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RESEARCH PAPER

Comparision of Copper Alloy Mesh with Conventional Nylon Nets in Offshore Cage Farming of Gilthead Seabream (*Sparus aurata*)

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Abstract

In the present study, a new net technology of copper alloy mesh (CAM) was tested and compared with traditional nylon nets either with (TNN_{+AF}) -or without antifouling coats (TNN_{-AF}) in a long-term growth experiment on gilthead seabream (*Sparus aurata*). Fish performed better growth and feed utilization in the CAM pen over the TNN_{+AF} and TNN_{-AF}, with a survival rate over 85% in all cage environments after the 7 months growth period. Relative wet weight gain of seabream in the CAM and TNN_{+AF} pens were 25% and 15% higher compared to the TNN_{-AF} cage, respectively. Surface of the TNN_{-AF} mesh remained clean for two months after sea water deployment, while the mesh size in the TNN_{+AF} pen remained effective for four months, and shrinking in mesh size thereafter. Dissolved oxygen was highest inside the CAM followed by the TNN_{+AF} and TNN_{-AF} cages, respectively. The results of the present study demonstrate that CAM might be beneficial for cage farming in offshore conditions, however further studies are encouraged to evaluate leaching of metals into the marine environment as well as toxic influences on fish tissues and health risks to human consumers.

Introduction

The expansion of cage aquaculture facilities to more exposed marine ingenerates new challenges for fish farmers such as net panel deformations and reduction in cage volume due to drag forces in high currents, risks of fish escapes by net failures in storms or predator attacks, or the development of biofouling on fish nets, creating significant problems such as reduced water flows. The settlement and development of marine organisms significantly increase operational costs of farms because cleaning or replacing the nets with new ones is expensive, time consuming and stressful to the fish (Hodson, Lewis, & Burke, 1997; Solberg, Saethreb, & Julshamn, 2002; Chambers, Bunker, Watson, & Howell, 2012). Fouled fish nets may reduce drag resistance of the pens, resulting in a less water flow in the cage environment and reducing oxygen levels leading to an increased stress environment, hence a lower fish welfare (Braithwaite & McEvoy, 2005; Braithwaite, Carrascosa, & McEvoy, 2007; Nys & Guenther, 2009; Fitridge, Dempster, Guenther, & de Nys, 2012; Bloecher, Olsen, & Guenther, 2013; Klebert, Lader, Gansel, & Oppedal, 2013). In order to fight this problem, cage farmer impregnate the fish nets with antifouling coats (Nys & Guenther, 2009; Fitridge *et al.*, 2012), which contain active ingredients such as cuprous oxide, cadmium and zinc (Hodson *et al.*, 1997; Voulvoulis, Scrimshaw, & Lester, 1999). However, active compounds eventually leach out over time (6-8 months) (Braithwaite *et al.*, 2007; Bloecher *et al.*, 2013; Castritsi-Catharios, Neofitou, & Vorloou, 2015), resulting in accumulation of the active ingredients in the water column as well as the sediments under fish cages that may have toxic

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effects on non-target marine life (Katranitsas, Castritsi-Catharios, & Persoone, 2003; Nys & Guenther, 2009; Burridge, Weis, Cabello, Pizarro, & Bostick, 2010).

As a new approach to overcome these problems encountered in cage farms under high energy conditions, copper alloy mesh is reported as a new technology with a potential to improve fish welfare in lower stress conditions (Wilks, Michels, & Keevil, 2006; Lowell, 2012; González, Hurtado, Gace, & Augsburger, 2013), prevent risks of fish losses in storms (Moe, Gaarder, Olsen, & Hopperstad, 2009), reduce environmental impacts compared to traditional nylon nets (Dwyer & Stillman, 2009; Ayer, Martin, Dwyer, Gace, & Laurin, 2016; Buyukates, Celikkol, Yigit, DeCew, & Bulut, 2017), avoids biofouling development on the mesh (Tuthill, 1987; Braithwaite & McEvoy, 2005; Lowell, 2012; Drach *et al.*, 2013; Chambers *et al.*, 2012; Carvalho, Doma, Sztyler, Beech, & Cristiani, 2014), and improves economic benefits in aquaculture facilities (Chambers *et al.*, 2012; González *et al.*, 2013; Yigit, Ergün, Bulut, Celikkol, & Yigit, 2017). To our knowledge so far, there are only few studies available regarding the performance of copper alloy mesh versus nylon pens with reports from British Columbia (Dwyer & Stillman, 2009; Gray *et al.*, 2013), Hawaii (Lowell, 2012), Chile (González *et al.*, 2013; Ayer *et al.*, 2016), the USA (Chambers *et al.*, 2012), Turkey (Yigit *et al.*, 2016), and Greece (Kalantzi *et al.*, 2016).

