**Cultivable bacterial communities from purse-seined small pelagic fish, fishing nets and storage tanks**

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**Abstract**

 Sardines and anchovies are the most important small pelagic species throughout the Mediterranean, caught mainly by purse-seiners. Their rapid bacterial degradation after capture and storage limits their commercial use and shelf life. Gills are the most vulnerable tissue in fish, harboring bacterial communities which have not been previously investigated in these species. This study examined cultivable bacteria from gills of harvested sardines and anchovies, tanks in which they are stored aboard, and fishing nets on four occasions (twice for both warm and cold seasons). A total of 471 bacteria were isolated, belonging to 74 genera and 163 species by MALDI-TOF MS identification. A number of genera and species identified from fish and surfaces occurred only in the warm or cold season, but a fraction occurred in both seasons. Sardine gills harbored most of the isolated bacteria (254), belonging to 53 genera and 111 species, as opposed to anchovy with 120 strains, belonging to 30 genera and 64 species. A diverse cultivable bacterial community consisting of 17 species was isolated exclusively from the polyethylene surfaces of nets and tanks. This finding is significant regarding their potential migrations between planktonic and sessile lifestyles. Possible contamination and cross-contamination are likely to increase with the rise in ambient and seawater temperature during the hauling and sorting of fish in the tanks. The food safety risks stem from pathogens and fish spoilage bacteria on both the fish and the surfaces of fishing nets and storage tanks.

*Keywords*: Anchovy, Sardine, Gills, Bacteria, Purse-seine vessel, Polyethylene, Microbial contamination, Marine ecology

**Introduction**

 In the eastern Adriatic, purse-seiners comprise a relatively small proportion of the fishing fleet, although they account for the highest percentage of the catch, over 94 % (Soldo et al. 2019). They are the most efficient vessels for catching small pelagic shoaling species, predominantly sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*). Sardine and anchovy are the most important small pelagic fish species in the Adriatic Sea fished by purse-seiners (Janči et al. 2023). Both species are widely distributed throughout the Mediterranean and are known to be short-lived and fast-growing fish with a protracted spawning: Adriatic sardines spawn from October till April, peaking between November and February, depending on the temperature (Zorica et al. 2020). The anchovies begin their spawning season from March to October and reach their peak between April and July (Zorica et al. 2020). Both species largely ensure the functioning of the marine ecosystem by providing energy transfer from lower to higher trophic levels, although their biomass varies considerably over the years (Cury 2000; Zorica et al. 2019; Hure and Mustać 2020). In addition, they are part of small pelagic fish which account for 47% of the Mediterranean fishery, giving them a prominent role in the fishing industry and related economy (Zorica et al. 2019).

Given their importance, all aspects affecting the quality parameters of their meat, such as handling, storage, transportation and processing, may have a consequence along the fish supply chain. Small pelagic fish have a delicate body structure, vulnerable chemical composition and neutral pH, which makes them susceptible to rapid quality deterioration due to oxidation processes, autolysis and microbial activity (Ababouch et al. 1996; Janči et al. 2023). The rapid bacterial decomposition to which both species are prone, as well as the difficulty of their preservation at low temperatures limit their commercial use and shelf life (Campos et al. 2005). Bacteria from the aquatic environment interact mainly with the intestinal tract, gills and skin of the fish. The gills are the most subjected to bacterial proliferation and spoiling promoted by microbial activities. They contain a microbiota determined by the surrounding environment, which occurs from the larval stage of fish (Leroi and Joffraud 2011). At the fish death, microorganisms from the gills may contaminate the meat by mobilization over muscle tissue, depending on their spoilage potential (Leroi and Joffraud 2011). In the course of storage, the physicochemical properties of the fish meat changes dramatically due to various intrinsic and extrinsic factors, making it critical to determine the key species of their microbial community (Kaszab et al., 2022).

Therefore, the microbial degradation of the catch largely depends on the procedures used by the vessel crew to handle the fish before and during hauling, as well as onboard. The purse-seiners surround the shoal with a deep curtain of seine and tighten (purse) the bottom of the net underneath it (Soldo et al. 2019). The catch is harvested by hauling the net or bringing it alongside the vessel, loading or pumping the fish onboard, and storing it in tanks with flake ice or mixture of chilled slurry and ice (Janči et al. 2023). Rough handling of fish, elevated water temperature, tank surface condition and density of stored fish may affect their bacterial degradation (Leroi and Joffraud 2011).

