

Research Article

Seasonal variations in texture, lipid content and vitamin A and E content in cultured olive flounder (*Paralichthys olivaceus*) muscle

Shim K.B.^{1*}, Yoon N.Y.², Kim H.C.³

1 Department of Food Science and Technology, Pukyong National University, Busan, Korea

2 Food Safety and Processing Research Division, National Institute of Fisheries Science, Busan, Korea

3 Genetics and Breeding Research Center, National Institute of Fisheries Science, Geoje, Korea

*Correspondence: kbshim@pknu.ac.kr

Keywords

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Abstract

This study investigated the seasonal variation in the nutrient composition of four groups (with different average weights) of cultured olive flounder (*Paralichthys olivaceus*) to determine the optimal season and harvest size. The yield of 1 kg olive flounder did not vary throughout the seasons. The texture of the muscle tissue, levels of crude lipids, and vitamin E content in the 1 kg fish were highest in April and lowest in August. However, the levels in the 2, 3, and 4 kg fish were lowest in April, during the reproductive period, and highest in December. The vitamin E content in the fish muscle may correspond with high levels of crude lipids, possibly influenced by the spawning period. The 4 kg group had lower levels of crude lipid, texture, and vitamin E content than other fish, potentially owing to repeated spawning. The optimal harvest size for olive flounder was 3 kg based on nutrient evaluation.

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Introduction

The olive flounder (*Paralichthys olivaceus*) is an important commercial species in Korea and is used to provide the market with sliced raw fish. Aquaculture production accounted for approximately 90% of the total production of olive flounder in 2023, and the capture of wild stocks contributed to the remaining 10% (Korea Statistical Information Service (Okorie *et al.*, 2014; KOSIS, 2024).

The most important qualities of sliced raw fish in Korea are texture, color, odor, and flavor. Therefore, the white muscle tissue of marine fish, such as olive flounder, rockfish and rock bream, is preferred over the red muscle tissue of fish such as yellowtail and tuna (Shim and Cho, 2014). The texture of muscle tissue in fish is related to the lipid content, collagen content and size of muscle fibers (Listrat *et al.*, 2016). When fish have a high lipid content, it improves the texture and overall eating quality of their muscle tissues. The relationship between the lipid content and muscle hardness has been previously investigated in horse mackerel (Deka *et al.*, 2012). Changes in lipid content and fish quality are varied by season and body size. Moreover, a significant linear relationship was identified between crude lipid content and total length, as well as between lipid content and body weight of chub mackerel in Korea from July to December. The proximate composition of chub mackerel (*Scomber japonicus*) varied significantly between seasons in female fish that were more than 35 cm long and had started to mature productively (Shim *et al.*, 2017).

The effects of different diets and seasons on nutrient levels, including lipids, have

been investigated in many marine fish and shellfish species (Nisa and Asadullah, 2008; Nisa and Asadullah, 2011; Ozogul *et al.*, 2011). In many species, the lipid content increase during the feeding season and then decreases during spawning (Chrisolite *et al.*, 2016). The lipid content in such fish varies widely with season and sexual maturity (Shim *et al.*, 2017). Proteins and lipids in muscle tissue are mobilized during the reproductive period and transferred to the gonads, resulting in a change in the proximate composition (Boran and Karaçam, 2011; Ashwini *et al.*, 2016; Chrisolite *et al.*, 2016).

Jeong *et al.* (1999) identified a relationship between lipid content and the spawning period of 12 Korean fish species. When the lipid content in the muscle was high, the fish were of good eating quality. There is also a positive correlation between lipid content and the long-chain fatty acids, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Mohanty *et al.*, 2016).

Generally, the body size of fish influences the quality, texture, and nutrient content of muscle tissue and, therefore the taste (Cheng *et al.*, 2014; Shim *et al.*, 2017). Cultured olive flounder is supplied annually, and the fish commonly weighs 1 kg or less (Stieglitz *et al.*, 2021). To date, there have been no studies on the influence of body size or season on the texture or nutrient content of the muscle tissue of cultured olive flounder.

This study aimed to assess the effects of season and reproduction on the texture, proximate composition, and fat-soluble vitamin content of the muscle tissue of cultured olive flounder.

Materials and methods

Sample collection

Cultured olive flounder (*Paralichthys olivaceus*) was grown at the Genetics and Breeding Research Centre (GBRC), at the National Institute of Fisheries Science, located in Geoje City, South Korea. The fish were consistently fed a moisture pellet diet. Fish grown for approximately one year weighed round 1 kg. After two years, fish weighed approximately 2–3 kg, and after more than three years, fish weighed approximately 4 kg.

Ten fish from each of the four groups with different average weights (1 kg, 2 kg,

3 kg, or 4 kg) were sampled across various seasons (summer, autumn, winter, and spring) as well as during the reproductive season for this species (February, April, June, August, and December) from 2015–2016. Each fish was washed, and its length (cm) and weight (g) were recorded (Table 1). The samples were dissected to remove the skin, gills, and viscera, and the muscle tissue for analysis was removed from the dorsal, ventral, and fin regions (Fig. 1). A portion of the muscle was immediately analyzed for texture. Each sample was immediately placed on ice and taken to the laboratory for analysis (Nakayama *et al.*, 2017).

