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Nutritional Composition of Duckweed (*Lemna minor*) Cultured with Inorganic Fertilizer and Organic Manure in Earthen Ponds

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ABSTRACT

Duckweed (*Lemna minor*) have been used for several years to recover nutrients from wastewater and as feed ingredient for livestock, including fish and poultry. This study aimed at assessing the effect of different manure sources; inorganic fertilizer (a combination of DAP and Urea), and organic sources (chicken manure and cow dung manure) on the proximate composition, amino acid and fatty acid profile of *L. minor* under culture conditions. Results indicated that *L. minor* cultured with chicken manure had significantly higher crude protein level (36.8%) ($P < 0.05$) and lower crude fat content (8.10%) compared to the ones cultured with inorganic fertilizer and cow dung manure. Essential amino acids proportion was 50% in *L. minor* cultured with inorganic fertilizer, 45.4% in chicken manure and 44.8% in cow dung manure with lysine and phenylalanine being the most abundant amino acids. The total polyunsaturated fatty acids (PUFA) were significantly higher ($P < 0.05$) in *L. minor* cultured using inorganic fertilizer (43.05 mg/100 g) with linoleic acid being the most dominant PUFA. The presence of high levels of amino acids and PUFA in the *L. minor* cultured with organic and inorganic fertilizer respectively indicates that it can provide quality protein and PUFA required for fish growth and well-being.

Keywords: Organic manure, inorganic fertilizer, nutrients, *Lemna minor*, composition

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Introduction

Duckweed (*Lemna minor*) is a free-floating freshwater macrophyte belonging to the family *Lemnacea* and is found in freshwater ponds, lagoons, ditches and streams in both tropical and subtropical climates (Culley et al. 1981; Hassan and Edwards 1992; Young et al. 2006). In most cases, duckweed has been used for wastewater treatment, as food for humans, fish and terrestrial animals (Culley et al. 1981; Chakrabarti et al. 2018; Nesan et al. 2020). Previous studies have shown that *L. minor* is a source of protein, minerals and vitamins important for cultured fish (Yılmaz et al. 2004). The application of this aquatic macrophyte in fish nutrition requires continuous and sustainable production which is not achievable through collection from natural water bodies and wastewaters. Furthermore, macrophytes collected from natural/wastewater systems may contain contaminants that may affect fish or animal production and/or the quality of these products for human consumption.

Duckweed is readily consumed by Nile tilapia (*Oreochromis niloticus*), Common carp (*Cyprinus carpio*) and other herbivorous fish

(Hassan and Edwards 1992; Yılmaz et al. 2004). It is reported that it contains up to 45% crude protein (CP) of the plant's dry weight and can be easily cultured in nutrient-rich waters in tropical and subtropical countries (Culley et al. 1981; Hassan and Edwards 1992; Hasan and Chakrabarti 2009; Chakrabarti et al. 2018). However, conditions for the growth of duckweed are not generally possible in semi-intensive and intensive aquaculture systems which are used for fish, hence, it is important to culture them separately and incorporate them in fish feeds for fish to get the benefits of their nutrients (Bag et al. 2011).

The nutritional composition of cultured aquatic plants depends on the culture medium and can differ from the natural water bodies to the controlled environments with known fertilization rates. Inorganic and organic manures have been used in the production of duckweed in Bangladesh (Chakrabarti et al. 2018; Sharma et al. 2019). However, their nutritional composition normally varies depending on the type of manure used and the nitrogen content of the water used which arises from fertilization (Chakrabarti et al. 2018). The present study analyzed the proximate, amino acid and fatty acid composition of *L. minor* cultured with locally available organic and inorganic fertilizers, to evaluate their suitability for utilization in fish feeds.

Materials and Methods

Culture Unit Preparation

The study was carried out in earthen ponds at Kenya Marine and Fisheries Research Institute (KMFRI) Sangoro Aquaculture Station, Kisumu County, Kenya. Nine ponds measuring 169 m^2 each were drained off, dried and limed using agricultural lime at 100 g/m^2 to minimize unwanted macrophytes growth and nutrient load management. The ponds were filled with water to a depth of 40 cm (0.4 m) and were assigned fertilizer treatments randomly with T1 (inorganic fertilizer), T2 (chicken manure), and T3 (Cow dung manure) according to the procedures documented by Chakrabarti et al. (2018). Initial fertilization with organic manure was done at 1052 g/m^2 using chicken manure and cow dung, respectively. After every 10 days, fertilization was done at ¼ dose of the initial treatment. The manures were mixed with water and allowed to decompose for 3 days before applying to the culture units. Fertilization by a combination of DAP and urea was done at the rate of 1 g/m^2 for DAP and 1.5 g/m^2 for urea, respectively. After every 10 days, fertilization was repeated at a ¼ dose of the initial treatment. The nutritional content of the fertilizers used is summarized in Table 1. Nitrogen (N) and phosphorus (P) analyses of inorganic and organic fertilizers used in *L. minor* culture were performed according to standard methods. Available N was determined by extracting 5 g (dry weight equivalent) of each fertilizer with 25 mL of 2 M KCl (Mulvaney 1996). Available P was determined by extracting 2.5 g (dry weight equivalent) of each fertilizer with 25 mL of Mehlich-3 extracting solution (Frank et al. 1998).

Table 1. Nutritional composition of inorganic and organic fertilizers used in *L. minor* culture

Fertilizer	Nitrogen (%)	Phosphorus (%)
Diammonium phosphate (DAP)	18	46
Urea	46	-
Cow dung	2.03	1.54
Chicken manure	2.57	1.58

L. minor inoculation and culture

L. minor was collected from the Ahero Irrigation Scheme, Kisumu, County, Kenya and transported to KMFRI, Sangoro aquaculture station. The macrophytes were introduced to the culture system at a rate of 5 g (wet weight) per m^2 for the 3 treatments; T1 (inorganic fertilizer (DAP + Urea)), T2 (chicken manure), and T3 (cow dung manure) in triplicates (Chakrabarti et al. 2018). The duckweed was harvested after 60 days and washed with clean water to remove soil particles and any other impurities before drying under a shed.

Nutritional composition of *L. minor*

Biochemical (proximate) compositions of *L. minor* cultured in ponds using different fertilizers were determined by standard methods (AOAC 1990). Crude protein (CP) was analyzed by the copper catalyst Kjeldahl method. Analysis was done by taking 1g of each sample and 2 tablets of catalyst (Kjeldahl tablets) which were digested in 15 ml concentrated sulfuric acid (H_2SO_4) at 420 °C. The samples were cooled and automatically distilled in Kjeldahl equipment with 40% NaOH and ammonia gas trapped in 4% boric acid and reverse titrated using 0.2N HCl. The nitrogen content was determined and converted to crude protein content using a nitrogen factor for the crude protein calculation of 6.25. Ash was determined by expressing the weight of 2 g of the ground sample burnt at 600°C for 3 hours in a muffle furnace as a percentage of the un-burnt sample weight. Crude fat (CF) was extracted by heating 3 g of the sample in diethyl ether under reflux at 105 °C for 30 minutes in a VELP Solvent Extraction unit. The ether extract was calculated as the difference between the original sample and the ether extract residue. Crude fibre (CF) was determined gravimetrically by chemical digestion and solubilization, and quantified by: CF (%) = dried sample (g) – ashed sample (g)/initial sample weight × 100). Carbohydrate content was

determined by subtraction of protein, lipid and ash values.

Amino acid analysis

Amino acids were analysed from the *L. minor* using standard methods (Otter 2012). Analysis was done on 1 g of pooled finely ground *L. minor* from each treatment using ion exchange liquid chromatography via continuous flow chromatography. The compounds were identified and quantified using an authentic sample mixture (amino acid standard solution (AAS 18) from Sigma-Aldrich (Chemie GmbH, Munich, Germany).

Extraction of lipids and fatty acid analysis

Lipid extraction was performed according to the procedure of Bligh and Dyer (1959). Total lipid was extracted from 0.5 g samples of pooled finely ground *L. minor* by homogenization in 10 ml chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant and 2 ml cold isotonic saline, 0.9% Sodium chloride (NaCl). Homogenates were mixed vigorously and allowed to stand for 20 minutes before being centrifuged at 3000 rpm for 10 minutes and the upper aqueous layer aspirated before the lower organic/chloroform layer was transferred to a 100 ml reflux flask and evaporated to dryness under a vacuum. Fatty acid methyl esters (FAME) were prepared from the extracted total lipid and fatty acid standards by acid-catalyzed transmethylation. Briefly, 5 ml of 1% H_2SO_4 (v/v) in methanol was mixed with 1 ml of extracted total lipid in a 50 ml reflux flask and refluxed at 70°C for 3 hours. FAME was extracted into 750 ml of distilled water and 10 ml of hexane and then dehydrated using anhydrous Sodium sulphate (Na_2SO_4). The extracted FAME was concentrated at 0.5 ml in a vacuum evaporator and transferred into gas chromatography (GC) vials before GC analysis.

Gas chromatography analysis

FAME was separated and quantified by gas chromatography (GC) (Shimadzu Model GC14B, Japan) fitted with on-column injection and equipped with a fused silica capillary column (SUPELCO Column Omega wax^{tm} 530, 30 m x 0.5 mm x 0.5 µm) with nitrogen as a carrier gas. Temperature was increased from 170 °C to 220 °C at 1.8 °C min^{-1}, then 220 °C for 47 minutes and a total run time of 75 minutes. Injection and detection temperatures were 240 °C and 260 °C, respectively. All GC analyses were performed under the same conditions. Individual methyl esters were identified by comparing the retention times of FAME with known FAME standard (Supelco 37 Component FAME Mix standard (Sigma-Aldrich, Munich, Germany) obtained from Kobian© Kenya Chemicals. Fame analysis and identification were done according to Indarti et al. (2005).

Statistical analysis

All data were presented as mean ± SE. One-way analysis of variance (ANOVA) was used for mean comparisons and Tukey post hoc analysis for multiple comparisons of means (Bhujel, 2011). The significance level was set at $P < 0.05$. All statistical analyses were carried out using Statistical Package and Service Solutions (SPSS version 23).

Results

Proximate composition of *L. minor*

The proximate composition analysis indicated there were differences in the composition of *L. minor* cultured with inorganic and organic fertilizers. *L.minor* cultured with chicken manure had significantly higher crude protein levels (36.8 ± 0.05%), and lower fat content (8.10 ± 0.05%) compared to the ones cultured with inorganic manure which had (31.4 ± 0.02%) and (8.7 ± 0.53%) for protein and fat respectively ($P < 0.05$) (Table 2). Among the organic manure used, cow dung manure produced *L. minor* with a relatively higher percentage of ash (13.67%) ($P < 0.05$). Carbohydrates were also significantly higher in *L. minor* cultured with inorganic fertilizer ($P < 0.05$).

Table 2. Proximate composition of *Lemna minor* cultured with inorganic and organic fertilizer

Parameter (% dry matter)	Inorganic fertilizer (DAP+Urea)	Chicken manure	Cow dung manure
Crude protein	31.4 ± 0.02^a	36.8 ± 0.05^b	35.34 ± 0.03^c
Crude fat	8.7 ± 0.30^a	8.1 ± 0.05^b	8.36 ± 0.01^c
Crude fibre	5.3 ± 0.04^a	4.6 ± 0.02^b	5.35 ± 0.01^a
Ash	11.6 ± 0.24^a	11.8 ± 0.01^a	13.67 ± 0.01^b
Carbohydrates	43.1 ± 0.6^a	38.5 ± 0.13^b	37.25 ± 0.06^b

*Values are reported as mean ± SE. Values followed by different superscript letters across one row are significantly different (one-way ANOVA, Tukey post hoc test, P<0.05)

Amino acid profile of *L. minor*

The amino acid profile of *L. minor* cultured with different fertilizers is shown in Table 3. The essential amino acids and non-essential amino acids were present in *L. minor* cultured with both inorganic and organic manure. Essential amino acids were present in the following proportions: inorganic fertilizer (50%), chicken manure (45.4%) and cow dung manure (44.8%). The proportion of essential amino acids was significantly higher in *L. minor* cultured with inorganic fertilizer ($P < 0.05$). Most of the essential amino acids apart from methionine, isoleucine, threonine and tryptophan were significantly higher in *L. minor* cultured with chicken manure and cow dung manure but were significantly lower in *L. minor* cultured with inorganic fertilizer ($P < 0.05$). Lysine levels were not significantly different

in the *L. minor* cultured with inorganic fertilizer and cow dung manure (P > 0.05). The proportion of non-essential amino acids was significantly lower in *L. minor* cultured with inorganic fertilizer (50%) compared to chicken and cow dung manure.

Table 3. Amino acid profile (µg/100 mg) of *Lemna minor* cultured with inorganic and organic fertilizer
*Values are reported as means. Values followed by different superscript letters across one row are significantly different

Amino acid (µg/100 mg)	Inorganic fertilizer (DAP +Urea)	Chicken manure	Cow dung manure
Essential			
Lysine	1.6[a]	2.08[b]	1.8[a]
Histidine	0.3[a]	0.39[b]	0.35[c]
Leucine	3.3[a]	0.39[b]	0.35[c]
Valine	2.1[a]	2.3[b]	2.3[b]
Methionine	0.1[a]	0.13[a]	0.11[a]
Isoleucine	2.2[a]	2.26[a]	2.2[a]
Phenylalanine	2.5[a]	3.25[b]	3.2[b]
Threonine	0.1[a]	0.13[a]	0.12[a]
Tryptophan	0.1[a]	0.13[a]	0.12[a]
Non-essential			
Glycine	0.2[a]	0.26[a]	0.26[a]
Alanine	1.2[a]	1.56[b]	1.46[c]
Glutamic acid	5.2[a]	5.26[a]	5.16[a]
Proline	1.2[a]	1.56[b]	1.5[b]
Tyrosine	1.3[a]	1.39[b]	1.35[b]
Cysteine	0.1[a]	0.13[a]	0.13[a]
Arginine	3.1[a]	3.13[a]	3.13[a]
***Essential* (%)**	**50.0[a]**	**45.4[b]**	**44.8[b]**
***Non-Essential* (%)**	**50.0[a]**	**54.6[b]**	**55.2[b]**

(one-way ANOVA, Tukey post hoc test, $P < 0.05$)

Fatty acid profile of *L. minor*

In the current study, 21 fatty acids were identified in the dried *L. minor* 14 of them were saturated fatty acids. Palmitic acid (C16.0) was the dominant saturated fatty acid. The total saturated fatty acids (∑SFAs) were significantly higher (P < 0.05) in the *L. minor* cultured with chicken manure (35.90 mg/100 g). *L. minor* cultured with inorganic fertilizer had significantly higher palmitic acid values compared to the *L. minor* cultured with chicken manure and cow dung manure (P < 0.05). Among the monounsaturated fatty acids (MUFAs), Oleic acid (C18.1) was significantly higher in the *L. minor* cultured in inorganic fertilizer (19.33 mg/100 g). In addition, Oleic acid, (C18:1) was the dominantnounsaturated fatty acid across all treatments. Two polyunsaturated fatty acids were detected namely linoleic acid (C18:2) and linolenic acid (C18:3). Linoleic acid (C18:2) was significantly higher (P < 0.05) in the *L. minor* cultured with inorganic fertilizer (41.12 mg/100 g) and was the dominant polyunsaturated fatty acid across the treatments. Eicosapentaenoic acid, (C20:5) was the only highly polyunsaturated fatty acid (HUFA) detected in the cultured *L. minor* and was significantly lower (P < 0.05) in *L. minor* cultured in cow dung manure (Table 4). The total polyunsaturated fatty acids were significantly higher (P < 0.05) in *L. minor* cultured with inorganic manure (43.05 mg/100 g).

Table 4. Composition of fatty acids (mg/100 g) of *Lemna minor* cultured with inorganic and organic fertilizers

Fatty acid (mg/100 g)	Chemical structure	Inorganic fertilizer (DAP +Urea)	Chicken manure	Cow dung manure
Butanoic acid	C4:0	1.03 ± 0.02[a]	1.43 ± 0.01[b]	1.43± 0.02[b]
Caprylic acid	C8:0	1.77 ± 0.01[b]	3.01 ± 0.02[b]	2.96 ± 0.02[b]
Capric acid	C10:0	0.93 ± 0.11[b]	1.90 ± 0.3[b]	1.77 ± 0.31[b]
Undecanoic acid	C11:0	0.07 ± 0.02[a]	0.13 ± 0.02[b]	0.13 ± 0.02[b]
Lauric acid	C12:0	0.74 ± 0.19[a]	0.93 ± 0.24[b]	0.93 ± 0.24[b]

Table 4. Continues

Myristic acid	C14:0	1.03 ± 0.45[a]	1.98 ± 0.47[b]	1.82 ± 0.50[c]
Pentadecanoic acid	C15:0	1.74 ± 0.09[c]	2.26 ± 0.23[b]	2.25 ± 0.23[b]
Palmitic acid	C16:0	21.12 ±0.59[a]	15.03 ± 0.60[b]	14.83 ± 0.60[b]
Margaric acid	C17:0	1.26 ± 0.04[a]	2.25 ± 0.35[b]	1.19 ± 0.35[c]
Stearic acid	C18:0	0.55 ± 0.23[a]	2.85 ± 0.14[b]	3.48 ± 0.13[c]
Nonadecanoic acid	C19:0	0.52 ± 0.09[a]	0.73 ± 0.13[b]	0.67 ± 0.12[b]
Heneicosanoic acid	C21:0	0.25± 0.01[a]	1.68 ± 0.06[b]	1.69 ± 0.03[b]
Dodecenoic acid	C12:1	0.13 ± 0.13[a]	1.10 ± 0.13[a]	0.93 ± 0.11[b]
Myristoleic acid	C14:1	1.21 ± 0.06[a]	0.63 ± 0.04[b]	0.45 ± 0.05[c]
∑SFAs		**32.35 ± 0.01[a]**	**35.90 ± 0.02[b]**	**34.54 ± 0.01[c]**
Palmitoleic acid	C16:1	0.83 ± 0.56[a]	5.95 ± 2.01[b]	6.12 ± 1.60[c]
Palmitelaidic acid	Trans C16:1	2.31 ± 0.20[a]	1.06 ± 0.50[b]	1.31 ± 0.45[c]
Oleic acid	C18:1	19.33± 0.37[a]	15.43 ± 0.31[b]	14.97 ± 0.21[c]
Eicosenoic acid	C20:1	2.01± 1.15[c]	2.05 ± 0.39[b]	1.96 ± 0.29[a]
∑MUFAs		**24.48 ± 0.11[a]**	**24.50 ± 0.27[a]**	**24.36 ± 0.37[a]**
Linoleic acid	C18:2	41.12 ± 1.83[a]	37.13 ± 1.47[b]	38.42 ± 1.32[b]
Linolenic acid	C18:3	1.64 ± 0.93[a]	1.68 ± 0.32[a]	1.51 ± 0.22[b]
Eicosapentaenoic acid	C20.5	0.29 ± 0.14[a]	0.28 ± 0.01[a]	0.26 ± 0.02[b]
∑PUFAs		**43.05 ± 0.96[a]**	**39.08 ± 0.96[b]**	**40.19 ± 0.22[c]**

*∑SFA, total saturated fatty acids; ∑MUFA, total monounsaturated fatty acids; ∑PUFA, total polyunsaturated fatty acids. Values are reported as mean ± SE. Values followed by different superscript letters across one row are significantly different (one-way ANOVA, Tukey post hoc test, $P < 0.05$)

Discussion

The protein content of *L. minor* cultured in both organic and inorganic manures was higher than earlier reported values of 18% for *L. minor* collected from raceways, and other aquatic macrophytes like *Salvinia molesta* which had 17.3% crude protein (Yılmaz et al. 2004; Moozhiyil and Pallauf 2016). The protein content for *L. minor* cultured with chicken manure was similar to the results obtained by Chakrabarti et al. (2018) when organic manure containing chicken manure was used in the culture of *L. minor* in concrete ponds. The high crude protein level could be due to the culture environment which contained the required levels of nutrients as a result of fertilization, which is similar to the environment found in natural eutrophic waters where *L. minor* has traditionally been collected for fish feeds (Yılmaz et al. 2004). In addition, the high protein content of *L. minor* cultured with chicken manure could be related to the ability of the macrophyte to accumulate nitrogen for a longer period. The crude protein and crude fibre reported in this study are lower than the values reported for duckweed (*Lemna gibba*) grown in wastewater (Landesman et al. 2002). The carbohydrates were higher in *L. minor* cultured with inorganic fertilizer. Previous studies have reported higher carbohydrate levels (51.2%) in *L. minor* cultured in nutrient-enriched waters (Al-snafi 2019). This is higher than the results obtained from the present study in which 43.1% was recorded in *L. minor* cultured with inorganic manure. Similarly, organic manure (Chicken manure) resulted in lower carbohydrates in *L. minor* compared to inorganic fertilizer (Chakrabarti et al. 2018).