The Adriatic marine environment has mostly been investigated for bacterial contamination related to anthropogenic factors in coastal areas (Basili et al. 2021; Džal et al. 2021; Fonti et al. 2021; Kraus et al. 2022; Penna et al. 2023; Purgar et al. 2023). There has been less research on Adriatic bacteria in relation to plastic and other surfaces (Viršek et al. 2017; Biagi et al. 2021; Kapetanović et al. 2023), or pelagic sea and fish unrelated to aquaculture (Čož-Rakovac et al. 2002; Jakšić et al. 2002; Scicchitano et al. 2022). Anchovy and sardine are superior sources of n-3 PUFA and can also be used as supplements in functional food manufacturing (Farabegoli et al. 2019). However, there is a scarcity of studies regarding microbiota of their gills and surfaces post-catch (Gennari 1988; Gennari et al. 1989, 1999).

The presence of diverse bacterial species on harvested sardines and anchovies, on the tanks in which they are stored on board and on the fishing nets, can be valuable indicators of the potential microbial contamination of tissues during the post-catch and storage period on purse-seiners. The aim of this study was thus to investigate cultivable bacterial communities associated with the gill tissues of freshly harvested sardines and anchovies. In order to assess food safety risks, these findings were compared with bacterial communities isolated from the surfaces of the tanks in which they are transported to shore and from the surfaces of fishing nets. More specifically, we examined: (i) the cultivable bacteria swabbed from the gills of anchovy and sardine upon their pumping onboard during the operation of a fishing purse-seine vessel; (ii) the presence of diverse cultivable bacterial species on the surfaces of tanks where the fish are stored onboard, as well as on the surfaces of fishing nets; (iii) the correlation of bacterial genera and species between different fish species and different surfaces for each season; and (iv) we identified bacteria common to fish gills and plastics, as well as bacteria unique to anchovy, sardine, or solely to tank and net surfaces. The study was carried out in both warm and cold seasons.

**Materials and methods**

***Sample collection***

 Sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) were sampled on a commercial pelagic fishing seine-haul vessel operating in the mid-eastern Adriatic Sea, upon loading the fish onboard, during a cold season (March and November 2022) and a warm season (April 2021 and June 2022).

After spotting the aggregated fish schools by sonar, the purse-seiner crew harvested the surrounded fish by bringing the net alongside the vessel and loaded the mixed catch of sardine and anchovy onboard using pumps. The fish (anchovies and sardines together) were pumped onboard onto a selection grid, where smaller specimens passed through the grid and were immediately returned to the sea, while the adult fish were carried with the water stream toward tanks. The tanks (capacity 400 L) used for sampling of fish were filled with pumped seawater. The fish introduced into these tanks with the water stream were alive and began swimming immediately after introduction. These tanks, like the other tanks onboard, are normally used by the vessel crew for storing fish and are then filled with flake ice or a mixture of chilled slurry and ice for that purpose. The fish were sampled immediately and as quickly as possible. After sampling, they were returned to tanks with ice where the commercial catch of mixed anchovies and sardines was unloaded.

A total of 120 sardine and 120 anchovy were sampled, 30 per species, per sampling-point. Samples were taken in the form of gill swabbing. Before swabbing, a brief clinical assessment of potential gill pathology was performed. The clinical assessment was performed visually by observing abnormal color changes, macroscopic lesions, or the presence of parasites.

Gill arches on the left side of each specimen were swabbed. Operculum of each fish was held open with sterile instruments. A sterile swab (Copan, Zagreb, Croatia) was introduced on the outside and inside of the first gill arch, both sides of second and third arches and outer surface of the fourth arch. The inserted swab was gently rotated from the arch to the tip of the gill filaments, and returned to its tube containing the SRK Neutralizing Transport Medium (Copan).

With the same technique, samples were also collected from surfaces of the still moist fishing nets and fish storage tanks upon their usage, and upon unloading the fish onshore. Both tanks and nets were made of synthetic polymeric fibers, recyclable high-density polyethylene (HDPE) with a high strength-to-density ratio. For these surfaces, a sampling area of 10 cm2 was defined with a sterile template (autoclavable plastic square frame) where possible, while round and uneven surfaces were swabbed without a template, but with the approximation of a 10 cm2 area. Samples were swabbed from surfaces that come into contact with fish.

All swabbing procedures were carried out consistently by a trained researcher, with sterile sampling tools. Swabs were not exposed to air or other surfaces, in order to reduce the possibility of sample contamination and to minimize technical variations.

***Sample analyses***

 Swabs were transported to the laboratory in their corresponding tubes containing the SRK Neutralizing Transport Medium (Copan) stored in a portable cooler at 4 °C. The analyses were performed 48 h after swabbing. Samples were streaked onto Marine Agar and 1.5 % (w/v) NaCl-supplemented Tryptic Soy Agar (Oxoid Ltd, Basingstoke, England UK), and incubated at 25 °C for 48 h up to 5 weeks. Representative colonies (based on morphological characteristics and growth patterns) deriving from each of the surfaces were isolated and subcultured on fresh media until purity was attained. Colonies were subsequently Gram-stained and fresh bacterial biomass was subjected to identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS).

