# Evaluation of hygienic quality and labelling of fish distributed in public canteens of Northeast Italy

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## Abstract

Over the past few years, the demand for the introduction of fish products in public canteens (schools, hospitals and nursing-homes) has grown due to their good nutritional proprieties. The particular health conditions and sensitivity of some groups of consumers exposes them to greater risks of food poisoning. It is therefore important to monitor the raw materials that end up in mass catering implementing strategies of mass catering control, both with self-monitoring strategies and with regular controls performed by the competent health authorities. The purpose of this study is to assess the overall quality of seafood dealt out from public catering services located in Northeast Italy. In this paper we illustrate the results of microbiological analysis performed on 135 fish samples (58% of samples were raw fishes, 27% cooked fishes, 6% raw fish products, 9% cooked fish products) and species identification performed on 102 fish samples. Additionally, 135 environmental swabs were collected to determine the effectiveness of cleaning and sanitation of food contact (cutting boards, cooking equipment and food processing surfaces) and non-contact (refrigerator wall and handle, tap lever) surfaces. Of raw seafood samples, 24% had total aerobic mesophilic bacteria count >$10^5$ CFU/g and for *Enterobacteriaceae* the faecal contamination was excluded since no *Salmonella* spp. and *Escherichia coli* were isolated. Just 3.8% of raw seafood samples resulted positive for *Listeria monocytogenes*. The results of swab samples of cooking utensils and surfaces showed that sanitation practices should be improved. Molecular analysis for fish species identification revealed a mislabelling for 25% of sampled fishes. The results of this survey can provide valuable information for monitoring and surveillance programmes for the control of quality of fish and fish products.

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## Introduction

Fish (finfish and shellfish) and fish products have become increasingly required in hospitals, nursing homes and school canteens because of the growing awareness of their high nutritional properties and potential health benefits (Mozaffarian and Rimm, 2006). However, along with the benefits, potential risks associated with consumption of contaminated seafood must be considered. Finfish and shellfish are very perishable: the high water content, non-protein nitrogen concentration and relatively high pH of fresh seafood make them more sensitive to microbial attack (Gram and Huss, 1996; Gram and Dalgaard, 2002). Fish, crustaceans and mollusks can acquire microorganisms from different sources: surface or tissue contamination can occur directly in the marine environment or during handling, processing and preparation of the products. Contributing factors may include storage and transportation at inappropriate temperatures, contamination by an infected food handler, or cross-contamination through contact with contaminated seafood or seawater (Iwamoto *et al*., 2010).

Seafood is responsible for an important proportion of food-borne illness and outbreaks worldwide. As transmission of food borne pathogens mostly occurs through the fecal-oral route, it is crucial to apply strict hygiene rules throughout the entire production process. It is therefore important to assess the hygienic conditions in the production environments through the analysis of microbial indicators of fecal contamination. In the current study aerobic mesophilic bacteria, *Enterobacteriaceae* and *Escherichia coli* were analyzed to assess the hygiene of food and food processing equipment. Furthermore, the examination of pathogens is required to assess food safety. Excluding autochthonous pathogens belonging to the genus Vibrio, other bacteria may be responsible for seafood-associated infections, like *Salmonella* spp. as at source contamination (*i.e.* in the sea), *Staphylococcus aureus* and *Listeria monocytogenes* as cross contamination (Lee and Rangdale, 2008).

Species substitution of fish must also be included in the list of potential health hazards.

Major fraud concerned high value species substituted by species with lower commercial value. Lower commercial values species could have also a lower nutritional value, moreover as observed by Filonzi *et al*. (2010) in many cases of substitution, fish products come from extra European areas, without the same standards of sanitary controls of farming sites, pathogens and bioaccumulation of heavy metals. Among the methods of identifying commercially imported fish species, molecular genetics is gaining increasing attention (Lockely and Bardsley, 2000) and molecular barcoding has been proposed as the favorite methodology in forensic taxonomy (Dawnay *et al*., 2007). For species identification, the sequence of the evidence item must be matched to a reference sequence (Altschul *et al*., 1997). DNA barcoding uses the mtDNA gene cytochrome c oxidase I (COI) as a *barcode* (Hebert *et al*., 2003a, 2003b).