Table 1: Sample weight group, total length, body length, and body weight of cultured olive flounder samples.

Weight group	Total length (mm)	Body length	Body weight	No.
1kg	435-469 (422.4)	360-400 (355.8)	986.5-1,175.5 (1038.1)	50
2kg	525-560 (514.1)	425-500 (440.9)	1,851-2,467.5 (1,995)	50
3kg	608-665 (587.1)	505-585 (505.6)	2634-3273 (2,782.1)	50
4kg	655-760 (647.5)	555-650 (553.8)	3362-4727.5 (3,693.5)	50

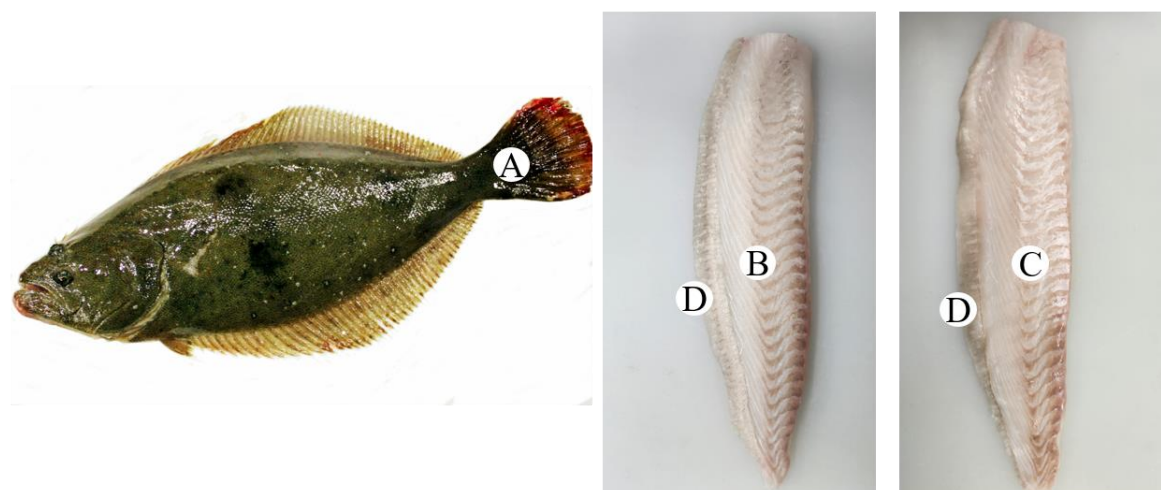


Figure 1: Location of the 3 different olive flounder muscle portions sampled. A, Olive flounder (*Paralichthys olivaceus*); B, dorsal muscle; C, ventral muscle; D, fin muscle.

Fillet yield

Fillet yield (%) was calculated relative to the whole-body weight and gutted weight as follows (Yasemi *et al.*, 2011):

$$\frac{\text{fillet weight}}{\text{body weight}} \times 100$$

Texture

Texture was measured using a rheometer (Compac-100; Sun Scientific Co., Ltd., Tokyo, Japan), by Shim and Cho (2014). The muscle was sliced into 20 mm×20 mm×10 mm cubes using a cutting knife. The elevation speed of the sample table was maintained at 3 cm min⁻¹, and a 10 mm cylindrical plunger was used. The results are presented as the mean values from ten samples.

Crude lipid content

Crude lipid content was determined using the method described by the Association of Official Analytical Chemists (AOAC, 1995) using a Soxhlet system (ST243 Soxtec, FOSS, Hillerød, Denmark).

Vitamin A and E content

Vitamin A and E content in the muscle tissue were analyzed according to Lee *et al.* (2011) and sample preparation was performed using the Ministry of Food and Drug Safety (MFDS, 2024) method. The fish tissue (3 g) was weighed and placed into a 250 mL flask. The sample was dissolved by adding 30 mL of ethanol, 1 mL of 10% pyrogallol-ethanol, and 3 mL of 60% potassium hydroxide solution. The sample solution was mixed well, and saponification was allowed to occur for 30 min in a water bath (90 °C). After rapidly cooling to 20–23°C, the sample solution

was transferred to a brown separation funnel containing 30 mL of water. Fifty milliliters of petroleum ether were added, and the funnel was shaken for 10 min. The separation funnel was allowed to stand for 30 min. The bottom layer was transferred to another funnel, and the sample was extracted twice with 30 mL of petroleum ether. All extracted petroleum ether layers were added to a separation funnel and washed with 50 mL of water until the red color of the phenolphthalein indicator disappeared. Water was removed from the funnel, and anhydrous sodium sulfate was placed in the petroleum ether layer for dehydration. The petroleum ether layer was transferred to a brown flask. The petroleum ether solution was dried at 40°C using a vacuum evaporator. The dried residue was dissolved in 5 mL of insert ethanol and the samples were filtered through a membrane filter (0.2 µm) before analysis. Retinol-acetate was used as a standard for vitamin A and α-tocopherol as a standard for vitamin E. A high-performance liquid chromatography (HPLC) (Surveyor Plus HPLC system; Thermo Fisher Scientific, USA) equipped with a photodiode array detector was used. A Develosil RPAQUEOUS C30 (4.6×250 mm, 5 µm, Phenomenex, Japan) column with isocratic elution (methanol: water=95:5) separated the analytes. The flow rate was 1.2 mL⁻¹, and the injection volume was 10 µL. The wavelength of the photodiode array detector was 298 nm (Lee *et al.*, 2011).

