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Monitoring of trace metals, biochemical composition and growth of Axillary seabream (Pagellus acarne Risso, 1827) in offshore copper alloy mesh cages

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Abstract

The study was conducted to assess trace metal contents, biochemical composition and growth performance of axillary seabream (*Pagellus acarne* Risso, 1827) cultured in a copper alloy mesh cage. A total of 400 axillary seabream (initial mean weight: 176.0±14.0 g), a new candidate species for the Mediterranean aquaculture, were stocked into a high-density polyethylene frame gravity cage and fed a commercial seabream diet for a period of 6 months. At the end of the feeding trial, fish reached a final weight of 264.8±16.8 g with a weight increase of 88.8 g and a feed conversion rate of 2.51. Overall, relative growth rate, specific growth rate and feed conversion ratio were satisfactory and comparable to the pelagic fishes such as gilthead seabream or European seabass, which are presently the main fish species for the Mediterranean aquaculture industry. Trace elements in fish grown in copper alloy net cages over a 6-month period showed satisfactory results, as the metal concentrations in fish tissues such as liver, skin, muscle and gills were below the reported upper limits for human consumption, indicating that copper alloy net is an acceptable and safe material for finfish cage aquaculture. Furthermore, from the growth performance data obtained in the present study, it can be concluded that axillary seabream showed potential for cage farming, and thus is a promising new candidate for the Mediterranean aquaculture industry.

Keywords: Axillary seabream, *Pagellus acarne*, trace metals, copper alloy mesh, growth performance, cage aquaculture.

Introduction

The current world population of 7.3 billion is in a rapid increase with daily births of around 15000 a day (Worldometer, 2015). Over the next 35 years the world population is expected to reach about 9.6 billion (FAO, 2012). The increasing demand of food for the increasing world population is an important challenge for the global food industry. Europe, with its marine and inland water resources and rapidly increasing aquaculture industry, has the capability to meet this increasing demand with high quality protein from aquaculture.

Pelagic marine fish aquaculture is mainly conducted in fish cages and the production in the Mediterranean countries is mainly focused on a few fish species such as the gilthead seabream (*Sparus aurata*), European seabass (*Dicentrarchus labrax*) or rainbow trout (*Oncorhynchus mykiss*). Among European countries Greece and Turkey are the main producers of gilthead seabream and European seabass with a total production of 211.055 tons in 2012, while Turkey is the main producer for rainbow trout with an annual production of 114.569 tons in 2012 (FAO, 2014).

For the future generations, fish farmers need to be supported with knowledge of new applications and new candidate species or alternative materials for the sustainable development of the fisheries industry. Besides the current aquaculture fish species, the introduction of new fishes to the aquaculture industry may increase the economic benefits of fish farms by expanding markets and improving supplies to meet the increasing market demand. Axillary seabream (also known as Spanish seabream, belonging to the Sparidae family) has a potential for farming. Information regarding growth, feed utilization, biochemical composition or trace elements of the fish body is scarce for this species. Hence, new information may support the cage culture of this species.

Another improvement that may help expand Mediterranean finfish aquaculture involves innovation in the aquaculture pens themselves. Biofouling of cage nets is one of the main problems in cage aquaculture facilities, which causes serious problems in terms of blockage of water flow through the net mesh and decreasing oxygen content in the water. Reducing biofouling on nets would have overall benefits due to better fish growth induced by increased feeding rate, reduced fish stress and lower labor costs from net cleanings and changes (Yigit *et al.*, 2013). The antimicrobial properties of copper alloys in health care applications are well documented (Grass *et al.*, 2011). Recently, copper alloys have been fabricated into wire mesh materials for use in cage nets in place of polymer netting. These mesh materials have demonstrated reduced biofouling on Mediterranean pens and improved fish health due to a more sanitary environment (Yigit *et al.*, 2013). For Atlantic salmon aquaculture, the adoption of copper alloy meshes, vs. conventional nylon meshes, have demonstrated both improved economic benefits (Gonzalez *et al.*, 2013) and reduced environmental impacts (Ayer *et al.*, 2016). Further, the biofouling resistance of copper alloy meshes reduces the drag of these nets, improving the durability of pens in high-energy offshore environments (Tsukrov *et al.*, 2011). The adoption of copper alloy meshes to Mediterranean aquaculture thus would be a great interest to fish farmers. Copper alloy meshes hold the promise of increased profitability with a reduction of maintenance costs and environmental concerns, due to minimizing the biofouling in marine cage systems. Thus, the present study aimed to evaluate trace metal levels, body biochemical composition and growth performance of axillary seabream (*Pagellus acarne* Risso, 1827) raised in in these innovative copper alloy mesh cage systems.

