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Aims and Scope

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Aquatic Research 7(3), 121-130 (2024) • https://doi.org/10.3153/AR24011 *Research Article*

Length-weight relationships and growth parameters of axillary seabream *Pagellus acarne* (Risso, 1827) from the Didim coast in the Southern Aegean Sea

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ABSTRACT

The length-weight relationships and growth parameters of the axillary seabream *Pagellus acarne* caught from the Didim coast in the southern Aegean Sea were examined. A total of 667 axillary seabream individuals, of which 295 were female (44.23%) and 372 were male (55.77%), were collected by commercial trawlers and gillnets from November 2021 to December 2023. The length-weight relationships were calculated for females, males, and sexes as $W = 0.0052 \times L^{3.2378}$, $W = 0.0065 \times L^{3.1521}$, and $W = 0.0058 \times L^{3.1965}$, respectively. The growth of *P. acarne* on the Didim coast was determined as positive allometric. The maximum age was found to be 4 for both females and males. The von Bertalanffy growth parameters were $L_\infty = 28.55$ cm, $k = 0.213$ year^{-1}, $t_0 = -2.011$ years for females; $L_\infty = 22.13$ cm, $k = 0.489$ year^{-1}, $t_0 = -0.862$ years for males; $L_\infty = 23.75$ cm, $k = 0.373$ year^{-1}, $t_0 = -1.203$ years for both sexes. The growth performance index (φ') for females, males, and sexes were 2.239, 2.379, and 2.324, respectively. This study provides the first contribution to the basic growth parameters of *P. acarne* along the Didim coast, southern Aegean Sea.

Keywords: Axillary seabream, *Pagellus acarne*, Length-weight relationship, Growth, Southern Aegean Sea

Introduction

The axillary seabream *Pagellus acarne* (Risso, 1827), one of the commercially significant species belonging to the family Sparidae, is distributed along the coasts of Madeira, the Canary Islands, and Cape Verde, from the Bay of Biscay to Senegal in the eastern Atlantic but is rare in the British Isles. It has a wide distribution in the Mediterranean along all coasts except the Black Sea (Froese & Pauly, 2023). Juveniles are generally found closer to shore, while adults particularly inhabit seagrass beds and sandy bottoms in various layers to depths of 500 m. However, they are more commonly found between 40 and 100 m (Bauchot & Hureau, 1986). Despite feeding omnivorous, their dietary tendencies predominantly lean towards a carnivorous diet, preying on small teleosts, arthropods, molluscs, echinoderms, and worms (Fehri-Bedoui et al., 2009). The species is protandric hermaphrodite. Typically, individuals start as males and then transform into females over 2 to 7 years (Bauchot & Hureau, 1986). The maximum total length reported is 36 cm (Bauchot & Hureau, 1986; Froese & Pauly, 2023). *P. acarne* is categorised as Least Concern (LC) in the IUCN Red List (IUCN, 2023).

Considering the dynamic nature of variations in length and weight of fish over time, the length-weight relationship (LWR) and the von Bertalanffy growth function (VBGF) provide dynamical parameters that lead to important mathematical inferences about stock assessments of species in a certain geographical region (Sparre, 1998). This makes it possible to determine the dynamic structure of fish populations, mathematically explain the growth rates of fish according to their age, interpret the current stock situation, develop long-term sustainable fishery strategies, and determine catch limits. Length-weight relationships of *P. acarne* have been examined in various studies conducted in different areas of the world (Pajuelo & Lorenzo, 2000; Velasco et al., 2011; Akel, 2016; Bensahla Talet et al., 2017; Cetkovic et al., 2018; Bentata-Keddar et al., 2020; Falsone et al., 2022; Ali-Basha et al., 2023) and Turkish marine waters (Tosunoğlu et al., 1997; Özaydın et al., 2007; İlkyaz et al., 2008; Cengiz, 2013; Bilge et al., 2014; Akalın et al., 2015; Altın et al. 2015; Soykan et al., 2015; Öztekin et al., 2016; Tünay, 2017; İlhan, 2018; Yedier et al., 2019; Kara et al., 2020; Gül et al., 2021; Acarlı et al., 2022). However, the growth parameters of the species have been determined in a limited number of studies such as Phan & Kompowski (1972), Pajuelo & Lorenzo (1994), Dominguez (2000), Pajuelo & Lorenzo (2000), Coelho et al. (2005), Velasco et al. (2011), Bentata-Keddar et al. (2020), and Ali-Basha et al. (2023) for the world and Tosunoğlu et al. (1997), Soykan et al. (2015), İlhan (2018), and Gül et al. (2021) for Türkiye.

P. acarne, a commercial and exploited species captured with trawl and gill nets, was harvested in 2018 with 3654 tons, the highest catch data. There were gradual decreases in 2019 and 2020, with 2913 and 2713 tons harvested, respectively. Then, in 2021, a harvest of 2728 tons was achieved, increasing slightly compared to the previous year (FAO, 2023). However, there are no specific catch data of *P. acarne* in the fisheries statistics of the Turkish Statistical Institute (TURKSTAT, 2022). Although the literature provides various studies on the population parameters of axillary seabream in different regions of Turkish marine waters, there is a lack of research on the species' basic biological parameters in Didim, an important coastline of southern Aegean. Didim, extending from the Büyük Menderes Delta in the north to Akbük Bay in the south, is a coastal area that stands out in terms of both fishery and aquaculture activities. Therefore, this study provides the first information on the length-weight relationship and growth parameters, aiming to contribute to the optimal exploitation of *P. acarne* stocks along the Didim coast in the southern Aegean Sea.

Materials and Methods

Between November 2021 and December 2023, the specimens of *Pagellus acarne* were collected by commercial trawlers and gillnets with various mesh sizes along the coast of Didim (Figure 1). The total length of the samples was measured to the nearest 0.1 cm and the weight to the nearest 0.01 g.

The length-weight relationships (LWRs) were independently estimated for all individuals with the formula $W = a \times L^b$ (Ricker, 1975). This equation can be expressed logarithmically as $\log W = \log a + b \log L$, where W is total body weight (g), and L is total length (cm). A is a coefficient relative to body form, and exponent b is the allometry coefficient of the linear regression equation expressing isometric (= 3), positive allometric (> 3) and negative allometric (< 3) growth in length. The significance of the linear regression coefficients obtained from length-weight data was tested by analysis of variance ANOVA (Zar, 1999). Student's t-test with a ±95% confidence interval was applied to verify whether the b values obtained in the linear regressions were significantly different from the null hypothesis of isometric growth (H_0: $b = 3$), using the equation $t_s = (b - 3) / sb$, where t_s is the t-test value, b the slope and sb the standard error of the slope (b) (Sokal & Rohlf, 1987).

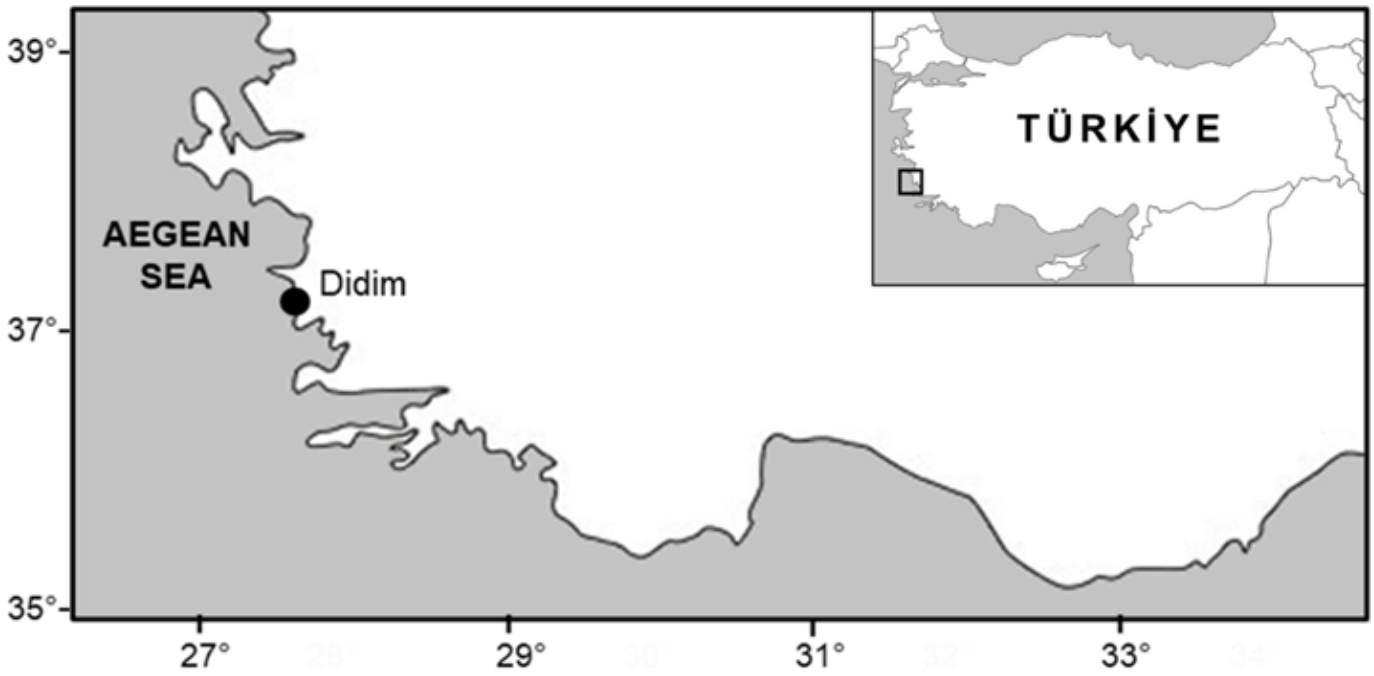

Figure 1. Sampling location of *Pagellus acarne*

Age readings were taken by counting growth annuli from all sagittal otoliths, as Holden and Raitt (1974) suggested. All otoliths were cleared in ethanol and then immersed in glycerine for examination. A reflected light binocular microscope was used to determine age.

Theoretical growth patterns for all individuals were calculated using the von Bertalanffy growth function (VBGF) (Beverton & Holt, 1957) $L_t = L_\infty [1 - e^{-k(t-to)}]$, where L_t is the fish length (cm) at the time t (year), L_∞ is the mean asymptotic length (cm), k is the growth coefficient (year^{-1}), and t_o (year) is the theoretical time at which the length equals to zero.

The growth performance index (φ') was estimated using the formula $\varphi' = \log k + 2 \log L_\infty$ (Munro & Pauly, 1983).

Results and Discussion

Descriptive Characteristics of the Sampling

During the research, 667 P. acarne samples, 295 female (44.23%) and 372 male (55.77%), were collected from the Didim coast. The overall sex ratio (F: M) was determined as 1:1.26. The chi-square test (χ^2) showed that the sex ratio was significantly different from the expected 1:1 ratio (χ^2, $P < 0.05$). Total length intervals were 12.0-21.8 cm for females, 11.6-21.0 cm for males, and weight intervals were 16.00-116.58 g for females, 14.66-99.86 g for males. The mean length and weights were 17.89 ±2.47 cm and 63.24 ±26.20 g for females and 16.43 ±2.44 cm and 47.91 ±21.53 g for males, respectively.

The maximum length and weight obtained from *P. acarne* individuals was 21.8 cm and 116.58 g. A statistically significant difference was found in overall length and weight values between male and female individuals (t_{test}, $P < 0.05$). Most individuals were between 16.0-16.9, 17.0-17.9, and 18.0-18.9 cm in total length, accounting for 53.07% of all samples (Figure 2).

Length-Weight Relationships

LWRs were independently calculated in logarithmic form as $log\ W = -2.2887 + 3.2378\ log\ L$ for females; $log\ W = -2.1838 + 3.1521\ log\ L$ for males; $log\ W = -2.2399 + 3.1965\ log\ L$ for both sexes.

Table 1 outlines the LWRs computed for males, females, and sexes of the axillary seabream sampled from the Didim coastline. The mean length and weight of females were higher than those of males, and all differences were statistically significant (t-test, $P < 0.05$). The exponent of the b parameter of females, males, and sexes demonstrated positive allometry and was statistically significant ($P < 0.05$). A strong correlation was obtained between the length and weight in the individuals of female, male, and both sexes ($P < 0.001$; $r^2 > 0.97$). A summary of the comparison between the length-weight relationships identified in this study and those from previous studies is presented in Table 2.

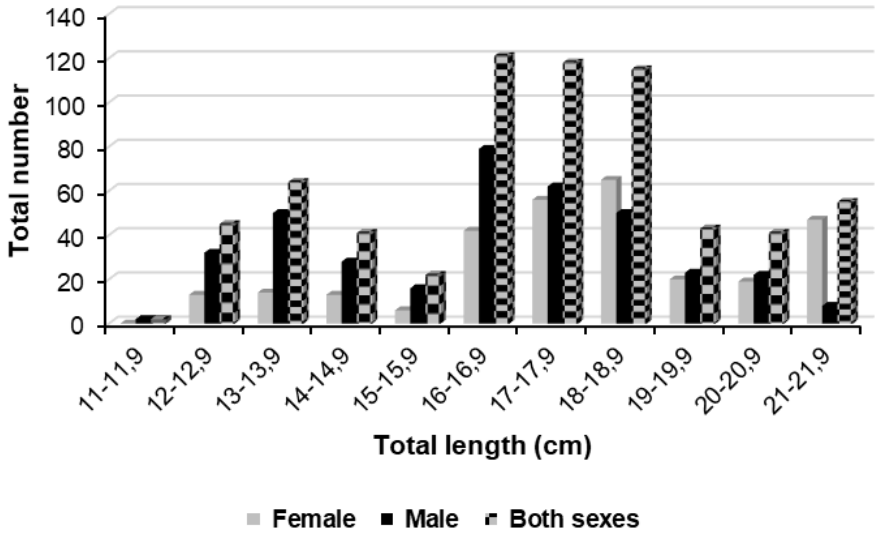

Figure 2. The length frequency distribution for females, males, and sexes of *Pagellus acarne* sampled from the Didim coast.

Table 1. General population statistics, LWR parameters and the isometric growth probability of *Pagellus acarne* were analysed with Student's *t*-test

Sex	N	Range of TL (cm) (L_{mean} ± SD)	Range of W (g) (W_{mean} ± SD)	LWR parameters		SE of *b*	95% CI of *b*	r^2	*t*-test
				a	*b*				
♀	295	12.0–21.8 (17.89 ±2.47)	16.00–116.58 (63.24 ±26.20)	0.0052	3.2378	0.0154	3.2102–3.2712	0.9922	15.55*
♂	372	11.6–21.0 (16.43 ±2.44)	14.66–99.86 (47.91 ±21.53)	0.0065	3.1521	0.0128	3.1263–3.1769	0.9925	11.77*
♀ + ♂	667	11.6–21.8 (17.08 ±2.56)	14.66–116.58 (54.69 ±24.89)	0.0058	3.1965	0.0096	3.1812–3.2191	0.9926	20.72*

♀, female; ♂, male; N, sample number; TL, length; W, weight; SD, standard deviation; *a*, intercept; *b*, slope; SE, standard error; CI, confidence interval; r^2, coefficient of determination; * $t > t_{0.05,N>250} = 1.65$

Determination of the von Bertalanffy Growth Function (VBGF) Parameters

Sagittal otolith examinations revealed age patterns ranging from I to IV age classes for both females and males. Table 3 displays the total length frequencies of *P. acarne* from the Didim coast based on age classes. In the female age class, III (43.04%) was dominant, followed by IV (24.42%), II (18.30%), and I (14.24%) classes, while in the male age class, II (49.19%) was dominant, followed by I (27.96%), IV (13.17%) and III (9.68%) classes.

The growth pattern in mean lengths from each age group was estimated for females, males, and both sexes of *P. acarne* from the Didim coast using the VBGF. The von Bertalanffy growth parameters were calculated as L_∞ = 28.55 cm, k = 0.213 year^{-1}, t_0 = -2.011 years for females; L_∞ = 22.13 cm, k = 0.489 year^{-1}, t_0 = -0.862 years for males; L_∞ = 23.75 cm, k = 0.373 year^{-1}, t_0 = -1.203 years for both sexes. The growth performance index (φ') was estimated for females, males, and both sexes as 2.239, 2.379, and 2.324, respectively. A summary of comparisons of von Bertalanffy growth parameters for *P. acarne* distributed in different geographical areas is presented in Table 4.

Overall, information on the age and growth parameters of *P. acarne* in southern Aegean Sea populations is limited. The present study is the first contribution to the biological parameters of *P. acarne* along the Didim coast (southern Aegean Sea). Thus, the LWR and VBGF parameters for the species have been compared using data from various locations within

its distribution area (Tables 2 and 4). The observed sex ratio of 1:1.26, favouring males, aligns with those obtained by Tünay (2017), İlhan (2018), Bentata-Keddar et al. (2020), and Ali-Basha et al. (2023). In contrast, it contrasts with the findings obtained by Pajuelo & Lorenzo (2000), Soykan et al. (2015), and Gül et al. (2021) in favour of females. These discrepancies in sex ratio could potentially be attributed to species-specific protandric hermaphroditism.

This study showed that the total length range of all *P. acarne* individuals from the Didim coast varied between 11.6-21.8 cm, which is similar to the ranges along the Aegean Sea determined by İlkyaz et al. (2008), Cengiz (2013), Bilge et al. (2014), Soykan et al. (2015), İlhan (2018), and Çolakoğlu (2021); however, it is inconsistent with the ranges estimated by Altın et al. (2015), Öztekin et al. (2016), Tünay (2017), Kara et al. (2020), and Acarlı et al. (2022). Kara & Bayhan (2015) emphasised that one of the important reasons for the fluctuations resulting from the similarities and differences in the total length range of the species in a region is the selectivity of the fishing gear used in sampling studies, while the other is the sampling performance carried out in different years.

Table 2. The LWR comparisons of *Pagellus acarne* from different geographical areas

Locations	N	Sex	*a*	*b*	GT	Author(s)
Gülbahçe Bay, Central Aegean Sea (Türkiye)	107	♀+♂	0.082	3.2887	A+	Tosunoğlu et al. (1997)
Canarian Archipelago, Northern Atlantic (Spain)	968	♀	0.0062	3.2416	A+	Pajuelo & Lorenzo (2000)
	556	♂	0.0065	3.2813	A+	
Izmir Bay, Central Aegean Sea (Türkiye)	303	♀+♂	0.0071	3.353	A+	Özaydın et al. (2007)
Izmir Bay, Central Aegean Sea (Türkiye)	334	♀+♂	0.0104	3.06	I	İlkyaz et al. (2008)
Gulf of Cadiz (Spain)	461	♀+♂	0.0048	3.3207	A+	Velasco et al. (2011)
Alboran Coast of Spain	406		0.0093	3.1132	A+	
Gallipoli Peninsula and Dardanelles (Türkiye)	228	♀+♂	0.0119	3.03	I	Cengiz (2013)
Southern Aegean Sea (Türkiye)	472	♀+♂	0.0121	3.2114	A+	Bilge et al. (2014)
Çandarli Bay, Aegean Sea (Türkiye)	83	♀+♂	0.0078	3.281	A+	Akalın et al. (2015)
Gökçeada Island, Northern Aegean Sea (Türkiye)	908	♀+♂	0.004	3.594	A+	Altın et al. (2015)
Izmir Bay, Central Aegean Sea (Türkiye)	281	♀	0.011	3.055	I	Soykan et al. (2015)
	80	♂	0.008	3.155	I	
Mediterranean Coast of Egypt	468	♀+♂	0.0348	2.6244	A-	Akel (2016)
Gallipoli Peninsula, Northern Aegean Sea (Türkiye)	53	♀+♂	0.0331	2.629	A-	Öztekin et al. (2016)
Oran Bay, Western Mediterranean (Algeria)	844	♀+♂	0.0089	3.10	A+	Bensahla Talet et al. (2017)
Gulf of Edremit, Northern Aegean Sea (Türkiye)	338	♀+♂	0.0145	2.911	A-	Tünay (2017)
Mediterranean Coast of Libya	100	♀+♂	0.00004	2.76	A-	Cetkovic et al. (2018)
Izmir Bay, Central Aegean Sea (Türkiye)	861	♀	0.0109	3.066	A+	İlhan (2018)
	905	♂	0.0153	2.943	A-	
Tekirdağ Coast of Marmara Sea (Türkiye)	294	♀+♂	0.02	2.8142	A-	Yedier et al. (2019)
Mediterranean Coast of Algeria	795	♀	0.0131	2.9716	A-	Bentata-Keddar et al. (2020)
		♂	0.0095	3.0756	I	
Izmir Bay, Central Aegean Sea (Türkiye)	525	♀+♂	0.0083	3.15	A+	Kara et al. (2020)
Saros Bay, North Aegean Sea (Türkiye)	246	♀	0.005	3.277	A+	Gül et al. (2021)
	115	♂	0.004	3.364	A+	
Gökçeada Island, Northern Aegean Sea (Türkiye)	1323	♀+♂	0.0170	2.8946	A-	Acarlı et al. (2022)
Southern Sicily, Central Mediterranean (Italy)	1250	♀+♂	0.0097	3.1093	A+	Falsone et al. (2022)
Lattakia Coast, Eastern Mediterranean (Syria)	465	♀	0.00564	3.290	A+	Ali-Basha et al. (2023)
	582	♂	0.00607	3.263	A+	
Didim Coast, Southern Aegean Sea (Türkiye)	295	♀	0.0052	3.2378	A+	Present Study
	372	♂	0.0065	3.1521	A+	