Statistical analyses

Data were analyzed using analysis of variance (ANOVA) with a general linear model. Differences between significant

means were identified using Duncan's multiple-range test. All statistical analyses were performed using SAS for Windows (SAS Enterprise guide ver. 4.3)

Results

Seasonal variation in fillet yield

The annual average fillet yield of cultured olive flounder with an average weight of about 1 kg, was 48.8–54.1% (Fig. 2). There were no significant differences in the yield between months, except for August. The average annual yields of cultured olive flounder with average weights of 2 kg, 3 kg, and 4 kg, were 43.9–54.1%, 42.6–60%, and 42.8–51.7%, respectively. The fillet yield

decreased gradually throughout the year and was lowest in April: 43.9% (2 kg), 42.6% (3 kg) and 42.8% (4 kg). The variation in fillet yield among individuals was high in fish from the 2 kg and 3 kg groups. Fillet yield was highest in fish from the 3 kg group, but there were no significant differences in yield among the four groups in February and August. In April, the yield was highest in the 1 kg group and lowest in the 4 kg group. There were no significant differences in yield among the different groups during June. During December, the yield was lowest in the 4 kg group, but there were no significant differences among the remaining groups.

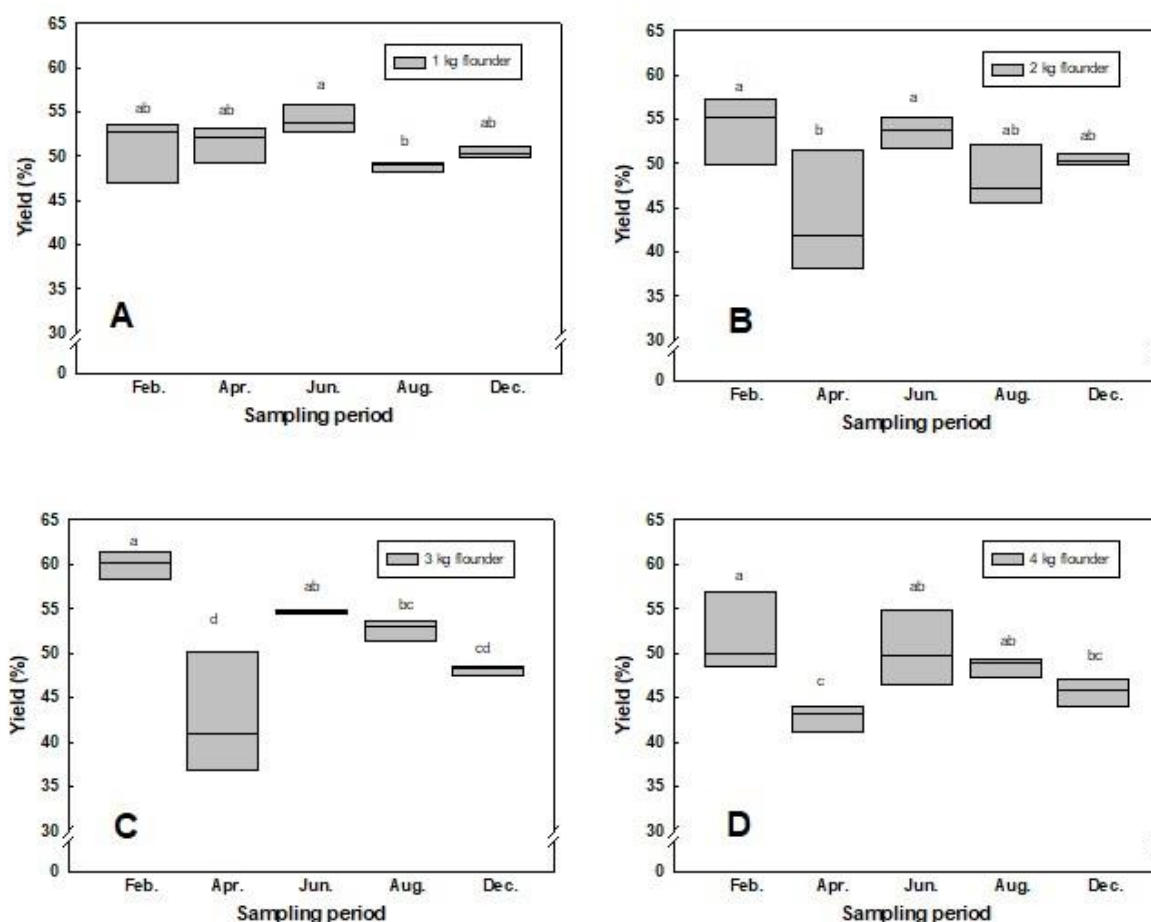


Figure 2: Seasonal variation in fillet yield (%) in the muscle tissue of olive flounder. A, 1 kg; B, 2 kg; C, 3 kg; D, 4 kg. ¹⁾ Different superscripts indicate significant differences based on Duncan's multiple range test at $p < 0.05$.

