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Assessment of the microbiological quality of three frozen seafood products of the Moroccan Atlantic

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Abstract
To assess the microbiological quality of frozen seafood products onboard deep-sea fishing vessels, samples of three species *Sepia officinalis*, *Solea vulgaris* and *Parapenaeus longirostris* were taken during the landing of these marine products at the port of Agadir, Morocco. All samples were analyzed for Aerobic Germs at 30°C, *Staphyloccocus aureus*, Enterobacteria, Coliforms at 30°C, Coliforms at 44°C, *Escherichia coli*, Anaerobic sulphitoreducer germs, *Listeria monocytogenes,* and *Salmonella spp*.
Absence of Salmonella and L. monocytogenes is noted in all samples of the three species, (0 CFU/25g). For S. officinalis, S. vulgaris and P. longirostris, the concentration of E. coli, Enterobacteria and Coliforms at 30°C counts were all below the regulatory threshold (10 CFU/g). S. aureus was less than 100 CFU/g for the three species. Our results also showed that the concentration of Coliforms at 44°C and the anaerobic sulphitoreducer germs (ASR) were less than 10 CFU/g for the three species.
Aerobic germs at 30°C counts ranged between 40 CFU/g and 1100 CFU/g in S. officinalis, between 45 CFU/g and 5000 CFU/g in S. vulgaris and between 55 CFU/g and 3800 CFU/g in P. longirostris. These results are below the thresholds recommended by national and international regulations, which are 105 CFU/g, 106 CFU/g and 107 CFU/g respectively for P. longirostris, S. officinalis and S. vulgaris.
This study shows that the seafood products landed by the vessels considered in this study are of good microbiological quality and show no contamination.

Keywords: microbiological assessment; bacteria; seafood; agadir; dakhla; Morocco

1. Introduction

Fish and seafood products are considered an important part of a balanced diet, they contain high levels of many nutrients that are not commonly found in other foods [12]. Seafood provides high-quality proteins, minerals essential traces elements, fat-soluble vitamins (vitamin D) and essential fatty acids [22, 15]. Aquatic products, especially fish, are sources rich in long-chain polyunsaturated fatty acids of the omega 3 series, such as eicasopentanoic acid or docasohexanoic acid [19, 10]. This high nutritional value means that these products remain the most perishable food item, and consumers are extremely sensitive to possible damage. Fish, crustaceans and mollusks can produce toxins or concentrate in their tissues toxins made by marine organisms that they feed on [25]. In their natural environment, seafood products are exposed to different microbiological and chemical contaminants, from natural or human origin. Microbiological contaminants are mainly found in the gills, coetaneous mucus and viscera, the muscles are theoretically not contaminated by pathogenic germs [16].
Microbial flora plays an important role in the degradation of fishery products, and the improvement of hygiene conditions can optimize the quality of fish [27], hence the importance of assessing the contamination of these products. Since 1962, a study at the Pasteur Institute on the microbial flora of West African fishes has made it possible to isolate several germs belonging in large part to the families of *Pseudomonadaceae, Enterobacteriaceae* and *Bacillaceae* [13]. As early as 1980, there was an international willingness to adopt preventive measures and HACCP safety and quality systems [1].
Studies of the microbiological quality (microbial indicators) of the flesh of fish taken at the point of harvest often reveal low levels of bacterial contamination. However, when fish are sampled on the market, there are frequently higher levels of contamination in their edible flesh [16]. Fish contamination by pathogenic bacteria reflects the use of unsanitary handling practices [5]. Wild or farmed seafood products are highly perishable, their quality is rapidly degraded due to the high moisture content and readily digestible macro-components. They are sensitive to the rapid deterioration and post-harvest loss due to both microbial growth and biochemical reactions exacerbated by high temperatures. Other problems are caused by contaminants present in the environment where seafood products are grown and harvested [4]. A major goal for the food processing industry is to provide safe, to meet this goal, the control of microorganisms is essential. This control is partly exerted through processing and preservation techniques that eliminate microorganisms or prevent their growth [26].
Along the chain, from catch to landings and processing, the fish are in contact with the surfaces of the handling equipment, as well as the storage and washing water in the