Materials and Methods

Experimental station and cage type

The experiment was conducted in the Strait of Canakkale (formerly, *the Dardanelles*) off the coast of Canakkale City in Turkey (40°03’42” N - 26°20’36” E, 40°03’51” N - 26°20’45” E, 40°03’45” N - 26°20’55” E, 40°03’36” N - 26°20’48” E). The research location is an exposed area that experiences about 10 to 12 storms each year, resulting in three to five meter-high waves. Additionally, the water flows in both directions along the strait, from the Sea of Marmara to the Aegean Sea, forcing a surface current in one direction and an undercurrent in the opposite direction. Due to the strong weather conditions in the area, the use of a durable material with reduced drag performance is necessary for successful marine aquaculture. Hence, Thus, copper alloy mesh netting, a strong and biofouling-resistant material, was tested in this experiment.

The offshore copper alloy mesh cage system used in the experiment was designed and deployed with the collaborative research efforts between the International Copper Association (ICA-NY, USA), the University of New Hampshire (UNH, USA) and Canakkale Onsekiz Mart University (COMU, Turkey). The offshore surface gravity type octagonal HDPE fish cage was designed to have a volume of 150 cubic meters, with a net enclosure of 5 meter depth and a diameter of 6 meters (Fig. 1).

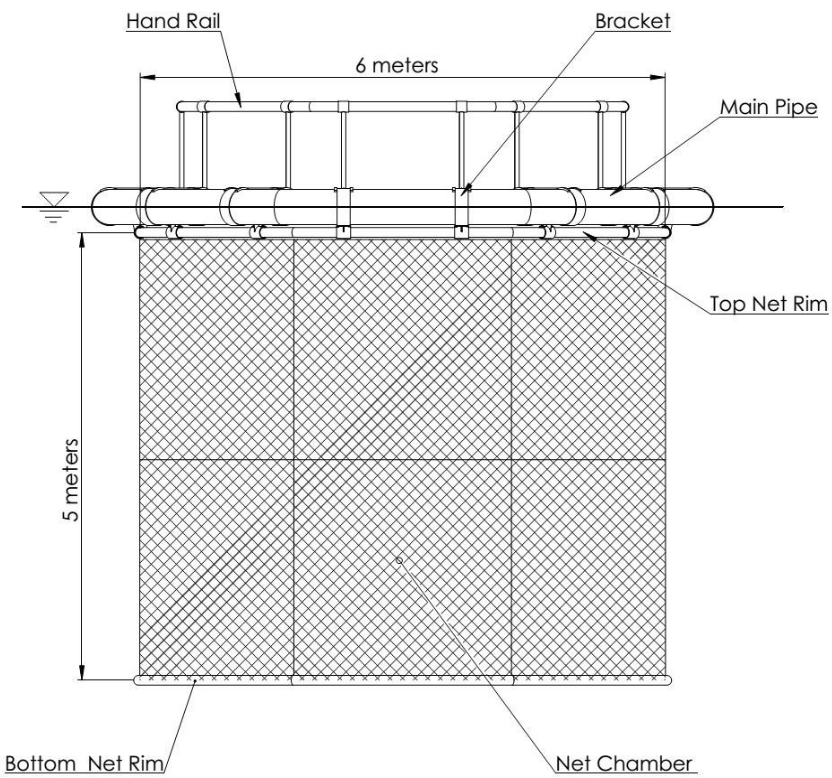

Fig. 1: Design of the HDPE gravity-type offshore cage system with copper alloy mesh used in the study.

