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AQUATIC RESEARCH



Aquatic Research 1(2), 50-54 (2018) • DOI: 10.3153/AR18006

Original Article/Short Communication

DETERMINATION OF SAGITTAL OTOLITH BIOMETRY AND BODY SIZE OF *Serranus cabrilla* (Linnaeus, 1758) DISTRIBUTED IN SOUTHERN AEGEAN SEA

Gökçen Bilge , Halit Filiz 

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Bilge, G., Filiz, H. (2018). Determination of Sagittal Otolith Biometry and Body Size of *Serranus cabrilla* (Linnaeus, 1758) Distributed in Southern Aegean Sea. Aquatic Research, 1(2), 50-54. DOI: 10.3153/AR18006

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ABSTRACT

Fish otoliths are generally used to determine taxon, age and size of the teleost fishes and are useful tools for studies of prey-predator relationships, population dynamics and ichthyo-archaeology. In the present study, the equations for the relationships were calculated between fish length (TL), weight (W) versus sagittal otolith length (OL), height (OH) and weight (OW) in comber (*Serranus cabrilla*, Linnaeus, 1758) specimens (N=310, 95–225 mm in TL and 7.54–111.27 g) captured via bottom trawl vessels from off the Güllük Bay (Southern Aegean Sea) between January and December 2013. Since there has been no statistical differences between left and right otoliths ($p>0.05$), left otoliths were used for calculations. Regression formulas were calculated as follows: $TL=28.75*OL-22.31$, $TL=64.36*OH-6.808$, $TL=2380*OW^{0.640}$, $W=0.056*OL^{3.618}$, $W=2.029*OH^{3.254}$, $W=5168*OW-28.33$, $OH=0.414*OL-0.054$, $OW=0.0000053*OL^{1.542}$, and $OW=0.003*OH^{1.382}$. The aim of this study is to fill in the missing data concerning otolith and fish size relationships of the species in the southern Aegean Sea, thereby providing researchers studying food habits of top predators to determine the size and weight of prey fish from length or weight of recovered otoliths.

Keywords: Comber, Serranidae, Sagittae, Otolith size

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Introduction

Otoliths have distinctive shape which is highly species specific, but varies widely among species (Maisey, 1987). Thus, several identification guides and keys has been published by Smale et al (1995) for south African Fishes, Harkönen (1986) for northeast Atlantic Sea, Morrow (1976) for Bering Sea, Campana (2004) for northwest Atlantic Ocean, Tuset et al. (2008) for the western Mediterranean, north and central eastern Atlantic, and Nolf (1985) for fossil fishes. The prey specimens are partially or totally digested and the hard remains in stomachs, intestines, faeces and scats (of piscivorous fishes, marine mammals, sea birds) are only diagnostic features that can be considered; among those hard remains, otoliths are quite resistant to digestion and they are important tools for prey classification (Al-Mamry et al., 2010). The comber *Serranus cabrilla* (Linnaeus, 1758) is a demersal fish species found at depth ranges between 5-500 m on the continental shelf and upper slope over rocks, *Posidonia* beds, sand and mud bottoms (Tortonese, 1986). It distributes in temperate and tropical regions, Eastern Atlantic: English Channel southward round the Cape of Good Hope to Natal, South Africa (Heemstra&Randall, 1986), including Azores, Madeira and the Canary Islands (Tortonese, 1986). Also in the Mediterranean and western Black Sea. This study represents the first information on the otolith-fish size relations of *Serranus cabrilla*, collected from the Güllük Bay (the southern Aegean Sea).

Materials and Methods

In the present study, the relationship equations were calculated between fish length (TL), weight (W) versus sagittal otolith length (OL), height (OH) and weight (OW) in comber (*Serranus cabrilla*, Linnaeus, 1758) specimens captured via bottom trawl vessels from the Güllük Bay (Southern Aegean Sea) between January and December 2013.

Total lengths (TL) were measured to the nearest millimeter. Fish weight (W) were determined to the nearest 0.01 g on a digital balance. Sagittae were (N= 310) removed through a cut in the cranium to expose them then cleaned and stored dry in glass vials and the left and right otolith were considered separately. Each sagitta was placed with the sulcus acusticus oriented through the observer and otolith length (OL) and otolith height (OH) was determined using eyepiece micrometer under stereo zoom microscope (Olympus SZX-16) and defined as the longest dimension between the rostrum and postrostrum axis (nomenclature of Smale et al., 1995 and Tuset et al., 2008) through the focus of the otolith (Al-Mamry et al., 2010) (Figure 1). Individual sagittal otolith weight (OW - in 0.1 mg) was determined using an electronic balance. Firstly, the *paired t-test* was used to check

any differences between left and right otolith pairs. When significant differences ($P < 0.05$) were not found, the H_0 hypothesis ($b_{\text{right}} = b_{\text{left}}$) was accepted and a single regression was used for each parameter (OL, OH and OW). Linear, $Y = ax + b$ and non-linear, $Y = ax^b$ regression equations were fitted to determine which equations (OW-W, OL-OW, TL-OH, OH-W, OW-OH, OL-W, OL-TL, OH-OL, and OW-TL) are best describing various relationships between otolith and fish size (Tarkan *et al.*, 2007).

Results and Discussion

The sagittal otoliths of 310 *S. cabrilla* specimens were examined. Table 1 shows the descriptive statistics regarding length and weight of the species and its sagittal otoliths (with otolith width): the average total length (\pm S.D) was 14.589 (\pm 2.909) mm (95-225 mm), and the length of otoliths ranged from 3.8 to 8.3 mm, height from 1.5 to 3.6 mm, and weight from 0.0077 to 0.0251 g.

Relations between fish and otolith measurements were given in Table 2, and the relationships between otolith morphometric parameters and fish sizes of *S. cabrilla* were given in Figure 2. No significant differences (t-test for paired comparisons, $P > 0.05$) were found between left and right otolith length and weight data. Therefore, the left sagittae measurements were used for the calculation of equations. Calculated regressions were displayed a high coefficient of determination ranging between 0.793 - 0.938. A linear regression model was used to determine the relationship between the fish length and otolith sizes, but an exponential regression model was used to describe the relationships between lengths and weights of otolith and fish for the species.

S. cabrilla is a discard or by-catch species by trawl landings in Turkey. Despite it has not any commercial value, ecological importance come from to be found in predators' diet. Otoliths are used extensively in stomach content analysis because they are one of the last species-specific features to be digested (Smale et al., 1995). This "powerful" taxonomic feature of earstones, allowed fish species identification. Even though there is some reference identification guides and keys for western Mediterranean, Atlantic Ocean and adjacent waters, this case is very limited, just for some species specific papers, for eastern Mediterranean. At this point, studying the marine predators' feeding habits is to fill the gap of information on the fish otolith morphology and on the estimation of specific equations, which is useful to calculate the size and mass of preys (Battaglia et al., 2010).

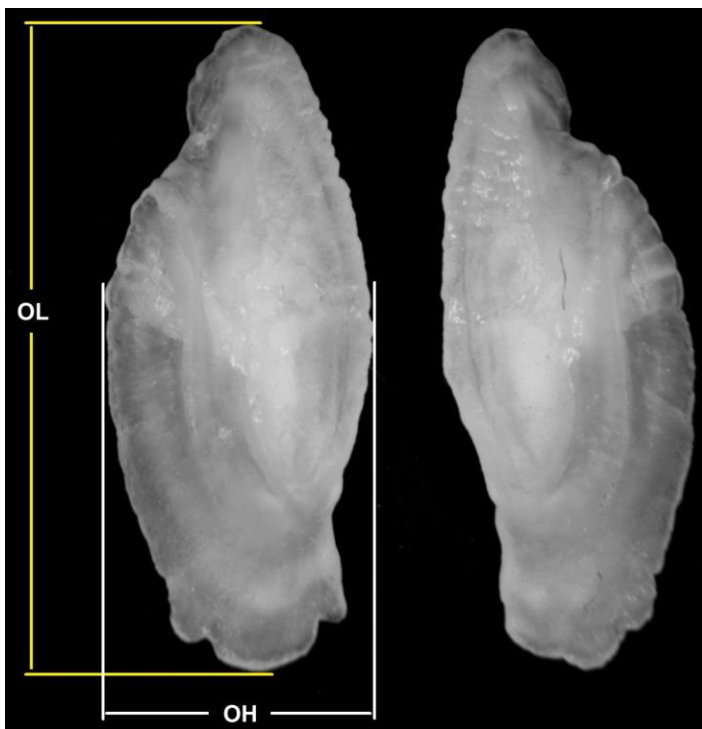
Table 1. Descriptive statistics of length and weight data of specimens and their otoliths obtained from the Southern Aegean Sea.

	Range	Average (\pm S.D.)
TL (mm)	95-225	14.589 (\pm 2.909)
W (g)	7.54-111.27	38.569(\pm 22.34)
OL (mm)	3.8-8.3	5.849(\pm 0.980)
OH (mm)	1.5-3.6	2.37(\pm 0.430)
OW (g)	0.0077-0.0251	0.0129(\pm 0.0039)

Total length (TL), Fish weight (W), Otolith length (OL), Otolith weight (OW) and Otolith height (OH), Standart deviation (S.D.)

Table 2. Intercept values (a), regression slope (b) and coefficients of determination (r^2) for linear (L) and exponential (E) relationships between otolith morphometric parameters, fish length and weight of *S. cabrilla*.

	Relationship	Regression	a	b	r^2
Fish Length	TL vs. OL	L	28.75	-22.31	0.938
	TL vs. OH	L	64.36	-6.808	0.908
	TL vs. OW	E	2380	0.640	0.838
Fish Weight	W vs. OL	E	0.056	3.618	0.917
	W vs. OH	E	2.029	3.254	0.887
	W vs. OW	L	5168	-28.33	0.822
Otolith	OW vs. OL	E	0.000053	1.542	0.825
	OH vs. OL	L	0.414	-0.054	0.891
	OW vs. OH	E	0.003	1.382	0.793

**Figure 1.** Sagittal otolith of the comber, *Serranus cabrilla*, sampled from the southern Aegean Sea in 2013 (Total length of the fish = 225 mm, otolith length = 8.3 mm; OL = otolith length, OH = otolith height).

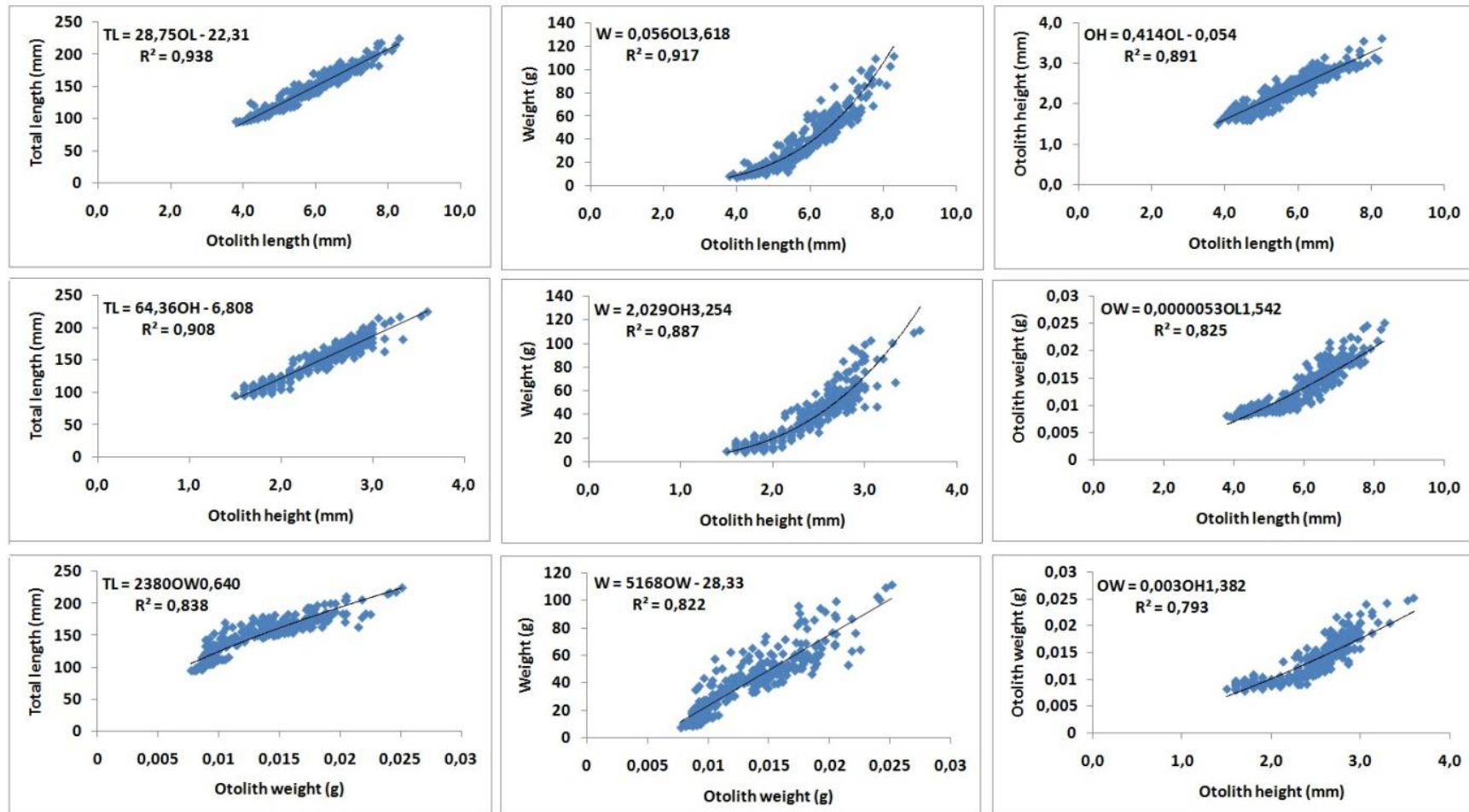


Figure 2. Relationships between otolith morphometric parameters and fish sizes of *S. cabrilla*.

Tuset *et al.* (2008) reported a % ratio relationships between the length of *S. cabrilla* (70, 177 and 255 mm TL, n = 3) and Sagitta sizes as OL/TL= 3.4-4.2 and OH/OL= 39.9-47.1; in the present study (95-225 mm TL, n = 310) these ratios were calculated as OL/TL=3.3-4.5 and OH/OL= 33.9-48.1.

Conclusion

The growth of individuals belonging to the same species may show some differentiations for different areas and stocks (Campana and Casselman, 1993). Therefore, a species had been studied its distribution area, separately, on this object. To meet this need, actually, small part of this lack, this study is providing OW-W, OL-OW, TL-OH, OH-W, OW-OH, OL-W, OL-TL, OH-OL, and OW-TL relationships for the species.

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AQUATIC RESEARCH



Aquatic Research 1(2), 55-63 (2018) • DOI: 10.3153/AR18007

Original Article/Full Paper

ANALYSIS OF MERCURY (HG) IN FOUR HOLOTHURIANS SPECIES (PHYLUM-ECHINODERMATA) FROM KARACHI COAST-NORTHERN ARABIAN SEA

Quratulan Ahmed¹ , Levent Bat² , Qadeer Mohammad Ali¹ 

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Ahmed, Q., Bat, L., Ali, Q.M. (2018). Analysis of Mercury (Hg) in Four Holothurians Species (Phylum-Echinodermata) From Karachi Coast-Northern Arabian Sea. Aquatic Research, 1(2), 55-63. DOI: 10.3153/AR18007

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ABSTRACT

Nowadays metal bioaccumulation in holothuroids is increasing and is a cause of worry owing to toxicity. In the present study the concentrations of mercury (Hg) were measured in holothuroids (*Holothuria (Thymiosycia) arenicola* Semper, 1868, *Holothuria (Lessonothuria) pardalis* Selenka, 1867, *Holothuria (Lessonothuria) verrucosa* Selenka, 1867 and *Holothuria (Halodeima) atra* Jaeger, 1833) collected during different seasons (pre-monsoon, monsoon, and post-monsoon) in 2015 at Buleji and Sunehri coasts of Karachi, Pakistan. Hg concentrations of tentacles were higher than those in muscle, tentacle and gut tissues of the pre-monsoon, monsoon and post-monsoon. The lowest Hg concentrations were found in muscle tissues at all seasons in Buleji and Sunehri coasts. The mean levels in the muscle tissues were 0.018 mg/kg dry wt. for *H. arenicola*, 0.024 mg/kg dry wt. for *H. verrucosa*, 0.026 mg/kg dry wt. for *H. pardalis* and 0.036 mg/kg dry wt. for *H. Atra*. The results indicate that according to the European Union legislation the Hg amounts in all tissues of holothurians is much lower than permitted levels. In terms of Estimated Weekly Intakes, this current work also proved that there was no likely health hazard to consumers on account of intake of sea cucumbers under the current consumption rate in Buleji and Sunehri coasts of Karachi, Arabian Sea.

Keywords: Mercury, Holothuroids, Buleji, Sunehri, Karachi coast, Estimated Weekly Intakes, Estimated Daily Intakes, Northern Arabian Sea

Introduction

In terms of industry Karachi in Pakistan is the most important city where its coastal water is contaminated with domestic and industrial effluent. Karachi coast is impacted principally by human activities including shipyards, industrial factories, touristic and fishing activities. Therefore research on metal pollution in Karachi coasts has been increased during the recent years. Most studies have dealt with metal concentration in fish (Ahmed et al., 2014, 2015; Ahmed and Bat, 2015a,b,c,d; Ahmed and Bat, 2016a,b; Ahmed et al, 2017a) and very few study concern the holothuroids (Ahmed et al, 2017b) in Karachi coasts of the Arabian Sea. Marine organisms, especially benthic invertebrates have the ability to absorb metals from their living environment. Because of the ecological importance of holothuroids and their potential as bio-indicators, recently they are used to study heavy metal pollution. They are deposit-feeder ingesting surface sediments and feeding on detritus and associated microorganisms and well distributed in the Northern Arabian Sea (WoRMS Editorial Board, 2018). Sea cucumbers also were accepted as biomonitor organisms (Warnau et al., 2006).

Heavy metals are considered the important chemicals in marine environments, and since they are not biodegradable, they can accumulate at the top of the food chain. Many inorganic elements that are non-essential for people enter the diet via seafood. Indeed, most of them are present in aquatic environment, usually in trace amounts that may be toxic under certain conditions. One of the most important non-essential inorganic elements in seafood that have no known or accepted biological function in organisms is mercury. Hg is very toxic metal and its harmful effects are well documented. Most inorganic Hg compounds are toxic, but organic Hg compounds, formed when Hg compounds combine with certain organic molecules, are even more so. These organic Hg compounds are soluble in fat, which means they accumulate easily in the body and so penetrate readily into the nerves and brain. Hg occurs naturally in marine environment and can also be released into the air thanks to industrial pollution. Its vapour is easily transported in the atmosphere and falls from the air and can pile up in the aquatic environment (Bat and Özkan, 2015).

Seafood is a good source of nutrition. It is high in protein, vitamin B and good fatty acids as omega 3. The health benefits of eating seafood outweigh the potential risks. Hg is a contaminant that can be found in edible tissues. Hg amounts differ from coasts to other coasts and may be owing to human activities or to natural causes. Hg poisoning is generally

counted as the maximum extensive environmental health threat and its absorption may constitute a serious risk to human health. It is well known that in Minimata, Japan, hundreds of people died or became very heavily sick mostly from nervous system damage from eating seafood from coastal waters that were seriously contaminated with Hg from local industrial discharge.

Sea cucumbers are a pretty appraised as food in the very Asian countries. They are traditionally eaten up raw, dried, boiled (Özer et al., 2004), soups and salads (Jinadasa et al., 2014) as food for human consumption. Moreover they are considered to be a high medical value (Jinadasa et al., 2014). The current study aims to determine the concentrations of mercury (Hg) in muscle, tentacle and gut tissues of holothuroids *Holothuria (Thymiosycia) arenicola* Semper, 1868, *Holothuria (Lessonothuria) pardalis* Selenka, 1867, *Holothuria (Lessonothuria) verrucosa* Selenka, 1867 and *Holothuria (Halodeima) atra* Jaeger, 1833 collected during different seasons (pre-monsoon, monsoon, post-monsoon) in 2015 at Buleji and Sunehri coasts of Karachi, Pakistan to compare the results with those obtained in international regulations and to assess the potential health risk for consumers based on their intake.

Materials and Methods

A total of 144 specimens namely *H. arenicola*, *H. pardalis*, *H. verrucosa* and *H. atra* (6 individuals in each species and 6 individuals each season) were collected from Buleji and Sunehri coasts of Karachi in 2015 (Figure 1). After collection, individuals were put in seawater from the sampling stations and transferred to the laboratory. The muscle, tentacle and gut tissues of holothuroids were then separated carefully (modified from Bernhard 1976; UNEP 1984 and 1985). 5 grams of muscles, whole tentacles and whole gut of each sea cucumber samples were placed into the crucibles and were dried in furnace at 600°C temperature for 3 hours and then dry ash were weighted in digital balance. These ashes were digested in the flasks containing 10 ml of concentrated HCl. When the digestions were completed, the solutions were cooled to ambient temperature and the digest was filtered and made up to 100 ml in a volumetric flask using ultrapure water as the diluent. The analysis for total mercury was performed on Mercury Hydride system (cold vapor Technique) Atomic Absorption Spectrometer. The AA Analyst 700 atomic absorption spectrometer with 253.7 wavelengths was used to determine Hg concentration. Detection limit was 2 ng Hg (0.00004 mg/L in a 50 mL sample).