Seasonal variation in crude lipid content

The annual lipid content of the dorsal muscle of cultured olive flounder from the 1 kg group was 0.62–1.63 g 100 g⁻¹. The lipid content of samples from the 2 kg, 3 kg, and 4 kg groups was 0.82–3.28, 1.03–3.41, and 1.05–2.51 g 100 g⁻¹, respectively. The annual lipid content of the ventral muscle of samples from the 1 kg, 2 kg, 3 kg, and 4kg groups was 0.7–2.0, 0.93–3.74, 1.21–3.16, and 1.29–2.42 g 100 g⁻¹, respectively. The lipid content of the fin muscle was 23.1–35.8 (1 kg), 16.4–49.4 (2 kg), 19.3–49.6 (3 kg) and 22.0–37.5 (4 kg) g 100 g⁻¹.

There were no significant differences in the lipid content of the fin muscle from fish in the 1 kg group among the different months, except in February (Fig. 3A). Fish from the 2 kg, 3 kg, and 4kg groups had the highest lipid content in the fin muscle in February and December.

Lipid content was similar in the ventral and dorsal muscles (Fig. 3B, C). Lipid content in the muscle was highest in February and December for fish from the 2 kg, 3 kg, and 4 kg groups but was highest in April for fish from the 1 kg group.

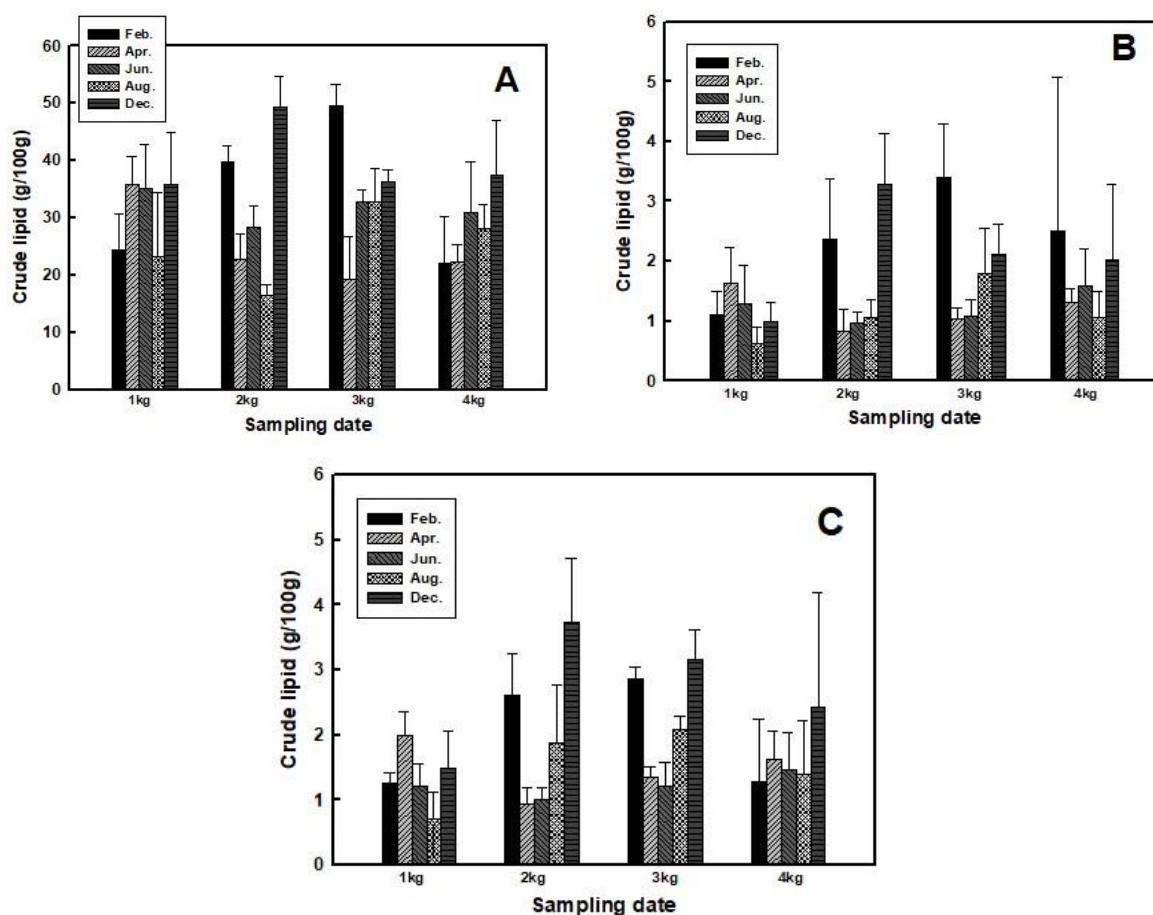


Figure 3: Seasonal variation in crude lipid content (g 100 g⁻¹) in muscle tissue of olive flounder. A: dorsal muscle; B, ventral muscle; C, Fin muscle. ¹⁾ Different superscripts indicate significant differences based on Duncan's multiple range test at $p < 0.05$.

