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# NITROGEN EXCRETION PATTERNS AND POSTPRANDIAL AMMONIA PROFILES IN BLACK SEA TURBOT *(SCOPHTHALMUS MAEOTICUS)* UNDER CONTROLLED CONDITIONS

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Key words: ammonia, Black Sea turbot, intensive fish culture, nitrogen excretion, *Scophthalmus maeoticus*

**Abstract**

Measurements of the rate of nitrogenous excretion were carried out in two batches of young Black Sea turbot (small 42 g, large 72 g) at 12±1°C under natural light conditions (10 h light:14 h dark and 13 h light:11 h dark, respectively). The ammonia nitrogen excretion rates of fish starved for 48 hours were 0.20±0.05 mg-N for the small fish and 0.18±0.09 mg-N/100g fish/h for the large. Fish were then fed a pellet diet containing 8.3% nitrogen at average rations of 0.67% and 0.59% of the body weight, respectively, for four days. On the fourth day, ammonia nitrogen excretion rates were evaluated. In both batches, the rates were 2-3 times higher immediately after feeding than in the starved fish, reaching a peak 3-6 hours after feeding and declining afterwards. For the small and large fish, respectively, 21% and 20% of the consumed nitrogen was excreted as ammonia nitrogen, 6% and 7% as urea nitrogen, and 8% and 4% as feces nitrogen within 24 hours after feeding.

## Introduction

The nitrogen excretion rate of fish is one of the most important factors governing stocking density in intensive fish culture, especially in closed re-circulating systems, due to the accumulation of nitrogenous waste products that can cause deterioration of water quality and threaten fish growth.

The Atlantic turbot, *Psetta maxima*, is one

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of the most important species in European aquaculture (Person-Le Ruyet, 1993) and its production is gradually increasing. The high market demand and interest in this species has caused many workers to study its biology, especially its nutritional requirements (Dosdat et al., 1995, 1996; Burel et al., 1996, 2000; Person-Le Ruyet et al., 1997, 2002; Regost et al., 1999, 2003; Pichavant et al., 2000; Imsland et al., 2001, 2002; Fournier et al., 2003, 2004). However little information is available on the Black Sea turbot (*Scophthalmus maeoticus*), an endemic subspecies and a new candidate for aquaculture in Turkey (Moteki et al., 2001; Sahin, 2001; Erteken and Nezaki, 2002; Yigit et al., 2003; Turker et al., 2005).

Protein intake is the major factor affecting nitrogenous end products based on both qualitative and quantitative criteria. In order to design an effective biological filtration system for closed culture, it is important to obtain information on the nitrogenous excretion rate after fish feeding. The effects of biotic and abiotic factors on nitrogenous waste production are well documented in flat fish species (Jobling, 1981; Kikuchi et al., 1992, 1995; Dosdat et al., 1995, 1996; Burel et al., 1996; Person-Le Ruyet et al., 1997, 2002; Pichavant et al., 2000).

According to our knowledge, data are lacking on the daily excretion pattern of the Black Sea turbot. In our previous study, ammonia nitrogen excretion rates were used as an index for protein quality evaluation of several feed fishes for the Black Sea turbot (Yigit et al., 2003). In the current study, daily patterns of the nitrogenous excretion rate of Black Sea turbot were investigated under controlled conditions. Such information is important for determining the optimal stocking density in intensive culture and for designing effective biological filtration in closed culture systems.

## Materials and Methods

*Fish and culture conditions*. In November 2001, Black Sea turbot ranging 20-35 g in body weight were obtained from the Central Fisheries Research Institute (CFRI) in Trabzon, Turkey, and transported to the facilities of the Faculty of Fisheries at the University of Ondokuz Mayis in Sinop, Turkey. The fish were reared in a 200-l tank with a flow-through system for acclimation to the new environmental conditions. During this period, sea water (ambient temperature 9-10°C , salinity 17 ppt) was supplied to the tank at a rate of 1.7 l/min. Fish were exposed to a natural light regime (10 h light:14 h dark) and fed a commercial diet containing 52% crude protein (8.3% nitrogen), 16% crude lipid, 9% nitrogen-free extract (NFE), 21 kJ gross energy/g feed, and 24.76 mg protein/kJ energy to satiation once a day until the start of the experiments. The experiments were carried out with two batches of Black Sea turbot (42 g and 72 g). The photoperiod was 10 h light:14 h dark during the experiments with the smaller fish, conducted in January-February 2002, and 13 h light:11 h dark during the experiments with the larger fish, carried out in March-April 2002. Water temperature was kept at 12±1°C during the course of the experiment.