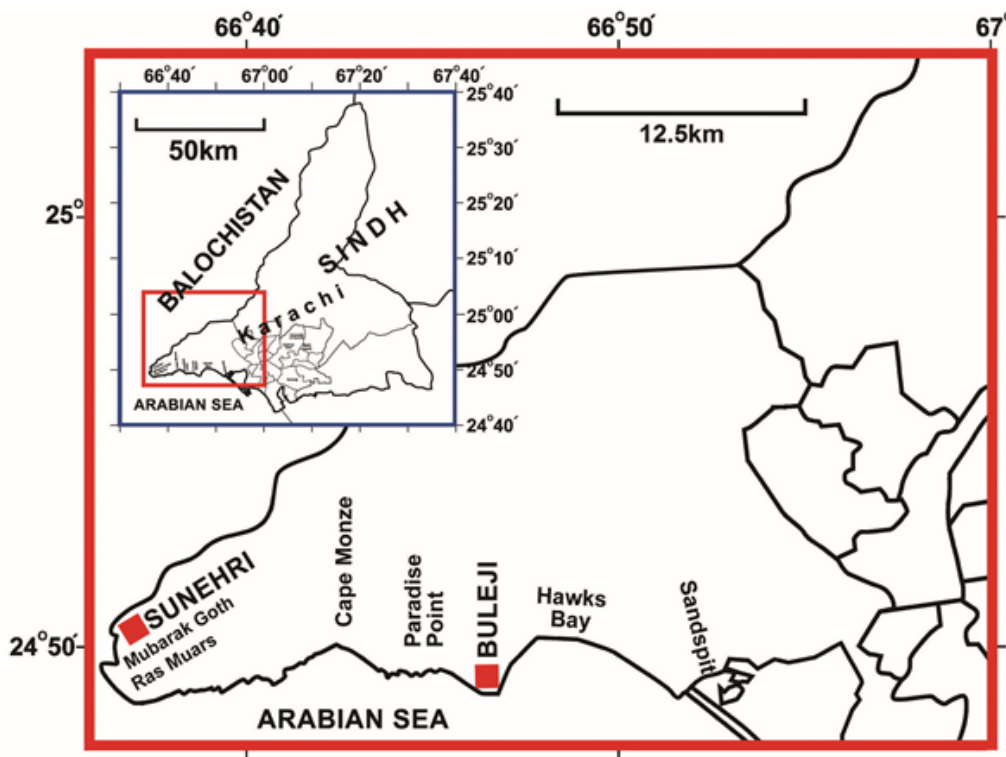


Figure 1. Sampling area from Buleji and Sunehri coasts of Karachi, Arabian Sea

Intake Levels Calculation

The weekly intake amounts were calculated using the highest Hg concentration in sea cucumbers EWI (Estimated Weekly Intakes) = the highest concentration of Hg (mg/kg) multiplied by holothuroids consumption (kg/70 kg body wt./week).

Statistical Analysis

Mean values of residues of Hg among sample locations of the holothuroids were compared using one-way analysis of variance (ANOVA) following Tukey post hoc comparisons (Zar, 1984). P-values less than 0.05 were considered significant. All analysis was carried out using the SPSS, version 21 and Excel 2010 to analyse the influence of pre-monsoon, monsoon and post-monsoon. All values were being expressed on mg/kg dry wt. basis.

Results and Discussion

The concentrations of Hg in muscle, tentacles and gut samples of the holothuroids are presented in Figures 2-5. Hg

concentrations of tentacles were higher than those in muscle, tentacle and gut tissues of the pre-monsoon, monsoon and post-monsoon as the differences were significant ($P < 0.05$), except in *H. verrucosa* from Buleji coasts in pre-monsoon as the difference was not significant ($P > 0.05$). The mean Hg levels in tentacles of *H. verrucosa*, *H. arenicola*, *H. atra* and *H. pardalis* were 0.077, 0.126, 0.127 and 0.170 mg / kg dry wt., respectively. However the mean Hg levels in gut of *H. verrucosa*, *H. arenicola*, *H. atra* and *H. pardalis* were 0.047, 0.029, 0.059 and 0.049 mg / kg dry wt., respectively. The lowest Hg concentrations were found in muscle tissues at all seasons in Buleji and Sunehri coasts. The concentrations of Hg also showed significant differences according to the sampling stations. The highest Hg concentrations in gut of *H. arenicola* and *H. pardalis* were usually observed in pre-monsoon and monsoon at Sunehri coasts. Hg levels were not constant in muscle, tentacle and gut tissues during pre-monsoon, monsoon and post-monsoon at both Buleji and Sunehri coasts. The sampling stations with the highest and lowest Hg levels varied according to the sampling periods and tissues.

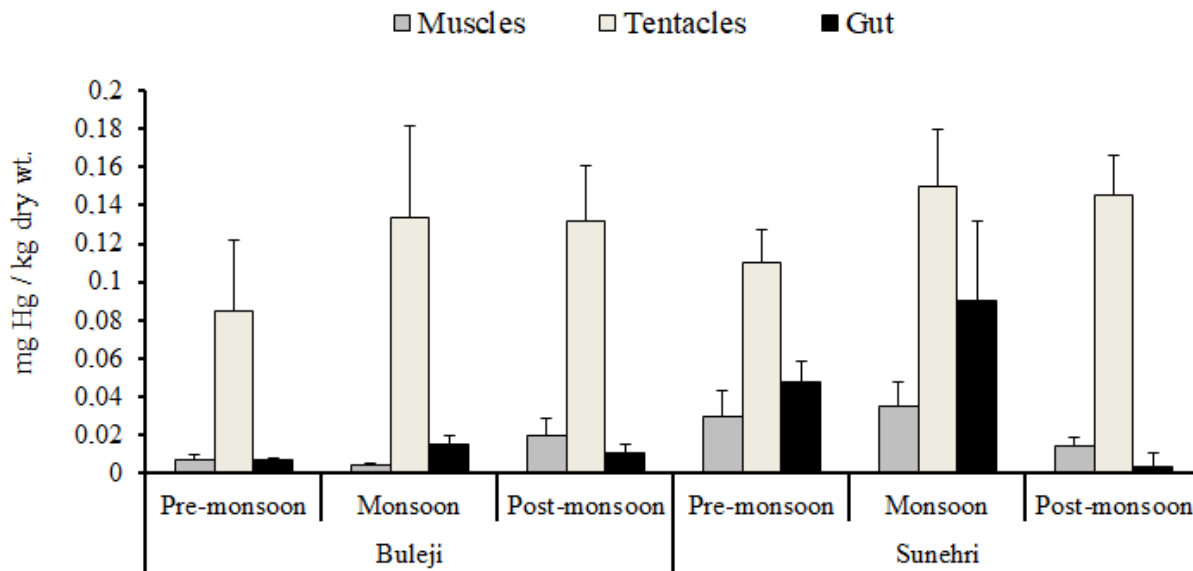


Figure 2. The means with standard errors (vertical lines) of Hg concentrations (mg/kg dry wt.) in muscle, tentacles and gut tissues of *H. arenicola* from Buleji and Sunehri coasts of Karachi, Arabian Sea.

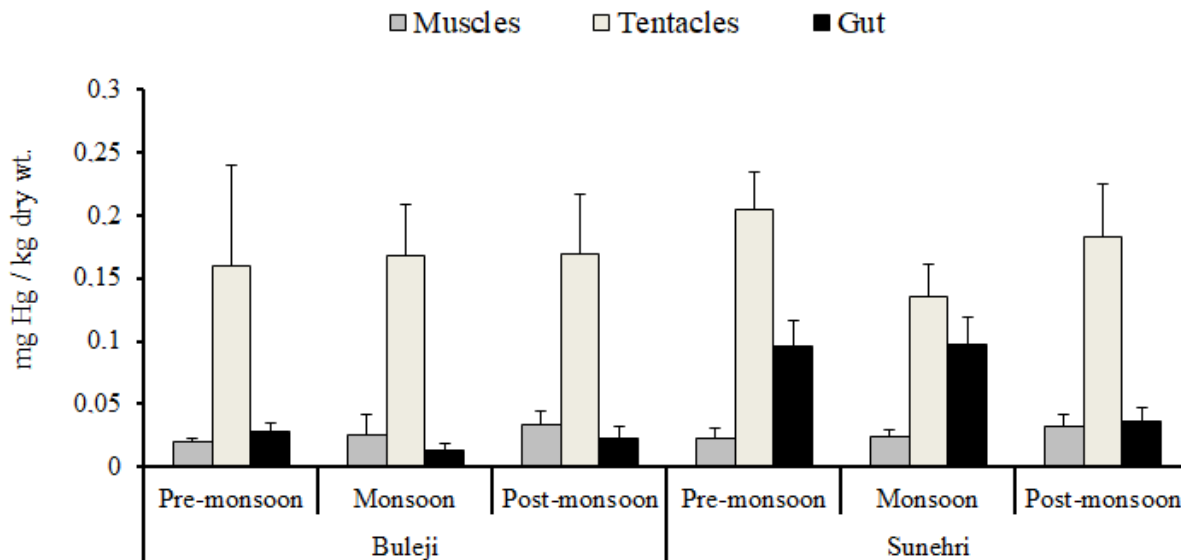


Figure 3. The means with standard errors (vertical lines) of Hg concentrations (mg/kg dry wt.) in muscle, tentacles and gut tissues of *H. pardalis* from Buleji and Sunehri coasts of Karachi, Arabian Sea.

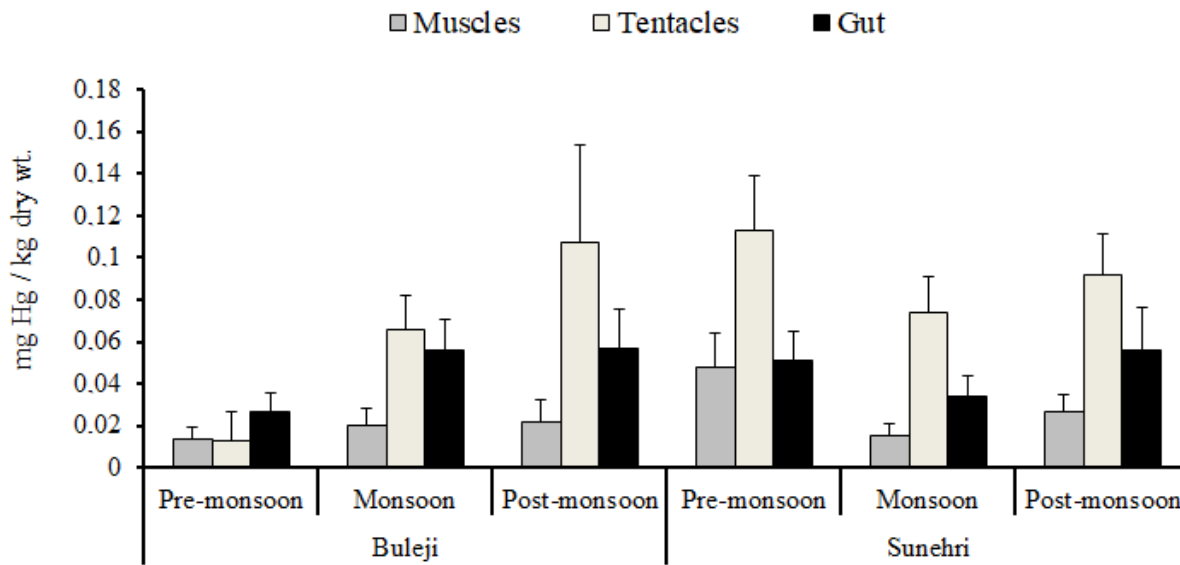


Figure 4. The means with standard errors (vertical lines) of Hg concentrations (mg/kg dry wt.) in muscle, tentacles and gut tissues of *H. verrucosa* from Buleji and Sunehri coasts of Karachi, Arabian Sea.

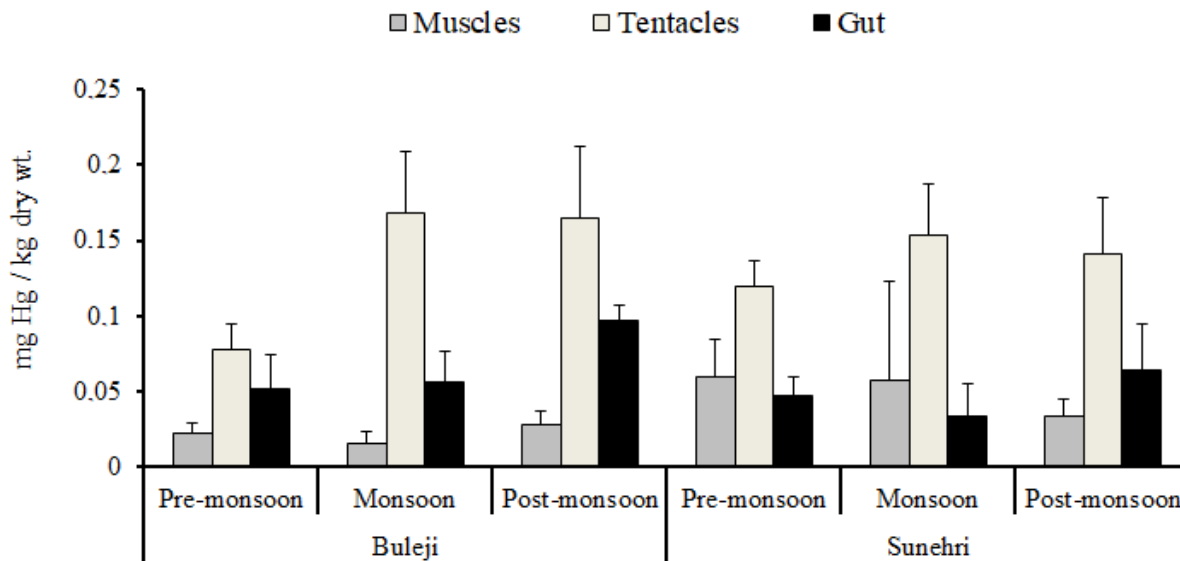


Figure 5. The means with standard errors (vertical lines) of Hg concentrations (mg/kg dry wt.) in muscle, tentacles and gut tissues of *H. atra* from Buleji and Sunehri coasts of Karachi, Arabian Sea.

In the current work statistical analysis of the data displayed significant differences ($P < 0.05$) among all of the holothuroids. The mean residue amounts of Hg varied amongst all species. The Hg concentrations (mg/kg dry weight) in muscle tissues of the sampled holothuroids are as follows: *H. arenicola* (0.018 mg/kg dry wt.) < *H. verrucosa* (0.024 mg/kg dry wt.) < *H. pardalis* (0.026 mg/kg dry wt.) < *H. atra* (0.036 mg/kg). Means of Hg in muscles of holothuroids are much lower than the maximum permissible limit (0.5 mg/kg wet wt.) set by the European Union and Turkish Food Codex (EU Commission Regulations, 2001 and 2006; TFC, 2002 and 2008). Environmental Quality Standards (EQS) for Hg in biota is given in European Union legislative acts as 0.02 mg/kg wet wt. (Official Journal of the European Union, 2013; European Union, 2014). Since our results were given in dry wt., mean concentrations of Hg converted to wet wt. dividing by 8.0 as factor. It was calculated that overall means of Hg in muscle tissues were between 4.4 and 9 times lower than EQS value (0.02 mg/kg wet wt.).

In the literature, Hg levels in the tissues of the holothuroids vary considerably among different studies. The obtained data illustrated that mean Hg accumulation in *H. atra* that is similar to Jinadasa et al. (2014) who found that the mean concentration was 0.031 (± 0.013) mg /kg dry wt. from North-Western Sea of Sri Lanka. The study of Jinadasa et al. (2014) from North-Western Sea of Sri Lanka indicated that Hg levels in ten Holothurians species ranged from 0.025 to 0.446 mg /kg dry wt., which is higher than those in the values detected in our study.

Holothuroids are deposit-feeders and live in close association with sediment, and feeds mainly detrital particles and associated microorganisms (WoRMS Editorial Board, 2018). Many studies (Bat et al., 2015 and 2017; Bat and

Özkan, 2015) indicated that heavy metals including Hg tend to accumulate in sediment. It is surprising that *H. arenicola*, *H. pardalis*, *H. verrucosa* and *H. atra*, which are benthic species and are associated with sediment, did not show the high levels of Hg. These results confirm that the both Buleji and Sunehri coasts of Karachi are not seriously contaminated with Hg. Slight differences in Hg levels in these holothuroids could considerably be attributed to the differences in seasons. It may be suggested that seasonal changes play basic and significant role in the control of Hg accumulation.

Hg is a non-essential element that can be toxic and carcinogenic at even low concentration and is known has no role in biological processes in living organisms. The European Food Safety Authority's revised the provisional tolerable weekly intake (PTWI) of inorganic Hg and set a PTWI of 0.004 mg/kg body wt. applies to seafood (EFSA, 2012). Special attention to well-being hazard, the permissible weekly intakes was computed by means of references for edible tissues of sea cucumbers eaten up by public. Pursuant to Food and Agriculture Organization (FAO) counts of seafood consumption in Pakistan remarked that the adult person eats the mean daily seafood consumption in Pakistan is 5 g per person (FAO, 2010), which is well-matched to 35 g/week. The tolerable weekly intake of elements as PTWI (Provisional Tolerable Weekly Intake), are set by the Food and Agriculture Organization/World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA). PTWI is the greatest of a pollutant to which a person can be exposed per week over a lifetime without an unacceptable risk of health effects (WHO, 1989 and 1996). Estimated Weekly Intake (EWI) and Estimated Daily Intake (EDI) for a 70 kg body weight of an adult person on basis of the current work outcomes were given in Table 1.

Table 1 Estimated Weekly Intakes (EWI) and Estimated Daily Intakes (EDI) of Hg as mg/kg wet wt. in edible tissues of holothuroids from Buleji and Sunehri coasts of Karachi, Arabian Sea.

Species	PTWI ^a	PTWI ^b	PTDI ^c	EWI ^d		EDI ^e	
				Buleji	Sunehri	Buleji	Sunehri
<i>H. arenicola</i>	0.004	0.28	0.04	0.0021	0.0030	0.00030	0.00045
<i>H. pardalis</i>	0.004	0.28	0.04	0.0028	0.0026	0.00040	0.00038
<i>H. verrucosa</i>	0.004	0.28	0.04	0.0023	0.0402	0.00033	0.00060
<i>H. atra</i>	0.004	0.28	0.04	0.0025	0.0039	0.00035	0.00055

^aPTWI (Provisional Tolerable Weekly Intake) in mg/week/kg body wt.

^bPTWI for 70 kg adult person (mg/week/70 kg body wt.)

^cPTDI (Permissible Tolerable Daily Intake) (mg/day/70 kg body wt.)

^dEWI (Estimated Weekly Intake) (mg/week/ kg body wt.)

^eEDI (Estimated Daily Intake) (mg/day/ kg body wt.)

From the people health point of sight, this current work proved that there was no likely health hazard to consumers on account of intake of sea cucumbers under the current consumption rate in Buleji and Sunehri coasts of Karachi, Arabian Sea.

Hg is consistent element in the marine ecosystem giving rise to grave disease in biota and human. Sources of Hg for marine water include atmospheric deposition, straight and via runoff, as well as direct discharges into seashores. Much of the Hg added to marine ecosystem accumulates in sediments where it has a risk to benthic biota and to people via consumption of these species. Sea cucumbers are much consumed seafood in the Asian countries (Özer et al., 2004), and they are good biomonitors for measurement the impacts of different environmental chemicals on marine ecosystem. However results of current work revealed that Hg amounts in the edible tissues were below the highest tolerable limits.

Conclusions

The outcomes of the current work are not detected health risk for the human population through seafood consumption. Hg in muscles of holothuroids are much lower than the maximum permissible limit (0.5 mg/kg wet wt.) set by the International Food Codex. Further investigates are needed to adequately evaluate mean amounts and the variability of Hg and other toxic elements in Pakistan food. It is also needed to determine the toxicological effects of non-essential metals on these congeners. Furthermore, the Hg also toxic a lifespan cancer risk to consumers of seafood collected from the Arabian Sea. Thus, continuous monitoring of heavy metal pollutants is needed to mitigate the impact of these contaminants on people health and the marine ecosystem.

Conflict of Interest Statement

We declare that we have no conflict of interest.