The amino acid levels in *L. minor* cultured with different manures were comparable to the ones collected from the wild and used for fish feeds (Yılmaz et al. 2004). The presence of all the essential amino acids was also reported by Landesman et al. (2002) in *L. gibba* cultured in wastewater even though the levels reported were higher than the levels in the present study. This is consistent with the reports that *L. minor* from natural water bodies and nutrient-enriched waters contains all the essential amino acids which make it suitable as a fish feed ingredient (Culley et al. 1981). Lysine and phenylalanine were the most abundant essential amino acid in this study contradicting the finding of Landesman et al. (2002) which had Leucine and valine as the abundant amino acids in *L. gibba*.

The low values of methionine in the present study are coherent with a previous study which had low

methionine of clones of duckweed in comparison with the requirement of 2.2 for fish feeds by FAO (Culley et al. 1981). *L. minor* has been previously reported to be having low levels of methionine, threonine, tryptophan and cysteine (Hammouda et al. 1995). Landesman et al. (2002) also reported the same deficiencies for *L. gibba* cultured in wastewater. *L. minor* cultured with chicken manure and cow dung manure were richer in the essential amino acid. Since fish requires the presence of 10 essential amino acids in their diets, *L. minor* cultured with organic manures can promote good growth, survival of fish and antibody production as they have high levels of essential and non-essential amino acids (Moyo et al. 2011). Additionally, previous studies have reported that the protein of duckweed is rich in certain amino acids that were often low in other plant proteins making it suitable for fish feed (Guha 1997).

The fatty acid profile of *L. minor* was significantly affected by the different manures. *L. minor* grown with inorganic fertilizers had higher levels of oleic acid, linoleic acid and a higher proportion of total polyunsaturated fatty acids (PUFA). The PUFAs were significantly higher compared to the saturated and monosaturated fatty acids across all the treatments. This is similar to previous studies which documented higher levels of polyunsaturated fatty acids in *L. minor* (Chakrabarti et al. 2018; Al-snafi 2019). Similar trends were reported for duckweed (*Spirodela polyrhiza*) cultured with inorganic manure (Sharma et al. 2019). Linoleic acid was the most predominant proportion of unsaturated fatty acids and has been reported to be essential for the growth and proper performance of fish (Mukherjee et al. 2010). Previous studies have documented that *L. minor* contains a larger proportion of polyunsaturated fatty acids mainly linolenic acid (41 to 47%) and linoleic acid (17–18%) (Chakrabarti et al. 2018). In this study, the saturated fatty acids were predominately palmitic and stearic acids in the *L. minor* cultured in chicken manure and cow dung manure. This agrees with the findings of Yan et al. (2013) which documented more than 70% PUFA and that palmitic acid and stearic acid were the predominant fatty acids in *L. minor*. The high level of PUFA indicates the enhanced nutritional value of the duckweeds cultured in inorganic manure.

The study indicates that duckweed cultured with chicken manure, contains high protein and can be suitable for use as fish feed. Large-scale production of duckweed using chicken manure should be encouraged for its sustainable use in aquaculture. The presence of high levels of essential amino acids and polyunsaturated fatty acids in the *L. minor* cultured with inorganic fertilizer indicates that it can provide the quality protein and polyunsaturated fatty acids required for fish growth and well-being. However, due to the high prices of inorganic fertilizers, organic manures are recommended for the production of *L. minor* for cost-effective fish-feed formulation.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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The First Ectohelminth Ichthyo-Parasitic Fauna of the Turkish Endemic Fish, Marmara Barbel, *Barbus oligolepis* Battalgil, 1941, with New Host and Geographical Record

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ABSTRACT

The aim of this study was to determine the ectoparasite helminth fauna of Turkey endemic fish, Barbus oligolepis (Battalgil, 1941), from Susurluk Stream in the village of Yıldız, Balıkesir. For this purpose, a total of 81 individuals of *B. oligolepis* were caught seasonally by electrofishing between the 2020 spring (April) and 2021 winter (February) and the ectoparasite helminth fauna was studied. Only two monogenean species, *Dactylogyrus carpathicus* (Zakhvatkin, 1951) and *Paradiplozoon homoion* (Bychowsky and Nagibina, 1959), were identified. As a result of this examination *P. homoion* was the most prevalent and the highest number in the host fish. This species was present throughout all seasons. A total of 126 specimens of *P. homoion* infected 29 of the 81 fish examined, with a prevalence and mean intensity of 35.8 (1.5 %), parasite/fish respectively. Additionally, infection parameters for two monogenean species were calculated in accordance with the season, host size and sex. This study is the first to report the presence of an ectoparasitic helminth for B. oligolepis in this location in Turkey. *P. homoion* and *D. carpathicus* are also new ectoparasitic helminth records from this host fish and location. In addition, sequence data of P. homoion from host fish were reported to GenBank for the first time with this study. As a result, new information about the geographical distribution and host range of two parasite species has been added.

Keywords: Platyhelminthes, Monogenea, *Dactylogyrus carpathicus*, *Paradiplozoon homoion*

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Türkiye Endemik Balıklarından Marmara Bıyıklısı, *Barbus oligolepis* Battalgil, 1941' in Yeni Konak ve Coğrafik Kayıt ile İlk Ektohelmint İhtiyo-Parazitik Faunası

Öz: Bu çalışmanın amacı, Balıkesir ili Yıldız Köyü Susurluk Deresi'nden *B. oligolepis* 'in ektoparazit helmint faunasının belirlenmesidir. Bu amaçla 2020 ilkbahar (Nisan) ile 2021 kış (Şubat) tarihleri arasında mevsimsel olarak elektroşoker ile toplam 81 *B. oligolepis* bireyi yakalanmış ve ektoparazit helmint faunası çalışılmıştır. Bu inceleme sonucunda sadece iki monogenean türü *Dactylogyrus carpathicus* ve *Paradiplozoon homoion* tespit edilmiştir. *P. homoion'* un konak balıklarda en yüksek yaygınlıkta ve en fazla sayıda olup, her örnekleme mevsiminde tespiti yapılmıştır. Toplam 129 *P. homoion* bireyi İncelenen 81 balık bireyinin 29'unu %35,8 enfeksiyon yaygınlığı ve 1,5 parazit/balık ortalama yoğunluk ile enfekte ettiği belirlendi. Bunlara ilaveten, iki monogenean türün enfeksiyon parametreleri mevsim, konak balık boy ve cinsiyet grupları açısından değerlendirilmiştir. Bu çalışma, Türkiye'de Susurluk Çayı'ndaki *B. oligolepis* için ektoparazitik helmint varlığını bildiren ilk çalışmadır. *P. homoion* ve *D. carpathicus* da bu konak balıktan ve bölgeden yeni ektoparazitik helmint kaydıdır. Ayrıca konuk balıklardan alınan *P. homoion*'un sekans verileri ilk kez GenBank'a bu çalışma ile bildirildi. Sonuç olarak, iki parazit türünün coğrafi dağılımına ve konak yelpazesine yeni bilgiler eklenmiştir.

Anahtar kelimeler: Plathelminthes, Monogenea, *Dactylogyrus carpathicus*, *Paradiplozoon homoion*

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Introduction

In Türkiye, the genus *Barbus* has 12 valid species of Cyprinid fishes. As well as *Barbus oligolepis,* at least five species are endemic to Türkiye (Çiçek et al. 2020). *B. oligolepis* is known to inhabit the following rivers that drain to the southern shore of the Marmara Sea in Türkiye: namely, Kocasu, Nilüfer River; Kocaçay, Susurluk River; Hançay, Gönen and Narlıca Stream, İznik (Turan et al. 2009). Generally, this species habits in swift-flowing water, with a stone and pebble bottom (Froese and Pauly 2022).

To the best of our knowledge, only two studies have investigated the presence of helminth parasites in endemic fish species of the genus *Barbus* in Turkey. Aydogdu et al. (2017) studied helminth parasites of *B. niluferensis* (Turan et al. 2009) from the Nilüfer stream*;* similarly, Önalan et al. (2022) studied the endohelminth parasites of *Barbus ercisianus* (Karaman, 1971) from the Nemrut Crater Lake**.** However, no ichthyo-helminthological studies have been performed for *B. oligolepis* so far. As a result, this study represents the first ichthyo - helminthological survey of helminth parasites in this endemic fish in Türkiye. In addition, various studies have been conducted on the helminth fauna of native fish species of the genus *Barbus* in Turkey (see, for example, Koyun 2001; Develi 2008; Turgut et al. 2011; Turgut and Özgül 2012; Koyun et al. 2015). Only three of these studies Develi (2008), Turgut and Özgül (2012), Koyun et al. (2015), were able to record the ectoparasitic helminth species parasitizing on *Barbus* spp.

In the studies mentioned above, ectoparasitic helminth species parasitized in this genus are represented in three genera, *Dactylogyrus*, *Gyrodactylus* and *Dogielius*. They reported on the occurrence of ectoparasitic helminth species in native fish species of the genus *Barbus* in Türkiye, including, *Dactylogyrus goktschaicus* (Gussev, 1966), *D. lenkorani (Mikhailov, 1967), Dactylogyrus malleus* (Linstow, 1877*), Gyrodactylus elegans* (von Nordmann, 1832), *Gyrodactylus hemibarbi (Ergens, 1980), Gyrodactylus* sp. and *Dogielius mokhayeri* (Jalali and Molnar,1990*)*. In addition to these, no previous study has found *Paradiplozoon* spp. in fish species of the genus *Barbus*. Therefore, the present study is the first in Turkey to present data on diplozoid species in *Barbus* spp. and to report sequence data to GenBank.

So, the current study aimed to isolate, identify and provide additional information on the ectoparasitic helminth species of *B. oligolepis* from freshwater ecosystems of the Susurluk stream in Türkiye, as well as to determine their dependence on the season, host sex and host length. Thus, it will contribute to increasing the diversity of ectoparasitic helminth species recorded in *Barbus* spp. in previous studies. As a result of this investigation, we will provide the first records of the ectoparasitic helminth of host fish for Türkiye as well as contribute to our understanding of the geographical distribution and host range of the parasite species identified from the host fish.

Materials and Methods

Overall, 81 individuals of Marmara barbell, *Barbus oligolepis* were collected from Susurluk Stream, the village of Yıldız, Balıkesir ($39^0$48’973’’K and $28^0$10’14’’ D) between spring 2020 (April) and winter 2021 (February), with seasonal intervals (Figure 1). Fish were collected from the stream by electrofishing. The individuals were placed in plastic containers filled with stream water and immediately transported to the research laboratory as live subjects. They were kept in aerated fish tanks in the laboratory and necropsied for ectoparasitic helminth approximately 3-4 hrs after collecting. Dissections were performed within 3-4 hours. The fish were killed by severing the spinal cord behind the head, and their total length were measured and divided into three groups based on length, to the nearest 10 cm. During dissection, the sex of each fish individual was determined; 40 females and 41 males. During dissection, all internal organs, gill filaments, eyes, fins and the body surface of each fish individual were examined for ectoparasitic helminth under an Olympus stereomicroscope with 16x-40x magnification. Two monogenean species were collected from the gills and either prepared using glycerin ammonium picrate or stored in ethanol solution (Malmberg 1957). Data on each fish individual was categorized according to seasons, host fish size and sex groups. Morphological identification of the monogenean species was performed using the identification keys developed by Gussev (1985, 1987), Khotenovsky (1985) and available references; Prevalence, intensity and abundance of infections were calculated according to Bush et al. (1997) and the mean and standard deviation of each parameter was determined using Microsoft Excel (Office 2000). Kruskal-Wallis (more than two groups) tests were applied to find significant differences in the mean intensity of the parasite species for host fish size and seasons. The Mann-Whitney U test (two groups) was used to determine the correlation between the intensity of each helminth species infection and the host sex. The significance level of $\alpha \leq 0.05$ was used. All statistics analyses were performed using SPSS v. 23.

Furthermore, the molecular analysis was performed to confirm the morphological identification of diplozoid species as previously described by Aydogdu et al. (2020a, 2020b).

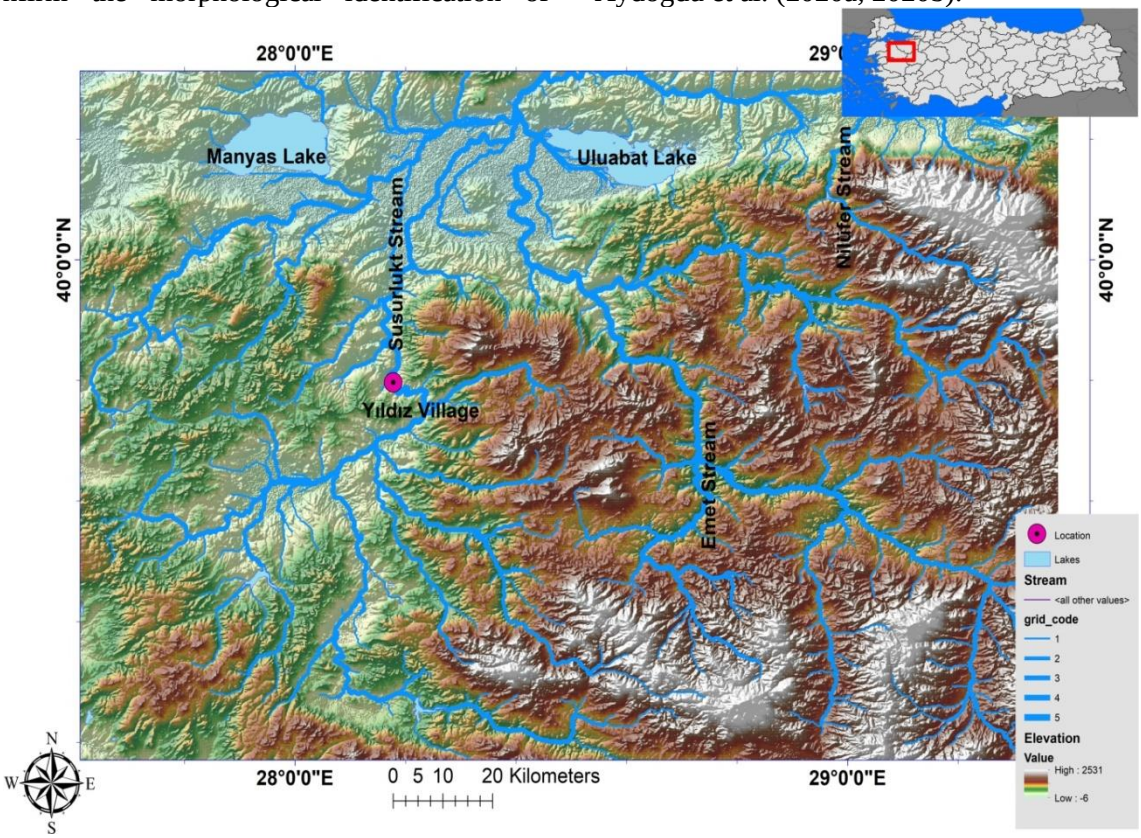

Figure 1. Sampling locality of *B. oligolepis* in the Susurluk Basin Map

Results

This study investigated ectoparasites of *Barbus oligolepis* in Susurluk Stream (Balıkesir) between the 2020 spring and 2021 winter. This examination revealed that 45 of 81 fish (55.5%) were infected with one or two monogenean species. According to the morphology observed in the collected parasite samples, only two monogenean species were identified, i.e., *Dactylogyrus carpathicus* (Figure 2, 3 and 4) and *Paradiplozoon homoion* (Figure 5) and we also confirmed *P. homoion* based on molecular analysis (Genbank assession number: OP558585).

