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Comparison of Antimicrobial Activities of *Sparus aurata* Skin and Mucus Extracts with *Laurencia papillosa* and *Carollina officinalis* Algae Dry Extracts

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ABSTRACT

The present study aimed to determine the antimicrobial activity of methanol, acetone, ethanol, or heptane extracts from *L. papillosa* or *C. officinalis* with the skin and mucus extracts from *S. aurata*. The inhibition zone (IZ) and minimum inhibitory concentration (MIC) of the extracts against *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida tropicalis*, and *C. parapsilosis* were determined by well diffusion and spectrophotometric broth microdilution methods, respectively. The highest antimicrobial activity of *L. papillosa* was against *C. tropicalis* with 4.98 mm, and the highest activity of *C. officinalis* was against *E. faecalis* with 7.84 mm. Among *S. aurata* mucus and skin extracts, the highest activity was on *C. parapsilosis* with 13.82 mm. The antimicrobial effect of *S. aurata* mucus extract on *E. faecalis* was found to be almost the same as *L. papillosa* extracts (6.14 mm and 6.43 mm). The inhibitions of *S. aurata* aqueous phase extract on *K. pneumoniae* and *C. parapsilosis* (7.09 mm) were much greater than the effects of *L. papillosa* and *C. officinalis* extraction. That *S. aurata* mucus and skin extracts were very effective, especially on *K. pneumoniae*, *A. baumannii* and *Candida* sp., was showed for the first time with this study. As a result, *S. aurata* mucus content is more effective on *K. pneumoniae* and *C. parapsilosis* than the phenolic content of both algae.

KEYWORDS: *Sparus aurata*, *Laurencia papillosa*, *Carollina officinalis*, Antimicrobial activity

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1. Introduction

Algae are of great importance in that they meet the nutritional needs of many living creatures, produce two-thirds of the world's photosynthetic carbon needs, contribute to the oxygen production in the atmosphere by 70-90%, and constitute approximately 90% of marine plants (Özdemir & Erkmén, 2013; Chapman, 2013; Baytaşoğlu & Başusta, 2015; Akyıl et al., 2016). Studies show that algae are rich in protein, fat, sterol, and water-soluble fiber content and have a high nutritional value in terms of minerals such as magnesium, potassium, and zinc (El-Sheekh et al., 2006; Alçay et al., 2017; Durmaz et al., 2002). Algae are the most encouraged biological resources in medicine, pharmacy, and cosmetics industries, agriculture, fertilizer production, and biodiesel production. In particular, it is widely used in the food industry as a gelling and thickening agent, such as agar-agar, alginate, and carrageenan (Kaba & Çağlak, 2006; Polat & Özoğul 2008; Carvalho et al., 2011). In addition, agar obtained from species belonging to the Rhodophyceae (Red algae) family is very important in bacteriology and biomedical studies (Baytaşoğlu & Başusta, 2015). It is known that the natural metabolites contained in algae are effective in defense against various pathogens (Kavita et al., 2014). As a result of studies on algae, many studies are determining their antimicrobial, cytotoxic, antimitogenic, anticancer, and antitumoral activities (Kandhasamy & Arunachalam, 2008; Silva et al., 2020). Studies show that the antibacterial effect of species belonging to Rhodophyta is more effective (Kolanjinathan & Stella 2009).

In aquaculture units with high fish density, diseases caused by many pathogens are encountered. Fish epidermal mucus is a barrier, providing primary defense against pathogenic microorganisms (Guardiola et al., 2014). The mucus layer contains innate immune components secreted by goblet cells (Spitzer & Koch, 1998; Dash et al., 2018). Mucus composition varies among fish

species and is affected by exogenous and endogenous factors (Jurado et al., 2015). The main compounds of skin mucus are water and glycoproteins containing high molecular weight oligosaccharide molecules called mucins (Guardiola et al., 2014; Jurado et al., 2015). Mucus also form a biochemical barrier containing enzymes such as antimicrobial proteins and proteases contributing to fish immunity (Fast et al., 2002). Immune molecules in mucus include immunoglobulin, lysozyme, lectin, interferon, histones, ribosomal proteins, proteolytic enzymes, antimicrobial peptides, and vitellogenin (Vasta et al., 2011; Ademek et al., 2013; Bergsson et al., 2005). Studies report that fish increase their mucosa or change their composition when exposed to pathogenic bacteria (Van der Marel et al., 2010; Gustafsson, 2013). Many complex molecules, such as lysozymes, phosphatases, esterases, lectins and immunoglobulins in mucus, try to eliminate pathogens and strengthen immunity in case of any infection (Reverter et al., 2018). Many researchers investigated raw fish mucus's antimicrobial properties against infectious pathogens (Hellio et al., 2000; Johansson et al., 2010; Gustafsson et al., 2013). The antimicrobial properties of raw mucus against pathogens were first studied in *Oncorhynchus mykiss* (Rainbow trout) (Austin and McIntosh, 1988).

It is estimated that discards from fishing in the world are approximately 20 million metric tons per year (hellio et al., 2002). In particular, the use of by-products such as mucus and skin obtained from various fish species in biotechnological research has become widespread. Because many bioactive substances can be extracted from fish processing waste (Cunha et al., 2023).

Indiscriminate use of antibiotics in the treatment of infections causes pathogenic bacteria to become resistant to drugs (Kandhasamy & Arunachalam 2008). Therefore, the decreasing effectiveness of antibiotics necessitates the development of new alternatives. In this study, the antimicrobial activities of methanol (M),

acetone (A), ethanol (E), or heptane (H) extracts (E) from *L. papillosa* or *C. officinalis* and the skin and mucus extracts from *S. aurata* on *A. baumannii*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, *C. tropicalis*, and *C. parapsilosis* were investigated.

2. Material and Methods

2.1. Collection of Samples and Preparation for Experiments

Laurencia papillosa (G. Agardh, 1830) and *Carollina officinalis* (Linnaeus, 1758) were collected from Mersin Bay. *S. aurata* (Linnaeus, 1758) were obtained from aquaculture systems. The algae samples were washed with pure water and dried on filter papers. The drained algae samples were dried in the oven and transferred to the grinder. Powdered samples were transferred to falcon tubes and stored in the refrigerator at +4°C. The mucus layer of *S. aurata* was extracted as soon as they were obtained from the cage and transferred to falcon tubes with ice. *S. aurata* were placed in ice and brought to Mersin University, Faculty of Fisheries. They were cleaned from muscle tissue and scales and stored at -18°C.

2.2. Preparation of Algae, Fish Mucus, and Skin Extracts

4 g of dry-powdered *L. papillosa* or *C. officinalis* with 40 mL of methanol, acetone, ethanol, or heptane (Merck (Darmstadt, Germany) was mixed with a magnetic stirrer for 24 hours. The samples were filtered using 0.45 filter paper. Aqueous phase and acetic acid extract were prepared from the mucus and skin of *S. aurata* (Uyan, 2020). To obtain aqueous phase extract, 10 mL mucus and 10 mL NaCl (0.85%) were mixed in the tube. The samples were centrifuged at 10000 rpm for 10 minutes to ensure layer formation. After centrifugation, the upper layers of the mucus samples were taken into new tubes. To obtain acetic acid extract, 10g of skin sample and 50 mL of acetic acid (3%) were mixed and homogenized. The homogeneous sample

was kept in hot water for 5 minutes and cooled.

2.3. Antimicrobial screening of the extracts

The antimicrobial activity of some extracts of *L. papillosa*, *C. officinalis* and *S. aurata* were investigated using spectrophotometric broth microdilution and disc diffusion methods. Strains used in this study: *Acinobacter baumannii* (ATCC 02026), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus aureus* (ATCC 25925), *Enterococcus faecalis* (ATCC 29212) bacterial strains and *C. tropicalis* (ATCC 750) and *C. parapsilosis* yeasts. Before the experiment, bacteria were inoculated on TSA (Tryptic Soy Agar) and yeast were inoculated on SDA (Sabouraud Dextrose Agar) solid medium and incubated at 37°C for 18-24 hours. At the end of 1-day of incubation, the colonies were taken directly from single fallen colonies on the agar plate with a loop and the number of McFarland (~ 10⁸ CFU/mL) was adjusted with physiological saline. Ampicillin was used for bacteria and fluconazole for yeast as positive control antibiotics (Erdoğan Eliuz, 2021).

2.3.1. Determination of MIC (minimum inhibitory concentration) of the extracts

The sterile 96-well plates were prepared for the extracts of *L. papillosa* and *C. officinalis* with methanol, acetone, ethanol and heptane; and the crude, aqueous phase and acetic acid extracts of *S. aurata*. Serial dilutions for each sample were prepared in the horizontal well row of the plate. Starting from the first well of the plate, 50 µL of Mueller Hinton Broth (MHB) medium was added to all wells in the microplates. Then, 100 µL of the extract was placed in the first well and a double-fold dilution was made until the end of the first ten rows. Positive control, medium control and negative controls were prepared in the last two wells. Finally, 5 µL of microorganisms were added

to the wells containing extract and antibiotics and left for incubation. Spectrophotometric measurements (Thermo Scientific, MULTISKAN) were taken at 600 nm and inhibition-concentration graph was drawn. The % inhibition value was obtained using the formula below (Eq. 1). The experiments were repeated 3 times (Erdoğan Eliuz, 2021).

$$\text{Inhibition (\%)} = \left[1 - \frac{OD_{\text{test well}}}{OD_{\text{corresponding control well}}} \right] \times 100 \text{ Eq. 1}$$

2.3.2. Inhibition zone of the extracts

A certain amount of microorganism solution adjusted according to McFarland 0.5 was spread on the MHA agar petri dish and wells with a diameter of 6 mm were opened in the middle of the petri dish. Each well was filled with 50 μL of the extract and incubated at 37°C for 24 hours. While evaluating the results, the diameters of the inhibition zones were measured in millimeters using the Images program. Sterile distilled water was used as a negative control and all tests were repeated three times (Erdoğan Eliuz, 2021).

2.4. Statistical Analysis

IZ and MIC data obtained were statistically evaluated with One Way Anova (post-hoc Tukey HSD Test). Differences ($p \leq 0.05$).

3. Results

The antimicrobial activity results of algae, skin, and mucus extracts are comparatively given in Tables 1 and 2. In the ME, where the antimicrobial effects of *L. papillosa* and *C. officinalis* on bacteria and yeasts were investigated, it was determined that the highest inhibition was (5.87 mm) of *C. officinalis* against *E. faecalis*. It was determined that the highest inhibition of *L. papillosa* extracted with acetone was against *K. pneumoniae* (4.77 mm), and the lowest inhibition diameter was against *S. aureus* (2.80 mm). The highest inhibition diameter

of AE of *C. officinalis* was determined as 7.84 mm in *E. faecalis*, and the lowest level was 1.18 mm in *A. baumannii*. *C. officinalis* AE was ineffective against *C. tropicalis*. Both EA of *L. papillosa* and *C. officinalis* more inhibited *E. faecalis* with 6.43 mm and 5.67 mm, respectively, than other microorganisms. The highest inhibition of *L. papillosa* extracted with heptane was calculated as 5.04 mm and 4.59 mm in *C. parapsilosis* and *C. tropicalis* yeasts, respectively. The highest inhibition level in HE of *C. officinalis* was determined as 4.56 mm in *A. baumannii* bacteria. The only bacteria on which the CE prepared from the mucus of *S. aurata* showed an antimicrobial effect was *E. faecalis*, and the inhibition diameter was determined as 1.05 mm. The IZ of the aqueous phase extract prepared from *S. aurata* mucus was determined as 6.14 mm in *E. faecalis*, 7.09 mm in *K. pneumoniae*, and 8.75 mm in inhibition diameter in *C. parapsilosis*. In addition, all pathogens were resistant to acetone, methanol and water used as negative controls.

Interestingly, the aqueous phase extract prepared with *S. aurata* skin had a very high inhibition (13.82 mm) on *C. parapsilosis* (Figure 1).

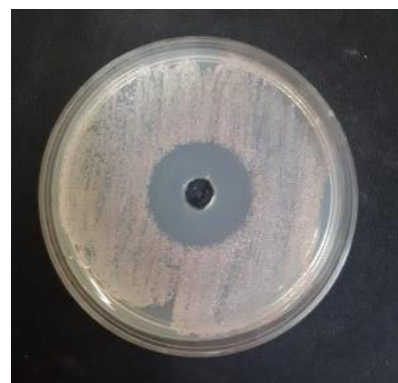


Figure 1. IZ (13.82 mm) of APE of *S. aurata* skin on *C. parapsilosis*.

MICs of *L. papillosa* and *C. officinalis* on gram positive, gram negative and yeast were reported in Table 2. When the MIC levels of *L. papillosa* and *C. officinalis* prepared by methanol were compared, it was determined that *C. officinalis* had a more effective MIC value on bacteria and yeasts.

Table 1. Inhibition zone diameters (mm) of algae, mucus and skin extracts against *A. baumannii*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, *C. tropicalis*, *C. parapsilosis*.

	<i>Sa</i>	<i>Ef</i>	<i>Ab</i>	<i>Kp</i>	<i>Cp</i>	<i>Ct</i>
Lp. ME	4.27±1.14 ^b	3.97 ±1.28 ^b	2.63±0.43 ^b	2.65 ±0.04 ^b	1.74 ± 0.00 ^b	4.98±1.49 ^b
C.o. ME	3.96±0.37 ^b	5.87±1.49 ^b	4.07±0.01 ^b	3.43±2.10 ^b	4.05±1.29 ^b	4.11±1.90 ^b
L.p. AE	2.80±0.51 ^b	3.98±0.01 ^b	3.32 ±0.23 ^b	4.77 ±0.57 ^b	3.87 ± 0.41 ^b	3.59±0.73 ^b
C.o. AE	3.83±1.05 ^b	7.84 ± 0.02 ^b	1.18 ± 0.00 ^b	3.78 ±0.00 ^b	1.52 ± 0.02 ^b	-
L.p. EE	5.01±1.79 ^b	6.43 ± 0.08 ^b	3.65 ± 0.04 ^b	4.61±0.75 ^b	2.72 ± 0.00 ^b	2.81±1.25 ^b
C.p. EE	3.76±1.49 ^b	5.67 ± 2.30 ^b	5.54 ± 0.03 ^b	3.08±0.01 ^b	4.14 ± 0.36 ^b	3.23±1.21 ^b
L.p. HE	2.84±0.01 ^b	-	3.05 ± 0.23 ^b	2.07 ± 0.00 ^b	5.04 ± 0.93 ^b	4.59±0.00 ^b
C.o. HE	-	1.50±0.00 ^b	4.56 ± 0.04 ^b	1.84 ± 0.03 ^b	3.79 ± 0.47 ^b	-
S.a. M-CE	-	1.05±0.01 ^b	-	-	-	-
S.a.M-PE	2.76±0.01 ^b	6.14±0.54 ^b	1.38 ± 0.01 ^b	7.09 ± 0.49 ^b	8.75 ± 0.55 ^b	-
S. a. S-CE	-	-	-	-	-	-
S. a. S-APE	-	-	-	-	13.82±0.01 ^a	-
Ant.	32.7±0.01	17.8±0.01	15±0.02	21.0±0.01	19.8±0.03	35.0±0.01

Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Ab: *Acinobacter baumannii*, Kp: *Klebsiella pneumoniae*, Cp: *Candida parapsilosis*, Ct: *Candida tropicalis*. Lp: *Laurencia papillosa*, Cp: *Carollina officinalis*, S.au: *Sparus aurata*; M; mucus, S; skin, CE: Crude Extract, APE: Aqueous Phase extract, Acetone extract: AE, Methanol extract: ME, Ethanol extract: EE. Ant: antibiotic (p≤0.05).

For *L. papillosa* ME was 37.41 mg/mL against *C. parapsilosis*, and the highest value was 735.88 mg/mL against *A. baumannii* bacteria. The highest MIC value of ME of *C. officinalis* was determined as 30.42 mg/mL against *E. faecalis*, and the highest value was determined as 86.63 mg/mL against *C. parapsilosis* (p≤0.05).

