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Effects of Feeding Frequency on Growth, Feed Consumption, and Body Composition in Juvenile Turbot (*Psetta maxima* Linnaeus, 1758) at Low Temperature

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Abstract: Juvenile turbot *Psetta maxima* (Linnaeus, 1758) (15.23 ± 2.99 g) were fed to satiation with a commercial extruded feed over a 60-day period. Feeding regimens were twice a day (TD), every other day (EOD), and 2-day intervals (TDI). Fish were kept in seawater (18 ppt) at 5-7.5 °C from January 21 to March 23, 2004. Feed conversion rate (FCR) and the protein efficiency ratios (PER) were within the ranges of 1.12-1.14 and 2.26-2.29, respectively. While the maximum relative growth rate (RGR), weight gain, and specific growth rate (SGR) were recorded in the TD group, they were lower in the EOD and TDI groups. Both the SGR and RGR increased as feeding frequency increased. The feeding frequency influenced the chemical composition of the fish. Lipid and protein content of the fish increased in the TD group. In terms of SGR and RGR, it can be concluded that the best growth rates of Black Sea turbot cultured at 5-7.5 °C were obtained from the fish in the TD and EOD groups.

Key Words: Growth, feed frequency, body composition, juvenile turbot, *Psetta maxima* (L.)

Yemleme Frekansının Düşük Sıcaklıktaki Kalkan Balığı (*Psetta maxima* Linnaeus, 1758) Yavrularında Büyüme, Yem Tüketimi ve Vücut Kompozisyonu Üzerine Etkileri

Özet: Yavru kalkan balıkları, *Psetta maxima*, (Linnaeus, 1758), ($15,23 \pm 2,99$ g) ticari ekstrüde yemle; her gün (TD), gūnaşarı (EOD) ve iki gūnde bir (TDI) olmak üzere, iki kez 60 gūn sūresince doyuncaya kadar yemlenmiştir. Balıklar 21 Ocak–23 Mart 2004 tarihleri boyunca deniz suyunda (%o 18), 5-7,5 °C'de tutulmuştur. Yem dönüşüm oranı (FCR) 1,12-1,14 ve protein verimlilik oranı (PER) 2,26-2,29 arasında kaydedilmiştir. Maksimum oransal büyüme oranı, ağırlık kazancı ve spesifik büyüme oranı TD grubunda kaydedilirken, bu deęerler EOD ve TDI gruplarında daha düşük bulunmuştur. Spesifik büyüme oranı (SGR) ve oransal büyüme oranının (RGR) ikisi de yemleme frekansının artışıyla artış göstermiştir. Yemleme frekansı balıkların kimyasal kompozisyonunu etkilemiştir. Balıkların yağ ve protein içerikleri her gün iki kez yemlenen TD grubunda artış göstermiştir. SGR ve RGR deęerlerine bakılarak, Karadeniz kalkan balığı için en iyi büyüme oranının, 5-7,5 °C su sıcaklığında, her gün iki kez (TD) ve gūnaşarı iki kez (EOD) yemlemeden elde edildięi sonucuna varılabilir.

Anahtar Sözcükler: Büyüme, yemleme frekansı, vücut kompozisyonu, yavru kalkan, *Psetta maxima* (L.)

Introduction

Black Sea turbot (*Psetta maxima* (Linnaeus, 1758)) is an important commercial fish in European, Mediterranean, and Black Sea regions. The genetic results in a recent report show that the Black Sea turbot is a single species, *Psetta maxima*, L. (1). About 450 million juveniles of the European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*), and 5 million juveniles of turbot (*Psetta maxima*) were produced in 1999.

The optimal temperature for growth of turbot juveniles is 16-20 °C (2). In 40-50 g turbot of the Atlantic strain, the optimum temperature range for growth is 16-19 °C. In 10 g turbot, the optimal range is 16-22 °C. The turbot growth rate decreases rapidly below 14 °C and above 20 °C. Water temperatures at which turbot stop feeding during the growth phase are 5 and 25 °C. Specific growth rates and food intake are related to water temperature (3).

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The nutritional requirements of the Atlantic turbot have been well documented (4-8); however, studies on the optimal feeding frequency of Black Sea turbot and its effects on growth and feed utilisation are scarce (9-11).

A study on feeding behaviour in several fish species suggested that adjustment of feeding times to match the natural feeding rhythm improves nutritional efficiency, food conversion efficiency, and affects the utilisation of certain nutrients (12).