production environment. During this contact, microbial contamination can be caused by water, personal hygiene and inadequate cleaning procedures. Just after capture and when fish arrive on the deck of the fishing boat, bacteria grow quickly and their skin no longer provides natural protection against bacteria [12]. It is at this stage that intervention is essential to maintain quality at the highest level by starting as soon as possible [5].
Morocco has an exclusive economic zone extending up to 200 nautical miles, characterized by a great diversity of resources of about 500 species. The fishing sector in Morocco is organized in three segments, artisanal fishing with 14225 boats, inshore fishing with 1835 coastal vessels and deep-sea fishing with 344 offshore vessels. The contribution of Morocco's fishing sector to the national GDP is 2% to 3% depending on the year. 70% of Moroccan turnover is made for export to various international markets including the European Union, USA, Asia and Africa (According to development strategy and competitiveness of the fisheries sector, Moroccan Ministry of Agriculture and sea fishing).
To ensure the safety of fishery products introduced on the market, regulations have been developed at national and international level, Order 624-04 and 293-19 on microbiological standards, Regulations (EC) No. 2073/2005 and (EC) No 1441/2007 concerning microbiological criteria for foodstuffs, Regulations (EC) No 1881/2007 and (EC) No 1005/2015 fixing maximum levels for certain contaminants in foodstuffs. In order for imported food products to be marketed in the EU, they must comply with its food safety requirements or with conditions recognized by it to be at least equivalent (Regulation 178/2002).
In Morocco, most seafood quality studies have so far been carried out on land and few people have been interested in source contamination. These studies are more concerned with bivalve molluscs harvested on the coasts [24, 7, 8, 20, 14, 2, 3]. However, no studies have been done on deep-sea products. This is the context of the work presented in this survey, which aims to assess, for the first time, the microbiological quality of three sea products caught by fishing vessels on the high seas, operating more than ten nautical miles from the Moroccan Atlantic and implementing a food safety management system respecting the rules of good hygiene practice. Our results are compared to the different national and international regulations in force. These results can also serve as a database for those wishing to set up a food safety management system on board fishing vessels.

2. Materials and methods

2.1. Species studied

In this study, three species were used as biological material, the fish (*Solea vulgaris*), the cephalopod (*Sepia officinalis*) and the shrimp (*Parapenaeus longirostris*). These three species are used as sentinel species in several studies [23, 17]. and also, are, amongst others, the most targeted by the fishing activity and are intended for the local and international market in large quantities.

2.2. Geographic origin

For the three species considered in this study, *S. vulgaris* and *S. officinalis* were caught in the open sea of Dakhla, while *P. longirostris* was caught in the open sea of Agadir. These products were landed at the port of Agadir by deep-sea fishing vessels that practice fishing beyond 10 nautical miles.
To represent the different fishing points of our analyzed samples, the ArcGIS geographic information software was used. This software allowed us to mark the exact geographical position of the fishing origin of our samples and to know the depth, which varies between 25 m and 220 m. As well as the distance between this point and the coast which varies between 21. 42 km and 72.37 Km. Figure 1 illustrates different positions that are marked by SO for *Sepia officinalis*; SV for *Solea vulgaris* and PL for *Parapenaeus longirostris*.

2.3. Sampling

2.3.1. Sampling method

Samples were taken monthly from December 2015 to January 2017 on frozen products landed by deep-sea fishing vessels in the Agadir port, except for the biological rest periods in which fishing is prohibited for the species considered.
When the boats are unloaded at the port of Agadir, the sample was taken and the sample information was recorded on a sample sheet. The temperature was measured using a calibrated thermometer, the fishing date was recorded, sampling time and the serial number of the body containing the sample were noted. Samples taken were immediately put in the refrigerator within a period not exceeding 10 minutes before sending it to the laboratory the same day of sampling. Upon arrival at the laboratory, the product temperature was measured and the sample was stored at -18°C until analysis.
In addition to the samples taken at the landing in the port of Agadir, a sample was taken aboard a boat just after received the fishing capture for *S. officinalis*, *S. vulgaris,* and *P. longirostris*. This sample was frozen immediately without undergoing any treatment, and whose analysis tells us about the initial bacterial load. This sample was marked (Control).

