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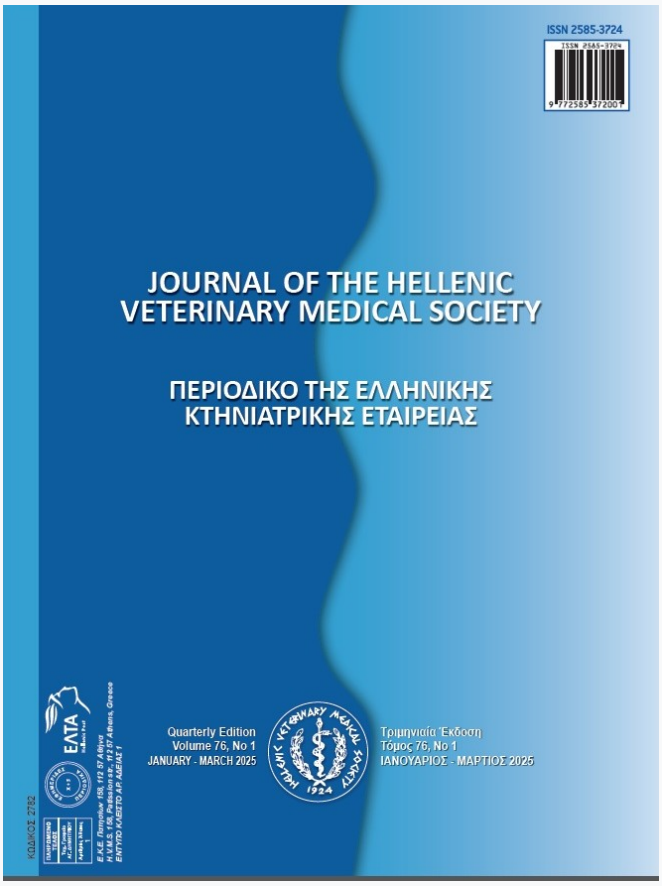

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Research article
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Assessment of microbiological status of fresh gilthead seabream (*Sparus aurata*) from aquaculture in Adriatic sea, Montenegro
Microbiology of gilthead seabream

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ABSTRACT: The aim of this research was to determine the microbiological status of gilthead seabream (Sparus aurata), which were caught in the Boka Kotorska Bay, for the first time in this part of Montenegro coast. Collected fish were analysed for hygiene indicator and pathogens microorganisms through the winter and summer season during one year. Total bacterial counts of aerobic mesophilic bacteria (AB), *Enterobacteriaceae*, *Salmonella* spp., *Escherichia coli*, coagulase-positive *Staphylococci* (CoPS) and sulphite-reducing Clostridia (SRC) were measured. Results show that all samples were suitable for human consumption in terms of microbiology status. The microbiological quality of individual samples varied widely between winter/summer seasons regarding total counts of aerobic mesophilic and *Enterobacteriaceae*. Samples obtained in summer showed significantly higher levels for aerobic mesophilic bacteria and *Enterobacteriaceae* than samples obtained in winter. *Salmonella* spp. and *E. coli* were not detected in fish samples, and also none of the samples tested for coagulase positive *Staphylococci* and sulphite-reducing *Clostridia* were above the detection limit (10 CFU/g). Results underline the relevance of preventing and the application of good hygiene and good manufacturing practice during fish handling to secure the quality and safety of sea bream. Also, results presented in this study is valuable in the assessment of conditions in aquatic environments and this method of testing can be applied in other locations worldwide.

Keywords: microbiological status; *Sparus aurata*; hygiene; Adriatic sea.

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INTRODUCTION

The consumption of fishery products in all world has increased thanks to better consumer information on the health benefits of fishmeat (Domingo, 2007). World fish production has increased to around 179 million tons in 2018, during the past 60 years, with a value of $401 billion (FAO, 2020). Aquaculture is an important industrial sector for Montenegro, where marine fish culture is dominated by white fish, especially gilthead seabream. Farming of gilthead seabream in this country implies a closed breeding cycle in floating cages in the sea. The annual production is 64 tons, still takes place in only two farms which are located in the area of Boka Kotorska Bay and has a declining trend in previous years (MONSTAT, 2020).