The particular health conditions of customers of hospitals, nursing homes and school canteens expose them more than other categories to food-borne diseases. It is therefore essential to ensure the safety of raw materials, the adoption of good hygiene practices and to maintain them strictly during all stages of food preparation until distribution.

The aim of this study was to provide data on microbiological contamination of seafood and food-working surfaces in hospitals, nursing homes and school canteens and to assess the conformity of seafood species with the label information.

## Materials and Methods

### Samples collection

A total of 79 raw fish, 36 cooked fish and seafood, 8 raw fish products and 12 cooked fish

products were collected from 65 public canteens located in the Northeast Italy. Selected public canteens were canteens of preschool and primary school (n=16), companies, universities, prisons and religious communities (n=19), nursing homes and facilities for disabled people (n=26) and hospital canteens (n=4).

A sanitary monitoring program allows to assess whether every stage of the management and delivery system of catering service is kept in check: from raw material purchase to meals distribution. It was therefore decided to collect not only food samples for the assessment of safety and hygiene microbiological parameters, but also environmental samples to assess good hygiene practices adopted by the staff. Using swabbing devices, 135 surfaces and utensils have been sampled: 25 food processing surfaces, 12 fridge handles, 31 fridge inner walls, 17 knifes, 35 cutting boards and 15 tap levers. An area of 100 $cm^2$ has been tested for each device. Samples of food non-contact surfaces have been included in the study because they may serve as a vehicle of cross contamination for food.

To assess the correspondence between the label information and the packaged product, this study focused on fish families most used in catering: *Merluccidae*, *Pleuronectidae, Salmonidae.* A total amount of 102 samples were submitted to DNA barcoding for differentiation of species.

All samples were transported in suitable thermal containers to ensure maintenance of the temperature between 0 and 4°C.

## Samples analysis

Samples were analyzed by an accredited laboratory (UNI CEI EN ISO/IEC 17025/2005; ISO, 2005). Microbiological analysis on food samples was carried out according to standard ISO methods as follow. Total aerobic mesophilic plate count was performed according to ISO 4833-1:2013 (ISO, 2013). Plates were incubated in aerobiosis at 30°C for 72 hours. *Enterobacteriaceae* were enumerated according to ISO 21528-2:2004 (ISO, 2004b). Plates were incubated in aerobiosis at 37°C for 24 hours. *Escherichia coli* glucuronidase positive at 44°C were tested following ISO 16649-2:2001 (ISO, 2001) incubating plates in aerobiosis at 44°C for 24 hours. Coagulase positive staphylococci count was performed according to ISO 6888-2:1999 Amd 1 2003 (ISO, 2003). Plates were incubated in aerobiosis at 37°C for 48 h.

Presence of *Salmonella* spp. was tested according to ISO 6579:2002/Cor 1:2004 (E) and *Listeria monocytogenes* to ISO 11290-1:2005 (ISO, 2002, 2005).

The sampling procedure for environmental swabs followed standard ISO 18593:2004 (ISO, 2004a). Succeeding analysis for total mesophilic aerobic count (ISO 4833-1:2013; ISO, 2013), *Enterobacteriaceae* (ISO 21528-2:2004; ISO, 2004b) and *Listeria monocytogenes* (ISO 11290-1:2005; ISO, 2005) were carried out accordingly to already cited standard ISO. For fish species identification, DNA from all samples was recovered using the Qiaamp® DNA Minikit (Qiagen, Venlo, The Netherlands) commercial kit.

DNA was amplified using COI universal primers (CoiFish F1: 5' TCAACYAATCAYAAAGATATYGGCAC3' and CoifishR1: 5' ACTTCYGGGTGRCCRAARAATCA3'). PCR products were sequenced and all sequences were analyzed using Ittiobase (http://90.147.123.23/ittiobase/), GenBank (http://www.ncbi.nlm.nih.gov/genbank/) and BOLD Identification System (http://www.boldsystems.org) databases for species identification.