- For the fish (*Solea vulgaris*): We take a block of 3 kg.
- For cuttlefish (*Sepia officinalis*): We take a block of 3 kg.
- For deepwater rose shrimp (*Parapenaeus longirostris*): we take one box of 1 kg.

2.3.2. Sample information

Using a traceability system installed onboard fishing vessels; we have determined a variety of environmental parameters that can influence the microbiological quality of our samples. They are the geographical location of sampling, the trawling time (TR), the quantity caught by the haul on the sample (Qty), the duration of onboard processing operations (DP), the duration of refrigeration (DR), the duration of freezing (DF), the freezing temperature (FT), the product temperature (PT) and the storage temperature (ST). We also determined for each sampling depth (DH) and distance from the coast (DC).

2.3.3. Microbiological analysis

In the laboratory, after defrosting the sample 25 grams of the flesh were blended with 225 ml of sterile water. After 15 min, we grind the solution in a stomacher bag for 60 seconds. 0.1 ml of the suspension was transferred to a test tube with sterile water to prepare different dilutions.
In this study, the various germs sought in the tree species are: Total aerobic germs at 30°C were determined by NM ISO 4833-1, *Staphylococcus aureus* determined by NF V

08 057-1, Enterobacteria by NF V 08 054, Coliforms at 30°C by NF V 08 050, Coliforms at 44°C by NF V 08 060, *Escherichia coli by* ISO 16649-2, Anaerobic Sulphitoreducer germs (ASR) by NF V 08 061, *Listeria monocytogenes* by ISO 11290-1 and *Salmonella spp by using* NF ISO 6579. The methods used are all recounted and validated.

2.4. Statistical analysis
A correlation test was performed with Statistica v6 at a significance level of $p<0.05$ to test the relationship between environmental parameters and the concentration level of aerobic germs in the muscles of the three species considered. Furthermore, for aerobic germs, we tried to assess the significant differences between months in one hand, and between each month with the control in the other hand. To do that, Statistical Software (*STATISTICA* and *SPSS*) were adopted.

3. Results
3.1. Environmental parameters
The environmental parameters monitored in this study are shown in Tables 1, 2 and 3 respectively for the three species *S. officinalis*, *S. vulgaris,* and *P. longirostris*. These are the fishing date, the sampling date, sampling depth (DH), distance of the coast (DC), the trawling time (TR) which is the time the fishing trawl remains in the water, the duration of the preparation steps onboard (DP), which is the time required for sorting, washing and sizing the product. The refrigeration time (DR), which is the time spent in the tunnel by the sample at a temperature ≤ 2°C, the freezing time (DF), the end-of-freezing temperature (FT), the storage temperature (ST) and the product temperature (PT), are also recorded. The knowledge of these parameters is useful to give information about which impacted the concentration of bacteria in the species cited above, since several environmental factors influence microbial growth in food [6].
Between *S. officinalis* and *S. vulgaris*, there are no notable differences in most of the parameters. While for *P. longirostris*, the profiles are different from the other two species. In general, the means of environmental parameters that exhibit significant fluctuations between the three species *S. officinalis, S. vulgaris* and *P. longirostris* are respectively: DH (m): 50, 49 and 155; TR (min): 115, 120 and 215; DR (min): 227, 228 and 0; DF (min): 490, 500 and 202; FT(°C): -43, -43 and -30; ST (°C): -29, -28 and -23 and finally, PT (°C): -26, -27 and -22. DH is linked to the type of fishery that requires more depth in the catch of shrimp than that of fish and cephalopods. The shrimp TR is larger than the TR of the other species, it is in relation with the technique adopted and the fishery nature. The shrimp does not undergo a refrigeration, that is why its DR equals zero, the other two species need a refrigeration of almost 227 min. *S. officinalis* and *S. vulgaris* freezing duration (DF) are high compared to *P. longirostris,* because of the difference in volume and texture. The higher negative freezing (FT), storage (ST) and processing (PT) temperatures leads to the drying of our shrimp, which is why they are low than *S. vulgaris* and *S. officinalis* DF, ST and PT.