The use of cost-effective feed, an appropriate feeding regime, and optimal feeding intervals for fish affects intensive farm activities due to their influence on profit, and both fish growth, and health. The objective of this study was to determine the effect of feeding frequency on growth, feed consumption, and body composition of juvenile Black Sea turbot at low temperature (5-7.5 °C).

Materials and Methods

Juvenile turbot, *Psetta maxima* (Linnaeus, 1758), with initial body weights of 15.23 ± 2.99 g (mean \pm s.d.; $n = 135$) were obtained from the Japan International Cooperation Agency (JICA) and the Central Fisheries Research Institute in Trabzon, Turkey. Juvenile turbot were stocked in 50-l rectangular polypropylene indoor rearing tanks filled with seawater. Water was pumped from the sea into each tank at a rate of 0.8 l/min. The seawater was obtained directly from natural sea conditions. Groups of 15 randomly chosen fish were stocked into each of 9 tanks. Fish were acclimated to the

experimental system, diet, and their respective feeding frequencies for 2 weeks prior to the beginning of the experiment. Fish were fed with a commercial extruded feed (3 mm) containing 42% crude protein, 18% crude lipid, 2.7% crude fibre, 6.9% ash, and 7.5% moisture (Gross energy: 4300 kcal; Digestible energy: 3800 kcal). Three study groups were created based on the following feeding regimens: twice a day (TD), every other day (EOD), and 2-day intervals (TDI). The feed P/E ratio was estimated to be about 20.2 mg/kJ. Feeding frequency regimens were randomly assigned to triplicate tanks. The experimental indoor tanks were kept in the natural photoperiod (about 9 h of light and 15 h of dark). The study was conducted between January 21 and March 23, 2004.

Fish were individually weighed to the nearest 1 g every 15 days. At the end of the study, fish were weighed 24 h after the final feeding. Specific growth rate (SGR: % day⁻¹), feed conversion ratio (FCR), weight gain, and protein efficiency ratio (PER) were calculated using formulae outlined by Riche et al. (13) and Yang et al. (14).

Water quality parameters during the experiment consisted of daily temperature recording, with a low of 5 °C and a high of 7.5 °C (average temperature 6.3 ± 0.07 °C) (Figure 1). Owing to the seawater being obtained directly from the sea, the water temperature changed between 5 and 7.5 °C during the study. Water quality measurements were as follows: oxygen concentration (range: 8.5-9 mg/l⁻¹); pH (range: 7.4-6.8); salinity (range 17.6‰-18‰).

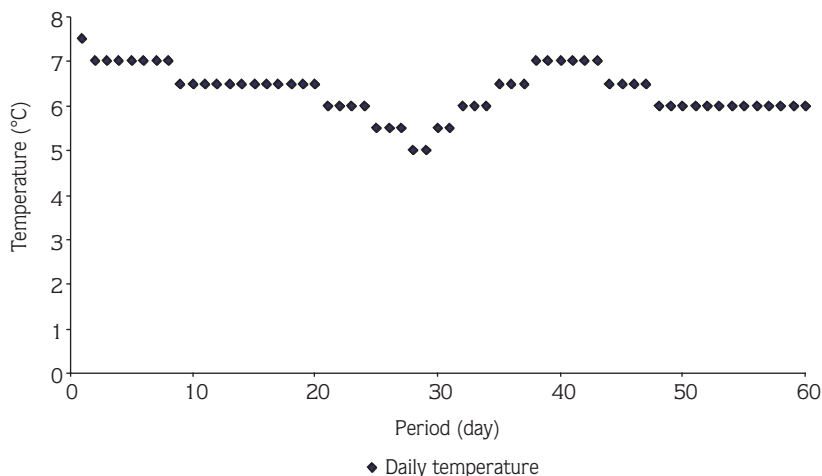


Figure 1. Temperature (°C) throughout the 60-day feeding period.

At the end of the experiment, 4 randomly chosen fish were collected from each replicate and frozen at $-30\text{ }^{\circ}\text{C}$ for subsequent determination of carcass proximate composition. Analyses were performed in triplicate. The proximate analyses of the carcasses and diet were performed before initiation of and after termination of the experiment.

Data were subjected to ANOVA and Tukey's procedure using Graphpad Prism (4.01 Trial Version) software. A P value < 0.05 was considered statistically significant.

