

A study of the microbiology of the intestinal tract in different species of Teleost fish from the Black Sea

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Abstract

This paper presents a study on the microbial status of different fish species and their habitats in the Bulgarian Black Sea area. The samples were collected in the period of January 2021 until March 2021. The fish species we used in this study were Black Sea turbot (*Scophthalmus maximus*), round goby (*Neogobius melanostomus*), shore rockling (*Gaidropsarus mediterraneus*) and European anchovy (*Engraulis encrasicolus*). The BIOLOG system was used for microbiological determination. From the different fish species, different species of microorganisms were isolated (using selective nutrient media). From the turbot, we isolated species *Enterococcus villorum* with 24×10^3 cells in 1 ml, *Moraxella nonliquefaciens* with 70×10^3 cells in 1 ml and *Pseudomonas synxantha* with 123×10^3 cells. *Pseudomonas putida* was isolated from the round goby with 20×10^3 cells in 1 ml. The species *Streptococcus entericus* with 123×10^3 cells in 1 ml was isolated from the shore rockling. *Pseudomonas fulva* with 60×10^3 cells in 1 ml was isolated from the European anchovy. A total of 223×10^3 cells in 1 ml of *Pseudomonas agarici* were isolated from *Trachinus draco*. *Pseudomonas tolaasii* with 145×10^3 cells in 1 ml were isolated from *Merlangius merlangus*. A different species of bacteria of the genus *Pseudomonas* was found for each of the investigated species of Black Sea fish. Apparently, the species *Pseudomonas* is characteristic of marine Teleostei and is important for the life and metabolism of these vertebrates. These microorganisms probably are resident species and developed not as result of pollution or environmental change.

Keywords

marine fish, microbial status, intestinal tract

Introduction

Fish are consumed in large quantities throughout the world and are considered one of the main sources of protein. According to FAO (2007), fish are the most important single source of high quality protein providing about 16% of the animal protein consumed by the world's human population. Fish have high nutritional values and are a good source of saturated fats, omega-3 fatty acids that cannot be synthesised by the human body. Fish are known to be low in fat and cholesterol and are easily digested and suitable for infants, children and the elderly. Unlike other local products, the price of fish is much lower and more affordable for most people. The microbiology of fish is closely related to human health because it can represent a risk depository, as fish carry inside and on their surface different types of microorganisms. On the other hand, the microbiology of fish is closely dependent on the state of the environment and can be used as an indicator of the water quality. Several bacterial genera, such as *Escherichia*, *Listeria*, *Pseudomonas*, *Klebsiella* and *Salmonella* were isolated from fish and can indicate multisource pollution (Sichewo et al. 2014; Manhondo et al. 2018). Banquero et al. (2008) described aquatic systems as genetic reactors or hotspots for AMR genes where significant genetic exchange and recombination can occur and can shape the evolution of future resistance profiles. Bacteria very quickly acquire resistance either through gene transfer or through mutations. In this way, antibiotic resistance genes can also be passed on. These processes occur mainly in the intestinal tract of fish (Levy and Marshall 2004). Random use of antimicrobials is associated with increased selection pressure on bacterial populations and favours the survival and reproduction of resistant bacteria. In Bulgaria, Stratev et al. (2015) conducted a study of 161 samples of frozen fish products collected from retail markets. High numbers of total viable counts (TVCs) were found in Black Sea roach (7.28 log cfu/g) and horse mackerel (5.90 log cfu/g). High amounts of *Aeromonas spp.* were found in the Black Sea horse mackerel (2.68 log cfu/g). *Aeromonas spp.* was not found in the Black Sea roach.

After their death, fish undergo rapid bacterial and microbiological changes, which are determining factors in their consumption by humans (Stratevaa et al. 2021). The expiry period of fish is determined by the content of protein, fat, water (Velioglu et al. 2015) and the amount of microorganisms (Fengou et al. 2019). Fresh fish have a high water quantity and this may be the reason for their rapid spoilage (Zotta et al. 2019).

To date, data on the microbiota of fish in the Black Sea are very limited. Gaining information in this field is important as the sea can be a source of species of bacteria that are potentially dangerous to human health. The main goal of the present work was to study the microbiological composition of the intestinal tract in different species of Teleost fish from the Black Sea.

Place and duration of the study

The study was conducted at the Department of Biology, University of Shumen, Bulgaria, January 2021 until March 2021. The samples were collected from the regions of Varna: 43.18 N, 27.91 E and Sozopol: 42.4317N, 27.70 E (Datum WGS 84) (Fig. 1).