3.2. Concentrations of microbiological germs
The results of the microbiological analyses carried out on the different samples of the *S. officinalis*, S. *vulgaris* and *P. longirostris* are regrouped in tables 4, 5 and 6. These results showed that *Salmonella* and *L. monocytogenes* were absent in 25g (0 CFU/25 g). *E. coli*, *Enterobacteria*, Coliforms at 30°C, Coliforms at 44°C and Anaerobic Sulphitoreducer germs counts were all less than 10 CFU/g. For *S. aureus*, it was less than 100 CFU/g.
For the sample taken before treatment (in situ) as a control, the concentration of Salmonella and *L. monocytogenes* were absent in 25 g, while Coliforms at 30°C, Coliforms at 44°C, *E. coli*, and the ASR concentrations are all less than 10 CFU/g in the muscles of the three species considered. For *S. aureus*, the concentration of these germs in the muscles of the three species is less than 100 CFU/g. For Enterobacteria, they were <10 CFU/g of sample for *S. officinalis* and *S. vulgaris* while for *P. longirostris*, 40 CFU/g of these bacteria were detected in analyzed product.

3.3. Concentrations of Aerobic germs at 30°C in *S. officinalis*, *S. vulgaris*, and *P. longirostris*
Figure 2 shows that the smallest concentrations of aerobic germs at 30°C for *S. officinalis*, and *S. Vulgaris* are 40 CFU/g and 45 CFU/g respectively while The greatest concentrations are 1100 CFU/g and 5000 CFU/g. The lowest concentration of aerobic germs at 30°C in *S. Vulgaris* was in June 2016 with 45 CFU/g, it corresponds to the lowest product temperature with -34.33°C. The highest concentration was in February 2016 with 5000 CFU/g, it corresponds to the highest refrigeration time with 685 min. The lowest concentration of aerobic germs at 30°C in *S. officinalis* was in June 2016 with 40 CFU/g, it corresponds to the lowest product temperature with -34°C. The highest concentration was in February 2016 with 1100 CFU/g, it corresponds to the lowest refrigeration time with 30 min. For *P. longirostris,* the highest concentration of aerobic germs at 30°C was in December 2015 with 3800 CFU/g while the lowest concentration was in December 2016 with 55 CFU/g.
For the sample taken before treatment (in situ) as a control, the concentration of aerobic germs at 30°C was about 310 CFU/g for *S. officinalis,* 460 CFU/g for *S. vulgaris* and 830 CFU/g for *P. longirostris*.

3.4. Correlation between environmental parameters and concentrations of aerobic germs at 30°C
Table 7 shows the values of the correlation coefficients between the environmental parameters and the concentrations of aerobic germs at 30 ° C. there is a positive correlation with the trawling time (TR) for *S. officinalis*, and with the refrigeration time (DR) for *S. vulgaris*. For *P. longirostris*, there is a negative correlation with trawling time (TR).

4. Discussion
Our investigations focused on the concentrations of different germs in the muscles of three species of seafood products from the deep-sea fisheries: the fish *Solea vulgaris*, the cephalopod *Sepia officinalis* and the shrimp *Parapenaeus longirostris*. These products are fished, handled and then frozen and stored onboard fishing vessels that adopt a Food Safety Management System (FSMS) gauged in accordance with the requirements of the international standard ISO 22000: 2005. The main purpose of the application of these requirements is to avoid the health risks caused by the consumption of seafood that does not meet the quality and safety requirements [1]. Consequently, most of the seafood-

borne diseases of microbiological origin are caused by the consumption of the contaminated product [4]. *S. vulgaris* and *S. officinalis* are caught in the Dakhla region, which is an area known by its large stock of fish and cephalopod. While *P. longirostris* was caught in the open sea of Agadir where the fishing of this species has been widely practiced.

The results presented in this study show that *Salmonella* and *Listeria monocytogenes* are not detected in 25 g for the three studied species of seafood, which confirms the application of the stringent requirements of the standard. Our results agree with the study realized in fish processing in India [26] which showed that *Salmonella spp* were absent in all samples analyzed for shark and tuna. Indeed the study carried out in Sri Lanka shows that *Salmonella spp* and *L. monocytogenes* were detected in large and small fish on nine occasions [5]. *L. monocytogenes* which is frequently isolated from ready-to-eat seafood products than from raw materials [15, 21] is considered a serious risk in relatively closed environments. This bacterium has the potential to grow at refrigeration temperatures and in high saline matrices such as seafood, it was isolated from 12.4% of fish in Serbia during 2013 [21].