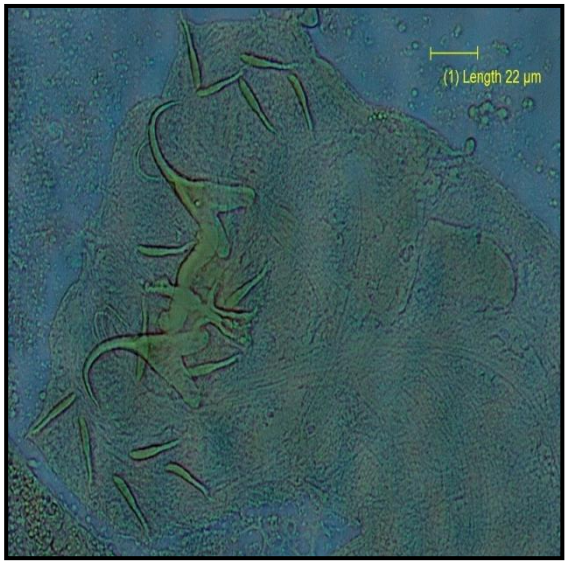

Figure 2. *D. carpathicus* haptor

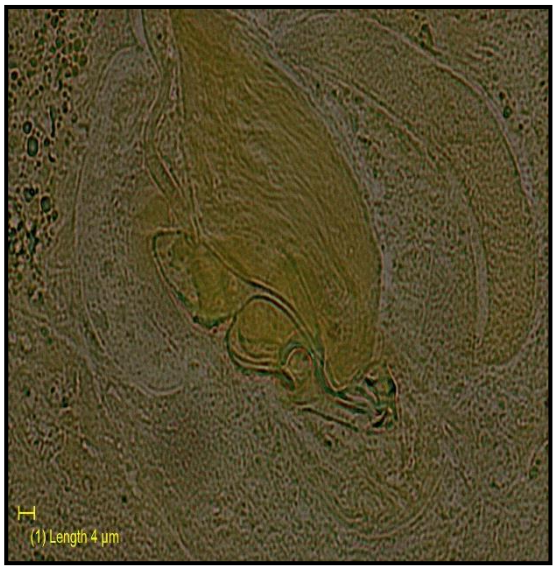

Figure 3. *D. carpathicus* copulatory organ

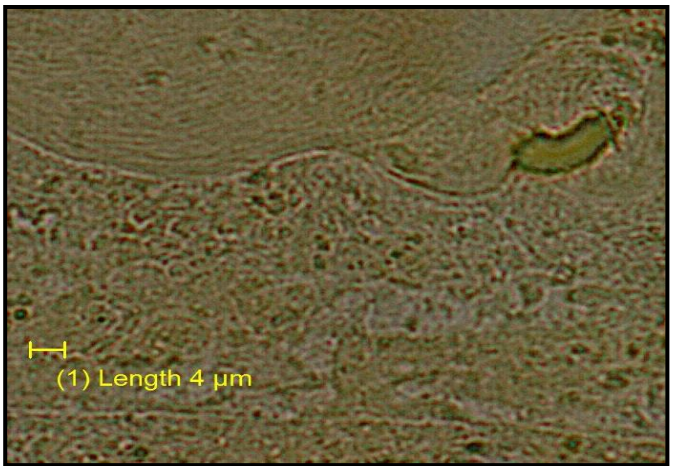

Figure 4. *D. carpathicus* vaginal tube

In our study, *P. homoion* (Figure 5) was the most prevalent and the highest number in the host fish. Moreover, it was detected in every sampling season. A total of 126 specimens of *P.homoion* (Figure 5) were found on 29 of the 81 fish examined. Infection variables were recorded as follows: prevalence 35.8 %, mean intensity 4.3 parasite/fish and mean abundance 0.6 (Table 1).

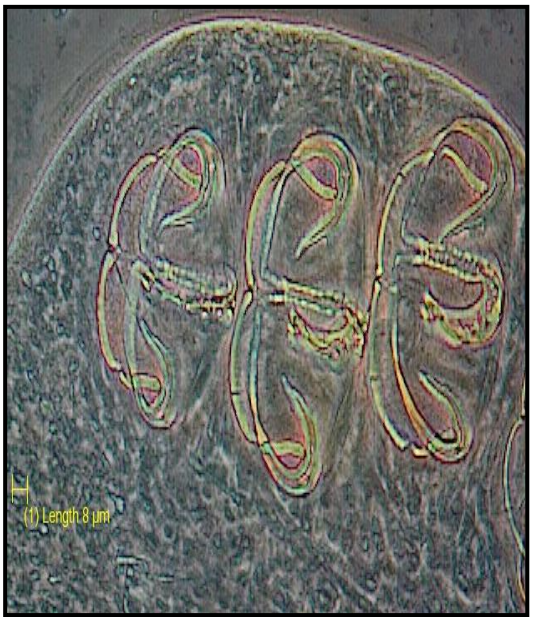

Figure 5. Structure of *P. homoion'*s clamps

Table 1. Distribution of infection value of ectohelminth parasites in *B. oligolepis* from Susurluk Basin, Balıkesir

Parasite species	Number of fish Infected	No. of parasites collected	Prevalence (%)	Mean intensity	Abundance
D. carpathicus	22	71	27.1	3.2	0.8
P. homoion	29	126	35.8	4.3	1.5

Seasonal prevalence of infection was highest in spring, 73.6 % ($p=0.016$, $p>0.05$). The highest abundance is also found in the spring, while the highest mean intensity (parasite/fish) was found in the winter season (Table 2).

Table 2. Distribution of infection value of ectohelminth parasites in *B. oligolepis* from Susurluk Basin, Balıkesir, according to seasons

Name of Parasites	Infection Parameters	Seasons			
		Spring 2020 (n: 19)	Summer 2020 (n: 20)	Autumn 2020 (n: 22)	Winter 2021 (n:20)
D. carpathicus	Infected Fish	10	3	9	
	Prevalence (%)	52.6	15	37.5	
	Mean Intensity	4	6.3	1.3	
	Abundance	2.1	0.9	0.5	
	Total parasite no	40	19	12	
P.homoion	Infected Fish	14	5	4	6
	Prevalence (%)	73.6	25	18.1	30
	Mean Intensity	4.5	3.4	3.7	5
	Abundance	3.3	0.8	0.6	1.5
	Total parasite no	64	17	15	30

A total of 71 specimens of *D. carpathicus* were recovered from 22 of 81 individuals of *B. oligolepis* examined (prevalence 27.1 %, mean intensity 3.2 parasite/fish, mean abundance 0.8 respectively) (Table 1). Seasonal prevalences varied between 15 and 52.6 %, highest in spring and lowest in summer and mean intensities of 1.3 to 6.3, highest in summer, varied significantly (Kruskal-Wallis p=0.135, p>0.05). The mean abundance of 0.5 to 2.1 was also highest in spring. This species was not found in any of the winter samples (Table 2).

The prevalence, intensity and abundance levels of *P. homoion* and *D. carpathicus* according to the size of the host fish are presented in Table 3. Both monogenean species were found in all fish size classes and infection values varied according to fish size classes (Table 3).

P. homoion was found on fish of all size classes, with prevalences varying between 27.2 and 44.4 %. The highest infection levels occurred in size class III (44.4 %) and its highest mean intensity levels were recorded in size class III (5.5 parasite/fish). However, there were no statistically significant differences between the size groups (p=0.726, p>0.05) in terms of the abundance level of this species. For *D. carpathicus*, the lowest prevalence was in size class I (15.3%), while the highest rate was in size class II (39.3%). Mean abundance of 0.2 to 1.6 was found to be highest in size class II (Table 3).

Table 3. Distribution of infection value of ectohelminth parasites in *B. oligolepis* from Susurluk Basin, Balıkesir, according to the host length

Fish Classes Groups (cm)	Parasite species	Infected Fish	Prevalence (%)	Mean intensity	Abundance	Total parasite no
10-20 (n=39)	*D. carpathicus*	6	15.3	1.6	0.2	10
	P. homoion	16	41	3.8	1.5	62
20.1-30 (n=33)	*D. carpathicus*	13	39.3	4	1.6	53
	P. homoion	9	27.2	4.6	1.2	42
30.1< (n=9)	*D. carpathicus*	3	33.3	2.6	0.8	8
	P. homoion	4	44.4	5.5	2.4	22

There were no statistically significant differences in abundance levels of this species between the size classes of the host fish (p=0.135, p>0.05). The prevalence, intensity and abundance levels of two monogenean species according to the sex of the host fish are presented in Table 4. For *P. homoion*, prevalence and mean intensity levels were both higher in females (37.5%, five parasite/fish) than in males (34.1%, 3.5 parasite/fish). The maximum parasite number was found in female fish specimens for this parasite species (Table 4). However, there was no statistical correlation between the numbers of *P. homoion* parasites between sex groups of the host fish (p=0.526, p>0.05). The prevalence and intensity levels of infection of *D. carpathicus* were both higher in females (30%, 3.5 parasite/fish) than in males (24.3%, 2.9 parasite/fish). Similarly, *D. carpathicus* was recorded in maximum numbers in female individuals of the host fish. In contrast to these findings, no correlation was found between the abundance of *D. carpathicus* and host sex (p=0.627, p>0.05).

Table 4. Distribution of infection value of ectohelminth parasites in *B. oligolepis* from Susurluk Basın, Balıkesir, according to the host sex

Fish Sex Groups	Parasite species	Infected Fish	Prevalence (%)	Mean intensity	Abundance	Total parasite no
Female (n=40)	*D. carpathicus*	12	30	3.5	1	42
	P. homoion	15	37.5	5	1.9	76
Male (n=41)	*D. carpathicus*	10	24.3	2.9	0.7	29
	P. homoion	14	34.1	3.5	1.2	50

Discussion

This study investigated ectohelminth ichthyo-parasitic fauna of the endemic fish, *Barbus oligolepis*, from Susurluk stream, the village of Yıldız, Balıkesir. Only two monogenean species were identified, namely *Dactylogyrus carpathicus* and *Paradiplozoon homoion*. In the present study, *D. carpathicus* were identified using morphological and anatomic assessment. The identifying feature of this species, which separate it from other closely related species in the genus *Dactylogyrus*, considered during the current study was that the anchors have nearly equally sized rots (dorso and ventro apical length), quite characteristic male copulatory organs, vaginal tube and ventral bars. (Figure 1, 2). Besides, the measurements of chitinoid structure parts served as the basis for identifying the species.

In the case of *P. homoion*, we described it based on morphology and confirmed it using molecular analysis. In addition to these, this is the first survey on the ichthyo-helminthological data for *B. oligolepis* in Türkiye. Furthermore, the host fish is the new host record for the two monogenean species. Moreover, *D. carpathicus* and *P. homoion* were found for the first time at Biotope Yıldız. Therefore, the present study adds new data to the geographical distribution and the host range of two parasite species. To date, ectoparasitic helminth species parasitizing freshwater fish in Turkey are mostly represented in two genera: *Dactylogyrus* and *Gyrodactylus*. *Dactylogyrus*, with 47 species, is the genus that representing the highest number of species parasitized in freshwater fish in Türkiye (Özer 2021). On the other hand, parasitic individuals of this genus recorded in 14 different freshwater fish species living in different habitats in Türkiye were not defined at the species level (Özer 2021). *D. carpathicus*, which we recorded in our current study, is one of the species defined at the species level. So far, *D. carpathicus* has only been reported from the Doğancı Dam Lake and Nilüfer Stream in the Marmara region in Türkiye (Aydogdu et al., 2002; Aydogdu and Kubilay, 2017). Therefore, the present study adds new data to the geographical distribution and the host range of *D. carpathicus*.

In the studies mentioned above, these authors have also investigated the effect of season on infection levels of this species. In this study, the highest infection levels of D. *carpathicus* in spring (52.6%) are similar to the findings of Aydogdu et al. (2002) in Doğancı Dam Lake. Aydoğdu and Kubilay (2017) found that the prevalence of infection with this species was highest in winter (92.3%) in *Barbus niluferensis* in the Nilüfer stream in Turkey's north west region. It is followed by the spring season, which has an 85.7 % prevalence. These findings are inconsistent with ours.

According to Özer (2021), the family Diplozoide consists of three genera: *Diplozoon*, *Eudiplozoon* and *Paradiplozoon*. To date, these three genera include eight named species of parasites and five parasites that have not been identified as a species in Türkiye. Among these genera, the genus *Paradiplozoon* is represented by the most species with six named parasite species in Turkey. Among these species, *P.*

homoion is the most recorded species so far in Turkey (see, for example, Aydogdu et al. 2020a, 2020b). According to these studies, this is the first record of *P. homoion* from this host fish and locality. Therefore, the present study increases the number of host fish and localities where *P. homoion* has been recorded in Türkiye.

The seasonal variation of the infection of *P. homoion* was also investigated in our study. The highest infection prevalence of this species was recorded in spring (73.6%) (Table 2). Infection intensity of the parasite peaked in winter (5 parasites/fish) (Table 2). Seasonal variation of *P.homoion* infection rates has also been studied in different fish species in Turkey so far (Koyun 2001; Öztürk 2005; Soylu 2007; Aydogdu et al. 2020a, 2020b). Aydogdu et al. (2020a, 2020b) from Manyas spirlin, *Alburnoides manyasensis* from Nilüfer stream, Bitterling fish, *Rhodeus amarus* (Bloch 1782) from Susurluk Stream, respectively and Soylu (2007) from flower fish, *Pseudophoxinus antalyae* (Bogutskaya, 1992) from Kepez, Antalya studied in seasonal variation of infection rates of this species. They recorded the highest infection prevalence value of *P. homoion* in the winter season. Contrary to these findings, Koyun (2001) did not find this species from bleak in winter, *Alburnus alburnus* (Linnaeus 1758). Similarly, while Öztürk (2005) recorded *P. homoion* from *Rutilus rutilus* (L., 1758) in all seasons except winter, the same researcher found this species only in *Chalcalburnus chalcoides* (Güldenstaedt, 1772) in summer in the same study. The findings of our study are similar to the findings of the studies mentioned above before our study. According to the authors, this situation could be due to different rates of parasite development in different waters. We also support this view.

Regarding the connection in this study, the highest prevalences and mean intensities of *D. carpathicus* were observed in fish 20.1-30 cm long (Group II) (Table 3), indicating a link between the infection level of two ectoparasitic helminth species and host fish size. The relationship between the infection levels of this species and host fish sizes in Türkiye was studied only by Aydogdu and Kubilay (2017). These authors investigated the relationship between infection rates of *D. carpathicus* and host length in *Barbus niluferensis.* In contrast to our study for *D. carpathicus*, they found the highest mean intensity of infection in the fish size classes of 15.1-20 cm (Group II) despite recording the highest prevalence levels of infection in the fish size classes of 10-15 cm (group I), *P. homoion*, was found in fish of all size classes. Infection prevalence levels and mean density for *P. homoion* were highest in the 30.1 < cm fish size classes (group III), (Table 3). According to current data, only four ichthyoparasitological studies in Türkiye have been conducted to determine the relationships between infection levels of *P. homoion* and host fish sizes Koyun (2001), Soylu (2007), Öztürk (2011), Aydogdu et al. (2020a). Among these four different studies, only Aydoğdu et al. (2020a) observed the highest prevalence levels of infection of this species in large-size classes (7.1- 12 cm). This study lends support to the findings of our study in this context.

In our study, the prevalence and abundance of *D. carpathicus* and *P. homoion* were higher in the female host than in the male host. However, there was no statistically significant difference in the number of *D. carpathicus* (p=0.627) and *P. homoion* (p=0.526) between the sexes of the host fish. In this context, in only one Aydogdu and Kubilay (2017) of the two studies in which *D. carpathicus* was found in our country, the preference of parasite infection values of this species based on sex was investigated and the prevalence, the mean intensity values of infection in female fish were found to be higher. Our study is in line with the findings of Aydogdu and Kubilay (2017). As for *P. homoion*, the highest prevalence and mean intensity levels of infection were the highest in female fish (Table 4). In their study, Aydogdu et al. (2020b) found the highest prevalence and mean intensity levels of this species in female fish in *Alburnoides manyasensis* (Turan et al. 2013). The results of the present study also confirm the finding of Aydogdu et al. (2020b)**.** In contrast to our study, Tunç and Koyun (2018) recorded the highest infection rate of this species in male fish.

The purpose of this study was to determine the ectoparasitic helminth fauna of Türkiye endemic fish, *B. oligolepis*, from the Susurluk Stream, in the village of Yıldız, Balıkesir. As a result of the conducted study of 81 individuals of *B. oligolepis*, only two monogenean species *D. carpathicus* and *P. homoion* were identified. Among these parasites, *P. homoion* was the most prevalent and had the highest number in the host fish. To our knowledge, *D. carpathicus* and *P. homoion* are the first records of this host fish. Additionally, Susurluk Stream was identified as a new locality record for two monogenean species. Furthermore, in the present study, the prevalence, mean intensity and abundance of helminth parasites were calculated in accordance with season, host fish length classes and sex. As a result, new information about the geographical distribution and host range of these helminth species has been gained.

Conflict of interest

The authors declare no conflict of interest

Compliance with ethical standards

During the study, no treatment/experiment was implemented on the live animal. All sampling and laboratory work on fish complies with the Republic of Turkey, Ministry of Agriculture and Forestry animal welfare laws.

Data availability statement

No data availability statement

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Low-Pressure Application Improved the Physicochemical and Microbiological Properties of Fresh and Stored Pikeperch (*Sander lucioperca*)

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ABSTRACT

In this study, low-pressure treatment was applied to pike-perch and the changes in quality criteria during the storage process were examined. L*, a*, and b* values in samples treated with low pressure were significantly different compared to the control. It was observed that the process had an improving effect on the textural properties of the fish. While the a_w value decreased to 0.822, the pH value decreased to 6.47. While TBARS values of the samples ranged between 0.014 and 0.031 mg MA / kg, TVB-N values ranged between 7.69 and 24.06 mg / 100 g. Total aerobic mesophilic bacteria, yeast/mold, total coliform group bacteria, and total aerobic psychrophilic bacteria counts of all samples exposed to low-pressure treatment were lower than the control during storage. As a result, the treatment positively affected the quality of pikeperch meat during the storage period.

Keywords: Pike-perch, low-pressure, quality, texture

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Düşük Basınç Uygulaması Taze ve Depolanmış Sudak Balığının (*Sander lucioperca*) Fizikokimyasal ve Mikrobiyolojik Özelliklerini Geliştirdi

Öz: : Bu çalışmada sudak balığına düşük basınç işlemi uygulanmış ve depolama sürecindeki kalite kriterlerindeki değişimler incelenmiştir. Düşük basınç işlemi uygulanmış örneklerde L*, a* ve b* değerleri kontrole kıyasla belirgin bir şekilde farklı çıkmıştır. İşlemin balığın tekstürel özellikleri üzerinde iyileştirici etkisi olduğu görülmüştür. a_w değeri 0,822 değerine kadar düşerken, pH değeri 6,47 değerine kadar düşmüştür. Numunelerin TBARS değerleri 0,014 – 0,031 mg MA/kg aralığında değişirken, TVB-N değerleri 7,69 – 24,06 mg/100 g aralığında değişmiştir. Düşük basınç işlemine maruz kalmış bütün örneklerin toplam aerobik mezofilik bakteri, maya/küf, toplam koliform grubu bakteri, toplam aerobik psikrofilik bakteri sayımları depolama süresince kontrole kıyasla daha düşük çıkmıştır. Sonuç olarak yapılan işlem depolama sürecinde sudak balığı etinin kalitesi üzerinde olumlu etki göstermiştir.

Anahtar kelimeler: Sudak, düşük basınç, kalite, tekstür

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Introduction

Manufacturers encounter several significant challenges while producing food products. One of the priorities is to provide affordable, high-nutrition, and reliable food that can meet the needs of the ever-growing population. Producers are developing new techniques to meet the increasing demand for minimally processed, perishable foods. Therefore, producers are developing different techniques to cope with these problems and trying to apply them commercially (Corradini 2018). Another problem with food products is that they are often transported and consumed outside their production location and timeframe. Since many food products are consumed in different places and over extended periods, storage becomes critical (Gökoğlu 2020).