The lowest MIC values of *L. papillosa* and *C. officinalis* AE were determined against *A. baumannii* as 25.34 mg/mL and 45.92 mg/mL, respectively. The lowest MIC values of *L. papillosa* and *C. officinalis* EA were determined as 31.81 mg/mL and 31.66 mg/mL against *E. faecalis*. In the HEs, the lowest MIC levels of *L. papillosa* and *C. officinalis* were found to be 54.62 mg/mL and 54.62 mg/mL against *C. tropicalis* (p≤0.05).

The most effective (low) MIC values of *S. aurata* were as follows; 88.25 mg/mL for mucus extract on *S. aureus*, 26.73 mg/mL for mucus aqueous phase extract on *C.*

parapsilosis; 26.91 mg/mL for skin crude extract on *C. parapsilosis*; 49.87 mg/mL for skin aqueous phase extract on *E. faecalis* (p≤0.05).

4. Discussion

Red algae contain many bioactive compounds with many pharmacological properties. For this reason, especially in recent years, the number of studies on red algae has increased to reveal antimicrobial components (Kolanjinathan & Stella 2009b). Many species, such as *G. edulis* (Kolanjinathan et al., 2009a), *Actinotrichia fragilis* (Salem et al., 2011); *Gracillaria folifera*, *Hypneme muciformis* (Kandhasamy and Arunachalam 2008); have been found to effectively inhibit Gram-positive and Gram-negative bacteria.

Table 2. MICs (mg/mL) of algae, mucus and skin extracts against *A. baumannii*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, *C. tropicalis*, *C. parapsilosis* microorganisms

	<i>Sa</i>	<i>Ef</i>	<i>Ab</i>	<i>Kp</i>	<i>Cp</i>	<i>Ct</i>
Lp. ME	107.99±4.72 ^a	104.55±8.25 ^a	735.88±1.7 ^b	94.104±1.94 ^a	37.41±1.20 ^a	64.59±17.8 ^a
C.o. ME	67.29±1.79 ^a	30.42±2.56 ^a	34.42±1.55 ^a	50.15±1.20 ^a	86.63±4.70 ^a	46.28±7.9 ^a
L.p. AE	78.56±3.61 ^a	515.43±9.73 ^b	25.34±0.5 ^a	59.89±1.59 ^a	93.73±7.04 ^a	96.47±3.42 ^a
C.o. AE	140.14±8.46 ^a	140.76±5.27 ^a	45.92±1.81 ^a	434.85±3.77 ^b	60.99±7.63 ^a	74.94±6.22 ^a
L.p. EE	56.35±2.01 ^a	31.81±0.24 ^a	94,17±5,92 ^a	41.93±7.09 ^a	1938.78±1.23 ^b	35.62±3.58 ^a
C.p. EE	101.22±8.02 ^a	31.66±3.78 ^a	38.79±4.18 ^a	60.90±9.96 ^a	88.05±3.54 ^a	37.40±2.10 ^a
L.p. HE	163.63±8.81 ^b	404.56±2.54 ^b	198.76±2.85 ^a	56.62±7.30 ^a	72.71±4.06 ^a	54.62±1.74 ^a
C.o. HE	214.90±9.50 ^b	45.53±3.44 ^a	607.96±7.91 ^a	133.22±1.05 ^a	125.87±4.46 ^a	54.62±1.74 ^a
S.a. M-CE	88.25±2.91 ^a	130.79±4.72 ^a	134.99±1.04 ^a	101.97±7.93 ^a	246.13±1.17 ^a	378.78±2.97 ^a
S.a.M-PE	86.61±2.69 ^a	240.20±2.07 ^a	93.78±4.41 ^a	76.36±4.64 ^a	26.73±2.86 ^a	756.69±6.99 ^b
S. a. S-CE	78.22±8.72 ^a	91.23±1.70 ^a	122.67±6.86 ^a	110.60±4.14 ^a	26.91±1.50 ^a	152.81±8.72 ^a
S. a. S-APE	50.97±4.25 ^a	49.87±1.30 ^a	143.27±7.17 ^a	311.79±1.27 ^b	246.13±1.17 ^a	67.34±2.44 ^a
Ant. (µg/mL)	68.7±0.01	12.7±0.02	92.1±0.01	88.1±0.01	128±0.03	48.7±0.02

Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Ab: *Acinobacter baumannii*, Kp: *Klebsiella pneumoniae*, Cp: *Candida parapsilosis*, Ct: *Candida tropicalis*. Lp: *Laurencia papillosa*, Cp: *Carollina officinalis*, S.au: *Sparus aurata*; M; mucus, S; skin, CE: Crude Extract, APE: Aqueous Phase extract, Acetone extract: AE, Methanol extract: ME, Ethanol extract: EE. Ant: antibiotic (p≤0.05).

A few antimicrobial activity studies with *L. papillosa* extracts have been found in the literature. Among them, Kavita et al., (2014) reported the inhibition zone of *L. papillosa* methanol extract on *E.coli* (Gram -), *S. aureus* (Gram +), *B. subtilis* (G +), *P. aeruginosa* (G -) bacteria as 12.33 mm, 14.33 mm, 11.66 mm, respectively. In our study, the most effective inhibition of *L. papillosa* was against *S. aureus* (G +), and *E. faecalis* (G +), with 6.43 mm and 5.01 mm, respectively. The difference in the level of inhibition may result from technical differences in the extraction step. In another study, the antimicrobial potential of *C. officinalis* on *E. faecalis* (G +), *E. aerogenes* (G -) and *E. coli* (G -) was revealed by Taşkın et al. The inhibition level of *C. officinalis* extract on *E. faecalis* and *E. coli* bacteria was reported as 21.66 mm and 32 mm, respectively. This study supports our finding that *C. officinalis* is effective on *E. faecalis*. The highest inhibition of *Corallina officinalis* on *E. faecalis* (G+) was realized with acetone (7.84 mm) and ethanol (5.67 mm) extracts.

It is known that the antimicrobial potential in macroalgae is generally due to the polyphenolic compounds it contains (Silva et al., 2020). In particular, green and red algae contain many secondary metabolites, including catechin, phlorotannin, phenolic acids and flavonols (Gómez-Guzmán et al., 2018; Cassani et al., 2020). *L. papillosa* has been found to be very rich in substances such as vanillin, hydroxytyrosol, urolithin A, phloroglucinol, 2-Hydroxy-2-phenylacetic acid, quercetin caffeoyl-glucoside, p-Coumaric acid methyl ester-p-Hydroxybenzoic acid, p-Hydroxybenzaldehyde and sinapic acid (Goksen 2023). The presence of p-hydroxybenzaldehyde in red algae has also been shown previously (Rajauria et al., 2016; Nørskov et al., 2021) and it has been determined to be an important antioxidant (Zhong et al., 2020) and antimicrobial (Taib et al., 2020). Similarly, phenolic acids such as salicylic acid, p-hydroxybenzoic acid, gentisic acid, protocatechuic acid, gallic acid, vanillic acid, and syringic acid were found

abundantly in *Gracilaria species* (Xu et al., 2015; Dhaouafi et al., 2023).

When the antimicrobial effects of algae were compared with *S. aurata* mucus, similar results were observed with the phenolic contents of macroalgae. The antimicrobial effect of *S. aurata* mucus extract on *E. faecalis* was found to be almost the same as *L. papillosa* (6.14 mm and 6.43 mm). The inhibitions of *S. aurata* aqueous phase extract on *K. pneumoniae* and *C. parapsilosis* (7.09 mm) were much greater than the effects of *L. papillosa* and *C. officinalis* extraction. This means that *S. aurata* mucus content is more effective on *K. pneumoniae* and *C. parapsilosis* than the phenolic content of both algae. It has been previously reported that mucus may have antimicrobial effects due to its structure and chemical content. A study reported that cupra skin mucus contained lower levels of lysozyme, alkaline phosphatase and protease, and higher esterase, peroxidase and antiprotease activities. (Guardiola et al., 2014). Additionally, that heavy metals in sea environment were affected the enzymatic changes and bactericidal activity in the mucus layer in *S. aurata* (Guardiola et al., 2015). Cordero et al. (2016) reported that a decrease was observed in the level of total sugar and protein residues, in protease, peroxidase and lysozyme activities compared to fresh samples during fresh and freezing of mucus samples in *S. aurata*. In another study, bioactive metabolites of *P. sophore* mucus extract were analyzed by HR-LCMS and reported that the mucus content consisted of compounds such as cysteamine, glucosamine, phytosphingosine, arachidonoyl amine, 2-amino-tetradecanoic acid, 2,4-dimethyl-tetradecanoic acid, dihydrosphingosine (Reid et al., 2020). In a study, Subramanian et al. (2008) conducted that high inhibition was reported on the *Salmonella enterica* strain by extracting (acidic, organic and aqueous solvents) mucus samples of various fish species (*Salvelinus alpinus*, *S. fontinalis*, *Cyprinus carpio*, *Melanogrammus aeglefinus*). In the same study, they did not detect any antimicrobial

effect in aqueous mucus extracts (Subramanian et al., 2008). In another study, Hellio et al. (2002) reported that extracts obtained from fish epidermis and epidermal mucus did not inhibited gram-negative and gram-positive bacteria. However, they detected antibacterial effects in ethanolic and dichloromethane fractions. It is understood that the antimicrobial effects of extracts prepared with fish mucus and skin samples may vary depending on the climate, type of fish, and technical conditions.

5. Conclusion

As a result, the antimicrobial activities of *L. papillosa* and *C. officinalis* red algae, which are rich in phenolic content, showed average activity on the microorganisms we studied. It is understood that *S. aurata* mucus and skin extracts are very effective, especially on *K. pneumoniae*, *A. baumannii* and *Candida* sp., which were studied for the first time. When compared to the phenolic richness in macroalgae, it is understood that the enzymes and protein structures that can be found in the mucus and skin of *S. aurata* were almost as effective as phenols. In future studies, extracts of both organisms can be studied by creating complex structures together in order to enrich each other with bioactive compounds.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no competing interests.

Author contribution

All authors' contributions are equal for the preparation of research in the manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Relationships between Otolith Dimensions and Total Length of Some Small-sized Fish Species from the Marmara Sea, Türkiye

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ABSTRACT

The small-sized adult and juvenile fish species have great ecological importance by means of the prey-predator relationship. Due to these species become prey of larger fish species, their otoliths are mostly seen in the stomach content of predators. The fish size-otolith size studies reveals robust data for estimating fish size from the otolith size. To contribute this aspect, the relationships between total length and otolith length (TL-OL), total length and otolith width (TL-OW), total length and otolith radius (TL-OR) of seven species (*Liza saliens*, *Pomatoschistus marmoratus*, *Pomatoschistus bathi*, *Pomatoschistus minutus*, *Synapturichthys kleinii*, *Chelidonichthys lucerna* and *Symphodus roissali*) were investigated. Species were obtained by beach-seine nets from 12 sampling stations in the shallow waters of the Marmara Sea. While calculating the relationships, linear regression analysis ($y = bx + a$) was used and the coefficient of determination (R^2) was identified. The R^2 values were usually seen as high in all equations. The highest values of R^2 for TL-OL and TL-OW were obtained from *P. minutus*, while the highest values of TL-OR detected were for *C. lucerna*. The lowest values of R^2 for TL-OW and TL-OR were obtained from *P. marmoratus*, while the lowest values of TL-OL were detected for *P. bathi*. This study revealed the first results for *P. minutus*, *S. kleinii*, *S. roissali* in the Türkiye Seas and for *P. bathi* in the worldwide by means of fish length – otolith size relationships.

KEYWORDS: Beach seine nets, Juvenile, Fish, Otolith size, Shallow waters

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1. Introduction

The inner ears of all teleost fishes contain calcified structures, which otoliths are balance and hearing organs (Popper et al., 2005). These structures serve as the life history of the species (Belchier et al., 2004). The otoliths continue to grow throughout the species life and do not resorb in times of stress (Yaremko, 1996; Mendoza, 2006). So, the analysis of the microstructure of the otolith is a reliable piece of information on the daily (Mendoza, 2006; Yazıcı et al., 2021), size (Campana and Jones, 1992), growth (Mendoza, 2006), sexual dimorphism (Cardinale et al., 2004; Yazıcı, 2023), stock identification (Campana, 2005), feeding ecology of predators (Campana and Casselman, 1993), and the determination of the migration direction (Kennedy et al., 2002) and ontogeny of fish species (Song et al., 2019). These informations contribute to fisheries management (Campana and Thorrold, 2001; McFarlane et al., 2010; Gerard and Malca, 2011). Also, otolith dimensions can be related to fish sizes (Pannella, 1971). These relationships are species-specific, but they may occur in different populations of species (Fey, 2006). According to Harvey et al. (2000), the relationship between fish length and otolith dimensions can be described by using a simple linear regressions.

Some valuable studies on fish size – otolith size relationships have been revealed in the literature (Harvey et al., 2000; Battaglia et al., 2010; Viva et al., 2015). These relationships were revealed for some species presented in this study in the Türkiye seas, as for *L. saliens* and *P. marmoratus* in

the Aegean Sea (Akyol and Kınacıgil, 2001; Altın and Ayyıldız, 2018), for *P. marmoratus* and *Chelidonichthys lucerna* in the Black Sea (Gümüş et al., 2021; Hasimoğlu et al., 2016) and for *C. lucerna* in the Mediterranean Sea (Başusta and Bıyıklı, 2022).

The small-sized adult and juvenile fish species have a great ecological importance by means of the prey-predator relationship. Due to these species become prey of larger fish species, their otoliths are mostly seen in the stomach contents of predators. The fish size-otolith size studies reveals robust data for estimating of fish size from the otolith size. Between the seven species examined in this study, *P. marmoratus*, *P. bathi*, *P. minutus*, and *S. roissali* were small-sized adults and *C. lucerna*, *S. kleinii* and *L. saliens* were juveniles and originated as an important components of the coastal biodiversity of the Marmara Sea. Thus, we aimed to reveal the otolith size-fish size relationship of these species, which some of exhibit first data for the Marmara Sea (all 7 species), for Türkiye seas (*P. minutus*, *S. kleinii*, *S. roissali* and *P. bathi*) and for worldwide (*P. bathi*) in the literature.

2. Material and Methods

Individuals were sampled with beach seine nets from 12 equally-spaced sampling locations around the Marmara Sea, Türkiye between November 2021 and March 2022. All beach seine net tows were realized with 2 replications from shallow waters (0-5 meters) in day hours (Figure 1).