In April and August, the lipid content of 1 kg of fish was higher than that of fish from the other groups. In December, the lipid

content was highest in the 2 kg fish, and there were no significant differences among the other groups.

Seasonal variation in texture

The texture for fish from all groups was lower in April compared with all other months: 0.83 (1 kg), 0.87 (2 kg), 0.86 (3 kg), and 0.84 (4 kg) kg. The texture in the 1 kg group was highest in February and August, ranging from 1.02–1.29 kg (Fig.

4). There were no other significant differences in texture among any of the other months for fish in the 2 kg and 3 kg groups. The highest value for texture in the dorsal muscle of the 4 kg group (1.15 kg) was measured in December.

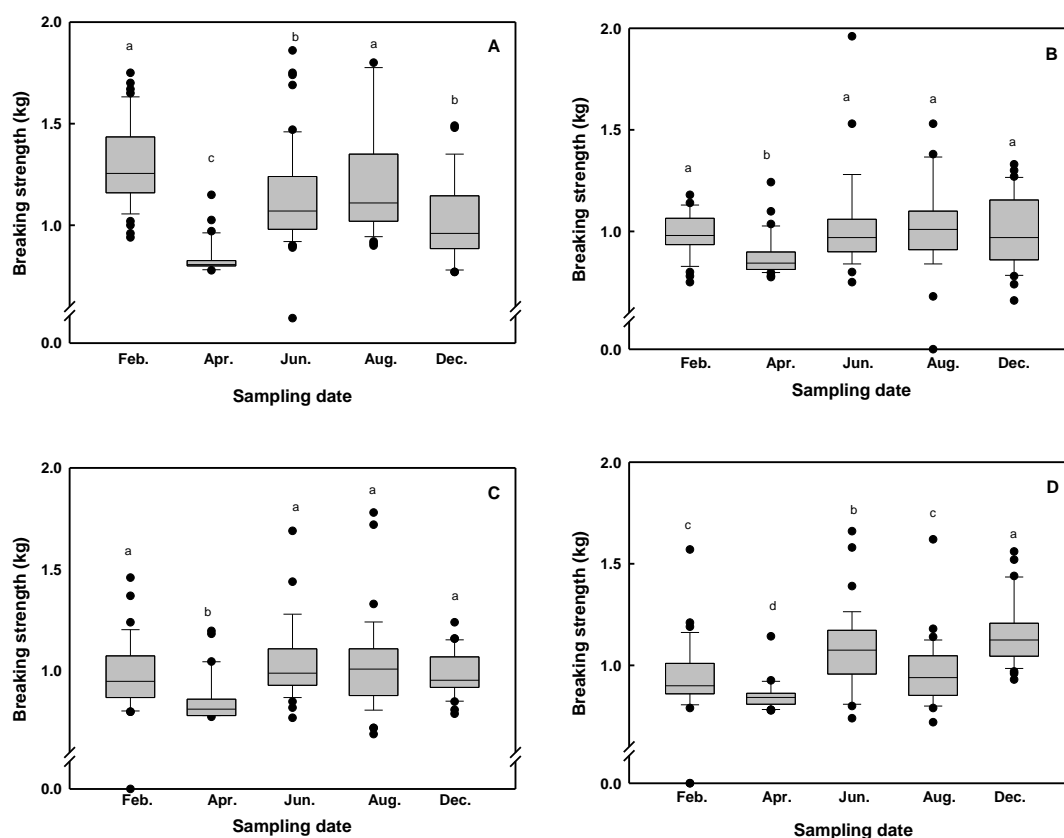


Figure 4: Seasonal variation in texture of dorsal muscle tissue in olive flounder. A, 1 kg; B, 2 kg; C, 3 kg; D, 4 kg. ¹⁾ Different superscripts indicate significant differences based on Duncan's multiple range test at $p < 0.05$.

The texture of the ventral muscle of olive flounder was similar to that of the dorsal muscle (Fig. 5). Values for the texture of the ventral muscle in 1 kg, 2 kg, 3 kg, and 4 kg olive flounder were lower in April than in any other month (0.81, 0.86, 0.86 and 0.86 kg, respectively). Breaking strength

ranged from 1.09–1.24 kg in 1 kg fish during all months except April. Other than in April, the values for the texture of the ventral muscle in 2 kg, 3 kg, and 4 kg fish were highest in August or December and lowest in February, although there were no significant differences.