***MALDI-TOF MS identification***

 MALDI-TOF MS was performed with a bench-top Bruker Microflex LT mass spectrometer equipped with the Bruker Biotyper 3.0 software (Bruker Daltonik, Bremen, Germany) system. Two biological replicates of each isolate were applied on a 96-spot polished stainless-steel target plate, at minimum in duplicates and up to quintuplicates, and prepared by the on-target extraction method. One colony loopful from each selected strain was smeared on a target plate (Bruker Daltonik). One μL of 70 % formic acid (Kemika, Zagreb, Croatia) was added to each spot overlying the bacterial colony and left to dry at room temperature. Subsequently, 1 μL of MALDI matrix as the saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile and 2.5 % trifluoroacetic acid (Bruker Daltonik) was added to each spot and allowed to air dry at 22 ˚C. The calibration was conducted with alpha spiked *Escherichia coli* DH5 with two additional pure proteins covering a mass range 4-17 kDa. Ions were captured in the positive linear mode and positive ions were extracted at accelerated voltage of 20 kV. Spectra with the sum of the respective ions were obtained by 240 laser shots in different regions of every target plate spot. All obtained mass spectra were matched up to the reference mass spectra in the database, and according to the obtained log scores, species identification results were classified as high (log score of 2.000 to 3.000), low (log score of 1.700 to 1.999), and inconclusive (<1.700). Confidence in genus identification of isolates was considered high if the same genus was identified in all replicates and was considered low if variable genera were returned.

***Limitations of MALDI-TOF MS identification and culture-dependent methods***

The most important limitation of MALDI-TOF MS identification is the incompleteness of databases, especially regarding environmental bacteria (Topić Popović et al. 2017). Another limitation is the needed optimization of measurement conditions of fastidious bacteria and identification of bacteria directly form tissues (Lavigne et al. 2013; Schumann and Maier 2014). Correct identification of the protein-based approach requires an intact colony, and is not reliable with polymicrobial plates (Lavigne et al. 2013). Although MALDI‐TOF MS is an excellent tool for bacterial identification, there are still several shortcomings, such as the lack of an optimal standardized protocol for sample preparation and culture conditions, salt content, and blood in the culture media (Topić Popović et al. 2023).

There are also limitations to methods that require culturing bacteria for identification. These include selective growth conditions, slow growth rates, specificity of culture media, polymicrobial samples, the viable but non-culturable state of some bacteria, and the risk of contamination. However, culture-based methods provide reproducible results with minimal error, and enable isolation of specific target organisms (McLain et al. 2016).

***Data analyses***

 All exploratory data analyses and visualizations were performed using R v4.3.2 (R: A language and environment for statistical computing. R Core Team (2023), URL: <https://www.R-project.org/>) in RStudio IDE v2021.09.0 (RStudio, PBC (2021), URL: [https://www.rstudio.com/](https://www.R-project.org/)). Bacterial diversity of the isolated community in anchovies, sardines, fish tanks, and fish nets was evaluated using observed occurrence counts, Shannon and Simpson indices for alpha-diversity within the sample types. Differences between sample types were tested using Kruskal-Wallis rank sum test with Wilcoxon rank sum test for multiple pairwise comparisons between groups. Bacterial beta-diversity between samples types were evaluated using principal coordinate analysis with Bray-Curtis distance of species abundance and tested using permutational multivariate analysis of variance (PERMANOVA) with 10000 permutations.

**Results**

 In this study, a total of 471 bacteria were isolated, belonging to 74 different genera and 163 species (Table 1), identified by the Biotyper system. Table S1 shows all bacteria identified by MALDI-TOF MS from gills of freshly harvested anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*), from fish storage tanks, and from fishing nets. Identification results are also presented according to the warm or cold season of sampling, when sea temperatures ranged from 20-23 °C and 11-14 °C, respectively.

There were approximately the same number of overall isolates in both warm (235) and cold seasons (236) belonging to 90 species and 47 genera (warm) and 92 species and 43 genera (cold). When observing the identified bacteria by their origin: anchovy gills, sardine gills, storage tanks, or fishing nets overall, sardine gills harbored most of the bacteria isolated. A total of 254 bacteria were isolated from sardine, belonging to 111 species and 53 genera, as opposed to anchovy, were a total of 120 bacteria were isolated, belonging to 64 species and 30 genera. Tanks surfaces had 56 isolates (26 species, 15 genera), while nets had 41 isolates (23 species, 16 genera). There was a number of distinctive species and genera identified from fish and surfaces which occurred only in warm or only in cold seasons, but only a fragment of these occurred in both seasons (Table 1), namely: *Lactobacillus gasseri, Vibrio gigantis* (anchovy); *Aeromonas media, A. veronii, Glutamicibacter bergerei, Pseudomonas anguilliseptica, P. antarctica, P. rhodesiae, Psychrobacter arcticus, Ps. namhaensis, V. anguillarum, V. gigantis, V. pomeroyi* (sardine); *Ps. alimentarius, Ps. maritimus, Ps. namhaensis, Ps. vallis* (tanks); *Ps. vallis,* and *V. gigantis* (nets).