Spoilage of food means that the product becomes unconsumable, and if consumed, it causes serious health problems. Especially products such as fresh fish meat, which have a high water activity value, are rich in nutrients, have weak connective tissue and pH values close to neutral, are considered easily

perishable foodstuff (Gökoğlu 2019). The main factors affecting spoilage in fish are biochemical reactions such as hydrolysis and oxidation of polyunsaturated fatty acids catalyzed by endogenous proteases (Zeng et al. 2023). To ensure the delivery of high-quality fish meat to consumers, it is crucial to preserve and process fish under appropriate conditions.

Today, consumers pay considerable attention to the quality and freshness of food products, as well as their minimal processing. Thermal treatment traditionally applied in food production causes a loss of quality and a decrease in the nutritional value of raw materials. Therefore, using new non-thermal technologies is very advantageous to reduce the heat-related loss of nutritional value and deterioration in sensory quality that occurs in traditional production methods (Chen et al. 2020). Foods with higher nutritional value, longer shelf life, and higher product safety can be produced with these technologies. Therefore, the demand for minimal and non-heat-treated food products has increased significantly in recent years. Because of this, as with other foods, research on the application of non-thermal methods for the preservation of fish has become very popular (Rathod et al. 2021).

The pikeperch (*Sander lucioperca*) is a freshwater fish that belongs to the family Percidae and is native to Asia and Europe. The pikeperch stands out with its meat being soft, white, delicious, and low in fat. It is appreciated and preferred by consumers as it is also one of the most popular freshwater fish due to its small number of intermuscular bones (Tönißen et al. 2022). Within the scope of our research, pikeperch was preferred because it is abundant in the inland waters of our country, is a fish species preferred by consumers, and its meat is suitable for use in the study. Upon reviewing the available literature, it has come to our attention that there are no existing studies on the utilization of low pressure in pikeperch fish. As such, our study marks a pioneering effort in this particular field.

Materials and Methods

Materials

The pikeperch (*S. lucioperca* (L.,1758)) used in the research was caught from Lake Eğirdir in May 2023. The average weight of the caught fish was 432.53 ± 101.24 grams and their length varied between 36.42 ± 3.72 cm. The fish were brought to the laboratory on the same day in ice-filled foam boxes. After use, they were promptly cleaned and stored in a freezer at a temperature of -20 ℃ until they were ready to be analyzed again.

Methods

Application of low-pressure treatment

A specially designed cabin was used for the low-pressure process applied to fish samples. The samples were subjected to two different low-pressure and time applications separately. The applications determined cabin interior conditions as pressure: -250/-500 mbar, temperature: 4 °C, humidity: 55.7 %, oxygen concentration: 0.06 %, carbon dioxide: 0.13 ppm.

Physicochemical analysis of samples

pH and a_w values

pH values of pikeperch samples were measured with a calibrated pH meter (Ohaus, starter 3100) (AOAC 2016). Water activity values of the samples were determined according to AOAC (2016) with the help of a water activity tester (Novasina Lab Touch-a_w Lachen, Switzerland).

Thiobarbituric acid (TBA) and total volatile basic nitrogen (TVB-N) values

TBA values of pikeperch samples during storage were determined by Tarladgis et al. (1960), while TVB-N values were determined according to the method specified in İnal (1992).

Color values

Color analysis of the samples was carried out using a Konica Minolta (Chroma meter CR-400) device. Color measurements were determined separately by making three parallel measurements on the interior section (flesh) and exterior (skin) surfaces.

Texture profile analysis (TPA) values

TPA values of fish samples were determined at room temperature using a 5 kg load cell texture analyzer (TA-XT2i; Stable Microsystems Ltd. Surrey, UK). Measurements were performed using a cylindrical aluminum probe (P/50, 50 mm diameter Stable Micro Systems LTD, Godalming, UK). Pre-test, test, and post-test speeds were set to 5, 1, and 5 mm/s, respectively. The type of deformation applied to the samples during the analysis was selected as strain and set as 50%. Measurements were made in triplicate to determine the TPA profile of each sample (Eroğlu et al. 2015).

Microbiological analysis

Microbiological analysis of the samples was performed according to the spread plate technique. For microbiological analysis, 10 g of sample was taken, and 90 mL of sterile Ringer's solution (Merck, 11525, Germany) was added and homogenized in a Stomacher (Lab Stomacher Blender 400-BA 7021, Seward Medical). Appropriate dilutions were prepared by taking 1 mL of this homogenate. In the research, all sowings were carried out in two parallel ways, and the results were given as log cfu/g (Sekin and Karagözlü 2004).

Total aerobic mesophilic bacteria (TAMB) and total aerobic psychrophilic bacteria (TAPB) counts were determined using plate count agar (PCA) (Merck 1.05463, Germany). The cultivated Petri dishes were incubated under aerobic conditions for 48-72 hours at 30°C for TAMB count and 5-7 days at 0-4°C for TAPB count (ISO 2008; ISO 2013a). For the total count of yeast/molds, rose bengal chloramphenicol agar (Merck 1.00467, Germany) (RBC) was used, and the cultivated Petri dishes were incubated at 22°C for 5-7 days under aerobic conditions (ISO 2013b). For total coliform group bacteria (TCGB) count, violet red bile agar (Merck 1.01406, Germany) was used, and Petri dishes were incubated under aerobic conditions at 30°C for 24-48 hours (ISO 1991).

Experimental design and statistical analysis

The experimental design was random with a factorial structure (3 x 6). Factors are storage time (days 1, 5, and 7) and fish samples (control, -250 mbar for 60 minutes, -250 mbar for 120 minutes, -500 mbar for 60 minutes, and -500 mbar for 120 minutes low-pressure treated samples). Two-way analysis of variance was used to determine differences ($P < 0.05$) between samples across sample type and storage time. The analysis results were subjected to the ANOVA procedure followed by Duncan's multiple range tests (SPSS, version 23). The design was completely randomized with replications.

Results

Color values

The color values determined by measuring

Table 1. Color values of pikeperch samples subjected to low-pressure treatment

		Sample	Storage Time		
			1st Day	5th Day	7th Day
L*	Interior	Control	43.89±0.65^{Ac}	40.83±0.69^{Bc}	35.62±1.20^{Cc}
		-250 mbar 60 min	45.01±0.19^{Ac}	43.90±0.25^{Bb}	40.61±0.36^{Cab}
		-250 mbar 120 min	47.09±0.72^{Ab}	42.19±0.50^{Bbc}	39.44±1.82^{Bbc}
		-500 mbar 60 min	46.67±0.49^{Ab}	43.61±1.57^{ABb}	39.89±2.23^{Bbc}
		-500 mbar 120 min	49.09±0.56^{Aa}	47.72±0.99^{ABa}	44.37±1.28^{Ba}
	Exterior	Control	39.90±0.31^{Ab}	36.47±1.34^{Ab}	31.27±1.62^{Bb}
		-250 mbar 60 min	41.91±1.05^{Ab}	39.69±0.63^{Aa}	34.29±1.65^{Bab}
		-250 mbar 120 min	43.99±0.38^{Aa}	40.79±0.45^{Aa}	35.54±1.84^{Bab}
		-500 mbar 60 min	44.76±0.78^{Aa}	40.40±1.48^{ABa}	35.95±1.83^{Bab}
		-500 mbar 120 min	45.75±0.69^{Aa}	41.43±1.37^{ABa}	38.69±2.04^{Ba}
a*	Interior	Control	4.60±0.03^{Ca}	6.74±0.11^{Ba}	8.99±0.06^{Aa}
		-250 mbar 60 min	4.28±0.07^{Cb}	5.24±0.12^{Bb}	7.01±0.25^{Ab}
		-250 mbar 120 min	4.22±0.0.04^{Cb}	5.22±0.18^{Bb}	6.79±0.20^{Ab}
		-500 mbar 60 min	4.16±0.07^{Bb}	4.71±0.39^{ABbc}	5.33±0.28^{Ac}
		-500 mbar 120 min	4.11±0.11^{Ab}	4.17±0.15^{Ac}	4.42±0.08^{Ad}
	Exterior	Control	2.93±0.77^{Aa}	2.26±0.78^{Ba}	2.04±0.18^{Ba}
		-250 mbar 60 min	2.49±0.78^{Ab}	2.25±0.28^{Aa}	1.82±0.23^{Aa}
		-250 mbar 120 min	2.28±0.08^{Ab}	1.95±0.13^{Ba}	1.88±0.07^{Ba}
		-500 mbar 60 min	2.26±0.21^{Ab}	2.12±0.16^{Aa}	2.07±0.08^{Aa}
		-500 mbar 120 min	1.73±0.19^{Ac}	1.50±0.06^{Ab}	1.38±0.04^{Ab}
b*	Interior	Control	3.20±0.15^{Ba}	3.24±0.17^{Ba}	4.47±0.11^{Aa}
		-250 mbar 60 min	2.08±0.30^{Ab}	2.49±0.06^{Ab}	2.88±0.33^{Ab}
		-250 mbar 120 min	1.10±0.05^{Cc}	1.97±0.26^{Bc}	2.56±0.17^{Abc}
		-500 mbar 60 min	1.91±0.17^{Bb}	2.18±0.21^{Bbc}	2.76±0.07^{Abc}
		-500 mbar 120 min	1.07±0.33^{Bc}	1.98±0.21^{Ac}	2.34±0.13^{Ac}
	Exterior	Control	-3.22±0.13^{Aa}	-3.43±0.13^{Aa}	-3.96±0.11^{Bd}
		-250 mbar 60 min	-3.86±0.07^{Bb}	-3.29±0.25^{Aba}	-3.08±0.21^{Ac}
		-250 mbar 120 min	-4.36±0.47^{Bbc}	-3.49±0.18^{Ba}	-2.05±0.31^{Ab}
		-500 mbar 60 min	-4.18±0.10^{Bbc}	-3.08±0.31^{Aba}	-2.54±0.52^{Abc}
		-500 mbar 120 min	-4.71±0.18^{Cc}	-2.75±0.42^{Ba}	-0.92±0.27^{Aa}

±: Represents standard deviations. A - C (→): Values with the different capital letters in the same line for each analysis differ significantly ($P < 0.05$). a - d (↓): Values with the different small letters in the same column for each analysis differ significantly ($P < 0.05$).

the interior and exterior of the samples are given in Table 1.

When the storage time was examined, it was observed that there was a time-dependent decrease in the L* value of each sample. Regarding the applied process parameters, the increase in pressure and time also caused an increase in the L* value. The highest L* (interior) and L* (exterior) values were detected in the first day sample (49.09 and 45.75) with -500 mbar pressure for 120 min treatment, while the lowest L* (interior) and L* (exterior) values were detected in the control sample on the seventh day (35.62 and 31.27).

While there was a time-dependent increase in a* interior values of all samples according to the storage time, there was a decrease in a* exterior values. The increase in pressure and time caused an increase in a* (interior) value and a decrease in a* (exterior) value. While the highest a* (interior) value was detected in the control sample on the seventh day (8.99), the lowest a* (interior) value was detected in the first-day sample (4.11) with -500 mbar pressure for 120 min treatment. While the highest a* (exterior) value was detected in the control sample on the first day (2.93), the lowest a* (exterior) value was detected in the seventh-day sample (1.38) with -500 mbar pressure for 120 min treatment.

There was a time-dependent increase in all samples' b* (interior) values according to the storage time. There was a general increase in the b* (exterior) values, except for the control sample. Parallel to the increase in pressure and time, there was an increase in b* (interior) values. When b* (exterior) values are examined, while there was a decrease in the control sample, there was an increase in all treated samples. While the highest b* (interior) value was detected in the control sample on the seventh day (4.47), the lowest b* (interior) value was detected in the first-day sample (1.07) with -500 mbar pressure for 120 min treatment. The highest b* (exterior) value was detected in the seventh-day sample (-0.92), where -500 mbar pressure for 120 min of treatment was applied.In contrast, the lowest b* (exterior) value was detected in the seventh-day sample (4.71), where -500 mbar pressure for 120 min of treatment was applied.

Textural values

Textural values of the samples are given in Table 2.

Table 2. Textural values of pikeperch samples subjected to low-pressure treatment

	Sample	Storage Time 1st Day	5th Day	7th Day
Hardness	**Control**	730.69±6.33^{Ba}	1227.44±22.38^{Aa}	1261.63±45.85^{Aa}
	-250 mbar 60 min	225.75±9.06^{Cb}	512.32±14.97^{Bc}	581.87±22.10^{Ac}
	-250 mbar 120 min	118.60±8.66^{Cd}	554.61±9.00^{Bb}	634.28±16.65^{Abc}
	-500 mbar 60 min	141.39±11.24^{Cc}	587.37±14.32^{Bb}	664.04±22.35^{Ab}
	-500 mbar 120 min	117.86±3.38^{Ce}	362.91±10.82^{Bd}	511.61±14.96^{Ad}
Adhesiveness	**Control**	-5.59±0.65^{Aa}	-7.02±0.16^{Ba}	-9.78±0.33^{Ca}
	-250 mbar 60 min	-20.49±0.79^{Ad}	-25.07±1.90^{Bc}	-25.09±1.37^{Bb}
	-250 mbar 120 min	-18.44±1.69^{Acd}	-19.09±2.10^{Ab}	-31.28±0.88^{Bc}
	-500 mbar 60 min	-16.25±0.55^{Abc}	-19.48±0.09^{Bb}	-26.22±1.23^{Cb}
	-500 mbar 120 min	-14.59±0.78^{Ab}	-16.33±0.08^{Ab}	-32.81±1.86^{Bc}
Springiness	**Control**	0.908±0.01^{Aa}	0.817±0.02^{Ba}	0.694±0.02^{Ca}
	-250 mbar 60 min	0.676±0.03^{Ab}	0.598±0.02^{Bb}	0.519±0.02^{Cb}
	-250 mbar 120 min	0.609±0.01^{Ac}	0.557±0.01^{Bc}	0.447±0.02^{Cc}
	-500 mbar 60 min	0.589±0.01^{Ac}	0.560±0.01^{Ac}	0.427±0.02^{Bcd}
	-500 mbar 120 min	0.538±0.02^{Ad}	0.498±0.01^{Ad}	0.395±0.01^{Bd}
Cohesiveness	**Control**	0.676±0.02^{Aa}	0.638±0.02^{ABa}	0.623±0.01^{Ba}
	-250 mbar 60 min	0.592±0.02^{Ab}	0.579±0.01^{Ab}	0.496±0.01^{Bb}
	-250 mbar 120 min	0.557±0.01^{Ac}	0.517±0.02^{Ac}	0.456±0.01^{Bc}

(table continues)	**-500 mbar 60 min**	0.554±0.01^{Ac}	0.527±0.02^{Ac}	0.445±0.01^{Bc}
	-500 mbar 120 min	0.536±0.01^{Ac}	0.500±0.01^{Bc}	0.432±0.01^{Cc}
Gumminess	**Control**	494.01±17.71^{Ba}	783.60±1.27^{Aa}	785.73±14.29^{Aa}
	-250 mbar 60 min	133.74±9.83^{Bb}	296.92±10.48^{Ab}	288.52±7.41^{Ab}
	-250 mbar 120 min	66.09±5.83^{Bc}	286.82±15.43^{Ab}	289.29±12.07^{Ab}
	-500 mbar 60 min	78.37±7.22^{Bc}	310.03±22.92^{Ab}	295.94±16.06^{Ab}
	-500 mbar 120 min	63.23±1.56^{Cc}	181.52±10.03^{Bc}	221.12±13.70^{Ac}
Chewiness	**Control**	448.63±20.27^{Ca}	640.58±13.91^{Aa}	545.57±2.85^{Ba}
	-250 mbar 60 min	90.54±10.24^{Cb}	177.63±10.89^{Ab}	149.68±1.77^{Bb}
	-250 mbar 120 min	40.24±3.17^{Cc}	159.96±10.94^{Ab}	129.58±11.33^{Bb}
	-500 mbar 60 min	46.25±5.31^{Cc}	173.59±12.39^{Ab}	126.67±12.93^{Bb}
	-500 mbar 120 min	34.04±2.36^{Bc}	90.56±7.69^{Ac}	87.54±8.39^{Ac}
Resilience	**Control**	0.386±0.01^{Ca}	0.467±0.01^{Ba}	0.513±0.01^{Aa}
	-250 mbar 60 min	0.359±0.01^{Bb}	0.441±0.01^{Aa}	0.478±0.01^{Ab}
	-250 mbar 120 min	0.339±0.01^{Ccd}	0.399±0.01^{Bb}	0.478±0.01^{Ab}
	-500 mbar 60 min	0.355±0.01^{Cb}	0.407±0.01^{Bb}	0.463±0.01^{Abc}
	-500 mbar 120 min	0.328±0.01^{Cc}	0.381±0.01^{Bb}	0.448±0.01^{Ac}

±: Represents standard deviations. A - C (→): Values with the different capital letters in the same line for each analysis differ significantly ($P < 0.05$). a - e (↓): Values with the different small letters in the same column for each analysis differ significantly ($P < 0.05$).

The low-pressure process was effective on all texture values. The highest hardness value was detected in the control sample on the seventh day (1261.63), while the lowest hardness value was detected in the first-day sample (117.86) with -500 mbar pressure for 120 min treatment. The highest adhesiveness value was determined in the control sample on the first day (-5.59), and the lowest adhesiveness value was determined in the seventh day sample (-32.81), which was applied at -500 mbar pressure for 120 min of treatment. The lowest springiness value was found in the seventh-day sample (0.395) with -500 mbar pressure for 120 min treatment, while the highest springiness value was found in the first-day control sample (0.908). Similarly, the lowest cohesiveness value was determined in the seventh-day sample (0.432), where -500 mbar pressure for 120 min treatment was applied, and the highest cohesiveness value was determined in the first-day control sample (0.676). When the gumminess value was examined, the lowest value was detected in the first-day sample (63.23) with -500 mbar pressure for 120 min treatment, and the highest value was found in the control sample on the seventh day (785.73). When looking at the chewiness values, the highest value was found in the control sample on the fifth day (640.58), and the lowest value was found in the first-day sample (34.04) with -500 mbar pressure for 120 min of treatment. Regarding resilience value, the highest value was determined in the control sample on the seventh day (0.513), and the lowest value was determined in the first-day sample (0.328) with -500 mbar pressure for 120 min treatment.

a_w, pH, TBARS and TVB-N values

a_w, pH, TBARS and TVB-N values of the samples are given in Table 3.

In general, a_w values tended to decrease according to the storage time of the samples and the applied process parameters. While the highest a_w value was determined in the control sample on the first day (0.900), the lowest a_w value was determined in the seventh-day sample (0.822), which was applied at -500 mbar pressure for 120 min of treatment. Considering the pH values, the low-pressure treatment did not cause much change in the samples; only an increase was observed in the control sample over time.