Experimental fish and feed

The offshore HDPE fish cage with a copper-alloy mesh enclosure was stocked with 400 axillary seabream (*Pagellus acarne*) (initial mean weight: 176 g) (40°03'42" N - 26°20'36" E). Prior to the start of the feeding trial, experimental fish, obtained from local fishermen, went through an adaptation period for one month to the cage conditions. During the acclimatization period, fish were hand-fed 2 times a day and fish behavior was monitored during feed distribution. After the adaptation period for one month, 15 fish from the experimental cage were randomly sampled, weighed and thereafter the feeding trial was initiated. Ambient water temperature ranged from 12 to 25 °C during the course of the study. Fish were handfed twice a day at 09:00 and 17:00 using a commercial seabream diet with optimum protein and lipid levels (P/E ratio) (Table 1) (Commercial company feed chart). Daily feed intake data were recorded and used for the calculation of feed utilization values. Since the study location was an exposed area with strong water currents, feeding activity was carefully monitored to ensure an even distribution of the feed to all fish in the cage. Besides, underwater monitoring for biofouling on copper alloy mesh nets were periodically recorded by diving activities.

Calculation of the specific growth rate (SGR), relative growth rate (RGR), and feed conversion rate (FCR) were performed as described by Burel *et al.* (2000) and Yigit *et al.* (2006, 2010).

Fish sampling and analytical methods

Prior to the start of the trial, 15 fish from an initial pool of fish were anesthetized with clove oil at 20 mg L^{-1}, body temperature lowered in a freezer, stored in polyethylene bags and frozen (20 °C) for analysis of muscle composition (dry matter, protein, lipid, ash), fish body indices, and tissue metal concentrations. In order to reduce the metal contamination during tissue dissections and sampling, non-metallic tools such as scissors and tweezers were used. The same protocol of sampling was followed for each sampling period and also at the end of the study. All analyses were conducted in triplicate. Muscle tissues sampled between the lateral line and the dorsal fin from both sides of the fish were prepared for analyses by homogenizing the muscle tissue in a blender.

Chemical analyses of diets and fish muscle tissue were performed according to AOAC (1984) guidelines as follows: dry matter after drying in an oven at 105 °C for 24 h until constant weight, protein (N 6.25) by the Kjeldahl method after acid digestion, lipids by ethyl ether extraction in a Soxhlet System, ash by incineration in a muffle furnace at 550 °C for 12 h, and NFE was calculated by difference. Viscerasomatic index (VSI), Hepatosomatic index (HSI), lipid accumulation around the viscera (Mesenteric fat index, MFI) and Spleen somatic index (SSI) were also determined and recorded for bioassay evaluations using the following formulae: Viscerasomatic index (VSI, %) = (Viscera weight / Body weight) x 100; Hepatosomatic Index (HSI, %) = (Liver weight / Body weight) x 100; Mesenteric fat index (MFI, %) = (Lipids weight around viscera (g) / Body weight) x 100; Spleen somatic index (SSI, %) = (Spleen weight / Body weight) x 100.

Metal contents such as Copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) in muscle, skin, liver and gills of fish were determined using Atomic Absorption Spectrophotometry (AAS) at the Laboratories of COMU, Faculty of Marine Science and Technology in Canakkale, Turkey. Muscle and skin analyses were used for investigating possible transfer of metals to humans through fish consumption. Liver and gill analyses were performed for the determination of metal accumulations in fish body. Fish were terminally anaesthetized with clove oil at 20 mg L^{-1} and dissected for tissue metal analysis. Initially, tissues (muscle, skin, liver and gills) were rinsed and oven-dried until constant weight, digested in 5 ml of concentrated nitric acid, and diluted to 20 ml with de-ionized water for metal analyses. Blank digest was also carried out in the same way. For the AAS the following wavelength lines were used: Cu 324.754, Zn 206.191, Mn 259.373, Fe 259.941. Dogfish muscle certified reference material for metal analysis (DORM-2) was used to calibrate the AAS prior to metal analysis of fish samples. DORM-2 and lobster hepatopancreas reference material for metals (TORT-2) were purchased from the National Research Council (NRC), Canada. The concentrations found were within 90–115% of the certified values for all measured elements. Percentage tissue moisture content was calculated from wet and dry tissue weights. All metal concentrations were expressed as µg g^{-1} dry weight.

Table 1. Nutritional composition of experimental diets used in the feeding trial (% dry basis, except for moisture).