As for the MALDI-TOF MS, the identification success was better at the higher (genus) than at the lower (species) taxonomical levels. For genus identification confidence, the ratio between the high identification and inconclusive identification confidence was: anchovy 57/38 (%), sardine 43/54 (%), tanks 70/20 (%), and nets 51/39 (%). For MALDI-TOF MS species identification confidence, the ratio between the high identification and inconclusive identification confidence changed in favor of the inconclusive results: anchovy 29/43 (%), sardine 18/57 (%), tanks 20/36 (%), and nets 7/68 (%). The presence of identified bacterial species from gills of anchovy and sardine, from tanks and nets in warm or cold season of sampling that had at least low confidence in genus assignment is presented in Table 2. Taking only isolates with high confidence in genus assignment, the sharing of bacterial genera between different fish species and surfaces is presented for genera and species levels in Figure 1. The overall relative abundance and alpha- and beta-diversity plots are available in Supplementary materials (Fig. S1-3). There were no significant differences in alpha-diversity indices between sample types with observed counts (*p*=0.17), and Shannon (*p*=0.08) index. The significant association of Simpson (*p*=0.04) index and sample type was not supported by *post-hoc* multiple pairwise comparisons. In all these indices the fish nets had the lowest and anchovies the highest alpha-diversity. Fish nets were shown to be the most clustered and separated sample type in evaluation of beta-diversity but significant separation of sample types was not supported by PERMANOVA results (*p*=0.15).

Of all isolated bacteria, only *Ps. vallis* appeared on both fish species and also on tanks and nets in all seasons. Species isolated from anchovy and from tanks, independent of seasonal occurrence, were *Exiguobacterium artemiae, Ex. mexicanum, Ex. oxidotolerans, Filifactor villosus, Psychrobacter* spp, *Ps. luti, Ps. maritimus,* and *Ps. namhaensis*. Species isolated from anchovy and nets were *Microbacterium* spp, *Microbacterium luticocti, Photobacterium damselae, Psychrobacter* spp, *Ps. immobilis, V. alginolyticus*, and *V. gigantis*. Species isolated from sardine and from tanks were *Ex. mexicanum, F. villosus, Ochrobactrum intermedium, Ps. alimentarium*, *Ps. luti, Ps. maritimus* and *Ps. namhaensis.* Species isolated from sardine and from nets were *Lacticaseibacillus paracasei, Lactobacillus reuteri, Microbacterium* spp, *Pseudomonas veronii, Ps. alimentarius, V. alginolyticu*s and *V. gigantis*.

Of all isolated species, only *V. gigantis* occurred in anchovy and sardine in both warm and cold season. *Lactobacillus gasseri* was identified from anchovy in both seasons*.* Species identified from sardine in both seasons were *A. media, A. veronii, Glutamicibacter bergerei, P. anguilliseptica, P. antarctica, P. rhodesiae, Ps. arcticus, Ps. namhaensis* and *V. anguillarum*.

**Discussion**

 Fish flesh, due to a number of factors, particularly favors microbial development. Despite a low carbohydrate percentage, it is rich in non-protein nitrogenous molecules, which along with the high postmortem pH and the high polyunsaturated fatty acids, enable rapid growth of psychrotrophic spoilage bacteria (Leroi and Joffraud 2011). Bacterial communities responsible for spoilage of fish meat depend mainly on storage temperature (Kim et al. 2023). The principal fish spoilage bacteria at low temperatures of up to 5 °C are *Shewanella putrefaciens, Aeromonas* sp., *Acinetobacter* sp. and *Pseudomonas* sp., while their communities change toward Gram positive bacteria, *Enterobacteriaceae,* and *Vibrionaceae* with increasing temperature (Leroi and Joffraud 2011; Zhengkayi et al. 2023).