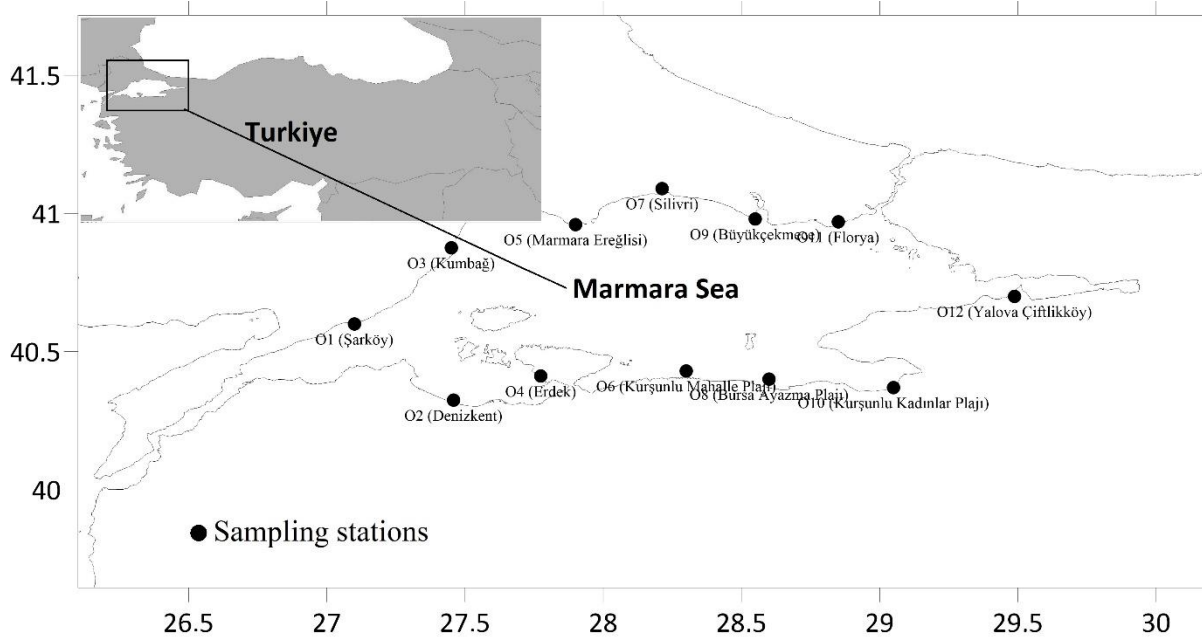


Figure 1. Study area and the sampling stations

The mesh size of the beach seine net was used as a 6.5 mm bar length in the wings and 4 mm bar length in the bag. The wings of this net had 15 m length, and the bag had $2 \times 2 \times 2$ meters dimensions. So, the total length of this net was determined as approximately 32 m. The hanging ratio of the bag and wings were arranged as 0.85 and 0.66, respectively. In order to haul, 15 m of long warp was rigged for each wing. Synthetic polypropylene twine diameter ropes were used in hanging as 9 \varnothing , leadline and floatline as 6 \varnothing and net as 2.5 \varnothing . The individuals were kept in an ice-box and transported to the laboratory, immediately. Species identification was realized according to Whitehead et al. (1986). The total length (TL) of individuals were measured with a digital caliper (Mitutoyo CD-15 APX) and the larger individuals were measured with a measuring board to the nearest 0.01 mm.

Sagittal otolith pairs of species were removed, cleaned, dried and stored in eppendorf tubes. These otoliths of species were photographed then measurements were made. The otolith length (OL), otolith width (OW) and otolith radius (OR) of individuals were measured to the nearest 0.001 mm with using stereomicroscope and Q-Capture digital imaging program, each right and left sagitta of each individual. The otolith length

(OL) was recorded as the distance from the midpoint of the rostrum through the primordium to the posterior edge. The otolith width (OW) was recorded as the distance perpendicular to the length passing through the primordium (Javor et al., 2011). The otolith radius (OR) was determined by the distance from the otolith core to its margin (Zabel et al., 2010). These measurements were tested to identify whether the right and left otoliths were different. The student t-test (Zar, 1999) was used to compare between length, width and radius of the right and left sagittal otoliths. No significant differences of measurements were detected for all species ($p > 0.05$). These statistical analyses were done using the PAST Version 2.17 program (Hammer et al., 2001). So, right otoliths were used in regression analyses.

Linear regression analysis ($y = bx + a$) was used to determine the relationship between otolith dimensions and fish length (TL-OL; TL-OW and TL-OR) and the coefficient of correlation determination (R^2) were calculated.

3. Results

In this study, a total of 111, 113, 107, 6, 61, 7 and 8 individuals of *Liza saliens*,

Pomatoschistus marmoratus, *Pomatoschistus bathi*, *Pomatoschistus minutus*, *Synapturichthys kleinii*, *Chelidonichthys lucerna* and *Symphodus roissali* were analyzed, respectively. Sample sizes for species ranged between 6 and 113 individuals. The individual number (n), minimum (min), maximum (max) and mean of TL, OL, OW, and OR of these species can be seen in Table 1. The mean otolith length and otolith radius of *S. kleinii* was smaller than other species. Also, the mean otolith width of *S. roissali* was smaller than other species. The small sizes were measured for *L. saliens* and *P. marmoratus* in OL (Table 1).

The equations of relationship between the total length and otolith dimensions of seven species are given in Table 2. Also, the equation curves of each species can be seen in Figures 2, 3, 4, 5, 6, 7 and 8, respectively. The R² values were detected usually high in all equations. The highest values of R² were

obtained from *P. minutus* for the equations of TL-OL and TL-OW, while the highest values of R² were detected for *C. lucerna* in the equation of TL-OR.

The lowest values of R² were obtained for *P. marmoratus* for equations of TL-OW and TL-OR, while the lowest values of TL-OL were found for *P. bathi*. Although TL-OR coefficient relationships were weak, higher coefficient determination values were found for *P. minutus* from the equations of TL-OL and TL-OW. The highest R² values for all relationships were recorded for *L. saliens* (0.91, 0.88 and 0.91, respectively), while the lowest R² values were detected for *P. marmoratus* (0.76, 0.80 and 0.65, respectively) (Table 2). Although both two species were represented with the highest sample number, the R² values varied relatively higher. Thus, it was understood that the sample size was not a major determinant for R².

Table 1. The min, max and mean of TL, OL, OW, and OR of seven fish species

Species	N	Total length (mm)			Otolith length (mm)			Otolith width (mm)			Otolith radius (mm)		
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
<i>L. saliens</i>	111	18.86	123.19	51.7	0.497	4.033	1.822	0.311	2.302	1.139	0.228	2.009	0.828
<i>P. marmoratus</i>	113	24.81	79.9	50.82	0.81	1.994	1.542	0.71	1.849	1.329	0.467	0.963	0.749
<i>P. bathi</i>	107	25.75	78.12	55.11	0.958	1.969	1.653	0.747	1.639	1.365	0.473	1.016	0.805
<i>P. minutus</i>	6	49.41	63.91	55.5	1.509	1.957	1.724	1.252	1.573	1.374	0.78	1.029	0.924
<i>S. kleinii</i>	61	26.57	125.16	73.02	0.543	1.942	1.193	0.42	1.654	1.058	0.251	1.005	0.607
<i>C. lucerna</i>	7	67.63	87.92	77.23	1.643	1.961	1.803	1.398	1.649	1.525	0.639	0.993	0.809
<i>S. roissali</i>	8	42.77	96.82	68.46	1.189	2.107	1.74	0.839	1.267	1.024	0.541	1.021	0.767

Table 2. The relationships between TL-OL, TL-OW, TL-OR of seven fish species

Species	TL-OL	R ²	TL-OW	R ²	TL-OR	R ²
<i>L. saliens</i>	TL=0.0381OL-0.1461	0.91	TL=0.0174OL+0.2434	0.88	TL=0.0177OL-0.0885	0.91
<i>P. marmoratus</i>	TL=0.0179OL+0.6298	0.76	TL=0.0151OL+0.5635	0.80	TL=0.007OL+0.3933	0.65
<i>P. bathi</i>	TL=0.0176OL+0.6817	0.75	TL=0.0142OL+0.5821	0.82	TL=0.0088OL+0.3186	0.81
<i>P. minutus</i>	TL=0.0294OL+0.0924	0.95	TL=0.0236OL+0.0638	0.97	TL=0.0147OL+0.1058	0.74
<i>S. kleinii</i>	TL=0.013OL+0.2465	0.92	TL=0.0111OL+0.2577	0.87	TL=0.0077OL-0.0021	0.87
<i>C. lucerna</i>	TL=0.0138OL+0.7395	0.86	TL=0.0097OL+0.7737	0.90	TL=0.0169OL-0.4964	0.92
<i>S. roissali</i>	TL=0.0165OL+0.6105	0.88	TL=0.0085OL+0.4438	0.91	TL=0.0084OL+0.1929	0.85