N, number of sample studied; *a*, intercept; *b*, slope; ♀, female; ♂, male; ♀+♂ = females + males (consist of unsexed individuals); GT, growth type; A+, allometric positive; A-, allometric negative; I, isometric

Table 3. Length key of female and male *Pagellus acarne* individuals depend on age classes from the Didim coast

	Age classes							
	♀				♂			
Range of TL (cm)	**I**	**II**	**III**	**IV**	**I**	**II**	**III**	**IV**
11-11.9					2			
12-12.9	13				32			
13-13.9	14				46	4		
14-14.9	12	1			24	4		
15-15.9	3	3				16		
16-16.9		40	2			79		
17-17.9		10	46			56	6	
18-18.9			47	18		24	20	6
19-19.9			16	4			10	13
20-20.9			13	6				22
21-21.9				47				8
Total	42	54	124	75	104	183	36	49
Coverage	14.24%	18.30%	43.04%	24.42%	27.96%	49.19%	9.68%	13.17%
Mean (TL)	13.44±1.05	16.52±0.47	18.35±0.95	20.61±1.24	13.28±0.91	16.81±1.00	18.56±0.60	20.15±0.78
Mean (W)	24.11±5.85	45.04±2.78	65.30±13.06	94.85±16.99	23.22±4.85	47.92±9.01	67.18±7.70	86.08±9.85

♀, female; ♂, male; TL, total length; W, weight

Table 4. The growth comparisons of *Pagellus acarne* from different geographical areas

Locations	**Sex**	L_∞	k	t_o	φ'	**Author(s)**
West Africa Coasts, Northern Atlantic	♀+♂	36.0	0.23	-0.97		Phan & Kompowski (1972)
Canarian Archipelago, Northern Atlantic (Spain)	♀+♂	32.09	0.232	-0.919		Pajuelo & Lorenzo (1994)
Gülbahçe Bay, Central Aegean Sea (Türkiye)	♀+♂	21.00	0.171	-3.222		Tosunoğlu et al. (1997)
Alboran Coast of Spain	♀+♂	29.62	0.27	-1.36		Dominguez (2000)
Canarian Archipelago, Northern Atlantic (Spain)	♀	33.90	0.21	-0.99		Pajuelo & Lorenzo (2000)
	♂	27.98	0.27	-0.67		
Algarve Coast, Northern Atlantic (Portugal)	♀	32.30	0.18	-2.56		Coelho et al. (2005)
	♂	28.82	0.29	-1.47		
Gulf of Cadiz (Spain)	♀+♂	31.65	0.21	-1.76	2.32	Velasco et al. (2011)
Alboran Coast of Spain		32.14	0.17	-2.69	2.24	
Izmir Bay, Central Aegean Sea (Türkiye)	♀+♂	22.66	0.315	-1.202	2.21	Soykan et al. (2015)
Izmir Bay, Central Aegean Sea (Türkiye)	♀	27.75	0.201	-2.347	2.190	İlhan (2018)
	♂	22.45	0.341	-1.554	2.235	
Mediterranean Coast of Algeria	♀+♂	29.97	0.41	-0.34	2.57	Bentata-Keddar et al. (2020)
Saros Bay, North Aegean Sea (Türkiye)	♀+♂	30.63	0.26	-0.95	2.39	Gül et al. (2021)
Lattakia Coast, Eastern Mediterranean (Syria)	♀	22.97	0.18	-0.239	1.97	Ali-Basha et al. (2023)
	♂	21.50	0.32	-0.238	2.17	
Didim Coast, Southern Aegean Sea (Türkiye)	♀	28.55	0.213	-2.011	2.239	Present Study
	♂	22.13	0.489	-0.862	2.379	

♀, female; ♂, male; L_∞, asymptotic length; k, growth coefficient; t_o, theoretical age at length equal to zero; φ', growth performance index

The *b* value of the LWR obtained from the present study indicated positive allometry for both females (3.2378) and males (3.1521), which generally agrees with the previous calculations outlined in Table 2. In contrast, Akel (2016), Öztekin et al. (2016), Tünay (2017), Yedier et al. (2019) and Acarlı et al. (2022) reported the negative allometric *b* value of *P. acarne* as 2.6244 (Mediterranean Coast of Egypt), 2.629 (Gallipoli peninsula), 2.911 (Edremit Bay), 2.8142 (Sea of Marmara), and 2.8946 (Gökçeada Island), respectively. The *b* value, indicating the natural growth of fish, varies between 2 and 4 (Tesch, 1971). Apart from protandric hermaphroditism contributing to different length distributions and variations in both sexes, a combination of factors such as sampling site, habitat, season, maturity, sex, age, diet, and differences in length ranges of fish samples may give rise to observed differences in length-weight relationships (Ricker, 1975).

Age estimations obtained from otolith readings of *P. acarne* individuals collected from the Didim coast revealed that samples from both sexes were between I to IV age classes. The dominant age class of females is III, where the most common total length ranges are 17.0-17.9 and 18.0-18.9 cm. As for males, the predominant age class is II, where the most frequent total length range is 16.0-16.9 cm. Tosunoğlu et al. (1997) determined that the dominant age classes of all individuals were I and II. The total length groups were 11 and 12 cm. Pajuelo & Lorenzo (1994, 2000) found the dominance of age class II for all individuals and determined the most prevalent size in this class as 15 and 16 cm. Coelho et al. (2005) detected the most dominant age class of females and males as VI and IV and found the size range with the highest number of individuals 25.0-27.7 and 22.4-25.5 cm, respectively. Velasco et al. (2011) estimated the dominance of age classes V and III for all Gulf of Cadiz and Alboran Sea individuals. They obtained the frequent length groups as 24 and 22 cm, respectively. İlhan (2018) found the dominant age classes of females to be I and II. The total length ranges with the highest number of individuals to be 11.9-15.5 and 14.9-17.1 cm, while for males, the dominant age classes were also I and II, with the most common length ranges being 11.1-15.3 and 13.5-18.5 cm. Using fishing equipment such as longlines, gill nets, and beach seines, capable of capturing larger specimens compared to trawling, might have led to the observed variations in age classes based on the size ranges suggested various studies in different regions.

The asymptotic length (L_∞) of *P. acarne* derived from this study was 28.55 cm for females, 22.13 for males, and 23.75 cm for both sexes, which is generally around the previous studies, except for Phan & Kompowski (1972), Pajuelo & Lorenzo (1994), Pajuelo & Lorenzo (2000), Coelho et al. (2005), Velasco et al. (2011), and Gül et al. (2021) (Table 4). Phan & Kompowski (1972) analysed the population structure of *P. acarne* from West African coasts and found L_∞ to be 36 cm for both sexes. Pajuelo and Lorenzo (1994, 2000) studied the biological parameters of *P. acarne* in the Canarian Archipelago**,** and their computation of L_∞ was 32.09 and 32.98 cm for all individuals, respectively. Coelho et al. (2005) studied some parameters of the *P. acarne* population from the Algarve coast and calculated L_∞ for all samples as 32.05 cm. Velasco et al. (2011) revealed the age and growth features of *P. acarne* in the Gulf of Cadiz and the Spanish coast of the Alboran Sea and found L_∞ to be 31.65 and 32.14 cm for all individuals, respectively. Gül et al. (2021) examined the population structure of *P. acarne* from Saros Bay and found L_∞ to be 30.63 cm for combined sexes. Wotton (1990) suggested that significant differences in growth characteristics can be observed in fish populations of the same species in different geographical regions. Additionally, differences in growth characteristics can be attributed to the potential changes in food quality and water temperature (Santic et al., 2002). In particular, variations in the estimated asymptotic length could be linked to phylogeographic diversity and factors such as fishing pressure, global climate change, and pollutants (Uyan et al., 2020).

The growth coefficient (*k*) was found to be 0.213 for females and 0.489 for males, around the several calculations provided in Table 4, pointing out higher growth performance in males than females. The present study attributes this observed phenomenon to protandric hermaphroditism, a suggestion supported by several studies (Pajuelo & Lorenzo, 2000; Coelho et al., 2005; İlhan, 2018; Ali-Basha et al., 2023). Furthermore, İlhan (2018) proposed that this can be substantiated by *P. acarne* females exhibiting a lower *k* and a higher L_∞.

The growth performance index (φ'), considering the correlation between L_∞ and *k*, of axillary seabream on the coast of Didim was 2.239 for females, 2.379 for males, and 2.324 for both sexes. These findings generally align with the growth performance index presented in Table 4 obtained from Gulf of Cadiz and Spanish coast of the Alboran Sea (Velasco et al., 2011), Izmir Bay (Soykan et al., 2015; İlhan, 2018), Mediterranean coast of Algeria (Bentata-Keddar et al., 2020), Saros Bay (Gül et al., 2021), Lattakia coast (Ali-Basha et al., 2023). This consistency provides evidence that the growth of seabream in these different regions is similarly affected by ecological conditions.

Conclusions

Ensuring effective fisheries management and enforcement is crucial for the protection and sustainable exploitation of natural resources. Rational and effective management of fisheries resources is possible through self-assessment of the investigated species and their regional stocks (Turan, 2021). The present study has provided the first contributions to the basic growth parameters of *P. acarne*, distributed along the Didim coast of the southern Aegean Sea. The information obtained is considerable for establishing collaborations between stakeholders, researchers, and policymakers and improving sustainability in the face of increasing predictable demand for seafood. This proactive approach will pave the way for the establishment of ecosystem-based sustainable fisheries for commercially important species in the future. Further studies investigating the populations of *P. acarne* in Turkish marine waters using genetic and morphological markers will contribute to a more detailed interpretation of the stock status and provide significant information from the perspective of sustainable fisheries management.

Compliance with Ethical Standards

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

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

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Investigate the quality parameters of fish crockets manufactured using different proportions of Jerusalem artichoke fibre

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ABSTRACT

This study determined the physico-chemical, microbiological and sensory properties of rainbow trout (*Oncorhyncus mykiss*) croquettes prepared with different proportions of Jerasalem artichoke fibre. For this purpose, three different concentrations of Jerusalem artichoke fibre (2%, 4% and 6%) were used, and a total of 4 groups of fish croquettes were prepared, including the control group without fibre. The prepared fish croquettes were packaged in styrofoam plates, covered with stretch film, and stored in the refrigerator (4 ±1°C) for 25 days. In terms of physicochemical properties between groups, water and fat contents were found to be significant ($p<0.05$), but protein, ash, carbohydrate and pH values were found to be insignificant ($p>0.05$). TVB-N and TBARS values were very significant ($p<0.01$). According to the microbiological analysis results, the bacterial counts of trout croquettes prepared with Jerusalem artichoke fibre were lower than the control group, and an increase was detected in all groups in parallel with storage. The most liked sensory group was the group B croquettes. In light of all these results, it was concluded that it is possible to use Jerusalem artichoke fibre in rainbow trout croquettes.

Keywords: Crocket, Fibre, Jerusalem artichoke, Quality

Introduction

Recently, consumers have started to show interest in ready meals, and demands have increased for foods that are easy to prepare and consumed quickly. In addition, showing awareness of healthy nutrition has caused consumers to prefer healthy and low-calorie foods (Kılınççeker & Karahan, 2019).

With rapid urbanisation and the increase in working women, fast food's popularity is increasing daily, and consumer preferences are shifting significantly towards such products. In addition, young people are now more interested in fast food style products. Fish croquettes, among the new generation fast food products, are especially popular among the young generation due to their delicious taste, unique texture and colour, and high nutritional quality. The fact that it is easy to prepare increases its popularity among employees (Emir Çoban, 2020; Emir Çoban, 2021).

Seafood is important for consumption because it contains significant amounts of protein, essential amino acids, and unsaturated fatty acids. In this respect, seafood and products prepared with seafood are important in fast and ready-meal consumption. Fish croquettes are a nutritious and delicious ready food and appear as a ready-to-use alternative for consumers. By using fish in the form of croquettes, the consumption rate can be increased, and fish with a short shelf life can be kept longer. In addition, the added additives contribute positively to the taste. Croquettes, marketed as cooked and ready for consumption, provide consumers convenience and offer delicious and nutritious foods (Bilgin & Metin, 2022).

Seafood processing in different forms is among the preferred products, especially in luxury hotels and restaurants. Using fish with low economic value, such as fish balls, croquettes, cakes, and sausages, will support large-scale factories (Lin et al., 2019). Croquettes are also consumed for breakfast, as a hors d'oeuvre, as a second course or as a side dish with fried meats. The main ingredients remain unchanged: chicken, fish, game, mushrooms, potatoes, artichokes, ham, shrimp, etc. Croquettes can be made with many food items (Patır et al., 2009).

Although fish products have many functional properties, such as high-quality and easily digestible proteins, health-beneficial polyunsaturated fatty acids, and vitamins and minerals necessary for human nutrition, they do not contain fibre. In this context, the use of dietary fibres in aquatic products is important in terms of not only improving functionality but also creating functional foods that are beneficial to health. In addition to increasing the nutritional value of products, dietary fibres also bring many advantages, such as reducing the total amount of fat, water binding, volumising, improving emulsion capacity and gelling properties, increasing product yield by reducing cooking loss and cost, increasing storage stability and improving textural properties (Jayasinghe et al., 2013; Kerimoğlu, 2020).

Nowadays, diseases such as celiac, diabetes, heart and digestive disorders are quite common, and the food industry has tended to offer alternatives to people with these diseases by producing different products in this context (Keskin & Kaplan Evlice, 2015). The meat industry is focused on producing healthier meat and meat products by reducing commonly perceived "negative" ingredients and/or using health-promoting ingredients (Fernández-López, 2021). One of these functional substances is "dietary fibres" (Jayasinghe et al., 2013).

Root vegetables are essential components of human food. Jerusalem artichoke (*Helianthus tuberosus* L.) is a plant that grows naturally in the central regions of North America. It was introduced to Europe by the French in the 17th century and has been used as human food and animal feed since then. Another name is the Jerusalem artichoke plant. It is known that yams have spread to many regions in Turkey, especially the Central Anatolia and Aegean regions, but are grown in very small areas for fresh consumption (Atlıhan, 2011; Tian & Liu, 2019; Yılmaz Acar, 2021).

Many studies have shown that Jerusalem artichoke has anticancer, antioxidant, antirheumatic and antidiabetic activities. Jerusalem artichoke is an important source of inulin. Inulin is used as a functional food source. It is also a good source of dietary fibre due to the presence of inulin. It is reported that Jerusalem artichoke roots contain inulin between approximately 7% and 30% of fresh weight (Atlıhan, 2011; Çetin Babaoğlu et al., 2021). When its chemical composition is examined, it is 80% water, 1%-2% protein and high amounts of mineral substances iron (0.4-3.7 mg), calcium (14-37 mg), potassium (420-657 mg) and sodium (1,8-mg-4.0 mg) content. They are rich in lysine and methionine. It contains all the necessary amino acids in appropriate proportions. Tubers are a good source of vitamins, especially vitamin B complex (thiamine, riboflavin, niacin, B6, pantothenic acid, biotin and cobalamin), vitamin C (ascorbic acid) and β-carotene, relatively high in folate or folic acid (13-22 $\mu g \cdot 100\ g^{-1}$) are available. Jerusalem artichoke tubers are low-calorie products. (Takeuchi & Nagashima, 2011; Baltacıoğlu, 2012; Harmankaya et al., 2012).

A literature review revealed a limited number of studies investigating the effect of dietary fibre addition on fish

fingers. Additionally, no study has found that Jerusalem artichoke, a rich source of inulin, was used in fish fingers. Our work is also important in developing a new product; in this context, it will contribute to the literature. At the same time, it has developed a functional product that is healthy for the consumer and producer and is thought to be important in increasing product diversity. This study investigated the usage possibilities of different ratios of Jerusalem artichoke fibre in rainbow trout (*O. mykiss*) croquettes.

Materials and Methods

Rainbow trout fillets, with an average weight of approximately 10 kg, were supplied from Atatürk University Faculty of Fisheries, and the products required for croquettes and Jerusalem artichoke were procured from a local market.

Jerusalem artichokes (*Helianthus tuberosus*) were washed with tap water and cut into slices with a knife. It was then dried in an oven at 60°C for 2 days, crushed into powder using a kitchen robot, and passed through the screen. The minced fish meat for making croquettes was added with various additives (10% wheat flour, 10% breadcrumbs, 2% salt, 5% granulated onion, 0.5% black pepper, 0.5% red pepper, 1% granulated garlic) and kneaded until a homogeneous mixture was obtained and shaped into croquettes. After shaping the dough, the coating stage was started. For this purpose, the croquettes were first breaded with a mixture containing 70% egg white, 12% baking soda and 2% salt, then with a mixture containing 50% wheat flour and 50% breadcrumbs (Çankırılıgil & Berik, 2017) and divided into 4 groups. It was prepared as a control (without Jerusalem artichoke fibre), and application groups were enriched with Jerusalem artichoke fibre in different amounts (2%, 4% and 6%). The prepared croquettes were fried, packed in styrofoam plates with stretch film and stored at +4°C for 25 days.

Physico-Chemical Analyses

Moisture and dry matter

After drying in the oven at 100°C for 2 hours, 10 g of the sample will be weighed and placed in aluminium drying containers, which will be placed in a desiccator, cooled and tared on a precision scale. The containers were taken to the desiccator, cooled, and weighed, and the %moisture value was calculated. The dry matter value was calculated by subtracting the moisture content from 100. (Gökalp et al., 2001).

Crude protein content

The protein content of the samples was determined using a Kjeldahl system. First, the nitrogen content of the samples was determined, and then the crude protein content (N × 6.25) was calculated (AOAC, 2000).

Fat content

The lipid extraction process of samples was made according to Folch et al. (1957).

Carbohydrate

Total carbohydrates were calculated using the numerical formula (Duman, 2022).

$$\text{Carbohydrate} = 100 - (\text{moisture} + \text{protein} + \text{fat} + \text{ash})$$

pH

Approximately 10 g of sample was taken, 100 mL of pure water was added, it will be homogenised with the help of ultra-turrax and measured using a pH meter (Gökalp et al., 2001).

Thiobarbituric acid reactive substance (TBARS)

TBARS content was determined according to Lemon (1975) and Kilic and Richards (2003). Approximately 2 g of the croquette sample was taken, and 12 mL of trichloroacetic acid was added to it, homogenised and then filtered with Whatman 1 filter paper. 3 mL of the resulting filtrate was taken, and 3 mL of 0.02 M thiobarbituric acid was added. Then, it was left to cool by keeping it in a water bath at 100ºC for 40 minutes and centrifuged at 2000 g and a spectrophotometric (530 nm) reading was taken. The TBARS content was expressed as µmol MA (MDA) kg^{-1} fish muscle.

Total volatile basic nitrogen (TVB-N)

TVB-N content was determined, as reported by Malle and Tao (1987). The TVB-N contents were expressed as mg 100 g^{-1} fish muscle. 40 g of the croquette sample was taken, and 80 mL of 7.5% trichloroacetic acid was added, homogenised and centrifuged. Filtering was done with the Whatman 3 filter paper. 5 mL NaOH was added to the resulting filtrate, placed in the distillation device, and the distillate was titrated with 0.1 N H_2SO_4 until a pink colour was obtained.

$$\text{TVB-N} = \text{n x } 16.8 \text{ mg nitrogen}$$

Microbiological Analysis

For microbiological analysis, 10 grams of samples were taken from the crocket, transferred to sterile stomacher bags, and homogenised using a stomacher device by adding 90 mL of sterile saline. The surface spreading method was used, and each microorganism was incubated at the appropriate temperature and time (Table 1).

Sensory Analysis

Ten panellists analysed the croquettes' sensory properties, including appearance, odour, texture, colour, flavour, and overall acceptability. They scored the samples during storage from 1 to 9 (Choi et al., 2014).

Statistical Analysis

Statistical analyses of the results obtained were made in the SPSS program, and the obtained results were compared with Duncan's multiple comparison tests ($p<0.05$).

Results and Discussion

Physicochemical Analysis Results of Samples

The nutritional composition of seafood varies from species to species or among the same species, depending on gender, fishing region, season and age (Karslı & Çağlak, 2021). The proximate composition results of rainbow trout croquettes are given in Table 2. The lowest moisture value was determined in sample C at 49.22%, followed by samples B at 49.70%, A at 51.11% and K at 51.90%. The difference between the groups was statistically significant ($p<0.05$). Similarly, Fuchs et al. (2013) reported that the moisture value was higher in raw control croquette compared with other groups (fried control croquette, raw and fried enriched with flaxseed flour croquette).