*Vibrionaceae* are considered among the most abundant bacteria in marine aquatic environments worldwide, exhibiting seasonal fluctuations in relation to sea temperature, salinity, pH and organic matter (Kapetanović et al. 2022). They are frequently found on living fish tissues, and many vibrios have been described as fish pathogens or opportunistic invaders (Topić Popović et al. 2014). Some species, such as the pathogen *V. anguillarum*, have a worldwide distribution (Austin and Austin 2016). *V. anguillarum* in this work was found on sardine gills only, in both warm and cold season. This is consistent with literature describing the strain growing at 15-37 °C (Austin and Austin 2016), making it a good candidate to proliferate on the fish meat during the storage in the tanks. It has previously been found on anchovies in Japan (Tajima et al. 1985), but this work is its first finding on pelagic fish in the Mediterranean. This study revealed 12 different strains of vibrios from anchovy and sardine gills. However, their preference for HDPE was low, as none was revealed on storage tanks, and only *V. algynoliticus, V. gigantis* and *V. pelagius* were retrieved from fishing nets as well. Moreover, *V. pelagius* was isolated only from nets. Interestingly, *V. algynoliticus* was the prevalent vibrio also identified by MALDI-TOF MS from the sea bass rearing plastic tanks, followed by *V. harveyi* and *V. pelagius* in low prevalence (Mougin et al. 2021). On the other hand, *V. gigantis* is a known colonizer of plastics (Borre and Sonnenschein 2021).

The best known fish spoilage bacteria are *Shewanella* sp. owing to their extracellular proteases (Zhengkayi et al. 2023). Although they are strong biofilm producers, with adhesion or attachment ability on plastics and PVC (Jayalekshmi et al. 2022), the four *Shewanella* species in this work (*S. baltica, S. gaetbuli, S. profunda, S. xiamenensis*) were retrieved only from fish gills. This could be due to the fact that the HDPE surfaces in this work were swabbed immediately upon their disengagement from netting and storage functions, and the adhesion of shewanellas was still loose, as it was shown that *S. putrefaciens* forms biofilm on plastics and PVC upon 48, 72 and 96 hours of incubation (Jayalekshmi et al. 2022). Possibly *Shewanella* may not be present on these surfaces due to other bacterial colonizers or environmental conditions (Jayalekshmi et al. 2022).

The other strong spoilage bacteria associated with low storage temperature, *Aeromonas* sp., *Acinetobacter* sp. and *Pseudomonas* sp. were isolated in abundance in this work with 7, 2, and 25 different species, respectively. They were almost exclusively retrieved from fish gills, while only *P. veronii* was isolated from nets. That finding was rather unexpected, as both aeromonads and pseudomonads are known biofilm producers on plastic surfaces. They produce slime as a protective mechanism against external stressors, serving as an indicator of a high-risk contamination (Sechi et al. 2002). This is particularly interesting as the nets used on the fishing vessel in question are not sanitized. Besides, aeromonads are considered primary fish pathogens able to survive at refrigerator temperature and also acting as a major source of food contamination and cross-contamination (Abdulhakeem et al. 2023). Their attachment and colonization on surfaces derives from their extracellular enzymes that produce adhesins and facilitate nutrient acquisition in aquatic environments (Barger et al. 2021). Nevertheless, the increase of bacterial biofilm formation on plastics was found to be correlated with the changes of HDPE structure. In particular, marine *Pseudomonas* sp. and *Lysinibacillus* sp. have lower biofilm formation on weathered HDPE, especially over 24 h, while *P. aeruginosa* has a high rate of biofilm formation on non-weathered HDPE over 24 h (Oliveira et al. 2021), which might partly explain their lack on our repeatedly used tanks and nets.

Bacteria can move from the plastic surfaces into the water medium because they have the ability to switch between planktonic and sessile lifestyles (Nikolopoulou et al. 2023). Although the forms attached to plastics provide protection from environmental pressures, various top-down or bottom-up factors can trigger a shift in habitat and bacterial movement back into the sea or onto fish (Nikolopoulou et al. 2023). It is not unusual that plastics harbor a bacterial community different from that found in the surrounding waters, and it can be scarcer than that of the free-living community (Nikolopoulou et al. 2023). That is consistent with our findings, as a number of bacteria were found on surfaces of nets and tanks, but not encountered on fish. Among the microbial species detected in this study, *Achromobacter xylosoxidans* and *Bacillus* (*licheniformis*) act in polymer degradation (Saeed et al. 2022), *Bacillus*, *Halomonas* and *Brachybacterium* species are potent producers of degradable polymeric biomaterials (Mandragutti and Sudhakar 2023), corynebacteria are active polyamine producers (Schneider and Wendisch 2011), *Citrobacter* (*amalonaticus*) is an effective low-density polyethylene-degrading bacteria (Montazer et al. 2018), *Exiguobacterium* are also capable of plastics degradation (Gao and Sun 2021), esterases produced by *Bacillus* and *Nocardia* act in PLA-PET recycling and bioconversion (Tamoor et al. 2021), and *Paracoccus* has a strong potential for PET degradation (Cheng et al. 2022). Generally, various bacterial genera show selectivity toward the specific polymer type and tend to colonize their preferred substrate (Li et al. 2019). The biofilm-forming bacteria adhere more firmly and better degrade polyethylene compared to bacteria that cannot form biofilm. Once the bacteria attach to the polyethylene surface, they use its polymer as their sole carbon source to continue to proliferate (Yao et al. 2022), which may be the reason we retrieved these bacteria solely from the nets and tanks surfaces, and not from the fish gill tissues. Whether these bacteria prevent colonization of some species such as *Shewanella* sp. that we have expected to occur (see above) remains to be investigated and, if so, this could be of value for food hygiene research.