	Proximate composition (g / 100g)
Moisture	12.0
Crude Protein	42.0
Crude Lipid	24.0
Crude Ash	8.0
Crude Fiber	3.9
Nitrogen free extracts[a]	14.0
Phosphorus	0.9
Gross energy (kJ g^{-1} diet)[b]	21.8
P:E (mg kJ^{-1})	19.3
PE:GE	0.46

[a] Calculated by difference.

[b] Calculated according to 23.6 kJ g^{-1} protein, 39.5 kJ g^{-1} lipid, 17 kJ g^{-1} nitrogen free extract.

Statistics

Values were expressed as mean ± SD for each of the measured variables. The statistical significance ($P < 0.05$) of body indices proximate composition, and metal concentrations in fish tissues were tested using one-way ANOVA followed by a Duncan's multi-comparison test (Duncan, 1955) with SPSS 17.0 (SPSS, Chicago, IL, USA) software package . Prior to analyses all data were checked for homogeneity of variance and normality, and not normally-distributed or non-homogenous data were transformed.

Results

Growth performance and feed utilization

After a feeding period of 180 days, fish stocked in offshore copper alloy mesh cages showed a weight increase of about 89 g during the course of the study, with and FCR of 2.51, and reached a final weight of 264.8±16.8 g at the end of the trial. Growth performance and feed utilization results are given in Table 2.

Table 2. Growth performance and feed efficiency of axillary seabream cultured in copper alloy mesh cage for 180 days (means ± SD).

	Axillary Seabream *(P. acarne)*
Initial body weight (g)	176.0±14.0
Final body weight (g)	264.8±16.8
Wet weight gain (WWG, g)	88.80
Relative growth rate (RGR, %)	50.47
Specific growth rate (SGR, % day^{-1})	0.23
Feed conversion ratio (FCR)	2.51

WWG (individual) = final weight – initial weight
RGR (percent increase in weight) = (final wet weight - initial wet weight/initial wet weight) x 100
SGR (% growth day^{-1}) = ((ln Final weight, W2 – ln Initial weight, W1) / (total days, t2-t1)) x 100
FCR = feed intake (g) / weight gain (g)

Biochemical composition of fish body

Protein and lipid contents of fish muscle tissue in axillary seabream increased with the increase of fish growth during the course of the study. However, the differences between the initial and final fish body compositions were not statistically significant ($P > 0.05$; Duncan, 1955). As expected a negative relation was observed between moisture and lipid contents in fish body (Table 3). Similar to the protein contents, ash levels in fish body also increased with the increase of fish weight, but these were also not significantly different ($P > 0.05$; Duncan, 1955). Body indices of fish grown in copper alloy mesh have also shown seasonal variations. Among body indices recorded in the present study, VSI, MFI and SSI significantly increased ($P < 0.05$; Duncan, 1955) with the increase of fish weight during the course of the study. Fish body proximate composition and several body indices of experimental fishe grown in copper alloy meshting are given in Table 3.

Trace elements in experimental fish

The level of Zn in the liver of axillary seabream grown in copper alloy cage for 180 days significantly increased ($P < 0.05$; Duncan, 1955) over the growth period with the increase of fish size. In the skin, muscles and gills however, Zn levels did not differ significantly ($P > 0.05$; Duncan, 1955) with the increase of fish size. In contrast to tissue Zn levels, Mn concentrations in skin, muscle and gills of axillary seabream tended to decline significantly ($P < 0.05$; Duncan, 1955) with the increase of fish size, which was not the case for Mn contents in the liver of fish. Cu concentrations in liver of axillary seabream increased significantly ($P < 0.05$; Duncan, 1955) with the increase of fish weight during the course of the study. Similarly, Cu levels in the muscle tissue of fish increased to three-fold ($P < 0.05$; Duncan, 1955) over the initial value at the end of the 180-day experiment. In contrast, Cu concentrations in the skin and gills of axillary seabream decreased sinificantly ($P < 0.05$; Duncan, 1955) with the increase of fish weight. The levels of Fe found in the tissues of axillary seabream grown in the copper alloy mesh cage tended to increase with the increase of fish size at the end of the growth period of 180 days, except for the Fe levels in the gills. Results of the metal contents (Zn, Mn, Cu and Fe) in various tissues (liver, skin, muscle and gills) of axillary seabream cultured in copper alloy mesh cage are given in Table 4.