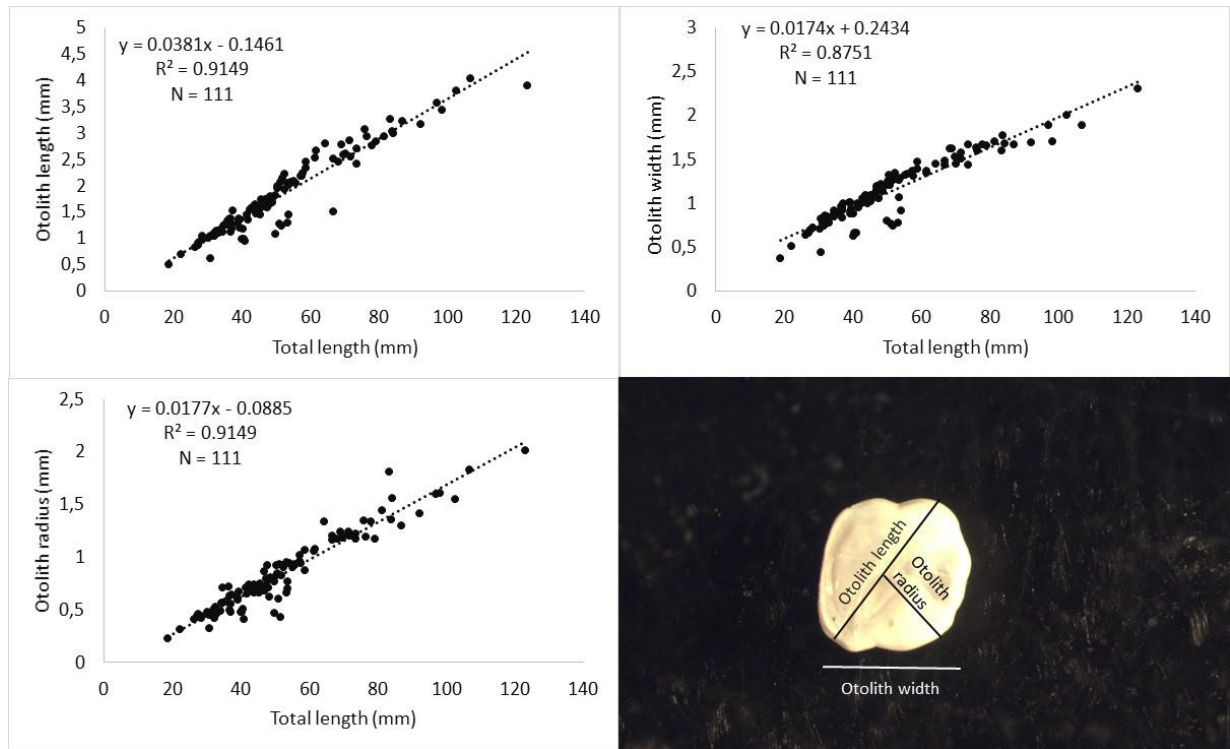


Figure 2. The relationships between TL-OL, TL-OW and TL-OR of *L. saliens*

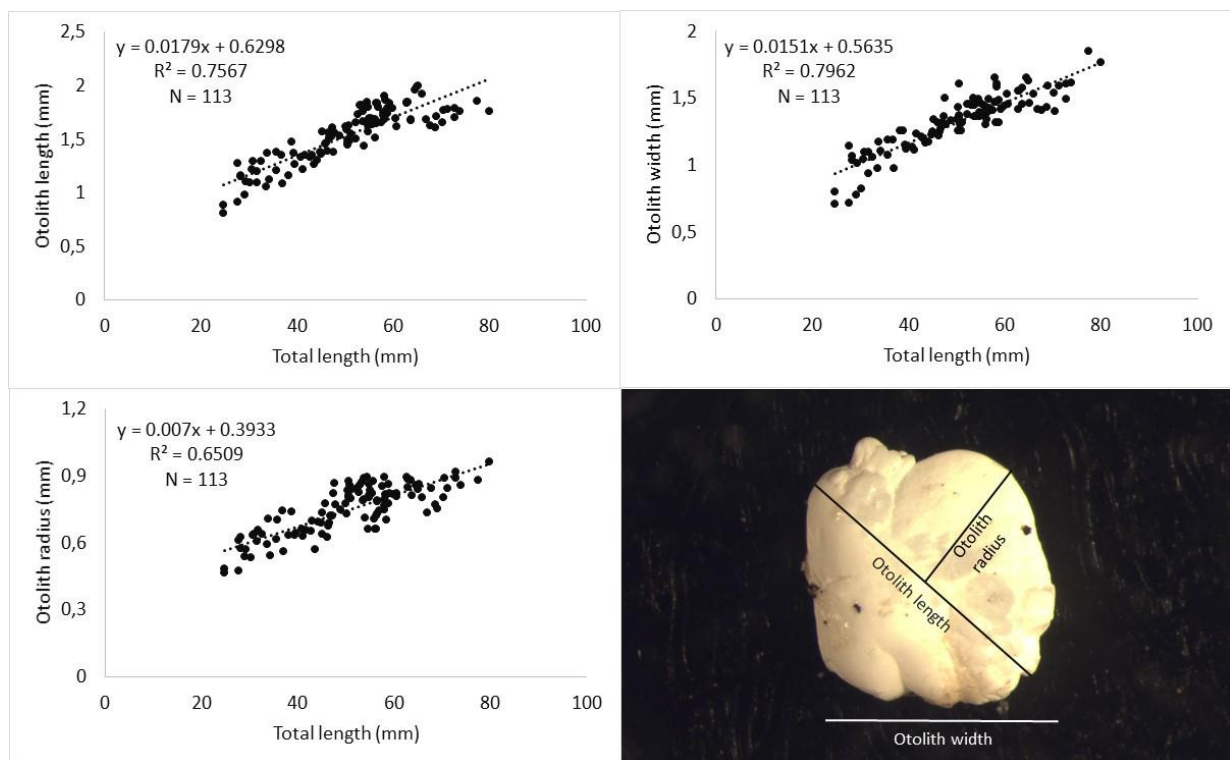


Figure 3. The relationships between TL-OL, TL-OW and TL-OR of *P. marmoratus*

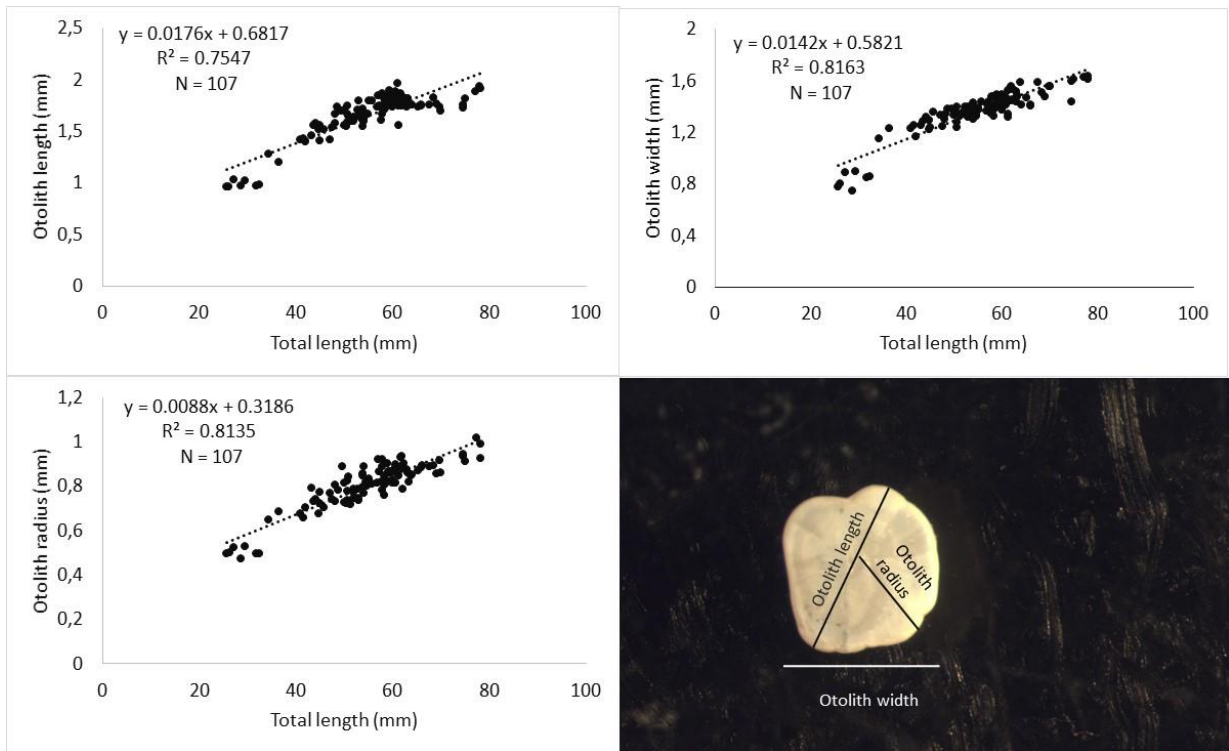


Figure 4. The relationships between TL-OL, TL-OW and TL-OR of *P. bathi*

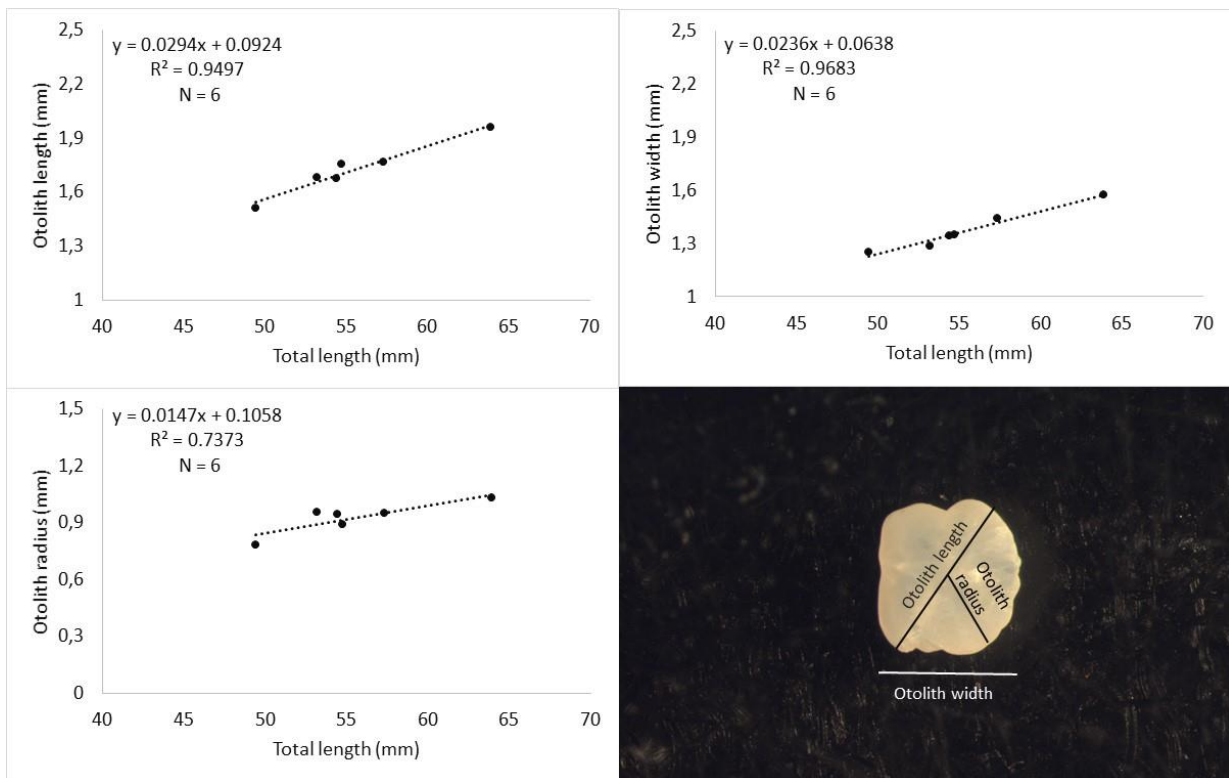


Figure 5. The relationships between TL-OL, TL-OW and TL-OR of *P. minutus*

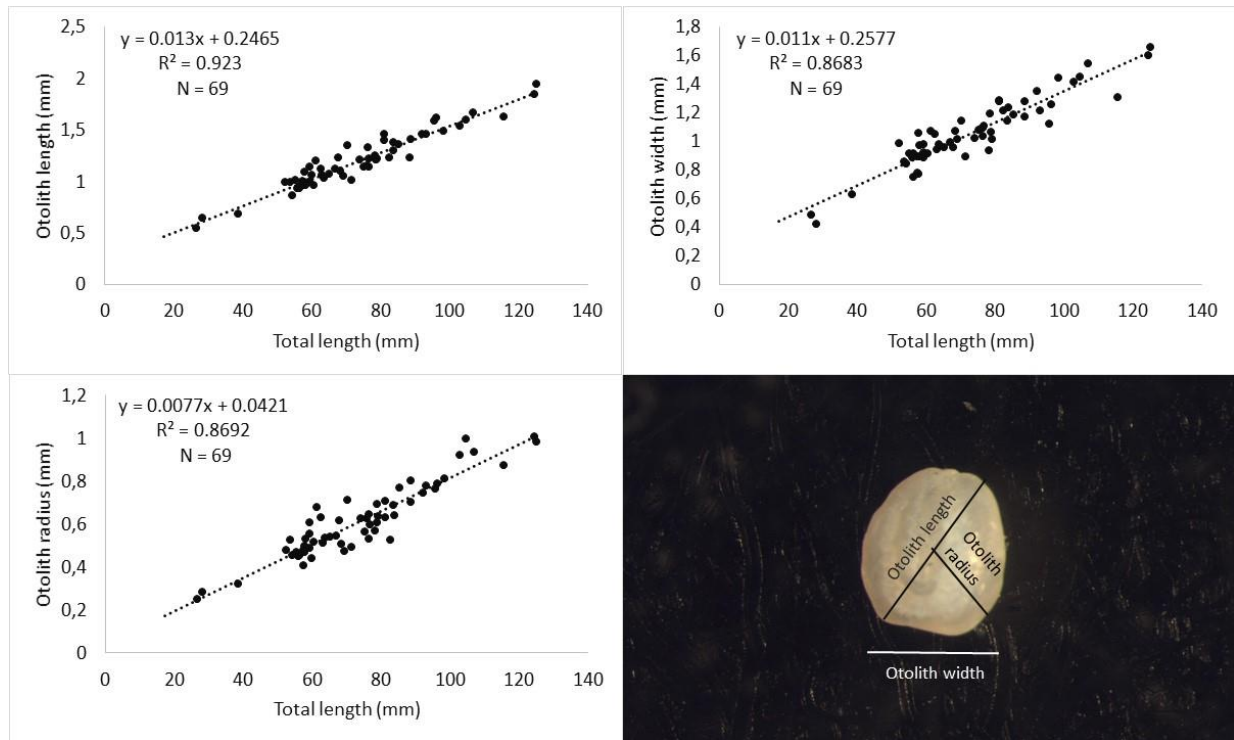


Figure 6. The relationships between TL-OL, TL-OW and TL-OR of *S. kleinii*

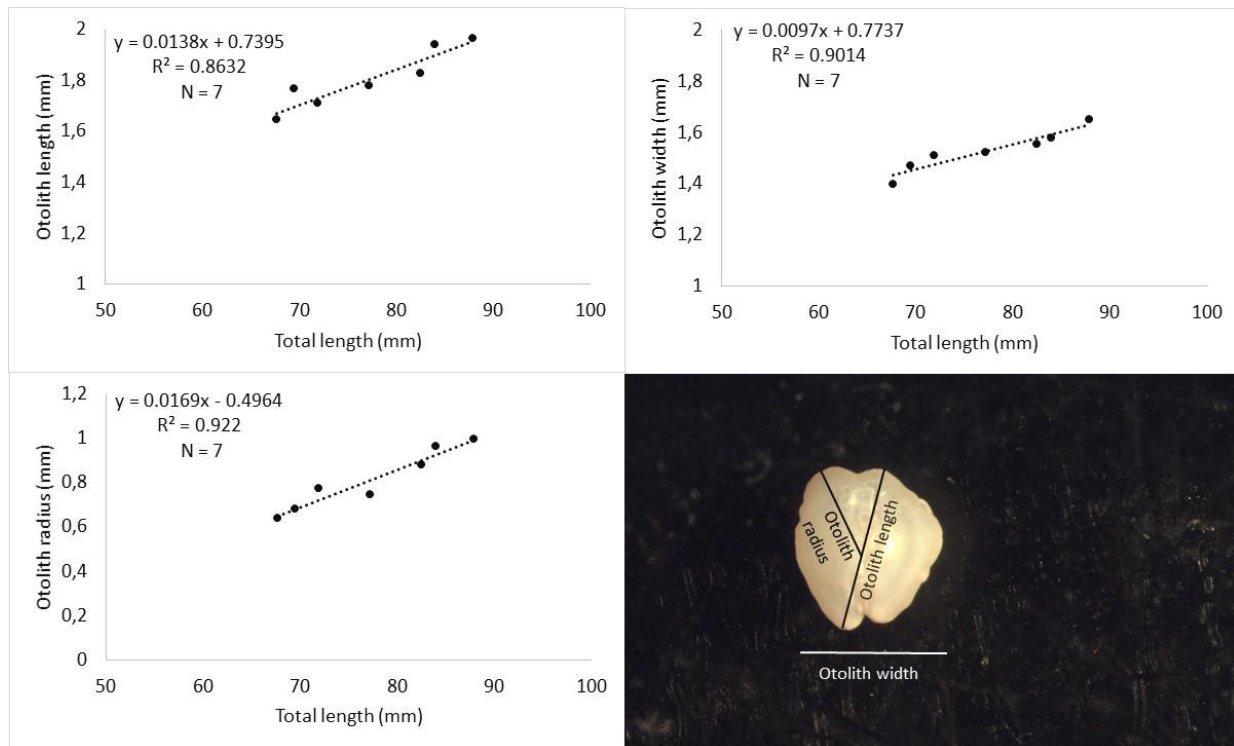


Figure 7. The relationships between TL-OL, TL-OW and TL-OR of *C. lucerna*

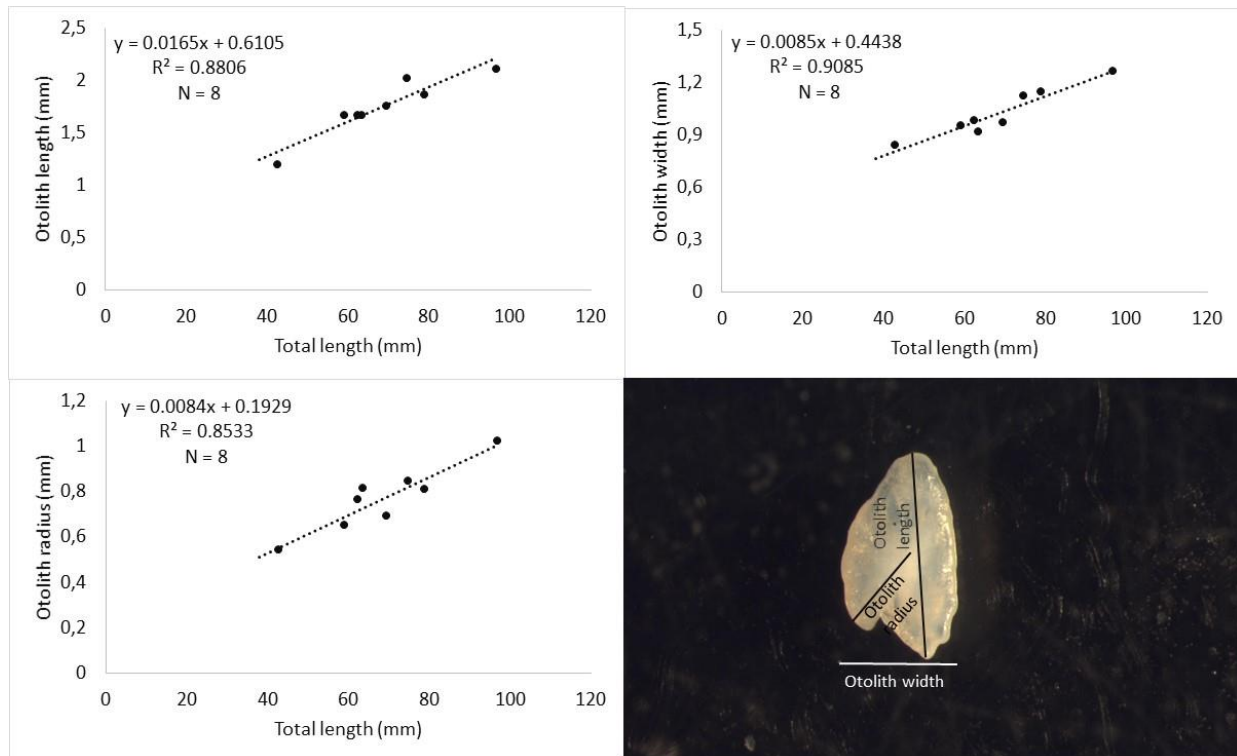


Figure 8. The relationships between TL-OL, TL-OW and TL-OR of *S. roissali*

4. Discussion

The newly settlers and juveniles of demersal fish species mostly distribute in shallower waters. Beside, some paleogic juvenile shoals such as Sardines, Anchovies etc. can be seen in the coastal areas. In addition to these, small-sized adult fish species such as Gobiidae, Labridae etc. family members mostly distribute these areas to avoid larger predators. Thus, coastal areas constitute important growth and nursery areas for these species, and embodies important biodiversity (Sheaves et al., 2015). So, the commercial use of beach seine nets is forbidden in the Turkiye Seas. For scientific purposes, it can be used after permission from authorized institution. However, the most appropriate method to collect coastal fish species is known as beach seine sampling with the scientific purposes. Due to small sized adults and juveniles constitute the upper levels of the food chain, a great majority of the prey of carnivorous species arised from these species (Lukoschek and McCormick, 2001). The studies related to otolith sizes and shapes are valuable tools for stomach content analyses. Beside, otolith

size-fish size relationships are important outputs for estimating fish size from the otolith size (Morley and Belchier, 2002; Hüsey et al., 2016; Więcaszek et al., 2020). These relationships give a chance to estimate prey size from otolith size and prey identification from otolith shape. Thus, seven small-sized aduly and juvenile fish species, which were dominant species of beach seine sampling were selected to reveal fish size-otolith size relationships in the present study.

The mean OL, OW, OR and TL of *P. marmoratus* were found to be 1.264 mm, 1.133 mm, 0.637 mm and 34 mm, respectively in Gökçeada Island, North Aegean Sea (Altin and Ayyildiz, 2018), which were smaller sizes than the results of the present study. But they determined higher coefficient of determination value for TL-OL, TL-OW, TL-OR of this species. The belief is that a beach seine net with specific mesh size is associated with effective catching larger individuals than specific size individuals. Conversely, Akyol and Kınacıgil (2001) compered to this study detected larger mean fork length (225.19 mm), OL (6.61 mm), and OW (3.35 mm) of *L. saliens* in the Aegean Sea. They did not calculate length-

length relationships. Whereas, Gümüş et al. (2001) found relatively the same sizes as 43 mm total length, 1.494 mm otolith length and 2.008 mm otolith width for *P. marmaratus* in the Black Sea. Although *P. marmoratus* was sampled from coastal areas in all areas, individuals were collected with varied fishing gear in the Aegean Sea and the sea water characteristics were relatively different from the Marmara Sea and the Black Sea. Also, L'Abée-Lund (1988) and Dawson (1991) emphasized that otoliths are used to differentiate stocks of the same species. Thus, it can be said that lots of variables such as the varied sea water characteristics, prey type, prey biomass, sampling gear type and season may be affected the varied mean sizes from the distinct geographical areas.

The OL, OW and OR measurements of the three *Pomatoschistus* species presented in this study were close to each other. Whereas, these otolith sizes of three species showed variations and these equations can be benefit for stomach content analyses when back-calculation of the size and species from the otolith. The TL-OL relationship of adult *C. lucerna* was estimated as $y=0.1325x+0.9428$ ($R^2=0.686$) in the Iskenderun Bay, Southeastern part of the Mediterranean Sea (Başusta and Bıyıklı, 2022). The parameters “a” and “b” in the equation were estimated as 0.0138 and 0.7395 in this study. Although the life phase and sampling location were relatively different between these two studies, the TL-OL relationship was found to be similar. Conversely, the TL-OW equation of these two studies showed great variations for *C. lucerna*.

