 978-1-78064-136-2. https://doi.org/10.1079/9781780641362.0000 **(if DOI number has)**

A book chapter

Craddock, N. (1997). Practical management in the food industry A case study. In Food Allergy Issues for the Food Industry; Lessof, M., Ed.; Leatherhead Food RA: Leatherhead, U.K., pp 25-38. ISBN: 4546465465

A webpages

CDC (2020). Rift Valley Fever | CDC. https://www.cdc.gov/vhf/rvf/index.html (accessed 20.08.2020).

Revisions

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 15 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be canceled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.