Gilthead seabream is one of the most cultured and commonly consumed marine fish species in Europe and seabream grow-out is practiced in offshore cages with double nets since net biting is a behavioral characteristic of seabream, creating holes through which the fish may escape into the natural waters (Moe, Dempster, Magne Sunde, Winther & Fredheim, 2007; Jackson *et al.*, 2015). Escapes of aquaculture fish from cages have been significant problem with consequences of slowed expansion of the marine aquaculture industry in Norway (Jensen, Dempster, Thorstad, Uglem, & Fredheim, 2010; Thorvaldsen, Holmen, & Moe, 2015) and in Scotland (Ellis, Turnbull, Knowles, Lines, & Auchterlonie, 2016). Hence, it is clear that alternative containment materials capable of reducing biofouling and fish escapements might be beneficial for the development of the aquaculture industry.

The aim of the present study was to compare the growth performance and survival of gilthead seabream cultured in copper alloy mesh and traditional nylon net cages with antifouling paint at industry standards in order to assess the potential of copper alloy mesh in marine aquaculture facilities.

Materials and Methods

Experimental Cages

Offshore gravity type high density polyethylene cages, two of them with a volume of 150 m^3 and one of 5 m^3, were designed and deployed into a 2-bay grid system moored with 12 anchors of 500 kg deadweight in the Strait of Canakkale (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), 0.6 nautical miles off the coast of Dardanos town (40°03’42”N - 26°20’36”E). The depth of the sea location was approximately 45 m and the cages had a diameter of 6 m and a net chamber depth of 5 m. The third gravity-type cage with a diameter of 1.5 m and a net chamber depth of 3 m served as a control cage for the biofouling monitoring of non-antifouling treated nylon net material in seawater. An antimicrobial wrought copper-zinc brass alloy with the ASTM designation of C44500 (https://www.kupferinstitut.de/en/arbeitsmittel/kupferschluessel.html) has been formed into a mesh of 3.0 cm for the CAM pen. The results of the analyses of the copper-alloy material given by the German Copper Institute are presented in Table 1. The second test cage was equipped with a traditional nylon net of 3.0 cm diameter, coated with a commercial antifouling paint (Flexgard™), a cuprous oxide based antifouling paint (http://www.trademarkia.com/company-flexabar-corporation-451575-page-1-2)(TNN_{+AF}),

Table 1. Copper-alloy material, proportional contents of metals*

Contents	Min (%)	Max (%)	mean ± SD (%)
Cu	70.00	73.00	71.50 ± 2.12
Zn	29.18	25.57	27.38 ± 2.55
Sn	0.80	1.20	1.00 ± 0.28
P	0.02	0.10	0.06 ± 0.06
Pb	N/A	0.07	0.07
Fe	N/A	0.06	0.06

*Antimicrobial wrought copper-zinc brass alloy with the ASTM designation of C44500, data from German Copper Institute: https://www.kupferinstitut.de/en/arbeitsmittel/kupferschluessel.html
N/A: not available

whereas the control cage was also fitted with a traditional nylon net of 3 cm diameter but without any antifouling treatment (TNN_{-AF}).