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AQUATIC RESEARCH



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Original Article/Full Paper

BİYOKÜTLE İÇİN MİKROALG VE SİYANOBAKTERİ'NİN BÜYÜK ÖLÇEKLİ ÜRETİMİ

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ÖZ

Mikroalgler ve siyanobakteriler, binlerce yıldır ilaç, gıda ve su ürünleri yetiştiriciliği endüstrisi için yüksek değerli bileşiklerin doğal bir kaynağı olarak kullanılmaktadır ve büyük ölçekli mikroalg yetiştirilmesi, yarım yüzyılı aşkın bir süredir yapılmaktadır. Yakın zamanda yeni mikroalg ve siyanobakteri türleri tanımlanmış ve çeşitli ürünler için algal biyokütle yetiştiriciliği ticari ölçekli sistemlere geçilmiştir. İlk olarak, steril şişede (2L, 5L ve 10L) laboratuvar koşullarında yetiştirilen *Arthrospira (Spirulina) platensis* ve *Phaeodactylum tricorutum* kültürlerinin spesifik büyüme hızları ve klorofil-a analizleri yapılmıştır. Ardından ticari uygulama için büyük ölçekli siyanobakterler ve mikroalg biyokütlesi üretimi için açık ve kapalı sistemler kuruldu. Alg biyokütlesi üretimi için çevresel koşullar altında bir açık karıştırma tankı ve iki farklı tipte kapalı-kültür sistemi veya fotobiyoreaktör (polipropilen torbalar ve plexiglas tüpleri) kullanıldı. Bununla birlikte, *P. tricorutum*, düşük sıcaklığa karşı iyi direnç göstermiş ve düşük ışık şiddeti koşullarında bile büyüebilmiştir. Açık havuz sistemlerinin performansı karşılaştırıldığında, entegre sıcaklık düzenlemesine sahip dış mekan sistemlerinin, daha iyi iklim koşullarına sahip bölgelerdeki açık havuzlarda yetiştirilen kültürlerle benzer bir biyokütle üretimi elde edildiği gösterilmiştir. Ülkemizde yetiştirilen mikroalg ve siyanobakterilerin biyokütle verimliliğinin artırması, ileri tekniklerle tasarlanan ve düşük maliyetli teknolojilerle geliştirilen fotobiyoreaktör ile sağlanabilir.

Anahtar Kelimeler: *Arthrospira (Spirulina) platensis*, *Phaeodactylum tricorutum*, Biyokütle, Fotobiyoreaktör

ABSTRACT

LARGE-SCALE PRODUCTION OF MICROALGAE AND CYANOBACTERIA FOR BIOMASS

Microalgae and cyanobacteria have been used a natural source of high-value compounds for pharmaceutical, food and aquaculture industry for thousands of years, and the large-scale cultivation of microalgae has existed for over half a century. More recently novel species of microalgae and cyanobacteria have been identified and the cultivation of algal biomass for various products is transitioning to commercial-scale systems. Firstly, *Arthrospira (Spirulina) platensis* and *Phaeodactylum tricorutum* cultures grown in the sterile bottle (2L, 5L and 10L) in the laboratory conditions were studied by means of specific growth rate and chlorophyll-a analysis. Then, open and closed systems were installed for large scale production of cyanobacteria and microalgae biomass industry for commercial application. For the production of algae biomass, one open stir tank and two different types of closed-cultured systems or photobioreactors were used (Polypropylene bags and Plexiglas tubes) under environmental conditions. However, *P. tricorutum* has a good resistance to low temperature and they can grow even under low light intensity conditions. Comparing the performance of open pond systems, it was shown that the outdoor systems with integrated temperature regulation resulted in a biomass production similar to that for cultures grown in outdoor open ponds in regions with better climatic conditions. Grown in our country increasing microalgae and cyanobacteria biomass productivity of can be achieved by designing advanced and developing low cost technologies photobioreactors.

Keywords: *Arthrospira (Spirulina) platensis*, *Phaeodactylum tricorutum*, Biomass, Photobioreactor

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Giriş

Mikroalgler ve siyanobakterilerin çoğu fotototrofik olarak yaşayabilirken az bir kısmı ise heterotrofik olarak, doğada çok geniş (tatlı su ve denizel, karasal) yaşam alanlarında hayatlarını sürdürebilmektedirler. Eskiden mikroalgler özellikle akuakültürde kullanılmasına rağmen günümüzde protein, klorofil, karotenoid ve lipidler gibi çeşitli yüksek değerli molekülleri içermeye yetenekleri sayesinde çalışmalarda tercih edilmektedirler. Ticari olarak mikroalgler ve siyanobakteriler, gıda, yem, farmastötik ve enerji sektöründe sürdürülebilir seçeneklerin geliştirmesi nedeniyle yakın gelecekte umut vaat eden organizmalar arasında yer almaktadır.

Arthrospira (Spirulina) platensis çok hücreli, filamentli fotosentetik bir siyanobakteri türüdür. Ticari olarak besin ve gıda takviyesi olarak üretimi yapılmaktadır. Bu özelliklerinin yanında *Spirulina* biyolojik işlevleri bakımından antiviral, anti-enflamatuar ve antioksidan aktiviteye de sahiptir. Yaygın şekilde yetiştirilmesinin sebebi bazı hastalıkların (artrit, anemi, kardiyovasküler hastalıklar, alerjiler, tümörler ve kanser) tedavisinde gıda takviyesi olarak kullanılmasındandır. *Spirulina* fikosiyanın proteini gibi fonksiyonel bileşiklere sahip olmasından dolayı gıdalarda renklendirici ve emülgatör olarakta kullanılmaktadır (Madkour ve diğ., 2012).

Phaeodactylum tricornutum en çok çalışılan denizel bir diyatom türüdür. Eikosapentanoik asit (EPA)in önemli potansiyel kaynağı olarak düşünülen tür, essansiyel yağ asitleri ve karotenoidleri (fukoksantin) insan ve akuakültürde hayvanlarının beslenmesinde kullanılmaktadır. Günümüzde *Phaeodactylum* biodizel üretimi içinde önemli adaylar arasında yer almaktadır (Benavides ve diğ., 2013).

Fukoksantin kahverengi yosunlar ve diyatomlarda bulunan majör (baskın) karotenoiddir. Bu pigment formları, klorofil (Chl) a, Chl c ve bir apoprotein ile birlikte çalışmaktadır. Işığı fukoksantin hasat ederek klorofil a/c kompleksi ile birlikte fotosentez için fotosentez reaksiyon merkezlerindeki ışık enerjisini transfer eder. Bu karotenoidin güçlü antioksidan, anti-inflamatuar, anti-obezite, antidiyabetik, antikanser ve antihipertansif aktiviteler sergilediği bilinmektedir. Fukoksantin, kanatlı hayvan ve su ürünleri yetiştiriciliği endüstrisinde hayvan yemi içerisine katkı maddesi olarak da ilave edilmektedir (Xia ve diğ., 2013).

Karotenoidler ve fikobiliproteinlerin hücredeki fonksiyonları ya ışık hasat pigmentleri ya da ışık koruyucu ajanları olarak görev alarak, fototrofik türlerde sentezlenmektedir. Karotenoidler ve fikobiliproteinler yem ve gıdalarda renklendirici olarak kullanılabilir, ancak en önemli özellikleri fonksiyonel sağlık içerikleri nedeniyle. Fikobiliprotein-

ler, bazı karotenoidler ve diğer biyolojik olarak aktif moleküller mikroalg ya da siyanobakteriler tarafından sentezlenir. Günümüzde β -karoten, astaksantin ve fikosiyanın elde edilmesi için üretim yöntemlerinin belirlenmesiyle büyük ölçekli mikroalgal kültürleri yetiştirilmektedir (Eriksen, 2016).

Dünya da mikroalgler ve siyanobakterilerin yetiştirilmesinde en çok açık havuz ve kapalı fotobiyoreaktör üretim sistemleri kullanılmaktadır. Fotobiyoreaktörler, besin maddelerinin ilavesiyle büyüme, sıcaklık, çözünmüş CO₂ ve pH gibi ekim parametrelerinin kontrol edildiği kapalı sistemlerdir. Tercih edilmesinin sebebi kontaminasyonu engelleyen, kolay kontrol edilebilen sistem sunmasındandır. Açık havuz sistemlerinde kontaminasyonların sınırlandırılmasının zor olması nedeniyle daha az tercih edilen sistemlerdendir. Bununla birlikte, fotobiyoreaktörler yüksek başlangıç maliyetine sahiptirler ve seçilen mikroalg türünün üretimi için özel fizyolojiye sahip olması gerekmektedir. Bu nedenle, üretim tesisinde mikroalg türlerine spesifik sistemlerin gerekliliği önemli bir faktördür (Harun ve diğ., 2010).

Ülkemizde mikroalg ve siyanobakterilerin üretimleri büyük çapta özellikle akuakültür, gıda takviyesi, gübre, kozmetik ve gıda ürünleri içine katkı maddesi olarak ilave edilmesi ile gerçekleşmektedir. Bu kapsamda ülkemizde üretilen büyük çap üretimleri için türler genellikle havuzlarda veya poşetlerde üretilmektedir. Bu çalışma ile *Arthrospira (Spirulina) platensis* ve *Phaeodactylum tricornutum* için ergonomik, kolay kontrol edilebilen sistemlerinin kurulması ile rahat işletilebilecek üretim sistemlerin geliştirilmesi hedeflenmektedir. Yapılan bu çalışma, Ege Üniversitesi Biyomühendislik Mikroalg Biyoteknoloji Laboratuvarının da küçük çapta üretilen mikroalg (*P. tricornutum*) ve siyanobakterinin (*A. platensis*) büyük çapta üretimi için Egert Doğal Ürünler Üretim Hayvancılık Gıda Yem İthalat İhracat Pazarlama San. Tic. Ltd. Şti. seraları ve üretim imkânlarından yararlanılmıştır. *P. tricornutum* kapalı (fotobiyoreaktörler) ve *A. platensis*' in açık (karıştırmalı tanklar) sistemlerde ticari üretiminin gerçekleştirilmesi için ucuz, dayanıklı, kolay kurulabilir ve üretim kolaylığı sağlayan sistemlerin kurulması hedeflenerek, üretim sonrası mikroalgal biyokütle miktarlarının artırılması sağlanmıştır.

Materyal ve Metot

Materyal

Arthrospira (Spirulina) platensis (EGEMACC 38) ve *Phaeodactylum tricornutum* Bohlin (EGEMACC 70) türleri Ege Üniversitesi Mikroalg Kültür Koleksiyonundan (Anonymus 2018a) temin edilmiştir.

Mikroalg ve Siyanobakterinin Üretimi ve Kurulan Sistem Özellikleri

Stok kültürlerden inokulumlarının hazırlanmasında 2L, 5L ve 10L'lik steril lenmiş şişeler kullanılmıştır. Zarrouk ve F/2 ortam içerisinde $22 \pm 2^\circ\text{C}$ sıcaklıkta, flüoresans beyaz ışık altında ($50 \mu\text{E m}^{-2}\text{s}^{-1}$) sürekli aydınlatmalı ve havalandırmalı (3 L dak^{-1}) olarak 15 gün boyunca üretilmiştir (Şekil 1).



Şekil 1. Stok kültürün 2L, 5L ve 10L'lik havalandırmalı steril şişelerdeki kontrollü üretimi

Figure 1. The stock cultures grown in 2L, 5L and 10L sterile bottles under control conditions

A. platensis ve *P. tricornutum* kültürlerin 2L, 5L ve 10L'lik üretimlerindeki optik yoğunluk değişimleri ve klorofil a analizleri 15 günlük üretim boyunca yapılarak, spesifik büyüme hızı ve ikilenme süreleri klorofil-a miktarından hesaplanmıştır.

Optik yoğunluk: Spektrofotometre (Ultrospec1100 pro UV-Visible Spektrofotometre, Amersham Biosciences) ile *A. platensis* için 560 nm (Cisneros ve diğ., 2004) ve *P. tricornutum* için 680 nm (Yongmanitchai ve Ward, 1991) kullanılarak kültürlerin optik yoğunlukları belirlenmiştir.

***A. platensis* için klorofil a tayini:** 5 mL *A. platensis* kültürü GF/C filtreden süzülüp, filtrat üzerine 5 mL metanol eklenmiştir. Klorofil-a'nın ekstraksiyonu için 70°C 'lik su banyosunda 2 dakika bekletilip ardından ekstrakt, 5000 devir/dak'da 5 dakika santrifüjlenerek hücre artıkları uzaklaş-

tırılmıştır. Daha sonra üst fazın 665 ve 750 nm'lerdeki absorbansları spektrofotometre yardımıyla ölçülerek, absorbans değerleri aşağıdaki eşitliğe yerleştirilerek *A. platensis* için klorofil-a miktarları Eşitlik 1'e göre mg/L cinsinden hesaplanmıştır (Boussiba vd., 2004).

$$\text{Klorofil-a (mg/L)} = 13,9 \times (OD_{665} - OD_{750}) \quad (1)$$

***P. tricornutum* için klorofil a tayini:** 5 mL *P. tricornutum* kültürü, 5 dakika boyunca 6000 rpm'de santrifüjlenip ve hücre pelleti üzerine 1 mL DMSO ilave edilmiştir. Hazırlanan örnekler, 5 dakika boyunca (HF frekansı 20 kHz) sonikasyona tâbi tutularak hücre parçalanması gerçekleştirilmiştir. Ekstraksiyon için parçalanmış örnekler karanlıkta 30 dakika boyunca 55°C de inkübe edilmiştir. 4500 rpm'de santrifüje edildikten sonra spektrofotometrede 665 nm absorbansı ölçülerek Eşitlik 2 'ye göre hesaplanmıştır (Seely ve diğ., 1972).

$$\text{Klorofil-a (mg/L)} = OD_{665} / 73,6 \quad (2)$$

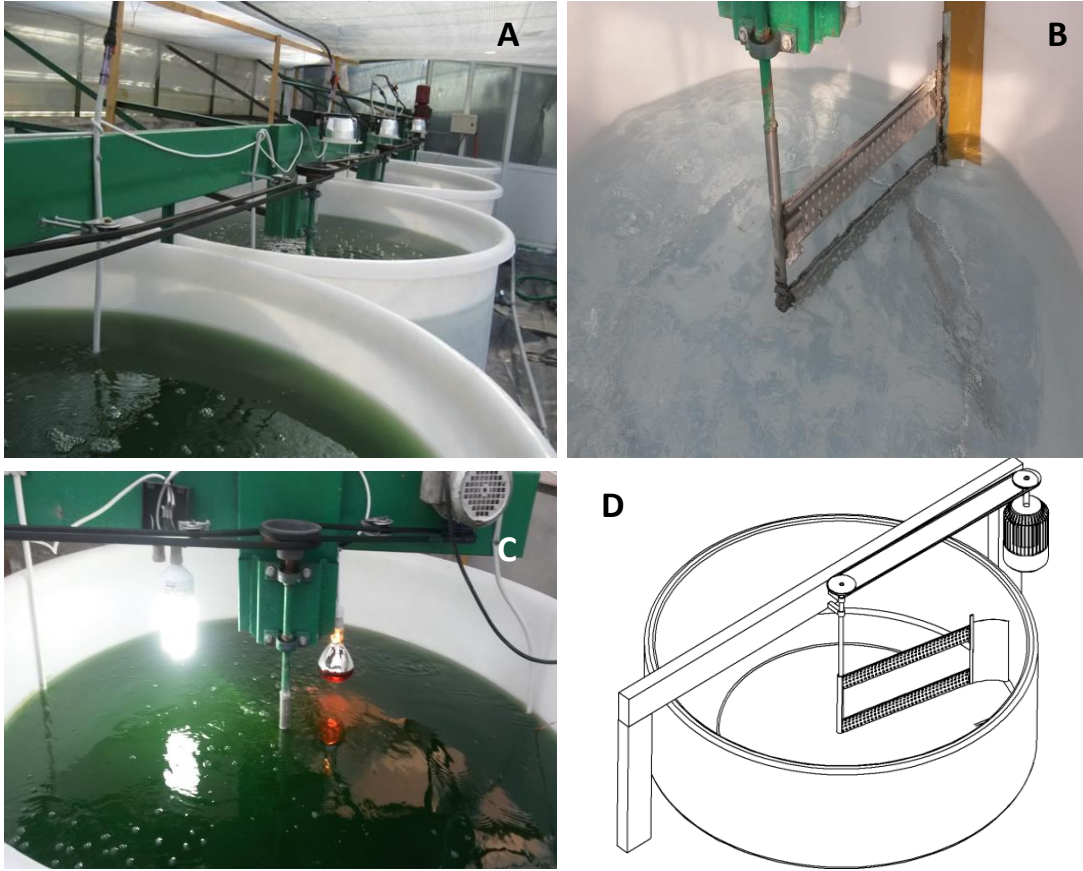
Spesifik Büyüme Hızının belirlenmesi: *A. platensis* ve *P. tricornutum* 'un spesifik büyüme hızı Eşitlik 3'e göre hesaplanmıştır (Tomaselli, 1997).

$$\mu = \ln x_2 - \ln x_1 / t_2 - t_1 \quad (3)$$

Burada: μ ; Spesifik büyüme hızı (gün^{-1}), x_2 ; t_2 (gün) anındaki konsantrasyon x_1 ; t_1 (gün) anındaki konsantrasyondur.

A. platensis büyük çap üretimi için karıştırmalı tank sistemleri hazırlanmış ve bu sistem içerisine Zarrouk ortam kimyasallarından sadece iz metaller ilave edilmeden, havuz içerisinde tamamen çözüldürülen kimyasallar ile kültür ortamı hazırlanmıştır (Şekil 2). Kullanılan kimyasallar gıda sınıfı (food grade) kalitesinden seçilmiştir. Hazırlanan ortam üzerine belli oranda inokulum ilave edilmiştir (Madkour ve diğ., 2012).

Polietilen karıştırmalı tanklar 1 tonluk su kapasitesine sahip olup, üretimler için 750 L'lik tank alanı kullanılmıştır. *A. platensis* bikarbonatça zengin alkali kültür ortamda üremesi nedeniyle kullanılan malzemeler pH 9-10 a dayanıklı ürünler arasından seçilmiştir. Havuzlar alttan havalandırmalı olarak tek taraflı yerleştirilen pervane yardımıyla karıştırılmıştır. Sera içerisinde yıl boyunca üretimin devamı için havuzların etrafı serpantin benzeri boru ile sarılarak, ceket sistemi hazırlanmış ve sistemin tüm yıl kullanılması sağlanmıştır. Tankların ısısı ve karıştırma hızı kontrol paneli sayesinde kontrol edilmiştir. Ayrıca havanın kapalı olduğu zamanlarda sentetik ışık yardımıyla havuzlar aydınlatılmıştır. Sistem paralel karıştırmalı olarak kurulmuş ve tahliye muslukları havuzların altına yerleştirilmiştir.



Şekil 2. *Arthrospira platensis*'in yetiştirilmesi için kullanılan karıştırılmalı tank havuzlar, A; Paralel üretim sistemi, B; Ortam ilave edilerek kullanılan sistem, C; Aydınlatmalı üretim sistemi, D; Sistemin teknik çizim ile gösterimi

Figure 2. The open stir tanks used for the cultivation of *Arthrospira platensis*, A; Parallel production system, B; The system used by adding the medium C; Illuminated production system, D; Schematic diagram of the system

P. tricornutum'un büyük çapta üretimindeki kültür ortamının hazırlanmasında Çiğli tuzludan alınan yıkanmış deniz tuzuna sodyum nitrat, sodyum fosfat ve sodyum silikat ilave edilerek sentetik F/2 kültür ortamı hazırlanmıştır (Guillard ve Ryther, 1962). Yıkanmış deniz tuzu litreye 20 gram olarak kullanılmıştır. Ana besleme tankı içerisine tuz ve kimyasallar ilave edilip çözündürüldükten sonra, asılı poşet ve karıştırılmalı havalandırılmalı fotobiyoreaktör sistemlerine tank yardımıyla eşit bir şekilde pompa edilerek aktarılmıştır.

Sera içindeki polietilen plastik torbadaki üretim için 75 L'lik hacimde hazırlanan torbalar V-şeklinde asılmış ve sabitlemek için tahta platform kullanılmıştır. Poşetler asıldıktan sonra delik, yırtık kontrolü yapılmış ve ana tank içerisinde

hazırlanan ortam Şekil 3 1b de görüldüğü gibi yukardan besleme ile poşetlere ilave edilmiştir. Bu sistemde kullanılan polietilen şeffaf poşetler 1 mm kalınlığında Gıda Çarşısından (İzmir) sulama hortumu olarak satın alınmıştır. Tahta platform üzerine Şekil 3 1a da görüldüğü gibi hava dağıtıcısı yerleştirilerek, her bir poşet için hava hattı yukardan ilave edilerek kültürlerin karışması ve hava ihtiyacı sağlanmıştır.

Sera içine kurulan diğer sistem karıştırılmalı havalandırılmalı fotobiyoreaktörün yaklaşık 450 L hacimde hazırlanmıştır. Sistemler demir platform üzerine yerleştirmiştir. Reaktörlerin havalandırma, karıştırma ve besleme hızı kontrolü platform üzerine yerleştirilen pano üzerinden kontrol edilmektedir (Şekil 4-2).



Şekil 3. *Phaeodactylum tricornutum*'un yetiştirilmesi için hazırlanan sistemler. 1; *P. tricornutum* yetiştirirken 75 L' lik V asılı torbalarda

Figure 3. The prepared systems for the cultivation of *Phaeodactylum tricornutum*, 1; *P. tricornutum* cultivation in vertical hanging plastic bags of 75 L



Şekil 4. *Phaeodactylum tricornutum*'un yetiştirilmesi için kullanılan sistemler. (2) Suşun dikey kolon ve karıştırımlı fotobioreaktörde üretimi

Figure 4. The systems used for cultivation of *Phaeodactylum tricornutum*, 2; The species cultivation in vertical column and stirred photobioreactor

Karıştırmalı Tankların ve Fotobiyoreaktörün Hasatı

A. platensis ve *P. tricornutum* üretimi kesikli ve yarı kesikli üretim sistemlerinde yaklaşık 15-20 günlük kültürlerin optik yoğunluklarına ve hücre sayıları belirlendikten sonra istenilen yoğunluğa erişen kültürlerin hasat işlemi gerçekleştirilmiştir.