Although four of the seven species were adults, the OL, OW and OR were close to each other. The three species were belong to the same genus, whereas the remaining species were belong to varied families. Thus, the primary factor affecting the otolith size of coastal species may be TL.

5. Conclusion

As a result of the seven fish species investigated for the first time the total length-

otolith dimensions relationships in the Marmara Sea and *P. bathi*, *P. minutus*, *S. kleinii*, *S. roissali* studied for the first time in the Türkiye Seas with this study. Also, otolith dimensions *P. bathi* contribute to the first results in the literature. This research provides new information because of the lack of data regarding the relationships between otolith dimensions and fish length for seven species. The fish length-otolith dimensions studies provide necessary information on species identification and size estimation of fish species in predator-prey studies. Also, these results are helpful for stock differentiation studies due to reveal detailed data.

Conflict of interest

The authors declare that there are no conflicts of interest or competing interests

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript.

Ethical approval

Ethics committee approval is not required.

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Rheological Properties, Moisture Content, and Carrageenan Yield of Macroalga *Kappaphycus alvarezii* Using Freshwater and Marine Water as Pre-Treatment

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ABSTRACT

A variety of seaweed post-harvest techniques have been developed using the same method of washing the seaweed with marine and drying it under the sun; however, a method of washing the seaweed with freshwater has yet to be developed. This study investigates the effect of freshwater and marine water as soaking solutions on the moisture content, carrageenan yield, and rheological properties of macroalga *K. alvarezii*. The seaweed was immersed in freshwater and marine water for 10 minutes with triplicates before being dried in a solar dryer for seven days. Extraction of seaweed was done after drying. Results revealed that the dried *K. alvarezii* soaked in freshwater had significantly lower ($p \leq 0.05$) moisture content than *K. alvarezii* soaked in marine water. Additionally, the carrageenan yield of *K. alvarezii* significantly increased ($p \leq 0.05$) by 14.48% when soaked in freshwater compared to the yield in marine water. Considering the rheological properties of the seaweed, the gelling temperature and melting temperature of *K. alvarezii* soaked in freshwater did not differ significantly ($p \geq 0.05$) from those of *K. alvarezii* soaked in marine water. However, other rheological properties such as the syneresis, viscosity, and gel strength of *K. alvarezii* greatly improved ($p \leq 0.05$) when they were soaked in freshwater with significant increases of 2.21%, 1.84 cPs, and 13.22 g cm⁻², respectively. Thus, this study indicates that macroalga *K. alvarezii* immersed in freshwater showed substantial improvements in their carrageenan quality.

KEYWORDS: Carrageenan yield, *Kappaphycus alvarezii*, moisture content, seaweeds, rheological properties

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1. Introduction

Traditionally, seaweeds have been used as a source of food, fodder, and fertilizer, as well as being a medicinal drug source. Moreover, they are the raw materials for making substances such as agar, algin, and carrageenan, which are used in the food industry (Kolanjinathan et al., 2014; El-Beltagi et al., 2022; Yangson et al., 2022). Approximately 28% of the world's seaweed production was attributed to eucheumatoid seaweeds, such as *Eucheuma* and *Kappaphycus* species (FAO, 2022). The seaweeds found in the Philippines can be considered remarkably diverse, with more than 800 species recorded in the country (Silva et al., 1987; Tahiluddin and Terzi, 2021; Valderrama, 2012). Additionally, according to the Fisheries Statistics Organization, the Philippines ranks fourth among the major seaweed producers worldwide in 2020 (FAO, 2022). Among the major income sources for the Philippine economy, seaweeds, particularly *Eucheuma* and *Kappaphycus* species, are the most important aquaculture species (Tahiluddin and Terzi, 2021). Moreover, carrageenan is a hydrocolloid extracted primarily from red seaweeds such as *Eucheuma* and *Kappaphycus* species (Loureiro et al., 2017). A wide variety of raw and semi-processed food products made from seaweed have been developed for use in food manufacturing (Kaliaperumal, 2003). The use of carrageenan is widespread in several industries, including the binding, gelling, and thickening of foods, pharmaceuticals, cosmetics, and commercial products (Vairappan, 2006; Naguit et al., 2009; Necas and Bartosikova, 2013; Rupert et al., 2002). In general, seaweeds collected from the sea are dried before they are used in nutritional studies or industrial purposes either for research or production (Chan et al., 1997; Pereira, 2011). Moreover, drying seaweeds reduces their water activity, which inhibits microbial growth, preserves the desirable qualities, and reduces the storage volume

(Gupta et al., 2011; Vorse et al., 2013). Researchers stated that, in the case of properly dried seaweeds, the gel content of the product can be preserved for an extended period of time without affecting its quality (Chan et al., 1997; Ling et al., 2015). Poncomulyo et al. (2006) have conducted studies on seaweed post-harvest techniques by washing and drying the seaweed with marine water. Additionally, choosing the most suitable post-harvest technique for seaweeds determines the quality of the finished product (Badmus et al., 2019; Vorse et al., 2013). Hence, post-harvest techniques (after cultivation) of seaweed cultivation are essential to ensuring seaweed quality. This study investigates the effect of freshwater and marine water as immersing solutions on the moisture content, carrageenan yield, and rheological properties of seaweed *Kappaphycus alvarezii*.

Material and Methods

2.1. Study Site

The study was conducted at the Seaweeds Post-Harvest Laboratory, College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), Philippines (Latitude 5.037107°, Longitude 119.743357°). The sample of macroalga *K. alvarezii* was obtained from seaweed farmers of Pasiagan, Bongao, Tawi-Tawi, Philippines. The systematic classification of macroalga *K. alvarezii* is also given below (Figure 1).

2.2. Drying Preparation

A two-step post-harvest technique was followed: one sample of *K. alvarezii* was thoroughly washed with marine water, while the other sample of *K. alvarezii* was thoroughly washed with freshwater. Following this, the samples were immersed in freshwater and marine water separately for 10 minutes and then dried in a solar dryer for seven days. The dried macroalga *K. alvarezii*



Kingdom: Plantae

Phylum: Rhodophyta

Class: Florideophyceae

Order: Gigartinales

Family: Solieriaceae

Genus: *Kappaphycus*

Species: *alvarezii*

Figure 1. Systematic Classification of *Kappaphycus alvarezii* (Photograph was using Huawei nova 7i)

sample immersed in marine water was darker than the dried macroalga *K. alvarezii* sample in freshwater (Figure 2).

2.3 Moisture Content Analysis

Each dried seaweed sample from the four corners of the pile and one at the center was mixed thoroughly. Each treatment with three replicate samples of 3 g weight was analyzed using a moisture analyzer. Moisture content was calculated using the formula below:

$$\text{Moisture Content} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Final Weight}} \times 100$$

2.4 Carrageenan Quality Analysis

Extraction for the carrageenan quality was followed by the method of Romero et al. (2000). The rheological properties such as gelling temperature, melting temperature, viscosity, syneresis index, and gel strength were determined after the extraction of carrageenan yield. Carrageenan yield was calculated using the formula below:

$$\text{Carrageenan Yield} = \frac{\text{Weight of Carrageenan}}{\text{Weight of Dried Seaweed}} \times 100$$

In the experiment, the gelling temperature was determined using a laboratory thermometer.

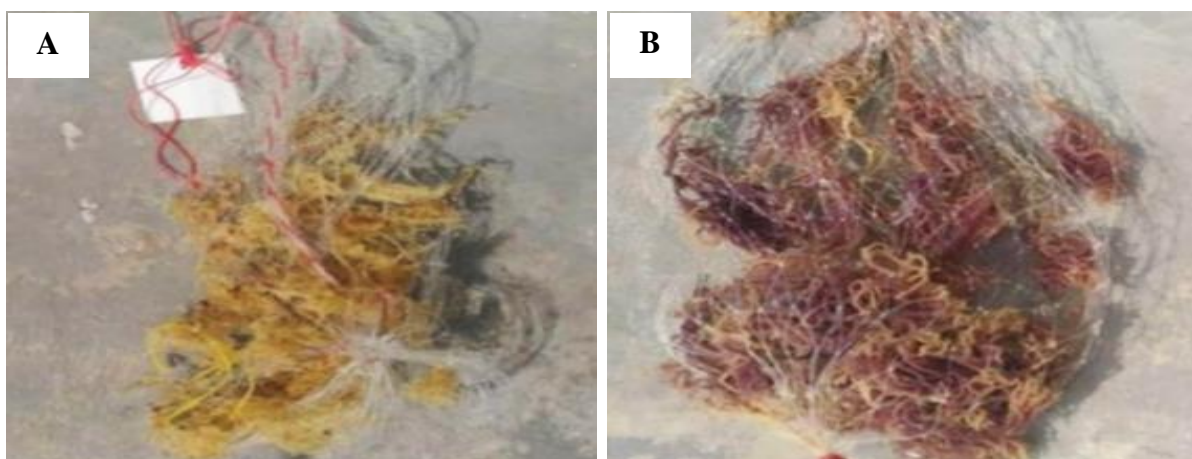


Figure 2. Dried sample of macroalga *K. alvarezii* after 7 days. A, *K. alvarezii* immersed in freshwater; B, *K. alvarezii* immersed in marine water.

It is determined when glass beads with a diameter of 2.85 mm and a weight of 30 mg fail to sink to the bottom at an interval of 0.5°C. In addition, the viscosity was determined using a Brookfield Viscometer (Model LVF) at 75°C running at 30 rpm in an electrolytic beaker measuring 110.3=46 mm in diameter. In the syneresis index, the percent of water released from the cylindrical gels (2.2=3.5cm) in the filter paper after two hours of weight loss was considered. Moreover, a gel strength measurement was performed on the carrageenan solution after it had been allowed to gel at room temperature for 15 hours.

2.5 Statistical Analysis

IBM SPSS software version 20 was used to analyze the collected data of moisture content, carrageenan yield, and rheological properties of macroalga *K. alvarezii* at $p \leq 0.05$ significance level. Data were

presented as mean \pm standard error of the mean (SEM). Determination of significant differences was computed through *t*-test.

3. Results

Figure 3 shows the moisture content and carrageenan yield of macroalga *K. alvarezii* immersed in freshwater and marine water. A sample of *K. alvarezii* immersed in marine water showed a moisture content of $10.39 \pm 0.82\%$, significantly lower ($p \leq 0.05$) than a *K. alvarezii* immersed in freshwater, which showed a moisture content of $16.67 \pm 0.49\%$. The yield of carrageenan from *K. alvarezii* immersed in freshwater ($55.04 \pm 1.33\%$) was significantly higher ($p \leq 0.05$) than that from *K. alvarezii* immersed in marine water ($40.20 \pm 0.97\%$).

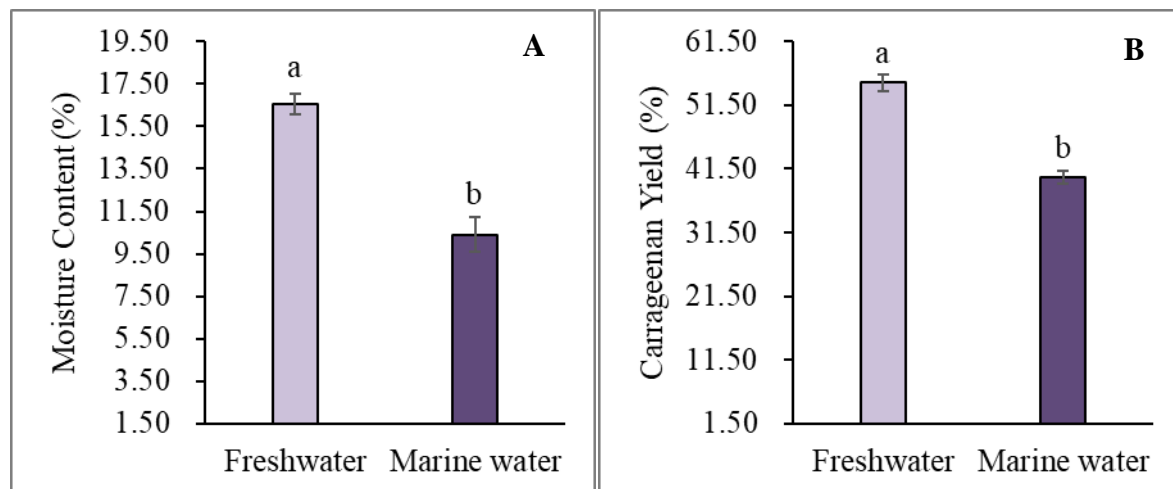


Figure 3. Macroalga *K. alvarezii* immersed in freshwater and marine water. A, Moisture content: B, Carrageenan yield. Differences in the letter are significantly different ($p \leq 0.05$). Error bar in SEM (standard error mean), $n=6$.

Moreover, Figure 4 shows the rheological properties of *K. alvarezii* immersed in freshwater and marine water. The gelling temperature of *K. alvarezii* immersed in freshwater (37.33 ± 0.33 °C) did not significantly differ ($p \geq 0.05$) from *K. alvarezii* immersed in marine water (37.00 ± 0.58 °C). *K. alvarezii* immersed in freshwater showed a melting temperature of 42.33 ± 6.67 °C, did

not significantly different ($p \geq 0.05$) than *K. alvarezii* immersed in marine water, which showed a melting temperature of 48.33 ± 1.35 °C. Additionally, the viscosity of *K. alvarezii* immersed in freshwater (5.67 ± 0.44 cPs) was significantly higher ($p \leq 0.05$) than *K. alvarezii* immersed in marine water (3.83 ± 0.17 cPs). The syneresis index from *K. alvarezii* immersed in freshwater ($8.31 \pm$

0.14%) was significantly lower ($p \leq 0.05$) than that from *K. alvarezii* immersed in marine water ($10.52 \pm 0.52\%$). Moreover, macroalga *K. alvarezii* immersed in freshwater showed a gel strength of $37.22 \pm 0.46 \text{ g cm}^{-2}$, significantly improved ($p \leq 0.05$) than *K. alvarezii* immersed in marine water, which showed a gel strength of $24.00 \pm 0.67 \text{ g cm}^{-2}$.

4. Discussion

Carrageenan is primarily derived from red seaweeds and is used for gelling, thickening, and stabilizing purposes (Husin, 2014). Several researchers have stated that carrageenan is an important ingredient in foods, pharmaceuticals, cosmetics, personal care, and other products (Thirumaran et al., 2009; Hayashi et al., 2011).