# Results

## Food samples

The sampling, carried out in different facilities, allowed the identification of the most commonly used fish genera by catering services. Among raw fish, the most represented genera were *Pleuronectiformes* (30% of samples), *Gadiformes* (28%), *Perciformes* (10%), *Salmoniformes* (9%), *Carcharhiniformes* (7%) and *Sepiida* (6%). Among cooked fish *Pleuronectiformes* were the most represented too (44%), followed by *Gadiformes* (19%), *Perciformes* (14%) and *Salmoniformes* (14%).

The microbiological analysis on raw and cooked fish, raw and cooked fish products are presented in Table 1.

Regarding the distribution of microbial population 31% of raw fish and seafood presented an aerobic mesophilic bacteria count in the range of $10^3$-$10^4$ CFU/g, 34 % in the range of $10^4$-$10^5$ CFU/g, and 24% higher than $10^5$ CFU/g. Contamination with *Enterobacteriaceae*

**Table 1. Results of aerobic mesophilic colony count, coagulase positive staphylococci, *Enterobacteriaceae* and *Escherichia coli* β glucuronidase positive in raw and cooked fish, raw and cooked fish products.**

| Analysis in different sample types | N | <10 (CFU/g) | 10 to <$10^2$ (CFU/g) | $10^2$ to <$10^3$ (CFU/g) | $10^3$ to <$10^4$ (CFU/g) | $10^4$ to <$10^5$ (CFU/g) | >$10^5$ (CFU/g) |
|---|---|---|---|---|---|---|---|
| Raw fish | | | | | | | |
| Aerobic mesophilic colony count | 79 | 1 | 08 | 26 | 26 | 18 | |
| Coagulase positive staphylococci | 77 | 77 | 00 | 0 | 0 | 0 | |
| *Enterobacteriaceae* | 77 | 74 | 01 | 1 | 0 | 1 | |
| *Escherichia coli* β glucuronidase positive | 78 | 78 | 0 | 0 | 0 | 0 | |
| Cooked fish | | | | | | | |
| Aerobic mesophilic colony count | 36 | 19 | 10 | 6 | 1 | 0 | 0 |
| Coagulase positive staphylococci | 36 | 36 | 00 | 0 | 0 | 0 | |
| *Enterobacteriaceae* | 35 | 33 | 11 | 0 | 0 | 0 | |
| *Escherichia coli* β glucuronidase positive | 30 | 30 | 00 | 0 | 0 | 0 | |
| Raw fish products | | | | | | | |
| Aerobic mesophilic colony count | 8 | 0 | 01 | 2 | 5 | 0 | |
| Coagulase positive staphylococci | 8 | 8 | 00 | 0 | 0 | 0 | |
| *Enterobacteriaceae* | 8 | 6 | 01 | 1 | 0 | 0 | |
| *Escherichia coli* β glucuronidase positive | 8 | 8 | 00 | 0 | 0 | 0 | |
| Cooked fish products | | | | | | | |
| Aerobic mesophilic colony count | 11 | 3 | 43 | 0 | 0 | 1 | |
| Coagulase positive staphylococci | 10 | 0 | 00 | 0 | 0 | 0 | |
| *Enterobacteriaceae* | 10 | 9 | 10 | 0 | 0 | 0 | |
| *Escherichia coli* β glucuronidase positive | 9 | 9 | 00 | 0 | 0 | 0 | |

N, number of samples analysed; CFU, colony forming unit.

occurred only in 3 cases and the values of the three samples were: $10^2$ to $10^3$ CFU/g, $10^3$ to $10^4$ CFU/g and $>10^5$ CFU/g. In all the three cases the samples belonged to *Pleuronectiformes*: 2 plaices (*Pleuronectes platessa*) sampled in two school canteens, and a common dab taken from a nursing home canteen. *Enterobacteriaceae* in the remaining samples were always under the detection limit (<10 CFU/g). Concerning cooked fish, 55% of the samples showed an aerobic mesophilic bacteria count under the detection limit (<10 CFU/g). In the majority of samples analyzed (94%) *Enterobacteriaceae* were below the sensitivity method threshold (<10 CFU/g), just one sample, a grouper (*Epinephelus marginatus)* fillet, contained 140 CFU/g *Enterobacteriaceae*. The grouper was sampled in collective canteen. In all samples of raw fish products analyzed, the aerobic colony count ranged from $10^2$ to $<10^5$ CFU/g and *Enterobacteriaceae* were always <10 CFU/g except for a cod stick sample, which contained 4700 CFU/g *Enterobacteriaceae.*

Concluding with cooked fish products, in 90% of the samples analyzed the aerobic colony count was below $10^3$ CFU/g and *Enterobacteriaceae* were under the detection limit (<10 CFU/g).