A. platensis biyokütle elde edilmesinde filtrasyon sistemi kullanılmıştır. Farklı gözenek çapına sahip fitreler yardımıyla eleme işlemi gerçekleştirilmiştir. Eleme için pompanın verdiği besleme hızı sabit tutularak her tank başına yaklaşık 1 saat eleme işlemi gerçekleştirilmiştir. Eleme sonunda elde edilen sulu biyokütle filtre üzerinden toplanıp preslendikten sonra ıslak (paste) olarak tartılıp dondurucuda (-20°C) kullanılabilecek kadar saklanmıştır.

P. tricornutum biyokütle eldesinde de çanaklı santrifüj separatör (GEA Westafalin GmbH) kullanılmıştır. Poşet üretimindeki hasat işlemi için havalandırma kapatılıp hücreler doğal sedimentasyona bırakıldıktan sonra peristaltik pompa yardımıyla sistemden uzaklaştırılarak, çanaklı santrifüj yardımıyla ıslak (paste) biyokütle elde edilmiştir. Aynı işlem fotobiyoreaktör içinde tekrarlanmıştır. Karıştırma ve havalandırma durdurulduktan sonra çökmeye bırakılan kültür tahliye musluğundan toplanıp, çanaklı santrifüj yardımıyla biyokütle elde edilmiştir.

Bulgular ve Tartışma

Türkiye de farklı amaçlar doğrultusunda mikroalg ve siyanobakterin üretimleri gerçekleştirilmektedir. Örneğin, akuakültür için yem ve yeşil su hazırlanmasında, gıda takviyesinde, gübre, kozmetik ve gıda ürünleri içine katkı maddesi olarak kullanılmaktadır.

Türkiye de büyük çapta siyanobakteri “Spirulina” üretimi ilk kez üniversite sanayi işbirliği ile 1999 yılında Ege Üniversitesi ile Egert Doğal Ürünler Üretim Hayvancılık Gıda Yem İth. İhr. Paz. San. Tic Ltd. Şti. arasında “Ürün Ticarileştirme Anlaşması”yla hayata geçirilmiştir. Gıda takviyesi ve akuakültürde akvaryum balıkları için yem üretimi gerçekleştiren Egert Doğal Ürünler Üretim Hayvancılık Gıda Yem İth. İhr. Paz. San. Tic Ltd. Şti. (Anonymus 2018b), İzmir de kurulmuştur. *Arthrospira (Spirulina) platensis* üretimini Manisa ilinin Turgutlu ilçesindeki tesislerde kanallı havuzlarda gerçekleştiren şirket Spirulina adı altındaki ürünü gıda takviyesi amacıyla ticari pazara kazandırmıştır.

Günümüzde TC Gıda Tarım ve Hayvancılık Bakanlığı'nın kayıt altına aldığı Su Ürünleri Yetiştiricilik Tesisleri olarak

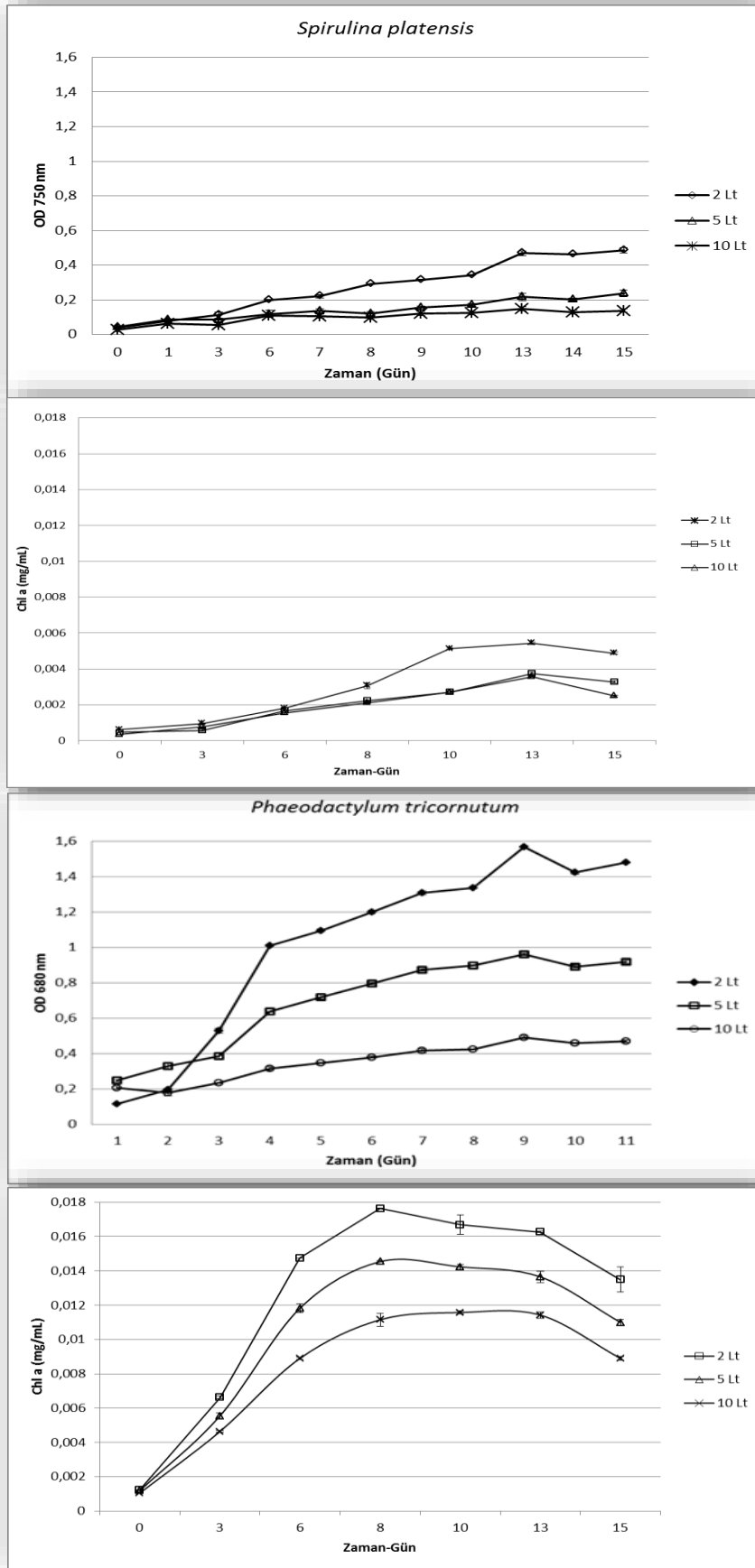
3 adet Adana ve 1 adet Manisa ilinde olmak üzere toplamda 4 adet Spirulina (su yosunu) yetiştirici şirketin bulunduğu 09.02.2018 tarihli raporda bildirilmektedir (Anonymus 2018c).

Yalova Üniversitesi Armutlu Meslek Yüksekokulunda yaklaşık 3 senedir sürdürülen çalışmalarının ardından laboratuvar ortamında üreten Spirulina cinsi yosun, yapay ortamda üretilip yoğurt, peynir ve ayran üretilmesi için çalışmalar sürdürmektedirler ve 2015 yılında "yosunlu ayran"ı üretmişlerdir (Anonymus 2018d; 2018e).

Gübre ve tarım uygulamalarında *Chlorella* spp. üreten Mikroalg Gıda Tarım Sanayi Anonim Şirketi, İzmir (Anonymus 2018f) tarafından ticari olarak *Chlorella* spp. li ürünü TerraDoc Gübreyi satışa sunmuştur. Aynı şekilde GPA Mühendislik, Isparta (Anonymus 2018g) da yosunlu gübre ve mikroalg tür (*Chlorella vulgaris*, *Botryococcus braunii*, *Scenedesmus obliquus* ve *Spirulina* sp.) satışını gerçekleştirmektedir. Algome (omega-3 yağ asitleri içeren, kuru mikroalg biyokütlesi-*Schizochytrium* sp.) ürününü, Aydın ilindeki (Anonymus 2018h) MarinBio Şirketinde üretimini gerçekleştirmektedir. Adana da 2005 yılında AB destekli, TÜBİTAK, ÜSAM, İŞKUR ve Ç.Ü. Su Ürünleri Fakültesi işbirliği ile istihdam sağlamak amaçlı açılan, Spirulina (mavi-yeşil alg) üretim ve pazarlama eğitim kursunu gerçekleştirmiş. 2006 yılında Akuatik Su Ürünleri ve Kozmetik Ltd. Şti. (Anonymus 2018i) en geniş ürün portföyü ile mikroalg türlerini besin takviyesi (ALGAMAX) ve kozmetik ürün (ALGEE) formlarında üretimini gerçekleştirerek, ticaretini yapmaktadır.

Mikroalgler ayrıca CO₂ salınımının azaltılması ve elde edilen biyokütlenin enerji sektöründe biyodizel, biyoetanol olarak kullanılmasını araştırma ve geliştirmesini inceleyen şirketler arasında ise Egebiyoteknoloji A.Ş (Anonymus 2018i); Mikroalg Gıda Tarım Sanayi Anonim Şirketi (Anonymus 2018f) yer almaktadır.

Bu çalışma, “Mikroalgler ve Siyanobakterilerden Doğal Renk Maddesi Fikosiyenin ve Fukoksantin Ekstraksiyonu, Saflaştırılması, Enkapsülasyonu ve Gıda Maddeleri İçinde Stabilitesinin Test Edilmesi” isimli Uluslararası (ES1408 numaralı European network for algal-bioproductions (EUALGAE) başlıklı COST aksiyonu) TÜBİTAK projesi (Proje No: 1150578) kapsamında *Arthrospira (Spirulina) platensis* ve *Phaeodactylum tricornutum* hem laboratuvar koşullarında küçük çapta hem de sera da tasarlanan özel üretim sistemleri içerisinde büyük çapta üretimlerinin gerçekleştirilmesi ile mikroalg biyokütle eldesi sağlanmıştır.



Şekil 5. *A. platensis* ve *P. tricornutum* büyüme grafikleri
 Figure 4. Growth curves of *A. platensis* and *P. tricornutum*

Küçük çapta *A. platensis* ve *P. tricornutum* kültürlerin 2L, 5L ve 10L'lik sterillemiş şişelerde, Zarrouk ve F/2 ortam içerisinde $22 \pm 2^\circ\text{C}$ sıcaklıkta, flüoresans ışık altında ($50 \mu\text{E m}^{-2}\text{s}^{-1}$) sürekli aydınlatmalı ve havalandırılmalı (3 L dak^{-1}) olarak 15 gün boyunca üretimi sonucunda hücresel artış hem optik yoğunluktaki hem de klorofil a miktarlarının değişimleri Şekil 5 de verilmektedir. Elde edilen sonuçlardan klorofil a miktarı kullanılarak spesifik büyüme hızı ve ikilenme süreleri hesaplanmıştır (Tablo1).

Büyük çapta üretim *A. platensis* ve *P. tricornutum* türleri için Egert Doğal Ürünler Üretim Hayvancılık Gıda Yem İth. İhr. Paz. San. Tic Ltd. Şti.'nin Ege Üniversitesi Teknopark içerisinde yer alan serasına kurulmuş ve şirket uzmanlığında gerçekleştirilmiştir. Tasarlanan sistemlerin ilk amacı biyokütle eldesi olduğu için türler için en uygun üretim sistemleri dizayn edilmeye çalışılmıştır. Bu çalışmada yapılmak istenen diğer birçok çalışmada olduğu gibi laboratuvar ve dış mekan yetiştiriciliği arasındaki tutarsızlıkların ortaya çıkması ve laboratuvar teknolojileri kullanılarak elde edilen sonuçların, saha çalışmalarında gözlemlenen kazançlarla paralellik göstermemesindedir (Schoepp ve diğ., 2014). Bu sebepler göz önüne alındığında, mikroalgleri ile siyanobakterilerin araştırma ölçekli dış mekân üretimlerindeki amaç düşük bakım gerektiren sistemler ile süreç geliştirilmesidir. İş gücü ve sistem maliyetlerini düşürmek için ucuz ve dayanıklı materyallerin kullanılması tercih edilmiştir. Ülkemizde mikroalglerin türe özgü üretimlerinin büyük çapta gerçekleştirilmesi için optimum proseslerin geliştirmesi, kurulacak sistemlerin kullanılabilirliklerinin araştırması, türleri kontaminasyondan uzak tutarak, düşük maliyetli yetiştirilmeleri için uygun üretimlerin yapılması hedeflenmiştir.

Mikroalg yetiştirme sistemlerinin endüstriyel uygulamasında kültürler sığ büyük havuzlar (raceway), tanklar, yuvarlak havuzlar ve kanallı havuzlarda yetiştirilirken, FAO verilerine göre *Spirulina platensis*'in ticari ekimi açık havada ve açık sistemlerde yapılmalıdır. Siyanobakterinin alkali (bazik pH=9,5 ve 9,8) çevrede yetişmesi nedeniyle diğer mikroalg kültürleri ile karşılaştırıldığında, dışsal kontaminasyonun engellemesi ile çevresel uygulamalarda rahatlıkla tercih edilmektedir (Papadaki ve diğ., 2017). *Spirulina*

yetiştirilmesi ve biyokütle üretimi besin maddelerinin kullanımı, sıcaklık ve ışık gibi faktörlere bağlıdır (Madkour ve diğ., 2012). Güler ve Gülmez tarafından 2008 yılında *Spirulina*, üretiminde en önemli sınırlayıcı parametrenin kış döneminde sıcaklık, yaz döneminde ise ışık olduğu bilinirken üretimleri için bikarbonat miktarı yüksek olan alkali suların tercih edilmesi gerektiğini bildirmişlerdir (Güler ve Gülmez, 2008; Kumar ve diğ., 2011).

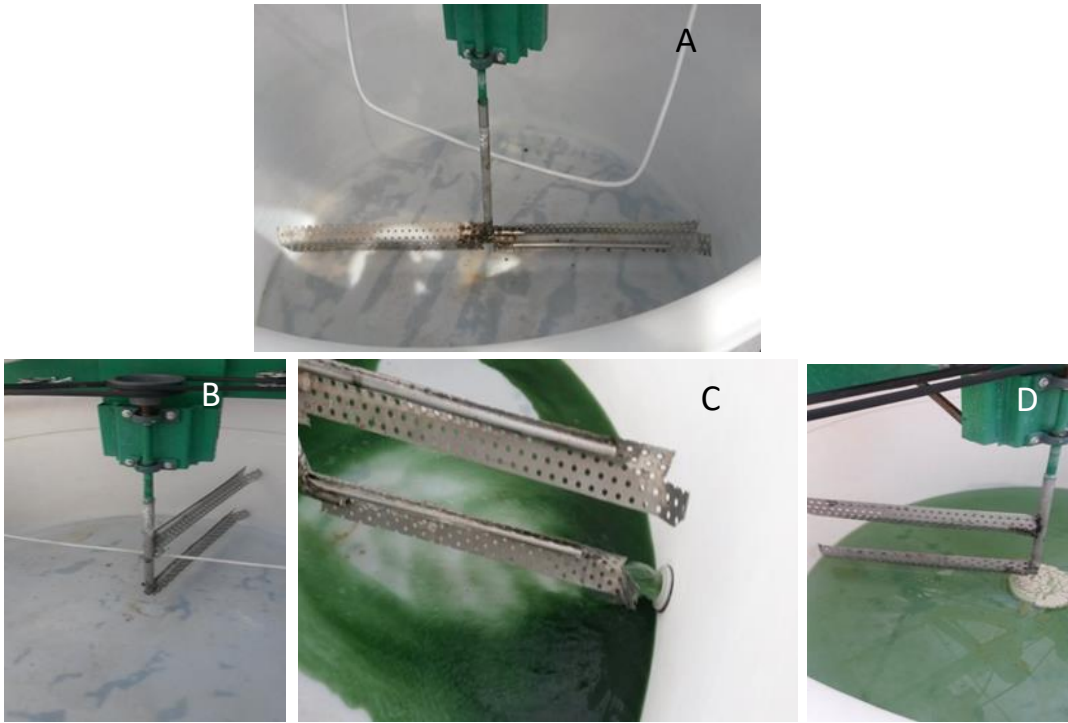
Bu bilgiler ve Egert Egert Doğal Ürünler Üretim Hayvancılık Gıda Yem İth. İhr. Paz. San. Tic Ltd. Şti.'nin bilgi birikiminden yararlanılarak tank sistemlerinin kış aylarında da kullanılması amacıyla tankların etrafına borulu ceket sistemi yapılmıştır. Bu sarılan borular içerisinden sıcak su geçirilmesiyle tankların kış aylarında ortalama ısının $22 \pm 2^\circ\text{C}$ de sabit tutulması sağlanarak, üretimler gerçekleştirilmiştir. Tank pervanesi ilk olarak iki yönlü teklî pervaneli ve aşağıdan karıştırmalı yapılmış, havalandırma ise yukardan verildiğinde hava kabarcıkların tank içerisinde uzun süre kalmadan hızlıca uzaklaştığı tespit edilmiştir. Pervane bütün kültürü karıştırmayı için hızlandırıldığında havuzun ortasında girdap oluşmuş ve *A. platensis*'in çoklu hücre filamentlerin de parçalanmalar meydana gelmiştir. Fragmente olan hücreler optik yoğunluğun artması sağlarken, biyokütle miktarını düşürmüştür. Karıştırma hızının artırılmasında hücrelerin zarar görmeye başladığı ve spirallerin kısa olarak çoğaldığı mikroskopik gözlemler ile belirlenmiştir (Şekil 7.1). O nedenle akışın düzenli ve 100-150 rpm hızını geçmeyecek şekilde, karıştırmanın tek yönlü olarak yapılmasına karar verilmiştir. Tek taraflı ve aradan havalandırmalarda (Şekil 6.B) Şekil 6.D de görüldüğü gibi hücre ve besin artıkları pervane ortasında birikmesine ve pervane altında ölü bölge oluşturmasını gerçekleştirmesi nedeniyle havalandırmada spiral olarak havuz dibine yerleştirilmiştir.

Diğer bir problem ise, tank kenardan ısıtılması olduğu için kışın kenarlardaki hücresel birikimin engellenmesi için pervane kenarına silikondan şerit ilave edilerek tank kenarındaki birimlerin engellenmesi sağlanmıştır (Şekil 2.B). Üretimde pervanenin ikili olarak tek yönden kullanımına karar verilmesi ile tank içerisindeki vorteks oluşumu engellenmiştir.

Tablo1. *A. platensis* ve *P. tricornutum* spesifik büyüme hızı ve ikilenme süreleri**Table 1.** Specific growth rate and doubling time of *A. platensis* ve *P. tricornutum*

Hacim (L)	<i>S. platensis</i> Spesifik Büyüme Hızı (gün ⁻¹)	İkilenme süresi (gün)	<i>P. tricornutum</i> Spesifik Büyüme Hızı (gün ⁻¹)	İkilenme süresi (gün)
2	0,2395±0,013	2,894	0,1996±0,003	3,544
5	0,1885±0,008	3,677	0,1928±0,005	3,595
10	0,1775±0,005	3,905	0,1756±0,004	3,947

±Standart sapma

**Şekil 6.** Karıştırılmalı tank sisteminin kurulumu A. İki yönlü tekli pervane ve yukardan havalandırma, B. Tek yönlü ikili pervane ortadan havalandırma, C. Tankın boşaltılması, D. Havalandırmasız tankta üretim sonrası kalan kalıntıların birikimi**Figure 6.** Construction of the open stir tank system A. Bidirectional propeller and aeration from above, B. Unidirectional propeller aeration of middle, C. Discharging the tank, D. Accumulation of remaining residues in non-aired the tank



Şekil 7. *Arthrospira platensis* hücrelerinin (1) fragmentasyonu sonucu kısa filamentler ve (2) fragmente olmayan hücrelerin görüntüsü 40X ve 60X

Figure 7. Cells of *Arthrospira platensis* (1) Fragmentation of filamentous and (2) Not fragmentation of filamentous

Spirulina hücreleri yarı kesikli üretimde yaklaşık 15-20 günün sonunda elenerek hasat edilmiş ve her 3 ayın sonunda havuzlar kesikli üretim ile tamamen elenerek boşaltılıp, temizlenip yeni üretimler için sistem hazırlanmıştır.

Spirulina tankların üretimi ve satışını yapan İzmir ilinde 2 şirket (Polidaş Polietilen Mam. Kim. San. Tic. Ltd. Şti. (Anonymus 2018j) ve Fibrolpol Cam Takviyeli Plastik İth. İhr. San ve Tic. Ltd. Şti. (Anonymus 2018k)) bulunmaktadır.

Phaeodactylum üretim optimizasyonunda özellikle sıcaklık ve ışığa dikkat edilmelidir. *Phaeodactylum* gibi bazı türlerin biyokütle üretiminde kapalı fotobiyoreaktörlerin kullanılması zorunludur, çünkü 20-25 °C civarında büyüme sıcaklığının sürekli kontrol altında tutulması gerekmektedir. Açık havuzlarda, özellikle yaz aylarında, bu sıcaklık aralığını korumak genellikle zordur (Benavides ve diğ., 2013). O nedenle *P. tricornutum* üretimi ilk olarak 75 L' lik V şeklinde asılı polietilen şeffaf poşetlerde hava kaldırmalı olarak yarı kesikli üretim yönteminde, Eylül- Mayıs ayları arasında üretimler gerçekleştirilmiştir. Soğutma maliyeti artıracığı için sistemlerin soğutulması denenmemiştir.