Experimental Fish and Feeding

A total of 2000 hatchery-reared gilthead seabream, obtained from a commercial marine hatchery (Pinar Marine Fish Hatchery, Izmir-Turkey), were transported to Dardanos Marine Aquaculture Research and Development Center of the Faculty of Marine Science and Technology at Canakkale Onsekiz Mart University (COMU) in Canakkale, Turkey (40°04'37"N - 26°21'39"E). After an acclimation period of one month, two of the 150 m^3 volume cages were stocked with 500 randomly selected seabream. The third cage with non-antifouling treated nylon net was stocked with 20 randomly selected seabream. Initial biomass in each of the experimental cage was arranged as 0.4 kg/m^3 stocking rate. Experimental fish were fed twice a day and fish behavior in cages was monitored during the adaptation period of one month and throughout the study. Well-adapted fish in each cage environment were weighed (initial mean weights; 120.5±21.6, 122.3±26.1 and 123.8±25.4 g for the CAM, TNN_{+AF} and TNN_{-AF} cages, respectively) and the feeding trial was initiated. During the feeding trial, fish were hand-fed two times a day at 09:00 and 17:00 for a period of 7 months (210 days) with a commercial seabream diet (42% protein, 24% lipid, 14% nitrogen free extract, 21.8 kJ/g diet gross energy). Feed intake data were recorded daily and used for evaluations of growth performance and feed utilization of fish in different net materials. Feeding activity was carefully monitored to ensure an even distribution of the feed to all fish in the cage.

Water Quality and Analytical Methods

Water quality parameters such as temperature, salinity, dissolved oxygen (DO), and pH in the three experimental cages were measured at 2 m depth from surface every three days during the course of the study using YSI 6600 MPS multi-parameter analyses device. Nitrite+nitrate (NO^-_2+NO^-_3), and ammonia (NH_4) was performed by spectrophotometric determination according to Strickland and Parsons (1972). Analysis for soluble reactive phosphate (PO_4) was conducted spectrophotometrically according to Parsons, Maita, and Lalli (1984).

Statistical Analyses

Growth data were expressed as mean±SD and statistical significance ($P<0.05$) were evaluated by one-way ANOVA with the SPSS 17.0 software package. Prior to analyses, Kolmogorov-Smirnov normality test and Levene's test were used to present normality and homogeneity of variance of the data, respectively.

Results

During the 7 months of study period, water temperature measured at 2 m depth ranged between 11.4 and 24.6 °C, and salinity between 22.20 and 32.30 ‰. The pH values were almost constant during the course of the study and ranged between 8.02 and 8.35. Ammonia, nitrite+nitrate, and phosphate in the experimental research site were recorded between 0.0003 and 0.05 mg/L, 0.0001 and 0.04 mg/L, and 0.0091 and 0.63 mg/L, respectively. Dissolved oxygen (DO), measured periodically at 2 m depth in all three cage environments, changed among the experimental cage environments, ranging between 7.05 and 10.82 mg/L, with the highest DO of 8.9 ± 1.9 mg/L in the CAM cage, which was followed by the TNN_{+AF} (8.1 ± 1.0 mg/L) and the TNN_{-AF} cages (7.97 ± 0.9 mg/L), respectively (Table 2).

Despite the feeding trial was conducted for 210 days, the recorded actual feeding days were 130, since the experimental site experienced around 10 storms during the course of the study, and feeding was interspaced for 6-8 days during stormy sea conditions. Survival rates of fish in all three cage environments were over 85%, indicating that cage environment did not affect mortality. Experimental fish grown in the CAM cage demonstrated better performance in terms of individual wet weight gain (223.4 g) over the traditional nylon mesh cages with - or without antifouling coats (TNN_{+AF}, 204.3 g; TNN_{-AF}, 178.7 g). The relative wet weight gain (RWWG) of fish grown in the CAM and the TNN_{+AF} pens were about 25% and 15% higher compared to those grown in the TNN_{-AF} cage (Figure 1). Similarly, better SGRs were found for fish grown in the CAM and TNN_{+AF} cages with about 16% and 10% higher relative specific growth rates, respectievly over those cultured in the TNN_{-AF} pen (Figure 2). The feed conversion rates (FCRs) followed a similar trend, with the best FCR (1.48) in the CAM pen, followed by the TNN_{+AF} (1.69) and TNN_{-AF} (2.09) cages, respectively (Table 3).