Table 3. a_w, pH, TBARS and TVB-N values of pikeperch samples subjected to low-pressure treatment

	Sample	Storage Time 1st Day	5th Day	7th Day
a_w	**Control**	0.900±0.03^{Aa}	0.883±0.02^{Ba}	0.872±0.03^{Ca}
	-250 mbar 60 min	0.876±0.01^{Ab}	0.859±0.01^{Bc}	0.850±0.01^{Bb}
	-250 mbar 120 min	0.868±0.03^{Abc}	0.844±0.03^{Bd}	0.826±0.02^{Cc}
	-500 mbar 60 min	0.877±0.03^{Ab}	0.865±0.01^{Bb}	0.853±0.02^{Cb}
	-500 mbar 120 min	0.864±0.01^{Ac}	0.843±0.01^{Bd}	0.822±0.01^{Cc}
pH	**Control**	6.65±0.01^{Ca}	6.81±0.03^{Ba}	7.03±0.02^{Aa}
	-250 mbar 60 min	6.61±0.01^{Bb}	6.65±0.01^{ABb}	6.70±0.03^{Ab}
	-250 mbar 120 min	6.57±0.01^{Ac}	6.54±0.01^{Ac}	6.58±0.02^{Ac}
	-500 mbar 60 min	6.55±0.01^{Ac}	6.50±0.01^{Bcd}	6.53±0.01A^{Bcd}
	-500 mbar 120 min	6.51±0.01^{Ad}	6.47±0.02^{Ad}	6.52±0.02^{Ad}
TBARS (mg MA/kg)	**Control**	0.026±0.002^{Ca}	0.029±0.001^{Ba}	0.031±0.002^{Aa}
	-250 mbar 60 min	0.024±0.001^{Bb}	0.024±0.001^{Bb}	0.026±0.001^{Ab}
	-250 mbar 120 min	0.019±0.001^{Bc}	0.019±0.001^{Bc}	0.021±0.001^{Ac}
	-500 mbar 60 min	0.023±0.001^{Bb}	0.023±0.001^{Bb}	0.025±0.001^{Ab}
	-500 mbar 120 min	0.014±0.001^{Bd}	0.015±0.001^{Bd}	0.018±0.001^{Ad}
TVB-N (mg/100 g)	**Control**	10.78±0.50^{Ba}	19.67±0.91^{Aa}	24.06±3.69^{Aa}
	-250 mbar 60 min	9.92±0.15^{Ca}	14.76±0.77^{Bb}	18.56±1.75^{Aab}
	-250 mbar 120 min	8.57±0.19^{Cb}	12.09±0.30^{Bc}	15.24±0.24^{Abc}
	-500 mbar 60 min	7.69±0.94^{Cb}	10.07±0.28^{Bd}	15.62±0.76^{Abc}
	-500 mbar 120 min	7.82±0.17^{Bb}	8.77±0.34^{Bd}	12.01±0.49^{Ac}

±: Represents standard deviations. A - C (→): Values with the different capital letters in the same line for each analysis differ significantly ($P < 0.05$). a - d (↓): Values with the different small letters in the same column for each analysis differ significantly ($P < 0.05$).

The highest pH value was detected in the control sample (7.03) on the seventh day, while the lowest was in the fifth-day sample (6.47) with -500 mbar pressure for 120 min treatment. When TBARS and TVB-N values are examined, the values detected in the low-pressure treated samples were detected in lower amounts than the control samples. The highest TBARS value was determined in the control sample on the seventh day (0.031 mg MA/kg), and the lowest TBARS value was determined in the first-day sample (0.014 mg MA/kg) with -500 mbar pressure for 120 min treatment. Similarly, the highest TVB-N value was found in the control sample on the seventh day (24.06 mg/100 g), and the lowest TVB-N value was found in the first-day sample (7.82 mg/100 g) with -500 mbar pressure for 120 min treatment.

Microbiological analysis results

Microbiological analysis results of the samples are given in Table 4. When the effect of low-pressure treatment on different groups of microorganisms was examined, it was seen that the counts in all treated samples were lower compared to the control samples. The total count of aerobic mesophilic bacteria was lowest in the fifth-day sample (3.67 log cfu/g) applied at -500 mbar pressure for 120 min treatment. The lowest value in terms of yeast/mold count was detected in the first-day sample (2.00 log cfu/g) at -500 mbar pressure for 120 min of treatment. When the total coliform group bacterial counts were examined, the lowest value was determined in the first-day sample (1.85 log cfu/g), where -500 mbar pressure for 120 min of treatment was applied. Finally, the lowest count of the total aerobic psychrophilic bacteria count was determined in the first-day sample (2.57 log cfu/g) at 500 mbar pressure for 120 min treatment.

Discussion

The effect of low-pressure treatment on the color values of the samples was found to be statistically significant ($P < 0.05$). The increased applied treatment parameters also led to an increase in L* values. However, all samples observed a decrease in L* values over time. The increase in treatment parame ters led to a decrease in both a* (interior) and a* (exterior) values. Considering the duration of storage, an increase in a* (interior) value was observed, while a decrease in a* (exterior) value was

Table 4. Microbiological analysis results of pikeperch samples subjected to low-pressure treatment

	Sample	Storage Time		
		1st Day	5th Day	7th Day
Total Aerobic Mesophilic Bacteria Count (log cfu/g)	**Control**	4.04±0.06^{Ca}	5.19±0.03^{Ba}	6.90±0.04^{Aa}
	-250 mbar 60 min	3.97±0.03^{Bab}	4.11±0.03^{Bb}	5.47±0.08^{Ab}
	-250 mbar 120 min	3.94±0.01^{Bab}	4.04±0.17^{ABb}	4.38±0.07^{Ac}
	-500 mbar 60 min	3.87±0.03^{Bb}	3.90±0.02^{Bbc}	5.24±0.12^{Ab}
	-500 mbar 120 min	3.75±0.06^{Bc}	3.67±0.08^{Bc}	4.15±0.07^{Ac}
Yeast/Mold Count (log cfu/g)	**Control**	2.76±0.04^{Ca}	4.21±0.17^{Ba}	5.93±0.03^{Aa}
	-250 mbar 60 min	2.61±0.03^{Cb}	3.00±0.01^{Bb}	4.61±0.06^{Ab}
	-250 mbar 120 min	2.41±0.02^{Bd}	2.59±0.07^{Bd}	4.30±0.09^{Ac}
	-500 mbar 60 min	2.52±0.01^{Cc}	2.67±0.05^{Bc}	4.46±0.04^{Ac}
	-500 mbar 120 min	2.00±0.05^{Ce}	2.42±0.04^{Be}	4.00±0.06^{Ad}
Total Coliform Group Bacteria (log cfu/g)	**Control**	2.94±0.04^{Ca}	4.96±0.03^{Ba}	5.34±0.05^{Aa}
	-250 mbar 60 min	2.67±0.05^{Cb}	3.69±0.05^{Bb}	4.09±0.06^{Ab}
	-250 mbar 120 min	2.15±0.03^{Cc}	3.29±0.04^{Bc}	3.77±0.09^{Ac}
	-500 mbar 60 min	2.23±0.03^{Cc}	3.38±0.03^{Bc}	3.86±0.05^{Ac}
	-500 mbar 120 min	1.85±0.12^{Cd}	2.41±0.11^{Bd}	2.77±0.02^{Ad}
Total Aerobic Psychrophilic Bacteria (log cfu/g)	**Control**	3.18±0.06^{Ca}	4.47±0.07^{Ba}	5.66±0.01^{Aa}
	-250 mbar 60 min	2.93±0.04^{Cb}	3.73±0.09^{Bb}	4.59±0.03^{Ab}
	-250 mbar 120 min	2.78±0.04^{Cbc}	3.53±0.01^{Bc}	4.44±0.02^{Ad}
	-500 mbar 60 min	2.76±0.09^{Cc}	3.73±0.03^{Bb}	4.51±0.01^{Ac}
	-500 mbar 120 min	2.57±0.04^{Cd}	3.13±0.02^{Bd}	3.99±0.02^{Ae}

±: Represents standard deviations. A - C (→): Values with the different capital letters in the same line for each analysis differ significantly ($P < 0.05$). a - e (↓): Values with the different small letters in the same column for each analysis differ significantly ($P < 0.05$).

observed.

When the effect of process parameters on b* values was examined, it resulted in a similar decrease in b* (interior) and b* (exterior) values. During the storage process, an increase was detected in all but a* (interior) values, while an increase was also detected in a* (exterior) values, except for control. When all color parameters were evaluated as a whole, it was seen that the low-pressure process had different effects on the flesh and skin of the pikeperch.

In a study (Bou et al. 2023), L*, a*, and b* values in vacuum-treated sea bream fillets were determined as 52, -4.6, and -0.8 on the first day, and 51, -4.6, and 0.3 on the fifth day, respectively. It is postulated that the dissimilarities in these estimations when compared to the outcomes of our research, are attributable to the variances in the species of fish utilized.

The low-pressure treatment applied to pikeperch was observed to have a significant effect, especially on texture values ($P < 0.05$). Hardness values in all treated samples decreased compared to the control. The increased applied pressure and time significantly caused a further decrease in hardness values. Depending on the storage process, hardness values also increased as the storage time increased. Adhesiveness values were also determined to be lower in treated samples compared to the control. A decrease in adhesiveness values was observed depending on the storage time. Notably, applying pressure at lower values caused lower adhesiveness values. In general, both the increase in the applied treatment parameters and the increase in storage time led to a decrease in springiness and cohesiveness values. As the applied pressure and time increased, both values decreased. Gumminess values in all treated samples were lower than the control. However, the change in treatment parameters had different effects on the samples. The gumminess values generally increased as the storage time increased depending on the process. Chewiness values of low-pressure treated samples decreased compared to the control. The increasedapplied pressure and time led to a general decreasing trend in chewiness values. During the storage process, chewiness values increased from the first day to the fifth day and decreased again on the seventh. Compared to the control, resilience values decreased due to increased treatment parameters. An increase in resilience values was observed as storage time in-

creased.

When all texture values are evaluated as a whole, it is worth noting that low-pressure treatment has been shown to have a positive contribution to the textural properties of fish. It was determined that our study results were different from the results of Bou et al. (2023). It is thought that the differences in textural values arise from the differences in the type of fish and treatment parameters.

The effect of low-pressure treatment and storage time on the a_w, pH, TBARS, and TVB-N values of the samples was found to be statistically significant ($P < 0.05$). a_w values were determined to be lower in the treated samples compared to the control sample. A decrease in a_w values was observed as storage time increased. It is thought that the water in the samples evaporates more quickly due to the decrease in external pressure with the low pressure applied. The pH values in samples treated with low pressure remained at lower levels compared to the control's. Especially, during the storage period, the pH value increased more in control samples. During the storage process, the formation of TMA and other volatile compounds due to the action and metabolism of endogenous and microbial enzymes causes the pH to increase (Olatunde and Benjakul 2018). The TBARS value, an indicator of lipid oxidation, was detected at lower levels in low-pressure treated samples than in the control. It can be said that the decrease in the amount of oxygen in the environment due to low-pressure treatment causes this value to be low. An increase in TBARS value was observed in all samples depending on the storage process. In a study (Muela et al. 2014), it was stated that the lipid oxidation value in terms of TBARS in *Thunnus obesus* increased in the presence of O_2, and it was stated that this was caused by oxygen causing the release of free radicals. TVB-N, a part of the non-protein nitrogen portion of fish muscle, has been reported in many studies as one of the indicator components in fish spoilage (Nikzade et al. 2019). A decrease in TVB-N values was detected in all samples treated with low pressure compared to the control. This decrease became greater as the applied pressure and treatment time increased. TVB-N values of all samples increased depending on the storage process. It has been reported that the activity of spoilage bacteria and internal enzymes causes the increase in TVB-N value (Nikzade et al. 2019). The increase in TVB-N values is thought to be due to the increase in microbial activity depending on the storage time.

The effect of low-pressure treatment and storage time on the microbial load of all samples was found to be statistically significant ($P < 0.05$). The microbial load of treated samples was lower than the control. While the count of microorganisms decreased with the increase in applied pressure and time, the count of microorganisms also increased as the storage time increased. At the end of the 7-day storage period, the total count of aerobic mesophilic bacteria in the control sample was determined to have the highest microbial count value with 6.90 log cfu/g. This value was within the range of 6 – 7 log cfu/g, considered the acceptability limit for freshwater and marine fish (Silbande et al. 2016). Microbial count values in all other samples remained below this value. The type and count of microorganisms that cause spoilage vary depending on storage conditions, especially temperature and atmospheric composition (Parlapani et al. 2014).

Following the exposure of pikeperch to low-pressure treatment, very significant differences were detected between the quality criteria in the storage process and the quality criteria in the storage process of control samples that were not exposed to any treatment. It was observed that color, texture, a_w, pH, TBARS, TVB-N, and microbial values were at superior levels in fish to which this process was applied. Notably, the changes in applied pressure and time played a decisive role in the quality characteristics of the fish. Upon evaluation of the study data as a whole, it can be concluded that fish stored using a low-pressure process is of superior quality. The results of this study will lead to similar applications in different aquatic food products.

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Influence of the host sex, size, and season on *Ergasilus lizae* infestation of Thicklip Grey Mullet (*Chelon labrosus*, L., 1758) in Beymelek Lagoon Lake (Antalya, Türkiye)

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ABSTRACT

The aim of the study was investigation of the changes in infestation levels of *Ergasilus lizae* on economically important fish species, thicklip grey mullet (*Chelon labrossus*) from Beymelek Lagoon Lake located on the western Mediterranean coast (Antalya, Türkiye). In this context, the infestation levels of *E. lizae* with respect to fish sexes, fish sizes and season were evaluated statistically. The research was conducted seasonally between May 2008 to April 2009, and the gills which belong to 103 fish samples were examined. The overall infection prevalence and mean intensity values were 60.2% and 15.95, respectively. The prevalence and mean intensity of *E. lizae* for thicklip grey mullet male and sexually unidentified fish samples were higher than that for females. Prevalence and mean intensity were determined the highest in size class I with 69.6% and 26.3, and almost the same values in size groups II and III. *E. lizae* on host fish were observed in every season. The highest prevalence and mean intensity of *E. lizae* was recorded in spring with 100%, 24.88. The lowest prevalence of *E. lizae* was recorded in summer at 12% and the lowest mean intensity of *E. lizae* was in the summer and the winter with 9, 8.67 respectively. The infestation level of *E. lizae* was statistically significant with host sex and season, but there was no effect on the fish size groups.

Keywords: *Ergasilus lizae*, *Chelon labrosus*, Infestation, Beymelek Lagoon, Türkiye

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Beymelek Lagün Gölü'ndeki (Antalya, Türkiye) Kalın Dudaklı Kefal (*Chelon labrosus*, L., 1758)'de *Ergasilus lizae* Kroyer, 1863 (Copepoda: Ergasilidae) Enfestasyonu Üzerine Konak Cinsiyeti, Büyüklüğü Ve Mevsimin Etkisi

Öz: Bu çalışmada, Batı Akdeniz kıyısında (Antalya, Türkiye) yer alan Beymelek Lagün Gölü'nde yaşayan ekonomik öneme sahip bir balık türü olan kalın dudaklı kefal (*Chelon labrossus*) üzerindeki *Ergasilus lizae* enfestasyon seviyesindeki değişimler araştırılmıştır. Konak balık cinsiyeti, konak balık büyüklüğü ve mevsime göre *E. lizae*'nin enfestasyon seviyesi istatistiksel olarak değerlendirilmiştir. Çalışma mevsimsel olarak Mayıs 2008 ile Nisan 2009 tarihleri arasında yürütülmüş ve 103 balık örneğine ait solungaç incelenmiştir. Toplam yaygınlık ve ortalama yoğunluk değerleri sırasıyla % 60,2 ve 15,95 olarak bulunmuştur. Erkek ve cinsiyeti belirlenemeyen balık örneklerinde *E. lizae* yaygınlığı ve ortalama yoğunluğu dişilerden daha yüksek bulunmuştur. Boy gruplarına göre, yaygınlık ve ortalama yoğunluk % 69,6 ve 26,3 ile I. boy sınıfında en yüksek, II. ve III. boy gruplarında ise hemen hemen aynı değerlerde tespit edilmiştir. Konak balık üzerindeki *E. lizae* her mevsimde gözlenmiştir. En yüksek *E. lizae* yaygınlığı ve ortalama yoğunluğu %100 ve 24,88 ile ilkbaharda kaydedilmiştir. En düşük *E. lizae* yaygınlığı %12 ile yaz mevsiminde, en düşük ortalama *E. lizae* yoğunluğu sırasıyla 9 ve 8,67 ile yaz ve kış mevsimlerinde kaydedilmiştir. *E. lizae*'nin enfestasyon seviyesi konakçı cinsiyeti ve mevsim ile istatistiksel olarak önemli bulunurken, balık büyüklük gruplarının etkisi görülmemiştir.

Anahtar Kelimeler: *Ergasilus lizae*, *Chelon labrosus*, Enfestasyon, Beymelek Lagoon, Türkiye

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Introduction

Ergasilidae von Nordmann, 1832 is one of the major families within the parasitic cyclopoid copepods and of the 13 valid genera of the family

Ergasilidae. The genus *Ergasilus* von Nordmann, 1832 contains 66 valid species (Walter and Boxshall 2023a). To date, four genera and nine species belonging to the family were recorded from Türkiye; *Ergasilus, Nipergasilus* Yamaguti, 1939 and *Paraergasilus* Markewitsch, 1937, *Neoergasilus* Yin 1965 comprising marine, brackishwater and freshwater species (Altunel 1983,1990; Öztürk 2002; Öktener and Trilles 2004; Oğuz and Öktener 2007; Öktener et al. 2007; Koyun et al. 2007; Akbeniz and Soylu 2008; Öktener et al. 2008; Soylu and Soylu 2012; Özer and Kırca 2013; Öztürk 2013; Soylu et al 2013, Alaş et al. 2015; Özer and Acar 2022).

E. lizae Kroyer, 1863 and *E. gibbus* belong to the genus *Ergasilus* the type genus of the family was recorded on Turkish marine fish, mullets, and European Eel (Altunel 1983, 1990; Öktener & Trilles 2004; Oğuz and Öktener 2007; Öztürk 2013; Soylu et al 2013, Özer and Acar 2022). *E. lizae* Kroyer, 1863 reported as *E. nanus* Beneden, 1870 by Altunel (1983) and Öktener and Trilles (2004) who recorded on mullets. *E. nanus*, is invalid species and should be accepted as *E. lizae* (Ben Hassine and Raibaut 1980; Kabata 1992; Walter and Boxshall 2023b).

Pathogens like fish parasites can cause significant diseases in wild and cultured fish populations but the effect of diseases on wild fish is less known than that of cultured fish populations (Hedrick 1998; Kır 2007; Özbek and Öztürk 2010). High levels of *Ergasilus* spp. infestation on cultured and wild fish can cause serious health problems, mortalities, and significant economic loss (Paperna and Overseet 1981; Johnson et al. 2004; Jithendran et al. 2008; Özer and Kırca 2013).

Although there are a few parasitological studies on thicklip grey mullet *Chelon labrosus* L., 1758 in marine and inland waters of Türkiye (Alaş et al. 2015; Koç et al. 2018) only one study on helminth parasites of thicklip grey mullet was conducted by Aydoğdu et al. (2015) in Beymelek Lagoon Lake. In addition, the helminth and parasitic copepod fauna of sea bass fish except thick-lipped mullet, were studied in our study area (Emre and Kubilay 2014; Yalım et al. 2014).