Karıştırmalı havalandırılmalı fotobiyoreaktör (yaklaşık 450 L) içerisinde deniz tuzuna hazırlanan ortam kullanılması nedeniyle tüm malzemelerin korozyona dayanıklı ürünlerden olmasına dikkat edilmiştir. Fotobiyoreaktörlerin ana gövdesi ışık geçiriminin sağlanması için pleksiglas şeffaf malzemedir yapılmıştır. Karıştırma ünitesi, sistem içerisinde oluşacak olan vorteksi engellemek ve verilen havanın fotobiyoreaktör içerisinde daha uzun süre kalmasını sağlamak için tasarlanmıştır. Hava difüzür ile verildiği için içeriye eşit olarak dağılması sağlanmıştır. Difüzür hem havalandırma hem de hücresel çökmelerin dipte birikmesini engellemektedir. Diatom hücreleri geliştikçe çökme eğiliminden olması nedeniyle sistemin altı konik şeklinde hazırlanmıştır. Bu yapının içerisine difüzür yerleştirilirken dış kenarlarına pleksiglas gövde yerleştirilmiştir (Şekil 8).

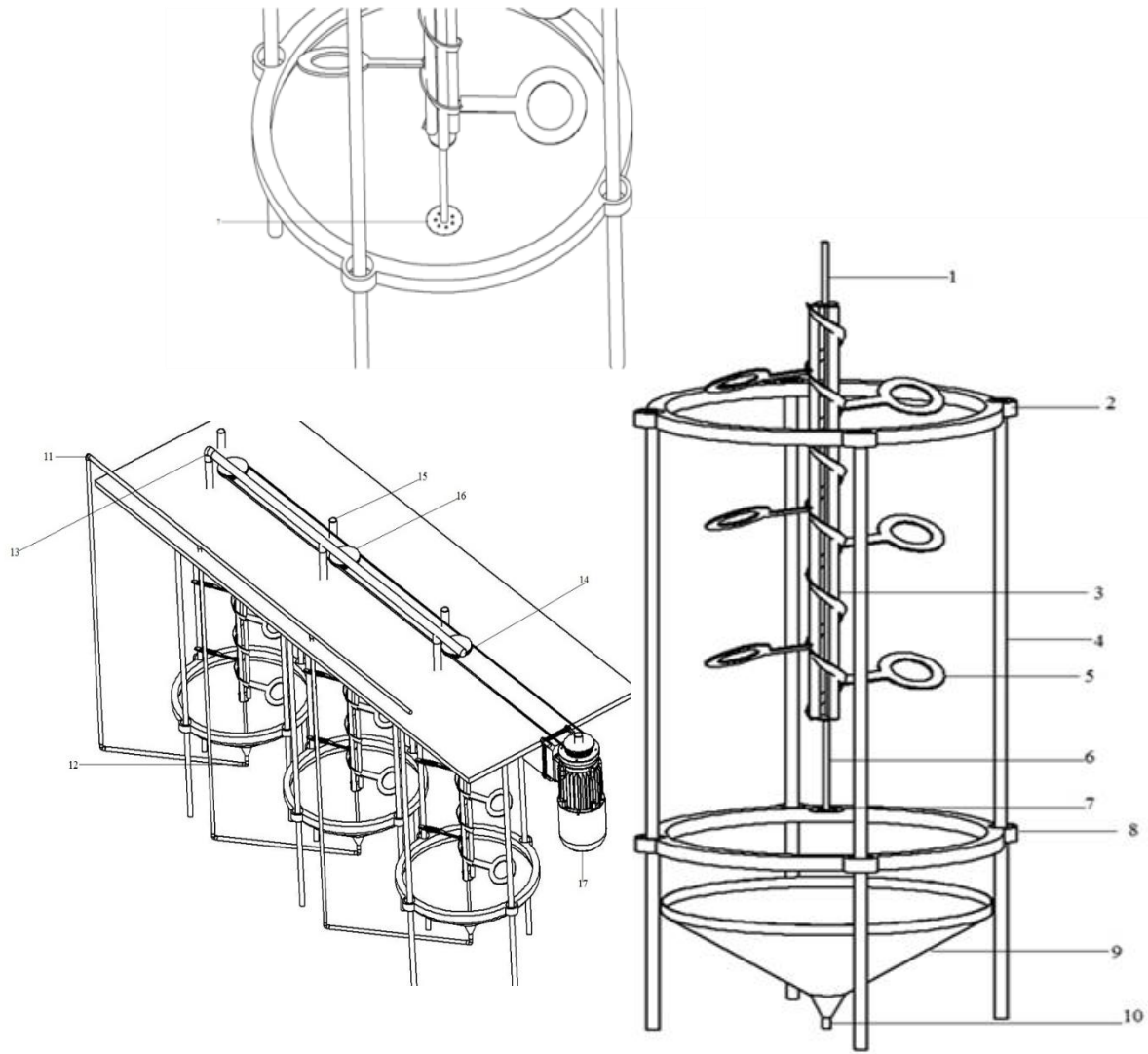
Yapılan sistemler türlere özel, kontaminasyonu engelleyici ve maksimum biyokütle eldesi düşünülerek tasarlanmıştır. Ülkemizde mikroalgler için farklı biyoreaktör ve fermentörler üretilerek, satılmaktadır. Örneğin, **Pikolab Mühendislik Biyoteknolojik Ürünler ve Lab. Hiz. San. Tic. Ltd. Şti. tarafından BioSIS** (Anonymus 2018l) marka bioreaktörler ve Nanosis Laboratuvar ve Test Sistemleri San. ve Tic. Ltd. Şti. (Anonymus 2018m) mikroalg üretim tesisi kurulumu ve satışı yapılmaktadır.

Sonuç

Ülkemizde mikroalg ve siyobakterilerden elde edilen ürünler gün geçerken artarken, alglerin biyokütle üretimi için kolay kontrol edilebilen, verimli, kontaminasyondan uzak ve düşük maliyetli teknolojilerle üretilen sistemlerin kurulumlarının gerçekleştirilmesi ile dış ülkelere bağımlılık azaltılarak ülke ekonomisine katkı sağlanacaktır. Bu kapsamda yapılan çalışma ile türlere özel büyük çap üretimler için uygun sistemler geliştirilmeye çalışılmıştır. Elde edilen biyokütlelerin gıda, kozmetik, gübre, hayvan ve akuakültür yemi olarak kullanılması ile sentetik ürünlere kıyasla doğal ve sağlıklı ürünlerin kullanılması sağlanacaktır.

Teşekkür

1150578 numaralı, "Mikroalgler ve Siyanobakterilerden Doğal Renk Maddesi Fikosiyanın ve Fukoksantin Ekstraksiyonu, Saflaştırılması, Enkapsülasyonu ve Gıda Maddeleri İçinde Stabilitesinin Test Edilmesi" isimli Uluslararası (ES1408 numaralı ve "European network for algal-bioproductions (EUALGAE)" başlıklı COST aksiyonu) TÜBİTAK projesine maddi desteği nedeniyle teşekkür ederiz. Emre Taylan DUMAN'a teknik çizimler için verdiği destekten dolayı teşekkür ederiz. Egert Doğal Ürünler Üretim Hayvancılık Gıda Yem İth. İhr. Paz. San. Tic Ltd. Şti. verdiği teknik destek ve sabırlı yaklaşımından dolayı proje çalışanları olarak teşekkür ederiz.



Şekil 8. Karıştırılmalı ve havalandırılmalı fotobiyoreaktör 1: Karıştırma mili, 2: Pleksiglas şeffaf ana gövdeyi tutan kelepçe, 3: Spiral düzenlenmiş karıştırma çarkı, 4: Kelepçe ayakları, 5: Karıştırmada ortamının karıştırılmasında kullanılan eğimli dairesel yapılar, 6: Karıştırma milinin difüzör bağlantısı, 7: Difüzör, 8: Ana gövdenin sabitlendiği kelepçe, 9: Çelik alt taban, 10: Hava girişi ve tahliye musluğu, 11: Hava hattı, 12: Deşarj musluğu, 13: Besleme Hattı, 14: Karıştırma çarkı, 15: Gaz çıkışı, 16: Çarkları paralel bağlayan kayış, 17: Karıştırma motoru

Figure 8. The stirred and aerated photobioreactor 1: Mixing shaft, 2: Holding clamp of the Plexiglass transparent main body, 3: Spiral regulated mixing ring, 4: Clamp feet, 5: Curved circular structures used to mix the mixing medium, 6: Diffusive connection of the mixing shaft, 7: Diffuser, 8: Steel bottom plate, 9: Steel bottom plate, 10: Air inlet and drain tap, 11: Air duct, 12: Discharge tap, 13: Feeding line, 14: Mixing ring, 15: Gas outlet, 16: parallel connecting belt, 17: Mixing motor

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Original Article/Full Paper

SPATIAL AND TEMPORAL DISTRIBUTION OF MARINE CLADOCERAN SPECIES IN THE SURFACE WATERS OF ISKENDERUN BAY

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ABSTRACT

Cladocerans are important member of pelagic ecosystem and they serve as good sources of food for fish and fish larvae during the warm periods. The aim of this study was to determine species composition of marine cladocerans and their temporal and spatial distribution in the surface waters of coastal and offshore waters of Iskenderun Bay. The study was conducted at the four stations in Iskenderun Bay between November 2005 and August 2006. The samples were collected horizontally with WP-2 net (200 mesh size). Six species of cladocerans (*Penilia avirostris*, *Evadne spinifera*, *Pseudoevadne tergestina*, *Evadne nordmanni*, *Pleopis polyphamoides* and *Podon intermedius*) were found. Among these, *Evadne nordmanni* was observed for the first time in Iskenderun Bay. With regard to annual abundance of cladoceran species, *Evadne spinifera* was the most abundant and followed by *P. avirostris* and *P. tergestina*. While the maximum abundance of cladoceran was observed in May-06, the minimum abundance was in November-05. It can be concluded that results of this study could provide a significant contribution to the future studies on cladoceran diversity in the region.

Keywords: Marine cladocerans, Iskenderun Bay, Abundance, Temporal distribution

Introduction

Having nearly six hundred species, cladocerans are represented by eight species in marine environments (Onbè 1999). This group is distributed in nearly all oceans and seas of the world. Some of its members are distributed widely in open seas (Gieskes, 1971), while some are densely distributed in coastal areas (Sherman, 1966), especially in bights and bays with river inputs (Bosch and Taylor, 1968, 1973). They have an important role for carnivores which are in the higher level of food web and, thereby, make a significant contribution to the energy and matter cycles. Cladocerans are found less densely in unstratified waters. Their vertical distribution is limited with surface waters and they are densely found above 15m (Tregouboff 1963). In addition, they need proper conditions in near-surface waters in order to reproduce and spread successfully (Moraitou-Apostolopoulou and Kiortsis 1973). Although they have an important place in food chain and show significant temporal changes in the plankton, there are not sufficient studies on the cladocerans in Turkish seas compared to other planktonic groups (Aker and Özel 2006; Büyükkateş and İnanmaz 2007; Büyükkateş and İnanmaz 2010, Terbıyık and Polat 2013, 2017). In the previous studies conducted in Iskenderun Bay, six cladoceran species were recorded (Dönmez 1998; Toklu and Sarihan 2003; Toklu-Alıçlı and Sarihan 2016; Terbıyık and Polat 2013, 2017). The abundance of cladocerans increases in spring and summer, and they comprise the great majority of zooplanktons in these periods (Terbıyık Kurt and Polat, 2014). Majority of the studies conducted in the İskenderun Bay comprised only species composition (Toklu and Sarihan 2003; Toklu-Alıçlı and Sarihan 2016). There are few data available regarding the abundance changes in the species (Terbıyık Kurt and Polat, 2013, 2014, 2017). But these studies conducted in the areas very close to coast and sampling depth of the stations area changed 5 to 10m. Apart from previously conducted studies, present study comprise species diversity and relative abundance data in offshore areas as well as coastal areas of İskenderun Bay. The aim is to determine the species composition, distribution and abundance changes of cladocerans in the surface waters of İskenderun Bay.

Materials and Methods

Study Area

Iskenderun Bay is formed due to the recession of Eastern Mediterranean Sea on its northeastern corner into the Anatolia in the direction of southwest-northeast (Figure 1). It is 65 km length, and 35 km width, and has an area of approximately 2275 km² and an average depth of 70 m, and the greatest depth which is approximately 100 m is found at the

entrance of the bay (Avşar 1999). The bay is affected much by bottom currents and winds since the width of the region where it is connected to the open sea is large (İyiduvar 1986). The largest river which flows into Iskenderun Bay is the Ceyhan River. Its time-averaged flow rate is 180 m³/sec. Due to all these factors, the bay has a hydrographically dynamic structure.

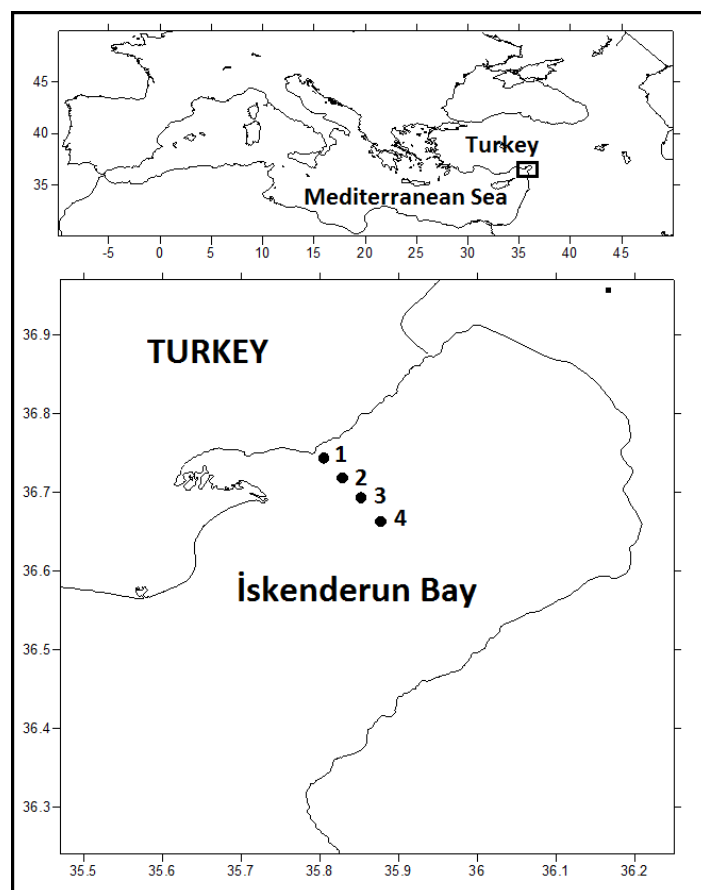


Figure 1. Study area and sampling stations

Methods

Samplings were conducted at four stations which were determined from the coast to offshore in Iskenderun Bay seasonally between November 2005 and July 2006 (Figure 1). Zooplankton samples were collected horizontally at each station using a WP-2 plankton net with a mouth area of 57 cm and mesh size of 200µm. The samples were preserved in the 4% borax buffered formaldehyde-seawater solution. At every station, temperature and salinity data were measured using CTD probe. Sub-samples were taken with Stempel Pipette depending on the cladoceran density in the sample. Identification and counting were performed under the Olympus SZX16 stereomicroscope. The identification

of cladoceran species was realized according to Onbè (1999). Cladoceran abundance was calculated on the basis of individual number per m³.

Results and Discussion

Seawater temperature and salinity were measured in the surface layer and the values were shown in Figure 2. Temperature showed significant temporal changes during the study. The lowest temperature values were recorded at near coastal stations (stations 1 and 2) in February-06 (15.7°C) and the highest at offshore station (station 4) in August-06 (30.8°C). Salinity values were partially homogeneous and significantly lower values were measured at all stations only in November-05 compared to the other months (station 1, 35.5 psu). These lower values were due to the rainy weather during the sampling period. On the other hand, the highest value was measured in February-06 (station 1, 38.7 psu).

In the study area, six cladoceran species which belong to five genera were recorded. These were *P. avirostris*, *E. spinifera*, *P. tergestina*, *E. nordmanni*, *P. polyphemoides* and *P. intermedius*. Among recorded species, *E. spinifera* was the dominant species in terms of abundance and followed by *P. avirostris* and *P. tergestina*, respectively. These three species comprised nearly 98% of cladoceran abundance (Figure 3). When temporal changes of the species was evaluated, *P. avirostris* were observed in all sampling times and *E. spinifera* and *P. tergestina* in February-06, May-06 and August-06, and *P. intermedius* in May-06 and August-06. On the other hand, *E. nordmanni* and *P. polyphemoides* were found only in February-06. Considering species abundance, *E. spinifera* was dominant in February-06 and May-06 while *P. tergestina* was dominant in August-06 (Figure 4).

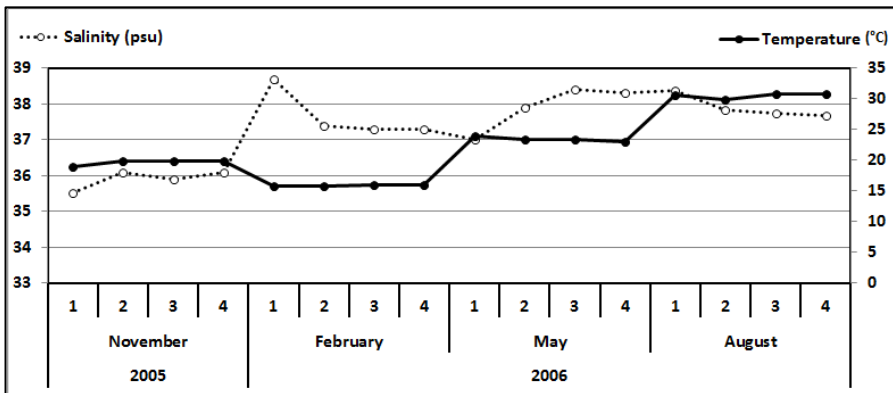


Figure 2. Changes in the surface temperature and salinity values during the sampling periods

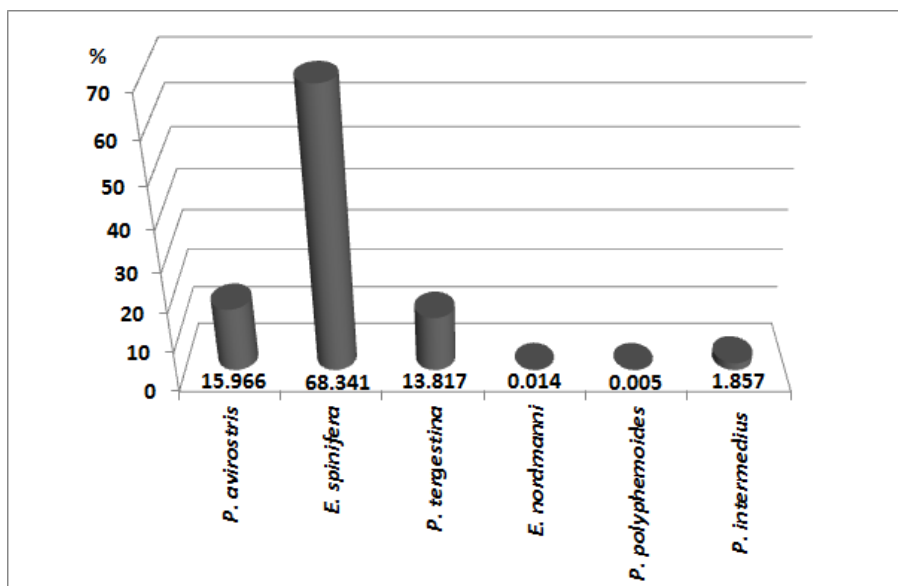


Figure 3. Proportion of the annual mean abundance of cladoceran species

Total cladoceran abundance showed temporal and spatial variations during the study. The lowest ($0.006 \text{ ind. m}^{-3}$) and the highest ($47.93 \text{ ind. m}^{-3}$) cladoceran abundance was observed at the offshore station (station, 4) in November-05 and in May-06, respectively (Figure 5). In May-06, higher abundance values were also observed at nearcoastal station (station 1). Especially in August-06 and May-06, significant differences were observed between stations in term of abundance (Figure 5).

Regarding the spatial distribution of cladoceran, there was no notable nearcoastal offshore difference. *P. intermedius*, *P. polyphemoides* and *E. nordmanni* were observed rarely in the study area. However, *E. spinifera*, *P. avirostris* and *P. tergestina* were the species that mainly affected cladoceran abundance and reached the highest abundance in May-06 (Figure 6). Cladocerans are densely found in the hyponeustonic layer and even over 30 cm depth (Moraitou-Apostolopoulou and Kiortsis 1973). *P. avirostris*, *E. spinifera* and *P. tergestina* are typical warm-water species (Onbè 1999; Marazzo and Valentin 2000). *P. avirostris* is a eurohaline and neritic species that mostly prefers low salinity waters (Moraitou-Apostolopoulou and Kiortsis 1973, Lakkis,

2011). In our study, this species was observed during the whole sampling period. *P. tergestina* has a higher temperature range compared to *E. spinifera* among cladoceran species recorded in this study, (Kiortis and Moraitou-Apostolopoulou 1975) and is found proportionately more densely than *E. spinifera* in August-06 when temperature was highest. On the other hand, *E. nordmanni* is known to be a cold-water species which is seen rarely in the months with lower temperatures (Onbè et al. 1996) and was observed only in February-06 when temperature was lowest, which is consistent with its ecology. There are contradictory reports about temporal distribution of *P. polyphemoides*. Onbè (1999) and Della Croce and Venugopal (1972) defined this as a warm-water species. On the other hand, Büyükaş and İnanmaz (2007) stated that this species showed negative correlation with temperature. In addition, Kiortis and Moraitou-Apostolopoulou (1975) reported that its distribution was limited with January-March. This species was recorded in spring (cold period) and summer (warmer period) in İskenderun Bay by Terbıyık Kurt and Polat (2014). In present study, we similarly observed it in February-06 when the temperature is lower.