Gilthead seabream belongs to the bream family Sparidae, to the order of the Spariformes and class of the Ray-finned fishes (Chouliara et al., 2004). It is an economically cultured fish species, rich protein food source and one the most and increasingly preference cultured fish species worldwide (Caggiano, 2000). At the moment of capture, fish muscle tissue is usually free of microorganisms. Biological hazards related to fish meat may enter the product before, during fishing or later during transport and processing (ICMSF, 2002). However, raw or undercooked fish, have been involved in outbreaks related with bacteria and viruses, histamine or parasites (Galaviz-Silva et al., 2009).

The microbiological condition of fish may depend on cultivation techniques, the method by which fish are harvested, environmental conditions or intestinal tract of fish (Svanevik et al., 2015). Moreover, contamination with pathogenic bacteria can occur because the insanitary conditions in fish boats or when the fish is washed with contaminated water (Mokrani et al., 2018). The microbial ecology of Mediterranean fish, like as gilthead sea bream, can include diverse microorganisms such as coagulase positive *Staphylococci*, sulphite-reducing *Clostridia* (SRC) and *Salmonella*. These include also food hygiene indicator microorganisms, such as aerobic mesophilic bacteria and *Enterobacteriaceae*, coliforms and *E. coli*, which can be associated with an increased risk of exposure to a pathogen and a impaired product shelf-life because of microbial spoilage (Anihouvi et al., 2019).

Despite its economic importance, there is a shortage of information about microbiology status of this fish species in Montenegro mariculture. Because of that, the aim of this study was to determine the microbiological correctness of Gilthead sea bream from Adriatic sea (Montenegro), according to the directive of the European Commission, during the summer and winter period.

MATERIALS AND METHODS

Samples, transport and sample preparation

Fish were randomly caught in the coastal waters, located in Montenegro (South Adriatic Sea), in warm (July, August) and cold (December, January) months of the year. Two large farms located in Boka Kotorska Bay were selected as sampling sites (Fig 1.).

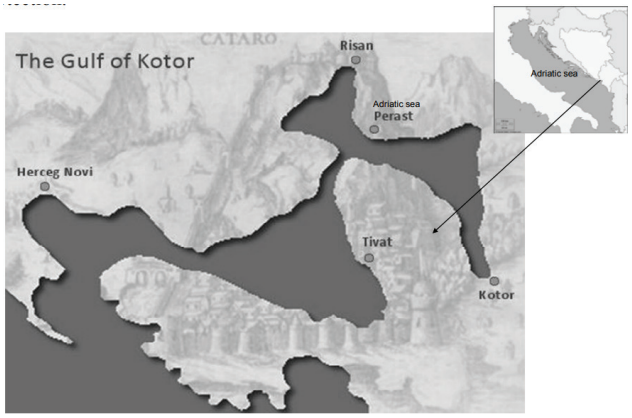

Figure 1. Geographical position of the sampling sites

A total of 30 individuals, at suitable commercial size (300g), were collected and slaughtered in liquid ice (15 samples per season). Individual laboratory samples were packed separately, with waterproof markings to allow traceability of the samples. Then, gilthead seabreams were transported immediately to the accredited laboratory in thermal boxes with ice (<4°C) (Diagnostic veterinary laboratory, Podgorica), and then subjected to microbiological analyses. Sample preparation for microbiological analyses is carried out according to EN ISO 6887-3 Microbiology of the food chain — *Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products*. In aseptic conditions, using sterile scissors and tweezers, two incisions (one removes the tail and the other a steak) and take a cube-shaped back muscle sample. Previously, the skin and all viscera were removed. Then, all samples were homogenized during 60 s at 1500 rpm using a stomacher with corresponding diluent (peptone water) to obtain a 1:10 dilution.