Discussion

Growth performance and feed utilization data of axillary seabream showed satisfactory results in terms of relative growth rates and feed conversion ratio. There is a lack of information regarding growth performance and feed utilization of axillary seabream (*Pagellus acarne*) in culture conditions, and to our knowledge so far, there are only two reports on growth and feed utilization of this species under culture conditions (Greco *et al.,* 1995 and Guner *et al.,* 2013). Greco *et al.* (1995), investigated growth performance and feed conversion efficiency of axillary seabream under different stocking rates. At the lowest stocking density of 10 fish m^{-3} (mean body weight of 11 g), Greco *et al.,* (1995) reported FCRs ranging from 4.7 to 8.5 after a growth period of 220 days for axillary seabream (*P. acarne*), which was over 2 to 3 fold higher than our findings for FCRs (2.51) in the present study.

Table 3. Body proximate composition (% dry basis, except for moisture) and biological indexes of axillary seabream reared in copper alloy mesh cage for 180 days. Values (means ± SD for triplicate groups) with different superscripts in the same line are significantly different at 5% level.

	Initial	Day 90	Day 150	Day 180
Fish weight (g)	176.0±14.0	223.7±17.2	240.5±18.6	264.8±16.8
Moisture	70.77±3.19[a]	70.12±2.88[a]	70.06±2.61[a]	70.01±2.93[a]
Lipid	18.25±4.89[a]	18.37±5.51[a]	18.39±5.46[a]	21.85±4.42[a]
Protein	66.72±7.29[a]	67.10±8.62[a]	69.02±4.83[a]	70.33±2.68[a]
Ash	4.53±0.68[a]	4.57±0.55[a]	4.64±0.50[a]	4.71±2.20[a]
VSI	N/A	8.25±1.50 [a]	8.80±0.98[a]	10.58±0.62[b]
HSI	N/A	1.45±0.22[b]	1.14±0.15[a]	0.99±0.16[a]
MFI	N/A	2.24±0.57[a]	5.25±1.13[b]	3.86±1.37[b]
SSI	N/A	0.02±0.00[a]	0.03±0.00[b]	0.03±0.01[b]
GSI	N/A	N/d	N/d	0.15±0.03

VSI= Viscerosomatic index, HSI= Hepatosomatic index, MFI= Mesenteric fat index, SSI= Spleen somatic index, N/A= not available, N/d= not detected.

In a more recent study, Guner *et al.* (2013) investigated growth and feed conversion rates of axillary seabream in sea cages in the Aegean Sea and reported an FCR of 3.3 after a feeding period of 13 month. Our finding for FCR of axillary seabream was also much lower than the reported value of Guner *et al.* (2013).

Besides the above mentioned reports on growth of axillary seabream, other studies focused on the age distribution, growth, reproduction and length of first maturity of axillary seabream naturally caught from the Canarian Archipelago (Pajuelo & Lorenzo, 2000) and the South Coast of Portugal (Coelho *et al.,* 2005).

Fish growth performance and feed utilization data may differ according to environmental conditions such as water temperature, salinity, dissolved oxygen or culture conditions such as fish stocking rate or feeding methods such as hand feeding, automatic feeding, demand feeding or restricted, etc. Closed recirculating aquaculture systems for example offer controlled rearing conditions where farmers can monitor fish behavior during feeding and make sure that all the fish in tank consume the feed supplied. However, the control of feeding behavior in fish cages especially in exposed locations is more difficult in terms of even distribution of feed in the cage and the loss of uneaten pellets is an important effect that may increase FCR. Due, it is important to compare growth performance and feed utilization data under similar culture conditions. Overall, RGR, SGR and FCR of axillary seabream in the present study were better than those reported by Greco *et al.* (1995) and Guner *et al.* (2013) for this fish species, and also comparable to those reported for gilthead seabream or European seabass, which are the main aquaculture species for the Mediterranean region.