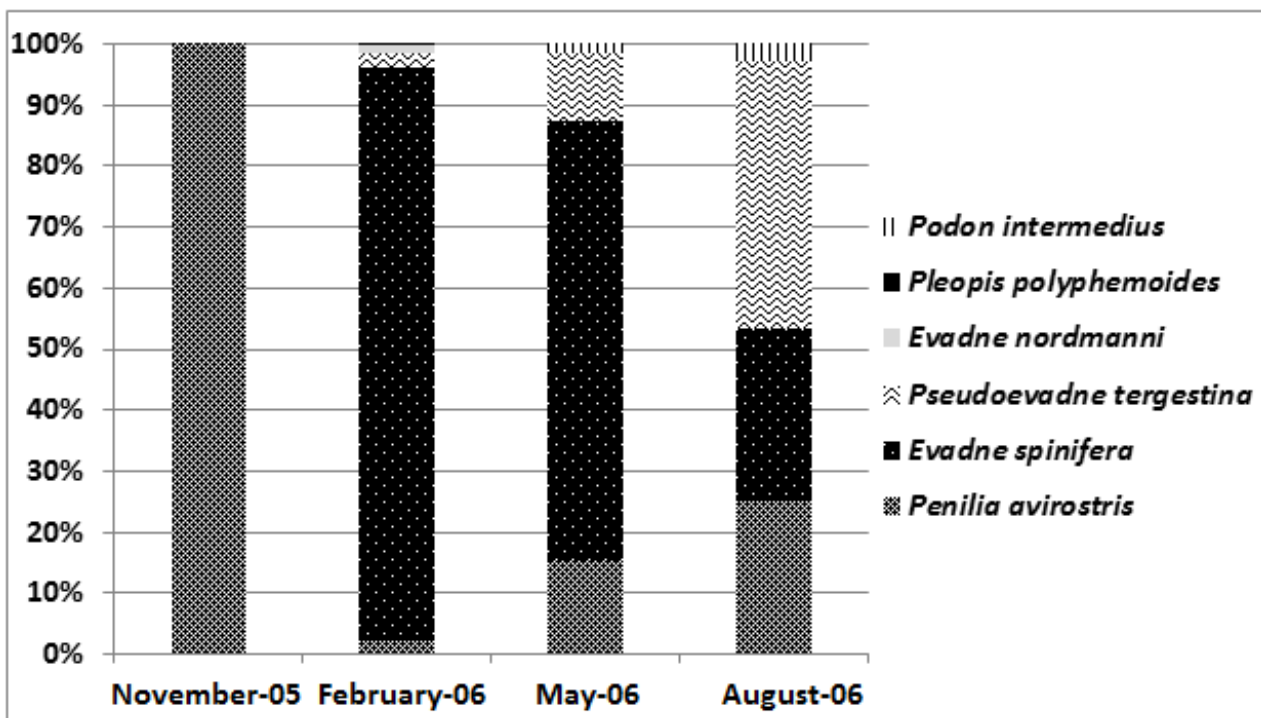


Figure 4. Availability of cladoceran species in percentage in the months when the study was conducted

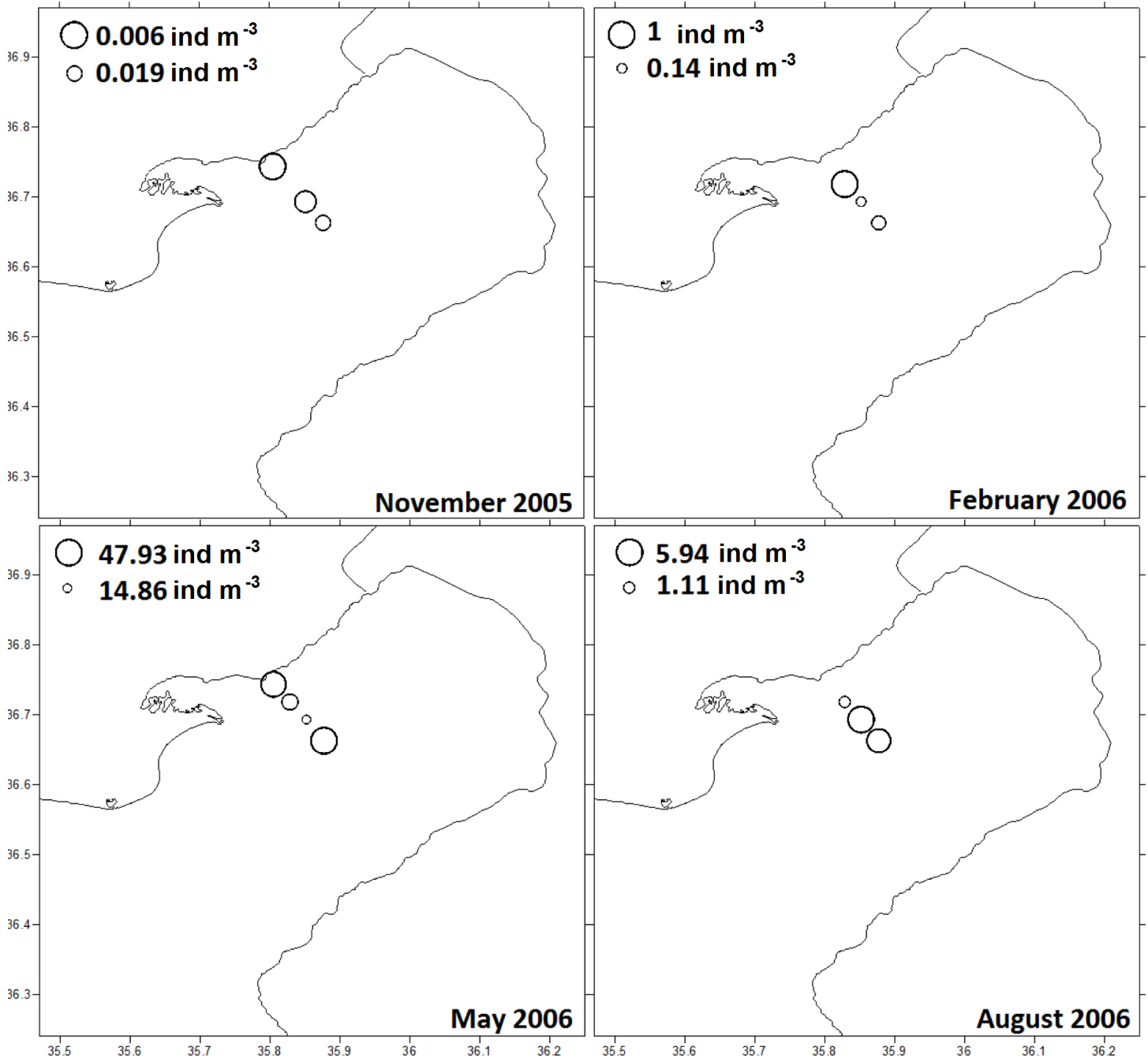


Figure 5. Monthly and spatial changes in the total abundance values of cladocerans

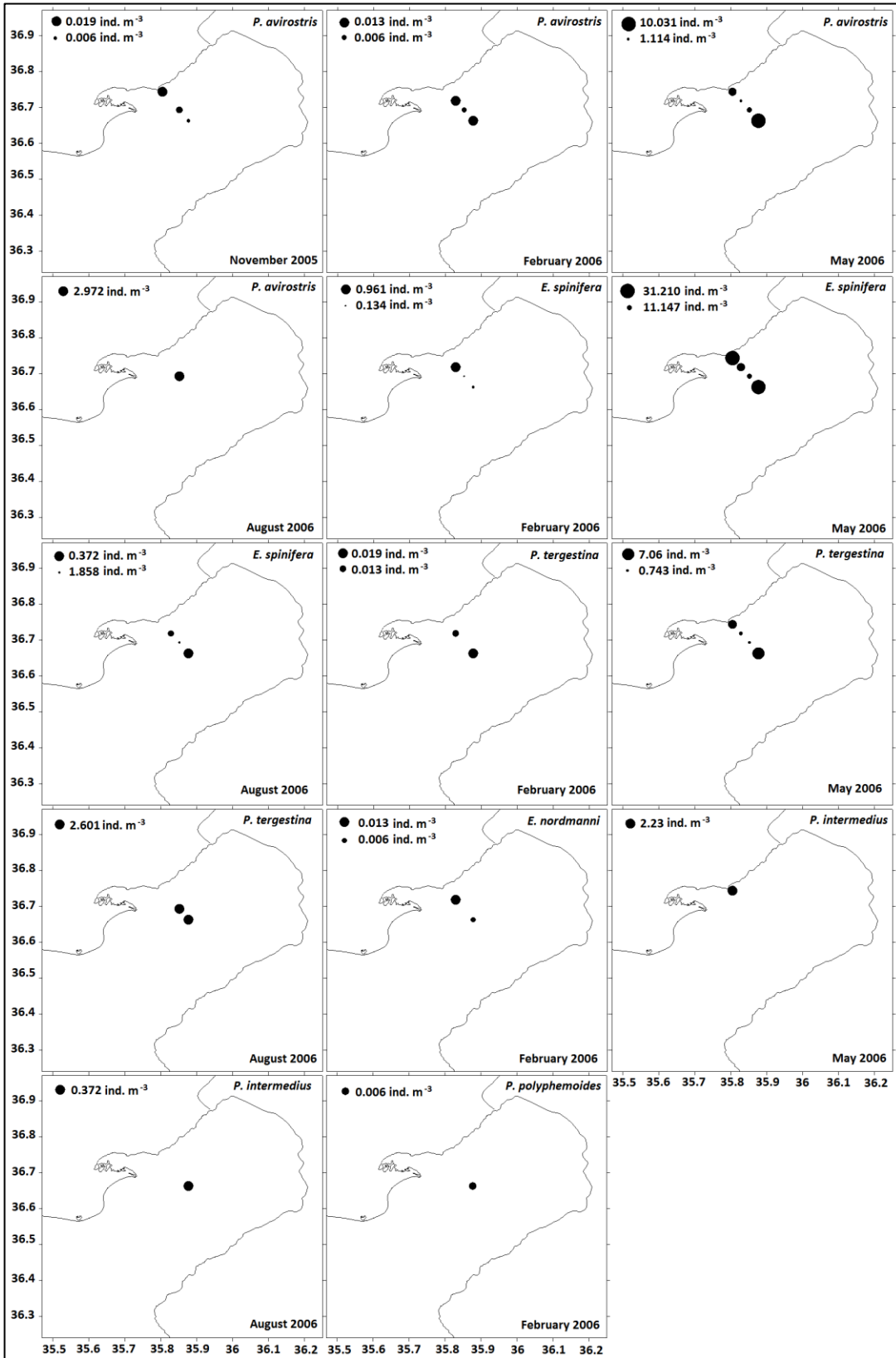


Figure 6. Monthly and spatial changes in the abundance values of cladoceran species

The abundance of cladocerans is much lower in surface area of İskenderun Bay when compared to other studies conducted in the same area (Terbıyık Kurt and Polat, 2013, 2014, 2017). However, it should be taken into account that different sampling methods were used in these studies. Moreover, cladocerans were collected by vertical tows and conducted in the area close to coast in these studies (Terbıyık Kurt and Polat, 2013, 2014, 2017).

In our study, we observed fluctuations in the distribution of cladocerans. The effect of temperature on cladocerans is a well-known phenomenon. However, besides temperature, the availability of food is remarkably important on the distribution. Although the temperature in May was lower than August, cladocerans were more abundant, which might be related to the amount of available food or the reproduction period of cladocerans as well as the hydrography of the environment where they were observed. Indeed, it is known that the highest chlorophyll-a concentration was observed in İskenderun Bay in May (Polat and Terbıyık, 2014). Besides seasonal changes, the changes at the stations might be due to land based inputs, mainly arising from the flow of Ceyhan River and circulation dynamics in the bay. İskenderun Bay has a hydrographically dynamic structure. On the other hand, the region is remarkably affected by terrestrial pollutants leading from agricultural activities, industrial and domestic wastes. The variable conditions in the bay which is caused by such factors is also affect distribution and abundance characteristics of the organisms.

Several studies on cladocerans were conducted in previous years in the region. Dönmez (1998) observed cladocerans only in summer and spring, while Terbıyık and Polat (2013) observed them in all seasons except November and reported the existence of four species which were *P. avirostris*, *E. spinifera*, *P. tergestina* and *Podon intermedius*. Toklu Alıçlı and Sarihan (2016) encountered cladoceran species in all seasons except winter and reported the existence of four species which were *P. avirostris*, *E. spinifera*, *P. tergestina* and *Pleopis polyphemoides*. Moreover, recently, *Pleopis schmackeri* was recorded in İskenderun Bay by Terbıyık Kurt and Polat (2017). In the present study, *E. nordmanni* was firstly observed in February 2006 and the number of the cladoceran species in İskenderun Bay raised to seven. The species showed distribution in Western Mediterranean Sea (Sampaio de Souza et al. 2011; Fernandez de Puelles et al. 2003) and various parts of Eastern Mediterranean Sea (Kiortis and Moraitou-Apostopoulou 1975; Siokou Frangou 1996; Brautovic 2001). This species is also distributed in Turkish coastal waters of the Black Sea (Demir 1955), Marmara Sea (Demir 1955; Büyükkateş and İnanmaz 2007) and

Aegean Sea (Aker and Özel 2006; Tarkan 2000). It is thought that the species had not been recorded before due to the scarcity or more local studies conducted in İskenderun Bay and the rare presence of the species.

Conclusions

In this study, the abundance changes, composition and distribution of cladocerans which is a important group in pelagic ecosystems were analyzed in surface waters of İskenderun Bay. The results of this study showed that there were clear seasonal changes in the distribution and abundance of cladocera species of the İskenderun Bay. The findings of this study might serve as a reference for future studies. In this respect, studies to be conducted in wider areas with more frequent sampling periods will be beneficial to determine changes in cladoceran population.

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Review Article

A REVIEW ON GROWTH OF SOME *DIPLODUS* SPECIES DISTRIBUTED WORLDWIDE

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ABSTRACT

Estimation of the growth parameters of fish are vital to understand their biology. For this purpose we collected studies that performed up to 2017 regarding the growth of species belonging to the *Diplodus* genus. Data were gathered from sources like Web of Science (webofknowledge.com), Scopus (scopus.com), Google Scholar (scholar.google.com), Researchgate (researchgate.com) and Academia (academia.edu). 79 datasets from 52 different studies belongs to 10 species were compiled. Reviewed studies were published between 1982 - 2017 and were performed in 26 different regions. It was determined that the most frequently studied species was *D. vulgaris* (n=23). Among growth parameters, it was determined that there is a negative relationship between K and L_{∞} , and K and t_{max} , there is a positive relationship between L_{∞} and L_{max} . It was also found that there is a negative relationship between K and L_{∞} vs latitude.

Keywords: Growth, *Diplodus*, Population dynamics, Life history parameters

Introduction

The *Diplodus* genus, distributed all around the world, have a significant economic importance (Gordoa and Moli, 1997; Pajuelo and Lorenzo, 2004; Soykan et al., 2015). Due to varied habitat preferences, these species can be found in different marine ecosystems such as rocky habitats and sandy bottoms. According to Fishbase, 21 species of this genus can be found in world seas (Froese and Pauly, 2017). While the main area of distribution for these species is the Mediterranean Sea and the Atlantic Ocean, they are also found in the Caribbean, Gulf of Mexico, the Indian Ocean, the Red Sea and the Persian Gulf (Figure 1, Sala and Ballesteros, 1997; Summerer et al., 2001; Froese and Pauly, 2017). Along with being a main target species for small scale, semi-industrial fisheries and sport fishing, a couple species belonging to this genus are also important with regards to aquaculture (Reina et al., 1994; Summerer et al., 2001). For this reason, their biology and population dynamics are essential.

While there are many studies regarding various biological characteristics of *Diplodus* species, studies conducted on age and growth are only available for 10 species (Appendix 1). Evaluating different species belonging to the same genus that show similar morphological and growth characteristics together offers significant advantages regarding population dynamics (Hilborn and Liermann, 1998; Helser et al., 2007). Compilation and reanalysis of growth studies help us for

better understanding the changes in growth characteristics (Pilling et al., 2002; Helser and Lai, 2004; Helser et al., 2007). For this purpose the aim of this study is to gather age-growth studies performed on species belonging to *Diplodus* genus and to establish the variability in growth between species and regions. Finally, growth variety between species was addressed based on the relationships between growth parameters.

Compilation of Data from References

Studies performed up to 2017 regarding the growth of species belonging to the *Diplodus* genus (Figure 2) were gathered from sources like Web of Science (www.webofknowledge.com), Scopus (www.scopus.com), Google Scholar (www.scholar.google.com), Researchgate (www.researchgate.com) and Academia (www.academia.edu). Collected studies were carefully classified and necessary information was extracted (See appendix). This information includes the following: the location the study (latitude, longitude), length type (LT), L_{∞} , K , t_0 , maximum age (t_{max}), minimum and maximum length (L_{min} , L_{max}), sex, age determination method (otolith reading (OR), scale reading (SR), length frequency method (LF)), sample size (N) and the year the study was performed (see Appendix).

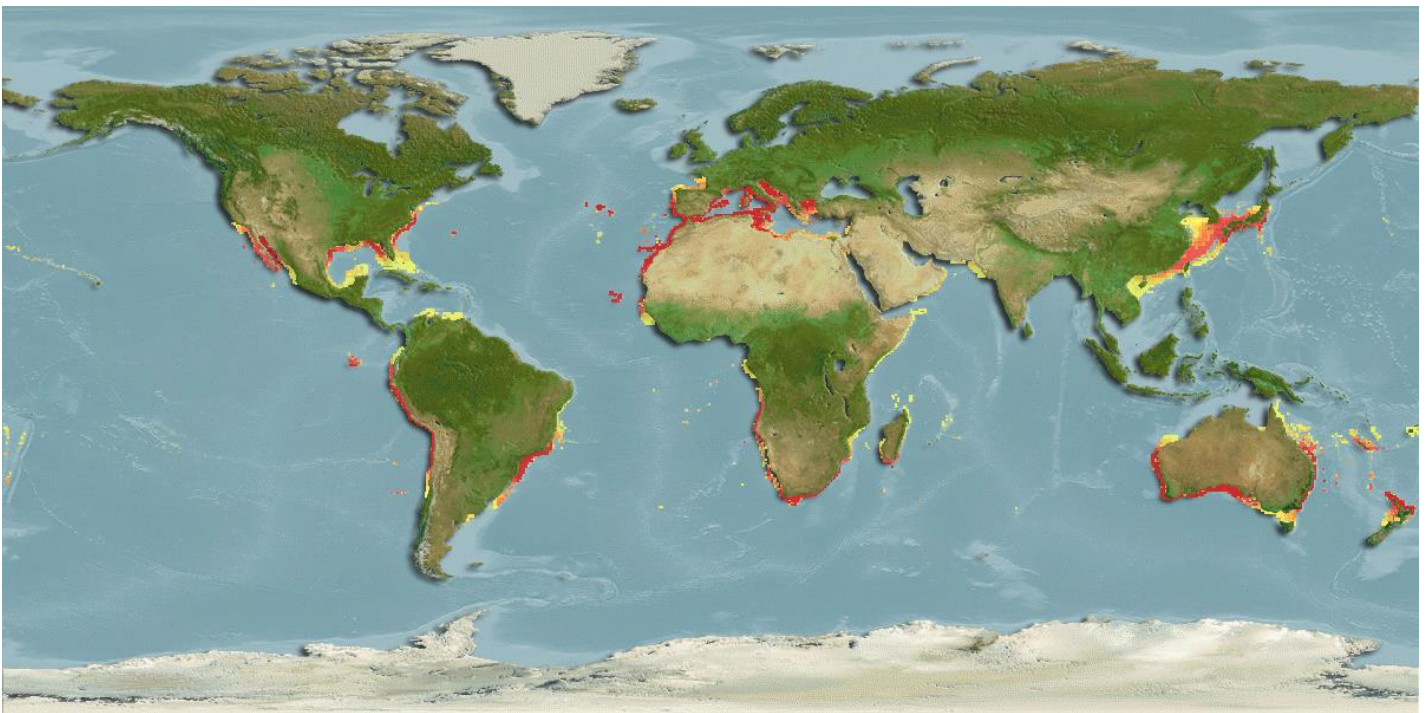


Figure 1. Distribution of the *Diplodus* genus (Fishbase 2017).