*Nitrogenous excretion of starved fish.* The starvation experiment was run with 14 fish of 30.6-48.5 g (avg 42.0±5.10 g) and seven fish of 64.9-77.9 g (avg 71.6±4.7 g), live weight. After 48 hours starvation, each small fish was placed in a 5-l plastic chamber with 3.5 liters of well-aerated and filtered sea water; large fish were placed in 8-l chambers with 6.5 liters of water. Twenty-four hours after the start of the experiment, water samples were taken from the chambers and ammonia-N concentrations were analyzed. Ammonia excretion was determined as the difference between the ammonia concentration of the filtered sea water and the concentration in the chambers after the 24-h period. Hourly excretion rates were calculated by dividing the excretion by 24 hours and were expressed as mg-N/100 g fish/h.

*Nitrogenous excretion of fed fish*. Fish of both sizes were fed a commercial diet once daily at 09:00 for 4 days until satiation in a 50-l rectangular tank. On the fourth day, fish were allowed to feed for 1 h and were immediately transferred (1 fish per chamber) to a 5 or 8-l plastic chamber with a 5 mm mesh base and

3.5 or 6.5 liters of water, depending on the weight of the fish as above. The chambers were placed in a 50-l tank with 12±1°C ambient water to maintain a constant water temperature in all chambers. Feeding rates were calculated on the basis of the ration level on the fourth day and were 0.67% of the body weight for the small fish and 0.59% for the larger fish. Ammonia excretion rates were determined from the difference in ammonia concentration of the filtered sea water and the concentration in the chambers with fish (Yigit et al., 2003). Water samples were taken from the chambers 1, 3, 6, 10, 12, and 24 h after feeding. Ammonia concentrations were analyzed and ammonia-N excretion rates were expressed as mg-N/100 g fish/h. At the end of the 24-hour period, the fish were removed and the sea water in the chamber was filtered through Whatman GF/A filters to collect feces, as described by Kikuchi et al. (1992) and Kikuchi (1995). The residue on the filter was analyzed for feces nitrogen. Fourteen fish of 31.4-49.2 g (avg 42.4±5.06 g, small fish) and seven fish of 65.2-78.2 g (avg 72.2±4.7 g, large fish) were used in the experiment.

*Analysis*. Ammonia concentrations were determined by the Nessler method with a HANNA C200 portable spectrophotometer (HANNA Instruments, Co., Italy). The ammonia-N excretion rate was calculated by determining the ammonia produced in each chamber after each sampling using the following formula for a static system (Almendras, 1994): $A = [(N_2\text{-}N_1) \times V_2]/W/T_{2\text{-}1}$, where A = ammonia excretion rate (µg total $NH_3$-N/g wet wt/h); $N_1$ = ammonia concentration at time 1 (µg total $NH_3$-N/ml); $N_2$ = ammonia concentration at time 2 (µg total $NH_3$-N/ml); $V_2$ = volume of the medium at time 2 (ml); W = wet weight of the fish (g); and $T_{2\text{-}1}$ = time interval between samplings 1 and 2 (h). Feces nitrogen (feces-N) was analyzed by the Kjeldahl procedure. Urea nitrogen (urea-N) was estimated according to Dostat et al. (1995): Urea-N excretion = 0.0041 (ingested N) + 1.33; n = 6; $r^2 = 0.96$.

## Results

Hourly ammonia-N excretion rates for the starved fish are given in Fig. 1. The average rates were 0.20±0.05 mg-N and 0.18±0.09 mg-N/100g fish/h for the small and large fish,