For Enterobacteria, Coliforms at 30°C, Coliform at 44°C, and E. coli, considered as an index of fish quality [22], and often used as an indicator of fecal contamination [16], the concentrations are all less than 10 CFU/g in the muscles of the three species considered in this study. This is due to the fact that compliance with the rules of good hygiene practices, cleaning and disinfection of equipment used as well as the work environment, is carried out in a methodical and uniform manner during the handling and preparation of the three sea products. Our results agree with those of the study carried out in Mandapam in India [26], which have shown that the high level of basic hygiene eliminate spoilage and pathogen bacteria. In contrary to the result reported by Ariyawansa *et al*., (2015) [5] in Sri Lanka, *E. coli* was present in 70% of small fish samples, the fecal Coliform counts about 90 MPN/g in large fish and 1100 MPN/g in small fish. According to these authors, the presence of pathogens and Coliform bacteria in fishes indicates a contaminant environment, poor post harvest processing and handling of fisherman.

For ASR germs, which are naturally present in the aquatic environment and which can be found both on live fish and on the raw material of fish [18], the concentrations are less than 10 CFU / g at the level of the three sea products studied. This result justifies the good initial quality of the samples analyzed, the absence of any cross-contamination, rapid cooling to a temperature <+ 2 ° C and compliance with the cold chain.

S. aureus, was found in all the samples of the three species but the concentrations were less than 100 CFU/g, because of the application of the walking forward principle, as well as freezing fast and conservation at temperatures < -18°C throughout the storage of these species. The study conducted in Mandapam by Prabakaran *et al.* in 2011 showed that the concentrations of *S. aureus* in Tuna and shark were also less than 100 CFU/g, because of the application of strict personal hygiene. Several germs included *S. aureus* has been isolated from the hands of workers in the food industry [22].

For the three species consider in this study, the low concentrations of these germs, does not eliminate the risk to consumer health, as these concentrations can be increased when distributing the product on the market. The study conducted by Chanpiwat *et al.* (2016) [9] in Vietnam found that *E. coli* and *L. monocetogenes* concentrations increase when the product was sampled on the market. Indeed, the bacterial flora in the local fish market is significantly higher than in the catchment point [12].

For the sample taken before treatment (in situ) as a control, the concentration of *Salmonella* and *L. monocetogenes* were absent in 25 g, while, Coliforms at 30°C, Coliforms at 44°C, *E. coli*, and the ASR concentrations were all less than 10 CFU/g in the muscles of the three species considered. For *S. aureus*, the concentrations were less than 100 CFU/g. These results are similar to those recorded in this study, for the samples taken on board the deep-sea fishing vessels which were processed on board. This confirms that contamination by these germs would generally result from non-compliance with good handling practices. For *P. longirostris*, the concentration of *Enterobacteria* recorded in the control sample (40 CFU/g) was higher than that of the other samples (<10 CFU/g). This result shows that the method used to wash the product immediately after fishing is effective and reduces the concentration of these germs.

Concerning the aerobic germs at 30°C, the statistical analysis showed that between species, sampling month and with the control sample there is a very high significant difference (p<0,001). For *S. officinalis*, the concentrations were between 40 CFU/g in June 2016 and 1100 CFU/g in February 2016. The concentrations of these germs are positively correlated with the trawling time. This means that the longer the trawl duration, the higher the concentrations of aerobic germs. In fact, just after capture, the species' defense against the proliferation of these germs decreases and their concentrations can increase during trawling.

For *S. vulgaris*, the concentration of aerobic germs at 30°C was between 45 CFU/g in June 2016 and 5000 CFU/g in March 2016, it correlates positively with refrigeration time and negatively with the frozen temperature. These results can be explained by the fact that the refrigeration and frozen methods affected significantly the growth and development of the aerobic germs at 30°C. These results show very low levels of contamination compared to the study carried out in Sri Lanka, which showed high concentrations of aerobic germs ranging from 2.10^2 to 2.10^6 and from 8.10^3 to 2.10^8 UFC/g in large and small fish respectively [5].