The ash values of the samples varied between 3.85 and 4.10%. Ash content increased in croquettes enriched with Jerusalem artichoke, and Çankırıgil & Berik (2017a) reported that the ash content in fried sardine croquettes increased compared to meat and croquettes. The increased crude ash content is due to the added ingredients and water loss depending on the frying process. The researchers' results are similar to the findings of this study.

The protein values of the samples were determined between 15.52% and 16.57%. Depending on the Jerusalem artichoke fibre addition rate, the protein contents of the samples tended to increase. Alkuraieef et al. (2020) found that after six months of storage, the protein content of Indian mackerel fish balls compared to fresh products decreased, while the protein content of Indian mackerel fish fingers increased. Altan et al. (2023), in their study with Atlantic salmon meatballs, determined that the crude protein and moisture content of the MTGase-added group was higher than the control group.

It was observed that the highest lipid content was in group C samples and the lowest in group control. Jerusalem artichoke tubers contain a small amount (0.02 g) of fat (Ünver Alçay, 2020). The difference between the groups in terms of lipid content was statistically significant ($p<0.05$). Çankırıgil & Berik (2017b) emphasised that there was a statistical increase in the crude oil amount of rainbow trout croquettes ($p<0.05$).

Carbohydrate values were found between 15.98-16.79%. The lowest carbohydrate value of the croquette samples was detected in sample A and the highest in sample B. Berik et al. (2011) emphasised that the carbohydrate value was higher in fried rainbow trout fingers compared to the control group.

Table 1. Incubation conditions for groups of microorganisms

Microorganisms	Medium	Incubation Conditions	References
Total Aerobic Mesophilic Bacteria	Plate Count Agar	30°C for 2 days	Baumgart et al., 1986
Psychrophilic Bacteria	Plate Count Agar	10°C for 7 days	Anonymous 1992
Yeast and Mold	Rose Bengal Chloramphenicol Agar	25°C for 5 days	Halkman 2005
Lactic Acid Bacteria	de Man, Rogosa Sharpe Agar	30°C for 2 days	Halkman 2005
Enterobactericeae	Violet Red Glucose Agar	30°C for 2 days	Gökalp et al., 2001

Table 2. Proximate composition results of samples

Analysis (%)	Samples			
	K	A	B	C
Moisture	51.90 ±1.11^{a}	51.11 ±0.55^{ab}	49.70 ±0.20^{bc}	49.22 ±0.38^{c}
Ash	3.85 ±0.09^{b}	3.99 ±0.03^{ab}	4.00 ±0.07^{ab}	4.10 ±0.02^{a}
Lipid	12.03 ±0.16^{a}	12.40 ±0.10^{a}	13.00 ±0.06^{b}	14.13 ±0.21^{c}
Carbohydrate	16.70 ±0.37^{ab}	16.48 ±0.36^{ab}	16.79 ±0.13^{a}	15.98 ±0.12^{b}
Protein	15.52 ±0.47^{b}	16.02 ±0.04^{ab}	16.50 ±0.20^{a}	16.57 ±0.45^{a}

Values shown with different letters are statistically different from each other (p≤0.05).

Chemical Analysis Results

Chemical analysis results of rainbow trout croquettes prepared with different proportions of Jerusalem artichoke fibre during cold storage (4 ±1ºC) are given in Table 3. TVB-N is an important criterion in determining the product's quality, and the fish's spoilage quality can be seen from the value of nitrogen bases (Febriani et al., 2023). At the beginning of storage, the lowest TVB-N value was 10.21 mg/100g in the C sample and the highest value was 12.20 mg/100g in the K sample. TVB-N values of all croquet groups increased significantly (p <0.05) according to storage time. According to Varlık et al. (1993), the limit value for TVB-N for aquatic products was determined as 35 mg/100g. Values above this may make the product unconsumable. This limit values were not exceeded in all groups during the storage period. Similarly, Balıkçı et al. (2022), in their study investigating the addition of rosemary, thyme and basil herbal extracts to mackerel meatballs, reported that TVB-N values in all groups remained below the acceptability limits during storage. Çorapçı et al. (2023) emphasised that although no statistical difference was observed between the groups on the 0th day in rainbow trout balls prepared by adding different concentrations of MTGase (0.5% and 0.8%), the TVB-N values of Group B (0.8% MTGase added trout ball) were different from the other groups at the end of storage (p<0.05). Nguyen et al. (2023) emphasised that the TVB-N values of fishballs obtained from knifefish and striped catfish increased gradually from the beginning to the end of storage.

TBARS value is the analysis method used to determine oxidation in aquatic products, and this value below 3 mg/kg indicates that the product is of very good quality (Özturan, 2022; Çorapçı et al., 2023). In our study, the highest TBARS value was observed at 8.36 µmol MA/kg in the K sample, and the lowest value was found to be 6.45 µmol MA/kg in C samples at the end of storage. Compared to the control group, a lower TBARS value was determined in croquettes prepared with Jerusalem artichoke. Chen et al. (2014) found the Jerusalem artichoke plant to have antioxidant activities. The results of our study also support this. Statistical differences were observed between groups (p<0,05). Similarly, Sa´nchez-Alonso et al. (2007) found that the TBA values of grape antioxidant dietary fibre added to minced fish muscle were lower. Özpolat (2022) reported that the TBA values of fish balls prepared from Capoeta trutta with different concentrations of liquid smoke increased with the storage time but did not exceed the consumable limit value in any group. Vidyarthi et al. (2022) emphasised that fruit powder slowed the lipid peroxidation of fish nuggets. Abdel-Wahab et al. (2020), in their study investigating the antioxidant potential of clove, sage and kiwi peel extracts and mixtures on fish fingers, stated that the lipid oxidation rate was delayed and remained below the standard level.

The pH value for fresh fish meat is between 6.0-6.5 and rises slowly depending on storage time. The consumability limit value for fish meat is between 6.8 and 7.0. However, pH value is not an absolute criterion and should always be supported by sensory and chemical tests (Güngör, 2011). It was determined that there were fluctuations in the pH values of the storage samples. The highest pH value was determined in group K, while the lowest was in group C croquettes. Statistically significant differences between the samples in pH values (p<0,05) were detected. Dalbosco et al. (2010) found that the pH values of goldfish crockets increased significantly after cooking. Ajik-Cerbas et al. (2022) observed that while the pH value of the crab balls obtained from blue swimming crab was 9.04, it gradually decreased to almost neutral on the 35th day.

Tablo 3. Chemical analysis results of fish croquettes produced using different proportions of Jerusalem artichoke fibre

Analysis	Storage time (days)	Samples			
		K	A	B	C
TVB-N (mg/100 g)	0	12.20 ±0.12^{h}	11.17 ±0.12^{h}	10.94 ±0.08^{h}	10.21 ±0.12^{h}
	4	13.74 ±0.05^{g}	12.14 ±0.15^{g}	11.90 ±0.01^{g}	11.14 ±0.12^{g}
	7	15.34 ±0.15^{f}	13.79 ±0.06^{f}	13.05 ±0.11^{f}	12.67 ±0.16^{f}
	11	17.08 ±0.08^{e}	15.74 ±0.10^{e}	15.12 ±0.19^{e}	14.35 ±0.28^{e}
	14	18.88 ±0.05^{d}	16.73 ±0.20^{d}	16.00 ±0.08^{d}	15.20 ±0.07^{d}
	18	20.65 ±0.18^{c}	18.63 ±0.06^{c}	18.07 ±0.15^{c}	17.11 ±0.16^{c}
	21	21.52 ±0.19^{b}	19.79 ±0.07^{b}	18.82 ±0.04^{b}	18.04 ±0.10^{b}
	25	23.10 ±0.07^{a}	20.52 ±0.09^{a}	20.00 ±0.02^{a}	19.77 ±0.14^{a}
TBARS (µmol MA/kg)	0	1.24 ±0.05^{h}	1.20 ±0.02^{g}	1.14 ±0.02^{g}	1.07 ±0.01^{g}
	4	1.92 ±0.05^{g}	1.65 ±0.13^{fg}	1.64 ±0.23^{fg}	1.42 ±0.13^{fg}
	7	2.43 ±0.06^{f}	2.13 ±0.11^{f}	1.99 ±0.07^{f}	1.92 ±0.03^{ef}
	11	3.10 ±0.26^{e}	2.89 ±0.16^{e}	2.77 ±0.16^{e}	2.39 ±0.23^{e}
	14	3.93 ±0.21^{d}	3.60 ±0.26^{d}	3.46 ±0.28^{d}	3.04 ±0.14^{d}
	18	5.69 ±0.19^{c}	5.00 ±0.11^{c}	4.66 ±0.30^{c}	4.13 ±0.28^{c}
	21	6.32 ±0.19^{b}	5.94 ±0.09^{b}	5.77 ±0.30^{b}	5.40 ±0.35^{b}
	25	8.36 ±0.10^{a}	7.38 ±0.55^{a}	6.79 ±0.32^{a}	6.45 ±0.51^{a}
pH	0	6.80 ±0.01^{a}	6.50 ±0.01^{a}	6.43 ±0.04^{ab}	6.45 ±0.33^{b}
	4	6.86 ±0.02^{b}	6.62 ±0.20^{a}	6.34 ±0.09^{bc}	6.33 ±0.19^{b}
	7	6.70 ±0.06^{ab}	6.53 ±0.35^{a}	6.53 ±0.07^{a}	6.83 ±0.04^{a}
	11	6.38 ±0.02^{c}	6.23 ±0.01^{ab}	6.24 ±0.07^{c}	6.21 ±0.05^{bc}
	14	6.48 ±0.01^{bc}	6.43 ±0.07^{a}	6.38 ±0.08^{abc}	6.37 ±0.05^{b}
	18	6.40 ±0.16^{c}	6.23 ±0.15^{ab}	6.06 ±0.06^{d}	6.12 ±0.09^{bc}
	21	6.01 ±0.19^{d}	6.26 ±0.09^{ab}	5.99 ±0.00^{de}	5.87 ±0.03^{cd}
	25	6.00 ±0.02^{d}	5.91 ±0.02^{b}	5.85 ±0.01^{e}	5.74 ±0.02^{d}

Means shown with different letters are statistically different ($p<0.05$). A: control; B: 2% Jerusalem artichoke fiber; C: 4% Jerusalem artichoke fiber; D: 6% Jerusalem artichoke fiber

Microbiological Analysis Results

Microbiological analysis results of rainbow trout croquettes prepared with different proportions of Jerusalem artichoke fibre during cold storage (4±1ºC) are given in Table 4. Total aerobic mesophilic bacteria is an important parameter for determining microbial quality in foods and is widely used (Anonymous, 2024). According to ICMSF (1986), the limit value for the total seafood is recommended as 6-7 log cfu/g.

These limits were exceeded in the control group (K) on the 14th day of storage. The total number of mesophilic aerobic microorganisms in the control group samples, which was 3.36 log cfu/g on day 0, increased to 8.20 log cfu/g at the end of storage of the croquet samples. An increase in the total number of mesophilic aerobic microorganisms was observed with storage in all groups. Similarly, Cadun et al. (2015) reported that the total number of aerobic bacteria increased during storage in whiting fish balls prepared by adding different fibre types (wheat and apple fibre). Bacterial counts of trout croquettes prepared with Jerusalem artichoke fibre were lower than the control group. It is thought to be due to the antimicrobial effect of *Jerusalem artichoke.* Studies have reported that the crude extract of Jerusalem artichoke leaves has antifungal or antimicrobial activities (Chen et al., 2013). The use of high concentrations of fibre further increased its positive effect. Çağlak & Karslı (2023) reported that the number of total aerobic mesophilic in rainbow trout croquettes enriched with dill extracts was lower than in the control group.

The number of psychrotrophic bacteria in the control group was 3.27 log cfu/g at the beginning of storage, and an increase

was detected in all groups in parallel with storage. The lowest number of bacteria was determined in group C samples. The differences between groups and storage days were statistically significant ($p<0.01$). Similarly, Gürel İnanlı & Amin (2022) observed increases in the number of psychrophilic bacteria in all groups depending on the time during storage in fish fingers coated with the addition of goji berry. İzci & Ümüt (2023) found that the total number of psychrophilic aerobic bacteria in bogue meatball samples was determined as 4.67 ±0.01 log cfu/g, and it was emphasised that the increase in the number of microorganisms was significant ($p<0.05$) during storage.

Yeast-mold numbers were determined as 2,00 log cfu/g in all groups at the beginning of storage, and an increase was observed in all groups during the storage period. The differences between groups and storage days were statistically significant ($p<0.01$). Lower yeast and mould counts were observed in fish croquettes with Jerusalem artichoke addition. Chen (2013) demonstrated that leaf extracts could exhibit antifungal capacities based on the structural properties of Jerusalem artichoke phenolics. The results of our study also support this. Altan (2020) determined the total yeast and mould numbers of trout balls on the first day of storage as 3.22 log cfu/g in the control group, 3.05 log cfu/g in the 0.5% MTGase group and 3.15 log cfu/g in the 1% MTGase group.

Lactic acid bacteria (LAB) are characterised as non-aerobic, Gram-positive cocci and rods (Nath et al., 2013). LAB is naturally dominating the microflora of many foods (Ghanbari et al., 2013). It is also part of the natural microbiota of fish fillets. LAB counts were determined as 2,00 log cfu/g in all groups at the beginning of storage (day 0). The highest LAB count was determined as 4.11 log cfu/g in the control group at the end of storage (25th day). Storage time and sample x storage time interactions on the number of lactic acid bacteria were significant ($p<0.01$). In their study, Uçak & Afreen (2022) found that the number of lactic acid bacteria was higher in the control and chitosan coating applied fish meatballs than in the group treated with peppermint essential oil emulsion containing chitosan.

Enterobacteriaceae is an indicator microorganism that is part of the microflora of seafood products and is considered an indicator of hygiene in fish and fish products (Uçak & Afreen, 2022). Yi et al. (2011) reported that the number of Enterobacteriaceae was highest in the control group in their study with fish balls prepared with different concentrations (0, 0.10, 0.15, 0.20, 0.25, and 0.30 g kg^{-1}) of tea polyphenols.

Sensory Analysis Results

Sensory analysis results of rainbow trout croquettes prepared with different proportions of Jerusalem artichoke fibre during cold storage (4 ±1°C) are given in Figure 1. Sensory properties are crucial in consumer food choices (Li et al., 2024). According to the sensory analysis results, a decrease was observed in all sample groups in parallel with storage. The panellists especially liked sample B regarding colour, texture, smell, taste, and appearance. As seen in Figure 1, the croquette samples with Jerusalem artichoke fibre were liked by most panellists regarding sensory properties. For acceptability, panellists gave the highest value to sample A and the lowest value to the control sample. According to their taste parameter scores, the ranking was B>C>A>K. Colour and smell are important parameters in sensory evaluation, and the panellists gave higher scores to the croquette samples prepared with Jerusalem artichoke compared to the control group. Çankırıgil & Berik (2018) emphasised that shrimp croquettes were the group's favourite regarding the sensory properties of the fish fingers they produced from different seafood such as deep-water rose shrimp, sardines and rainbow trout. Budaraga et al. (2021) reported that according to the sensory analysis results of red tuna and white oyster mushroom meatballs, the best storage time of 0 hours was using wrap packaging. İnanlı & Amin (2022) reported that fish burgers prepared using gojibery received better sensory scores than the control group. The study results are similar to those of our study. Gonal (2023) observed that adding broccoli flour to carp fish cake processing significantly affected consistency, taste and texture but did not affect aroma.

Tablo 4. Microbiological analysis results (log cfu/g) of fish croquettes produced using different proportions of Jerusalem artichoke fibre.

Samples	Storage time (days)	TMAB	TPAB	TYM	LAB	ENTERO
K	0	3.36 ±0.11^{g}	3.27 ±0.08^{h}	2.00 ±0.00^{f}	2.00 ±0.00^{e}	2.00 ±0.00^{d}
	4	4.13 ±0.14^{f}	4.19 ±0.09^{g}	2.00 ±0.00^{f}	2.00 ±0.00^{e}	2.00 ±0.00^{d}
	7	4.68 ±0.01^{e}	4.50 ±0.07^{f}	2.17 ±0.14^{f}	2.15 ±0.09^{e}	2.00 ±0.00^{d}
	11	5.39 ±0.18^{d}	5.31 ±0.02^{e}	2.90 ±0.02^{e}	2.00 ±0.00^{e}	2.10 ±0.06^{d}
	14	6.16 ±0.14^{c}	5.90 ±0.12^{d}	3.23 ±0.14^{d}	2.60 ±0.08^{d}	2.00 ±0.00^{cd}
	18	6.97 ±0.16^{b}	6.63 ±0.12^{c}	3.94 ±0.08^{c}	3.03 ±0.12^{c}	2.23 ±0.14^{c}
	21	7.92 ±0.04^{a}	7.19 ±0.26^{b}	4.77 ±0.16^{b}	3.72 ±0.21^{b}	3.00 ±0.07^{b}
	25	8.20 ±0.12^{a}	8.04 ±0.07^{a}	5.80 ±0.15^{a}	4.11 ±0.09^{a}	3.70 ±0.05^{a}
A	0	3.00 ±0.11^{h}	3.14 ±0.19^{g}	2.00 ±0.00^{e}	2.00 ±0.00^{d}	2.00 ±0.00^{d}
	4	3.57 ±0.16^{g}	3.46 ±0.17^{g}	2.00 ±0.00^{e}	2.00 ±0.00^{d}	2.00 ±0.00^{d}
	7	4.26 ±0.02^{f}	4.19 ±0.09^{f}	2.11 ±0.09^{e}	2.00 ±0.00^{d}	2.00 ±0.00^{d}
	11	5.00 ±0.03^{e}	5.22 ±0.09^{e}	2.85 ±0.10^{d}	2.09 ±0.12^{d}	2.13 ±0.18^{d}
	14	5.90 ±0.03^{d}	5.87 ±0.11^{d}	3.02 ±0.03^{d}	2.07 ±0.09^{d}	2.00 ±0.00^{d}
	18	6.50 ±0.07^{c}	6.46 ±0.28^{c}	3.63 ±0.11^{c}	2.74 ±0.06^{c}	2.40 ±0.10^{c}
	21	7.23 ±0.14^{b}	7.37 ±0.12^{b}	4.05 ±0.09^{b}	3.18 ±0.14^{b}	2.86 ±0.12^{b}
	25	7.93 ±0.05^{a}	7.94 ±0.09^{a}	5.12 ±0.19^{a}	3.93 ±0.04^{a}	3.34 ±0.14^{a}
B	0	2.67 ±0.14^{h}	2.90 ±0.06^{g}	2.00 ±0.00^{d}	2.00 ±0.00^{d}	2.00 ±0.00^{d}
	4	3.09 ±0.05^{g}	3.14 ±0.19^{g}	2.00 ±0.00^{d}	2.00 ±0.00^{d}	2.00 ±0.00^{d}
	7	3.94 ±0.07^{f}	4.06 ±0.07^{f}	2.07 ±0.09^{d}	2.00 ±0.00^{d}	2.00 ±0.00^{d}
	11	4.48 ±0.12^{e}	4.60 ±0.22^{e}	2.12 ±0.16^{d}	2.04 ±0.06^{d}	2.00 ±0.00^{d}
	14	5.37 ±0.16^{d}	5.29 ±0.23^{d}	2.50 ±0.08^{c}	2.00 ±0.00^{d}	2.02 ±0.03^{d}
	18	6.03 ±0.06^{c}	6.17 ±0.12^{c}	3.01 ±0.04^{b}	2.39 ±0.07^{c}	2.31 ±0.11^{c}
	21	7.01 ±0.04^{b}	7.07 ±0.09^{b}	3.07 ±0.09^{b}	3.00 ±0.10^{b}	2.75 ±0.09^{b}
	25	7.84 ±0.09^{a}	7.54 ±0.16^{a}	3.66 ±0.15^{a}	3.64 ±0.10^{a}	3.13 ±0.12^{a}
C	0	2.20 ±0.17^{h}	2.24 ±0.16^{h}	2.00 ±0.00^{e}	2.00 ±0.00^{c}	2.00 ±0.00^{d}
	4	2.79 ±0.14^{g}	3.29 ±0.20^{g}	2.00 ±0.00^{e}	2.00 ±0.00^{c}	2.00 ±0.00^{d}
	7	3.33 ±0.16^{f}	3.93 ±0.21^{f}	2.00 ±0.00^{e}	2.00 ±0.00^{c}	2.00 ±0.00^{d}
	11	4.10 ±0.02^{e}	4.40 ±0.09^{e}	2.14 ±0.06^{de}	2.00 ±0.00^{c}	2.00 ±0.00^{d}
	14	5.01 ±0.04^{d}	5.06 ±0.08^{d}	2.18 ±0.07^{d}	2.08 ±0.04^{c}	2.00 ±0.00^{d}
	18	5.88 ±0.13^{c}	6.02 ±0.04^{c}	2.70 ±0.09^{c}	2.20 ±0.28^{c}	2.17 ±0.10^{c}
	21	6.39 ±0.08^{b}	6.60 ±0.24^{b}	2.99 ±0.01^{b}	2.99 ±0.02^{b}	2.40 ±0.01^{b}
	25	7.28 ±0.19^{a}	7.76 ±0.12^{a}	3.51 ±0.11^{a}	3.44 ±0.15^{a}	3.00 ±0.02^{a}