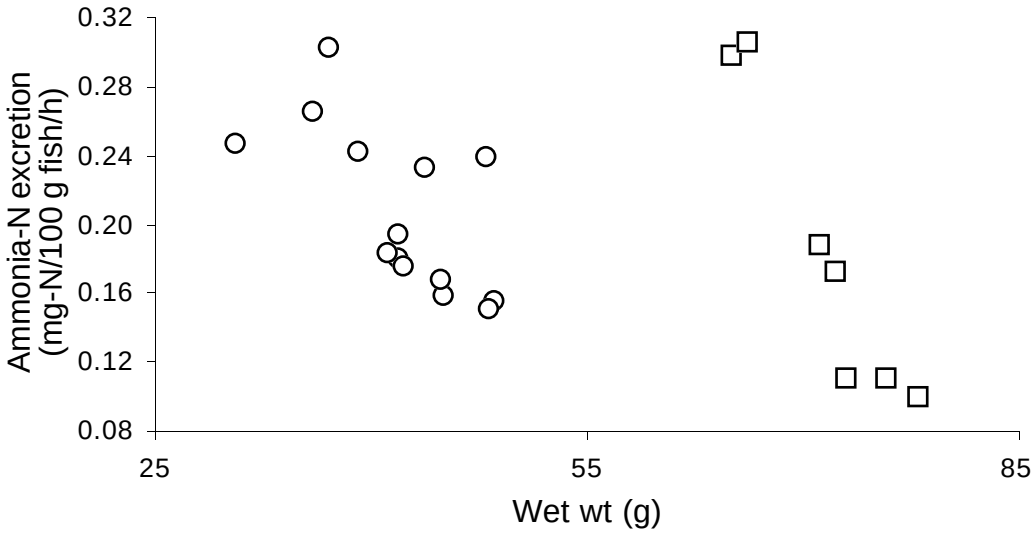


Fig. 1. Hourly rates of total ammonia-N excretion of starved Black Sea turbot (n = 14 for small fish and 7 for large).

respectively. Diurnal rates for fish after feeding are shown in Fig. 2, together with the average rates of the starved fish. In both groups, the ammonia-N excretion rate was about three times higher immediately after feeding than that of the starved fish. The rates peaked during the 3-6 hour period, remained almost constant during the next two periods, and dropped to a low that was still higher than that of the starved fish in the 12-24 h period. Throughout the 24 hours, the rate was higher in the smaller fish. Maximum values were 4-4.5 times higher than those of the starved fish in both fish groups. Daily rates of major nitrogenous end products are shown in Table 1. Daily rates of ammonia-N excretion and feces-N production are given in Fig. 3 and Fig. 4, respectively. The rates per weight were slightly higher in smaller fish for all nitrogenous substances.

## Discussion

As far as we know, this is the first study of the daily pattern of nitrogenous excretion of Black Sea turbot stock. The ammonia excretion rate of starved fish considerably differed between the two size classes, in agreement with reports on Japanese flounder in which endogenous nitrogen excretion at 20°C was 180 mg per kg body weight per day in fish of about 3 g, 55 mg in fish of 31 g, and 48 mg in fish of 400 g (Kikuchi et al., 1991, 1992; Kikuchi, 1995). The low excretion rate of the starved Black Sea turbot is similar to reports for Atlantic turbot (Dosdat et al., 1995) and Japanese flounder (Kikuchi et al., 1991, 1992; Kikuchi, 1995), but comparison with other species is difficult as metabolic rates rise with decreasing individual weight and increasing temperature (Jobling, 1981; Tatrai, 1981; Kikuchi et al., 1995; Burel et al., 1996).

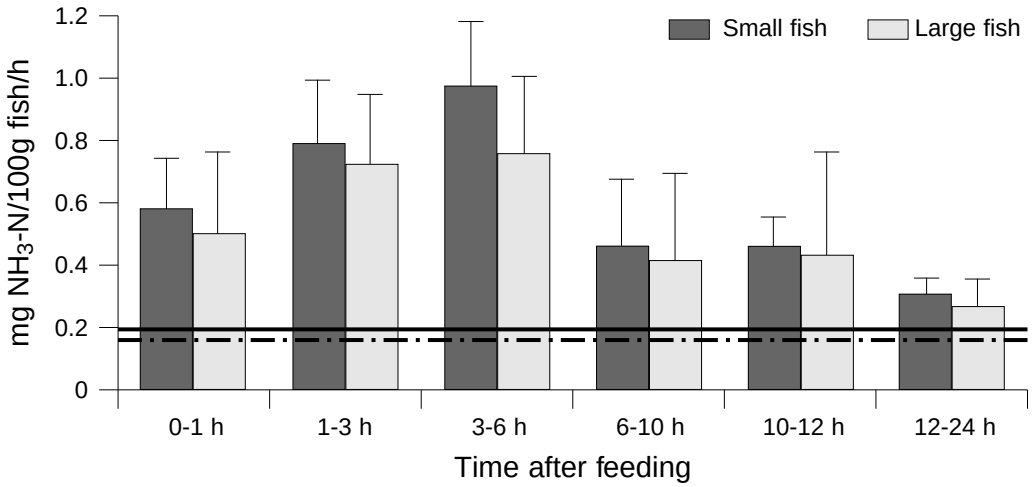


Fig. 2. Diurnal total ammonia-N excretion rates of Black Sea turbot after feeding. Solid line represents average rate of excretion of small starved fish, dashed line of large starved fish. Bars represent means plus standard deviations. Light regime for small fish was 10 h light:14 h dark and for large 13 h light:11 h dark.