Microbiological Analysis

Microbiological analysis on samples was carried

out according to standard ISO methods in accredited laboratory. All gilthead seabreams were analysed for aerobic mesophilic bacteria (AB) counted as colony forming units (CFU/g) according to the ISO 4833:2003. Using the standard method, ISO 21528-2:2004, the number of *Enterobacteriaceae* in samples was determined. Enumeration and detection of *Enterobacteriaceae* was carried out by counting colonies on a Violet Red Bile Glucose Agar. The coagulase-positive *Staphylococci* (CoPS) were enumerated according to the ISO 6888-1:1999/Amd 1:2003. This method prescribes the use of rabbit plasma, which confirms that the detected staphylococci are coagulase positive. The number of *E.coli* were determined according to the ISO 16649-2:2001. The use of this highly selective microbiological medium allows the detection of betaglucuronidase-positive *E. coli* without additional identification tests, which speeds up the testing process and makes it more efficient. Determination of *Salmonella* spp. was conducted from samples diluted in 225 ml buffered peptone water incubated at 37°C for 18 hours and with secondary selective enrichment in Rappaport-Vassiliadis peptone broth (41°C for 24 h) and Muller-Kaufmann tetrathionate broth with Novobiocin (37°C for 24 h), and plating on XLD agar and Rambach agar, XLT-4 agar (37°C for 24 h), according to the ISO 6579-1:2017/Amd 1:2020. Primary procedure in analysis of sulphitereducing *Clostridium* spores was to eliminate vegetative cells with heat treatment of the tubes containing the first decimal dilution in water bath at 75°C/20 min. This first, as well as all other steps were carried out in accordance with the standard method ISO 15213:2003.

Statistical analysis

Statistical analysis of the results was elaborated using software GrapfPad Prism version 5.00 for Windows, GrapfPad Software, San Diego, California USA, www.graphpad.com i MS Excel. Descriptive statistics were used to express the mean log, standard deviation and coefficient of variation. Data were analysed statistically using the non-parametric test of Kruskal-Wallis for multiple comparisons. The statistical test assessed whether season exerted a significant effect ($p<0.01$) on the microbiological parameters. Microbial counts obtained in the collected samples were transformed logarithmically (log cfu/g).

RESULTS

Average total counts of aerobic mesophilic bacteria (AB) in summer/winter samplings are presented in Fig 2. In summer samples, the mean value of AB was 3.63 log CFU/g, while in the winter samples the mean value of AB was 2.41 log CFU/g. The comparison of this samples revealed statistical differences in AB values between summer/winter (Tables 1). The average AB values of the fresh fish sampled in summer were significantly higher than average AB values of fish sampled in winter ($p<0.01$).

Out of 15 collected summer samples 13 (86.67%) showed positive isolation of *Enteriobacteriaceae*, above the sensitivity method threshold (10 CFU/g). Contrary to that, in winter samples the number of *Enterobacteriaceae* above 10 CFU/g were recorded in only 4 (26.67%) samples (Fig 3).

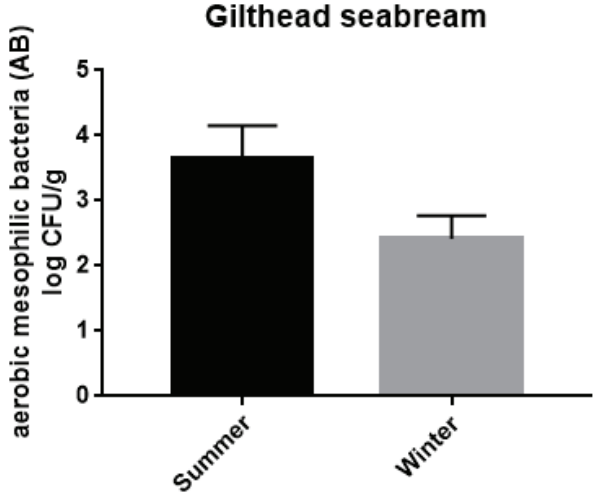

Figure 2. Aerobic mesophilic bacteria values for Gilthead seabream samples collected at the summer and winter during one year in Montenegro.

Table 1. Statistical data of total counts of aerobic mesophilic bacteria (AB) (log CFU/g) in Gilthead seabream samples in summer/winter sampling

	n	$\bar{x}$	SD	SE	CV (%)	X max	X min
Summer	15	3,63[a]	0,4997	0,129	13,68	4,32	2,9
Winter	15	2,41[a]	0,3614	0,0933	14,97	2,87	1,7