We also found a number of bacteria both on fish gills and HDPE surfaces of tanks and nets, however only *Ps. vallis* was retrieved from anchovy and sardine, and also from both tanks and nets in all seasons. Species isolated from anchovy and sardine and from tanks mostly belonged to similar bacterial communities of *Exiguobacterium, Filifactor,* and *Psychrobacter* genera. Interestingly, species isolated from anchovy and sardine and from nets were more diverse between fish, having in common species from *Microbacterium, Psychrobacter* and *Vibrio* genera, with further differences regarding to seasons. Of these, only *Psychrobacter* and *Vibrio* species are associated with spoilage of fish tissues (Leroi and Joffraud 2011), and both were also retrieved from plastic litter collected by trawlers in the Adriatic Sea (Kapetanović et al. 2023). *Filifactor* species are a rare finding on marine fish, and are found mainly in the intestinal tract (Liu et al. 2022), yet there are no reports on their associations with plastic. Notably, bacteria from the Actinobacteriaceae and Microbacteriaceae families were previously only isolated from gills of the flatfish (Ghotbi et al. 2022), just as in our case from anchovy and sardine gills. However, they have an affinity for polyethylene as they degrade polymers in the relevant rate of duration (Sharma et al. 2022), which further explains their finding on the fishing nets in our study. Besides, exiguobacteria can be also found on marine plastic debris with high abundance after a two-week exposure (Li et al. 2019). A peak of the abundance of bacteria from fish gills and HDPE was found in the warm season, as in the work of (Mougin et al. 2021). Indeed, seasonal variation of microbial communities in the sea water column results in the seasonal variation of their abundance on plastics, as temperature significantly shapes the occurrence of bacterial species and affects biofilm formation on plastics (Zhi Xiang et al. 2023). Temperature change can also impact a shift in the normal bacterial communities on fish, leaving them vulnerable to infections, but also adding to symbiotic microbial associations on fish tissues contributing to protective mechanisms against infections (Chelsea Black et al. 2021).

Gills are of utmost immunological importance where many bacteria from the water column ingress due to their environmental exposure (Clinton et al. 2021). A core bacterial community, different from that of the surrounding environment, can be observed on fish gills, suggesting that fish under healthy conditions can enrich and regulate host specific assemblages (Legrand et al. 2018). Interestingly, it was shown that gut health status is an important factor defining the gill bacterial assemblages of fish (Legrand et al. 2018). Although fish gill microbiota is influenced by environmental factors, its distinctiveness from bacterial populations of the surrounding sea is partly due to host factors and partly to existing microbial interactions (Rosado et al. 2019).

The core microbiota for the external epithelial tissues such as gills of anchovy and sardine yet has to be established, and we hope to have contributed to the resolution of that issue. It is also known that a wide range of bacteria are present on fish, but only a small number can survive and participate in fish spoilage after hauling, under favorable storage conditions (Zhengkayi et al. 2023). Therefore, the potential for microbial contamination of anchovy and sardine tissues during post-catch and storage results from their harvesting conditions and preservation processes that support the spread of microorganisms. Furthermore, cautious processing methods significantly reduce bacterial load in the fish tissues to be used as food.

**Conclusions**

 In this study, the abundance of cultivable bacteria in the gills of harvested sardines and anchovies, in the tanks in which they are stored on board, and in the fishing nets was investigated in relation to the warm or cold season. The related food safety risks derive from the findings of pathogens and fish spoilage bacteria on both the fish and the HDPE plastic surfaces of the fishing nets and storage tanks. This risk may surge as the temperature of the seawater and the ambient temperature rise during the hauling and sorting of fish in storage tanks. In addition, contamination and cross-contamination may increase due to the likely presence of contaminants in bacterial biofilms on plastic surfaces, such as *Vibrio, Bacillus, Psychrobacter, Salinicoccus, Halomonas* and other microorganisms of Gammaproteobacteria and Firmicutes known to be associated with plastic surfaces (Moyal et al. 2023). Bacteria common to fish gills and plastics have been identified, as well as bacteria characteristic solely for anchovies or sardines or solely for tanks and net surfaces, which is a novel key finding, particularly with respect to the importance of prospective bacterial migrations between planktonic and sessile lifestyles.