Collection of the samples

The fish individuals were randomly sampled from trawl catches using pelagic Midwater otter trawl (7 × 7 mm mesh size of the codend) from two localities of the Bulgarian Black Sea aquatory (Fig. 1). On board, the fish samples were shock frozen for best preservation and transported to the laboratory for further analyses.

Microbiological analysis

The fish were scrubbed free of dirt, washed in hypochlorite solution (20 mg/l), rinsed with sterile distilled water and shucked with a sterile knife. Tissue liquor samples (about 100 g) were homogenised (Maffei et al. 2009). Faecal coliforms (FC) were enumerated through a five tubes per dilution most probable number (MPN) series (Ignatova-Ivanova et al. 2018). After 3 h at 37 °C plus 21 h at 44 °C, gas positive tubes were recorded for FC. From each FC gas positive tube, 0.1 ml were transferred in tubes with 10 ml of Tryptone Water (Oxoid, Basingstoke, UK) and then incubated for 24 h at 44 °C. *E. coli* were enumerated by MacConkey agar (Merck, Darmstadt, Germany). The plates very incubated aerobically at 35–37 °C for 18–24 hours. *E. coli* developed matte dark pink to tile red, surrounded by an opaque area due to the precipitation of bile salts in this environment. *Pseudomonas* sp. were enumerated by Cetrimide Agar (Merck KGaA, 64271 Darmstadt, Germany).



Figure 1. Sampling locations at the Black sea coast.

Microbial Identification Databases for the “BIOLOG” System

The microbial identification was performed by the BIOLOG Microbial Identification System VIO45101AM. The isolated strains were screened on BL4021502 Tryptic Soy Agar (TCA), cultured for 24 hours at 37 °C and then subjected to Gen III plaque identification to identify Gram positive and Gram negative aerobic bacteria. The microscopic pictures were performed using the stereomicroscope OPTIKA (Italy) with a DinoEye, Eyepiece camera with 5 megapixels. The photographs were taken by using a Canon EOS 60D camera. The GEN III MicroPlate test panel provides a standardised micromethod using 94 biochemical tests to profile and identify a broad range of Gram-negative and Gram-positive bacteria. BIOLOG's Microbial Identification Systems software (e.g. OmniLog Data Collection) is used to identify the bacterium from its phenotypic pattern in the GEN III MicroPlate. The BIOLOG system allows us to quickly and accurately identify more than 2900 species of aerobic and anaerobic bacteria, yeasts and fungi. BIOLOG's advanced phenotypic technology provides valuable information on the properties of the strains, in addition to species-level identification. BIOLOG's carbon technology identifies the environment and pathogenic microorganisms by producing a characteristic pattern or “metabolic fingerprint” of discrete test reactions performed in a 96-well microplate. The culture suspensions are tested with a panel of pre-selected assays, then incubated, read and compared with extensive data-bases. <https://www.biolog.com/productsportfolio-overview/microbial-identification>.

Results

Using some classical microbiological methods, we investigated probes from the gastrointestinal tract of different species of fish from the Bulgarian Black Sea aquatory. After 24 h of cultivation on different media, the number of cells in 1 ml were obtained. The species of microorganisms were confirmed not only on selective media, but also by the results of the BIOLOG system. Data are represented in Table 1 and Fig. 2.

For the present study, fresh Black Sea fish were used, which were dissected in laboratory conditions. The intestinal tract was used for microbiological analysis. Different species of microorganisms were isolated from the fish species using selective nutrient media (Table 1) – *E. villorum* species (Fig. 2a) were isolated from *S. maximus* with 24×10^3 cells in 1 ml, *M. nonliquefaciens* with 70×10^3 cells in 1 ml and *P. synxantha* with 123×10^3 cells in 1 ml. The species *P. putida* was isolated from the Round goby with 20×10^3 cells in 1 ml. The species *S. entericus* with 123×10^3 cells in 1 ml was isolated from *P. flesus*. *P. fulva* with 60×10^3 cells in 1 ml was isolated from Sardine. A total of 223×10^3 cells in 1 ml of *P. agarici* (Fig. 2b) were isolated from *T. draco* and *P. tolaasii* with 145×10^3 cells in 1 ml were isolated from *M. merlangus*.

Table 1. Number of cells in 1 ml on the different media.