Statistical significance is shown with the same letters: a - $p<0.01$

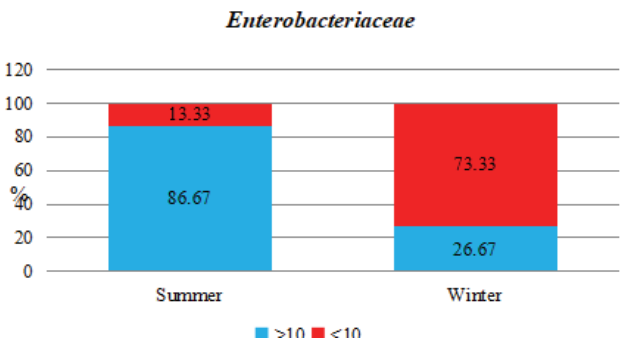

Figure 3. The percentage of summer/winter samples in which the number of *Enterobacteriaceae* were below and above the sensitivity method threshold (10 CFU/g)

In all tested samples, both in summer and winter months, we did not detect pathogenic microorganisms, *Salmonella* spp. and *E. coli.* As for sulphite-reducing *Clostridia* and coagulase-positive *Staphylococci*, their number was below the detection threshold (10 log CFU/g) in all sea bream samples tested.

DISCUSSION

The flesh of live healthy fish is sterile and one of the food categories with the shortest shelf life, and its quality is affected by many factors as the origin, cooling methods, treatment and storage conditions. Microorganisms are only present on the exterior surfaces (gills, skin) and in the gastro-intestinal tract of fish, and they are reflecting the types of bacteria which are in their environment (Stratev et al., 2015). There are only a few researches that included the study of food hygiene indicator microorganisms and pathogenic bacteria in Gilthead sea bream from aquaculture (Haldar et al., 2010; Floris et al., 2013; Parlapani and Boziaris, 2016). The results of this study constitute an indicator of the microbiological quality of gilt-head sea bream (*Sparus aurata*) caught off the Adriatic coast of Montenegro, for the first time in this part of the coast. Total bacterial counts of aerobic mesophilic bacteria (AB), *Enterobacteriaceae, Salmonella* spp., *Escherichia coli*, coagulase-positive *Staphylococci* (CoPS) and sulphite-reducing *Clostridia* (SRC) were used as microbial indices to evaluate the microbiological quality of the gilthead seabream. The general indicators of microbiological quality, which are widely used food hygiene indicator microorganisms in fish products comprise aerobic mesophilic bacteria and *Enterobacteriaceae* (Anihouvi et al., 2019). According to the microbiological upper limit for fresh fish, proposed by ICSMF (1986) for aerobic mesophilic plate counts at 30°C (7 log CFU/g) all samples were acceptable and did not exceed the maximum level for gilthead seabream regardless of the season harvested. The mean value of mesophilic count (3.63 log CFU/g in summer samples, and 2.41 log CFU/g in winter samples) in the present study is in correlation with results which were obtained in Spain (Andrés-Bello et al., 2015; Costa et al., 2020), Turkey (Şengör et al., 2021) and Croatia (Topić-Popović et al., 2020). The microbiological quality of individual samples varied widely between the winter and summer seasons. In our research, median values for AB counts of the fresh fish sampled in summer were significantly higher than AB counts of the fresh fish sampled in winter ($p<0.01$). Another index of fish quality is the number of *Enterobacteriaceae*, because it is connected to fish storage and handling (*Zambuchini et al.,* 2008) and it can indicate if these procedures have been performed following the honor of the HACCP norms (Hazard Analysis Critical Control Point) related to the hygiene and to the foods safety (*Directive 02/99/EC, 2003; Regulation 04/853/EC, 2004*). Concentration of *Enterobacteriaceae* strictly dependent on the degree of water pollution, increases with the spring/summer season when urban waste increases due to tourism activities. In this study also, only 13,33% of the summer samples showed an count under the detection limit. Concerning winter samples, 73,33% were below the sensitivity method threshold (<10 CFU/g). This results are expected, considering the tourist season, and a much larger number of people during the summer months in this place.

Besides this microorganisms, *Salmonella* spp. and *E. coli* are important for food safety. Fish can be infected by *Salmonella* through contact with contaminated water or inadequate hygiene when handling, but they are not their natural inhabitants (*Fernandes et al.,* 2018). The presence of this pathogen in marine environments may arise from the contamination by untreated sewage *(Lee et al.,* 2008). The presence *E. coli* in food indicates directly or indirectly fecal contamination, depending on the fecal origin. According to the microbiological analysis results in this study, *Salmonella* spp. and *E. coli* were not detected in fish samples. That is an indicator that the consumption of gilthead seabream was suitable for food safety.