Means shown with different letters are statistically different ($p<0.05$). A: control; B: 2% Jerusalem artichoke fiber; C: 4% Jerusalem artichoke fiber; D: 6% Jerusalem artichoke fiber

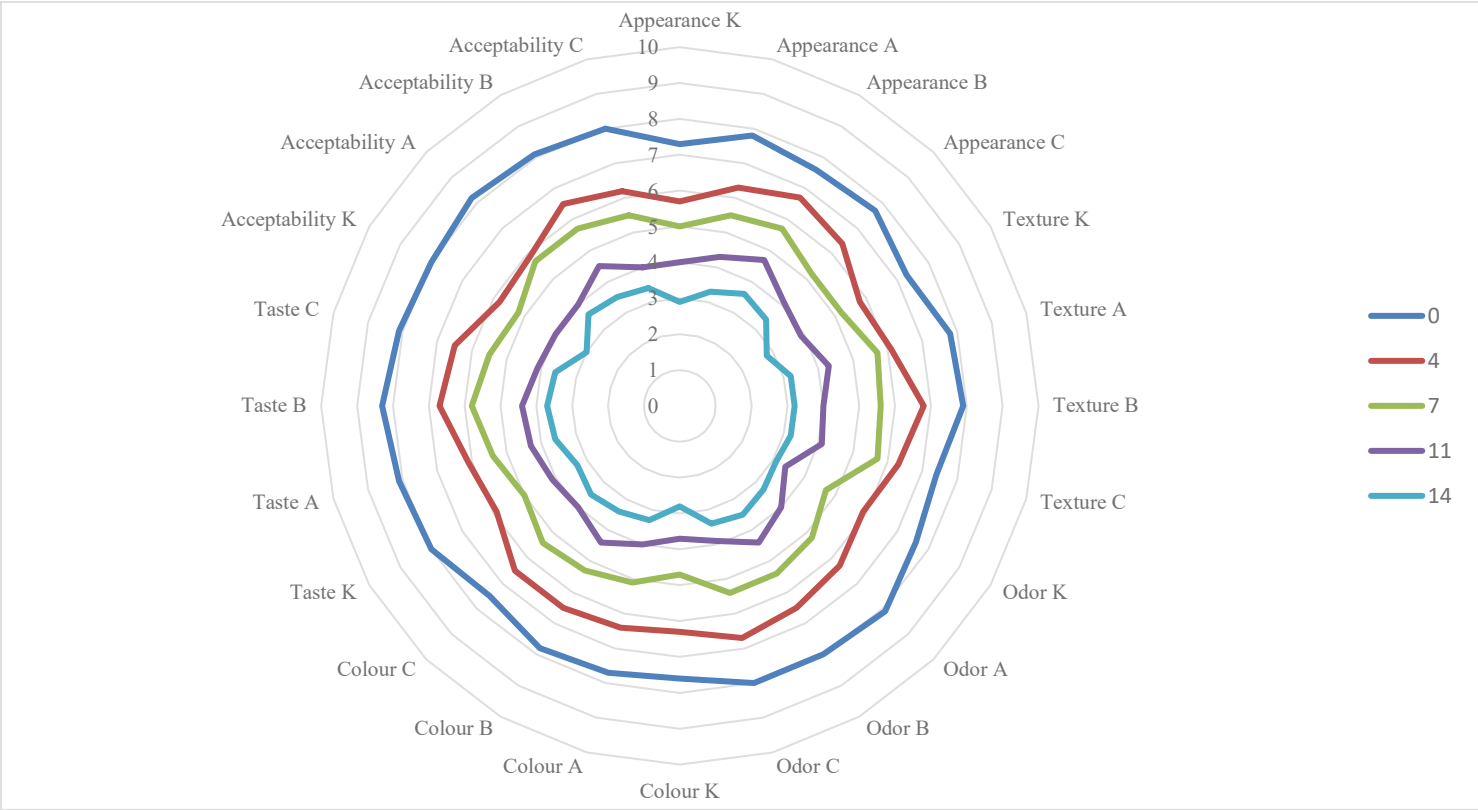

Figure 1. Sensory analysis results of fish croquettes produced using different proportions of Jerusalem artichoke fibre

Conclusion

With the spread of diseases, consumers have started to turn to healthy foods. Dietary fibres and fibre-rich products have therapeutic and curative properties for common diseases such as heart disease, cancer, and obesity. In addition, since plant-based flours contain high protein, starch and fibre, the shelf life of fish and fish products can be extended. Jerusalem artichoke is a low-calorie product. In addition to being rich in inulin, it is a good source of dietary fibre. Many studies have shown that Jerusalem artichoke has anticancer, antioxidant, antirheumatic and antidiabetic activities. In light of all the analyses, it was determined that the croquettes prepared with yam fibre gave positive results regarding physical, chemical and microbiological properties. It was determined that it gave better results than the control group croquettes in terms of sensory evaluation. It is thought that this study can be further developed by shedding light on further studies.

Compliance with Ethical Standards

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

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

Data availability: Data will be made available on request.

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Effect of different trophic cultures on the amount of total carbohydrate and chlorophyll of *Oscillatoria* sp.

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ABSTRACT

Cyanobacteria (blue-green microalgae) is a gram-negative prokaryotic autotroph found in natural waters that plays a pivotal role in biochemical cycles. The present investigation proposed to study the potential of using different concentrations of glucose as the carbon substrate to produce microalgal biomass and biochemical components, such as photosynthetic pigments and total carbohydrates (C.H.) by *Oscillatoria* sp. The cyanobacteria were collected, and the isolated colony was found to be *Oscillatoria* sp., and it was grown in BG-11 medium for mass cultivation. Then, the centrifuged biomass was weighed and used to extract bioactive compounds. *Oscillatoria* sp. cells were cultured in three different tropic cultures (phototrophic, heterotrophic and mixotrophic) under controlled laboratory conditions with continuous light illumination or unillumination and aeration. Chl–a and total C.H. contents were also evaluated after 120 hrs. The recorded optical density of *Oscillatoria* was increased from 0.6798 ±0.01 at 660 nm and 0.5847 ±0.01 at 750 nm after 24 hrs to 1.2174 ±0.002 at 680nm and 1.0243 ±0.01 at 730nm at the end of 120hrs of the experiment. According to analysis results, the mean amount of Chl-a and Total C.H. of *Oscillatoria* sp. biomass was determined as 0.5132 µg L^{-1} and 3.5715 mg mL^{-1} under the phototrophic culture (absence of glucose), respectively. Under the mixotrophic culture (presence of light), the experimental results showed that the chl-a content was calculated as 0.1770, 0.3380 and 0.7098 µg L^{-1}. In contrast, the total C.H. was calculated as 3.6150, 7.9129 and 11.3191 mg mL^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively. Under the heterotrophic culture (absence of light), the results showed that the chl-a content was 0.2366, 0.2456 and 0.2346 µg L^{-1} while the total C.H. was 4.2969, 8.0990 and 11.5861 mg m L^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively. The experimental results showed that the total C.H. content was increased from 3.5715 to 11.58 61 mg mL^{-1} in the heterotrophic (the absence of light and the presence of 10 g L^{-1} glucose) BG-11 culture conditions. The chlorophyll-a content was increased from 0.1770 µg L^{-1} to 0.7098 µg L^{-1} in the mixotrophic (the presence of glucose and light) BG-11 culture conditions. As a result of the experiment, it was determined that the most suitable culture in terms of total carbohydrate and growth rate was mixotrophic and heterotrophic BG-11 (10 g L^{-1} glucose) culture condition, and in terms of chl-a was mixotrophic culture (10 g L^{-1} glucose).

Keywords: Cyanobacteria, *Oscillatoria*, Glucose, Mixotrophic cultivation, Heterotrophic cultivation

Introduction

Microalgae are photosynthetic organisms found in pelagic and benthic environments (cyanobacteria, diatoms, dinoflagellates, and green algae) (Bhuyar et al., 1990; Abad et al., 2011; Bhuyar et al., 2020). They consist of various biomolecules, majorly including lipids, proteins, pigments, chlorophyll, and carbohydrates, which are found in the form of cellulose and soluble polysaccharides such as starch or glycogen (Paroz & Izquirerdo, 2011).

Cyanobacteria, found in many different habitats, from fresh to marine, hyper-saline, and terrestrial ecosystems, are gram-negative prokaryotic autotrophs that use chlorophyll for photosynthesis. In addition to photosynthesis, cyanobacteria, an incredible ancient group of prokaryotic organisms, are wealthy in numerous treasured compounds like pigments (astaxanthin, lutein, phycobiliprotein), antibiotics, vitamins, and essential nutrients (proteins, carbohydrates, and lipids) (Lau et al., 2015).

Oscillatoriaceae species (*Spirulina* sp., *Oscillatoria* sp., *Phormidium* sp., *Lyngbya* sp.) produce many pharmaceutical and nutraceutical ingredients with varying bioactivities, including antibacterial, antifungal, antioxidant, antialgal, antiviral, anticancer, and anti-inflammatory effects. This is one of the most important orders in cyanobacteria (Singh et al., 2022; Sultan et al., 2016).

A transition from phototrophic growth to mixotrophic growth can be observed in many species and genera of microalgae. Some microalgae species like *Chlorella vulgaris* (Mitra et al., 2012), *Haematococcus pluvialis* (Kobayashi et al., 1992), *Spirulina platensis* (Marquez et al., 1993), *C. sorokiniana* (Wang et al., 2012), *Botryococcus braunii* (Zhang et al., 2011), and *C. zofingiensis* (Li et al., 2011) have been observed under autotrophy, heterotrophy, and mixotrophy conditions. Many algal organisms can use either metabolic process (autotrophic or heterotrophic) for growth, meaning that they can photosynthesise and ingest prey or organic materials (Lau, 2015). The ability of mixotrophism to process organic substrates means that cell growth is not strictly dependent on photosynthesis. Therefore, light energy is not a limiting factor for growth and light, or organic carbon substrates can support the alga growth. Hence, there is less biomass loss during the dark phase. Mixotrophic and heterotrophic microalgae cultivation provides higher biomass and lipid productivities than cultivation under photoautotrophic conditions; the cost of the organic carbon substrate is estimated to be about 80% of the total cost of the cultivation medium (Choi & Lee, 2015).

In this study, it was investigated in which trophic culture environment *Oscillatoria* cells produce more chlorophyll and carbohydrates. The present work, therefore, investigates the effects of different trophic conditions (phototrophic, heterotrophic and mixotrophic) and different glucose substrate concentrations on total carbohydrate, chlorophyll-a total protein contents of *Oscillatoria* sp. studied under laboratory conditions. Optical density, carbohydrate and chlorophyll-a content of *Oscillatoria* cells were measured by spectrophotometric method. *Oscillatoria* sp. cells were cultured under controlled laboratory conditions with continuous light illumination and aeration. Optical density, chl-a and carbohydrate contents were also evaluated after 120hrs.

Materials and Methods

Sample Collection Followed by Identification

The samples were collected from Gediz River (38°39′40.7″ N, 27°18′44.2″ E), Manisa, Türkiye in August 2023. The algae samples were collected with microalgae by plankton net (55 µm mesh) and brought to the laboratory. Serial dilution was carried out to get isolated colonies. The algae were observed and identified directly under the microscope (Olympus Cover-015) between lam and lamel. The photographs were taken using normal microphotography techniques. Current literature sources were used for the determinations. The morphological observations referred to the blue-green algae group that belongs to *Oscillatoria* sp, as shown in Figure 1. (Desikachary, 1959; Komárek & Anagnostidis, 2005; Guiry & Guiry, 2015).

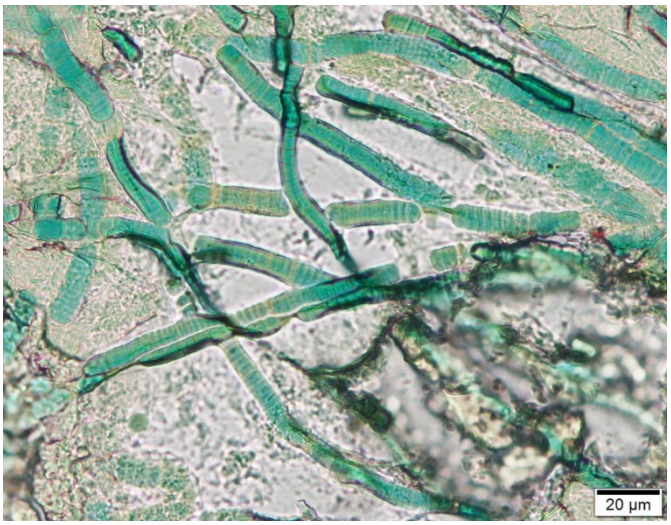

Figure 1. *Oscillatoria* sp. microalgae under microscopic view (x40)

Cultivation

A standard initial inoculum of the isolated algae was inoculated to culture flasks (200 mL each) that contained 100 mL of BG-11 medium, which consists of macronutrients (1.5 g $NaNO_3$ L^{-1}, 0.04 g K_2HPO_4 $3H_2O$ L^{-1}), inorganic salts (0.036 g $CaCl_2.7H_2O$ L^{-1}, 0.075 g $MgSO4.7H_2O$ L^{-1}, 0.02 g Na_2CO_3 L^{-1}), pH conditioners (0.001 g Na_2EDTA L^{-1}, 0.006 g $C_6H_8O_7$ L^{-1}, 0.006 g $C_6H_8O_7{\cdot}xFe^{3+}{\cdot}yNH_3$ L^{-1}), and trace elements (0.222 g $ZnSO4.7H_2O$ L^{-1}, 1.81 g $MnCl.4H_2O$ L^{-1}, 0.390 g Na_2MoO_4. $2H_2O$ L^{-1}, 0.079 g $CuSO_4.5H_2O$ L^{-1}, 2.86 g H_2BO_3 L^{-1}, and 0.0494 g $Co(NO_3)_2.6H_2O$ L^{-1}).

The culture media were sterilised by autoclaving at 121 °C and 1 bar for 15 min and incubated at 26 ± 1ºC under 16:8 h photoperiod (light intensity = 40 µE/m2 S), with aeration (1.2 L min^{-1}) and magnetic stirring (110 rpm). The pH value was adjusted to 6–7 using 1 M NaOH and 1 M HCI (Vijayabaskar & Shiyamala, 2011; Bhuyar et al., 2019). The growth of algae and biomass concentration was monitored by measuring optical density at a wavelength of 660 nm and 730 nm for 20 days. After the lag phase, the algal cells got into their logarithmic growth phases.

Experimental Conditions

Phototrophic culture (in the presence of light and absence of glucose)

Oscillatoria adapted to phototrophic growth conditions as described in the cultivation section. Cultures in the log (exponential) phase were used in this study. The culture was transferred as inoculum into 500 ml Erlen Mayer containing 200 ml of medium and 50 ml of biomass for 120 h under 16:8 h photoperiod conditions. Experiments were conducted in sets of three. The temperature and pH were 25-26°C and between 6.14 ± 0.07 and 6.87 ± 0.03, respectively. Sterile and humidified air was provided by a pump to supply enough oxygen and distribute it homogeneously in the incubator (Biosan ES-20/60). The culture was continuously mixed using a magnetic stirrer.

Heterotrophic culture (in the absence of light and the presence of glucose)

Oscillatoria biomass was cultivated in BG-11 medium using 2.5, 5 and 10 g glucose L^{-1} as the initial glucose concentration in the dark to identify the effect of different glucose concentrations on cell growth, chl-a and total CH—amount of the microalgae.

Mixotrophic culture (in the presence of light and glucose)

A series of experiments were conducted to assess the influence of glucose concentration (2.5, 5 and 10 g glucose L^{-1}) on mixotrophic microalgae growth in addition to chl-a and total CH. Amount of biomass.

Analysis of the Total Carbohydrate Contents (mg mL^{-1})

Total carbohydrate contents were measured using the phenol-sulfuric acid assay, a simple and rapid colourimetric method to determine total carbohydrates, and the d-glucose concentration scale was used to construct the standard curve by DuBois et al. (DuBois et al., 1956). Briefly, a 5% (w/v) phenol solution was prepared in distilled water. 50 µL of test samples and 2 mL of concentrated sulfuric acid were added to 50 µL of a phenol solution. The mixture was stirred for 30 min. 1 mL aliquots of the cultures were used to quantify spectrophotometrically at 490 nm.

Analysis of the Chlorophyll Content (µg L^{-1})

The spectrophotometric method, measured by the absorbance at 630 nm, 645nm, 665 nm, and 750 nm using a spectrophotometer with 90% acetone as blank, was used for the determination of pigment concentrations (Parsons & Strickland,1963). Briefly, 10 mL of culture was filtered using GF/C filters. An aliquot of the sample was centrifuged at 12000 rpm for 5 min, and the supernatant was discarded. The pellet was suspended in 10 mL of boiling acetone at 4°C, stored in the dark for 24 h, and measured using a spectrophotometer.

Determination of Dry Weight

A definite volume (10 mL) of algal suspension was filtered through a cellulose acetate filter membrane (47mm in diameter, 0.22 µm in pore size) and dried overnight in an oven at 105°C. Data were given as mg mL^{-1} algal suspension.

Growth Estimation

Growth was estimated in each sample by measuring the biomass turbidity of SP homogenised suspension at wavelengths 660 nm and 730 nm using a spectrophotometer (LW UV-200-RS); it was expressed in dry mass per litre of suspension (Seely et al., 1972).

Results and Discussion

The Analysis Results of Chl-a content (µg L^{-1}) of Biomass

Tables 1-3 show the effects of initial 2.5, 5, and 10 g L^{-1} glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria*

sp. during the different trophic incubation periods. The chlorophyll-a content (µg L^{-1}) of treated microalgae cells increased significantly with increased glucose concentrations under the mixotrophic culture (M.C.) conditions. The chl-a contents of cells were affected significantly, especially at 5 and 10 g L^{-1} glucose concentrations.

According to analysis results, the mean amount of Chl-a of *Oscillatoria* sp. biomass was determined as 0.5132 µg L^{-1} under the phototrophic culture (absence of glucose). Under the mixotrophic culture (presence of light), the experimental results showed that the chl-a content was calculated as 0.1770, 0.3380 and 0.7098 µg L^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively (Figure 2-4). Under the heterotrophic culture (absence of light), the results showed that the chl-a content was 0.2366, 0.2456 and 0.2346 µg L^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively.

Table 1. The effect of initial 2.5 g/L glucose concentration on the chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	chl- a (µg L^{-1})
1. Phototrophic (control-no glucose)	0.5079±0.002
2. Heterotrophic	0.2366±0.004
3. Mixotrophic	0.1770±0.001

Table 2. The effect of initial five g/L glucose concentration on the chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	chl- a (µg L^{-1})
1. Phototrophic (control-no glucose)	0.5201 ±0.002
2. Heterotrophic	0.2456 ±0.001
3. Mixotrophic	0.3380 ±0.002

Table 3. The effect of initial 10 g/L glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	chl- a (µg L^{-1})
1. Phototrophic (control-no glucose)	0.5116 ±0.002
2. Heterotrophic	0.2346 ±0.001
3. Mixotrophic	0.7098 ±0.001

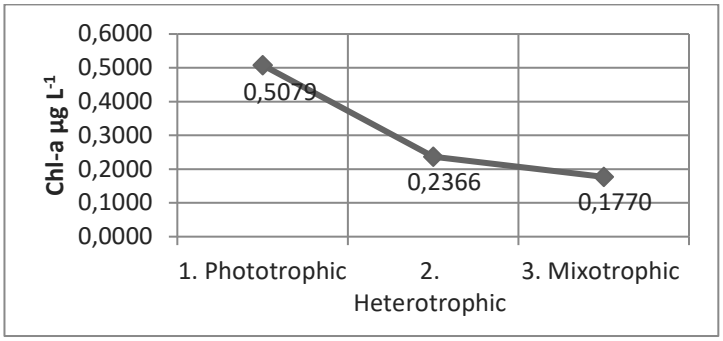

Figure 2. The effect of initial 2.5 g/L glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

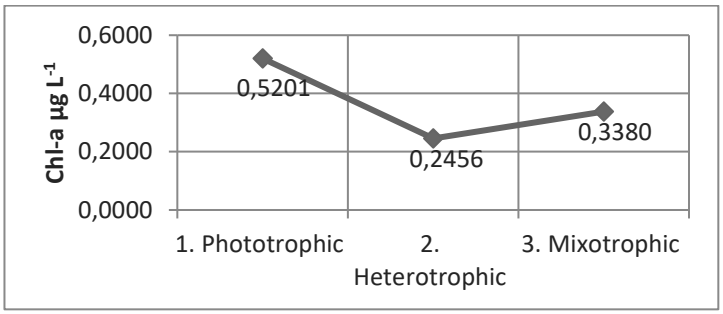

Figure 3. The effect of initial 5 g/L glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

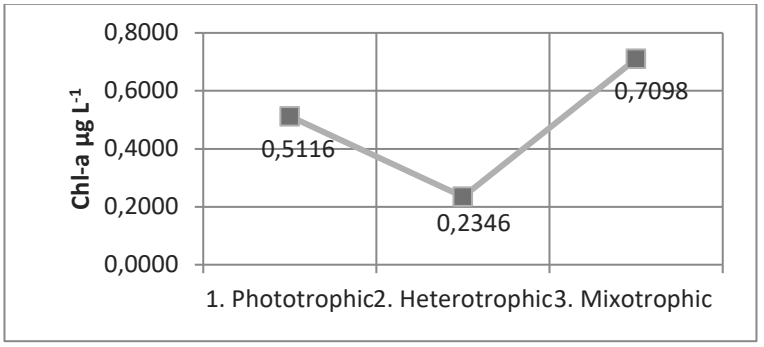

Figure 4. The effect of initial 10 g/L glucose concentration on chl-a amount (µg L^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

Since the chlorophyll concentration of cells is an indicator of photosynthesis, we can say that the decrease in chlorophyll in heterotrophic culture cells is related to the rate of photosynthesis. Due to low chlorophyll production, it can be concluded that the microalgae have preferentially used the available organic carbon as energy and carbon sources in heterotrophic metabolism rather than CO_2 in autotrophic metabolism (Cheirsilp & Torpee, 2012). According to the results of the study, we can say that the photosynthesis and trophic

mode of the algae were transformed into heterotrophic mode as a result of the high carbon source and lack of light in the environment.