Discussions

At the end of the feeding trial for a period of 7 months, SGRs in the present study (0.43-0.50% per day) fall in the range (0.32-1.04% per day) of earlier reports (Korkut & Balkı, 2004) for gilthead seabream in commercial cage farms using antifouling-coated nylon nets in the Aegean Sea. Similarly, our findings

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Table 2. Water quality parameters measured during the course of the study

Parameter	Unit	TNN_{-AF}	TNN_{+AF}	CAM
Oxygen (O_2)	mg/L	7.97 ± 0.9	8.1 ± 1.0	8.9 ± 1.9
pH		8.20 ± 0.21	8.15 ± 0.18	8.18 ± 0.15
Temperature	°C	18.1 ± 6.5	18.3 ± 6.6	18.2 ± 6.9
Salinity	‰	28.0 ± 6.4	27.4 ± 5.7	27.8 ± 6.1
Ammonia (NH_4)	mg/L	0.0005-0.05	0.0003-0.05	0.0003-0.04
Nitrite+Nitrate (NO^-_2+NO^-_3)	mg/L	0.0003-0.03	0.0001-0.04	0.0002-0.03
Phosphate (PO_4)	mg/L	0.0109-0.44	0.0121-0.38	0.0091-0.63

TNN_{-AF} (control group): traditional nylon net, no antifouling treatment; TNN_{+AF}: traditional nylon net, antifouling painted; CAM: copper alloy mesh

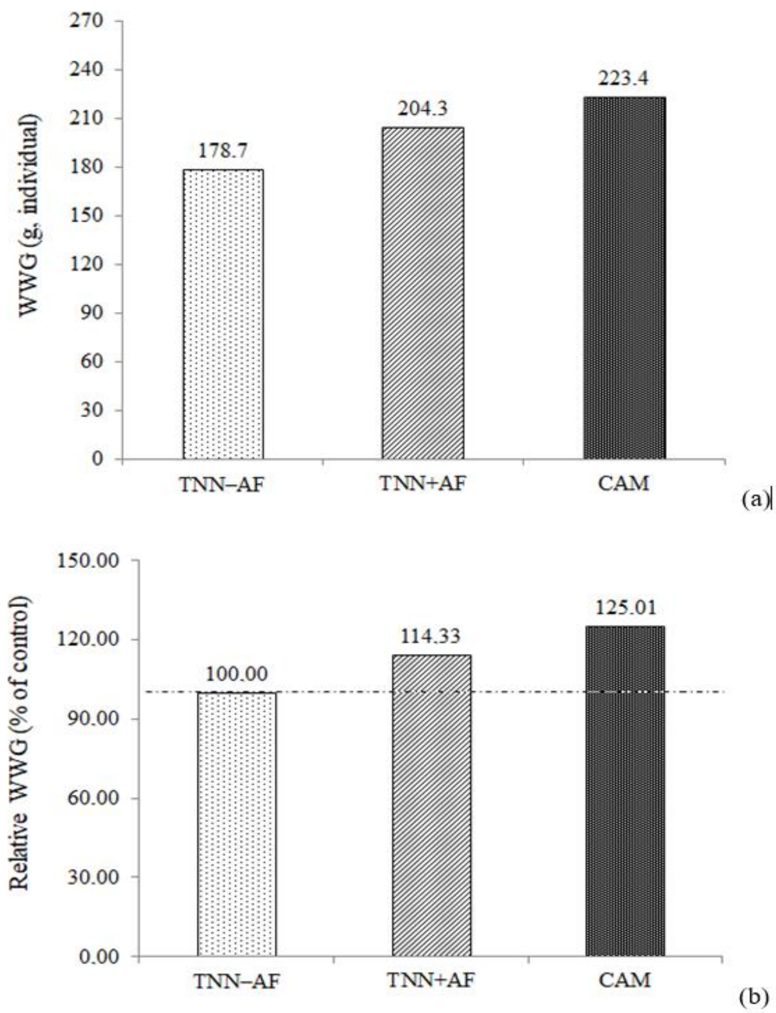

Figure 1. Individual wet weight gain of gilthead seabream in antifouling treated (TNN_{+AF}) and copper alloy mesh (CAM) pens (a) and WWG relative to the control group with non-antifouling treated polymer net (TNN_{-AF}) (b).
Wet weight gain (WWG, g) = final weight – initial weight
Relative WWG (RWWG) = WWG x 100 / WWG

for the FCRs (1.48-2.09) were in agreement and comparable with previous studies on seabream cultured in commercial nylon mesh systems (0.91-3.06, Korkut & Balkı, 2004; 1.14-3.73, Taher, 2007). In contrast, lower FCRs (1.1-1.2) were reported for gilthead seabream in recirculating aquaculture systems (Bischoff, Kube, Wecker, & Waller, 2005). In the present study, the offshore research site experienced around 10 heavy storms during the course of the study hence the feeding activity was weather-dependent. Thus, feeding was withheld for about a week during heavy storms, and the number of actual feeding days was determined as 130 out of the total period of 7 months (210 days) in the present