Table 1. Daily nitrogen intake and excretion rates of Black Sea turbot.

| | *Fish size* | |
|---|---|---|
| | *Small (42.4 g)* | *Large (72.2 g)* |
| Feed ration level (% body wt) | 0.67 | 0.59 |
| Ingested nitrogen (mg-N/100 g fish/day) | 56.02 | 49.41 |
| Excreted TAN (mg-N/100 g fish/day) | 11.6±1.5 | 10.1±2.0 |
| Excreted urea-N (mg-N/100 g fish/day) [1] | 3.63 | 3.36 |
| Produced feces-N (mg-N/100 g fish/day) | 4.3±2.7 | 2.2±1.4 |
| Urea-N/(TAN + urea-N) | 23.89 | 24.98 |
| Exogenous TAN (mg-N/100 g fish/day) [2] | 6.58 | 5.66 |
| Exogenous TAN per ingested N (%) | 11.76 | 11.46 |
| Exogenous urea-N (mg-N/100 g fish/day) [3] | 2.30 | 2.03 |
| Exogenous urea-N per ingested N (%) | 4.10 | 4.10 |
| *Nitrogen excretion/nitrogen intake (%)* | | |
| TAN | 20.63 | 20.40 |
| Urea-N [1] | 6.47 | 6.79 |
| Feces-N | 7.63 | 4.44 |
| Sum | 34.74 | 31.64 |

[1] estimated

[2] represents excreted total ammonia-N (TAN) less TAN excreted by starved fish

[3] represents excreted urea-N less urea-N excreted by starved fish

Although the hourly ammonia excretion rates immediately after feeding were three times higher than those of the starved fish, in both size groups the maximum rates were recorded 3-6 h after feeding and daily patterns were similar. The data indicate that the same metabolic process governing ammonia production from deamination occurs at the same time in both size classes under the employed experimental conditions. Similar to our findings, Dosdat et al. (1995) observed maximum hourly excretion rates about five hours after feeding in Atlantic turbot of 13 g at 12°C while Dosdat et al. (1996) reported that maximum post-prandial hourly excretion rates occurred 3-5 h after feeding in 10 g Atlantic turbot at 20°C and 5-8 hours after feeding in 100 g fish at 16°C. Our findings are also in close agreement with those of Kikuchi et al. (1991, 1995) who reported peak ammonia excretion 3-6 h after feeding Japanese flounder. Similar results were reported for other teleosts, namely, freshwater-adapted and seawater-adapted sea bass (Almendras, 1994), red sea bream (Kikuchi et al., 1996), and sea bass, sea bream, brown trout, and rainbow trout (Dosdat et al., 1996). Burel et al. (1996) reported that the post-prandial peak time might be affected by water temperature, as total ammonia nitrogen began to increase three hours after food intake, reaching a maximum three hours later for the three highest tested temperatures (14, 17, and 20°C) and four hours later for the two lowest temperatures (8 and 11°C).