Media/ fish	Pseudomonas agar	Streptococcus selective agar	Chromokult agar	MacConkey agar	strain BIOLOG
T26F1 <i>Scophthalmus maximus</i> – turbot–Varna				24.10 ³	<i>Enterococcus villorum</i>
<i>Scophthalmus maximus</i> – turbot – Varna		70.10 ³			<i>Moraxella nonliquefaciens</i>
<i>Scophthalmus maximus</i> – turbot – Varna				123.10 ³	<i>Pseudomonas synxantha</i>
T26F3 <i>Neogobius melanostomus</i> – round goby – Varna				20.10 ³	<i>Pseudomonas putida</i>
20T29-30F7 <i>Platichthys flesus</i> – shore rockling – Varna				123.10 ³	<i>Streptococcus entericus</i>
<i>Engraulis encrasicolus</i> – European anchovy Sozopol				60.10 ³	<i>Pseudomonas fulva</i>
20T10-11F2 <i>Trachinus draco</i> – greater wever – Varna	223.10 ³				<i>Pseudomonas agarici</i>
20T10-11F3 <i>Merlangius merlangus</i> – whiting–Varna	145.10 ³				<i>Pseudomonas tolaasii</i>

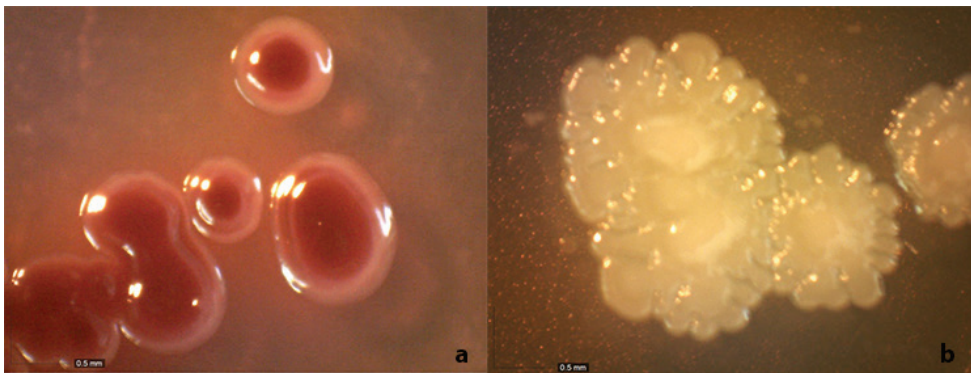


Figure 2. Stereomicroscope picture of the colonies of the isolated species in this case **a** *E. villorum* on media Chromocult agar and **b** *P. agarici* on media Pseudomonas agar. The picture was taken using the stereomicroscope OPTIKA (Italy) and DinoEye, Eyepiece camera, USB, 1.3 megapixel, up to 5 megapixels.

Discussion

The probable explanation for the difference in the species of microorganisms that were isolated from the intestine of the different fish species indicated that this may be due to the different habitats of fishes and their diet. In the winter months, the round goby lives at a depth of less than 60 m. The turbot is a predator and is found at a depth of 80 m buried in the sand. The flounder inhabits thin bottom layers at a depth of 50 m and feeds on crustaceans, worms and molluscs. The shore rockling feeds on “worms” and crustaceans, while the anchovy feed on plankton. According to Jorquera et al. (2001), some of the bacterial populations that inhabit the intestinal tract of fish include species

such as *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Photobacterium*, *Moraxella*, *Aeromonas*, *Micrococcus* and *Bacillus*. The bacterial species that are part of the intestinal tract of fish depend, on the one hand, on the diet of the fish and, on the other hand, on the state of the marine environment. The microbiota found in marine organisms can be considered in two aspects – the so-called “resident” microbiota, which is stable and unaffected by the environment and the “transitional” microbiota, which depend on environmental conditions. There are very little data in literature on the microbiological composition of the intestine of Black Sea fish. Some data were recently published (Orozova et al. 2010; Stratev et al. 2015; Stratevaa et al. 2021) and showed that these are mainly representatives of *Aeromonas* spp., which cause fish diseases, such as haemorrhagic septicemia and ulcers. This may lead to large economic losses in fish farms. To date, mostly freshwater fish were studied, such as carp, trout and silver carp. A study on turbot conducted in Turkey showed that the total number of microorganisms isolated on day 0 was 3.3 log cfu/g, but no concrete species were identified (Özogul et al. 2006). There are no data on the exact species composition of microorganisms in the intestinal tract of Black Sea fish to date.