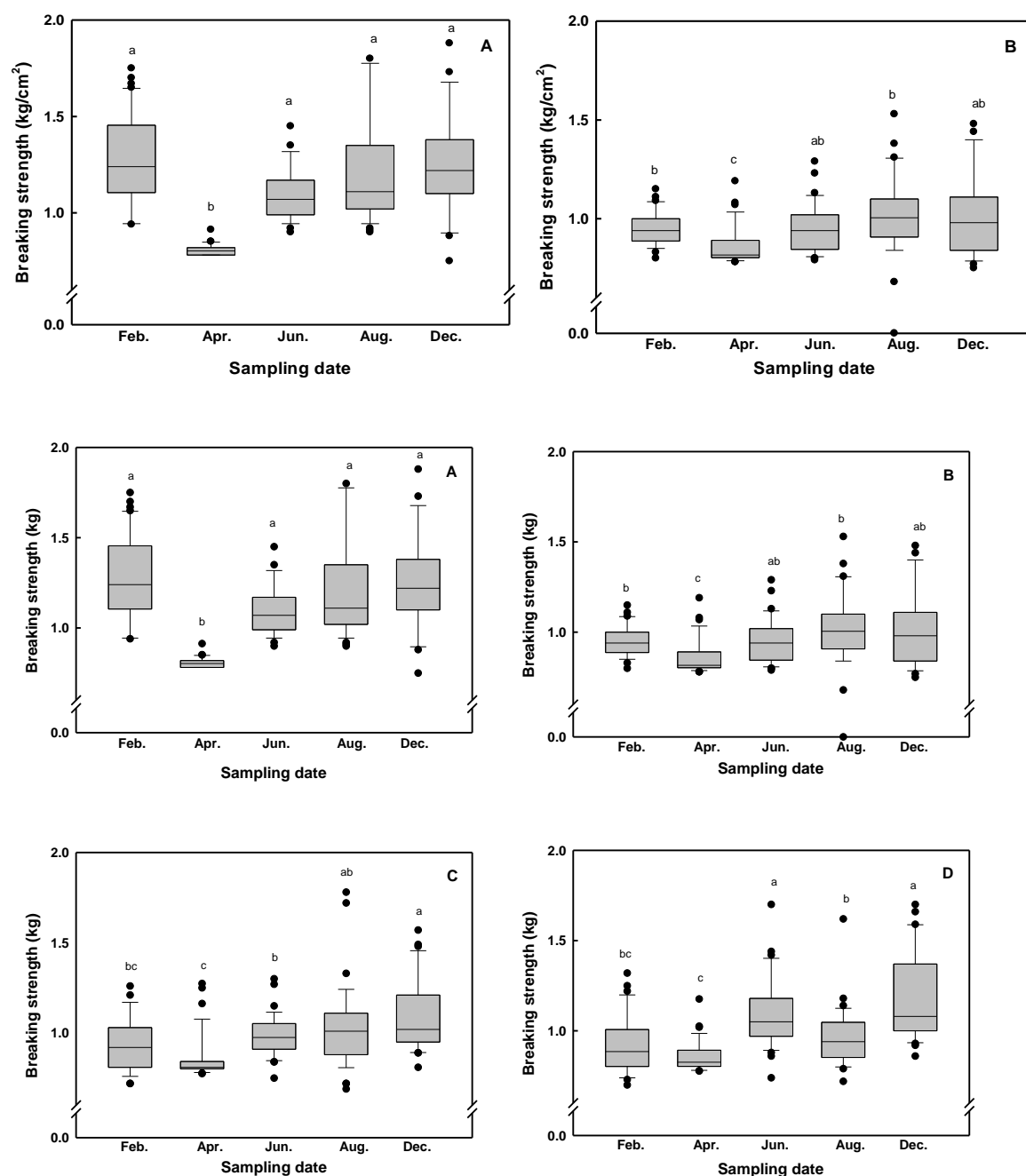


Figure 5: Seasonal variation in texture of ventral muscle in the olive flounder. A, 1 kg; B, 2 kg; C, 3 kg; D, 4 kg. ¹⁾ Different superscripts indicate significant differences based on Duncan's multiple range test at $p < 0.05$.

Seasonal variation in vitamin A and E contents

There were no significant differences in the vitamin A content of olive flounder between months in either the dorsal ($0-18.17 \mu\text{g } 100 \text{ g}^{-1}$) or ventral muscle tissue

($0-8.21 \mu\text{g } 100 \text{ g}^{-1}$) (Table 2). However, the vitamin A content differed between months and individuals from February to August in the 2 kg group. In December, high vitamin A levels were detected in the dorsal muscle of 2 kg ($10.36 \mu\text{g } 100 \text{ g}^{-1}$) and 4 kg fish

(18.1 $\mu\text{g } 100 \text{ g}^{-1}$). High vitamin A levels were also detected in the ventral muscle of 4 kg fish (1.79–7.44 $\mu\text{g } 100 \text{ g}^{-1}$). The vitamin A content detected in the fin muscle of the 1 kg group varied from June (23.8 $\mu\text{g } 100 \text{ g}^{-1}$) to August (67.96 $\mu\text{g } 100 \text{ g}^{-1}$, respectively). The vitamin A content in the fin muscle of the 2 kg, 3 kg, and 4 kg groups was lowest in February and increased from April to December.

The content of vitamin E in the dorsal and ventral muscles of fish was lowest in April (0.38–1.0 $\text{mg } 100 \text{ g}^{-1}$, and 0.4–0.99 $\text{mg } 100 \text{ g}^{-1}$) and highest in August (1.6–2.72 $\text{mg } 100 \text{ g}^{-1}$). However, the content in the ventral muscle was highest in December (1.27–3.59 $\text{mg } 100 \text{ g}^{-1}$). The vitamin E content detected in the fin muscle of 1 kg fish in June and August was 12.0 and 13.9 $\text{mg } 100 \text{ g}^{-1}$, respectively (Table 3).