**Statements and Declarations**

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**Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

**Author Contributions**

Conceptualization NTP; methodology NTP, SK, KB, MB, GB, SB; data curation ISP, RČR; formal analysis SK, KB, MB, GB, SB; investigation NTP, SK, KB, SB, MB, GB, ISP, RČR, DM; validation NTP, KB; writing—original draft preparation NTP; visualization KB; writing—review and editing NTP, KB; supervision, resources, project administration, and funding acquisition NTP, ISP, DM. All authors have read and agreed to the published version of the manuscript.

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**Ethics approval**

All experiments have been conducted as per the guidelines of the Institutional Animal Ethics Committee of the Ruđer Bošković Institute, Zagreb, Croatia. However, fish used in this study were captured for commercial purposes. Therefore, use of these animals in research does not require ethical approval.

**Data availability**

The data is available upon request from the corresponding author.

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**Figures**



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 **Fig. 1** The number of bacterial genera (top) and species (bottom) with high identification

 confidence between different fish species and surfaces

**Tables**

**Table 1** The number of distinctive bacterial species and genera identified from all fish and surfaces according to (warm/cold) seasons

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Distinctive species | Overall | Overall warm | Overall cold | Only warm | Onlycold | Both seasons |
| Anchovy | 64 | 41 | 25 | 39 | 23 | 2 |
| Sardine | 111 | 44 | 78 | 33 | 67 | 11 |
| Tanks  | 26 | 26 | 4 | 22 | 0 | 4 |
| Nets | 23 | 14 | 11 | 12 | 9 | 2 |
| Total  | 163 | 90 | 92 | 71 | 73 | 19 |
| Distinctive genera | Overall | Overall warm | Overall cold | Only warm | Onlycold | Both seasons |
| Anchovy | 30 | 19 | 18 | 12 | 11 | 7 |
| Sardine | 53 | 22 | 38 | 15 | 31 | 7 |
| Tanks  | 15 | 15 | 1 | 14 | 0 | 1 |
| Nets | 16 | 11 | 9 | 7 | 5 | 4 |
| Total  | 74 | 47 | 43 | 31 | 27 | 16 |

**Table 2** The presence/absence of bacterial species identified by MALDI-TOF MS from gills of freshly harvested anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*), from fish storage tanks and fishing nets in warm or cold season of sampling, when sea temperatures ranged from 20-23 °C and 11-14 °C, respectively