For *P. longirostris* the concentrations of aerobic germs at 30°C was between 55 CFU/g in December 2015 and 3000 CFU/g in December 2016. These concentrations correlate negatively with the trawling time (TR), the thing that we could explain by the drop of water temperature during the night when the TR is important.

For the three species, the concentrations of aerobic germs at 30 °C in the control sample, were always lower than the maximum values found in the other samples, but there are concentrations that are lower than the control. This result can be explained by the fact that the three species are in a very good microbiological quality just after the capture and the concentrations of aerobic germs at 30°C could decrease, after a fast washing for example, as it can also increase during various handling operations on board.

We note, in this study, that in most sampling months and also in the control sample, the concentrations of aerobic germs at 30 °C found in *P. longirstris* caught in the offshore of Agadir are higher than those recorded in *S. officinalis* and S. *vulgaris* caught in the open sea of Dakhla. This could be

related to the cancellation of refrigeration step (DR=0), the perishability and vulnerability of the shrimp as well as its external texture which can be a shelter and a refuge for germs to avoid elimination.

Our results show that for all the samples analyzed, the results are all in accordance with the current regulations. Whereas, the study carried out by Ariyawansa *et al.* (2015) [5] in Sri Lanka showed that the microbiological quality of a large proportion of fish from fishing vessels do not comply with regulatory requirements. The low quality of fish indicates poor handling practices along fish supply chains. In addition, in the Norwegian pelagic fishery, which the hygiene conditions at some critical points at an early phase of the production chain, 51% of all fishing vessels and fish-processing factories did not respect the limits in force [27]. Our results can be used to implement environmental seafood supply chain management systems that should include all stages of the supply chain [11].

5. Conclusion

This study presents a first overview of the concentrations of aerobic germs at 30°C, enterobacteria, total and fecal coliforms, *S. aureus*, ASR, *Salmonella*, *E. coli* and *L. monocetogenes* in the muscles of three seafood products. *(S. vulgaris, S. officinalis,* and *P. longirostris*) landed in the port of Agadir by deep-sea freezer vessels adopting a food safety management system (FSMS) certified according to the international standard ISO 22000: 2005. For all the samples analyzed for the three species, the concentrations of the different germs were well below the regulatory thresholds. Our results confirm that the introduction of preventative food safety techniques based on a comprehensive hazard analysis helps to maintain the microbiological quality of seafood products from the high seas fishery at a higher level and to prevent harvested products potential contamination.

We recommend the generalization of the application of this management system on board for boats in the national offshore fishery.