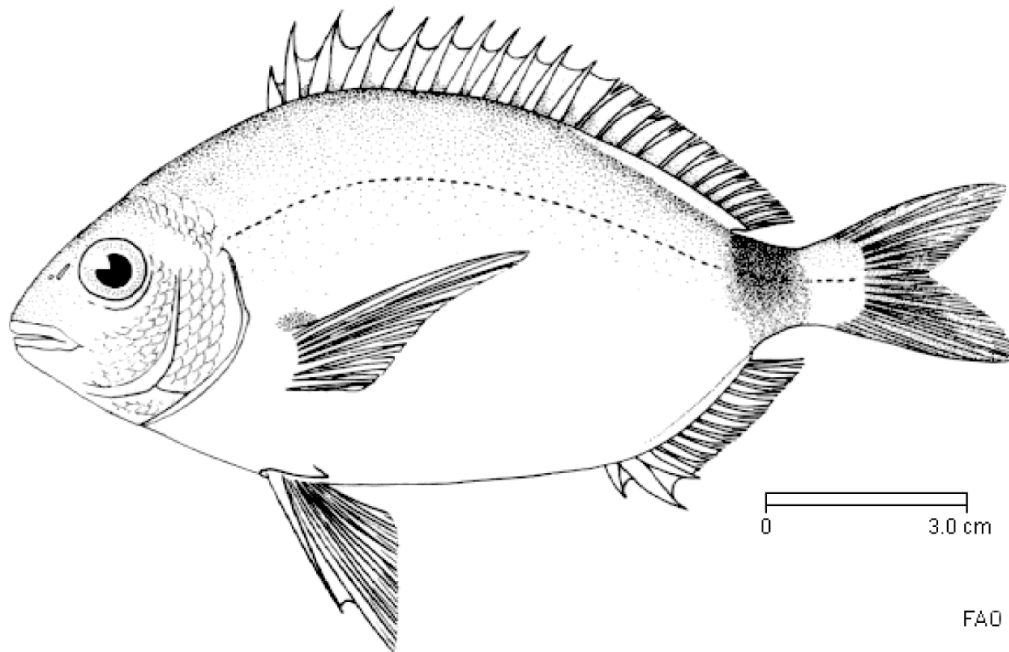


Figure 2. General appearance of *Diplodus* (*Diplodus annularis*, Source: Bauchot, (1987)

In cases where the studies compiled used different size types, the size-size relationship formula for the species involved presented in Fishbase (Froese and Pauly, 2017) was used for all size groups and L_{min} , L_{max} and L_{∞} values were transformed (Stergiou and Karachle, 2006; Froese, 2006; Gündoğdu and Baylan, 2016; Gündoğdu et al., 2016). For species where fork lengths were reported and to convert the fork lengths given to total length, the following formula taken from Fishbase (Froese and Pauly, 2017) were used.,

$$D. annularis: TL = 0 + 1.09FL$$

$$D. bellottii: TL = 0 + 1.093FL$$

$$D. capensis: TL = 0.2554 + 1.163FL$$

$$D. hottentatus: TL = 0.2628 + 1.161FL$$

$$D. sargus sargus: TL = 0 + 1.088FL$$

$$D. vulgaris: TL = 0 + 1.15FL$$

For studies where maximum length was not reported, if present the L_{max} value given in other studies from the same region, and if not the average L_{max} value given in Fishbase was used

In literature, fish growth is mostly expressed based on the von Bertalanffy growth model. Since this was the case for all literature collected, the parameters used in this model were taken directly without any recalculations. Latitude and

longitude information for the study areas given in gathered studies were reproduced as averages. This way, the same latitude and longitude information is given for studies performed in the same area. It was thought that if done otherwise, taking close latitudes and longitudes for studies performed in the same area would increase the difficulty of the analysis and reduce the significance of the results.

The change of growth parameters and other life history parameters taken from the compiled studies relative to each other and latitude was analyzed using the Tableau 10.0 software. Separate and joint growth formula of all species were recalculated using the median values of all parameters and regression constants (slope) were analyzed using an independent sample t-test with SPSS v20 package software.

Assessment of Data and Discussion

79 datasets from 52 different studies were compiled in this study. This data set belongs to 10 different species. Studies were published between 1982 - 2017 and were performed in 26 different regions (Figure 3; Appendix 1).

Most studies were from around Canary Islands (n=14, 18%). Gathered studies are most frequently on the biology of *D. vulgaris* (n=23, 29%), *D. annularis* (n=17, 22%) and *D. sargus sargus* (n=15, 19%) species (Appendix 1). Age reading method was used in 71 studies (otolith reading in 45, scale reading in 26), while in 6 studies estimation was done using

the length frequency method, and in 2 studies no information regarding this was given. Length measurements were done as total length (n=65) and fork length (n=14). It was determined that there was a significant amount of variation between number of observations in studies where growth parameter estimations were performed. In 7 estimates, >1000 individuals were used, while in 59 estimates the number of individuals used was <1000. It was determined that in 10 data sets the number of observations was not reported (Figure 4).

t_{max} value (1 year to 33 years) was reported in 71 data sets and L_{max} value (9,3 cm to 56,5 cm) was reported in 69 data sets. K , L_{∞} and t_0 values were reported in all studies (Appendix 1). It was determined that the K value varied between 0.073 year^{-1} and $0,56 \text{ year}^{-1}$, the L_{∞} value varied between 13.32 cm and 68.83 cm and t_0 value varied between -5.33 years and -0,02 years (Table 1).

It was determined that the L_{max}/L_{∞} ratio varied between 0.52 and 1.84 for all studies, with an average of 0.95 (Table 1). The relationship between L_{max} and L_{∞} was calculated together for all species and a positive and statistically significant correlation was discovered between them ($r=0.827$, $P<0.05$, $L_{\infty} = 4.42 + 0.95 * L_{max}$; Figure 4). It was determined that the relationship between t_{max} and K is negative and statistically significant ($r=-0.41$, $P<0.05$, $Ln(K) = -0.72 - 0.38 * Ln(t_{max})$; Figure 5). It was also determined that the relationship between K and L_{∞} is negative and statistically significant ($r=-0.71$, $P<0.05$, $Ln(K) = 1.66 - 0.92 * Ln(L_{\infty})$; Figure 5).

The relationship between latitude and von Bertalanffy parameters was determined to be negative for K and L_{∞} , and positive for t_0 (Figure 6). However, the relationships for all three parameters were found to be statistically insignificant (t-test, $P>0.05$; Figure 6).

Table 1. Descriptive statistics of the parameters belonging to the compiled studies

Parameters	Mean	Std.Error	Minimum	Median	Maximum
K	0.24	0.01	0.07	0.21	0.56
L_{∞}	35.3	1.48	13.32	33.3	68.8
t_0	-1.31	0.11	-5.33	-0.98	-0.02
L_{max}	32.6	1.29	9.30	32.0	56.5
t_{max}	10.0	0.60	1.00	9.00	33.0
L_{max}/L_{∞}	0.95	0.02	0.52	0.93	1.84

Table 2. Recalculated models using the median values taken from the compiled studies

Species	Estimated Model
<i>D. annularis</i>	$L_t = (20.37 * (1 - e^{-0.25(t+0.89)}))$
<i>D. bellottii</i>	$L_t = (28.42 * (1 - e^{-0.27(t+0.19)}))$
<i>D. capensis</i>	$L_t = (27.7 * (1 - e^{-0.31(t+1.05)}))$
<i>D. cervinus</i>	$L_t = (60.9 * (1 - e^{-0.15(t+0.76)}))$
<i>D. holbrooki</i>	$L_t = (33.28 * (1 - e^{-0.24(t+0.99)}))$
<i>D. hottentotus</i>	$L_t = (46.24 * (1 - e^{-0.15(t+2.15)}))$
<i>D. puntazzo</i>	$L_t = (36.84 * (1 - e^{-0.2(t+0.98)}))$
<i>D. sargus cadenati</i>	$L_t = (47.65 * (1 - e^{-0.14(t+1.98)}))$
<i>D. sargus sargus</i>	$L_t = (40.71 * (1 - e^{-0.18(t+0.86)}))$
<i>D. vulgaris</i>	$L_t = (33.3 * (1 - e^{-0.22(t+0.96)}))$
Total	$L_t = (33.3 * (1 - e^{-0.21(t+0.98)}))$



Figure 3. The distribution of the compiled studies

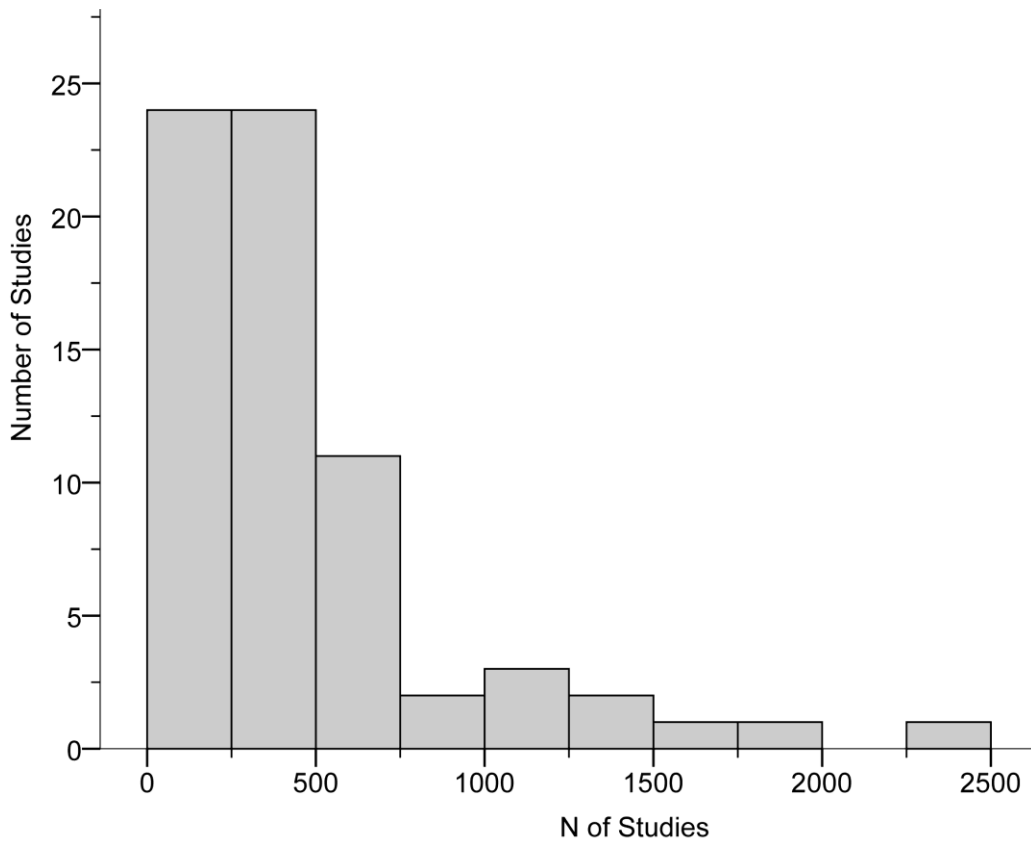


Figure 4. Frequency distribution of the sample sizes on which the biological parameters presented here were based (see Appendix 1).

The growth formula created based on the median values calculated using the entire data set is given in table 2. Estimated growth curves created with the help of these shared formulas are given in figure 7. As seen in figure 7, for the first three years, all species except for *D. annularis* demonstrate similar growth. It was seen that curves that match the initially rapid and later slowing growth with age posited in the general fish growth theory.

In this study, 52 studies performed in different regions around the world that include the biological parameters of 10 different species belonging to the *Diplodus* genus (Appendix 1). This study is one of the rare studies that considers the growth of the entirety of a certain genus at once, and it is the first study that considers the species belonging to the *Diplodus* genus together. Another similar study was performed by Helser et al. (2007) for the *Sebastes* genus, found in the Pacific Ocean. All studies other than our study and the Helser et al. (2007) study are studies where the growth parameters of more than one genus and species were considered together. These are Pauly (1978), Pauly (1980), Paul (1992), Stergiou (2000), Frisk et al. (2001), Stergiou and Karachle (2006), Apostolidis and Stergiou (2012), Apostolidis and Stergiou (2014) and Gündoğdu and Baylan (2016).

Aside from coloring, fish belonging to the *Diplodus* genus show similarities regarding many characteristics and have similar habitat demands (Summerer et al., 2001). This causes their feeding habits to be similar as well (Ventura et al., 2015). For this reason, considering the growth characteristics of fish belonging to the *Diplodus* genus together and in a comparative manner is quite reasonable.

Growth is the most studied subject, as it affects many life history parameters and involves a lot of basic information for fishing management (Helser and Lai, 2004). However, as stated above, the number of studies that consider different populations belonging to the same species or genus in a comparative manner is quite limited. Consequently, this study attempts to establish the relationship between various biological parameters and between some parameters and latitude through the compiled studies. Among these relationships, one of the most important is the relationship between K and L_{∞} . It is known that there's a negative relationship between these two parameters (Beverton and Holt, 1959; Adams, 1980; Pauly, 1980; Munro and Pauly, 1983; Pauly and Munro, 1984; Wootton, 2012). The negative correlation (-0.71) found in this study matches this general assumption (Figure 5). However, despite the presence of this negative relationship, in reality there's no direct evidence in natural

populations regarding this negative correlation (Pilling et al., 2002; Helser and Lai, 2004). It is thought that the negative relationship between these two parameters arises from the mathematical nature of the von Bertalanffy growth model (Stergiou, 2000). The negative relationship between the K value and the t_{max} value (-0.41) was found to be similar to the value found in a multi-species study performed by Stergiou and Karachle (2006) (-0.37). If we consider Taylor (1958)'s $K = 3/t_{max}$ equation a general equation, it can be seen that this study has a result that is close to this value (Table 1).

L_{max}/L_{∞} ratio (0.95) and the correlation between them (0.82) was found to be similar to the studies performed among different species (Stergiou and Karachle, 2006 (0.99); Apostolidis and Stergiou, 2014 (0.87); Gündoğdu and Baylan, 2016 (0.96)). And this shows that there's a relationship between these two parameters in general terms that is independent of species (Froese and Binohlan, 2000). Taylor (1958), Pauly and Munro (1984) and Froese and Binohlan (2000) state that fish usually live for 95% of the L_{∞} value. And this shows that there's a relationship like $L_{\infty} \approx L_{max}/0.95$ between these two parameters, which fits the results we have found in this study.

Helser and Lai (2004) state that there's a relative relationship between growth parameters and latitude that is independent of statistical significance. According to this, K and L_{∞} have a negative relationship with latitude, while t_0 has a positive relationship. Our findings are in the same direction. When Figure 6 is examined, it can be seen that K and L_{∞} values have a negative relationship with latitude, while t_0 value has a relatively positive relationship.

Feeding habits, genetic relationships, food available in the environment, competition and temperature are the basic factors that determine the growth performance of a species (Jobling, 1981; Helser et al., 2007). For this reason, growth trends of species that are similar to each other with regards to these factors would be similar as well. Consequently, the results expressed in table 2 and figure 7 support this conclusion. The mtDNA based relationship study performed by Summerer et al. (2001) on the *Diplodus* genus partially supports the estimated growth model curves we have presented here. Moreover, in Summerer et al. (2001)' study, *D. cervinus* in a different cluster than other species. Similarly, *D. annularis* and other *Diplodus* species are considered separate to a point. Furthermore, the same reports put all species other than *D. annularis* and *D. cervinus* in *D. sargus* clades (Figure 8).

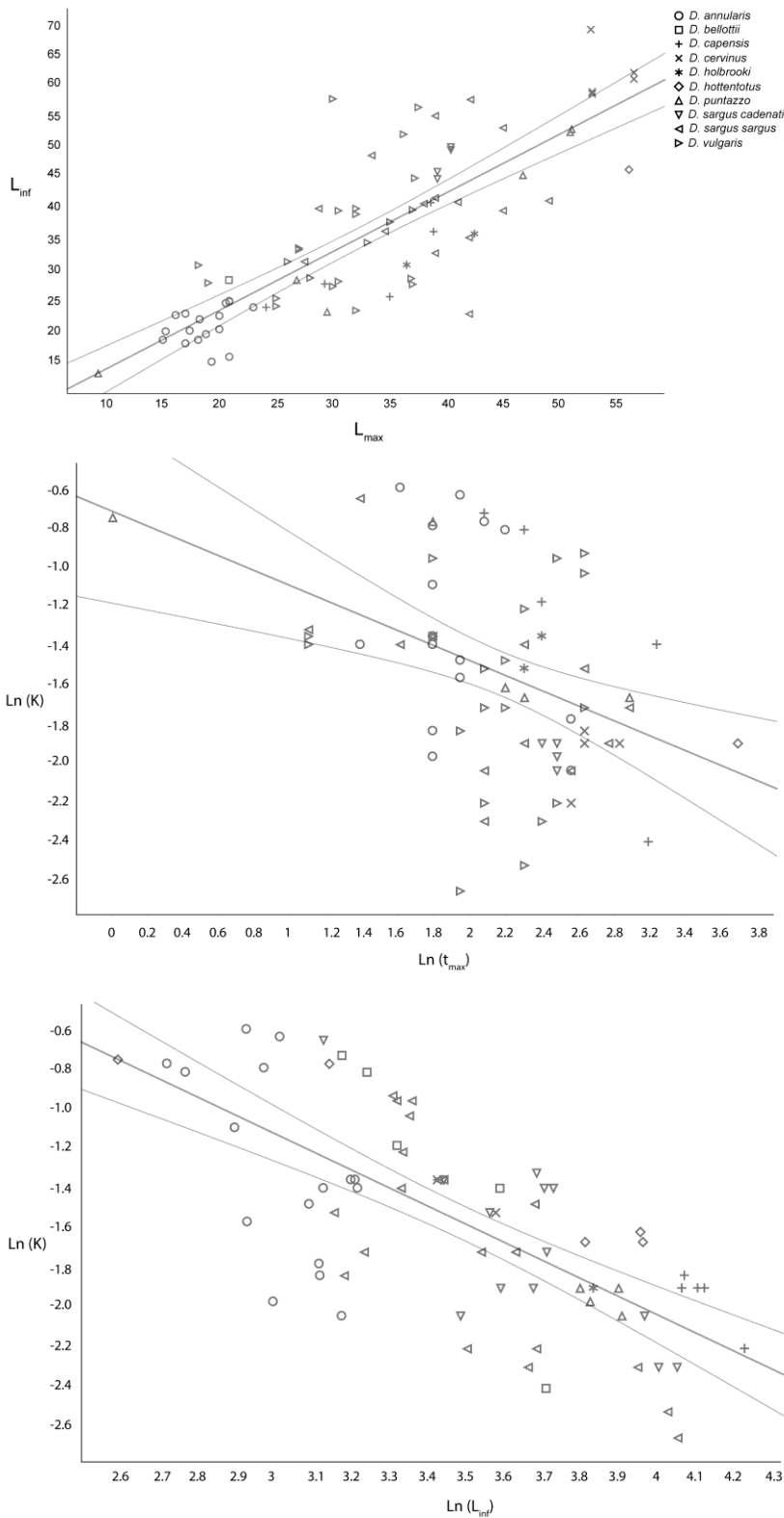


Figure 5. Relationships between von Bertalanffy growth parameters (a- L_{∞} vs L_{max} , b- $\ln(K)$ vs t_{max} , c- $\ln(K)$ vs t_0) and the fitted curves belonging to those. The middle curve in each graph represents the fitted curve, and the other two represent the 95% confidence limits.