Table 2: Seasonal variation in vitamin A content in the muscle tissue of olive flounder .

Part	Weight group	Feb.	Apr.	Jun.	Aug.	Dec.
Dorsal	1kg	ND ¹⁾	ND	ND	3.77	ND
	2kg	1.29±0.65	ND	ND	ND	10.36
	3kg	ND	ND	ND	ND	ND
	4kg	ND	ND	ND	ND	18.17
Ventral	1kg	ND	ND	ND	ND	1.79
	2kg	1.77	ND	ND	ND	4.76±2.85
	3kg	5.20	ND	ND	8.21	6.17±3.78
	4kg	ND	ND	ND	2.62	7.44±6.36
Fin	1kg	ND	ND	24.28±9.45 ^{b2)}	67.96 ^a	ND
	2kg	3.83 ^c	63.10±38.28 ^{ab}	22.09±11.30 ^b	94.12±6.19 ^a	62.00±40.33 ^{ab}
	3kg	72.58±26.99 ^{bc}	58.93±30.66 ^c	58.90±26.84 ^c	118.28±37.70 ^a	90.34±72.65 ^{ab}
	4kg	39.83±13.37 ^d	109.47±33.74 ^c	115.56±44.07 ^{bc}	121.78±30.95 ^b	195.09±51.66 ^a

¹⁾ ND: not detected.

²⁾ Different superscripts in same row indicate significant differences based on Duncan's multiple range test at $p<0.05$

Table 3: Seasonal variation in vitamin E content in the muscle tissue of olive flounder .

Part	Weight group	Feb.	Apr.	Jun.	Aug.	Dec.
Dorsal	1kg	0.84±0.09 ^{bc2)}	0.38±0.14 ^d	0.54±0.19 ^{cd}	1.60±0.54 ^a	1.02±0.26 ^b
	2kg	1.45±0.28 ^b	1.00±0.02 ^b	1.32±0.36 ^b	2.37±0.35 ^a	1.51±0.42 ^b
	3kg	1.12±0.32 ^c	0.53±0.32 ^d	1.84±0.41 ^b	2.72±0.69 ^a	2.22±0.52 ^{ab}
	4kg	1.45±0.87 ^{ab}	0.85±0.45 ^b	1.96±0.88 ^a	2.20±0.30 ^a	1.64±1.06 ^{ab}
Ventral	1kg	1.13±0.49 ^{ab}	0.40±0.09 ^c	0.78±0.26 ^{bc}	1.27±0.51 ^a	1.07±0.05 ^{ab}
	2kg	1.49±0.65 ^{ab}	0.63±0.21 ^c	1.16±0.34 ^b	2.83±1.21 ^a	3.32±0.10 ^a
	3kg	1.34±0.10 ^b	0.58±0.34 ^c	0.79±0.14 ^{bc}	3.37±0.98 ^a	3.59±0.60 ^a
	4kg	1.16±0.67 ^b	0.99±0.23 ^b	1.84±0.75 ^{ab}	1.84±0.89 ^{ab}	2.46±1.09 ^a
Fin	1kg	ND ¹⁾	ND	12.00±3.21 ^a	13.91±1.08 ^a	ND
	2kg	9.37±1.28 ^{bc}	2.62±1.63 ^c	7.79±1.32 ^{bc}	23.73±9.85 ^a	18.57±12.72 ^{ab}
	3kg	10.08±2.32 ^c	4.65±1.37 ^c	7.57±2.86 ^c	24.58±9.56 ^b	32.45±8.35 ^a
	4kg	23.21±9.10 ^b	23.14±5.79 ^b	31.04±6.68 ^{ab}	33.16±5.46 ^a	34.79±5.59 ^a

¹⁾ ND: not detected.

²⁾ Different superscripts in same row indicate significant differences based on Duncan's multiple range test at $p<0.05$.

Vitamin E in the 2 kg group was lowest in April (2.62 mg 100 g⁻¹) and subsequently increased in August (23.7 mg 100 g⁻¹) and then decreased in April (2.62–18.57 mg 100 g⁻¹). The content of vitamin E in the 3 kg group ranged from 4.65–32.5 mg 100 g⁻¹ and was lowest in April and highest in December.

Discussion

Many fish species spawn at different times of the year, most probably spawning in the spring or summer, coinciding with periods of low lipid content (Rasul *et al.*, 2021; Koca *et al.*, 2023). Hence, significant variations in the proximate composition of fish species reflect physiological processes and changes in environmental conditions that dictate spawning, migration, starvation, and heavy feeding activities (Boran and Karaçam, 2011; Ahmed *et al.*, 2022).

The spawning period of olive flounder along the Korean coast is from April to June, although there are differences in timing among habits. Spawning is generally limited to specific areas and times (Biggs *et al.*, 2021). Therefore, fishing during the spawning period may target different components of the population. The chance of catching the older (and larger) age classes may be higher during the spawning period as they gather on spawning grounds, which often affects the catchability of adults during the spawning period.