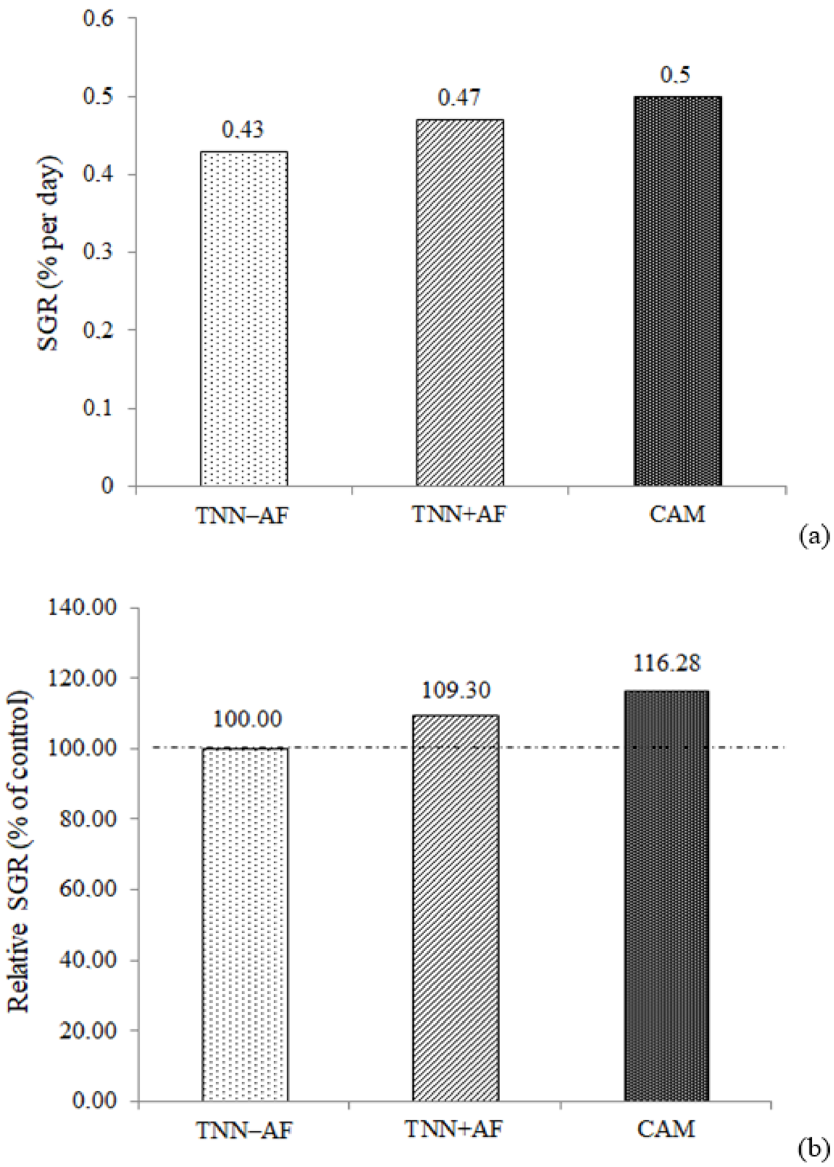

Figure 2. Specific growth rates of gilthead seabream in antifouling treated (TNN_{+AF}) and copper alloy mesh (CAM) pens (a) and SGR relative to the control group with non-antifouling treated polymer net (TNN_{-AF}) (b).
Specific growth rate (SGR, % growth/day) = $((\ln W_{final} - \ln W_{initial})/(t_2 - t_1) \times 100$
Relative SGR (RSGR) = SGR x 100 / SGR

Table 3. Growth performance and feed utilization of gilthead seabream reared in two different nettings for 7 months. Values (means ± SD) with no superscripts in the same line are not significant different at 5% level