Among marine fish species, flatfish seem

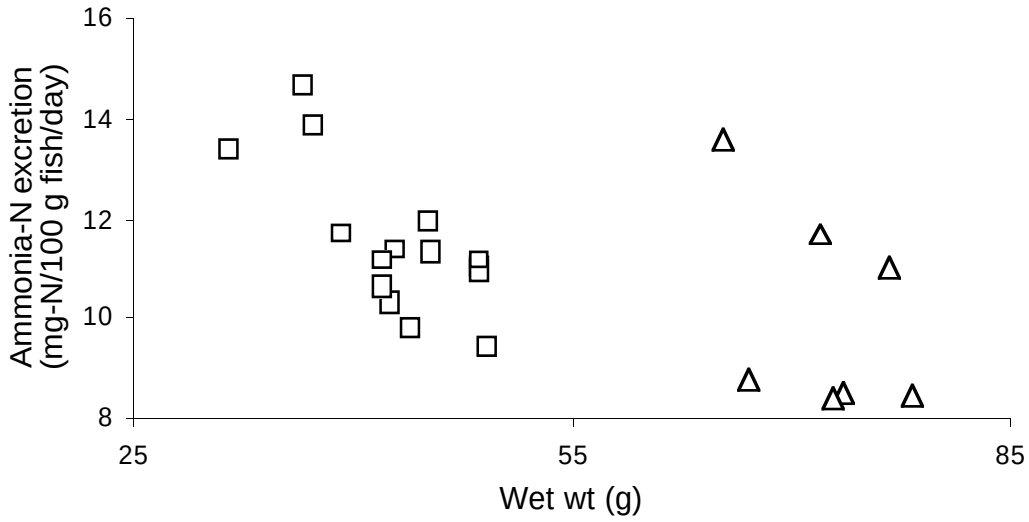


Fig. 3. Daily total ammonia-N excretion rates of small and large Black Sea turbot.

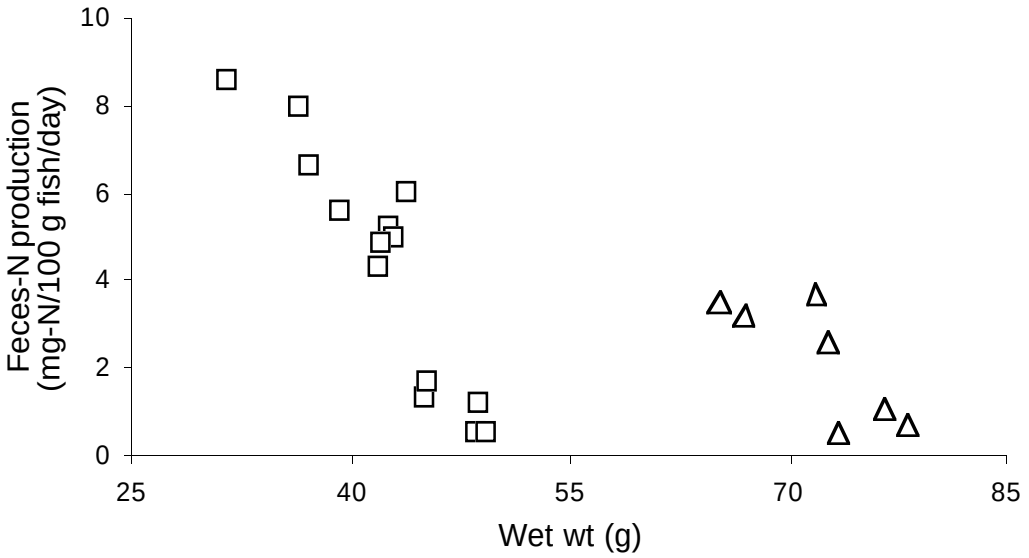


Fig. 4. Daily feces-N production rate of small and large Black Sea turbot.

to have lower ammonia production levels than pelagic species. Recent studies with sea bass (Ballestrazzi et al., 1994; Dosdat et al., 1996) and sea bream (Kikuchi et al., 1996) indicate that, depending on the quality of the diet, 30-35% of the ingested nitrogen was excreted as ammonia by 75 g or 100 g sea bass and about 30% by 20 g sea bream, respectively. Porter et al. (1987) reported that 35-37% of the ingested nitrogen was recovered as TAN in 3 g and 90 g sea bream. Similarly, Dosdat et al. (1996) found that 32-34% of the ingested nitrogen was recovered as TAN in 10 g and 100 g sea bream.

In contrast, Dosdat et al. (1996) reported lower values for 10 g and 100 g Atlantic turbot – 21% and 20% respectively. Similar values for Atlantic turbot were reported by Burel et al. (1996; 19-23% for fish ranging 72-108 g) and Fournier et al. (2003; 21-22% for 58-59 g fish). These data are in close agreement with our present findings of 21% and 20% excretion of ingested nitrogen by 42 g and 72 g Black Sea turbot, respectively. Our findings are also very similar to those reported by Kikuchi et al. (1992) for 1.8-5 g (23%) and 15-49 g (21%) Japanese flounder at 20°C. The significantly lower ammonia excretion rate in turbot (about 20%) compared to other marine fish species (30-37%) accords with the high protein efficiency (40%) of Black Sea turbot (Turker et al., 2005) and Atlantic turbot (Caceres-Martines et al., 1984; Regost et al., 1999, 2003; Imsland et al., 2001; Person-Le Ruyet et al., 2002; Fournier et al., 2004) and the low exogenous losses and activity that they demonstrate.