The microbiota of fish may be influenced by many factors, both endogenous and exogenous. Endogenous factors include the origin of the fish host (Miyake et al. 2015), genotype and diet (Wu et al. 2012), parasitic load (Llewellyn et al. 2017), immunological condition (Pérez et al. 2010), but also lifestyle (Stephens et al. 2016). Exogenous factors are habitat specific and include: environment condition, physicochemical parameters of water (Llewellyn et al. 2016; Sylvain et al. 2016) and bacterioplankton composition (Sylvain et al. 2017). Usually in fish intestines, microorganisms play a key role in nutrition, facilitating the breakdown and absorption of specific compounds present in the diet of the host fish.

According to data published very recently (Sylvain et al. 2020), the diverse microbiota community in fish skin mucus is suitable for the development of microbial biomarkers indicating the conditions of the environment, while the more stable and persistent intestinal microbiome is more suitable for studying long-term host-microbial interactions. The different fish species and their habitats showed differences in the taxonomic structure of the microbial community and this was confirmed for all studied species.

Conclusion

The question whether host-related microbiomes are primarily species-specific or depend on environmental factors still remains open. Our study is one of the few to focus on the intestinal microbiome of Black Sea fish species. It is interesting to note that the phylogenetic structure of the microbial communities of different fish is formed by species and habitat-specific factors. A different species of bacteria of the genus *Pseudomonas* was found for each of the investigated species of Black Sea fish. Apparently, *Pseudomonas* species are characteristic of marine Teleostei and are important for the

life and metabolism of these vertebrates. These microorganisms are probably resident species and have developed not as result of pollution or environmental change. The presence of the species *Enterococcus villorum* and *Moraxella nonliquefaciens*, which are pathogenic species, may be due to pollutants of seawater, which settled in the sediment and infested the turbot in its natural habitat.

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References

- Banquero F, Martínez JL, Canton R (2008) Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology* 19(3): 260–265. <https://doi.org/10.1016/j.copbio.2008.05.006>
- FAO (2007) Social issues in small-scale fisheries: how a human rights perspective can contribute to respective fisheries in Proceedings of the Presentation to FAO Committee of Fisheries No 27, Rome, Italy.
- Fengou LC, Lianou A, Tsakanikas P, Gkana EN, Panagou EZ, Nychas GJE (2019) Evaluation of Fourier transform infrared spectroscopy and multispectral imaging as means of estimating the microbiological spoilage of farmed sea bream. *Food Microbiology* 79: 27–34. <https://doi.org/10.1016/j.fm.2018.10.020>
- Ignatova-Ivanova TS, Ibrjmovska S, Stanachkova E, Ivanov R (2018) Microbiological characteristic of microflora of (*Mytilus galloprovincialis* Lam.) in the Bulgarian Black Sea aquatory. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 9(1): 199–205. [https://www.rjpbcs.com/pdf/2018_9\(1\)/\[29\].pdf](https://www.rjpbcs.com/pdf/2018_9(1)/[29].pdf)
- Jorquera MA, Silva FR, Riquelme CE (2001) Bacteria in the culture of the scallop *Argopecten purpuratus* (Lamarck, 1819). *Aquaculture International* 9(4): 285–303. <https://doi.org/10.1023/A:1020449324456>
- Levy SB, Marshall B (2004) Antibacterial resistance worldwide: Causes, challenges and responses. *Nature Medicine* 10(S12): S122–S129. <https://doi.org/10.1038/nm1145>
- Llewellyn MS, McGinnity P, Dionne M, Letourneau J, Thonier F, Carvalho GR, Creer S, Derome N (2016) The biogeography of the Atlantic salmon (*Salmo salar*) gut microbiota. *The ISME Journal* 10(5): 1280–1284. <https://doi.org/10.1038/ismej.2015.189>
- Llewellyn MS, Leadbeater S, Garcia C, Sylvain FE, Custodio M, Ang KP, Powell F, Carvalho GR, Creer S, Elliot J, Derome N (2017) Parasitism perturbs the mucosal microbiome of Atlantic Salmon. *Scientific Reports* 7(1): 43465. <https://doi.org/10.1038/srep43465>
- Maffei M, Vernocchi P, Lanciotti R, Guerzoni ME, Belletti N, Gardini F (2009) Depuration of Striped Venus Clam (*Chamelea gallina* L.): Effects on microorganisms. sand content