	Net Types – Culture Environments		
	TNN_{-AF}	TNN_{+AF}	CAM
IBW (g)	123.8±25.4	122.3±26.1	120.5±21.6
FBW (g)	302.5±19.4	326.6±30.5	343.9±63.9
RGR (%)	144.4	167.1	185.4
FCR	2.09	1.69	1.48
SR (%)	85	87	90

TNN_{-AF} (control group): traditional nylon net, no antifouling treatment; TNN_{+AF}: traditional nylon net, antifouling painted; CAM: copper alloy mesh; IBW: initial body weight; FBW: final body weight
Relative growth rate (RGR) = (final weight–initial weight/initial weight) x 100
Feed conversion rate (FCR) = feed intake (g) / weight gain (g)

study. It is well known that rearing conditions such as water temperature, dissolved oxygen, salinity, or fish stocking densities or feeding methods affect growth performance and feed utilization. This was also confirmed by Taher (2007), who reported that feed utilization appeared to be affected by the culture conditions in their study on gilthead seabream in gravity type cages.

In the present study, gilthead seabream grown to market size in the CAM cage reached higher final weight at harvest and demonstrated a better performance and feed utilization than those in the traditional nylon net pens either with -or without antifouling coats. This might be attributed to the biofouling-free mesh environment compared to the traditional nylon nettings with heavy biofouling development over time. Our findings regarding better growth of gilthead seabream in CAM versus nylon nettings was also supported in earliers reports on farmed fish (Fitridge *et al*., 2012; Gonzalez *et al*., 2013; Ayer *et al*., 2016). In contrast, Chambers *et al*. (2012) did not find significant differences in growth, survival, or feed efficiency in Atlantic cod (*Gadus morhua*) cultured in copper or nylon nettings, however the authors reported significantly more biofouling development on nylon nets with antifouling coats than the copper nets.

Underwater monitoring of cage net panels during the course of the study, showed that biofouling development on the nylon net without antifouling treatment was visible after two month of seawater deployment, whereas the surface of the nylon net with antifouling treatment remained clean for about four months, while thereafter underwent a reduction in the effective mesh size, restricting water exchange, a possible reason for the lower oxygen concentration in the non-antifouling treated nylon net. Findings from earlier reports pointed out that, antifouling paints may prevent biofouling on the nets for about six to eight months (Braithwaite *et al*., 2007; Bloecher *et al*., 2013; Castritsi-Catharios *et al*., 2015). The faster depletion of antifouling coating from the nylon net (4 month) compared to previous reports (6 to 8 month) could be atributed to the strong currents (0.22-2.32 m/s) recorded in our earlier studies (Tarhan *et al*., 2013; Buyukates *et al*., 2017) in the experimental site of Canakkale Strait, Turkey. Eventhough not significant, slightly higher O_2 levels were recorded in the CAM cage environment (8.9 ± 1.9 mg/L DO), compared to the nylon nylon nets either with (8.1 ± 1.0 mg/L DO) or without antifouling treatment (7.97 ± 0.9 mg/L DO), might be attributed to improved water exchange in the biofouling-free cage environment. In agreement to our findings in the present study, earlier reports pointed to a reduced water flow through the cage systems in fouled fish nets leading to depressed dissolved oxygen concentrations inside the pens, affecting fish welfare due to a possibly increased stress environment (Brooks & Mahnken, 2003; Braithwaite & McEvoy, 2005; Lader, Dempster, Fredheim, & Jensen, 2008; Nys & Guenther, 2009; Fitridge *et al*., 2012; Berillis, Mente, & Kormas, 2017). Apart from these findings, it is likely that the biofouling-free environment in the CAM pen with higher dissolved oxygen levels might have improved fish welfare by facilitating a reduced-stress environment with cleaner and more sanitary culture conditions, induced better utilization of diets for growth instead of stress accommodation over the traditional nylon pens as also reported by Braithwaite *et al*. (2007), Fitridge *et al*. (2012). In an earlier study, Gonzalez *et al*. (2013) indicated that CAM pens may not have direct influence on the amount of the feed used, however it may have an indirect effect through improving the culture environment that may enhance fish health and fish growth, instead of diverting some of the energy or nutrients from feed intake for stress responses by fish. These indirect influences may differ among locations due to factors known to affect fish growth, such as water temperature, feeding management, or fish species (Handeland, Bjornsson, Arnesen, & Stefansson, 2003). Furthermore, the one grow-out cycle may not be sufficient to measure changes in these particular performance characteristics and biofouling development on the nylon mesh can become more difficult to handle after multiple grow-out periods than it is over the course of one grow-out cycle (Ayer *et al*. 2016).