Consumption of organic carbon sources by photosynthetic microorganisms' heterotrophic cultivation can decrease chlorophyll content due to changes in photosystem activity (Caporgno et al., 2012). Organic carbon sources cause a decrease in the amount of excitation energy in the photosystem. As a result, a decrease in photosystem activity is observed (Liu et al., 2019). As a result, a decrease in the amount of chlorophyll is observed (Figure 5).

The Analysis Results of Total C.H. Content (mg mL^{-1}) of Biomass

Tables 4-6 show the effects of initial 2.5, 5, and 10 g L^{-1} glucose concentration on the total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. The total C.H. content (mg mL^{-1}) of treated microalgae cells increased significantly with increased glucose concentrations under the heterotrophic (H.C.) and mixotrophic culture (M.C.) conditions. The C.H. contents of cells were affected significantly, especially at 5 and 10 g L^{-1} glucose concentrations.

The analysis determined the mean amount of total C.H. of *Oscillatoria* sp. biomass as 3.5715 mg mL^{-1} under the phototrophic culture (without glucose). Under the mixotrophic culture (presence of light), the experimental results showed that the total C.H. was calculated as 3.6150, 7.9129 and 11.3191 mg mL^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively (Figure 6-8). Under the heterotrophic culture (absence of light), the results showed that the total C.H. was 4.2969, 8.0990 and 11.5861 mg m L^{-1} in the presence of 2.5, 5 and 10 g L^{-1} glucose, respectively (Figure 9).

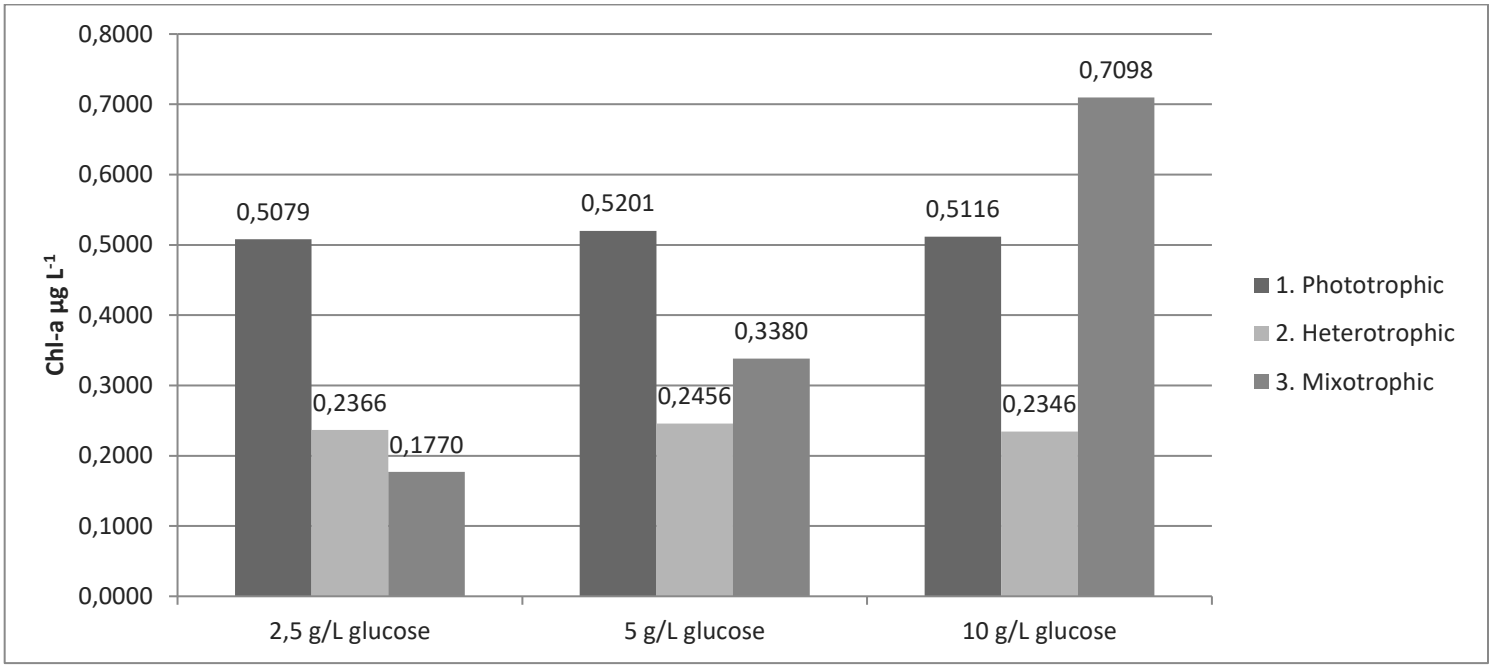

Figure 5. Chlorophyll-a contents of *Oscillatoria* cells grown in different trophic environments and glucose concentrations

Table 4. The effect of initial 2.5 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	Total CH. (mg mL^{-1})
1. Phototrophic (no glucose)	3.2927 ±0.002
2. Heterotrophic	4.2969 ±0.002
3. Mixotrophic	3.6150 ±0.001

Table 5. The effect of initial 5 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	Total CH. (mg mL^{-1})
1. Phototrophic (no glucose)	3.8836 ±0.001
2. Heterotrophic	8.0990 ±0.003
3. Mixotrophic	7.9129 ±0.001

Table 6. The effect of initial 10 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation periods. Data represent mean values ±SDM (n=3)

Cultures	Total CH. (mg mL^{-1})
1. Phototrophic (no glucose)	3.5382 ±0.002
2. Heterotrophic	11.5861 ±0.001
3. Mixotrophic	11.3191 ±0.002

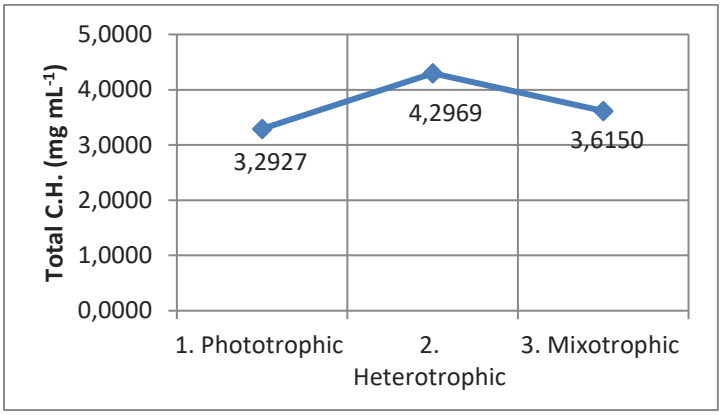

Figure 6. The effect of initial 2.5 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

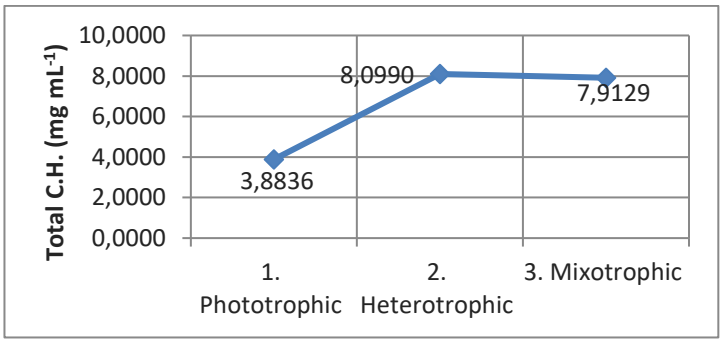

Figure 7. The effect of initial 5 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

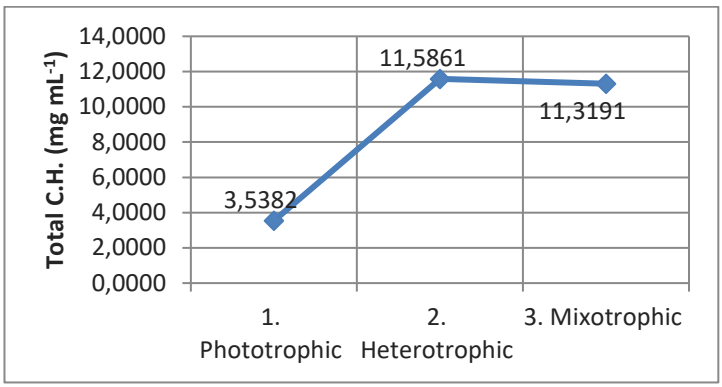

Figure 8. The effect of initial 10 g/L glucose concentration on total C.H. amount (mg mL^{-1}) of *Oscillatoria* sp. during the different trophic incubation period

Similar to the results of this study, Choi et al. (2019) have determined a high increase in carbohydrates and lipids in *Scenedesmus* cells grown in mixotrophic culture, compared with photoautotrophic and heterotrophic conditions. Glucose, often used as a carbon source for mixotrophic cultivation of various microalgae as it is easy to assimilate, can support *Oscillatoria* cell growth under heterotrophic culture conditions (absence of light). As growth occurs in the presence of glucose, a significant increase in carbohydrate concentration is observed compared to phototrophic control cultures. In this nutrition mode, the microalgae may assimilate different dissolved organic carbon (DOC) sources in addition to the inorganic carbon (CO2) fixed through photosynthesis (Abeliovich & Weisman, 1978; Chojnacka & Marquez-Rocha, 2004; Heredia-Arroya et al., 2010).

The Analysis Results of Growth Estimation (660 -730 nm)

Growth was estimated in each sample by measuring the biomass turbidity of SP homogenised suspension at wavelength 660 nm and 730 nm using a spectrophotometer (Table 7). *Os-*

cillatoria sp. were cultured under controlled laboratory conditions with 16:8 light illumination and aeration. With the cultivation of cells under 25-26 °C, all cultures had an obvious growth except the control group. The recorded optical density of *Oscillatoria* was increased from 0.6798±0.01 at 660 nm and 0.5847±0.01 at 750 nm after 24 hrs to 1.2174±0.002 at 680nm and 1.0243±0.01 at 730nm at the end of 120hrs of the experiment.

Results presented in Figure 10-11 and Table 7 demonstrate the effects of different concentrations of glucose on the biomass growth curves of *Oscillatoria* cells under mixotrophic (M.C) and heterotrophic (H.C) cultivation (120h). The O.D. of microalgae cells increased with the supplement of glucose and the glucose concentration under the H.C. and M.C. conditions. The samples supplied organic carbon source (glucose), especially at 10 g L^{-1} glucose concentrations, displayed superior growth compared with the phototrophic control. In particular, the growth rate of cells grown in M.C. and H.C. cultures was higher than that under phototrophic (control) culture. According to the experimental results, we can say that, compared to photoautotrophic culture, adding carbon sources such as glucose to mixotrophic or heterotrophic environments supports algal growth.

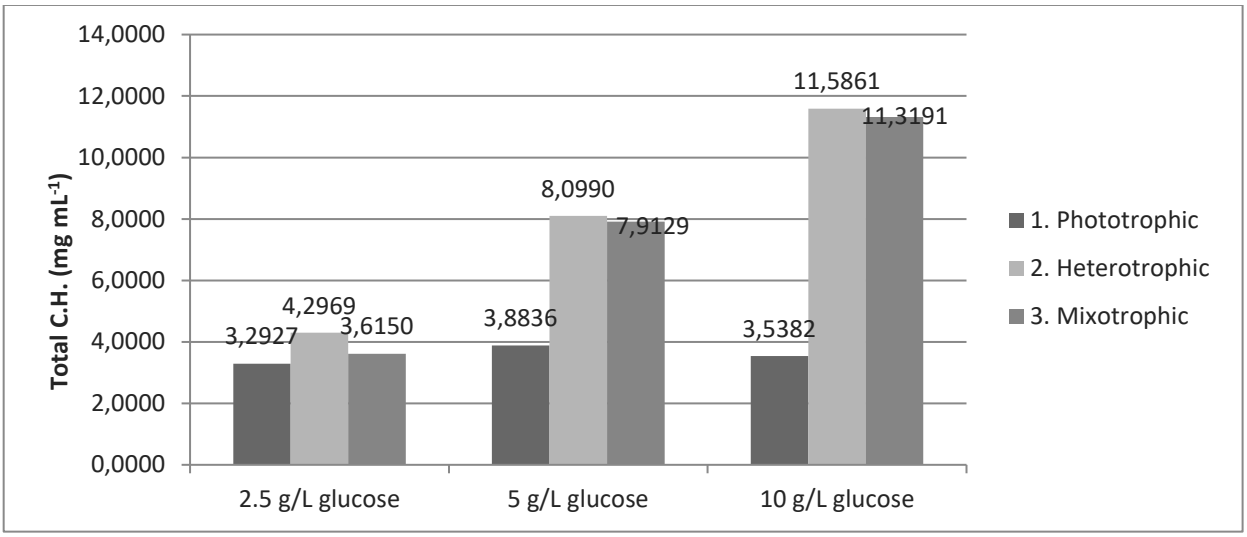

Figure 9. Total C.H. contents of *Oscillatoria* cells grown in different trophic environments and glucose concentrations

Table 7. Mean values of optical density (at 660 and 730 nm) for *Oscillatoria* growth with different concentrations of various glucose supplements at 1 th, 3 th and 5 th days of culture

O.D (660nm)	1 th day	3 th day	5 th day
Control	0.6798	0.6957	0.8412
Mixotrophic culture (2.5 g/L glucose)	0.7488	0.7496	0.7510
Mixotrophic culture (5 g/L glucose)	0.7497	0.7498	0.7563
Mixotrophic culture (10 g/L glucose)	0.8753	0.9586	1.1147
Heterotrophic culture (2.5 g/L glucose)	0.8214	0.8426	0.8515
Heterotrophic culture (5 g/L glucose)	0.8355	0.9551	0.9957
Heterotrophic culture (10 g/L glucose)	0.9597	1.1075	1.2174
O.D (730nm)	1 th day	3 th day	5 th day
Control	0.5847	0.5957	0.6982
Mixotrophic culture (2.5 g/L glucose)	0.6232	0.6863	0.7044
Mixotrophic culture (5 g/L glucose)	0.7411	0.7423	0.7433
Mixotrophic culture (10 g/L glucose)	0.7743	0.8186	1.0177
Heterotrophic culture (2.5 g/L glucose)	0.8022	0.8193	0.8215
Heterotrophic culture (5 g/L glucose)	0.8469	0.8561	0.9025
Heterotrophic culture (10 g/L glucose)	0.8604	0.9757	1.0243

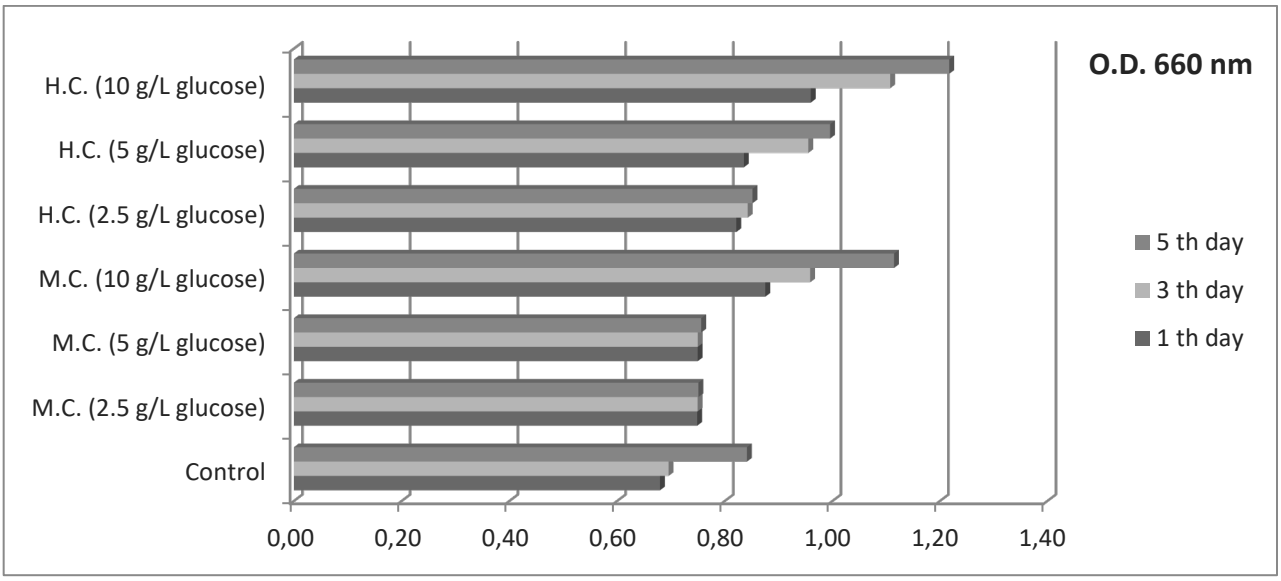

Figure 10. Mean optical density values at 660 nm for *Oscillatoria* growth with different concentrations of various glucose supplement at 1th, 3th and 5th days of culture

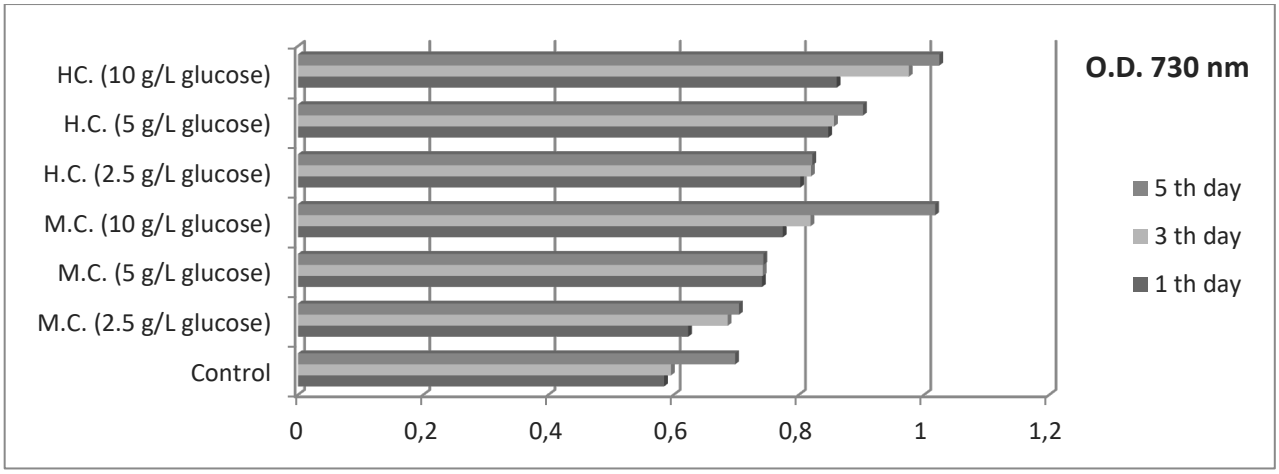

Figure 11. Mean optical density (O.D) values at 730 nm for *Oscillatoria* growth with different concentrations of various glucose supplements at 1 th, 3 th and 5 th days of culture.