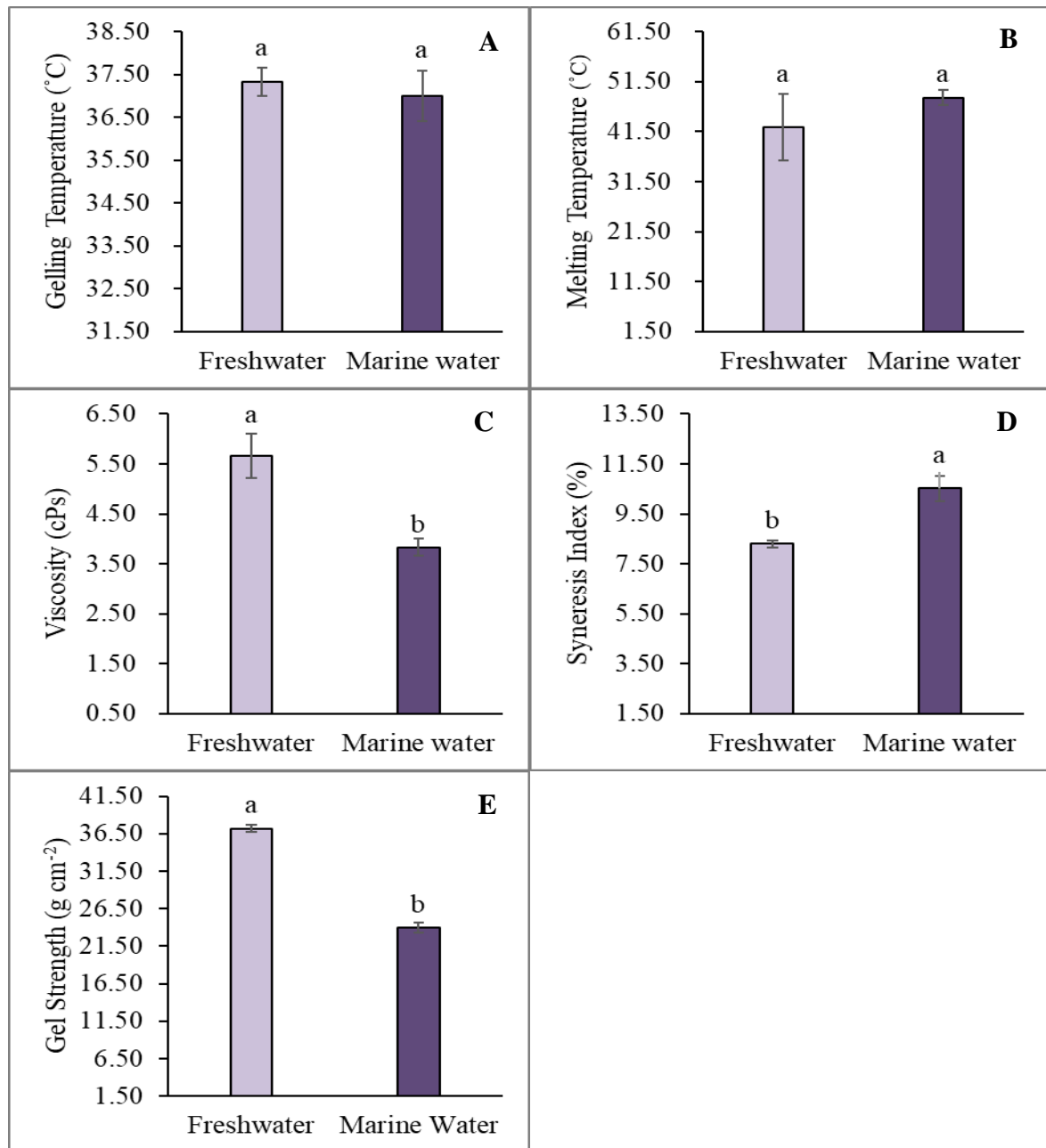


Figure 4. Rheological properties of macroalga *K. alvarezii* immersed in freshwater and marine water. A, Gelling temperature: B, Melting temperature: C, Viscosity: D, Syneresis index: E, Gel strength. Differences in the letter are significantly different ($p \leq 0.05$). Error bar in SEM (standard error mean), $n=6$.

It is common practice to dry seaweeds such as *Kappaphycus* sp. directly after they have been harvested without first being washed with freshwater (Sarri et al., 2022; Tahiluddin et al., 2022). The present study examined the effect of immersing farmed *K. alvarezii* in marine and freshwater on carrageenan quality in seaweed. A moisture content measurement was conducted before extracting macroalgae *K. alvarezii* for the quality of carrageenan. Results revealed that the moisture content from *K. alvarezii* immersed in marine water ($10.39 \pm 0.82\%$) was significantly lower than that from *K. alvarezii* immersed in freshwater ($16.67 \pm 0.49\%$). As reported by Tiroba (2006), seaweed of acceptable quality should not contain more than 35% moisture. Hence, the moisture content of the present study was within acceptable limits in terms of moisture content.

Moreover, the carrageenan yield from *K. alvarezii* immersed in freshwater achieved $55.04 \pm 1.33\%$ was significantly improved than the carrageenan yield from *K. alvarezii* immersed in marine water, which obtained $40.20 \pm 0.97\%$. In other studies, it has been shown that macroalga *Kappaphycus* sp. was directly dried after harvesting without being washed or immersed in freshwater, and then extracted, resulting in a carrageenan yield of 43% (Luhan et al., 2014), 38% (Loureiro et al., 2014), 36% (Sarri et al., 2022), 34% (Robles, 2020), 33% (Loureiro et al., 2014), and 32% (Tahiluddin et al., 2022). In contrast to the present study, in which macroalga *K. alvarezii* immersed in freshwater increased the amount of carrageenan yield, the results suggest that seaweeds should be immersed in freshwater before drying to maximize carrageenan yield production.

In this study, various rheological properties of macroalga *K. alvarezii* immersed in freshwater and marine water have been investigated, such as their gelling temperature, melting temperature, syneresis index, viscosity, and gel strength. It has been revealed that the gelling and melting temperature of macroalga *K. alvarezii* immersed in freshwater was not significantly

different than macroalga *K. alvarezii* immersed in marine water. However, the syneresis index, viscosity, and gel strength of macroalga *K. alvarezii* immersed in freshwater improved considerably than the macroalga *K. alvarezii* immersed in marine water. In another study, Robles (2020) investigated that the gelling and melting temperatures of farmed macroalga *K. alvarezii* were $35\text{ }^{\circ}\text{C}$ and $47\text{ }^{\circ}\text{C}$, respectively. Compared to the present study, which achieved gelling and melting temperatures of $37.33\text{ }^{\circ}\text{C}$ and $42.33\text{ }^{\circ}\text{C}$, respectively, for *K. alvarezii* immersed in freshwater, while it reached gelling and melting temperatures of $37.00\text{ }^{\circ}\text{C}$ and $48.33\text{ }^{\circ}\text{C}$, respectively, for *K. alvarezii* immersed in marine water. The syneresis index refers to the quantity of water exuded from a given amount of gel (Bryant et al., 1996; Robles, 2020). A lower syneresis index was obtained in macroalga *K. striatus* immersed in freshwater (8.31%), indicating that it improved the quality of carrageenan. Moreover, in the present study, the gel strength and viscosity of carrageenan quality from macroalga *K. alvarezii* immersed in freshwater were significantly improved, reaching 5.67 cPs and 37.22 g cm^{-2} , respectively. This was lower than the other study in which the viscosity and gel strength were achieved at 3.50 cPs and 31.77 g cm^{-2} , respectively (Robles, 2020).

5. Conclusion

In conclusion, macroalga *Kappaphycus alvarezii* immersed in freshwater before drying demonstrated substantial improvements in their carrageenan quality, including their rheological properties. In light of the results of this study, basic information can be given to seaweeds farmers that it is recommended to wash or immerse the seaweeds in freshwater before drying, as this will improve the quality of carrageenan that can be harvested from the seaweeds.

Compliance with Ethical Standards

Acknowledgement

The abstract of this study was presented at the 4th International Congress on Engineering and Life Science (ICELIS) on November 17-19, 2023 at Comrat, Moldova.

Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Data are available upon request.

Consent for publication

Not applicable.

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Larval Development of the Blue Dolphin Cichlid (*Cyrtocara moorii* Boulenger, 1902): Morphological Changes

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ABSTRACT

In this study, the larval development of *Cyrtocara moorii* was examined morphologically and compared with other Cichlidae species. The important morphological changes and critical developmental stages that *C. moorii* larvae undergo were determined during the process from hatching up to 20 days. It was observed that the larvae had a large yolk sac, transparent bodies, and undeveloped fins in the first days. Important developmental events such as eye development, mouth opening, onset of free swimming behavior, fin formation, and increased pigmentation were recorded. It was determined that the larvae started free swimming between 6-9 days, the yolk sac was completely depleted on the 10th day, and the larval development was completed, reaching the juvenile form on the 15-20th days. When the larval development of *C. moorii* was compared with other Cichlidae species, species-specific differences were observed as well as some similarities. It is thought that these differences may be related to the ecological adaptations, reproductive strategies, and evolutionary history of the species. It is suggested that future research should comparatively examine the larval development processes of more Cichlidae species and elucidate the mechanisms underlying this diversity.

KEYWORDS: *Cyrtocara moorii*, Cichlidae, larval development, morphology.

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1. Introduction

The Cichlidae family is one of the most diverse and widespread groups among freshwater fish (Kocher, 2004). *Cyrtocara moorii* Boulenger, 1902, a species within this family, is endemic to Lake Malawi (Ribbink et al., 1983). In addition to playing a significant role in the lake ecosystem, *C. moorii* is also a widespread species in the aquarium trade (Stauffer & Hert, 1992). However, limited information is available on the larval development of this species. The aquarium fish trade is a significant industry on a global scale, with a market volume of billions of dollars annually (Livengood & Chapman, 2007). The Cichlidae family is one of the most popular and economically valuable groups in the aquarium trade (Helfman, 2007). Cichlid species obtained from the African Great Lakes region (Victoria, Tanganyika, and Malawi) are particularly interest to aquarium enthusiasts due to their color diversity, interesting behaviors, and reproductive strategies (Barley & Coleman, 2010). *C. moorii* is a cichlid species exported from Lake Malawi and commonly found in the aquarium trade (Stauffer et al., 1997). Understanding the larval development of this species is also crucial for aquarium breeding. Larval development in fish is a critical process from hatching to the juvenile stage (Balon, 1975). During this period, larvae grow rapidly, undergo organogenesis, and acquire the characteristics of adult individuals (Kendall et al., 1984). Larval development is of great importance for the survival, growth, and reproduction of fish (Fuiman & Werner, 2009). Furthermore, understanding larval development is fundamental for fish farming, fisheries management, and conservation efforts (Houde, 1987). Although studies have been conducted on the larval development of many species in the Cichlidae family (Holden & Bruton, 1994; Meijide & Guerrero, 2000), there is no detailed research on the larval development of *C. moorii*. Morphological examination of the larval development of this species will enable the understanding of

ontogenetic processes and comparison with other Cichlidae species (Balon, 1986). This information will provide important clues about the species' biology, ecology, and evolutionary relationships (Meyer, 1993). Additionally, the findings will contribute to the aquarium breeding of *C. moorii*. The aim of this study is to examine and document the larval development of *C. moorii* in morphological detail. The findings will provide comprehensive information about the ontogeny of the species and allow comparative analyses with other species in the Cichlidae family (Stiassny & Meyer, 1999). Moreover, this study will serve as a foundation for future research on the larval development of *C. moorii* (Noakes, 1991). In conclusion, this research will make significant contributions to both the accumulation of fundamental biological knowledge and the aquarium industry.

2. Materials and Methods

Fish Material and Rearing Conditions

In this study, the dolphin cichlid (*C. moorii*) species belonging to the Cichlidae family was used. Two-year-old adult individuals were selected as broodstock. For egg production, colonies were established in 100-liter aquariums with a ratio of 1 male to 8 females. The broodstock was fed commercial aquarium fish feed three times a day. In the broodstock tanks, the water temperature was kept constant at 29°C ($\pm 0.5^\circ\text{C}$), and the pH values were measured in the range of 7.6 - 8.3. During the production period, no water change was performed, and sponge filters were used for aeration. All broodstock was stocked simultaneously in the production tanks.

Egg Collection and Artificial Incubation

Spawning monitoring was carried out periodically between 08:00 and 18:00 during the day. When spawning was detected, the completion of spawning was awaited, and a few hours later, the female with a mouth full

of eggs was regurgitated. The eggs taken from the female's mouth were subjected to artificial incubation under the same water conditions. Artificial incubation was carried out in 500 ml glass containers by agitating the eggs with water flow. Using a small water pump, water was continuously pumped into the glass containers containing the eggs, and during the incubation period, especially when the eggs had not yet hatched, they were kept in constant motion. This method prevented the eggs from fungal growth or death. Dead eggs were removed from artificial incubation. No disinfectants were used in artificial incubation, only clean water was used. The water temperature in artificial incubation was kept constant at 29°C (\pm 0.5°C).

Morphological Examinations

Physiological changes in the embryonic and larval development stages of eggs and larvae were monitored by photographing from the first day onwards. For morphological examinations, an Olympus BX51 research microscope (Tokyo, Japan) was used, and larvae were photographed with a Q Imaging Micropublisher 3.3 RTV camera (Canada) attached to the microscope. After the photographing process was completed, live specimens were returned to the incubation unit.

3. Results

The morphological changes that the dolphin cichlid (*C. moorii*) larvae undergo from the first day after hatching (Days After

Hatching) until the juvenile stage are described below.

1 DAH (Days After Hatching): The newly hatched dolphin cichlid (*C. moorii*) larva possesses a large, egg-shaped yolk sac (Fig. 1). The body appears transparent, with the head proportionally larger than the body, and the eyes have not yet completed their development. Due to the body's transparency, the heart is visible externally. The fins are not formed and are in a primordial form (Fig. 1). The larva is on the bottom and can perform short-term tail movements.

2 DAH: The eyes have completed their development and have taken their normal form (Fig. 1). The larva still has a large yolk sac on the second day. The mouth has not opened. The formation of the pelvic fins can be observed. However, other fins have not formed (Fig. 1). The tip of the vertebra in the caudal fin is about to curve. The larva is still on the bottom today. Tail movements have increased compared to the first day. The body color is transparent. However, pigmentation has begun to increase in the head region.

3 DAH: The mouth has opened (Fig. 2). The notochord tip has curved. The ray formations in the caudal fin have become more distinct (Fig. 2). Free swimming has not started. The vertebral structure has further developed. Pigmentation continues to intensify, especially in the head region.



Figure 1. Larval development and morphological changes in the dolphin cichlid (*C. moorii*) at 1-2 days after hatching (DAH).

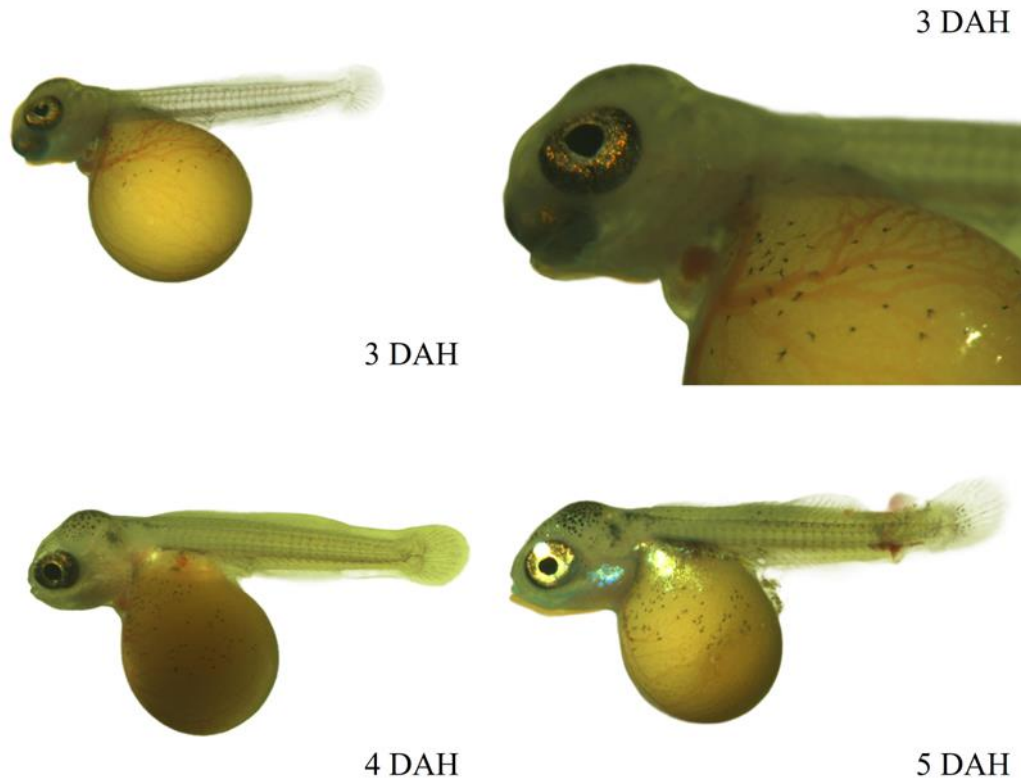


Figure 2. Larval development and morphological changes in the dolphin cichlid (*C. moorii*) at 3-5 DAH.

4-5 DAH: The larva still has a large yolk sac (Fig. 2). Dorsal and anal fin formations begin during these days (Fig. 2). The larva can perform short-term free swimming movements. The intensity of pigmentation in the head region has further increased (Fig. 2). Coloration in the remaining parts of the body is in the form of small black spots.