Beymelek Lagoon (30° 03' E, 36° 16' N), located on the southwest coast of Türkiye, is connected to a brackish water spring in the northwest by a small channel and to the Mediterranean Sea in the southeast by an inlet. The lagoon is a shallow lake with a mean depth of about 1.6 m and a surface area of 250 ha (Anonymous, 1984). It is one of the most productive and important lagoon lakes of Türkiye in which is identified 51 fish and crustacean species and 41 of these species are of commercial importance (Aydın et al. 2023). In the lagoon lake, the most common species are *Mugil cephalus, C. labrosus, Liza ramada, L. saliens, L. aurata, Sparus aurata, Dicentrarchus labrax, Lithognathus mormyrus* and *Diplodus sargus* (Emre et al. 2011).

Identifying parasite fauna in wild fish and monitoring studies are important to avoid spreading parasite infections from wild fish to cultured fish. In context, the infestation level of E. lizae in economically an important fish species thicklip grey mullet (*C. labrosus* L., 1758) in Beymelek Lagoon Lake was evaluated in relation to host sexes, host size, and season.

Materials and Methods

For the determining copepod parasites, the thicklip grey mullet samples were used from the research conducted by Aydoğdu et al. (2015). In this context, specimens of thicklip grey mullet were collected seasonally between May 2008 to April 2009 by fishing nets in Beymelek Lagoon Lake (30^0 04' E, 36^0 16' N). The fish samples were measured in total body length. Their sexes were determined with the examination of gonads. All fish samples were examined for the presence of gill lice under the Olympus SZ61 stereo microscopy. The copepod parasite samples were removed from the gill filaments and then dissected under the stereo microscope and eventually examined under the Olympus BX53 light microscope. The copepod parasites were identified according to Kabata (1979, 1992). The number of parasite samples and infected fish samples were counted. The prevalence and mean intensity levels of parasite infestation for host fish species were calculated according to Bush et al. (1997). Significant differences were detected using Fisher's exact tests for prevalence, the Kruskal-Wallis test (more than two groups), and the bootstrap t-test for mean intensity. Significance for all the statistical analyses was established with 95% confidence intervals. A statistical package the Jamovi program (Jamovi, 2019) for Windows and Quantitative Parasitology 3.0 (Rózsa et al., 2000; Reiczigel and Rózsa, 2005) were used for these statistical analyses.

Results

A total of 103 fish samples belonging to the thicklip grey mullet were examined. One copepod, *Ergasilus lizae* Kroyer, 1863 was determined on the gill flaments of the fish samples during the study period. Total lengths ranged from 25.8 to 50.8 cm for the host fish.

62 of 103 the thicklip grey mullet were infected by *E. lizae*. The prevalence and mean intensity of *E. lizae* were 60.2% and 15.95, respectively (Table 1). The intensity of *E. lizae* ranged from 1 to 99 parasites per fish sample and A total of 998 parasites were counted (Table 1).

The levels of infestation in thicklip grey mullet

male, female and sexually unidentified showed differences. The variation of infestation observed in host sexes was presented in Table 1. In general, the prevalence and mean intensity of *E. lizae* for thicklip grey mullet male and sexually unidentified fish samples (92%, 16.78; 55%, 21) were higher than that for female (48.3%, 13.61) respectively (Table 1). It is clearly indicated that the parasite on host male and sexually unidentified are highly aggregated than that for females (Table 1). Although the prevalence of *E. lizae* was significantly different between the sexes by Fisher exacts test ($P < 0.001$), the mean intensity was not Kruskal-Wallis test (P=0.84).

Table 1. Variations in infestations of *E. lizae* according to fish sexes

Host Sexes	No of host	No of infected host	Prevalence (%)	Mean intensity	Intensity range
Female	58	28	48.3	13.61	381 (1-41)
Male	25	23	92	16.78	386 (1-92)
Undetermined gender	20	11	55	21	231 (1-99)
Total	103	62	60.2	15.95	998 (1-99)

The thicklip grey mullet samples were infested with *E. lizae* in all size classes. The occurrence of infestation in different size classes was presented in Table 3. Prevalence was highest (69.6%) in size class I, followed by size class III (58.1%) and size class II (57.1%). Intensity was highest (26.31) in size class I, followed by size class III (13), and size class II (11.93) class (Table 2). The parasite is highly aggregated size class I of the host than other size classes (Table 2) but there were no significant differences in the prevalence and mean intensity of the parasite among the size class by Fisher exact's test and Kruskal-Wallis test (P=0.61, P= 0.37, respectively).

E. lizae on the gills of examined fish samples were observed in every season during the study period and the occurrence of the parasite showed a pronounced seasonal variation. The highest prevalence of *E. lizae* was recorded in spring with 100%, it was followed by winter with 75%, autumn with 50%, then finally dropped minimum in the summer with 12% (Table 3). Fisher's exact test showed seasonally significant differences in the prevalence of *E. lizae* on the thicklip grey mullet ($P < 0.001$). The highest mean intensity of *E. lizae* was recorded in the spring with 24.88, it was followed by 14.27, 9, and 8.67 during the autumn, the summer, and the winter respectively (Table 3). Comparing seasonal variations in the mean intensity of *E. lizae*, the Kruskal-Wallis test showed significant differences for the thicklip grey mullet (P=0.031).

Table 2. Variations in infestations of *E. lizae* according to fish length

Size Class	No of host	No of infected host	Prevalence (%)	Mean intensity	Intensity range
I	23	16	69.6	26.31	421 (3-99)
II	49	28	57.1	11.93	334 (1-28)
III	31	18	58.1	13	150 (4-38)

Table 3. Seasonal variations in infestation of *E. lizae* on host fish

Season	No of host	No of infected host	Prevalence (%)	Mean intensity	Intensity range
Spring	24	24	100	24.88	597 (4-99)
Summer	25	3	12	9	27 (4-14)
Autumn	22	11	50	14.27	157 (3-28)
Winter	32	24	75	8.67	208 (1-21)

Discussion

All parasite specimens, attributed as belonging to the genus *Ergasilus* based (a) two-branched leg IV with a 2-segmented exopod and 3-segmented endopod, (b) 6-segmented antennule, (c) well developed antenna with a single claw (Kabata 1979; Boxshall and Montú 1997). *E. lizae* was identified based on the violin body shape and narrower posteriorly, the grasping organ, antenna elongated and slender; basal segment short and small, the inner margin of the second segment with small outgrowth, subchelate with one small proximal and one distal setule, slightly curved claw. 2-segmented of leg 5; basal segment with one small seta, distal segment with two terminal setae, and one subterminal seta.

The present study is the report on the infestation of *E. lizae* in relation to season, host sexes, and length classes of thicklip grey mullet inhabited in Beymelek Lagoon Lake which is located in the West Mediterranean Sea coast of Türkiye. Gill lice, ectoparasite *E. lizae,* is widespread on host fish living in marine and brackish waters and also commonly reported on mullets (Kabata 1979, 1992; Knoff et al. 1994; El-Rashidy and Boxshall 1999; Marcogliese et al. 2001; Norris et al. 2002; Johnson et al. 2004).

The findings of the study were generally found to be higher compared to infestation levels of *E. lizae* observed on other hosts. Norris et al. (2002) recorded four ergasilid copepod species on *Acanthopagrus butcheri*. The prevalence and relative density of *E. lizae* ranged from 10 to 80% and 0.15 to 12.4, respectively. Marcoglise et al. 2001 determined the potential impacts of clear-cutting on parasites of the northern redbelly dace in four boreal lakes and the prevalence *E. lizae* was recorded between 7 and 23% in two lakes. Altunel (1983) determined that the infestation level of *E. lizae* (reported as *E. nanus*) was recorded on five species of mullets. These are *Mugil cephalus, Liza ramada, L. saliens, Chelon labrosus, Oedalechilus labeo,* and their parasite prevalences were 56%, 43%, 36.73%, 16.45%, 37.5%, respectively. Özer and Kırca (2013) investigated the natural parasite fauna of *Liza aurata* captured from Kızılırmak Delta. The prevalence of *E. lizae* was 50%. Öztürk (2013) determined the parasitic fauna of Juvenile golden grey mullet, *L. aurata* collected from Sarıkum Lagoon Lake (Sinop, Turkey) and evaluated diversity and the occurrence of parasites in relation to season and size classes of host fish. The prevalence was 14.2%. The occurrence of crustacean ectoparasites on white mullet, *Mugil curema* captured from the littoral waters of Rio Grande do Norte State, Brazil (Cavalcanti et.al., 2011). *E. lizae* occurred 8.33%, 3.23% during the rainy and the drought seasons, respectively. Aladetohun et al. (2013) carried out copepod parasites in the gills of *M. cephalus* and *L. falcipinnis* in Ganvie, Djdje and Zogbo regions of Lac Nokoue Lagoon. Three species of parasitic copepod were identified *Nipergasilus bora, E. latus* and *E. lizae*. *E. lizae* was 2.33%, 7.09%, 0.39% and 3.15% during the drought and rainy seasons in the Ganvie and Djdje respectively. The metazoan parasite fauna and infestation levels of 19 fish species caught from Mert Lake located in Igneada Wetland Ecosystem were examined between June 2015 and May 2017, seasonally (Kırcalar, 2018). *E. lizae* was determined in seven fish species, *A Alburnus chalcoides M. cephalus, C. ramada, Alosa sp., Lepomis gibbosus, C. saliens, Barbus* sp. The prevalence of *E. lizae* parasite was determined in *A.chalcoides* with 6.11 %, in *Alosa* sp. with %27, in *Barbus* sp. with 100 %, *Lepomis gibbosus* with %1.96 *C. ramada* with 34.33%, in *C. saliens* with 54.90 % and in *M. cephalus* with 53 %. Gueretz et. al. (2022) investigated the parasitic fauna of *M. curunema* in Parati River on the northern coast of the Santa Catarina State, Brazil. The prevalence of *E. lizae* was 77.66%. Özer and Acar (2022) investigated metazoan parasites in leaping mullets caught from Sinop coasts (Black Sea) and it was notified that the prevalence of *E. lizae* parasite was 4.2%.

In this study, the infestation level of *E. lizae* was affected by the host sex. Infestation of *E. lizae* for the thicklip grey mullet male and undetermined gender were higher than that for females. It was observed that *E. lizae* preferred thicklip grey mullet male over female of thicklip grey mullet. Twice the number of parasites found in only one host male and undetermined gender than that in females. We can say that the male and undetermined genders can be precise to parasitic infestation. Host sex is one important factor in interactions between the host and parasite (Lizama et al. 2005). The differences in parasitic load are related to physiological differences between the male and female hosts (Aloo et al. 2004; Lizama et al. 2005) and they notified that it might be related to the feeding habits and reproduction migrations of the fish hosts.

The parasite infestation levels were changed in relation to the size of the thicklip grey mullet. In general, a positive correlation was expected between parasite infestation (prevalence and intensity) and host fish size (Lo et al. 1998; Poulin 1999, 2000; Öztürk 2013) but, our findings showed a negative relation between host fish size and aggregation. The highest parasite accumulations were not found on the largest fish specimens, the parasitize loads decreased with the increase in fish length. The parasite is highly aggregated size class I of the host than other size classes. These results are consistent with the findings of several metazoan parasites studies (Bortone et. al. 1978; Meeûs et al. 1995; Öztürk 2002; Öztürk and Aydoğdu 2003; Kutlu and Özturk 2006; Ekanem

2011; Aydoğdu et al. 2015; Eyo and Effanga 2018; Özer and Acar 2022).

Differences in the prevalence and mean intensity of *E. lizae* were found significant seasonally. Seasonal occurrence of some species of the genus *Ergasilus* was reported to be higher in the spring, summer, and autumn compared to the winter by numerous authors (Altunel, 1983, Öztürk and Aydoğdu 2003; Öztürk 2013; Aladetohun et al. 2013; Garcia and Williams 1985). Öztürk and Aydoğdu (2003) reported that the infestation level of *E. sieboldi* on grey mullet in Karacabey Lagoon was higher during the summer and autumn than in the winter and early spring. Altunel (1983) notified that the highest infestation levels of *E. lizae* (reported as *E. nanus*) recorded on five species of mullets were determined in periods of increasing water temperature. Öztürk (2013) reported that the highest prevalence and mean intensity of *E. lizae* in juvenile the golden grey mullet was in autumn with 47.37%, 3.78, respectively and the lowest values were recorded in the winter with 2.72%, 3 for the prevalence and mean intensity, respectively. Aladetohun et al., (2013) researched the prevalence of parasitic copepods on *Mugil cephalus* and *Liza falcipinnis* captured from Lac Nokoue Lagoon during the dry (December-March, 2011) and the rainy season (April-July, 2012). They determined that the prevalence of *E. lizae* was higher in the rainy season than in the dry season. Garcia and Williams (1985) reported that the highest prevalence of *E. lizae* on the white mullet was recorded in winter and disappeared between April and September in Joyuda Lagoon. But the water temperature and salinity values of the lagoon were recorded at 24.8 ^{0}C, 19.9 ppt, respectively. They notified that the high prevalence of *E. lizae* was related to the migratory pattern of the adult fish than to seasonal hydrological conditions (Garcia and Williams, 1985). Obtained data for *C. labrosus* showed similarity to the above studies. *C. labrosus* the highest prevalence and intensity of *E. lizae* was recorded in spring. It was followed during winter and autumn and then finally dropped minimum in summer. The mean intensity of *E. lizae* was higher in the spring and autumn than in the summer and winter.

As reported in the studies above, the host size, host age, host sex, fish migration periods, spawning periods, and environmental conditions (temperature, rainfall, salinity, etc.) are the most important biotic and abiotic factors affecting the infestation levels of parasite species.

The fishes are also more susceptible to parasite infestations during the spawning period (Šimková et al. 2005; Lizama et al. 2006). The spawning period was from December to February for thicklip grey mullet in Beymelek Lagoon (Emre et al. 2011). As seen in Table 3, the heavily infestation of parasites was observed in the spawning period of fish (in winter), and spring period with increased water temperature. The present study showed that the infestation level of *E. lizae* was significantly changed with host sex and especially season, but there was no effect of fish size groups. It is important to carry out such studies continuously in the natural environment as well as in aquaculture conditions in order to prevent epizootic outbreaks. In conclusion, It is thought that parasitic copepod studies on wild fish could be useful determined, understood, and prevented in their effects on cultured fish.

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Türkiye'de *Dreissena* Türlerinin Dağılımı

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ÖZ

Palearktik Bölge'nin önemli türleşme bölgelerinden biri olan Türkiye *Dreissena* cinsinin yayılış merkezlerinden biridir. Yaklaşık 1800'lü yıllardan beridir özellikle Avrupalı araştırıcılar tarafından cinse ait birçok taksondan söz edilmektedir. Ancak son yıllarda yapılan moleküler çalışmalarla esas olarak üç taksonun varlığı belirlenmiştir *Dreissena caputlacus* (SCHÜTT 1993)' un, Ege ve Akdeniz, Güneydoğu Anadolu'nun özellikle lentik sistemlerinde; *D. polymorpha anatolica* (LOCARD 1893)'nın özellikle Göller Bölgesi ve Ege Bölgesi'nde ve *D. polymorpha gallandi* (LOCARD 1893)'nın Marmara ve Batı Karadeniz bölgelerinde yayılış gösterdiği bildirilmektedir. Bu sonuçlar doğrultusundan 2000 yıllarından beridir ve günümüzde topladığımız örnekler yeniden değerlendirilerek genel bir listeye ulaşılmıştır.

Bu çalışmada, son bulgular doğrultusunda Marmara, Ege, Akdeniz, Karadeniz, Güneydoğu ve Doğu Anadolu bölgelerden toplanan *Dreissena* örnekleriyle, cinsin yayılışı kısmen ortaya konulmaya çalışılmıştır. Bununla birlikte henüz yeterince araştırma yapılıp *Dreissena* taksonlarının bütünüyle açığa çıktığı söylenemez.

Anahtar Kelimeler: *Dreissena*, yayılış, Türkiye

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The Distribution of *Dreissena* Species in Türkiye

Abstract: Türkiye, which is one of the important speciation regions of the Palearctic Region, is one of the distribution centers of the genus *Dreissena*. Since the 1800s, many taxa belonging to the genus have been mentioned, especially by European researchers. However, in recent years, the existence of three taxa has been determined mainly in the lentic systems of *Dreissena caputlacus* (SCHÜTT 1993), Aegean and Mediterranean, Southeastern Anatolia; *D. polymorpha anatolica* (LOCARD 1893) especially in the Lakes Region and Aegean Sea. It has been reported that *D. polymorpha gallandi* (LOCARD 1893) is distributed in the Marmara and Western Black Sea Regions.

In this study, the distribution of the genus has been tried to be revealed partially with Dreissena specimens collected from at least certain regions in line with the latest findings. However, it cannot be said that *Dreissena t*axa have not been fully revealed yet.

Keywords: *Dreissena*, spread, Türkiye

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Giriş

Anadolu, tür çeşitliliği açısından zengin bir yarımada olması nedeniyle biyolojik kaynaklar açısından paleoarktik bölgede önemli bir konuma sahiptir. Türkiye geniş iç su kaynaklarına sahip olmaması ile birlikte, paleocoğrafik gelişmeler ve hidrocoğrafik bağlantılar nedeniyle zengin bir habitat ve tür çeşitliliğine sahiptir. Bununla birlikte bu durum, egzotik ve istilacı türler için de uygunluk sergilemektedir (Yıldırım vd. 2019).

Türkiye'nin iç sularında bilinen istilacı omurgasızların başında *Dreissena* cinsine ait türler gelmektedir. Birçok iç suyun bu cins için uygunluğunun yanı sıra, balıkçılık faaliyetleri ve

yeterli kontrollerin olmaması, bu cinsin bireylerinin yeni alanları kolaylıkla istila etmelerine olanak sağlamıştır. *Dreissena*'nın istilacılığı konusunda fikir birliği olmasına rağmen, *Dreissena*'nın hangi türlerinin istilacı olduğu konusunda farklı düşünceler bulunmaktadır (Geldiay ve Bilgin 1973; Yıldız vd. 2018; Yıldırım vd. 2019).

Son yapılan moleküler filogenetik çalışmalarda *Dreissena, Dreissena, Pontodreissena* ve *Carinodreissena* olmak üzere üç alt cinse ayrılmıştır. Bunlardan *Pontodreissena, D. caputlacus* ve *D. rostriformis* türlerini içermektedir. *D. caputlacus*'un ülkemizde yayılış gösterdiği bilinmektedir. *Carinodreissena* alt cinsi, *D. carinata* ve *D. blanci* taksonlarını içermekte olup; her ikisi de Balkan Yarımadası'ndaki antik göllerde yaşamaktadırlar. Ancak ülkemizdeki yayılışı ile ilgili bir kayıt bulunmamaktadır. *Dreissena* alt cinsi ise, *D. polymorpha anatolica* ve *D. polymorpha gallandi* olmak üzere iki alttür ile temsil edilmektedir. *D. polymorpha anatolica,* Akdeniz'in kuzeyindeki göllerde Türkiye'de endemiktir ve *D. polymorpha gallandi* Pontocaspian kökenli olup Avrasya ve Kuzey Amerika'nın büyük bölümünde yaygın bir şekilde yayılış göstermekte olduğu bilinmektedir (Gelembiuk vd. 2006).