Heterotrophic and mixotrophic cultures can present higher growth rates and biomass production than autotrophic ones. In this study results, the heterotrophic biomass productivity of *Oscillatoria* cells in glucose can be 1.74 times higher than the autotrophic one. Besides the reasons that limit biomass production in autotrophic cultivation systems, the bioenergetics of the heterotrophic metabolism can explain the better cultivation performance in dark conditions (Han et al., 2012).

In mixotrophic growth, there are two distinctive processes: photosynthesis (influenced by light intensity) and aerobic respiration (related to the organic substrate concentration). (Ben, 2012). The high cell density of mixotrophic cultures demonstrates that the growth-stimulating effects of light and CO_2 utilisation in mixotrophic cultures were as strong as the effects of glucose (Katarzyna & Andrzej, 2004). Mixotrophic growth offers the opportunity to increase microalgal cell concentration and volumetric productivity greatly. The increase in growth in mixotrophic culture may be because ATP formed in photochemical reactions accelerates anabolism from glucose in mixotrophic culture (Jaemin et al., 2022).

Conclusion

The main aim of the research was to determine the most suitable culture conditions among three different trophic growth environments that would increase the growth rates, chlorophyll-a and total carbohydrate of *Oscillatoria* cells. The experimental results showed that the total C.H. content was increased from 3.5715 to 11.58 61 mg mL^{-1} in the heterotrophic (the absence of light and the presence of 10 g L^{-1} glucose) BG-11 culture conditions. The chlorophyll-a content was increased from 0.1770 µg L^{-1} to 0.7098 µg L^{-1} in the mixotrophic (the presence of glucose and light) BG-11 culture conditions. As a result of the experiment, it was determined that the most suitable culture in terms of total carbohydrate and growth rate was mixotrophic and heterotrophic BG-11 (10 g L^{-1} glucose) culture condition, and in terms of chl-a was mixotrophic culture (10 g L^{-1} glucose). To generalise the results, we can say that microalgae grown in a mixotrophic culture are more effective, especially in increasing carbohydrate and chlorophyll concentrations. The finding suggests that the biomass growth curve, chl-a pigment, and C.H. production of *Oscillatoria* cells could be regulated by controlling the mixotrophic mode especially.

Compliance with Ethical Standards

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

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

Data availability: Data will be made available on request.

Funding disclosure: -

Acknowledgements: -

Disclosure: -

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Some biological parameters and cholinesterase enzyme profile of *Tilapia* sp. along Maragondon River, Cavite, Philippines

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ABSTRACT

This study evaluated *Tilapia* sp.'s biological parameters and cholinesterase enzyme activity along the Maragondon River. The biological parameters assessed were length-weight relationship and condition factor. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were measured in the brain, muscle, and hepatic tissues of *Tilapia* sp. Enzyme inhibition rates were then calculated at midstream and downstream stations relative to the reference site upstream. Results showed that *Tilapia* sp. exhibited negative allometric growth patterns ($b < 3$), supported by high correlation coefficients (0.86-0.94). The condition factor (K) values across sampling sites ranged from 1.94 to 3.82, indicating the overall fitness of *Tilapia* sp. However, AChE and BChE enzymes above the 20% threshold were observed at midstream and downstream stations of the river. Specifically, 49.03% and 48.41% inhibition in AChE and BChE of muscle tissue in midstream samples, 22.03% inhibition in the liver and 31.53% inhibition in muscle AChE at downstream station. The cholinesterase tissue localisation was also inferred, arranged from highest to lowest activity as follows: liver > brain > muscle. These findings provide valuable insights into the exposure of *Tilapia* sp. to cholinesterase inhibitors in Maragondon River, emphasising the importance of biomarkers in assessing the effect of environmental contaminants on aquatic organisms.

Keywords: Cholinesterase, Biomarkers, Pollution, Tilapia

Introduction

Freshwater ecosystems are sources of various biodiversities; they provide provisioning and regulating services but have been increasingly threatened in recent years due to overwhelming anthropogenic pressures. For instance, the intensified unsustainable agricultural production methods have made agriculture a prominent source of aquatic pollution (Catajan et al., 2023; Harisson et al., 2019; Liu et al., 2021; Zang et al., 2021). Pesticides have emerged as a major concern of all organic pollutants due to their extensive production and utilisation in agricultural countries and their high toxicity to non-target organisms (Neuwirthová et al., 2019). The unintentional release of pesticides into the environment often leads to adverse ecological impacts, affecting unintended organisms, such as fish (Mancini et al., 2019; Neuwirthová et al., 2019; Shah & Parveen, 2022). Some common pesticides are organophosphate (OPs), carbamates, and synthetic pyrethroids.

Organophosphates (OPs) and carbamates are major agrochemicals that strongly affect different neuroenzymes and the growth of various fish species (Ghazala et al., 2014). Fish are directly exposed to these pesticides by absorption through the skin, breathing, and oral intake of pesticide-contaminated water or pesticide-contaminated prey (Stanley & Preetha, 2016). Fish exposed to pesticides often experience alterations in haematological parameters and stress biomarkers (Santana et al., 2022). A notable biomarker in this organophosphate and carbamate exposure is the evaluation of cholinesterase (ChE) enzyme activities (Sepahi et al., 2023; Kaur et al., 2023). The cholinesterases (ChE) group of enzymes has been divided into two types: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Ghazala et al., 2014). AChE is a cholinergic enzyme mostly found in postsynaptic neuromuscular junctions and is important in removing acetylcholine. BChE catalyses the hydrolysis of esterscholine, including acetylcholine. The inhibitory effect of pollutants on cholinesterase may lead to acetylcholine accumulation at the synaptic cleft, disrupting normal neural transmission and causing paralysis and, ultimately, death (Colović et al., 2013).

As an agricultural country, the Philippines has relied on organophosphate (OPs) pesticides, commonly carbamates, and synthetic pyrethroids (Lu, 2022; Manuben et al., 2022). In addition, chlorinated pesticides, including aldrin, dieldrin, endrin, and heptachlor, were prevalent from the 1960s until the early 1980s (Santiago & Kwan, 2016). Following the recognition of the adverse impact of these substances on human health, the country prohibited several Persistent Organic Pollutants (POPs) in agricultural and pest control practices. By 1989, five out of twelve POPs had already been banned. Concurrently, the Department of Environment and Natural Resources (DENR) issued administrative order no. 2004-01 to regulate and eradicate PCBs nationwide. Additionally, the Philippines banned the use, sale, and import of chlorpyrifos and dichlorvos, two types of OPs, in accordance with Republic Act 19711. However, despite these regulations, the widespread utilisation of organophosphates, carbamates, and pyrethroids in agricultural pesticide applications continues to contaminate the environment and pose health risks to humans (Lu, 2010). Multiple studies have corroborated the presence and usage of POPs in various locations in the Philippines (Hallare et al., 2005; Carvalho et al., 2009; Santiago & Kwan, 2016; Villanueve et al., 2010).

The Maragondon River, situated in Cavite, Philippines, receives runoff from diverse agricultural areas throughout Maragondon, a predominantly agricultural town with the largest land area, cultivating a wide range of commodities (CLWUP-Maragondon, 2013). Studies have been conducted to evaluate the Maragondon River. Jalandoon (2018) reported heavy metals such as Pb and Cu Maragondon River levels. Another study (Pareja, 2015) highlighted the high nitrogen and phosphorus loads from households and urban runoff due to inadequate sewage treatments. Despite existing reports on contaminants like heavy metals and urban runoff, there is a notable absence of published studies investigating persistent organic pollutants in the Maragondon River. Given that the Maragondon River receives runoff from agricultural sources and considering the lack of research on cholinesterase inhibitors such as organophosphates and carbamates in the river, our study aimed to evaluate the exposure of *Tilapia* sp. to these contaminants using cholinesterase enzyme activity as a biomarker. This study provides baseline data on the aquatic health of the Maragondon River by evaluating the physiological response of fish populations in the ecosystem.

Materials and Methods

Site Description

Tilapia sp. were collected from the Maragondon River, a significant river basin in Cavite, Philippines. This river runs through the upland barangays (administrative districts) of Maragondon, culminating at Ternate, Cavite, where it empties to Manila Bay. This study divided the river into three sampling stations: upstream, midstream, and downstream.

In the Philippines, inland waters are categorised into different classes: AA, A, B, C, and D (DAO, 1994). The upstream station (N14°14'44.9" E120°46'55.6") is classified as Class B. It

features a rocky substrate, abundant vegetation, and a width of 18-20 m. Class B waters have good to excellent water quality, with allowable treated wastewater discharges. The midstream station (N14°16'23.3" E120°44'5.3") has an estimated 82-87 m width. Lastly, the downstream station (N14°17'01.6" E120°42'51.1") spans an estimated 132-135 m width. Both midstream and downstream stations are classified as Class C and are used for agriculture and aquaculture, where water quality may be impaired.

The samples from the upstream station were considered reference samples, given that they were collected from Class B water, which is characterised by excellent water quality.

Fish Sampling and Analyses

Juvenile tilapia samples (N=48) were collected from the three stations along the Maragondon River. Haphazard and random seine net collection was conducted, with samples not segregated by sex. This study's use of tilapia fish samples was predicated on its wide distribution and commercial significance in the Philippine waters (Guerrero, 2022). The sampling was conducted during the Philippines' dry season (April 2023). To account for the possibility of interspecific hybridisation and the lack of specificity in the feral fish samples' gene pool, the tilapia samples in this paper were referred to as *Tilapia* sp. Following capture, the fish were anesthetised and then carefully packed in ice. The specimens were transported to the Fish Diseases and Toxicology Laboratory, Cavite State University Naic, for analysis. In the laboratory, the biometric data, including total length (TL, cm) and wet weight (W, g), were examined and recorded. The TL (cm) measurements were taken from the tip of the mouth to the tip of the longer lobe of the caudal fin using a digital calliper, whereas the W (g) was measured up to 0.0001g accuracy using an analytical weighing balance. Additionally, each fish was dissected, and the hepatic tissues, brain, liver, and cut of muscles were separated. Tissues were then stored in a -18 ˚C laboratory freezer for subsequent analysis. Samples were analysed within 7 days of storage to avoid enzyme degradation.

Length-Weight Relationship and Condition Factor

The length-weight relationship (LWR) was estimated by using the equation. $W = aL^b$, where W = body weight (g), L = total length (cm), a is a scaling constant, and b is allometric growth. A logarithmic transformation ($log\ W = log\ a + log\ b\ L$) was used to make the relationship linear. A regression analysis estimated the intercept (Log a) and the regression coefficient or slope (b). Meanwhile, condition factor (K) was calculated following a previously defined equation as $K = \frac{W}{L^3}\ x\ 100$. Where by K = condition factor; W = the weight (g); L = the total length of the fish (cm). Isometric growth in a fish species is identified when the estimated regression coefficient value approaches 3. Conversely, if the coefficient deviates from 3, fish growth is categorised as either negative allometric ($b < 3$) or positive isometric ($b > 3$) (Froese, 2006).

Cholinesterase Enzyme Assay

Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity was determined using the rapid colourimetric method following a previously established standard (Ellman et al., 1961). Briefly, Tilapia sp.'s brain, liver, and muscle tissues were homogenised in phosphate buffer (pH 8.0) at a concentration of approximately 20 mg/mL. A 0.4 mL aliquot of the homogenate was mixed with 2.6 mL phosphate buffer, followed by 100 µL of dithiobisnitrobenzoic acid reagent. Absorbance was then recorded at 412 nm. Subsequently, either acetylthiocholine iodide (AChE) or butyryl thiocholine chloride (BChE), in a volume of 20 µL, was introduced as substrate. The enzyme activity, as indicated by the hydrolysis of acetylthiocholine and S-butyrylthiocholine, was monitored at 412 nm for 7 minutes. The inhibition rate of cholinesterase activity was then calculated using the equation:

$$[(A_{rs} - A_{ss})/100]\ x\ 100$$

Where büyük harf A. alt r s is the absorbance of the reference site (upstream), and A_{ss} is the absorbance of sampling sites midstream and downstream.

Statistical Analyses

All data in this study were presented as means ± standard deviation unless otherwise specified. One-way analysis of variance (ANOVA) was conducted to determine significant differences in AChE and BChE enzyme activities in different tissues of *Tilapia* sp. Subsequently, Tukey's honest significant difference (HSD) post hoc test was employed to identify statistically significant differences. In all cases, a significance level of $p < 0.05$ was considered. All analyses were conducted using Microsoft 365 software under appropriate licensing.

Results and Discussion

Length-Weight Relationship and Condition Factor

Tilapia sp. samples of larger size were obtained from the midstream site, exhibiting a mean length of 19.13 ±2.63 cm and a mean weight of 135.80 ±50.46 g. Subsequently, downstream samples displayed a mean length and weight of 94.96 ±33.53 cm and 16.76 ±2.09 g, while upstream samples were notably smaller, measuring 10.68 ±1.61 cm in length and weighing 46.64 ±10.90 g (Table 1).

In terms of growth patterns, *Tilapia* sp. displayed a negative allometric pattern along the Maragondon River, with *b* values of 2.45 (Upstream), 2.59 (Midstream), and 2.72 (Downstream). Regarding the condition factor, only *Tilapia* sp. samples collected from the upstream fell within the ideal range of *K* values, ranging from 2.90 to 4.80, with a value of 3.82. In contrast, the condition factors of samples from the midstream and downstream were outside the ideal range, with values of 1.94 and 2.01, respectively (Table 1).

The observed allometric growth pattern, where the fish become more rotund as their length increases, is supported by the high correlation coefficients (r^2) ranging from 0.86 to 0.94 across the sampling sites (Figure 1A-1C). This indicates a strong relationship between length and weight, confirming the negative allometric growth pattern.

Table 1. Descriptive statistics and some biological parameters of *Tilapia sp.* were collected from differ-ent sampling sites in the Maragondon River, Cavite, Philippines

Sampling Site	a	b	r^2	Mean Length (cm)	Mean Weight (g)	Condition Factor
Upstream	-2.60	2.45	0.94	10.68 ± 1.61	46.64 ± 10.90	3.82
Midstream	-2.76	2.59	0.86	19.13 ± 2.63	135.80 ± 50.46	1.94
Downstream	-3.13	2.71	0.90	16.76 ± 2.09	94.96 ± 33.53	2.01

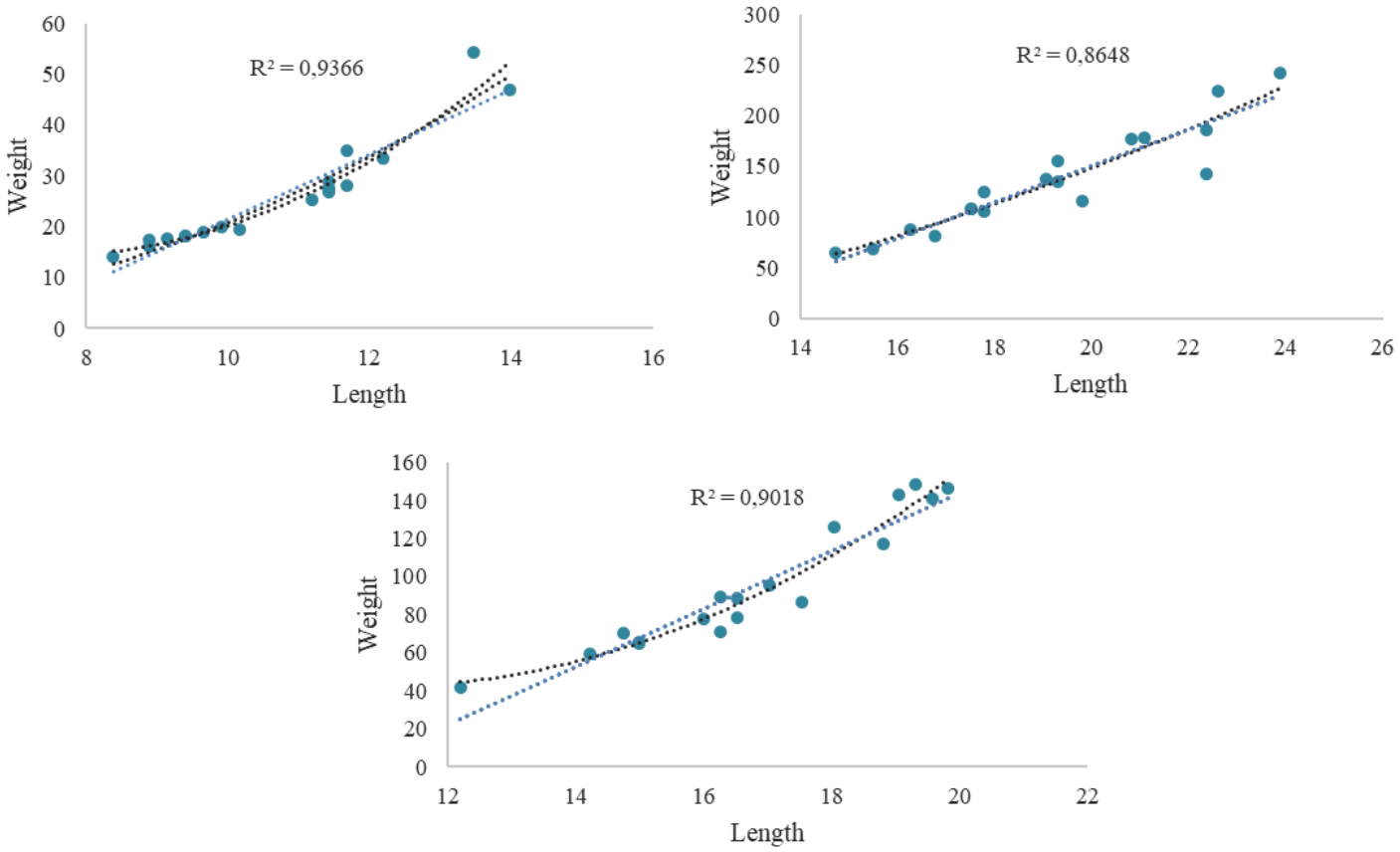

Figure 1. Length-weight relationship of *Tilapia* sp. along Maragondon River during warm months. (a) Upstream (b) Midstream (c) Downstream.

Cholinesterase Enzyme Activities of Tilapia sp. Along Maragondon River

Brain, liver, and muscle cholinesterase activities expressed in mol min^{-1} g^{-1} tissue of *Tilapia* sp. obtained from three sampling sites are presented in Figure 2.

The brain acetylcholinesterase (AChE) activity in *Tilapia* sp. was comparable along the Maragondon River. The mean AChE activity in samples from the upstream was 1.039 ±0.405 mol min^{-1} g^{-1}, comparable with mean values of 0.87 ±0.179 in the midstream station and 0.992 ±0.402 mol min^{-1} g^{-1} downstream. On the other hand, the butyrylcholinesterase (BChE) enzyme activities in the brain of *Tilapia* sp. exhibited a different pattern. The midstream shows higher BChE activity, with a mean value of 1.024 ±0.327 mol min^{-1} g^{-1}, compared to both the upstream (0.969 ±0.418 mol min^{-1} g^{-1}) and downstream (0.780 ±0.306 mol min^{-1} g^{-1}).

In the liver tissue, the enzyme activity of *Tilapia* sp. from the Maragondon River is lower in midstream than upstream and downstream (Figure 3). In midstream, the enzyme activity of AChE is 0.276 ±0.276, and BChE is 0.712 ±0.427 mol min-1 g-1, which is lower compared with 0.889 ±0.351, 0.914 ±0.364, and 1.312 ±0.50, 1.022 ±0.23 mol min^{-1} g^{-1} of upstream and downstream.

In muscle tissue, AChE enzyme activity is higher upstream of the river with a mean value of 0.151 ±0.083 mol min^{-1} g^{-1} compared with 0.077 ± 0.038 and 0.078 ±0.066 mol min^{-1} g^{-1} of midstream and downstream, respectively. Meanwhile, for BChE enzyme activity, higher mean values were observed in midstream with a mean value of 0.078 ±0.085 mol min^{-1} g^{-1} compared with 0.055 ±0.033 and 0.034 ±0.040 mol min^{-1} g^{-1} of upstream and downstream.