None of the four types of samples (raw and cooked fish, raw and cooked fish products) tested for coagulase positive *Staphylococci* and *E. coli* were above the detection limit (10 CFU/g). No *Salmonella* spp. has been found in any samples, whereas three samples were positive for *Listeria monocytogenes:* a frozen squid (*Dosidiscus gigas*), frozen fillets of blue shark (*Prionace glauca*) and halibut (*Hippoglossus hippoglossus*). Even though the isolation of *Listeria monocytogenes* has been verified, its concentration was always <10 CFU/g.

### Environmental swabs

Total counts of aerobic mesophilic bacteria are presented in Figure 1. In 50% of samples the total count of aerobic mesophilic bacteria was in the range of 1-10 CFU/cm$^2$, 25% in the range of 10-$10^2$ CFU/cm$^2$ and the remaining 25% had counts $>10^2$ CFU/cm$^2$. Only two samples had a total count of aerobic mesophilic bacteria $>10^5$ CFU/cm$^2$; both were samples of food processing surfaces of school canteens. *Enterobacteriaceae* were always below the sensitivity method threshold (<1 CFU/cm$^2$) and no *Listeria monocytogenes* was found.

### Fish species identification

Fish sampled for species identification belonged mostly to genera *Gadiformes* (31%), *Pleuronectiformes* (20%), *Perciformes* (11%), *Salmoniformes* (10%), *Squaliformes* (5%) and *Sepiida* (5%) (Figure 2). Out of 102 samples, 98 (96.1%) revealed valuable sequence results, while 4 samples (3.9%) did not give valid results due to poor DNA quality, and were therefore discarded. Results of molecular analysis for differentiation of species are reported in Table 2.

## Discussion

The results of this study constitute an indicator of the overall quality of seafood and fish products served by public catering services.

Fish is one of the food categories with the shortest shelf life, and its quality is influenced by many factors as the source, cooling methods, processing and storage conditions (Stratev *et al*., 2015).

The International Commission on Microbiological Specifications for Foods sets the limit for total aerobic plate counts in fresh and frozen fish at $10^7$ CFU/g and as stated by Broekaert *et al.* (2011), loads of $10^7$-$10^8$ CFU/g make spoilage organoleptically detectable. In this study, 24% of raw fish samples had total aerobic mesophilic bacteria count above $10^5$ CFU/g, but only two raw plaices (*Pleuronectes platessa*), sampled in two different canteens of nursery schools, had an aerobic mesophilic bacteria load of $10^6$ to $<10^7$ and $>10^7$ CFU/g. These two samples had also *Enterobacteriaceae* loads respectively of $2.1\times10^3$ CFU/g and $2.5\times10^5$ CFU/g. From these two samples, other samples that showed an *Enterobacteriaceae* contamination were a raw common dab (*Limanda limanda*) fillet ($3.6\times10^2$ CFU/g), a raw cod stik sample ($4.7\times10^3$ CFU/g) and a cooked grouper (*Epinephelus marginatus*) fillet ($1.4\times10^2$ CFU/g). The *Enterobacteriaceae* count is considered as a fish quality index indicator because it is related to storage on ice, washing, evisceration (Zambuchini *et al*., 2008) and handling of seafood. The *Enterobacteriaceae* contamination was found only in a small amount of samples in this investigation, but the concentration was unacceptable if compared to the limit of $10^2$ CFU/g established by Popovic *et al*. (2010) for fresh and frozen fish. No *E.coli* and *Salmonella* spp. were isolated, allowing to exclude a contamination by *Enterobacteriaceae* of fecal origin. In Italy, however, a two-year survey demonstrated a rate of *Salmonella* spp. in seafood of 0.5% (Busani *et al*., 2005).