Ülkemizde bugüne kadar yapılan çalışmalarda *D. caputlacus, D. p. anatolica* ve *D. p. gallandi* taksonlarının yayılış gösterdiği rapor edilmiştir (Gürlek vd. 2019). Ayrıca *D. bouldourensis* D'ARCIAC, 1866, *D. bouguignati* LOCARD, 1893, *D. hermosa* LOCARD, 1893 fosil türlerin de varlığı söz konusudur (Schütt, 1991, 1993). Ancak ülkemizde yapılan araştırmaların çoğunda rastlanılan *Dreissena* taksonları *D. polymorpha* olarak tanımlanmaktadır. Bunlardan bazılarının lokalite ve araştırıcıları aşağıda verilmiştir.

-Çıldır Gölü, Eğirdir Gölü ve beş göletten (Altınyazı Baraj Gölü, Keban Baraj Gölü, Küçükçekmece Gölü, Karpuzlu Baraj Gölü, Kadıköy Baraj Gölü) toplanılan örnekler (Berber vd. 2018),

-Karasu Çayı üzerinde bulunan Sarımehmet Barajı (Van) (Akkuş vd. 2019),

-Birecik ve Kesikköprü Baraj Gölü (Bobat 2019),

-Keban Baraj Gölü (Aksu vd. 2012),

-Sapanca Gölü ve Maşukiye deresi (Ercan vd. 2013).

Son yapılan çalışmalarda taksonlar kısmen açığa çıkarılmış olmalarına karşın, dar alanlarda farklılaşma ve türleşme imkanı veren habitat çeşitliği barındıran ülkemizde birçok sucul omurgasız türünde olduğu gibi bu cinse ait taksonların belirlenmesine yönelik moleküler çalışmalara ihtiyaç bulunmaktadır.

Materyal ve Metot

Bu çalışmada Türkiye'nin farklı su ünitelerinden standart toplama yöntemlerine uygun olarak (Schultes, 2012) örnekler elle toplanarak temin edilmiş ve Burdur Mehmet Akif Ersoy Üniversitesi Fen Edebiyat Fakültesinde saklanan eski ve yeni koleksiyonlar yeniden incelenmiştir.

Bulgular

Ülkemizin önemli iç su kaynaklarında (göller, göletler, bataklıklar, kaynaklar ve nehir havzaları) sürdürdüğümüz çalışmalarda *D. caputlacus, D. polymorpha anatolica* ve *D. polymorpha gallandi* türlerinin yayılış göstermekte olduğu belirlenmiştir. Ayrıca *D. bouldourensis* D'ARCHIAC, 1866, *D. bouguignati* LOCARD, 1893, *D. hermosa* LOCARD, 1893 fosil türleri de tespit edilmiştir.

1.*Dreissena caputlacus* SCHÜTT 1993

1993 *Dreissena caputlacus* SCHÜTT, Arch. Moll., 122: 329, T. 1 F. 14.

2006 *Dreissena caputlacus* --GELEMBIUK, et al.

2019 *Dreissena caputlacus* --GURLEK, et al.

İlk kez Hartwig Schütt tarafından Adıyaman Gölbaşı Gölü'nden belirlenmiş olan türün karakteristik olarak üzerinde beyaz renkli enine çizgiler bulunmaktadır. Yine Familya özelliği olarak kavkın birlikte yer almasını sağlayan menteşe üzerinde herhangi bir diş çıkıntısı yoktur bunun yerine kabukları birbirine bağlayan elastik menteşe bağı bulunmaktadır (Şekil 1).

Genel Yayılışı

Ülkemizin endemik midye türlerindendir. Önceleri Seyhan Nehri, Seyhan Barajı, Sır Baraj Gölü, Kurtağılı Baraj Gölü (Yozgat)'nde bilinmekte iken ayrıca tarafımızdan Menzelet Baraj Gölü, Almus Baraj Gölü (Tokat) ve Kartalkaya Baraj Gölü (Kahramanmaraş)'nde türün yayılış gösterdiği tespit edilmiştir. Muhtemelen türün evrimleşme merkezi tip lokalitesi olan Gölbaşı Gölüdür. Lokalitenin yer aldığı bölgede yaz ayları çok sıcak ve kurak geçmektedir. Bu yüzden türe ait canlı örneklere yaz aylarında ancak littoral zonda rastlanılmaktadır. Bununla birlikte, Gölbaşı Gölü ile hidrocoğrafik bağlantıları bulunan göl ve göletlerde türün oldukça yoğun ve büyük populasyonlarına rastlanılmaktadır. Özellikle Ceyhan baraj gölünde enerji üretim sistemlerinde biyofouling etkisi göstermektedirler.

Şekil 1. *Dreissena caputlacus* SCHÜTT 1993, Gölbaşı Gölü, Adıyaman

2. *Dreissena polymorpha anatolica* (LOCARD 1893)

1853 *Dreissena polymorpha anatolica* -- BOURGUIGNAT, (Beyşehir Gölü)
1893 *Dreissensia anatolica* -- [BOURGUIGNAT] LOCARD, (Beyşehir gölü).
1986 *Dreissena polymorpha anatolica,* -- KINZELBACH, (Bafa Gölü, Çavuşcu Gölü, Kovada Gölü, Fırat Nehri Birecik),
1993 *Dreissena polymorpha anatolica,* -- SCHÜTT
2006 *Dreissena polymorpha anatolica,* -- GELEMBIUK, et al.
2019 *Dreissena polymorpha anatolica,* -- GURLEK, et al.

Anadolu platosunda türleşmiş cinsin en küçük hacimli türlerindendir. Sırt bölgesi, S harfi şeklinde bir şeklinde bir omurgaya sahiptir. Esas yaşam yerleri genel olarak Göller Bölgesi olmasına karşın, antropojenik etkinliklerle hidrocoğrafik bağlantısı bulunan baraj gölleri ve göletlerde de yayılış göstermektedirler (Şekil 2).

Şekil 2. *Dreissena polymorpha anatolica* (LOCARD 1893) Beyşehir Gölü

Genel Yayılışı

Ülkemizin diğer bir endemik midyesidir. Genel olarak Göller bölgesinde yayılış göstermekle birlikte antropojenik etkilerle daha geniş bir alanda yayılış gösterdiği beklenilmektedir. Şu ana kadar yaptığımız çalışmalarda Beyşehir Gölü, Eğirdir Gölü, Karataş Gölü Burdur, Karaçal Baraj Gölü Burdur, Yortanlı Barajı Bergama İzmir, Sevişler Barajı Soma Manisa, Yortanlar Barajı Bergama İzmir, İlyas Deresi Bayramiç Çanakkale, Bayramiç Barajı, Fırat Nehri ve bu nehir ile bağlantılı barajlarda yayılış gösterdiği belirlenmiştir. Çalışmaların tamamen kabuk morfolojisi ağırlıklı olması nedeniyle, kesin tanımlamaların ancak moleküler çalışmalarla ortaya çıkacağına inanılmaktadır

3.*Dreissena polymorpha gallandi* (LOCARD 1893)

1893 *Dreissensia letourneuxi* -- [BOURGUIGNAT] LOCARD, (Sapanca Gölü].
1893 *Dreissensia lacunosa* -- [BOURGUIGNAT] LOCARD, (İznik Gölü).
1893 *Dreissensia gallandi* -[BOURGUIGNAT] LOCARD, (Apolyont Gölü = Uluabat Gölü]).
1893 *Dreissensia hermosa* --[BOURGUIGNAT] LOCARD, (İznik Gölü).
1897 *Dreissensia polymorpha* var. *gallandi*, --

ANDRUSOW
1897 *Dreissensia polymorpha* var. *hermosa*, -- ANDRUSOW
1986 *Dreissena polymorpha gallandi*, -- KINZELBACH, (Apolyont Gölü),
1993 *Dreissena polymorpha gallandi*, -- SCHÜTT
2006 *Dreissena polymorpha gallandi*, -- GELEMBIUK, et al.
2019 *Dreissena polymorpha gallandi*, -- GÜRLEK, et al.

Bu alt türe ülkemizde denize (Marmara ve Karadeniz) yakın bölgelerde rastlanılmaktadır. Nominal alt tür *Dreissena polymorpha polymorpha* ile büyük benzerlik göstermelerine karşın, zoocoğrafik özellikleri ve daha ince ve biçimli yapılarıyla farklılık göstermektedirler (Şekil 3).

Şekil 3. *Dreissena polymorpha gallandi* (Üst Uluabat Gölü, alt Terkos Gölü- İstanbul)

Ülkemizde günümüzde yayılış gösteren *Dreissena* türlerine ilaveten, Piliosen dönemine ait fosil alanlarında belirlenmiş taksonlar bulunmaktadır. Bunlar: Konya civarında *Dreissena iconica* SCHÜTT 1993, Erzurum ve Kars çevresinden *Dreissena diluvii* (ABICH 1859) (Schütt, 1989); Burdur Gölü havzasında *Dreissena bouldourensis* FISCHER 1866; Hatay civarında *Dreissena bourguignati* LOCARD, 1893; Antakya civarından *Dreissena bourguignati* (LOCARD 1883); Aras Nehri civarından *Dreissena diluvii* (ABICH 1859), Bolu civarından *Dreissena polymorpha arnouldiformis* SCHÜTT, 1993 türleridir. Bunlarla birlikte Fırat Nehri havzasında tespit edilen *Dreissena polymorpha siouffi* (LOCARD 1893) ve Ege Bölgesinde *Dreissena blanci* WESTERLUND 1890 taksonlarının da yayılışlarından söz edilmektedir (Schütt 1993). Ancak bu diğer araştırıcılar ve tarafımızdan henüz doğrulanmamıştır.

Tartışma ve Sonuç

Palearktik bölgenin önemli türleşme merkezlerinden biri olan ülkemiz dünya da geniş yayılışlar gösteren *Dreissena* cinsi içinde uygun habitatlara sahiptir. Bugüne kadar yapılan çalışmalardayer yer sinonim listesince verildiği üzere 1800 yıllardan beridir özellikle Avrupalı ve ülkemiz araştırıcıları tarafından bildirilen çok sayıda *Dreissena* cinsine ait takson bulunmaktadır. Gelembiuk ve arkadaşları ülkemiz tüm bölgelerini içermeyen yaptıkları moleküler çalışma ile en azından çoğu türün belirlenen 1 tür ve 2 alttürde toplamışlardır(Gelembiuk 2006). Bunlar Doğu Orta Anadolu ve Doğu Anadolu Bölgesinde *Dreissena caputlacus* SCHÜTT 1993, Ege, Akdeniz, Güneydoğu Anadolu özellikle lentik sistemlerde *Dreissena polymorpha anatolica* (LOCARD 1893) ve Marmara ve Batı Karadeniz bölgelerinde sucul habitatlarında *Dreissena polymorpha gallandi* (LOCARD 1893) taksonlarıdır. Ancak sıklıkla dile getirildiği gibi yapılan çalışmaların yeterli olduğu ve Türkiye *Dreissena* türlerinin açığa çıkarıldığı söylenemez. Bu yüzden geniş alanda saha çalışmalarına ve moleküler incelemelere ihtiyaç olduğu düşünülmektedir.

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Black Sea Salmon' (*Salmo labrax* Pallas, 1814) Journey: From Pond To Plate

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ABSTRACT

Black Sea salmon is from the Salmonidae family and is an endemic species distributed in the Black Sea. These fish, which exhibit anadromous behavior, prefer parts of rivers close to their source for reproduction and the Black Sea for feeding. The first cultivation study of this species, which has high consumer preference and economic value, was started by SUMAE in 1998. As a result of 24 years of work, F7 generation broodstock was created. In order to popularize the production of the species, the private sector was supported with broodstock. In this study, the breeding process of Black Sea salmon was examined and the introduction of the species was aimed. Today, 25 private sector enterprises have production licenses and many enterprises carry out commercial production of the species in the form of trial production with 1603 tons in total. Commercial production is concentrated in the Eastern Black Sea Region, which is the natural distribution area of the species. While enterprises that produce portion size in ponds and have restaurants prefer the red-spotted stream ecotype in production, enterprises that produce large-sized fish in dam lakes and the sea prefer the marine ecotype, which reaches sexual maturity late and has a better growth performance than other ecotypes. Since Black Sea salmon is our only endemic trout species showing typical salmon characteristics, its adaptation to the natural environmental conditions of our country for sea net cage and freshwater aquaculture is quite good.

Keywords: Black Sea Salmon, aquaculture, farming management, production, food

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Karadeniz Somonunun Yolculuğu: Havuzdan Sofraya

Öz: Karadeniz somonu, Salmonidae ailesinden olup Karadeniz'de dağılım gösteren endemik bir türdür. Anadrom davranış gösteren bu balıklar üremek için akarsuların kaynağına yakın kısımları, beslenmek için ise Karadeniz'i tercih ederler. Tüketici tercihi ve ekonomik değeri yüksek olan bu türün ilk kültür çalışması SUMAE tarafından 1998 yılında başlatılmıştır. 24 yıl devam eden çalışmalar sonunda F7 nesil damızlık stok oluşturulmuştur. Türün yetiştiriciliğinin yaygınlaştırılması amacıyla, damızlık balıklarla özel sektör desteklenmiştir. Bu çalışmada, Karadeniz somonunun kültür süreci irdelenmiş, türün tanıtımı amaçlanmıştır. Günümüzde 25 özel sektör işletmesi üretim ruhsatlı ve birçok işletme de deneme üretimi şeklinde toplamda 1603 ton olarak türün ticari üretimini yapmaktadır. Türün doğal yayılım alanı olan Doğu Karadeniz Bölgesinde ticari üretim yoğunlaşmıştır. Karada havuzlarda porsiyonluk üretim yapan ve restoranı olan işletmeler kırmızı benekli dere ekotipini üretimde tercih ederken baraj gölü ve denizde büyük boy balık üretimi yapan işletmeler ise geç cinsel olgunluğa ulaşan ve diğer ekotiplere nazaran daha iyi büyüme performansı gösteren deniz ekotipini tercih etmektedir. Karadeniz somonu tipik salmon karakteri gösteren tek endemik alabalık türümüz olduğundan deniz ağ kafes yetiştiriciliği ve tatlısu yetiştiriciliği için ülkemizin doğal çevre koşullarına adaptasyonu oldukça iyi durumdadır.

Anahtar kelimeler: Karadeniz Somonu, su ürünleri yetiştiriciliği, çiftlik yönetimi, üretim, yiyecek

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Introduction

Fish from the Salmonidae family have great importance economically as a food source, environmental in terms of species diversity and aquatic ecosystem health, as well as socially as they are preferred in angling (Latiu et al. 2020). Black Sea

salmon (*Salmo labrax*) is one of the less studied salmonid species compared to other species (Figure 1). This species was first identified by Peter Simon Pallas in 1814. Since then, it has been evaluated as a subspecies of *Salmo trutta* with the name *Salmo trutta labrax*. Black Sea salmon is a species with many synonyms (Froese and Pauly 2018). There are phenotypic differences between *Salmo trutta* populations found in Europe, the Mediterranean and the Adriatic. These taxonomic groups with different morphology have been defined by many species or subspecies names. It is possible to consider Black Sea salmon as a species, subspecies or local population (Çiftçi et al. 2007). Therefore, although the species name has been accepted as *Salmo trutta labrax* in studies on this species, this binomial nomenclature is now accepted as a synonym. Currently, the accepted name of the species is *Salmo labrax* (Black Sea salmon) according to Fishbase (Froese and Pauly 2018) and World Register of Marine Species (WORMS 2017) databases and it is one of the largest sized species among migratory salmonids (Nikandrov and Shindavina 2007).

Figure 1. Black Sea Salmon (*Salmo labrax*)

The natural distribution area of the Black Sea salmon is the Black Sea and the rivers flowing into the Black Sea briefly (IUCN 2022). It has been reported that anadromous forms of Black Sea salmon were previously found in many streams flowing into the Azov and Black Sea (Svetovidov 1984, Solomon 2000). It mainly spreads in the northeastern coasts of the Black, Azov and Caspian Sea basins (Okumuş et al. 2004). This species, which migrates to the Danube river for breeding (Dudu et al. 2011), has also been reported in the Balkan peninsula and the Czech Republic (Lusk et al. 2004, Georgiev 2011).

Many local databases of Bulgaria, Czech Republic, Georgia and Russia report that this species is endangered (GRID 1999, Lusk et al. 2004, Vassilev and Pehlivanov 2005, Peev et al. 2011). The stocks of this species in nature are endangered especially due to the pressure of overfishing in Türkiye as well(Çakmak et al. 2019). In this context, this species is stated as one of the important endangered species in the Black Sea Biodiversity and Environmental Protection Protocol(Black Sea Biodiversity and Landscape Conservation Protocol, 2003). While Black Sea salmon (*Salmo labrax*) is considered to be in the category of Least Concern according to the IUCN Red list (2022), a decreasing structure is observed in the natural stocks of the species due to human activities today. The species, which was mostly seen in the Fırtına and Çağlayan streams in the past, is almost impossible to come across in the region from the Georgian border to Giresun, including these rivers now.

In the countries neighboring the Black Sea (Russia, Ukraine, Romania, Bulgaria, Georgia, Türkiye), the bioecological characteristics and stock status of Black Sea salmon have been studied in general, but there is a lack of information (Solomon 2000, Nikandrov and Shindavina 2007, Makhrov et al. 2018). In our country, studies have been carried out mainly to determine the cultural characteristics and to produce them under farming conditions. In this context, the first cultivation studies were initiated in 1998 with the adaptation of individuals caught from the natural environment to farming conditions.

The cycle of the species was closed, some culture characteristics were determined, the broodstock of the species was brought to the private sector, the obtained culture line was used to support the natural stocks, the nutritional needs were determined and feeding studies were carried out with different feed additives with subsequent projects. The selective breeding program, which is continued with different projects in order to improve the culture characteristics after the adaptation of the species to the captivity, still continues with the breeding studies

with genetic approach.

Black Sea Salmon (*Salmo labrax*)

The population structure and life cycle of Black Sea salmon are similar to sea trout in Northwest Europe. The fish that hatch in freshwater stay in the river environment for one or three years and migrate to the sea after their smoltification is completed in the coastal region. Salmon, which develop rapidly in the Black Sea, return to the river to breed after being in the sea for one or two years (Solomon 2000, Tabak et al. 2001). Adult individuals with reproductive activity migrate to the sea without staying in a stream environment, as is the case with Atlantic salmon, and can reproduce several times during their lifetime (Barach 1962).