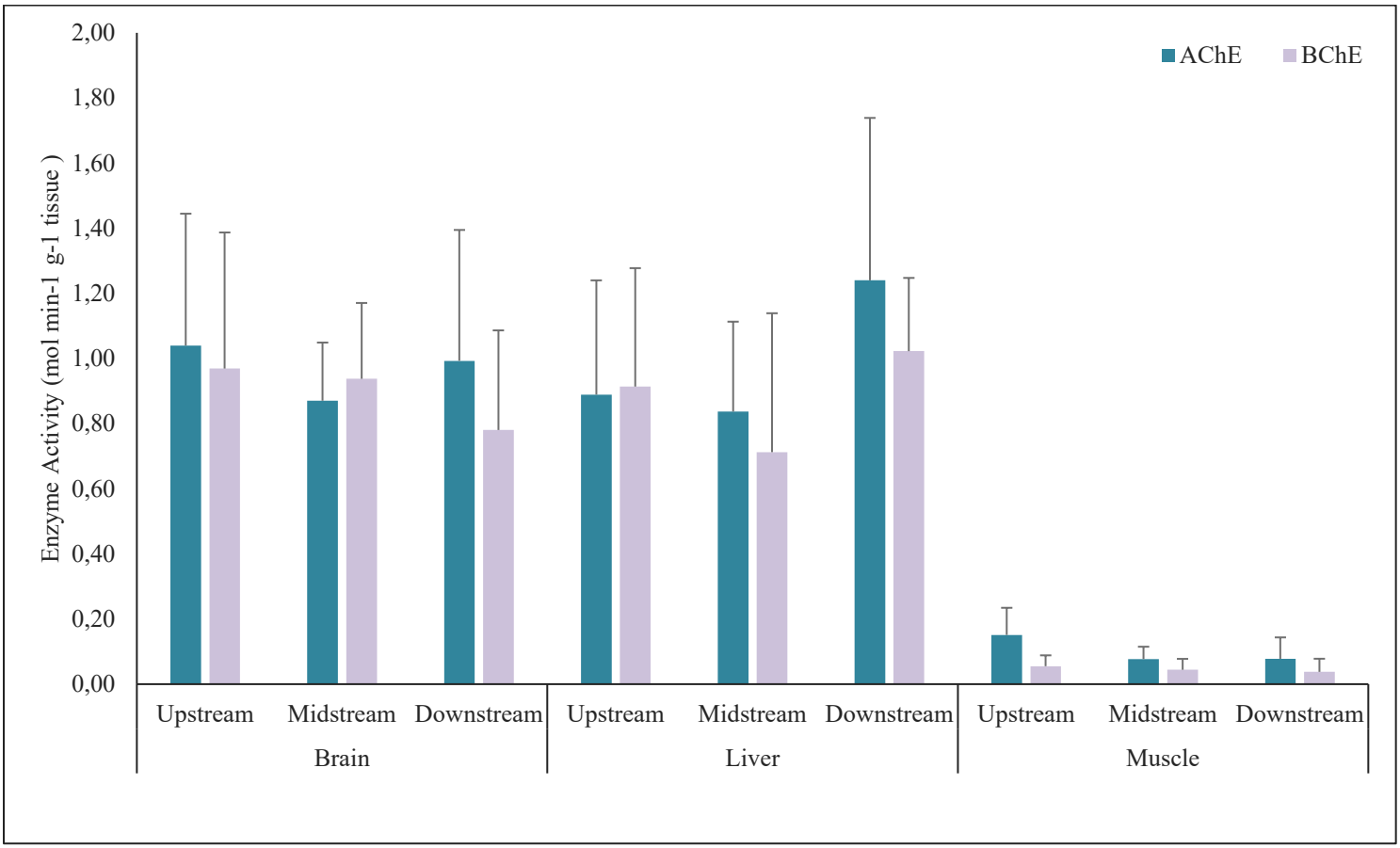

Figure 2. Acetylcholinesterase and butyrylcholinesterase enzyme activities in different tissues of *Tilapia sp.* collected along the Maragondon River

Inhibition Rates of Cholinesterase Enzyme Activity

The differences in enzyme activities between midstream and downstream samples were expressed as inhibition rates relative to the upstream station (Table 2). The highest inhibition rates for AChE and BChE were found in the muscle of midstream samples, measuring 49.03% and 48.41%, respectively. This was followed by the muscle in the downstream station, exhibiting inhibition rates of 19.96% and 31.53%. Meanwhile, negative inhibition rate values were observed in BChE activities in both midstream (-39.14%) and downstream (-11.91%) samples, indicating that the enzyme activities were higher than those in the upstream station.

Tissue Distribution of Cholinesterase Enzyme in Tilapia sp.

The distribution of cholinesterase enzymes in different tissues of *Tilapia* sp. exhibited significant differences. AChE and BChE enzyme activities were significantly higher in the hepatic and brain tissues than in the muscular tissue of *Tilapia* sp. (Figure 3). In the hepatic tissue, the AChE enzyme activity was measured to be 1.01 ±0.43 mol min^{-1} g^{-1}, while in the brain tissue, it was 0.92 ±0.34 mol min^{-1} g^{-1}. These values were significantly higher than the AChE activity observed in the muscular tissue, which was only 0.10 ±0.07 mol min^{-1} g^{-1}. Similar patterns were observed for BChE enzyme activities. The mean values of BChE activity in the hepatic tissue and brain were 0.88 ±0.36 mol min^{-1} g^{-1} and 0.92 ±0.34 mol min^{-1} g^{-1}, respectively. These values were significantly higher than the BChE activity observed in the muscular tissue, which was 0.06 ±0.06 mol min^{-1} g^{-1} ($p < 0.01$).

Table 2. Inhibition rate of acetylcholinesterase and butyrylcholinesterase in brain, liver, and muscle of *Tilapia* sp. collected in Maragondon River

	Midstream		Downstream	
	AChE	**BChE**	**AChE**	**BChE**
Brain	16.29	4.50	3.24	19.47
Liver	5.85	-39.14	22.03	-11.91
Muscle	49.03	48.41	19.96	31.53

***** The values represent the inhibition rates (%) for AChE and BChE activities in different tissues of *Tilapia* sp.

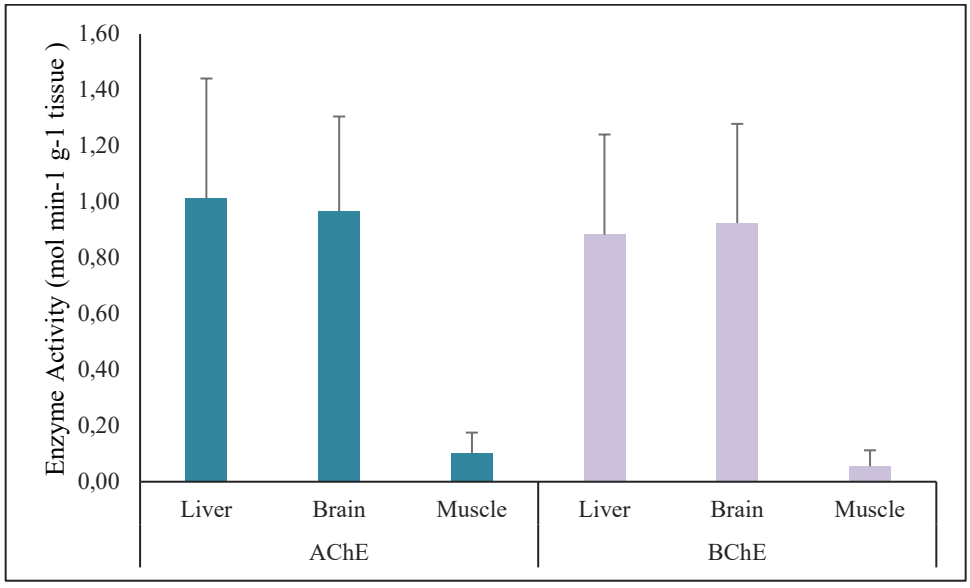

Figure 3. Tissue distribution of cholinesterase enzymes in *Tilapia* sp.

The length-weight relationship (LWR) provides valuable information for studying the growth dynamics of fish populations. Factors affecting differences in LWR among species include variations in environmental conditions, health of fish, food availability, and spawning period (Suquet et al., 2005). Moreover, the growth patterns of fish may be linked to the productivity of the aquatic habitat (Przybylski, 1996; Randall & Minns, 2000; Randall, 2002). In the current study, the LWR regression slope (b) value of *Tilapia* sp. samples along the Maragondon River continuum demonstrated a negative allometric growth pattern; fish grew with weight increasing at a slower rate ($b<3$) compared to their length. This result is in line with previous studies which also reported negative allometric growth patterns in different cichlid species (Peña Messina et al., 2010; Dalu et al., 2013; Zuh et al., 2019; Abdalla et al., 2023). The negative allometry observed in samples from the Maragondon River, congruent with previous reports, indicates heterogeneity, with body weights varying non-uniformly about the cube of total length (Kwikiriza et al., 2023).

The condition factor (*K*), on the other hand, reflects a species' robustness and overall health, specifically indicating its feeding status, health, sexual maturity, and adaptability to its environment. Fish species with a *K* value near or equal to one are generally considered to have a good overall condition (Datta et al., 2013). Moreover, *K* values above one indicates that fish species are adequately fed and thriving in optimal environmental conditions. In this study, *K* values were observed to be above the ideal value in the following order: upstream > downstream > midstream. These *K* values suggest that the Maragondon River provides favourable conditions for the growth of *Tilapia* sp.

Despite the good environmental conditions indicated by both the LWR and K values of *Tilapia* sp. in the Maragondon River, inhibition of cholinesterase enzyme activities was observed. A 20% inhibition of cholinesterase enzymes suggests organismal exposure to anticholinesterase compounds (Menéndez-Helman et al., 2015; Fajardo & Ocampo, 2018). In this study, significant inhibition was observed in midstream and downstream samples. In midstream samples, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were inhibited in the muscle, with inhibition rates of 49.03% and 48.41%, respectively. Meanwhile, in downstream samples, significant AChE inhibition was observed in the liver at 22.03%. AChE and BChE were also inhibited in the muscles of fish collected downstream, with 19.96% and 31.53% rates.

Cholinesterase enzyme activity is used as a biomarker for assessing neurotoxicity caused by organophosphates and carbamates (Sepahi et al., 2023; Kaur et al., 2023). Although cholinesterase, including AChE and BChE are considered a specific neurotoxic biomarker of organophosphorus and carbamates exposure, several studies also describe an alteration of their activities by other pesticides in fish including fungicides (Melefa & Nwani, 2021; Kovačević et al., 2023), synthetic pyrethroids (Martins-Gomes et al., 2022) and glyphosate (Bernal-Rey et al., 2020; Thanomsit et al., 2021; Cuzziol Boccioni et al., 2022). Due to the lipophilic characteristics of pesticides from the pyrethroid family, they may interact with the active site, causing inhibition of enzymatic activity (Martins-Gomes et al., 2022).

In Maragondon River, where inhibition of cholinesterase enzyme activity was observed in *Tilapia* sp. samples, there is a high likelihood that some, if not all, of these contaminants are present. Although the concentration of anticholinesterase compounds is still unknown, the midstream station is hypothesised to have the most contaminant levels, as suggested by the results of this study. This is also supported by the results of Jalandoon (2018), who found that midstream stations have the highest amount of nitrates and phosphates. Moreover, the phosphate concentration exceeded the set standards at 0.5-1.0 mg/L limit. The highest cholinesterase enzyme inhibition observed in the midstream station of the river might be attributed to the nearby farmlands, where pesticides and fertilisers might run off into the river.

The tissue distribution of cholinesterase enzymes can also be inferred from the results of this study. AChE and BChE tissue localisation patterns are arranged from highest to lowest enzyme activity as follows: liver > brain > muscle. The significant abundance of cholinesterase enzymes in the liver and brain compared to muscle can be attributed to their physiological functions. The abundance of cholinesterase activity in brain tissues can be attributed to its role in signal termination at cholinergic synapses through the rapid hydrolysis of the neurotransmitter acetylcholine. Cholinesterase activity is also abundant in the liver tissue of *Tilapia* sp. because these enzymes are synthesised in the liver (Weber et al., 1999). Moreover, cholinesterase enzymes play a role in detoxification. Many organophosphates require desulfuration (bioactivation) by cytochrome P450 (CYP) into their active oxon form to effectively inhibit cholinesterase in the liver (Sams et al., 2004; Ellison et al., 2012). Cholinesterase enzyme was also observed in the muscular tissue of *Tilapia* sp., albeit statistically lower compared to liver and brain enzyme activity. The presence of cholinesterase in muscular tissue is primarily due to cholinergic innervation (Lopes et al., 2014)

This study provides baseline information on the exposure of *Tilapia* sp. to anticholinesterase contaminants, such as organophosphates and carbamates, in the Maragondon River. It is noteworthy that although the *K* value of *Tilapia* sp. along the Maragondon River suggests the overall fitness of fish, the effect of contaminants on cholinesterase enzyme activities was not reflected in these values. This suggests that relying solely on biological parameters to conclude the overall health of fish might not be appropriate. For future research directions, it is recommended that studies be conducted on the levels of organophosphates and carbamates in the Maragondon River and their correlation with cholinesterase enzyme activity. Additionally, future studies should consider sorting and disaggregating the sex of samples to account for the sexual dimorphic characteristics of *Tilapia* sp. Finally, further exploration of other biological endpoints in model organisms should be pursued to gather valuable information for managing the Maragondon River.

Conclusion

Our findings reveal a negative allometric growth pattern ($b<3$) in the *Tilapia* sp. Additionally, the condition factor (K) indicates that *Tilapia* sp. are generally fit and thriving in optimal conditions for growth. However, inhibition of AChE and BChE enzymes in the river's midstream (muscle tissue) and downstream (muscle and liver) stations was observed. These results underscore the importance of considering multiple biomarkers and indices to assess organismal health comprehensively. The findings from this study provide valuable insights into the ecotoxicological status of *Tilapia* sp. in the Maragondon River and emphasise the significance of biomarker studies in monitoring and managing the impact of environmental contaminants on aquatic ecosystems.

Compliance with Ethical Standards

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

Ethics committee approval: The authors affirm that all international, national, and institutional guidelines for the care and use of laboratory animals have been diligently followed and adhered to throughout this study.

Data availability: Data will be made available on request.

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Disclosure: -

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An extensive review of human health benefits from consuming farmed or wild fish with special reference to gilthead seabream (*Sparus aurata*)

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ABSTRACT

In public, there is a significant concern regarding the safety and quality of farmed fish that poses problems for fish farmers in marketing. There is widespread recognition that farmed fish are less healthy than their wild equivalent, mostly attributed to unhealthy farm conditions or the ingredients used in artificial diets for aquaculture. However, the nutritional quality of farmed -or wild-caught fish may differ based on regional variation or a cultural environment's complex aspects. Whether farmed or wild fish provide better product quality is a long-standing matter for consumer preferences and marketing. Information was collected from a wide range of references through an extensive literature review, and detailed evaluations were made on the health levels of cultured fish and natural fish in human consumption. Therefore, the present study provides an extensive review to address the differences in the nutritional contribution of farmed and wild fish for human consumers. Addressing the questions arising from consumers' concerns will undoubtedly support farmers in their challenging marketing efforts.

Keywords: Consumer concern, Social awareness, Healthy food, Cultured fish, Food safety

Introduction

Limited freshwater resources and land available for agricultural production brought new challenges to seeking alternative food sources to meet the increasing demand from human consumers worldwide. With this respect, a common view is that the production of aquatic foods will need to expand globally to meet population-driven demand (Naylor et al., 2021). Capture fisheries can no longer meet the global demand for fish, making aquaculture the most possible alternative for supporting increased fish consumption (López-Mas et al., 2021). Stabilising fishing yields and using technological equipment such as sonars and eco-sounders in fishing vessels increase the pressure on wild populations that may not increase within the present state of fishing operations. World fish production was reported at around 180 million tons (Mt) in 2018, with a total first sale value estimated at USD 401 billion, of which 82 Mt (valued at USD 250 billion) was supplied by aquaculture production. Out of the total production, 156.4 Mt (FAO, 2020) were used for human consumption, equivalent to an estimated annual supply of 20.5 kg per capita (FAO, 2020) for a World population of 7.6 billion in 2018 (FAO, 2020). The production of aquatic animals in 2018 was dominated by finfish (54.3 Mt, valued 139.7 billion US$), harvested from inland aquaculture (46.9 Mt, valued 104.3 billion US$) as well as marine and coastal aquaculture facilities (7.3 Mt, valued 35.4 billion US$) (FAO, 2020). Cage aquaculture, the locomotive of the fish production sector, is in a rapid growth period and operates in more exposed sites nowadays (Bostock et al., 2010). Cage aquaculture in the Mediterranean was initiated in near-shore sites with wooden-framed floating constructions and shallow net depth in the early 80s with gilthead seabream and European seabass juveniles collected from the nature during the spring season when juveniles remain in shallow coastal waters. With the development of technological and innovative equipment, limitations of in-shore suitable sites, and increasing public concern about environmental impacts from fish farming, these farms have been moved to more exposed sites with the extension of cage size and net volumes. Since then, cage aquaculture has been in a rapid growth period and is considered the locomotive of the fish production sector that has recently been operating in more exposed sites (Bostock et al., 2010) with a production of over 7.3 Mt of fish in 2018 (FAO, 2021). Even though cage systems are operating in very exposed sites today, with strict control mechanisms of local governments in terms of environmental impact assessment and best management practices with the consideration of carrying capacity estimations, there is still remarkable concern in society with questions unclear for consumers in regards to the faster growth of farmed fish compared to the wild populations of same species and whether the farmed fish is safe for human consumption. The consumers' general belief that farmed fish grow faster than their respective equivalents caught from nature is mainly attributed to unfavourable conditions in the culture environment or even to dietary ingredients used in aqua-feeds, which is a set of problems for fish farmers in marketing. However, the nutritional quality of farmed -or wild-caught fish may depend on regional conditions or a complex of aspects in the cultural environment. Whether farmed or wild fish provide better flesh quality is a long-standing matter for consumer preferences and marketing challenges. The image of farmed fish among consumers is less positive compared to their respective wild-caught individuals, and images of food products are mainly affected by consumers' choices, which are highly advised to consider in marketing strategies of fishery products (López-Mas et al., 2021).

The gilthead seabream (*Sparus aurata*) is one of the main fish species dominating the Turkish marine aquaculture industry, with a harvest yield of over 130.000 tons out of a total of 467.048 tons in 2021 (Yigit et al., 2023). Therefore, the present review aims to provide information for understanding the differences in fish growth between farmed individuals, particularly in seabream versus natural fish populations, with a comparative evaluation of age-growth relation and supply helpful information for fish farmers in challenging marketing efforts as well as for consumers relieve in terms concerning food safety and food quality.

Developing Resistant Cage Nettings: Reduced Risks of Net Ruptures and Fish Escapes

Among different fish production systems, marine aquaculture operating in exposed sites uses flexible but durable materials resistant to heavy storms and typhoons. Different from the early 80s, cage farming operating at the very edge of limits today is considered one of the most dangerous industrial activities with high safety risks forced by heavy storms and hurricanes (Holmen et al., 2017). Therefore, the strength, durability, and flexibility of the material used in cage construction are very important and need high technology. Offshore cage aquaculture, a rapidly growing industry in exposed sea conditions (Bostock et al., 2010), supplies high-quality food of over seven million tons of fish (FAO, 2021), covering an important amount of the food demand for the growing world population. Resistant offshore cage systems, successfully used in Mediterranean aquaculture today, result from long-term experience and know-how. However, remarkable public concerns remain about fish escaping from cage nets due to operational failures or technical damage to nettings, cage

components and structures in heavy storms (Arechavala-López et al., 2012). However, based on technological developments, cage systems' strength and resistance have increased. Further, using ultrahigh molecular weight polyethene (UHMWPE) nets in aquaculture provided high performance in terms of resistance and strength. Nowadays, more and more farms replace traditional nylon nettings with strength UHMWPE nets (personal communications: Melih Geçgil, Operation and Logistics Director of Kılıç Co. 07.03.2021 (RIP 11.04.2021); Huseyin Cakir, Founder of Cakir Fishing Co. 20.10.2020; Huseyin Ek, CEO of Akuakare Co. 23.08.2021. Alternatively, during the last decade, research efforts have been made to introduce high-strength antimicrobial and anti-biofouling copper alloy mesh material for aquaculture economies (Yigit et al., 2017).

Growth Performance and Welfare of Farmed Versus Wild Fish: Conditions and Impacts

Numerous studies on age, growth, reproduction, feeding behaviour, etc., are available in farmed fish, in contrast to their respective wild-caught equivalents in the Mediterranean (Chaoui et al., 2006). Reports on farmed fish are more comprehensive, with consistent findings. In contrast, studies on wild-caught fish populations provide a wide range of results due to the characteristics of the environment at optimum -or near optimum levels in farm conditions versus year-around variations in the natural environment. A direct comparison of growth between farmed fish and wild populations might be misleading. It may not accurately focus on growth performance in two different environmental conditions.

Growth of fish, whether in farm conditions or the natural habitat, is a complex process depending on several biotic or abiotic factors of environmental conditions including photoperiod (Wendelaar-Bonga, 2011), temperature (Carriquiriborde et al., 2009), salinity (Tipsmark et al., 2004), dissolved oxygen, pH (Kestemont & Baras, 2001), elevated nitrogenous compounds of nitrite and ammonia (Schram et al., 2010), food intake, diet characteristics and quality, management practices, stocking densities, species and genetic differences (Lanari et al., 2002), predator effects, or a combination and interaction of all these factors (Imsland et al., 2001). Further, feed quality and feeding strategies may influence fish growth; in their comprehensive report, Hernández et al. (2003) underlined that ration size affected growth, reducing the time to reach market size. No significant differences were observed when fish were fed at 80 or 100% ration size. However, growth performance declined significantly when the feeding rate was reduced to 60% of the normal ration size. Further, the authors explained that the growth performance was strongly related to geographical areas. Regardless of the ratio size, it was noted that the time required for a seabream to reach any size was shorter in the Atlantic than in the Mediterranean area.