Poor hygiene or sanitation during fish capture can lead to presence of *Staphylococcus* spp. coagulase +, which is not part of the natural microbiota of fresh fish. Their enterotoxins are serious cause of gastroenteritis after consumption of fish and related prod-

ucts (*Al Shabeeb et al.*, 2016).

Sulphite reducing C*lostridia* are group of bacteria that reduce sulphate to sulphite, and their presence in water indicate the remote faecal contamination (*Borrego et al,* 2002). *C. perfringens* is a key species and an important cause of food poisoning. This group of bacteria can withstand the cooking temperature, because of that the hygienic handling practices have to be followed avoid the sulphite reducing clostridia/*C. perfringens* in fish. In our research, none of the summer/winter samples tested for coagulase positive *Staphylococci* and sulphite-reducing *Clostridia* were above the detection limit (10 CFU/g). It indicates the good microbiological quality of the fish samples.

CONCLUSION

This is the first complete examination of the microbiological status of sea bream in this part of Southern Adriatic Sea (Montenegro), which included both hygiene indicators and pathogenic microorganisms that can be found in fish. The results of the microbiological analyses of muscle of gilthead seabream caught off the Adriatic coast of Montenegro can be defined as quite satisfactory, given that the majority of samples complied with the reference standards. Both groups were found to be suitable for human consumption in terms of hygiene indicator microorganisms and pathogenic bacteria. Besides, the absence of *Salmonella* spp. and *E. coli* is the indication that fish are suitable for consumption in terms of food safety. This results also underline the relevance of preventing the microbiological contamination of water in fish production systems in order to assure the quality and safety of gilthead sea bream.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST None declared

REFERENCES

Al Shabeeb SS, Ibrahim MM & Ramadhan GA (2016) A comparative microbial quality assessment among fishes, prawns and cuttlefishes collected from dammam fish market. International Journal of Current Microbiology and Applied Sciences, 5(9):405-418.

Andrés-Bello A, De Jesús C, García-Segovia P, Pagán-Moreno MJ & Martínez-Monzó J (2015) Vacuum impregnation as a tool to introduce biopreservatives in gilthead sea bream fillets (*Sparus aurata*). LWT-Food Science and Technology 60(2): 758-765.

Anihouvi DGH, Kpoclou YE, Abdel Massih M, Iko Afe OH, Assogba MF, Covo M & Mahillon J (2019) Microbiological characteristics of smoked and smoked-dried fish processed in Benin. Food science & nutrition, 7(5):1821-1827.

Borrego JJ, Castro D & Figueras MJ (2003) Fecal streptococci/enterococci in aquatic environments. Encyclopedia of Environmental Microbiology.

Caggiano M (2000) Quality in harvesting and post-harvesting proceduresinfluence on quality. Fish freshness and quality assessment for seabass and seabream. Cahiers Options Méditerranéenns (CIHEAM),1022-1379.

Chouliara I, Savvaidis IN, Panagiotakis N & Kontominas MG (2004) Preservation of salted, vacuum-packaged, refrigerated sea bream (*Sparus aurata*) fillets by irradiation: microbiological, chemical and sensory attributes. Food Microbiology, 21(3): 351-359.

Costa JCCP, Floriano B, Villegas IMB, Rodríguez-Ruiz JP, Posada-Izquierdo GD, Zurera G & Pérez-Rodríguez F (2020) Study of the microbiological quality, prevalence of foodborne pathogens and product shelf-life of Gilthead sea bream (*Sparus aurata*) and Sea bass (*Dicentrarchus labrax*) from aquaculture in estuarine ecosystems of Andalusia (Spain). Food microbiology, 90: 103498.

Directive 2002/99/EC of 16 December 2002 laying down the animal health rules governing the production, processing, distribution and introduction of products of animal origin for human consumption. Official Journal, L, 18 (23/01/2003), pp. 11-20

Domingo JL (2007) Omega-3 fatty acids and the benefits of fish consumption: is all that glitters gold?. Environment International 33(7):993-998.

European Union. (2004). Commission Decision of 29 April 2004 concerning laying down specific hygiene rules for on the hygiene of foodstuffs, 04/853/EC.Official Journal, L 139, 30/04/2004 (2004)

Fernandes DVGS, Castro VS, Cunha Neto AD & Figueiredo, EEDS (2018) *Salmonella* spp. in the fish production chain: a review. Ciência Rural, 48.