It is suggested that there are three different ecotypes of the Black Sea salmon in the natural distribution area: sea, lake and stream (Tabak et al. 2001). The most obvious difference between marine and stream ecotypes is the silvery coloration resulting from the accumulation of guanine layer on the skin after smoltification. Lake ecotype (*natio lacustris*) individuals are trapped in a certain lake and spend their entire lives there. In other words, they do not migrate between the sea and freshwater during the breeding and feeding periods (Slastenenko 1956, Çelikkale 1994, Geldiay and Balık 1996). Great variations in color are seen in all three ecotypes of the *Salmo labrax*. Since the marine ecotype is migratory between sea and freshwater, there are great differences in morphology, especially in color and pattern, between juveniles and adults. Although the young offspring of this ecotype carry scattered black and red spots on the sides of their bodies in freshwater, when they return to the sea, they gradually lose this color and pattern, turning into a silvery-white color and take on the color of their sea parents (Slastenenko 1956, Svetovidov 1984). While such a situation is observed in the marine ecotype, there is no significant color and pattern difference between juveniles and adults in lake and stream ecotypes. Red spots, which are much more characteristic in juveniles, especially in the stream ecotype, remain the same when they become adults and do not disappear throughout their lives (Svetovidov 1984, Çelikkale 1994). It is possible to encounter individuals with different appearances according to color, form, mottling and smoltification in the same basin (Figure 2).

Figure 2. Black Sea Salmon ecotypes (a) lake, (b) stream, (c) sea

Economic Importance

Natural trout fishing production, which was 518 tons in 2011, decreased by 42% and reached 301 tons in 2019 in our country. Overfishing, pollution of rivers, climate change, interventions in the river valley (due to the establishment of hydroelectric power plants on rivers) have been effective in the reduction of natural stocks. Regulations of fisheries were issued in order to prevent the declining stocks from being completely extinct. Regional and seasonal time period bans were introduced, fishing gear restrictions were made and length ban was introduced with these regulations. SUMAE (Central Fisheries Research Institute) brought Black Sea salmon that was adapted to captivity, in the private sector through on-site practical training in 2010. Trial production, which continued until 2013, became official from this year and started to take place in the aquaculture statistics. Culture production, which started with 1248 tons in 2014, has reached 1603 tons/year today (Figure 3). The species was introduced to private enterprises and they were allowed to start breeding after the private sector project. The number of enterprises producing the species reached 25 in 2021. The total fry production capacity of these enterprises is 23,256,000 and the fish production capacity is 3,287,000 kg. These enterprises are located in the provinces of Trabzon, Rize, Artvin, Giresun, Gümüşhane and Muş (BSGM 2021, TUİK 2022).

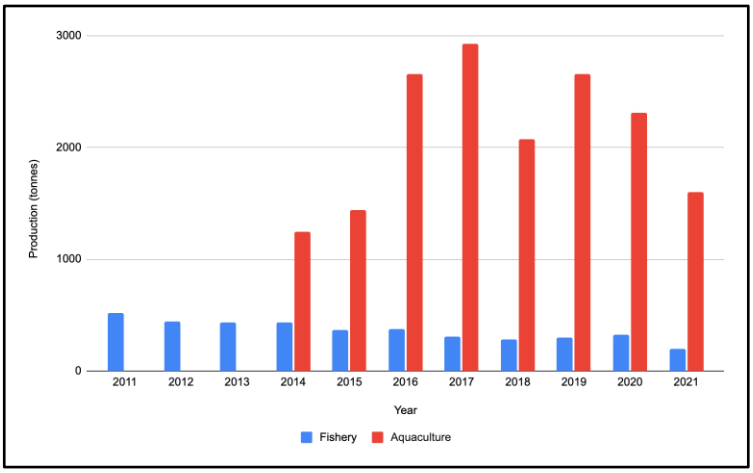

Figure 3. Trout fishery and aquaculture production statistics (ton/year) (TUİK, 2022).

Our native species has gained importance in culture conditions in recent years due to its resistance to local disease strains, rapid growth in Black Sea salinity, high adaptation to culture conditions, high commercial value, attractive in appearance and being consumed by the people of the region. It is especially preferred by trout producers with restaurants that appeal to domestic and foreign tourism, and even some businesses prefer only this species in production. Today, in the provinces of Artvin, Rize and Trabzon, portion size (200-250g) fish are sold at a price of 40-50 ₺/piece (2-3 $/piece), and in restaurants, they are cooked and served for about twice the price of rainbow trout.

Black Sea Salmon Characteristics

Broodstock Management

Ideally, broodstock should be kept as close as possible to the environmental conditions to which they are exposed in nature. However, in practice it may not be possible to provide ideal growing conditions for all factors. Water quality, feeding regime and feed formulation quality, stocking density, exposure to pathogens and stress factors during various treatments can be optimized with appropriate management and aquaculture practices (Okumuş 2002b).

Commercial trout feeds can be used in the feeding of Black Sea salmon broodstock apart from the reproduction period. However, it is essential to meet the nutritional needs of the species in order to obtain good quality eggs and good hatchability when gonad development begins in August. The use of diets containing 15%-20% fish oil, 45%-50% protein (anchovy meal) for broodstock in terms of polyunsaturated fatty acids, especially DHA, which is an important parameter in terms of hatching efficiency and egg quality affects the survival rate positively (Çakmak 2019).

Black Sea salmon will be exposed to stress at different levels from various practices (such as gonad development control, male-female separation, marking, transport and stripping) during the breeding period. This situation adversely affects the health of the broodstock and the viability of the progeny. In order to keep the stress at a minimum, reproductive period controls should be done at the appropriate time and as often as possible, taking into account the previous reproduction time, and anesthetics should be used if necessary. If the water quality is suitable, a stock density of 10 kg/m³ is optimum for Black Sea salmon broodstock, taking into account the welfare of the fish (Çakmak et al. 2010). The criterias such as reaching smolt height (11.5 cm) in the first November after stripping, reaching sexual maturity at 34 months, feed conversion ratio (FCR) close to 1, species-specific normal body shape and silver-spotted coloration (marine ecotype), not showing morphological deformation and skeletal anomaly and early adaptation to culture conditions should be considered while the broodstock were created. It is recommended that the broodstock should be created from at least 650 individuals in order to preserve genetic variation, the sex ratio should be kept as equal as possible, and broodstock older than 6 years old with low reproductive performance should be removed from the stock (Çakmak et al. 2018). It has been determined that the egg production of the broodstock varies between 2159-2629 eggs/kg depending on age in the conducted studies (Çakmak et al. 2018).

The dissolved oxygen in the water to be given to the ponds where the broodstock are stocked should be at least 6 mg/l. The CO_2 level should not exceed 25 mg/l. Total suspended solids in broodstock ponds should be reduced by using suitable filters. Fluctuations close to the lower or upper tolerance limits that may occur in the water temperature during

the day adversely affect the performance of the broodstock. Sudden changes (±5°C) should be avoided particularly. It has been experienced that Black Sea salmon broodstock are kept in ponds with a water temperature of 5-16°C throughout the year as much as possible in order to get healthy gonad development. Feeding should be stopped when the temperature rises above 20°C, especially in summer. The daily water change should be at a flow rate that will not drag the fish, and should not be below the level of 8 times/day In the broodstock ponds (Çakmak et al. 2010).

Hatchery Management

The breeding season of the Black Sea salmon in the natural environment starts from mid-October and continues until the end of December (Tabak et al. 2001). Taking into account the natural life cycle of the species, breeding controls of broodstock in culture conditions should be started in the first week of October every year. During the spawning season, egg maturity checks should be made at weekly intervals. Individuals with matured gonads should be taken into separate ponds for stripping. Broodstock to be stripped can be anesthetized by applying 50 ppm benzocaine (Oswald 1978) for height-weight measurements and easy stripping. Dry stripping method affects stripping efficiency positively (Billard and Cosson 1992).

The water temperature is required to be 7-12°C (average 10°C) during the incubation and hatching periods for Black Sea salmon eggs. Especially in the Black Sea salmon, since the incubation period is relatively long compared to other species, losses may increase if the eggs are kept in multiple layers. It is a correct approach to place a maximum of two layers of eggs in the incubation trays for Black Sea salmon. Detecting the dead eggs and removing them from the environment without causing any disease can give the larvae a chance to be kept in the same environment, especially during the period from hatching to free swimming and pre-feeding stages in this way. Dead eggs are removed from the tray by siphoning, collecting with forceps or other methods within the first 36 hours after stripping (at 10°C water temperature). After fertilization, eyed stage occurs in 28-32 days at 10°C, and hatching occurs in 38-41 days. Free swimming begins 67-77 days after hatching, thus the incubation phase is completed.

The most appropriate time to start feeding is when the larvae consume 60-70% of their yolk sacs. Fish should be fed with diets containing 15% fish oil and 45% fish meal protein during pre-feeding and feeding acclimation stages (Çakmak et al. 2019).

Stocking in the pre-feeding and fry stage according to the size of the floor area provides an advantage in reaching the planned survival and feed utilization performance. 7,000–10,000 larvae/m_2 is the appropriate stock density range in the larval tanks where pre-feeding will be made. When the fish reach an average weight of 600-750 mg, they should be taken into fry rearing tanks. If the water to be used in the pre-feeding ponds contains sufficient dissolved oxygen (> 7 mg/L), is clear and has a constant water temperature (average 10°C), it will be sufficient to change it 4 times per hour. Adjusting the water depth to 15 cm will prevent the larvae from having the problem of not being able to fill the air bladders (Çakmak et al. 2010). It was determined that the FCR ranged between 0.98-1.08 during the growth process from the pre-feeding stage to 2 g of fish kept in suitable rearing conditions in the studies carried out (Özel 2022).

It is important for the success of production that the fish are raised in hatcheries with optimum water quality until at least smolt size in Black Sea salmon farming. In fry rearing systems (tank, pool, etc.), it is recommended that the water is changed at a rate of 4 times/hour and its depth should not exceed 50 cm. (Çakmak et al. 2010).

Fry harvested as 2 g from nursery ponds are placed in rearing ponds where they will be grown up to smolt size, considering the average fish size of 10-15 kg/m3. The feed to be used to grow up fry to smolt size should have 10% fish oil and 47.5% protein (Çakmak et al. 2022). The FCR was determined as 0.73-1.08 (Özel 2022), 0.77-1.08 (Özel et al. 2021), 1.20-2.70 (Çakmak et al. 2022) in the studies performed. It takes an average of 10 months after the fertilization stage for the offspring to reach smolt size (11.6 cm, 18.72 g) (Özel 2022).

Fish Rearing to Portion Size

Pond and dam lake net cage enterprises are used in order to produce portion size or fillet candidate fish.It is sufficient to provide 2-3 times/hour water changes in portion size production pools. Pool dimensions, water inlets and outlets, ground slopes, pool depth should be such that they benefit the most from fresh water by fish and producers.

While the FCR ranges from 1.16 to 1.35 in the period from smolt to portion size (Çakmak et al. 2022, Özel 2022), this value may vary entirely depending on the business management. The stock density can be increased to 25-30 kg/m_3 considering the average harvest weight in this period (Çakmak et al. 2010). The fish reach a portion size (approximately 250 g) at the 16th month after the eggs are fertilized (Çakmak et al. 2018). In another study, it was determined that the weight of the broodstock caught as 300-350 g reached 2500-3000 grams when kept under controlled conditions for a year (Güven et al. 2016).

It was determined that the oxygen consumption was 95.2-140.0 mgO_2kg/hour at a water temperature of 10°C in the study using Black Sea salmon of different sizes. It has been determined that oxygen consumption increases in the light period (Akbulut et al. 2012).

It has been observed that the Black Sea salmon completes gastric emptying in 44 hours during the feed adaptation and pre-feeding stages, also in 68-72 hours during the breeding stage (Başçınar and Çakmak 2011). Feed formulation, feeding method, amount of feed and number of feeding are among the most important criterias affecting the growth of fish in culture production. The amount of feed and the number of feeding should be determined by considering the gastric digestion time in order for the whole culture stock to benefit equally from the given feed.

Fish Farming to Fillet Size in Sea Cages

Black Sea salmon is an opportunistic marine environment user in nature. It grows in marine water significantly faster than freshwater in aquaculture systems. It has been determined that the Black Sea salmon should be at least 12 cm in length and 15 g in weight (smolt length) for adapting to the Black Sea salinity. These sizes of fish can adapt physiologically in 7-8 days, and all blood values remain at normal levels after a period of 17 days in Black Sea (Çakmak et al. 2010).

Although daily feed consumption varies according to water temperature, fish size and flow rate in the area where the cages are located, feeding 1.5-2% of their live weight for fish has a positive effect on weight gain and feed utilization rate. A stocking density of 30 kg/m_3 can be reached depending on the suitability of the water quality in the net cages in the marine environment (Çakmak et al. 2010). When the Black Sea water is suitable for salmon/trout farming in November -the beginning of the season-, fish with an average weight of 40 g are transported to the cages and reach an average of 400 g at the June -end of the season-. Fish with an average weight of 1,500 g can be obtained from fish that are transported to sea cages with an average weight of 200 g at the end of the production season (Çakmak et al. 2010). For the production of fillet size (>2500 g), it would be appropriate to transfer larger fish (400-500 g) to sea water at the beginning of the production season.

Meat Yield and Quality

The body proportions of Black Sea salmon males are according to their live weight have determined as for fin 4.66%, head 13.04%, carcass 68.32%, gonad 5.15%, skin 7.17%, liver 1.04%, bone 2.33%, internal organs 13,98%, meat yield was 58,82%, body proportions of females were 4.02% fin, 11.37%, carcass 70.55%, gonad 4.21%, skin 6.22%, liver. 1.59%, bone 2.41%, internal organs 14.06% and meat yield 61.99%. It has been determined that the meat yield of female individuals is 3.17% higher than male individuals (Çakmak et al. 2008). Preferring female individuals in conventional production is important for business profitability.

The average protein, fat, water and ash ratios of Black Sea salmon fillets were found to be 17.69%, 6.13%, 73.34%, and 1.38%, respectively. In addition, the ratio of omega 3 (n-3) and omega 6 (n-6) fatty acids, which are known to be important in human nutrition, is 30.79% and 14.10%, respectively. Among the fatty acids in the omega 3 group, EPA is 5.10% and DHA is 1.39% (Çankırılıgil 2019).

A total of 250-1000 mg of EPA and DHA is recommended daily usage in adult human nutrition, but three billion people worldwide receive less than 100 mg per day (Panchal and Brown 2021). When at least 64 g of Black Sea salmon meat is consumed per person a day, the minimum daily requirement will be met, and the average of European fish consumption will be reached, and if this amount is up to 256 g, the average of Japan will be reached. However, our country consumes an average of 18 g of fish per day (TUIK 2022), which is below the minimum requirement and world standards.

It is known that consumption of fish oil in human nutrition contributes to heart, intestine, inflammatory diseases, joint rheumatism and brain development. In case of dietary deficiency, behavioral disorders, depression, bipolar disorder and cognitive impairment in advanced age have been observed. In addition, studies have shown positive effects against various types of cancer and diabetes (Sidhu 2003, Balami et al. 2019, Yıldız 2019, Tacon et al. 2020).

Widespread Impact

Twenty five enterprises producing the species after the private sector project of SUMAE are located in the provinces of Trabzon, Rize, Artvin, Giresun, Gümüşhane and Muş (BSGM 2021).

Fish farm enterprises with restaurants in the region serve Black Sea salmon as fried in butter, grilled and steamed. In addition, different presentations are made (Figure 4).

Figure 4. Various plates of Black Sea salmon served at Abu Trout Farm and Restaurant in Fındıklı, Rize

Figure 4. Various plates of Black Sea salmon served at Abu Trout Farm and Restaurant in Fındıklı, Rize(continue)

The commercial distribution of Black Sea salmon is also supported by the pictures below (Figures 5).

Figure 5. Various newspaper news in Turkish, translations were given below respectively from left to right; Black sea salmon instead of Norway salmon, 115 cm salmon in Fırtına river, Black sea salmon will grow faster by breeding studies.

The information about the Black Sea salmon is given in the İnan Kardeşler museum, which is visited by tourists coming to Trabzon, (Figure 6).

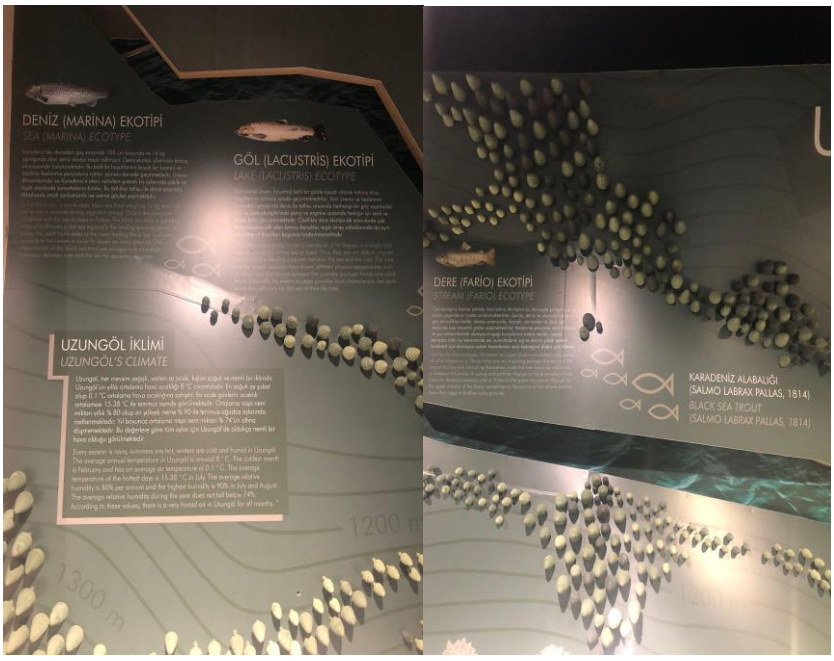

Figure 6. İnan Kardeşler Museum in Uzungöl, Trabzon

Conclusion

Today, 1603 tons of Black Sea Salmon is produced in our country by 25 private sector enterprises that have production licenses and many enterprises carry out commercial production in the form of trial production. Commercial production is concentrated in the Eastern Black Sea Region, which is the natural distribution area of the species. Enterprises that produce portion sizes in ponds on land and have restaurants prefer the red-spotted stream ecotype for production, while enterprises that produce large fish in dams and seas prefer the marine ecotype, which reaches late sexual maturity and shows better growth performance than other ecotypes. Since the Black Sea salmon is the only endemic trout species showing typical salmon character, our country's adaptation to natural environmental conditions is quite good for marine net cage farming and freshwater aquaculture. The point reached in the production of Black Sea salmon, which has similar nutritional values with other cultured salmonid species, shows that the new culture lines to be created may have the potential to compete with the Atlantic salmon. The contribution of this situation to the economy of our country will be very important.

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Data Availability Statement: There is no data availability for this study.

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