The excreted urea-N rate of the fed turbot was similar to that reported for other fish species. Small fish excreted more urea than larger fish, indicating that urea excretion in turbot is size dependant as reported by Kikuchi et al. (1992) for Japanese flounder and Dosdat et al. (1996) for sea bream, sea bass, and Atlantic turbot. Further, Burel et al. (1996) reported higher urea excretion in Atlantic turbot as the water temperature increased.

In the present study, estimated urea production in fed Black Sea turbot represents 6-7% of the ingested nitrogen, within the range reported for similar sized Atlantic turbot by Burel et al. (1996) and Dosdat et al. (1996), but higher than that of other fish species, i.e., 4-5% for sea bream and sea bass (Dosdat et al., 1996) and 4% for Japanese flounder (Kikuchi et al., 1992; Kikuchi, 1995). In contrast, Person-Le Ruyet et al. (2002) reported that 8% of the total consumed nitrogen was excreted as urea in Atlantic turbot of 56 g raised in 17°C water.

Estimated urea excretion in fed Black Sea turbot represents 24-25% of the TAN + urea-N production. This finding closely agrees with findings for Atlantic turbot of similar sizes, i.e., 24.3% for 72 g fish, 25.4% for 87 g fish, 26.4% for 102 g fish, and 26.9% for 111 g fish by Burel et al. (1996) and 22% in 100 g by Dosdat et al. (1996), but are relatively high compared to other findings for flatfish of similar sizes, namely, 15% in Japanese flounder (Kikuchi et al. 1992; Kikuchi 1995), 19% in Atlantic turbot (Person-Le Ruyet et al. 2002), and 12% in sea bream and 13% in sea bass (Dosdat et al., 1996). The relatively high value in turbot is a consequence of a comparatively lower endogenous ammonia production. In aquaculture facilities, urea is less toxic than ammonia. Due to its high urea to ammonia ratio, turbot culture is less harmful to the environment than culture of other teleosts. This is also very important for water quality, particularly in the case of high density rearing and recirculating systems.

Between size groups, there were only small differences in the proportions of excreted substances, namely, 20-21% of the consumed nitrogen was excreted as ammonia, 6-7% as urea and 4-8% as feces nitrogen, i.e., about 30% of the consumed nitrogen was excreted. This may show that small fish feed on more dietary protein and excrete more nitrogen than larger fish, but the utilization of consumed nitrogen in percent is similar for both sizes. The same trends were reported by Kikuchi et al. (1992) and Kikuchi (1995) for Japanese flounder and by Dosdat et al. (1996) for rainbow trout, brown trout, sea bass, and Atlantic turbot. Dosdat et al. (1996) found that in 100 g Atlantic turbot, the total excreted nitro-

gen (ammonia+urea+feces) represented 29% of the consumed nitrogen, similar to our finding (31%) for 72 g Black Sea turbot.

The largest proportion of excreted nitrogen was in the form of ammonia, which accounted for more than 60% of the total nitrogen excretion. Smaller fish excreted a higher proportion of ammonia-N and feces-N per nitrogen intake but larger fish had a higher urea-N excretion rate per consumed nitrogen. Although it is known that smaller fish consume proportionately more feed (and, thus, nitrogen) and excrete more nitrogen, there was only a small difference in utilization of the consumed nitrogen between the two groups in the present study. This was probably due to the small difference in weights of the two groups. Similar results were reported by Porter et al. (1987) and Dosdat et al. (1996) in sea bream, Kikuchi et al. (1992) in Japanese flounder, and Burel et al. (1996) and Dosdat et al. (1996) in Atlantic turbot.

In conclusion, Black Sea turbot stock, because of the relatively low proportion of ammonia in its total nitrogen excretion, has high potential for protein retention. It is a promising species in terms of protein productivity and environmental friendliness. Nitrogen metabolism has consequences in high density intensive fish culture. Especially in recirculating systems, total ammonia, urea, and feces nitrogen needs to be considered in treatment processes for developing effective closed aquaculture systems. It is important to examine the maximum nitrogen excretion rate of fish throughout the culture period. The low nitrogenous excretion rates in this study confirm a promising future for Black Sea turbot, which utilizes dietary protein very well. Further investigations are necessary to evaluate nutritional characteristics of this species, which presents very high protein utilization (Turker et al., 2005).

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