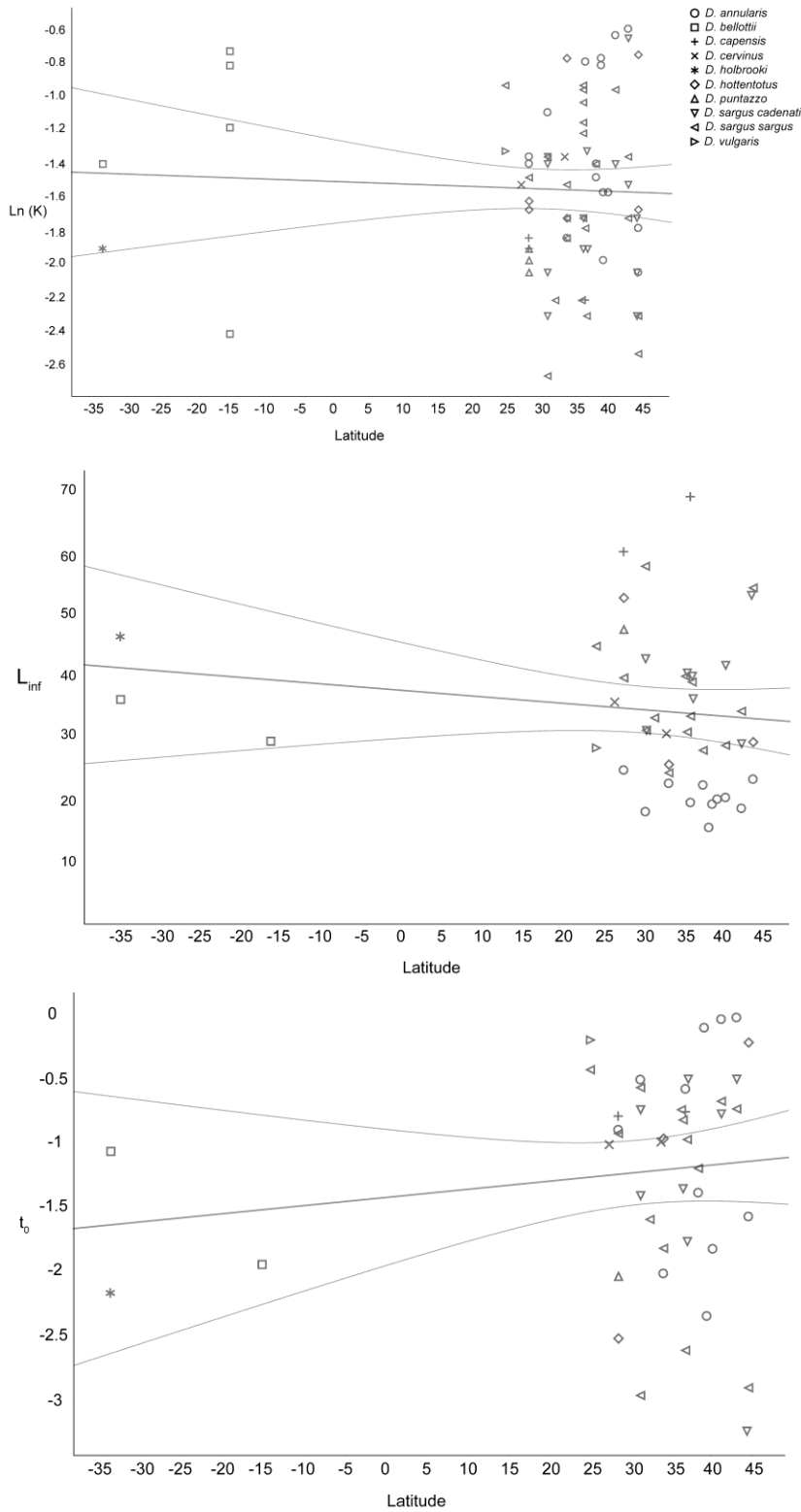


Figure 6. Relationships between growth parameters and latitude and the fitted curves belonging to those. The middle curve in each graph represents the fitted curve, and the other two represent the 95% confidence limits.

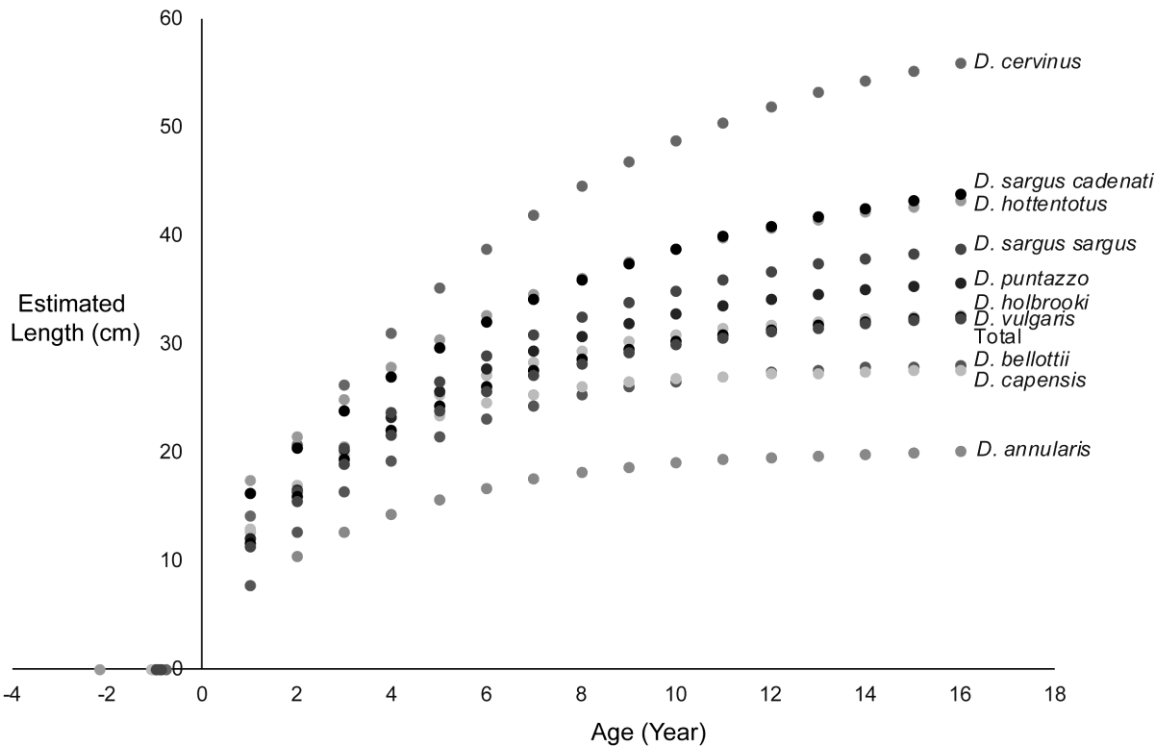


Figure 7. Estimated age-length curves the new models based on the median values provided

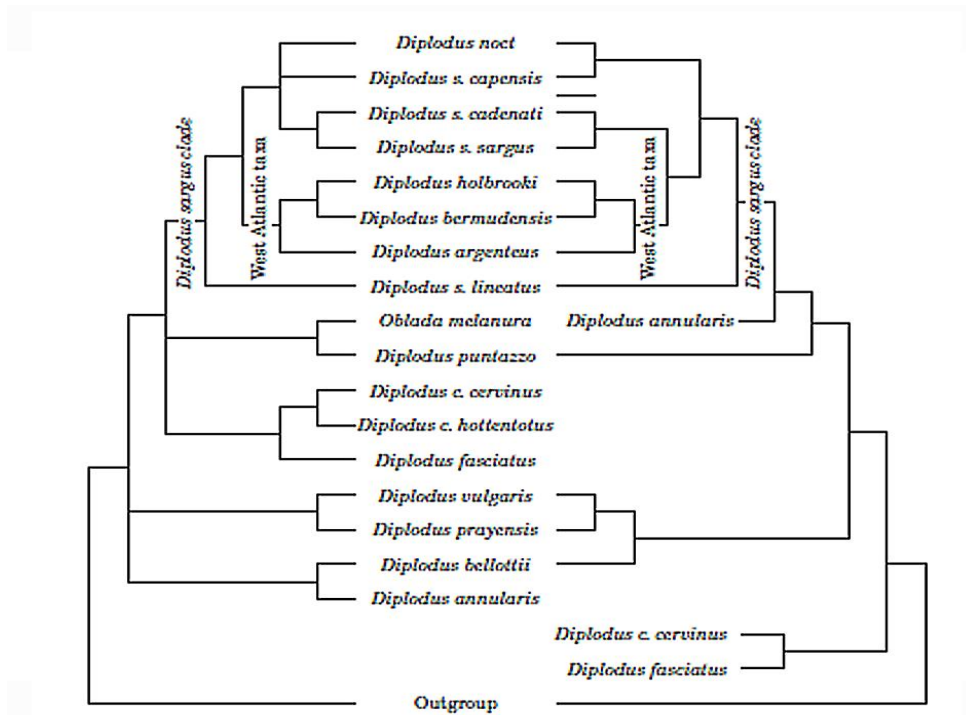


Figure 8. Relationship between genetic characteristics and morphology of genus *Diplodus*. The mtDNA (left) and morphological (right) comparison of species belonging to the *Diplodus* genus (Taken from Summerer et al. (2001)).

Conclusion

Establishing the variation of growth parameters between populations and species is to key for ecological studies. Comparing growth models and parameters both systematically and over other variables would help us in understanding the growth characteristics of the genus and species involved. Comparative studies like these carry great significance to understand the biology of species that can be considered target species for fishing.

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Appendix

Biological parameters for various *Diplodus* stocks in various seas [K in yr⁻¹, L_∞ in cm, and t₀ in yr. Sex (M=males, F=females, B=combined). N denotes the number of individuals used for parameter estimation. Method denotes the method used for the estimation of age (O=otoliths, S=scales, LF=length-frequencies). L_{max} and t_{max} denote maximum body length, in cm, and maximum age, in yr, respectively. LT denotes type of length used in the original study (TL=total, FL=fork,). nr: not reported.

Species	Location	Country	LT	Sex	L _∞	K	t ₀	t _{max}	L _{min}	L _{max}	L _{max} /L _∞	N	Method	Year	Author
<i>D. annularis</i>	Adriatic Sea	Coratia	TL	B	23,95	0,13	-1,66	13	3,3	23,0	0,960	786	SR	2000-2002	Matic-Skoko et al. (2007a)
			TL	B	22,60	0,17	-1,46	13	3,3	20,0	0,885	1872	SR	2000-2002	Matic-Skoko et al. (2004)
	Alexandria	Egypt	TL	B	18,10	0,34	-0,50	6	9,0	17,0	0,939	466	SR	1980-1981	Wassef (1985)
	Annaba Gulf	Algeria	TL	B	19,54	0,46	-0,57	6	12,6	18,8	0,962	648	SR	nr	Nouacher and Djebar (2007)"
			TL	M	24,57	0,26	-0,89	6	8,9	20,6	0,838	173	OR	1998	Pajuelo and Lorenzo (2002b)
	Canary Is-lands		TL	F	24,96	0,25	-0,89	6	9,4	20,9	0,837	139	OR	1998	Pajuelo and Lorenzo (2002b)
			TL	B	24,79	0,26	-0,88	6	8,2	20,9	0,843	194	OR	1998	Pajuelo and Lorenzo (2001)
			TL	B	20,37	0,54	-0,03	7	9,0	20,0	0,982	180	OR	nr	Gordoa and Moli (1997)
	Mallorca island		TL	F	15,93	0,45	-0,12	9	9,0	20,9	1,312	166	OR	2007	Alos et al. (2010)
			TL	M	15,17	0,47	-0,07	8	8,4	19,3	1,272	141	OR	2007	Alos et al. (2010)
	Edremit Gulf		FL	M	20,01	0,14	-2,93	6	8,2	15,3	0,763	330	OR	1997-1998	Torcu-Koç et al. (2002)*
			FL	F	18,76	0,21	-1,73	7	8,0	15,0	0,802	322	OR	1997-1998	Torcu-Koç et al. (2002)*
	İzmir Bay		FL	B	22,86	0,25	-1,45	4	8,5	17,0	0,744	160	LF	1997-1999	Kınacıgil and Akyol (2001)*
			TL	B	22,01	0,23	-1,30	7	7,7	18,3	0,831	2393	OR	2004-2007	Kınacıgil et al. (2008)
	Gulf of Gabes	Tunusia	TL	B	22,64	0,16	-2,00	6	8,4	16,1	0,712	nr	LF	nr	Bradai et al. (2001)
Thermoikos Gulf	Greece	TL	B	20,10	0,21	-1,81		6,3	17,4	0,866	135	nr	nr	Froese and Pauly (2017)"	
Gulf of Lion	France	FL	B	18,66	0,56	-0,02	5	3,3	18,2	0,973	nr	SR	nr	Girardin (1978)*"	
<i>D. bellottii</i>	Western Sahara	Morocco	FL	B	28,42	0,27	-0,19		8,9	20,8	0,733	nr	LF	1980-1982	Mennes (1985)*"
<i>D. capensis</i>	Tsitsikamma coast	South Africa	FL	B	36,19	0,25	-1,05	21	8,9	38,9	1,074	318	OR	1989-1990	Mann and Buxton (1997)*
			FL	F	40,84	0,09	-4,40	20	8,9	38,6	0,946	326	OR	2008-2009	Richardson et al. (2011)*
	South Angola	Angola	FL	M	27,70	0,31	-1,40	11	8,9	29,3	1,059	64	OR	2008-2009	Richardson et al. (2011)*"
			FL	F	25,61	0,45	-1,00	10	8,9	35,0	1,368	131	OR	2008-2009	Richardson et al. (2011)*
			FL	M	23,98	0,49	-0,90	8	8,9	24,1	1,005	57	OR	2008-2009	Richardson et al. (2011)*"

Species	Location	Country	LT	Sex	L_{∞}	K	t_0	t_{max}	L_{min}	L_{max}	L_{max}/L_{∞}	N	Method	Year	Author
<i>D. cervinus</i>	Canary Islands	Spain	TL	F	58,80	0,16	-0,80	14	16,0	52,8	0,898	327	OR	2000-2001	Pajuelo et al. (2003a)
			TL	M	60,90	0,15	-0,73	17	19,3	56,5	0,928	151	OR	2000-2001	Pajuelo et al. (2003a)
			TL	M	61,90	0,15	-0,84	17	19,3	56,5	0,913	114	OR	2000-2001	Pajuelo et al. (2003b)
			TL	F	58,40	0,15	-0,76	14	16,0	52,8	0,904	298	OR	2000-2001	Pajuelo et al. (2003b)
	Annaba Gulf	Algeria	TL	B	68,83	0,11	-0,75	13	9,8	52,7	0,766	190	OR	2001	Derbal and Kara (2013)
<i>D. holbrooki</i>	South Atlantic Bight	US	TL	B	35,80	0,22	-1,00	10	12,5	42,5	1,187	nr	OR	1971-1974	Darcy (1985)
	Off N. Carolina		TL	B	30,76	0,26	-0,98	11	5,5	36,5	1,187	349	OR	1993-1995	Manooch and Potts (1996)
<i>D. hottentotus</i>	Tsitsikamma coast	South Africa	FL	B	46,24	0,15	-2,15	33	1,6	56,1	1,213	281	OR	1989-1990	Mann and Buxton (1997)*"
<i>D. puntazzo</i>	Canary Islands	Spain	TL	M	52,70	0,19	-2,76	10	16,9	51,0	0,968	168	OR	2001-2003	Dominguez-Seoane et al. (2006)
			TL	F	52,30	0,20	-2,23	9	15,9	50,9	0,973	348	OR	2001-2003	Dominguez-Seoane et al. (2006)
	Gulf of Gabes	Tunusia	TL	B	28,39	0,18	-1,65		11,4	26,8	0,944	1335	OR	2008-2010	Chaouch et al. (2013)
	Gulf of Gabes		TL	B	23,19	0,47	-0,25	6	11,9	29,5	1,272	112	SR	nr	Bradai et al. (1998)
	Adriatic Sea	Coratia	TL	B	45,28	0,19	-0,31	18	13,3	46,7	1,031	598	SR	2004-2005	Kraljevic et al. (2007)
TL			B	13,32	0,48	-0,11	1	1,6	9,3	0,698	663	LF	1991-1992	Matic-Skoko et al. (2007b)	
<i>D. sargus cadenati</i>	Canary Islands	Spain	TL	F	49,90	0,13	-2,23	12	16,2	40,4	0,810	341	OR	2000-2001	Pajuelo and Lorenzo (2004)
			TL	M	44,70	0,15	-1,89	11	15,8	39,2	0,877	117	OR	2000-2001	Pajuelo and Lorenzo (2004)
			TL	F	49,40	0,15	-2,05	12	16,2	40,4	0,818	289	OR	2000-2001	Pajuelo and Lorenzo (2002a)
			TL	M	45,90	0,14	-1,91	12	15,8	39,2	0,854	97	OR	2000-2001	Pajuelo and Lorenzo (2002a)
<i>D. sargus sargus</i>	North Spain	Spain	TL	F	57,59	0,10	-5,33	8	26,0	42,1	0,731	102	OR	1983-1984	Pastor and Quadros (1996)
			TL	M	52,92	0,13	-3,73	8	24,5	45,0	0,850	91	OR	1983-1984	Pastor and Quadros (1996)
			TL	nr	48,48	0,18	-0,58		17,4	33,4	0,689	nr	nr	nr	Martinez-Pastor and Villegas-Cuadros (1996)"
	Catalan Coast		TL	B	41,70	0,25	-0,76	10	9,0	39,0	0,935	184	OR	nr	Gordoa and Moli (1997)
			TL	B	39,55	0,15	-1,89	16	16,9	45,0	1,138	331	SR	1992-1999	Abecasis et al. (2008)"
	South Portugal	Portugal	TL	B	40,93	0,18	-1,28	18	16,9	41,0	1,002	715	OR	1992-1999	Abecasis et al. (2008)"
TL			B	41,20	0,18	-0,86		16,9	49,0	1,189	nr	OR	nr	Erzini et al. (2001)"	
Beymelek Lagoon	Turkey	TL	B	39,90	0,27	-1,75	3	10,0	28,7	0,719	355	SR	2006-2007	Balik and Emre (2016)	

Species	Location	Country	LT	Sex	L_{∞}	K	t_0	t_{max}	L_{min}	L_{max}	L_{max}/L_{∞}	N	Method	Year	Author
<i>D. sargus sargus</i>	Gulf of Lion	France	TL	B	35,25	0,22	-0,84	14	6,0	42,0	1,191	484	SR	1980	Man-Wai and Quignard (1982) Girardin (1978)*"
			FL	B	22,86	0,53	-0,14	4	10,0	42,0	1,837	nr	SR	nr	
	Abu Qir Bay		TL	B	31,30	0,26	-0,73	6	7,5	27,5	0,879	746	SR	2008-2009	Mahmoud et al. (2010)
	Alexandria	Eygpt	TL	B	32,70	0,13	-1,84	13	11,2	39,0	1,193	nr	SR	nr	LahLah (2004)"
			TL	B	54,86	0,10	-2,06	8	11,2	39,0	0,711	604	SR	2008-2009	El-Maghraby et al. (1981)"
	North Sinai		TL	B	40,71	0,25	-0,28	5	11,0	38,0	0,933	991	SR	2010-2012	Al-Beak et al. (2015)
East Algeria	Algeria	TL	B	36,30	0,15	-0,49	10	12,2	34,6	0,953	241	OR	2005-2006	Benchalel and Kara (2013)	
<i>D. vulgaris</i>	Alexandria	Eygpt	TL	B	57,71	0,07	-2,94	7	11,2	30,0	0,520	410	SR	2008-2009	El-Maghraby et al. (1981)
	Abu Qir Bay		TL	B	31,30	0,26	-0,56	6	8,5	26,0	0,831	616	SR	1998-2008	Adam (2010)
	Adriatic Sea	Coratia	TL	M	56,25	0,08	-2,92	10	14,5	37,5	0,667	1620	SR	2005-2006	Dulcic et al. (2011)
			TL	F	51,96	0,10	-2,84	11	14,5	36,2	0,697	1333	SR	2005-2006	Dulcic et al. (2011)
	Gulf of Gabes	Tunusia	TL	B	24,14	0,16	-2,33	7	7,0	25,0	1,036	1097	SR	2006-2007	Hadj Taieb et al. (2013a)
			TL	B	25,40	0,18	-1,63	9	7,0	25,0	0,984	1097	OR	2008-2010	Hadj Taieb et al. (2013b)
	Gulf of Tunu- sia		TL	B	23,47	0,22	-1,45	8	10,8	32,0	1,363	97	SR	nr	Bradai et al. (1998)
			TL	B	39,90	0,11	-0,73	12	10,0	32,0	0,802	510	SR	2005-2006	Mouine et al. (2010)
			TL	B	39,00	0,10	-0,96	11	10,6	32,0	0,821	492	OR	2005-2006	Mouine et al. (2010)
			TL	B	28,10	0,30	-1,62	10	12,5	30,5	1,085	374	OR	1992-1994	Gonçalves (2000)
	South Portugal	Portugal	TL	B	39,60	0,32	-0,48		12,5	30,5	0,770	374	LF	1992-1994	Gonçalves (2000)
			TL	B	34,49	0,18	-1,27	14	9,0	33,0	0,957	377	SR	1992-1999	Abecasis et al. (2008)
			TL	B	27,40	0,40	-0,77	14	9,0	30,0	1,095	1076	OR	1992-1999	Abecasis et al. (2008)
			TL	M	28,60	0,36	-0,38	14	14,5	36,9	1,290	368	OR	1992-2000	Gonçalves et al. (2003)
	Gulf of Lion	France	TL	F	27,67	0,39	-0,34	12	13,8	37,0	1,337	440	OR	1992-2000	Gonçalves et al. (2003)
TL			B	37,80	0,18	-0,83	8	10,0	35,0	0,926	556	SR	nr	Man Wai (1985)	
FL			B	30,79	0,26	-0,61	3	9,0	18,2	0,590	nr	SR	nr	Girardin (1978)*"	
TL			B	39,70	0,23	-0,91	9	13,0	37,0	0,932	488	OR	2000-2001	Pajuelo and Lorenzo (2003)	
Catalan Coast	Spain	TL	B	28,78	0,39	-0,66	6	8,0	28,0	0,973	201	OR	nr	Gordoa and Moli (1997)	
Benghazi Coasts	Libya	TL	B	33,30	0,11	-1,58	8	11,0	27,0	0,811	290	SR	2005	Saeid et al.. (2016)	
Scilia Strait	Italy	TL	B	33,50	0,17	-2,59		14,0	27,0	0,806	603	OR	1997-1999	Beltrano et al. (2003)	
İzmir Bay	Turkey	TL	B	27,96	0,25	-1,18	3	7,0	19,0	0,680	709	OR	2004-2007	Soykan et al. (2015)	
Western Sa- hara	Morocco	FL	B	44,85	0,40	-0,42		9,6	37,2	0,829	nr	LF	1980-1982	Mennes (1985)*"	

* FL transformed to TL according to formula given in the manuscript

" L_{max} , L_{min} or both not reported in the original study. They are assigned from Fishbase or other studies carried at same location.

Appendix References

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