Results

Feeding frequency affected the growth of the fish. Feeding frequency for maximum growth was TD. Mean weight gains were $11.26 \pm 0.34\text{ g}$, $10.09 \pm 0.20\text{ g}$, and $4.89\text{ g} \pm 0.19\text{ g}$ for fish in the TD, EOD, and TDI groups, respectively. The mean weight increase of fish in the TD group was significantly higher than in the others ($P < 0.05$). Growth trend of fish during the course of the experiment is shown in Figure 2. Both the relative growth rate (RGR%) and specific growth rate (SGR%) were significantly higher ($P < 0.05$) in fish in the TD and EOD groups (Table 1).

The highest daily dry feed intake and daily dry energy intake were obtained from the fish in the TD group ($P < 0.05$). No statistically significant differences were found in FCR and protein efficiency ratio (PER) between the TD, EOD, and TDI groups ($P > 0.05$), whereas significant differences were observed in the apparent net protein retention (ANPR) between the TDI group and the others ($P < 0.05$) (Table 2). The feeding frequency influenced

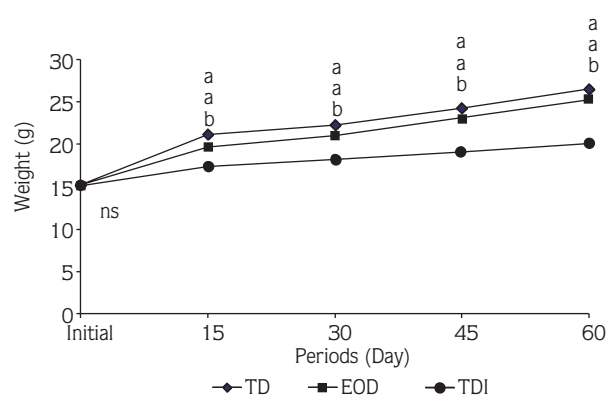


Figure 2. Mean weights of juvenile turbot fed twice a day (TD), every other day (EOD), and at 2-day intervals (TDI) for 60 days.

the carcass composition of the fish. The proportion of lipid and protein in growth increased in the TD fish. The initial and final carcass compositions of juvenile turbot are represented in Table 3.

Discussion

This study demonstrated a significant effect of feeding frequency on growth and weight gain in juvenile turbot. Turbot in the TD and EOD groups were able to consume sufficient feed to maintain more positive rates of growth than fish in the TDI group (Figure 2). These results are in agreement with an earlier study on halibut (15); however, growth rates in all groups were poorer than those reported in a previous study of turbot, which was carried out at higher temperatures (3). Feeding activity and food requirements are affected by water temperature (16). Fish age, size, and culture conditions,

Table 1. Growth performance and survival of juvenile turbot (*Psetta maxima*) fed at different frequencies after 60 days. Values (mean \pm SD) with different superscripts in the same row are significantly different at the 5% level.

	TD ¹	EOD ²	TDI ³
Initial wet weight (g)	15.22 \pm 1.15 ^a	15.22 \pm 2.08 ^a	15.24 \pm 2.52 ^a
Weight gain (g)	11.26 \pm 0.34 ^a	10.09 \pm 0.20 ^b	4.89 \pm 0.19 ^c
Relative growth rate (%)	73.99 \pm 2.10 ^a	66.33 \pm 1.26 ^b	32.10 \pm 1.55 ^c
Specific growth rate (% day ⁻¹)	0.92 \pm 0.02 ^a	0.84 \pm 0.01 ^b	0.46 \pm 0.02 ^c
Survival (%)	100	100	100

¹TD: Twice a Day

²EOD: Every Other Day

³TDI: Two-Day Intervals

Table 2. Efficiency parameters for juvenile turbot (*Psetta maxima*) fed to satiation twice a day (TD), every other day (EOD), or at 2-day intervals (TDI) for 60 days.

	TD	EOD	TDI
Daily dry feed intake (g/fish)	0.19 ± 0.01 ^a	0.17 ± 0.02 ^b	0.07 ± 0.0 ^c
Daily dry protein intake (g/fish)	0.08 ± 0.03 ^a	0.07 ± 0.01 ^b	0.04 ± 0.01 ^c
Daily dry energy intake (kJ/fish)	4.06 ± 0.19 ^a	3.66 ± 0.04 ^b	1.79 ± 0.01 ^c
FCR	1.12 ± 0.02 ^a	1.13 ± 0.01 ^a	1.14 ± 0.03 ^a
Protein efficiency ratio (PER)	2.29 ± 0.04 ^a	2.27 ± 0.03 ^a	2.26 ± 0.07 ^a
ANPR (%)	38.25 ± 1.3 ^a	37.90 ± 4.33 ^a	26.17 ± 0.52 ^b

Values (mean ± SD) with different superscripts in the same row are significantly different at the 5% level.