| Taxon | Confidence level\* | Anchovy(warm/cold) | Sardine(warm/cold) | Tanks(warm/cold) | Nets(warm/cold) |
| --- | --- | --- | --- | --- | --- |
| *Acinetobacter johnsonii* | SH | - / - | + / - | - / - | - / - |
| *Aerococcus viridans* | GH SL | + / - | - / - | - / - | - / - |
| *Aeromonas eucrenophila* | SH | + / - | + / - | - / - | - / - |
| *Aeromonas hydrophila* | GH SL | - / - | + / - | - / - | - / - |
| *Aeromonas media* | SH | - / - | + / + | - / - | - / - |
| *Aeromonas molluscorum* | SH | + / - | - / - | - / - | - / - |
| *Aeromonas sobria* | SH | - / - | + / - | - / - | - / - |
| *Aeromonas veronii* | SH | + / - | + / + | - / - | - / - |
| *Arthrobacter psychrolactophilus* | GH SL | + / - | - / - | - / - | - / - |
| *Arthrobacter stackebrandtii* | GH SL | + / - | - / - | - / - | - / - |
| *Bacillus amyloliquefaciens* | SH | - / - | - / - | + / - | - / - |
| *Brachybacterium conglomeratum* | GH SL | - / - | + / - | - / - | - / - |
| *Brachybacterium paraconglomeratum* | GH SL | - / - | + / - | - / - | - / - |
| *Chryseobacterium scophthalmum* | GH SL | - / - | + / - | - / - | - / - |
| *Comamonas spp.* | GH SI | - / - | + / - | - / - | - / - |
| *Corynebacterium flavescens* | GL  | - / - | - / - | + / - | - / - |
| *Cryptococcus flavescens* | GL  | - / - | - / + | - / - | - / - |
| *Dietzia natronolimnaea* | GL | - / - | + / - | - / - | - / - |
| *Exiguobacterium artemiae* | GH SL | + / - | - / - | + / - | - / - |
| *Exiguobacterium marinum* | GL  | - / - | - / - | + / - | - / - |
| *Exiguobacterium mexicanum* | SH | + / - | + / - | + / - | - / - |
| *Exiguobacterium oxidotolerans* | SH | - / + | - / - | + / - | - / - |
| *Exiguobacterium sibiricum* | SH | + / - | - / - | - / - | - / - |
| *Flavobacterium granuli* | SH | + / - | + / - | - / - | - / - |
| *Flavobacterium psychrolimnae* | GL  | + / - | - / - | - / - | - / - |
| *Glutamicibacter bergerei* | SH | - / - | + / + | - / - | - / - |
| *Halomonas aquamarina* | GL | - / - | - / - | - / - | + / - |
| *Janthinobacterium lividum* | SH | - / - | + / - | - / - | - / - |
| *Lacticaseibacillus paracasei* | GL | - / - | + / - | - / - | + / - |
| *Leucobacter triazinivorans* | SH | - / - | - / - | - / - | + / - |
| *Nocardia wallacei* | GL | - / - | - / - | - / - | + / - |
| *Paracoccus spp.* | GH SI | - / - | - / - | + / - | - / - |
| *Proteus mirabilis* | SH | + / - | + / - | - / - | - / - |
| *Providencia rettgeri* | SH | + / - | - / - | - / - | - / - |
| *Pseudoclavibacter helvolus* | GH SL | - / - | - / - | + / - | - / - |
| *Pseudomonas anguilliseptica* | GH SL | + / - | + / + | - / - | - / - |
| *Pseudomonas antarctica* | GH SL | - / - | + / + | - / - | - / - |
| *Pseudomonas chlororaphis* | GH SL | + / - | + / - | - / - | - / - |
| *Pseudomonas fragi* | SH | + / - | + / - | - / - | - / - |
| *Pseudomonas frederiksbergensis* | GH SL | - / - | + / - | - / - | - / - |
| *Pseudomonas gessardii* | SH | + / - | + / - | - / - | - / - |
| *Pseudomonas lundensis* | GH SL | + / - | - / - | - / - | - / - |
| *Pseudomonas rhodesiae* | GH SL | - / + | + / + | - / - | - / - |
| *Pseudomonas taetrolens* | GH SL | + / - | - / - | - / - | - / - |
| *Psychrobacter alimentarius* | GH SL | + / - | - / + | + / + | - / + |
| *Psychrobacter arcticus* | GH SL | + / - | + / + | - / - | - / - |
| *Psychrobacter celer* | GH SL | - / - | - / - | + / - | - / - |
| *Psychrobacter immobilis* | SH | - / + | - / + | - / - | - / + |
| *Psychrobacter luti* | GH SL | + / - | + / - | + / - | - / - |
| *Psychrobacter maritimus* | GH SL | + / - | + / - | + / + | - / - |
| *Psychrobacter namhaensis* | GH SL | + / - | + / + | + / + | - / - |
| *Psychrobacter spp.* | GH SI | + / - | - / - | + / - | + / - |
| *Psychrobacter vallis* | GH SL | + / - | + / - | + / + | + / + |
| *Rothia endophytica* | SH | - / - | - / - | + / - | - / - |
| *Salinicoccus roseus* | GH SL | - / - | - / - | - / - | + / - |
| *Shewanella baltica* | SH | + / - | + / - | - / - | - / - |
| *Shewanella profunda* | GL  | + / - | + / - | - / - | - / - |
| *Vagococcus fluvialis* | GL  | + / - | - / - | - / - | - / - |
| *Vibrio alginolyticus* | SH | + / - | + / - | - / - | + / - |
| *Vibrio anguillarum* | SH | - / - | + / + | - / - | - / - |
| *Vibrio cyclitrophicus* | GH SL | - / - | + / - | - / - | - / - |
| *Vibrio europaeus* | SH | - / + | - / - | - / - | - / - |
| *Vibrio fortis* | GH SL | - / + | - / - | - / - | - / - |
| *Vibrio gigantis* | SH | + / + | + / + | - / - | + / + |
| *Vibrio harveyi* | SH | + / - | + / - | - / - | - / - |
| *Vibrio pelagius* | GH SL | - / - | - / - | - / - | + / - |
| *Vibrio pomeroyi* | SH | + / - | + / + | - / - | - / - |
| *Vibrio tasmaniensis* | GH SL | - / - | - / + | - / - | - / - |
| *Vibrio xuii* | SH | - / - | + / - | - / - | - / - |

\* MALDI-TOF MS identification taxonomic confidence results: G for genus and S for species taxonomic levels and I (inconclusive), L (low), and H (high) for confidence levels. If genus is inconclusive or low, species level is inconclusive and is not indicated. Conversely, if species is high, genus is high and not indicated. Results are presented for isolates that had no inconclusive genus identification, see Supplementary Table S1 for full results