Feed conversion rate (FCR) of axillary seabream found in the present study fell within the range of earlier reports for gilthead seabream under similar temperature conditions. Bischoff *et al.* (2005) reported FCRs of 1.1 - 1.2 in gilthead seabream reared in recirculating aquaculture system tanks. In an experimentation conducted in a commercial fish farm in the Aegean Sea, Korkut & Balki (2004) reported FCRs of ranging from 0.96 to 3.06 for gilthead seabream fed at different feeding levels in floating cage systems. Taher (2007) investigated feed conversion and growth rates of gilthead seabream reared in floating cages at different densities and reported FCRs ranging from 1.14 to 3.73, 1.34 to 3.90, and 1.32 to 3.78 at densities of 50 kg m^{-3}, 100 kg m^{-3}, and 150 kg m^{-3}, respectively. Compared to the findings from previous studies, the FCR (2.51) observed in the present study is satisfactory in terms of the utilization of feed supplied in a floating fish cages.

Evaluation of growth performance and feed utilization of axillary seabream in copper alloy mesh cage has shown that fish growth was effective and beneficial in a demonstrably biofouling-free cage environment. No organic growth was observed on Copper alloy mesh material used in the present study over its full 180-day duration. Underwater monitoring showed a good fish condition with no disease symptoms, possibly due to a more sanitary environment and reduced stressful conditions in the cage, which is an important sign for improved fish welfare as also reported by Yigit *et al.* (2013)Burry et al. (2003) reported that copper has a role as a co-factor for a number of key proteins such as dopamine hydroxylase, cytochrome oxidase, superoxide dismutase, and ceruloplasmin. Similar to Iron, the flexible redox state of copper shows that it plays an important role in cellular respiration with cytochrome c oxidase as an important copper protein. Hence, copper is accepted as an essential element and the amount of daily requirements in the diets of fish are given as 15-60 µmol (1-4 mg) Cu kg^{-1} dry mass (Watanabe *et al.,* 1997). On the other side,

Table 4. Trace elements in body tissues of axillary seabream cultured in copper alloy mesh cage for 180 days. Values (means ± SD for triplicate groups) with different superscripts in the same line are significantly different at 5% level.

	Initial	Day 90	Day 180
Fish weight (g)	**176.0±14.0**	**223.7±17.2**	**264.8±16.8**
Zn			
Liver	67.26±9.15[a]	120.1±32.4[b]	171.2±34.2[b]
Skin	115.9±14.3[c]	119.3±25.7[c]	147.9±21.7[c]
Muscle	9.37±1.98[a]	11.24±1.93[a]	11.62±2.11[a]
Gills	49.24±6.77[a]	49.58±13.5[a]	49.53±3.66[a]
Mn			
Liver	N/d	2.96±0.45[b]	2.53±0.46[b]
Skin	7.22±1.16[c]	2.33±0.14[b]	0.50±0.19[a]
Muscle	N/d	0.52±0.12[b]	0.18±0.06[a]
Gills	6.13±2.57[b]	4.77±0.33[b]	3.77±1.60[b]
Cu			
Liver	10.41±2.36[a]	32.26±12.4[b]	49.80±14.8[bc]
Skin	6.92±1.69[d]	4.39±0.88[cd]	0.55±0.18[a]
Muscle	1.01±0.15[a]	1.53±0.26[b]	1.94±0.43[b]
Gills	6.20±1.32[c]	2.42±0.35[b]	0.78±0.13[a]
Fe			
Liver	100.5±10.4[b]	162.6±20.1[c]	178.3±54.5[c]
Skin	15.41±2.74[ab]	18.75±1.90[bc]	25.27±6.47[cd]
Muscle	3.77±0.55[a]	3.55±0.81[a]	11.80±1.82[c]
Gills	81.55±10.9[b]	116.2±12.9[c]	109.5±36.1[bc]

Concentrations (µg g^{-1} dry wt), N/d= not detected.

however, when copper is at excess levels, it can be toxic for fish and so for humans through the food chain. Hence, knowledge on limits of metal concentrations in food is vital for a safe food category. The maximum copper level permitted is given as 30 mg kg^{-1} by the World Health Organization (WHO, 1996) and 20 mg kg^{-1} for by MAFF (1995) and Turkish Food Codex (Anonymous, 2008).