Antifouling paints are commonly used in cage farms for the prevention of biofouling on nylon fish nets. The use of antifouling paints began in marine transportation industy, as well as oil and gas industries to control biofouling on surfaces of the materials used in marine environment (Yebra, Kiil, & Dam-Johansen, 2004; Nys & Guenther, 2009; Dürr & Watson, 2010). In cage aquaculture, it is a common approach to paint the fish nets by antifouling coats in order to limite biofouling development and maintain healthy culture environment in the traditional nylon net cage systems. However, biocidal compounds such as heavy metals and organic biocides may leach from these paints onto the surface, providing a thin layer preventing the onset of biofouling organisms but these chemicals are reported as hazardous to non-target marine live as well, with detrimental effects on the survival and growth of shellfish (Paul & Davies, 1986) and fish species (Short & Thrower, 1986; Bruno & Ellis, 1988). It is also reported that antifouling paints in nylon mesh pens may cause 50% increased potential of marine ecotoxicity impacts compared to the CAM systems (Ayer *et al*., 2016). Furthermore, copper used in antifouling paints is lost permanently by leaching into the marine ecosystem, while in contrast more then 98% of the copper used in the CAM nets may be reused at the end of the effective life of 3 to 4 years of grow-out cycles with high recyclability (Chambers *et al*., 2012; Ayer *et al*., 2016). These characteristics of copper to reuse can be considered as a dominant benefit for the sustainability of the material and environment-friendly marine farm activities.

Hence, the control of biofouling development on fish nets still remains a major concern with fish farming and one of the most challenging issues increasing production costs for cage aquaculture industry in the world. Concerns regarding initial investment costs discourage fish farmers from using new technologies such as copper alloy mesh pens. However, it was reported that the cost of 2 cm mesh nylon net, coated with Flexgard is around \$16.87/m^2 compared to a copper alloy mesh with 2.4 cm of \$39.69/m^2 (Chamber *et al*., 2012). Eventhough there is an initial cost differences, copper alloy mesh may perform economically benefitial in long term use, by decreasing operational costs such as net cleaning, repair or replacement with new mesh (Chamber *et al*., 2012; Yigit *et al*., 2017). Additionally, the better growth of fish and improved fish welfare in a less sressful environment may increase fish quality for a better product in the market. Further studies are encouraged to evaluate copper leaching into the environment over time and their effects on marine life around and under the cage farms, as well as toxic effects on fish tissues and human health risks when consuming these fish.

Additionally, the stability or the volumetric integrity of the cage volume in exposed sea conditions is another important factor for benefits to production. Turnbull, Bell, Adams, Bron, & Huntingford (2005) reported that any deformation on the net affects productivity and fish welfare, because reduced cage volume delimits swimming environment of the fish and mitigates water flow rates, which then force an increase of biomass density in the cage. Hence, in the CAM pens, a minimal reduction in cage volume can be expected due to lower drag forces and improved durability of the cages in highly exposed offshore sea conditions as also reported by Tsukrov, Drach, DeCew, Swift, & Celikkol (2011) and González *et al*. (2013). Further, Swift *et al*. (2006) and Lader *et al*. (2008) investigated the hydrodynamic characteristics of nylon nets in floating cages and found net panel deformations due to drag forces at different current speeds. In the present study, underwater monitoring of cage volume integrity was performed by SCUBA diving and net panel deformation in terms of shrinking cage volume and increased fish densities were observed in strong currents due to aggregation of the fish in a narrow area inside the shrunken pens. This might have influenced fish welfare due to the decreased cage volume, as also described by Swift *et al*. (2006), Lader *et al*. (2008), and Chambers *et al*. (2012).

As a result of this study, gilthead seabream showed a better performance in copper alloy mesh pens over their representatives in the traditional nylon net cages, leading to a conclusion that copper alloy mesh could be a promising solution to combat problems arising from biofouling development in marine fish farms and limitate the use of antifouling paints in order to prevent their hazardous effects on marine life, which obviously may support the sustainable growth of an environment-friendly aquaculture industry.

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