6-9 DAH: The yolk sac has continued to shrink day by day (Fig. 3). During these days, external food intake can be provided to the larvae. Free swimming has started on the 6th day. The duration of free swimming has extended with each passing day. The larva can now swim completely freely during these days.

10 DAH: The yolk sac has been completely absorbed (Fig. 4). The dorsal and

anal fin structures have become more distinct (Fig. 4). Pigmentation has spread throughout the body. The transparent appearance of the larva begins to disappear after these days.

11-13 DAH: The dorsal and anal fins are distinct (Fig. 4). However, they continue to develop. Body coloration is darker (Fig. 4). These days represent the transition stage between the larva and juvenile.

15-20 DAH: During these days, fin formations are completed, and the body is colored. The body form has transformed into the body form of the parents. Therefore, during these days, larval development is completed, and the transition from the larval stage to the juvenile stage has occurred.

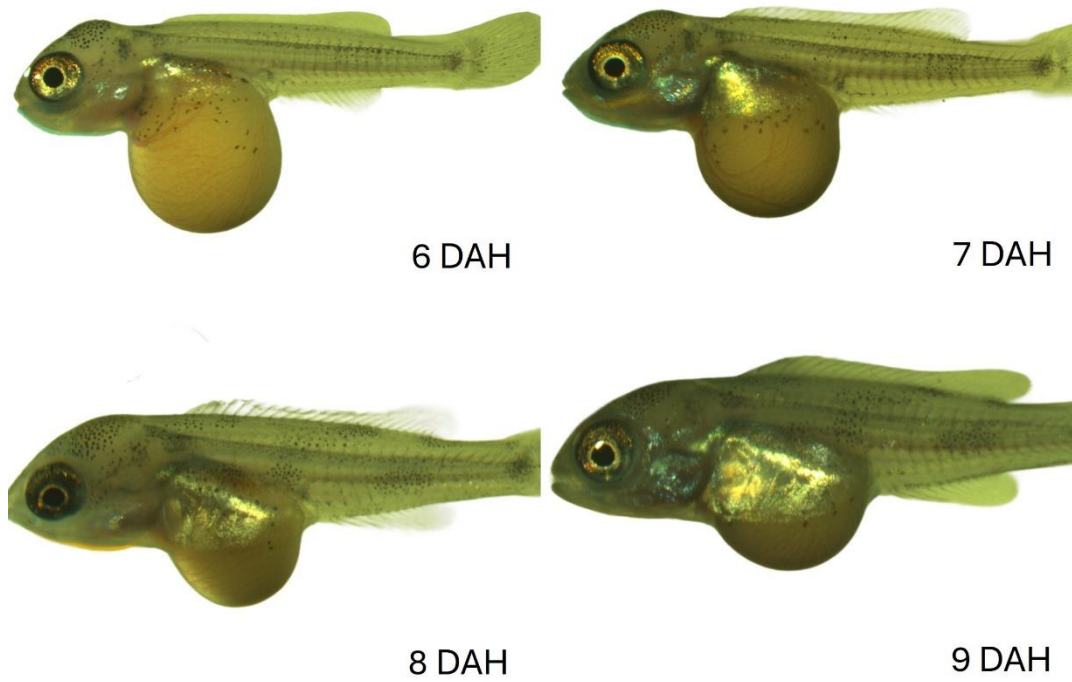


Figure 3. Larval development and morphological changes in the dolphin cichlid (*C. moorii*) at 6-9 DAH.

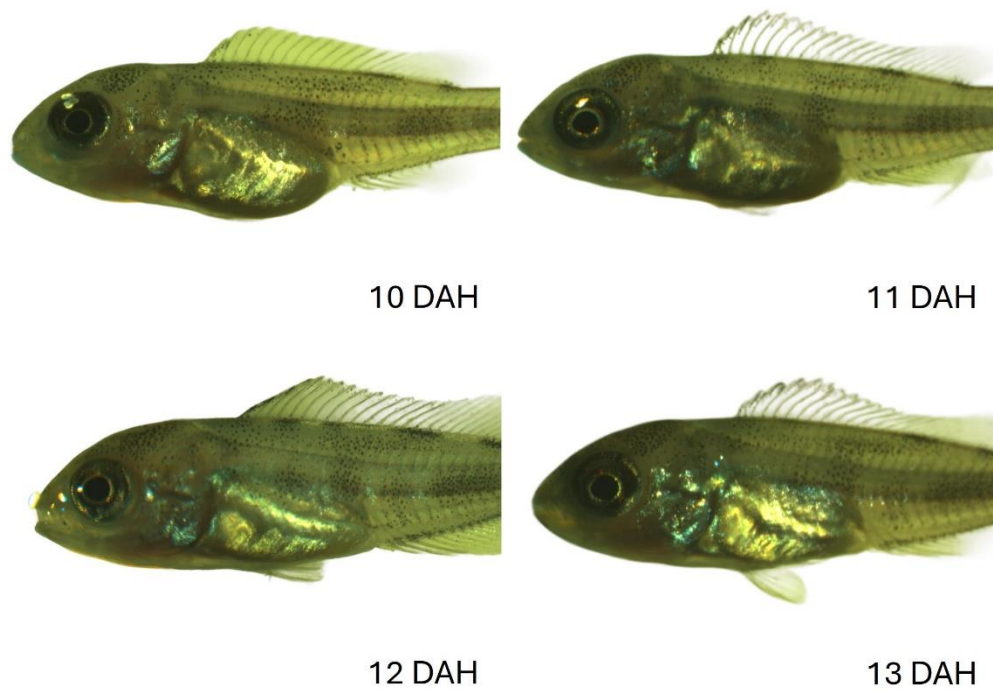


Figure 4. Larval development and morphological changes in the dolphin cichlid (*C. moorii*) at 10-13 DAH.

4. Discussion

The morphological development process of the dolphin cichlid (*C. moorii*) larvae after hatching shows similarities with the larval development processes observed in other cichlid species (Fujimura & Okada, 2007; Meijide & Guerrero, 2000). However, there are also some differences between species (Holden & Bruton, 1994).

In general, cichlid larvae exhibit common characteristics such as having a large yolk sac after hatching, transparent body structure, undeveloped eyes, and fins (Fujimura & Okada, 2007; Kratochwil et al., 2015). However, the rate and duration of development of these characteristics may vary among species (Meijide & Guerrero, 2000; Balon, 1999).

For example, in *Oreochromis niloticus* (Nile tilapia) larvae, mouth opening and free swimming behavior have been observed earlier compared to *C. moorii* (Rana, 1988). *O. niloticus* larvae typically exhibit free swimming behavior around 2-3 DAH, while in *C. moorii* larvae, this behavior starts between 6-9 DAH (Fujimura & Okada, 2008).

Moreover, pigmentation development may also differ among species (Stiassny & Meyer, 1999). In some cichlid species, such as *Amphilophus citrinellus*, larval pigmentation is observed earlier and more intensely (Kratochwil et al., 2015), while in *C. moorii* larvae, pigmentation development progresses more gradually (Baroiller et al., 2009).

In terms of fin development, the timing and rate of dorsal and anal fin formation can vary in different cichlid species (Sfakianakis et al., 2011). In some species, fin development is completed earlier, while in others, this process may take longer (Koumoundouros et al., 2001).

In Maylandia zebra (Zebra cichlid) larvae, the yolk sac is rapidly depleted during the first few days after hatching, and the larvae begin to feed externally (Holden & Bruton,

1994). *M. zebra* larvae exhibit free-swimming behavior at an earlier stage (approximately 4-5 DAH) compared to *C. moorii* (Fujimura & Okada, 2007).

In *Labidochromis caeruleus* (Yellow princess) larvae, the yolk sac is preserved for approximately 2-3 days after hatching, and the larvae remain motionless during this period (Balon, 1977). *L. caeruleus* larvae start free swimming at a later stage (7-8 DAH) compared to *C. moorii* and *M. zebra* (Fujimura & Okada, 2007).

In *Aulonocara jacobfreibergi* (Peacock cichlid) larvae, the yolk sac is rapidly depleted during the first few days after hatching, and the larvae begin free swimming around 3-4 DAH (Holden & Bruton, 1994). In *A. jacobfreibergi* larvae, pigmentation development starts earlier and progresses faster compared to *C. moorii* and *M. zebra* (Baroiller et al., 2009).

In *Satanoperca pappaterra* (Pappaterra cichlid) larvae, the yolk sac is rapidly depleted during the first few days after hatching, and the larvae begin free swimming around 3-4 DAH (Pandolfi et al., 2009). In *S. pappaterra* larvae, pigmentation development progresses gradually, similar to *C. moorii* and *M. zebra* (Meijide & Guerrero, 2000).

Astronotus ocellatus (Oscar cichlid) larvae are dependent on the yolk sac for approximately 3-4 days after hatching and have limited movement ability during this period (Shibatta & Dias, 2006). *A. ocellatus* larvae start free swimming at a later stage (8-10 DAH) compared to *C. moorii* and *S. pappaterra* (Shibatta & Dias, 2006; Pandolfi et al., 2009).

Pterophyllum scalare (Angelfish) and *Symphysodon discus* (Discus fish) larvae are protected and fed by their parents for an extended period (10-14 days) after hatching (Chellappa et al., 1999; Buckley et al., 2010). In these species, larvae do not exhibit free swimming behavior immediately after

hatching, and pigmentation development also progresses more slowly (Cacho et al., 2006; Buckley et al., 2010).

Crenicichla lepidota (Pike cichlid) larvae are dependent on the yolk sac for approximately 2-3 days after hatching and have limited movement ability during this period (Nakatani et al., 2001). *C. lepidota* larvae start free swimming around 5-7 DAH, similar to *C. moorii* and *S. pappaterra* (Nakatani et al., 2001; Pandolfi et al., 2009).

These comparisons reveal the diversity in larval development processes among different cichlid species. In some species (e.g., *P. scalare* and *S. discus*), parental care continues for a longer period, while in other species (e.g., *C. moorii*, *S. pappaterra*, *A. ocellatus*, and *C. lepidota*), larvae become independent at an earlier stage. The onset of pigmentation development and free swimming behavior also varies among species. These differences may have been shaped by each species' ecological niche, reproductive strategy, and evolutionary history (Salzburger & Meyer, 2004; Sefc, 2011).

Future studies comparing the larval development processes of more cichlid species may help elucidate the mechanisms underlying this diversity. Additionally, understanding how differences in larval development processes relate to species' adult morphological and ecological adaptations could be an important research topic (Henning & Meyer, 2014; Sefc, 2011).

In conclusion, although there are some similarities in larval development processes among different cichlid species, species-specific differences are also observed. These differences may be related to species' ecological adaptations, reproductive strategies, and evolutionary histories (Salzburger & Meyer, 2004; Takahashi 2003). Future research comparing the larval development processes of more cichlid species may help illuminate the mechanisms underlying this diversity (Henning & Meyer, 2014).

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Evaluating Shoreline Changes at Ayvacık Reservoir (Çanakkale, Türkiye) Through Remote Sensing and Geographic Information System Techniques: A Twelve-Year Assessment

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ABSTRACT

This study aims to determine the spatial and temporal changes occurring along the shoreline of the Ayvacık Reservoir in Çanakkale, Türkiye. Landsat 8 OLI/TIRS and Landsat 7 ETM+ satellite images were analyzed using remote sensing and geographic information system techniques. The dataset used in the study covers the period between the completion of the dam construction in 2008 and 2019. Preprocessing of the remote sensing satellite images and digital image processing analyses were carried out using ENVI and ArcGIS software. The shoreline was determined through manual digitization. Consequently, it was found that the shoreline length was 14.994 km in 2008 and increased to 22.293 km in 2019. These values represent the observed minimum and maximum shoreline lengths, respectively. The study period revealed an increase in shoreline length. Given that this study is the first to elucidate shoreline changes occurring at the Ayvacık Reservoir, it is anticipated to provide essential insights for water resource managers by contributing significantly to the literature.

KEYWORDS: Hydrological Changes; Satellite Imagery Evaluation; Shoreline Dynamics; Temporal Analysis.

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1. Introduction

The shoreline represents one of the most dynamic processes in coastal areas. Spatial and temporal alterations encompass geomorphological, tectonic, hydrodynamic, climatic, seismic, and sedimentation/erosion events along the shorelines, which can manifest gradually (Thom and Cowell, 2005) or rapidly (Scott, 2005). Changes occurring in the shoreline due to human-induced and/or natural processes hold significant environmental implications. Monitoring shoreline changes is crucial for water resource management, urban and coastal planning, as well as the determination of sediment accumulation and erosion.

Determining the shoreline using traditional ground survey techniques is challenging and time-consuming (Aedla et al., 2015). Previous assessments of shoreline changes have been conducted using aerial photographs and satellite images. Remote sensing plays a pivotal role in acquiring spatial data. Satellite imagery can be easily obtained and interpreted through remote sensing techniques. Recent advancements in Geographic Information Systems (GIS) and remote sensing have significantly overcome the challenges in shoreline delineation, providing more successful results in a shorter time compared to conventional methods (Kale and Acarlı, 2019a). Remote-sensed images are widely used for long-term shoreline change assessments due to their advantages over traditional methods, including cost-effectiveness, higher resolution, and extensive imaging capabilities (Mahapatra et al., 2013). Additionally, as water absorbs near-infrared wavelengths, vegetation and soil exhibit strong reflection (Alesheikh et al., 2007). Hence, remote-sensed satellite images are extensively utilized in shoreline mapping.

Frequent updating of coastal information related to water resources and continuous monitoring of morphological changes resulting from natural or artificial factors are

essential. There is currently no study available regarding the monitoring of the Ayvacık Reservoir's shoreline. Therefore, the aim of this study is to identify temporal changes occurring in the Ayvacık Reservoir's shoreline.

2. Material and Methods

2.1. Study Area

The Ayvacık Reservoir (Figure 1) is situated on the Tuzla River. It is a soil-filled dam constructed for irrigation and drinking water purposes, characterized by a clay-cored sand-gravel fill. The dam has a body volume of 1,200,000 m³, a height of 53 m from the riverbed, and a normal water level capacity of 39 hm³, providing a lake area of 3.42 km² at the normal water level (DSI, 2020). The dam serves an irrigation area of 3,419 hectares.

2.2. Data

This study utilized satellite images obtained from the Landsat 7 Enhanced Thematic Mapper Plus (ETM+) and Landsat 8 Operational Land Imager (OLI) and Thermal Infrared Sensor (TIRS) sensors to monitor changes in the Ayvacık Reservoir's shoreline. Both the ETM+ and OLI/TIRS sensors have a spatial resolution of 30 m. Additionally, the panchromatic band (band 8) in both sensors possesses a 15 m spatial resolution.

The satellite images used in this study cover the period between 2008 and 2019. To mitigate potential variations in shoreline changes within the year, the dataset used in this study was selected from available satellite images taken annually in May over the specified 1-year intervals. Landsat satellite images covering the study area were obtained from the United States Geological Survey (USGS) website (<https://earthexplorer.usgs.gov>).