In the present study, copper concentrations in the muscles and skin, which are the edible parts of fish, were found to be much lower than the above given upper limits for human consumption. Earlier studies on copper levels in fishes were reported between 0.32–6.48 mg kg^{-1} (Türkmen *et al.,* 2009) in the muscles of gilthead seabream, *Sparus aurata* from the Aegean Sea and the Mediterranean Seas. Cu levels in the liver of fish was reported between 5.29-14.9 mg kg^{-1} in gilthead seabream, *Sparus aurata* from the Marmara, Aegean and the Mediterranean Seas (Türkmen *et al.,* 2009) and between 0.35–12.0 mg kg^{-1} in fishes from Tuzla Lagoon (Dural *et al.,* 2007).

Similar to copper, zinc is also known as an essential element due to its vital structural or catalytic importance in over 300 proteins, playing important roles on fish growth and reproduction, as well as on the immune system (Watanabe *et al.,* 1997; Burry *et al.,* 2003). Previous studies, reported zinc levels around 56.3 mg kg^{-1} in the muscle tissues of gilthead seabream *Sparus aurata* from Black Sea and the Aegean Sea (Uluozlu *et al.,* 2007), and between 4.49-11.6 mg kg^{-1} in muscle tissues of gilthead seabream, *Sparus aurata* from Marmara, Aegean and the Mediterranean sea (Türkmen *et al.,* 2009).

Manganese is also considered as one of indispensably important essential trace elements. Manganese is reported to be an actor for the actions of some enzymes, being a structural component of some enzymes (Watanabe *et al.,* 1997). Uluozlu *et al.* (2007) a manganese concentration of 4.72 mg kg^{-1} in muscle tissues of *Sparus aurata* from the Black Sea and the Aegean Sea, while Türkmen *et al.* (2009) reported manganese levels between 0.1–0.99 mg kg^{-1} in muscles and 0.55–5.40 mg kg^{-1} in livers of *Sparus aurata* from Marmara, Aegean and the Mediterranean Seas.

Iron is known as an indispensable nutrient for living organisms. With the presence of iron in the haem moiety of hemoglobin improves oxygen binding andcarrying capacity for the oxygen transfer to the tissues of the organisms. A negative consequence of iron's redox flexibility is its production of oxygen free radicals which are toxic to the organism (Burry *et al.,* 2003). Excess iron levels in the tissues can be toxic and may have negative effects on fish health (Dalzell & MacFarlane, 1999). Iron levels in fish tissues have been reported between

7.46-40.1 mg kg^{-1} in muscle tissues and between 105-442 mg kg^{-1} in the liver tissues for gilthead seabream (*Sparus aurata*) from the Marmara, Aegean and the Mediterranean Seas (Türkmen *et al.,* 2009), whereas Uluozlu *et al.* (2007) reported a value of 69.7 mg kg^{-1} for muscle tissues of gilthead seabream from the Black Sea and the Aegean Sea. Results on iron concentrations obtained in our study show similar tissue concentrations with the previous reports.

The legal limits allowed for maximum copper level is reported as 30 mg kg^{-1} by the World Health Organization (WHO, 1996) and 20 mg kg^{-1} by the Turkish Food Codex (Anonymous, 2008). The upper limit for zinc in fish tissue for human consumption is reported as 50 mg kg^{-1} (Anonymous, 2008). Overall, metal concentrations in fish tissues of axillary seabream cultured in Copper alloy mesh cage were below the reported permissible upper limits for human consumption levels, showing that copper-alloymesh netting is a safe material for cage aquaculture, in terms of their minimal corrosive metal loss to the surrounding water environment and fish tissue accumulation.

As a result, trace metals in fish grown in copper alloy mesh cage were below the upper limits for human consumption, supporting the use of copper alloy mesh nets in cage farming. Growth performance of fish also was satisfactory leading to a conclusion that axillary seabream is a promising candidate fish species for the Mediterranean aquaculture industry. The present study monitored metal accumulation in fish grown in a copper alloy cage until market size. However, further studies are encouraged to investigate the long term effect of copper alloy mesh nets on metal accumulation in tissues of larger fish sizes as well as the nutritional requirements and dietary optimizations for this species.

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