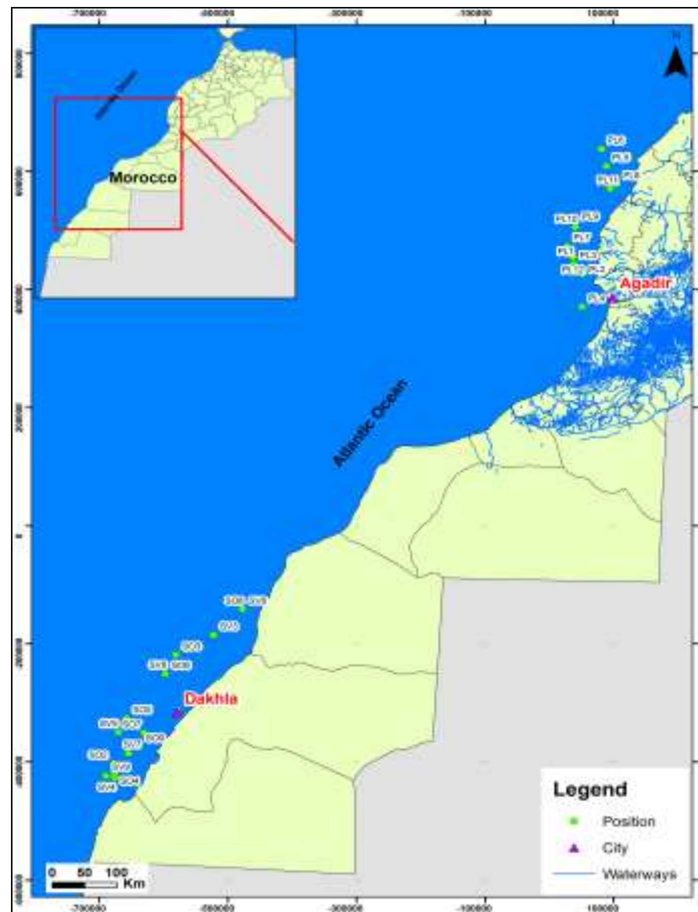

Fig 1: Representation of the geographic origin of biological samples

Table 1: Environmental parameters for *S. officinalis*

Fishing date	Sampling date	DC (Km)	DH (m)	TR (min)	DP (min)	DR (min)	DF (min)	FT °C	ST°C	PT°C
12/12/2015	28/12/2015	72.34	55	100	75	80	535	- 42	-31	-22.33
16/01/2016	02/02/2016	51.01	51	125	160	220	410	- 40	-27	-25.33
07/02/2016	22/02/2016	72.37	57	135	80	30	485	- 40	-29	-31
07/03/2016	05/04/2016	39.77	45	125	100	705	495	- 52	-30	-24.33
04/06/2016	11/07/2016	69.68	65	110	75	445	505	- 40	-30	-34.33
25/07/2016	29/08/2016	55	58	130	75	150	520	- 43	-21	-21.67
06/08/2016	29/08/2016	33.87	25	65	125	90	465	- 39	-28	-24
15/12/2016	16/01/2017	28.14	27	120	25	210	480	- 40	-31	-26.33
05/01/2017	16/01/2017	26.90	65	120	65	105	515	- 48	-31	-26

Table 2: Environmental parameters for *S. vulgaris*

Fishing date	Sampling date	DC (Km)	DH (m)	TR (min)	DP (min)	DR (min)	DF (min)	FT °C	ST°C	PT°C
13/12/2015	28/12/2015	67.41	47	140	135	45	495	-42	-31	-22.33
18/01/2016	02/02/2016	36.83	42	110	60	330	410	-42	-27	-26.67
13/02/2016	22/02/2016	59.33	42	120	105	30	485	-40	-29	-32.33
09/03/2016	05/04/2016	42.10	64	110	80	685	565	-52	-28	-25.33
06/06/2016	11/07/2016	66.34	62	110	90	515	475	-40	-28	-34.33
25/07/2016	29/08/2016	55	58	130	55	190	560	-44	-23	-21.67
07/08/2016	29/08/2016	36.30	26	80	100	80	430	-40	-28	-26
09/12/2016	16/01/2017	28.14	47	120	35	180	480	-40	-29	-25.33
10/01/2017	16/01/2017	52.02	50	160	55	0	600	-49	-31	-28

Table 3: Environmental parameters for *P. longirostris*

Fishing date	Sampling date	DC (Km)	DH (m)	TR (min)	DP (min)	DR (min)	DF (min)	FT °C	ST°C	PT°C
12/12/2015	06/01/2016	29.08	120	180	65	0	150	-28	-23	-22.73
13/01/2016	01/02/2016	21.42	140	200	70	0	375	-29	-22	-19.77
14/03/2016	23/03/2016	39.95	210	230	80	0	170	-37	-23	-21.47
27/04/2016	04/05/2016	41.69	200	285	135	0	180	-29	-23	-22.47
13/05/2016	23/05/2016	45.73	180	195	110	0	190	-29	-22	-21.13
11/06/2016	29/06/2016	57.07	110	225	105	0	210	-30	-24	-20.67
11/09/2016	20/09/2016	49.70	220	265	110	0	210	-28	-22	-22.6
11/09/2016	20/09/2016	25.30	138	190	75	0	180	-30	-22	-20.8
10/10/2016	12/10/2016	38.93	175	170	65	0	180	-29	-24	-20.27
07/11/2016	09/11/2016	38.20	70	205	50	0	180	-29	-23	-19.53
29/12/2016	03/01/2017	28.15	117	245	90	0	185	-30	-23	-21.83
26/01/2017	31/01/2017	40.78	190	190	90	0	210	-29	-23	-24.93