Pathogens could be transmitted to fish in water (*i.e. Salmonella* spp.) or during processing under bad hygienic conditions (Uddin *et al*., 2013), as *Listeria monocytogenes*. Contamination of fish with *Listeria monocytogenes* in the early stages of the production chain could follow the product throughout the production process (Svanevik *et al*., 2015).



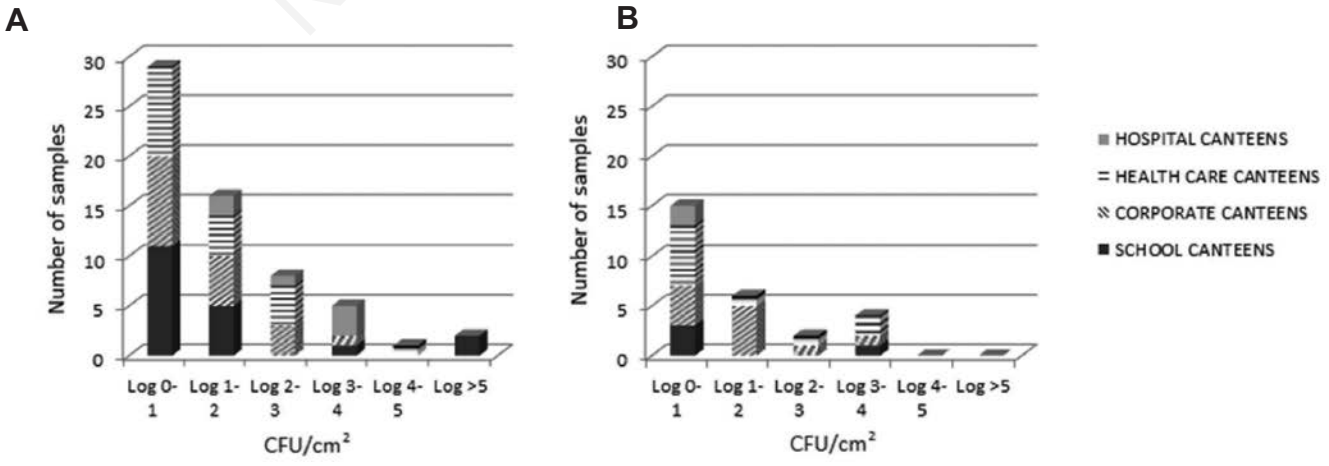


**Figure 1. Total counts of aerobic mesophilic bacteria (Log colony forming unit/cm$^2$) on: A) food contact surfaces (processing surfaces, chopping boards, knives) and B) food non-contact surfaces (fridge inner wall, fridge handles, tap lever).**

Once the pathogen is established in a processing environment, it can be a long-term source of contamination because of its ability to form biofilms on processing surfaces. Additionally, *Listeria monocytogenes* is known to tolerate low temperatures, including freezing temperature, which can reduce its chance of being eliminated from the product (Rocourt *et al*., 2000). *Listeria monocytogenes* was detected only in 3.8% of raw seafood samples, a considerably lower percentage in comparison with the 6.5% found by Busani *et al*. (2005). Furthermore, the quantitative analysis of these samples attested that the concentration of *Listeria monocytogenes* was always <10 CFU/g. Even though the detected concentration of *Listeria monocytogenes* was below the 100 CFU/g, accepted by the International Commission on Microbiological Specification for Foods, it is of major concern because these samples were collected from canteens mostly dedicated to a population particularly vulnerable to food-borne illness.