Feed conversion rate (FCR) = g wet feed intake/g wet weight gain

Protein efficiency rate (PER) = wet weight gain in g/protein intake

Apparent net protein retention = [(final weight in g x final body protein in %) – (initial weight in g x initial body protein in %)/protein intake in g] x 100

Table 3. Initial and final whole-body composition of *Psetta maxima* fed a commercial trout diet to satiation twice a day (TD), every other day (EOD), or at 2-day intervals (TDI).

	Moisture (%)	Crude Protein (%)	Crude Lipid (%)	Crude Ash (%)
Initial values	76.72	16.39	3.05	1.32
TD	75.32 ± 0.07 ^a	17.08 ± 0.24 ^a	4.92 ± 0.24 ^a	1.21 ± 0.06 ^a
EOD	76.25 ± 0.13 ^b	17.03 ± 0.73 ^a	4.07 ± 0.08 ^b	1.35 ± 0.1 ^{ab}
TDI	77.21 ± 0.1 ^c	15.45 ± 0.1 ^b	2.75 ± 0.19 ^c	1.53 ± 0.1 ^b

Values (means ± SD) in a column with different superscripts are significantly different at the 5% level.

including food quality, amount of feed provided, and water temperature affect the optimum feeding frequency for maximum growth of fish (17,18). In fish, water temperature below the optimal range results in reduced food consumption and growth rate (2,4).

Fish in the TD group consumed more feed than those in the EOD and TDI groups. Fish in the TDI group consumed more feed during the morning feeding than fish in the TD and EOD groups, as was observed in tilapia, *Oreochromis niloticus*, (13), halibut (19), and turbot (4,20). After severe food restriction, turbot become transiently hyperphagic when fed to satiation, but the observed compensatory growth could not completely compensate for lost growth (3).

Daily feed intake increased with increasing feeding frequency in this study and there were significant

differences between daily feed intake of the fish in the TD, EOD, and TDI groups ($P < 0.05$). The high growth rates of the fish in the TD and EOD groups were achieved by improved feed utilisation. Increased feeding frequency has been shown to improve the growth of various fish species (21,22). Two or three feedings a day have been found to be sufficient for maximum growth of a number of species, such as rainbow trout (23), halibut (16), and tilapia (13).

Fish growth and food intake were closely correlated and were generally highest in the TD and EOD groups. These results are similar to those obtained in other studies (13,22,24,25). The fish in the TDI group had the lowest RGR and feed intake when compared with the other groups. Fish in the TD and EOD groups had the highest RGR, feed intake, and energy intake.

The SGRs were 0.77% day⁻¹ and 0.71% day⁻¹ in the TD and EOD groups, respectively, which was better than that obtained by Iglesias et al. (26), Mallekh et al. (4), and Person Le Ruyet (3), who reported SGRs of about 0.66% day⁻¹, 0.60% day⁻¹, and 0.5 day⁻¹ for fish 40-50 g, respectively. In the TDI group, SGR was 0.38% day⁻¹, lower than in the above-mentioned studies. The specific growth rate parameters were similar to the results obtained by Imsland et al. (27) during the winter season for fish 34-44 g.

Feed conversion (1.12-1.14) was higher than reported for juvenile turbot by Person Le Ruyet (3) at 8 °C and Mallekh et al. (4) at 18-19 °C. In commercial fish farming practices, optimum water temperatures for the best growth are more important than upper or lower lethal ranges. Temperature affects the growth rate by affecting a variety of metabolic processes, including respiration, feeding, and digestion. Any divergence from the normal range of these processes may alter the optimal range for health and growth (28).

ANPR results suggested that TD and EOD feeding regimens were significantly better than TDI ($P < 0.05$). In this study, ANPR improved as the feeding frequency increased. The high values of ANPR indicated that digestible energy in the diet was sufficient for fish in the TD and EOD groups, whereas it was insufficient for TDI fish. This could have been due to the different feeding frequencies or food intake. Improved food utilisation, when fed at certain times of the day, could be explained by a coincidence with natural rhythms of secretion of or the activation or synthesis of digestive and/or metabolic enzymes (29).

In this trial, growth of 15 g juvenile turbot was significantly better in the fish in the TD, and EOD groups, compared to those in the TDI group. It was concluded that juvenile turbot between 15 and 25 g, in water 5-7.5 °C, could be fed every other day for the cost savings of the reduced labour associated with such a feeding schedule.

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