Spawning in the red snapper (*Lutjanus campechanus*) is related to the age and body size of the fish (Porch *et al.*, 2015). Spawning frequency also increases with size and age, which is consistent with the idea that it is important to conserve older individuals in the population (Hixon *et al.*,

2014). Chub mackerel from the Korean coast begin to mature in March, and spawning occurs primarily in May, and is complete by June. The proximate composition of chub mackerel, especially that of crude lipids, varies significantly with the season, and there is a relationship between lipids and the length of adult fish (Shim *et al.*, 2017). In Korea, an olive flounder farm that produces 100 metric tons of fish per year, typically yields fish with an average market size of 1 kg (Sim *et al.*, 2024). Most male olive flounder become reproductively mature between 1 and 3 years of age, although the majority mature at 2 years of age. Females start maturing from 2 to 4 years of age, although most reach reproductive maturity at 3 years (Ozawa *et al.*, 1996).

The crude lipid content in the dorsal and ventral muscle tissues of larger olive flounder was lowest during the spawning period in April. However, lipid levels in smaller, non-spawning fish (1 kg group) were not significantly different between the months (Fig. 2). The lipid content of the muscle affected the texture of the samples. There was no correlation between muscle lipid content and texture in the 1 kg group, contrasting the significant negative correlation between lipid content and texture observed in cultured fish from the 2 kg, 3 kg, and 4 kg groups.

The texture was lowest in April, corresponding with olive flounder's spawning/ post-spawning season. This suggests that reproductive maturation and spawning may have influenced the meat texture of these fish (Thakur *et al.*, 2009).

In an earlier study on yellowtail, we observed a soft meat texture in March,

which corresponded to the spawning season of this species (Thakur *et al.*, 2002). Because yellowtail spawns earlier in the season than amberjack, it is likely that the soft texture of the cultured amberjack observed in June was caused by spawning (Rodríguez-Barreto *et al.*, 2012). Touhata *et al.* (1998) reported that seasonal changes in the texture of red seabream were mainly dependent on sexual maturation and spawning. The texture becomes softer during the spawning season owing to structural changes in the pericellular connective tissue (Thakur *et al.*, 2003).

The primary function of vitamin E is to serve as an antioxidant, and it is by far the most physiologically important antioxidant in most vertebrates (Kelestemur *et al.*, 2012; Bilguven, 2014). Vitamin E plays an important role in protecting eggs during early development (El-Sayed and Izquierdo, 2021). Vitamin A is required for the growth, reproduction, maintenance of epithelial tissues, and embryonic development in fish. Similar to vitamin E, vitamin A and carotenoids cannot be synthesized by fish, and the quantity of these nutrients deposited into the eggs is a critical factor in determining reproductive fitness (Palace and Werner, 2006).

Vitamin E (α -tocopherol) content in olive flounder was higher in the fin muscle than in the dorsal or ventral muscle. The lowest vitamin E content was recorded in April at the beginning of the spawning season (Table 3).

The effects of vitamin E on the quality of fish fillets have been studied in several species, including rainbow trout, channel catfish, sea bass, Atlantic salmon, turbot, catfish, Nile tilapia, sunshine bass, and

hybrid tilapia (Niu *et al.*, 2014; Lozano *et al.*, 2017; Arslan *et al.*, 2021). The amount of α -tocopheryl acetate in the diet affected growth and increased vitamin E and lipid content in fish flesh. By measuring tocopherol content and weighing tissues, calculated the total amount of tocopherol in fish with either a vitamin E deficiency or sufficient vitamin E for normal functioning. The study found that approximately 35 mg of tocopherol in fish tissues was sufficient for healthy functioning, whereas 25 mg was insufficient. The liver is the primary storage organ of vitamin E, although the ovaries may also be important (Chaiyapechara *et al.*, 2003; Palace and Werner, 2006; Qiang *et al.*, 2019).

The olive flounder fed a regular diet, the vitamin E content was higher in the gonads and lower in the serum and liver of female fish, compared to male fish (Park *et al.*, 2021). The same pattern was evident in fish fed a diet containing vitamin supplements; however, the overall vitamin levels were higher in each tissue type. They found that tocopherol supplementation in the fish diet stimulated multiple spawning events. Females with supplements in their diet maintained their gonadal somatic index from the peak to the post-spawning period, spawning 1.5 times more eggs than fish on the unsupplemented diet.

In the present study, the vitamin E content in the dorsal muscle did not vary among months in any of the fish tested. However, the vitamin E content in the ventral and fin muscles was lower in April and June, corresponding to the spawning period for olive flounder. Vitamin E (tocopherol) content measured in different species varies depending on the species,

origin, physiology, and environmental factors (Bruni *et al.*, 2004; Szymańska and Kruk, 2008; Hosni *et al.*, 2017; Saheli *et al.*, 2021). A high tocopherol content in the fin muscle may correspond with high levels of crude lipids, which monthly changes due to reproductive development may directly influence (Røjbek *et al.*, 2012).

Olive flounder weighing 2 kg, 3 kg, and 4 kg had lower crude lipid content, texture, and vitamin E levels in April, the start of the spawning period. However, immature fish from the 1 kg group had the highest lipids, texture, and vitamin E levels in April and the lowest in August.

The values for crude lipids, texture, and vitamin E were lower in the 4 kg fish than in any other group, possibly owing to repeated spawning. Therefore, the optimal harvest size for fish consumption is when the fish weighs approximately 3 kg.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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