- and mortality. *Journal of Food Science* 74(1): M1–M7. <https://doi.org/10.1111/j.1750-3841.2008.00971.x>
- Manhondo P, Gono RK, Muzondiwa J, Mafa B (2018) Isolation and identification of pathogenic bacteria in edible fish: A case study of bacterial resistance to antibiotics at Lake Chivero, Zimbabwe. *African Journal of Agricultural Research*.
- Miyake S, Ngugi DK, Stingl U (2015) Diet strongly influences the gut microbiota of surgeonfishes. *Molecular Ecology* 24(3): 656–672. <https://doi.org/10.1111/mec.13050>
- Orozova P, Chikova V, Najdenski H (2010) Antibiotic resistance of pathogenic for fish isolates of *Aeromonas* spp. *Bulgarian Journal of Agricultural Science* 16(3): 376–386. <https://www.agrojournal.org/16/03-17-10.pdf>
- Özogul Y, Özogul F, Kuley E, Özkutuk AS, Gökbulut C, Köse S (2006) Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the Black Sea, during chilled storage 2006. *Food Chemistry* 99(4): 752–758. <https://doi.org/10.1016/j.foodchem.2005.08.053>
- Pérez T, Balcázar JL, Ruiz-Zarzuola I, Halaihel N, Vendrell D, de Blas I, Múzquiz JL (2010) Host-microbiota interactions within the fish intestinal ecosystem. *Mucosal Immunology* 3(4): 355–360. <https://doi.org/10.1038/mi.2010.12>
- Sichewo PR, Gono RK, Muzondiwa J, Mungwadzi W (2014) Isolation and identification of pathogenic bacteria in edible fish: A case study of rural aquaculture projects feeding livestock manure to fish in Zimbabwe. *International Journal of Current Microbiology and Applied Sciences* 3(11): 897–904. [https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.680.9981&rep=rep1&ctype \[=pdf\]](https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.680.9981&rep=rep1&ctype=[=pdf)
- Stephens WZ, Burns AR, Stagaman K, Wong S, Rawls JF, Guillemin K, Bohannon BJ (2016) The composition of the zebrafish intestinal microbial community varies across development. *The ISME Journal* 10(3): 644–654. <https://doi.org/10.1038/ismej.2015.140>
- Stratev D, Vashin I, Daskalov H (2015) Microbiological status of fish products on retail markets in the Republic of Bulgaria. *International Food Research Journal* 22(1): 64–69.
- Stratevaa M, Pencheva G, Stratev D (2021) Histological, physicochemical and microbiological changes in the carp (*Cyprinus carpio*) muscles after freezing. *Journal of Aquatic Food Product Technology* 30(3): 323–337. <https://doi.org/10.1080/10498850.2021.1882633>
- Sylvain FE, Derome N (2017) Vertically and horizontally transmitted microbial symbionts shape the gut microbiota ontogenesis of a skin-mucus feeding discus 646 fish progeny. *Scientific Reports* 7(1): 14. <https://doi.org/10.1038/s41598-017-05662-w>
- Sylvain FÉ, Cheaib B, Llewellyn M, Gabriel Correia T, Barros Fagundes D, Luis Val A, Derome N (2016) pH drop impacts differentially skin and gut microbiota of the Amazonian fish tambaqui (*Colossoma macropomum*). *Scientific Reports* 6: 64310. <https://doi.org/10.1038/srep32032>
- Sylvain FÉ, Holland A, Bouslama S, Audet-Gilbert É, Lavoie C, Val AL, Derome N (2020) Fish skin and gut microbiomes show contrasting signatures of host species and habitat. *Applied and Environmental Microbiology* 86(16): e00789–e20. <https://doi.org/10.1128/AEM.00789-20>

- Velioglu HM, Temiz HT, Boyaci IH (2015) Differentiation of fresh and frozen-thawed fish samples using Raman spectroscopy coupled with chemometric analysis. *Food Chemistry* 172: 283–290. <https://doi.org/10.1016/j.foodchem.2014.09.073>
- Wu S, Wang G, Angert ER, Wang W, Li W, Zou H (2012) Composition, diversity and origin of the bacterial community in Grass Carp intestine. *PLoS ONE* 7(2): e30440. <https://doi.org/10.1371/journal.pone.0030440>
- Zotta T, Parente E, Ianniello RG, De Filippis F, Ricciardi A (2019) Dynamics of bacterial communities and interaction networks in thawed fish fillets during chilled storage in air. *International Journal of Food Microbiology* 293: 102–113. <https://doi.org/10.1016/j.ij-foodmicro.2019.01.008>