Overall, the control of foraging and feed intake is related to interactions between the brain and the signal from the surrounding aquatic environment via sensory information transferred through hormones and nutrient molecules in the blood system that either stimulate or prevent food intake (Nguyen, 2015). Unlike wild individuals, fish in culture conditions are protected from the peripheral environment with easy access to abundant food and typically without predators (Arechavala-López, 2012). The wild individuals, however, have to protect themselves against circumjacent predators, leading to a significant amount of energy expenditure for sheltering or hiding efforts, tissue recovery and performance loss from wounds (Sinclair et al., 2011). Stressful conditions may decrease appetite and voluntary feed intake, which is reported as one of the main reasons for growth suppression in fish (Wendelaar-Bonga, 1997).

When estimating dietary energy requirements or tissue deposition and growth utilisation, a wide variation can be noted among different species or fish sizes within the same species based on tissue composition and utilisation efficiency (Lupatsch, 2005). Generally, fish in favourable conditions without stress, and the energy budget is partitioned into five parts (Tort, 2011). Removing the basic metabolic needs, the fish in a no-stress environment assign their energy to growth. Fish going through mating and reproduction may shift a significant part of the energy absorbed from food into use for reproduction rather than growth. When fish encounter stress, the requirement level for energy to maintain growth or reproduction will be increased (Tort, 2011). Reaching the highest growth rate at harvest with a minimum cost and per cent share of feed expenses, which comprises around 60% of the total operational and production costs (Ergün et al., 2020), is the main goal in aquaculture facilities. Hence, preventing stress factors or minimising stress levels is a challenge in aquaculture management for increased productivity on fish farms.

Unlike the wild populations, the only competition in farm conditions that fish encounter is due to the crowding effect strongly related to stocking densities, which is a matter of management practice. A fish stocking rate of around 20-25 kg per cubic meter is common in commercial seabream culture in Mediterranean cage farming. Sánchez-Muros et al. (2017) did not observe any differences in cortisol levels as an indicator of stress condition in fish kept at a biomass level of 20 kg/m^3 compared to those cultured at a lower density of 5 kg/m^3 stocking rate; however, fish reared at higher biomass

densities presented a significantly reduced growth performance compared to the lower biomass condition. Batzina et al. (2014) reported less aggressive behaviour in seabream juveniles with more even distribution at higher stocking rates of 9.7 to 29.9 kg/m^3, compared to those kept in lower densities of 4.9 to 14.7 kg/m^3, attributed to a more favourable social environment in higher stocking rates, that probably provides an ambient similar to the natural habitat in terms of socialised fish schooling behaviour for farmed individuals.

In nature, wild fish are challenged in a competitive environment in many aspects, not only in foraging but also in hiding from predators, breeding and mating, habitat settlement or migration behaviours, which are under the severe influence of various environmental conditions. These matters affect the growth progress of natural fish populations if the fishermen do not capture them. Among different environmental impacts, marine currents or the availability of sufficient food resources, for example, may influence biological aspects of migration behaviour or habitat use, which is still a significant area of investigation, and there is a lack of information about the structural knowledge in general (Rossi et al., 2006).

Several reasons, such as biomass control, improved fish welfare, good feeding practices, and management regimes, might be the key to parasite-free fish under farm conditions. Besides, the anti-microbial and anti-fouling properties of the nettings are also important to ensure a parasite-free cage environment. Parasites, with their deleterious effects, may invade, move around or grow in -or on the fish and influence growth performance, survival, or even the reproductive efficiency and fitness of the farmed fish (Barber et al., 2000). Biofouling development on cage mesh may host parasites and other pathogens, which, in time, pass over and invade the fish, especially at reduced welfare conditions of physiological imbalance or general malaise, resulting in disease and severe losses at extreme conditions. Hence, nets used in cage farms are treated with antifouling coats to prevent biofouling and attachment of sessile organisms such as algae or mussels on the mesh. Therefore, the aquaculture industry's rapid growth, challenging the sustainability of coastal ecosystems and economies, can only reach high profits with good farm management set forward to produce disease-free fish with improved welfare.

Various reliable data are available on wild seabream's age-length or length-weight relations. However, most of these studies are on a regional and local basis. Also, different fish species show different genetic patterns, such as the case of seabream compared to seabass (Arechavala-López, 2012). Some earlier studies reported that wild seabream displayed a slight genetic variation among the Mediterranean populations, but these differences were unrelated to geographical factors (Palma et al., 2001; Rossi et al., 2006). However, a genetic variation between the wild populations from the Atlantic and the Mediterranean and within the wild individuals from the Mediterranean has been identified using fine-scale differences (De Innocentiis et al., 2004). Seasonal migration of wild populations and consequences of inter-crossing between neighbour populations might be a reason for the slight genetic variation (Palma et al., 2001), which can explain the discrepancies among earlier studies. Despite some divergences, these studies prove the differentiation between wild populations and farmed specimens (Arechavala-López, 2012).

Considering the findings of Al-Zahaby et al. (2018), a 3-year wild seabream weighing around 390 g would correspond to the harvest size of a farmed seabream grown in a cage farm for a year and a half, depending on water temperature (Lupatsch, 2005). Clear evidence for the significant influence of water temperature on gilthead seabream growth was presented by Hernández et al. (2003). According to the growth prediction of Lupatsch (2005), seabream was reported to have higher growth potential than seabass. It may attain 380 g after 12 months, starting from an initial stocking size of 1 g. In contrast, seabass reached an average body weight of 325 g within the same production period while feeding on the same diet as the seabream (45% protein, 19% lipid, 21.2 gross energy). Similarly, in a comparative study carried out in a commercial earth pond facility supplied with ground brackish water of 7‰ salinity, Altan (2020) reported that both seabream and seabass with an initial body weight of 1.6 g reached over 300 g after 20 months at 19 °C water temperature, with slightly higher harvest weight for the seabream (369.1±24.1 g) compared to seabass (328.4±22.9 g).

Nutritional Quality -and Contribution of Farmed Versus Wild Fish

The nutritional quality of farmed -or wild fish differs according to the complex aspects of the cultural conditions and regional characteristics of the marine environment. Profiles of amino acids (AAs) and fatty acids (FAs) in fish meat are important sources of high-quality protein and lipid with health potentials for human consumers (Öztekin et al., 2018, 2020).

Nutritional Quality Level of Amino Acids

The protein quality can be addressed with the availability of amino acids, especially essential amino acids (Jiang et al., 2017). Fish feeds used in aquaculture facilities depend on fishmeal and fish oil processed from wild fisheries. These two

commonly used marine resources are scarce due to the increasing demand for aquaculture's rapid growth worldwide. The aquaculture sector is forced to replace fishmeal and fish oil with other sources rich in the amino acid pattern but low in cost, which can reduce feed expenses and increase competition in global marketing. Plant proteins are the strongest candidates for replacing fishmeal and oil (Dubois et al., 2007). Among them, soybean meal is one of the strongest alternatives for the replacement of fish-based protein and oil sources (Alam et al., 2014), which eventually helps to reduce feed costs comprising around 60% of the total production costs in fish farms, as stated earlier by Ergün et al. (2020). The substitution of fishmeal with plant-based proteins might differentiate the amino acid composition of the diet and change body composition as well as the nutritional quality of farmed fish in general.

A wide range of amino acid profiles in farmed fish are reported with discrepancies in several studies, that could be attributed to the different fish species (Mohanty et al., 2014), diet quality and processing techniques of ingredients (Kim et al., 2012; Wang et al., 2014), or even to the water quality changes in the culture environment, as well as feed storage conditions (Wang et al., 2014). Among essential amino acids, methionine, lysine, tyrosine, histidine, and tryptophan may show antioxidant effects (Saito et al., 2003). Others, such as glycine, proline, leucine, glutamine, and aspartic acid, play an important role in cytotoxic activity against cancer cells (Kim et al., 1999). Arginine improves disease resistance when fish are exposed to stress conditions (Costas et al., 2011), while methionine is a stimulator for protein synthesis, supporting cell survival (Belghit et al., 2014).

Earlier reports provided interesting findings in terms of higher concentrations of glutamic acid and serine and lower levels of methionine, lysine, isoleucine, valine, threonine, glycine, and aspartic acid levels in fish-fed soybean meal incorporated diets compared to those fed on fishmeal-based diets (Kim et al., 2012). Wild fish schooling around cage farms might change their body amino acid profiles by feeding on the abundant pellets around the cage systems (Skog et al., 2003). This was supported by Fernandez-Jover et al. (2008), who underlined that wild fish aggregating around fish farms significantly consume pellets lost from fish cages, which might differentiate their feeding behaviour and affect natural fish populations through a slight change in their amino acid profiles. Therefore, it is likely that wild fish schooling around fish cages consume remarkable amounts of pellets lost from the pens, which might change the amino acid profiles in wild fish captured from marine sites dominated by cage farms (Oztekin et al., 2020).

Higher levels of essential amino acids (valine, threonine, isoleucine, and phenylalanine) and non-essential amino acids (glycine, alanine, and tyrosine) were found in the muscles of wild seabream compared to the caged fish or those aggregating around the pens (Oztekin et al., 2020). However, these differences were insignificant ($p<0.05$) except for the methionine among all three populations, namely caged fish, cage-aggregated wild and wild captured from a distance from the cage facility. In contrast, the authors reported higher levels of non-essential amino acids of serine, glutamic acid, and hydroxylysine in caged -and cage-aggregated seabream than those captured from wild populations from a distant area (Oztekin et al., 2020).

Higher total essential amino acids (∑EAA) and lower total non-essential amino acids (∑NEAA) compared to farmed or farm-aggregated individuals axillary seabream (*Pagellus et al.* et al., 2020), in meagre (*Argyrosomus regius*, Saavedra et al., 2017), ussuri catfish (*Pseudobagrus ussuriensis*, Wang et al., 2014), beluga sturgeon (*Huso huso*, Hamzeh et al., 2015), and barramundi (*Lates calcarifer*, Manthey-Karl et al., 2016). However, higher levels of EAAs were found in farmed seabass, turbot (*Scophthalmus maximus*), and red tail (*Chanodichthys mongolicus*), compared to wild-caught fellows by Baki et al. (2015), Manthey-Karl et al. (2016), and Jiang et al. (2017), respectively. In fish species captured from different marine sites and culture conditions, Manthey-Karl et al. (2016) pointed out differences in amino acid profiles between turbot captured from wild populations in the Atlantic Ocean and those cultured in fish farms in Spain or Chile. Manthey-Karl et al. (2016) also investigated amino acid levels for wild-caught. They farmed barramundi in Australia and Vietnam, where the authors stated that the environmental conditions of water quality or feed storage conditions could affect amino acid profiles in fish meat.

Several earlier reports provided similar ratios of total essential amino acids to total amino acid levels (∑EAA/∑AA) for farmed versus wild marine fishes; namely, 43.02, 50.81, 39.84, 45.10, 44.3% for farmed -and 42.78, 51.35, 40.33, 45.77, 46.5% for wild unsure catfish (Wang et al., 2014), Beluga sturgeon (Hamzeh et al., 2015), red tail (Jiang et al., 2017), meagre (Saavedra et al., 2017), and axillary seabream (Öztekin et al., 2020) respectively. These ratios of ∑EAA/∑AA were comparable with the ratio given for Egg (50%) as a reference value in reports of FAO/WHO (1989) or even higher than the recommended reference value of 40% for ∑EAA/∑AA by FAO/WHO (1991).

The recommended reference value for the total essential amino acids to total non-essential amino acids (∑EAA/∑NEAA) has been reported as 40% by FAO/WHO

(1991). Oztekin et al. (2020) reported ∑EAA/∑NEAA ratio as 79.6% in cage-farmed seabream in the Northern Aegean Sea, whereas a slightly higher ratio of 86.8% was noted in wild-caught fish, which was higher than the which was higher than the recommended reference value of >60% in FAO/WHO (1991) reports, underlining that seabream either farmed in fish pens or caught from natural populations provide a remarkable high level of nutritional contribution in terms of a high-quality-protein-source for human consumers.

Any source of protein showing an amino acid score less than 1 (AAS <1), the reference amino acid level reported by FAO/WHO (1973), needs further supplementation from another protein source in order to meet the sufficient level of protein in the human diet. Overall, higher levels of AASs were recorded in wild unsure catfish (Wang et al., 2014) and red tails (Jiang et al., 2017). In axillary seabream, however, Oztekin et al. (2020) found AASs higher than "1" (>1.00) in both farmed and wild individuals, except for lysine and leucine, indicating that both farmed and wild seabream provide sufficiently high nutritional quality in terms of amino acid profiles as a favourable protein source, with the only exception for lysine and leucine, indicating that lysine and leucine are the "first limiting" amino acids in seabream and the requirements of lysine and leucine in human diets, estimated below "1", need to be supplied by another source of protein.

Farmed fish seems to be a reliable marine food in terms of a high-quality protein source for human consumers. Based on recommended reference levels by FAO/WHO (1973, 1991), its nutritional contribution of amino acid profiles is not less but even higher than that of their wild representatives.

Nutritional Quality Level of Fatty Acids

Higher lipid levels in caged axillary seabream compared to wild individuals were reported by Oztekin et al. (2018). Twice more lipid levels in farmed fish over their wild representatives were reported in axillary seabream. Similar findings were reported in farmed versus wild salmon (Johnston et al., 2006), seabream (Grigorakis, 2007), and meagre (Saavedra et al., 2017). Johnston et al. (2006) reported that the higher fat levels in farmed fish compared to the wild fellows could be linked to the higher fat concentrations in the aquadiets as well as the feeding frequency or a wide range of several factors such as plenty food availability in farm conditions, dietary ingredients, and the higher energy consumption of fish in culture conditions over the wild populations (Grigorakis et al., 2002), which was also supported by Öztekin et al. (2018). The fatty acid profile of fish can differ based on several factors, such as the lipid source used in the diet, water temperature, salinity, or seasonal changes (Yildiz et al., 2008), as well as a combination of all these factors (Öztekin et al., 2018).

In terms of polyunsaturated fatty acids (PUFAs), higher concentrations of linoleic acid (LA) were reported in caged axillary seabream compared to their wild representatives captured from the same area (Öztekin et al., 2018). Increased LAs were noted in fish fed diets supplemented with soybean oil (60%) compared to those fed diets prepared with fish oil as the sole source. Further, Fountoulaki et al. (2003) reported that substituting fish oil with soybean oil showed a significant increase in linoleic acid and linolenic acid contents with increased levels of n-6/n-3 ratio in the farmed fish. Nevertheless, when the fatty acids are converted into mg/100 g wet weight basis, all fatty acids presented higher levels in farmed axillary seabream, with twice higher arachidonic acid (ARA, C20:4n6) and eicosapentaenoic acid (EPA, 20:5 n- 3) in farmed fish compared to their wild representatives from the same aquatic environment, and 1.5 to 4 times higher alpha(α)-linolenic acid (ALA, C18:3n3), EPA, and docosahexaenoic acids (DHA, 22:6*n*-3) in cage-farmed fish over the wild-caught seabream (Öztekin et al., 2018).

When converting fatty acid levels into mg/100 g, based on lipid levels in fish meat, it was recorded that ARA, ALA, EPA and DHA were higher in farmed fish than in the wild-captured individuals from the same marine environment. Additionally, the nutritional contribution of LA and ALA from farmed seabream were higher than their wild representatives, which was also supported by Dubois et al. (2007), underlining that the dietary incorporation level of plant oil could result in an increased level of LA in fish meat, supporting the hypothesis of improved nutritional contribution of LA or ALA in cage-farmed seabream compared to the wild-caught individuals. Additionally, Öztekin et al. (2018) reported a higher nutritional contribution for EPA+DHA in farmed axillary seabream compared to the wild populations, which met the recommended daily intake levels of EPA+DHA (250 to 500 mg/day) reported by the European Food Safety Authority (EFSA, 2010) for the prevention of primary cardiovascular disease in human beings. This is in line with the report of the American Dietetic Association and Dietitians of Canada, suggesting 500 mg/day intake of EPA+DHA for the prevention of cardiovascular disease in humans, which is around 112 g per serving and could be gained by consuming oily fish twice a week (Kris-Etherton & Innis, 2007). In light of these recommendation levels and the nutritional contribution of EPA+DHA in seabream reported recently by Oztekin et al. (2018), 227 g of farmed seabream would be more than sufficient (140.3%) to meet the 500 mg level recommendation by EFSA (2010). In contrast, the wild-captured fish from the

same area was just enough (99.9%) to cover the recommended daily intake level of EPA+DHA with a serving of 227 g of fish to prevent human cardiovascular diseases. Similarly, Saavedra et al. (2017) reported that a daily intake of 160 g of farmed meagre (A. regius) could cover the recommended level of 500 mg for EPA+DHA. In contrast, wild meagre could meet some recommended daily intake levels daily intake levels, according to Saavedra et al. (2017).

Farmed fish seems promising and reliable marine food with high quality and health potential for human consumers. Its nutritional contribution of EPA+DHA is not less but even higher than that of its wild representatives, especially for people with coronary health risks.

Results and Discussion

The discrepancies among earlier reports regarding age-growth relations of seabream either in farm conditions or wild populations can be attributed to the complexity of fish growth performance irrespective of farm -or wild conditions (Wendelaar-Bonga, 2011), which is significantly correlated to various environmental factors of water temperature (Carriquiriborde et al., 2009), food availability, species and genetic differences, predator effects, habitat selection, sheltering or hiding conditions, breeding, mating etc. Other notable factors in the culture environment, such as photoperiod (Wendelaar-Bonga, 2011), temperature, salinity, dissolved oxygen, pH (Kestemont & Baras, 2001), nitrogenous compounds (nitrite, ammonia) (Schram et al., 2010), voluntary food intake and food quality (Lanari et al., 2002), feeding strategies (Hernández et al., 2003) and operational management practices, stocking densities, or a combination and interaction of all these factors together may influence the growth performance in farmed fish (Imsland et al., 2001). All these biological activities are linked to significant energy expenditure in fish. In farmed fish, a bigger part of the energy consumed is allocated for growth. In contrast, in wild individuals, higher energy expenditure stands for various efforts for competition in repeated food search and foraging, hiding, tissue recovery, performance loss, habitat selection, mating and reproduction efforts, or even area-specific short-term habitat change and seasonal migrations. Beyond all, it is important to consider that farmed fish are kept under controlled conditions, apart from all the risks and harsh competition that wild fish face. Fish welfare in farm conditions is highly linked to sufficient oxygen and water flow through the system, biomass control and optimum stocking density, and biofouling-free netting that prevents pathogen attachments. Hence, if environmental conditions are optimum, cultured fish probably compete with their fellows only during feeding.

Apart from cormorant or seagull attacks, owing to the development of high-strength net materials, fish in cage farms are no longer in danger of predator attacks except for a few incidents of monk aggressions (Güçlüsoy & Savas, 2003) or possible shark attacks in certain areas. High-technology new-generation cage systems and innovative net materials improve strength against net failures at storms and typhoons, preventing fish escapes. Considering the nature of the Mediterranean ecosystem, however, seabream is among the endemic species that naturally and locally inhabit the Mediterranean. Hence, negative consequences regarding genetic or ecological influences, such as interbreeding or predation effects, are still uncertain for Mediterranean escapees with few reports (Thorstad et al., 2008). An existing complex environmental interaction between escaped fish and wild individuals through food, habitat, and mating competition, or disease transmission to nearby farms or the transmission of pathogens to native fish stocks have been investigated by Thorstad et al. (2008), who indicated "low probability" for impact of genetic interaction between escapees and endemic wild populations (Arechavala-López, 2012). Some studies underlined that the escapees may aggregate around cages and swim away from one farm to nearby cage farms or even to local fishing grounds and coastal habitats for foraging (Arechavala-López et al., 2012). Alternatively, legal measures can be set at the earliest to encourage the use of high-strength nettings such as ultrahigh molecular weight polyethene (UHMWPE) nets or the innovative copper alloy mesh (CAM) nets in cage facilities in order to reduce or even prevent escapes from marine cage farms at all. In general, fish farms operating in exposed marine sites show reduced environmental impacts (Utne et al., 2015), and the level reached by the Mediterranean aquaculture sector today is promising for sustainable production of healthy seafood for human consumers.

Conclusion

The information provided in this review prepared based on currently available knowledge, underlines that the nutritional contribution of farmed fish in terms of amino acids and fatty acid profiles is not less but even higher than that of their wild representatives. Accordingly, it might be underlined that farmed fish is a reliable source of seafood with high nutritional value, just like natural fish.

Compliance with Ethical Standards

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

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

Data availability: Data will be made available on request.

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A book in print

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

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