Legend: Distance of the coast (DC); Sampling depth (DH); Trawling time (TR); Duration of onboard processing operations (DP); Duration of refrigeration (DR); Duration of freezing (DF); Freezing temperature (FT); Storage temperature (ST); Product temperature (PT)

Table 4: Concentration (CFU) of different germs in *S. officinalis*

Germs (CFU/g)	Dec-15	Jan-16	Feb-16	Mar-16	Jun-16	July-16	Aug-16	Dec-16	Jan-17	Control
Enterobacteria	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Coliform at 30°C	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Coliform at 44°C	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
E. coli	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
S. aureus	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
ASR	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Salmonella	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g
Listeria	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g

Table 5: Concentration (CFU) of different germs in *S. vulgaris*

Germs (CFU/g)	Dec-15	Jan-16	Feb-16	Mar-16	Jun-16	July-16	Aug-16	Dec-16	Jan-17	Control
Enterobacteria	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Coliform at 30°C	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Coliform at 44°C	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
E. coli	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
S. aureus	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
ASR	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Salmonella	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g
Listeria	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g

Table 6: Concentration (CFU) of different germs for *P. longirostri*

Germs (CFU/g)	Dec-15	Jan-16	Mar-16	Apr-16	Mai-16	Jun-16	Aug-16	Sep-16	Oct-16	Nov-16	Dec-16	Jan-17	Control
Enterobacteria	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	40
Coliform at 30°C	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Coliform at 44°C	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
E. coli	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
S. aureus	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100	<100
ASR	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Salmonella	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g
Listeria	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g	0/25g

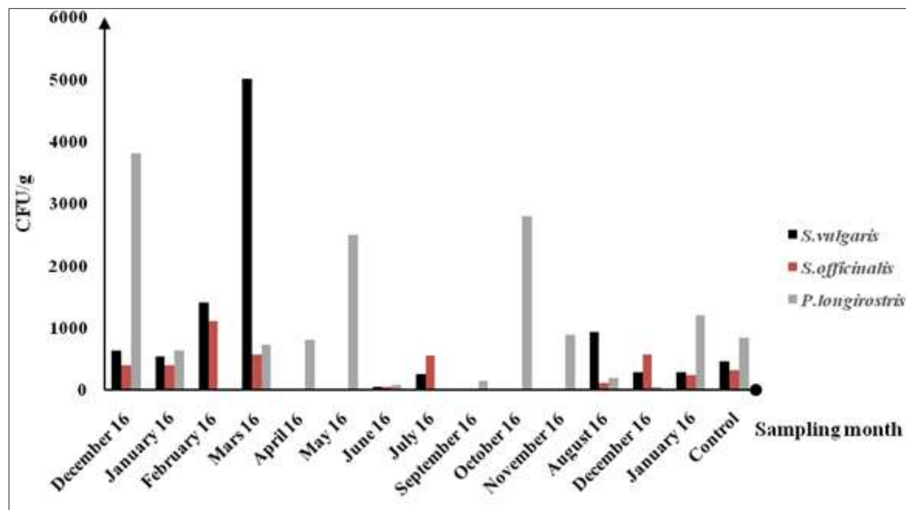

Fig 2: Concentrations of aerobic germs at 30°C *S. officinalis*, *S. vulgaris and P. longirostris*

Table 7: Correlation coefficients between concentrations of aerobic organisms at 30°C and environmental parameters

	Aerobic Germs (CFU/g)		
	S.O.	***S.V.***	***P.L.***
DC (Km)	0.27	-0.39	-0.35
DH (m)	0.02	0.36	0.02
TR (mn)	0.64	-0.24	-0.54
DP (mn)	-0.18	0.06	-0.13
DR (mn)	-0.02	0.67	-
DF (mn)	-0.02	0.31	-0.42
ST (°C)	0.12	0.02	0.34
PT (°C)	-0.01	0.23	-0.17
QT (Kg)	0.02	-0.30	-0.06

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