Floris R, Manca S & Fois N (2013) Microbial ecology of intestinal tract of gilthead sea bream (*Sparus aurata* Linnaeus, 1758) from two coastal lagoons of Sardinia (Italy). Transitional Waters Bulletin 7(2): 4-12.

Food and Agriculture Organization (FAO). (2020). The state of world fisheries and aquaculture 2020. Retrieved from http://www.fao.org/state-of-fisheries-aquaculture

Galaviz-Silva L, Goméz-Anduro G, Molina-Garza ZJ & Ascencio-Valle F (2009) Food safety Issues and the microbiology of fish and shellfish. Hoboken, NJ: John Wiley & Sons.

Haldar S, Maharajan A, Chatterjee S, Hunter SA, Chowdhury N, Hinenoya A & Yamasaki S (2010) Identification of *Vibrio harveyi* as a causative bacterium for a tail rot disease of sea bream *Sparus aurata* from research hatchery in Malta. Microbiological research, 165(8): 639-648.

ICMSF (1986). International commission on microbiological specifications for foods. Sampling plans for fish and shellfish. In ICMSF

(Ed.), ICMSF, Microorganisms in foods. Sampling for microbiological analysis: Principles and scientific applications (Vol. 2, 2nd ed.). Toronto, Canada: University of Toronto Press.

ICMSF, Microbiological testing in food safety management. Microorganisms in foods, Vol. 7, Kluwer Academic/Plenum Publishers, New York (2002), pp. 1-43

ISO 15213:2003 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions

ISO 16649-2:2001 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide

ISO 21528-2:2004 Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of *Enterobacteriaceae* — Part 2: Colony-count method

ISO 4833:2003 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of microorganisms — Colony-count technique at 30 degrees C

ISO 6579:2002 Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Salmonella* spp.

ISO 6887-3:2017 Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 3: Specific rules for the preparation of fish and fishery products

ISO 6888-1:1999/Amd 1:2003 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 1: Technique using Baird-Parker agar medium — Amendment 1: Inclusion of precision data

Lee RJ, Rangdale RE, Croci L, Hervio-Heath D, Lozach S (2008) Bacterial pathogens in seafood T. Borresen (Ed.), Improving Seafood Products for the Consumer, Woodhead Publishing, Cambridge, United Kingdom , pp. 1-70

Mokrani D, Oumouna M & Cuesta A (2018) Fish farming conditions affect to European sea bass (*Dicentrarchus labrax* L.) quality and shelf life during storage in ice. Aquaculture 490:120-124.

MONSTAT (2020): https://www.monstat .org/cg/page.php?id=265&pageid=265.

Parlapani FF & Boziaris IS (2016) Monitoring of spoilage and determination of microbial communities based on 16S rRNA gene sequence analysis of whole sea bream stored at various temperatures. LWT-Food Science and Technology 66:553-559.

Şengör GFÜ, Ceylan Z, Gürün S, Kalkan S & Hülyar O (2021) The visual characteristics and quality of cultured gilthead seabream (*Sparus aurata*) in earthen ponds and net cages in Turkey.

Stratev D, Stoev S, Vashin I & Daskalov H (2015) Some varieties of pathological changes in experimental infection of carps (*Cyprinus carpio*) with *Aeromonas hydrophila*. Journal of Aquaculture Engineering and Fisheries Research 1(4): 191-202.

Svanevik CS, Roiha IS, Levsen A & Lunestad BT (2015) Microbiological assessment along the fish production chain of the Norwegian pelagic fisheries sector-results from a spot sampling programme. Food Microbiology 51: 144-153.

Topić Popović N, Benussi Skukan A, Džidara P, Čož-Rakovac R, Strunjak-Perović I, Kozačinski L & Brlek-Gorski D (2010) Microbiological quality of marketed fresh and frozen seafood caught off the Adriatic coast of Croatia. Veterinarni Medicina 55 (5): 233-241

Zambuchini B, Fiorini D, Verdenelli MC, Orpianesi C & Ballini R (2008) Inhibition of microbiological activity during sole (*Solea solea* L.) chilled storage by applying ellagic and ascorbic acids. LWT-Food Science and Technology, 41(9): 1733-1738.