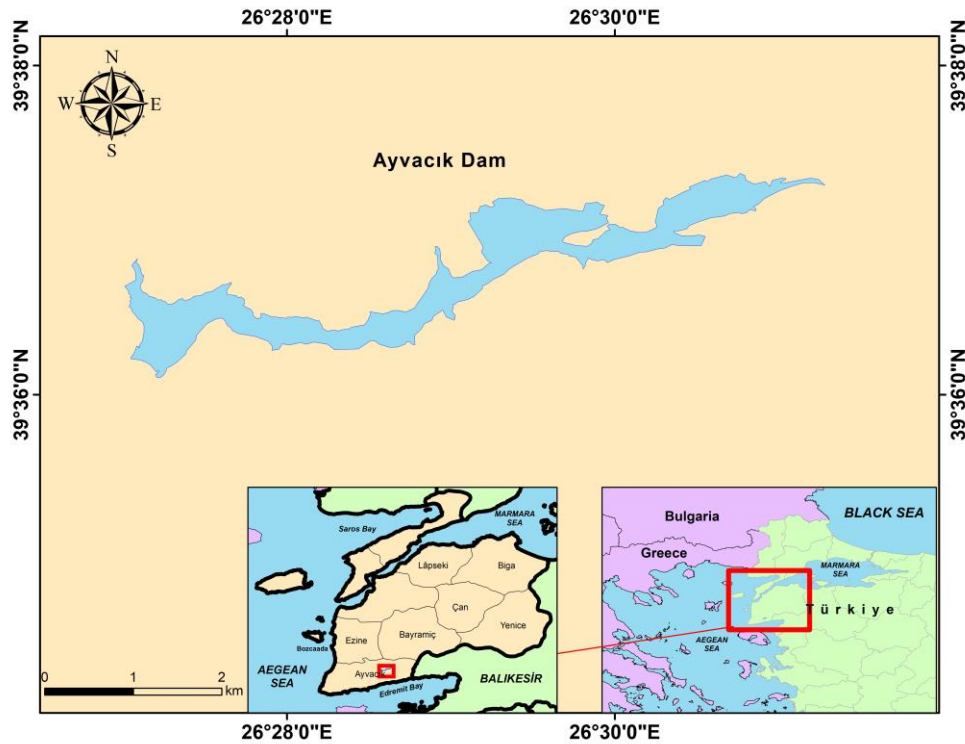


Figure 1. Map of the study area

2.3. Method

The processing and analysis of remote-sensed satellite images in this study were conducted using ENVI and ArcGIS software. Furthermore, auxiliary toolboxes and extensions within these software packages were employed as per the requirements of the processing and analysis.

Multiple methods exist for detecting and extracting the shoreline from satellite images (Dolan et al., 1991; Gao, 1996; McFeeters, 1996; Braud and Feng, 1998; Frazier and Page, 2000; Xu, 2006; Shen and Li, 2010; Feyisa et al., 2014). Some researchers have developed and recommended the use of indices and auxiliary systems such as the Normalized Difference Vegetation Index (NDVI), Normalized Difference Water Index (NDWI), Modified Normalized Difference Water Index (MNDWI), Normalized Difference Moisture Index (NDMI), Automated Water Extraction Index (AWEI), Water Ratio Index (WRI), Land Surface

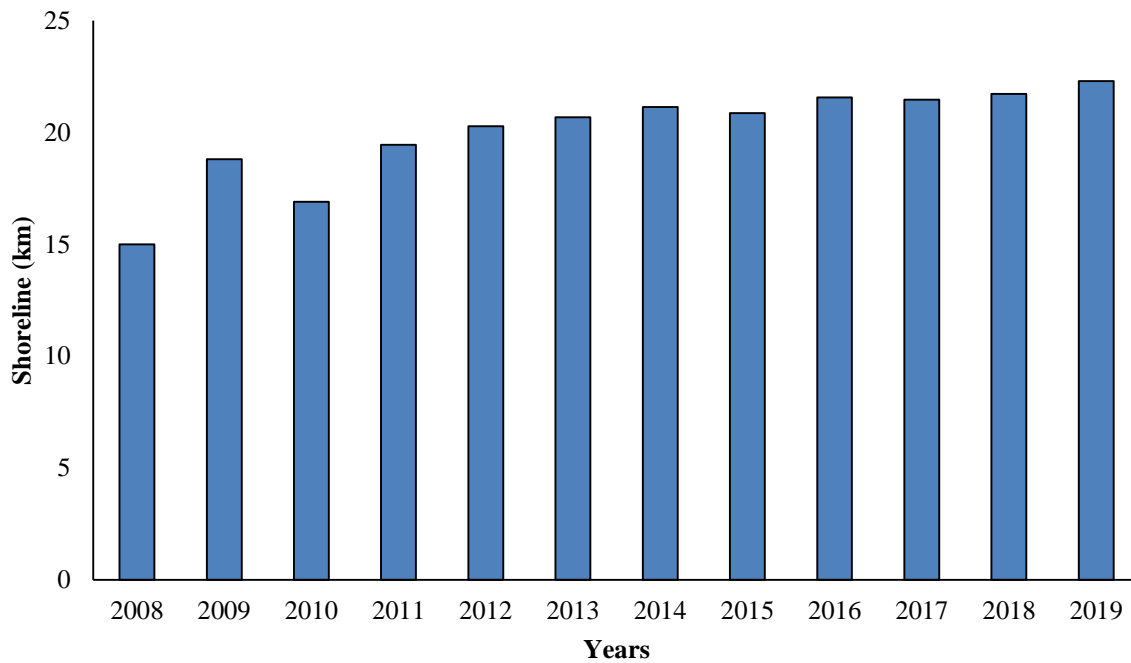
Water Index (LSWI), Tasseled Cap Wetness Index (TCWI), and Digital Shoreline Analysis System (DSAS) for the purpose of determining and automatically extracting the shoreline. Through remote sensing and GIS analyses applied to satellite images, these techniques are capable of semi-automatic or automatic extraction of shorelines. These recommended automatic extraction methods are frequently employed in the literature and have shown successful outcomes.

3. Results

The changes occurring in the shoreline of the Ayvacık Reservoir are presented in Table 1 and Figure 2. The total shoreline length was calculated to be 14.994 km in May 2008, the initial period when water retention began at the dam, and 22.293 km in 2019. Furthermore, the changes in the shoreline for each year are individually displayed in Figure 3.

Table 1. Changes in the shoreline of the Ayvacık Reservoir

Satellite	Image Date (YYYYMMDD)	Path/Row	Shoreline (km)	Change in Shoreline Since Establishment (km)	Shoreline Change Rate (%)	Shoreline Change Compared to the Previous Year (%)
Landsat 7 ETM+	20080528	181/033	14.994	0.00	0.00	
Landsat 7 ETM+	20090531	181/033	18.799	3.80	25.38	3.805
Landsat 7 ETM+	20100502	181/033	16.903	1.91	12.74	-1.895
Landsat 7 ETM+	20110630	181/033	19.444	4.45	29.68	2.541
Landsat 7 ETM+	20120507	181/033	20.276	5.28	35.23	0.832
Landsat 8 OLI/TIRS	20130518	181/033	20.682	5.69	37.94	0.406
Landsat 8 OLI/TIRS	20140521	181/033	21.135	6.14	40.96	0.453
Landsat 8 OLI/TIRS	20150524	181/033	20.861	5.87	39.13	-0.275
Landsat 8 OLI/TIRS	20160510	181/033	21.570	6.58	43.86	0.710
Landsat 8 OLI/TIRS	20170427	181/033	21.467	6.47	43.18	-0.103
Landsat 8 OLI/TIRS	20180430	181/032	21.721	6.73	44.87	0.253
Landsat 8 OLI/TIRS	20190519	181/032	22.293	7.30	48.68	0.572

**Figure 2.** Changes occurring in the shoreline of the Ayvacık Reservoir

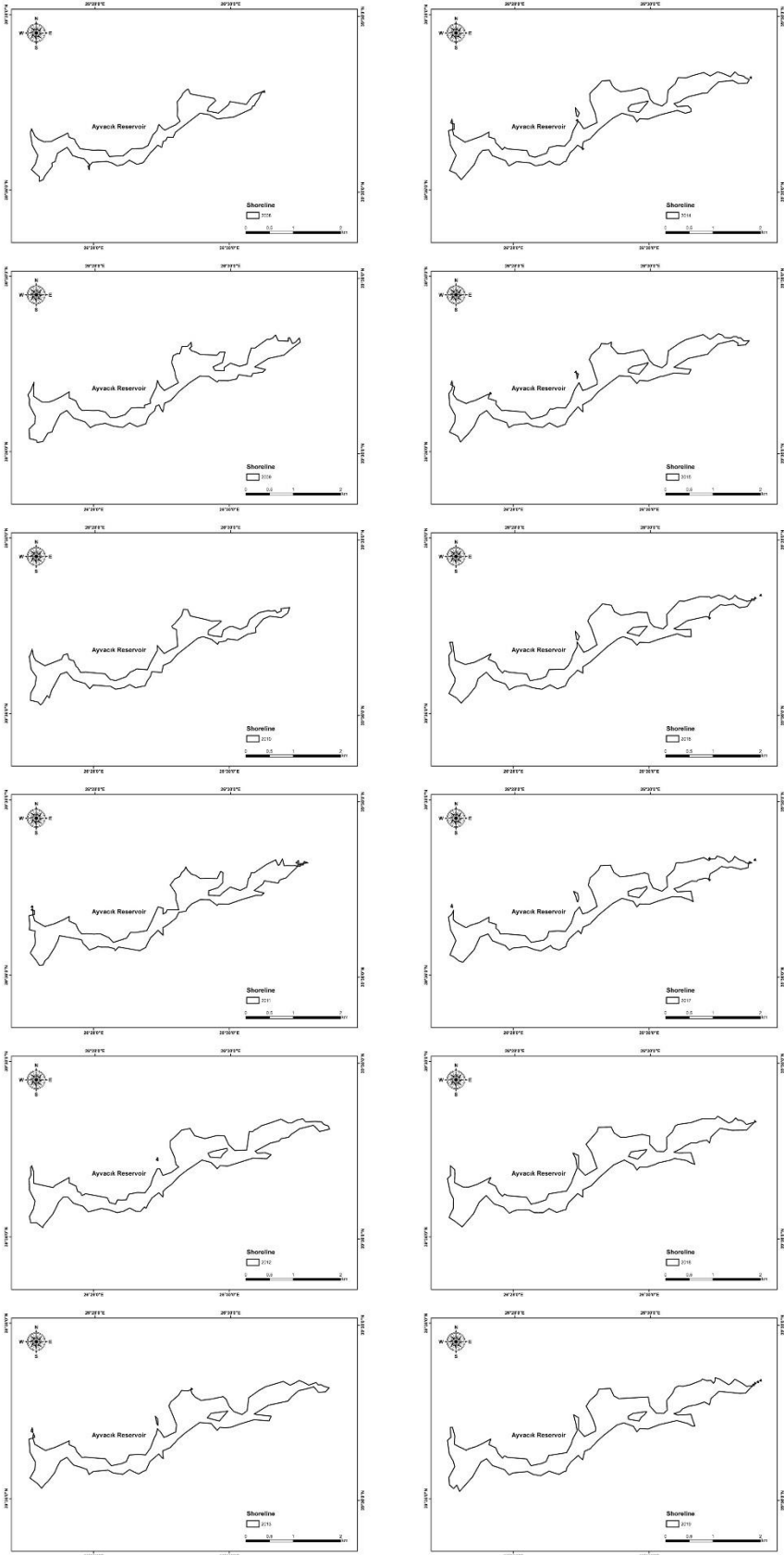


Figure 3. Shoreline changes identified in the Ayvacik Reservoir between 2008 and 2019

4. Discussion

Many researchers worldwide have engaged in studies aiming to identify and track alterations in shoreline. Alesheikh et al. (2007) investigated changes occurring in the shoreline of Lake Urmia between 1989 and 2001, reporting a 3-meter reduction in the shoreline. This change was noted to have led to a decrease in the lake's surface area by approximately 1000 km². Aedla et al. (2015) reported the highest shoreline advancement of 8.69 meters per year for the Netravati and Gurgur river mouths in India, along with the highest erosion rate of 4.31 meters annually. Erener and Shirzad (2016) applied remote sensing and GIS techniques to identify coastal changes in the part of the Amu Darya River within the boundaries of Kunduz city in northern Afghanistan, reporting that the banks along the river eroded due to continuous erosion, primarily caused by high river flow rates, wind, and waves. The changes along the river's shoreline not only presented environmental problems in the study area but also raised new potential political issues between Afghanistan's northern region and Tajikistan, as it forms the border between these regions.

Shoreline changes in various coastal areas of Türkiye have been extensively studied by many researchers. However, while coastal changes in marine coastal areas have been widely investigated, changes occurring in reservoirs and lakes have not been studied with the same intensity. Few studies focusing on monitoring shoreline changes in Turkish lakes have yet to reach a sufficient level. Temiz and Durduran (2016) reported a significant decrease in the shoreline of Lake Acıgöl between 1985 and 2015. Similarly, Kale (2018) noted shoreline regression in Lake Akşehir between 1990 and 2016. Kesikoglu et al. (2017) observed seasonal variations in the shoreline of the Yamula Reservoir Lake, reporting both increments and reductions throughout the year. Duru (2017) reported an average rate of shoreline change for Sapanca Lake between 1975 and 2016 as an advancement of 2.7 meters per year. Kale

and Acarlı (2019b) conducted research on monitoring shoreline changes in Atikhisar Reservoir Lake, noting significant variations throughout the monitoring period and fluctuations in shoreline length among the years. The current study highlights varying shoreline length that demonstrates a rising pattern, diverging from prior research findings.

Shoreline changes can be attributed to various natural and/or artificial factors. Sener et al. (2010) highlighted the influence of surface runoff, precipitation, and evaporation on shoreline changes. Similarly, Arkoc and Özşahin (2018) attributed changes observed in the Gala and Pamuklu Lakes' shorelines primarily to precipitation and evaporation. Duru (2017) indicated that the primary reasons for changes in the shoreline were natural fluctuations in rainfall and reductions in water due to excessive water consumption and development projects associated with human activities. On the other hand, Yıldırım et al. (2011) attributed observed shoreline changes to population growth, increased domestic and agricultural water consumption, construction of reservoirs and ponds, and irrigation system installations driven by human activities. Likewise, Bayram et al. (2013) emphasized the substantial impact of human-induced activities on shoreline changes and highlighted the significant effects of land use differences.

Kaya (2016) reported changes in the shoreline of Lake Terkos (Istanbul, Türkiye) without a specific identifiable cause, but suggested that excessive water consumption from the lake for domestic and agricultural irrigation and sand extraction could be influential. The current study suggests that due to relatively lower human activities and a lower population in the region, the shoreline is not negatively affected as the demand for domestic/agricultural water consumption might be lower compared to other studies.

Although Kale et al. (2018) reported decrease in the runoff of Tuzla River (dataset cover the period between 2006 and 2013) and precipitation, and increase in the evaporation over the region, our study revealed that the shoreline perimeter was increased. Kale et al. (2018) illustrated that the runoff of Tuzla

River and rainfall tended to decrease until 2013. However, the rainfall tended to increase and evaporation tended to decrease in the period between 2008 and 2013 as presented in their Figures 2 and 3. Our study analyzed data period between 2008 and 2019. And, after 2013, our data showed that the perimeter of the shoreline increased. On the other hand, there are several papers reporting decrease in the runoff of some rivers located close area to our study area (Kale et al., 2016a, 2016b; Ejder et al., 2016a, 2016b). However, each water resource may react in different way to the affecting factors.

The monitoring of these areas is necessary for achieving ecological balance, preserving biological diversity, sustainably utilizing natural resources, and executing planned urbanization and development. Additionally, it's crucial to assess potential future changes in these areas by considering both anthropogenic and natural alterations and making predictions about their possible consequences. To achieve this, monitoring shoreline changes is of vital importance. In this context, this study represents the first attempt to identify and monitor changes in the shoreline of the Ayvacık Reservoir, the sole source of drinking, domestic, and agricultural water for Ayvacık district. This study will enable future predictions by monitoring spatial and temporal changes in the dam. Changes in population and climatic events affect water demand, leading to an increase in the required amount of water. Considering these factors, continuous monitoring of changes in the dam is crucial to ensure sustainable water use and prevent the deterioration of societal welfare and the reduction of economic contributions derived from agricultural activities. In future studies, monitoring seasonal changes considering the variations within a year would be beneficial for detecting possible abrupt changes in the shoreline.

5. Conclusion

In conclusion, this study examined the shoreline changes occurring from the inception of the Ayvacık Reservoir in 2008 until

2019. Analysis of the spatial and temporal variations in the dam's shoreline revealed the preservation and expansion of water resources. Consequently, it is anticipated that the Ayvacık Reservoir will continue to fulfill its role in providing water supply services.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

SK: Conceptualization, Writing – original draft, Writing – review and editing, Data curation, Formal analysis, Methodology, Visualization

SB: Methodology, Investigation, Data curation

DA: Investigation, Writing – review and editing

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Consent for publication

Not applicable.

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