Food contact surfaces are a major concern for food service facilities in controlling the spread of food-borne pathogens (Cosby *et al*., 2008), thus the evaluation of their bacteriological quality has been included in this investigation. Henroid *et al*. (2004) suggested a standard of less than 1.3 $\log_{10}$ CFU/cm$^2$ as acceptable level for aerobic mesophilic bacteria count and for *Enterobacteriaceae* less than 1.0 $\log_{10}$ CFU/cm$^2$. Compared to this standard just 14% of surfaces samples were acceptable for aerobic mesophilic bacteria count, whereas the standards for *Enterobacteriaceae* count was met for all samples. The high percentage of unacceptable samples for aerobic mesophilic bacteria plate count indicates either inadequate sanitation or recontamination, but the satisfactory levels of *Enterobacteriaceae* reassure that human enteric pathogens have been

**Table 2. Data of polymerase chain reaction species identification.**

| Genus | Families | PCR species identification | Compliant samples (n) | Not compliant samples (n) | Label denomination for not-complying samples |
|---|---|---|---|---|---|
| *Gadiformes* | | | | | |
| | Gadidae | *Theragra chalcogramma* | 4 | 5 | 3 generic codfishes; 1 generic plaice; 1 generic crab |
| | | *Gadus morhua* | 1 | 1 | 1 generic codfish |
| | Merlucciidae | *Merluccius productus* | 0 | 1 | 1 generic codfish |
| | | *Merluccius capensis* | 3 | 1 | 1 generic codfish |
| | | *Merluccius paradoxus* | 0 | 2 | 2 generic South-African codfish |
| | | *Macruronus novazelandiae* | 3 | 1 | 1 generic plaice |
| | Merluccius | *Merluccius hubbsi* | 6 | 1 | 1 generic codfish |
| | | *Merluccius gayi* | 2 | 0 | |
| | | *Merluccius merluccius* | 0 | 1 | 1 generic South-African codfish |
| *Pleuronectiformes* | | | | | |
| | Pleuronectidae | *Pleuronectes platessa* | 10 | 0 | |
| | | *Reinhardtius hippoglossoides* | 5 | 5 | 4 generic halibut, 1 generic plaice |
| | | *Lepidopsetta polyxystra* | 0 | 1 | 1 generic limanda |
| *Perciformes* | | | | | |
| | Serranidae | *Acanthistius brasilianus* | 0 | 1 | 1 generic grouper |
| | | *Epinephelus sp.* | 0 | 1 | 1 generic Atlantic grouper |
| | Centropomidae | *Lates niloticus* | 0 | 1 | 1 Greenlandic halibut |
| | Cichlidae | Oreocromis niloticus | 1 | 0 | |
| | Moronidae | *Dicentrarchus labrax* | 1 | 0 | |
| | Scombridae | *Thunnus sp.* | 1 | 0 | |
| | | *Scomber scombrus* | 2 | 0 | |
| | Sparidae | *Sparus aurata* | 1 | 0 | |
| | Xiphiidae | *Xiphias gladius* | 2 | 0 | |
| *Salmoniformes* | | | | | |
| | Salmonidae | *Oncorhynchus mykiss* | 6 | 1 | 1 keta salmon |
| | | *Oncorhynchus keta* | 1 | 0 | |
| | | *Salmo salar* | 1 | 0 | |
| | | *Salvelinus fontinalis* | 1 | 0 | |
| *Scaridae Sepiida* | | | | | |
| | Scarus | *Scarus sp.* | 0 | 1 | 1 generic grouper |
| | Sepiidae | *Sepia officinalis* | 3 | 0 | |
| | | *Sepiella sp.* | 1 | 1 | 1 Sepia pharaonis |
| *Squaliformes* | Prionace | *Prionace glauca* | 5 | 0 | |
| *Clupeiformes* | Clupeidae | *Sardina pilchardus* | 3 | 0 | |
| *Mugiliformes* | Mugilidae | Liza ramada | 2 | 0 | |
| *Siluriformes* | Pangasiidae | *Pangasius hypophtalmus* | 2 | 0 | |
| *Scorpaeniformes* | Triglidae | *Chelidonichtys cuculus* | 1 | 0 | |
| *Zeiformes* | Zeidae | *Zeus faber* | 1 | 0 | |
| *Mytiloida* | Mytilidae | *Mytilus sp.* | 1 | 0 | |
| *Atheriniformes* | Atherinidae | *Atherina boyeri* | 1 | 0 | |
| *Lamniformes* | Lamnidae | *Isurus oxyrhincus* | 1 | 0 | |
| *Veneroida* | Veneridae | *Paphia undulata* | 1 | 0 | |

PCR, polymerase chain reaction.

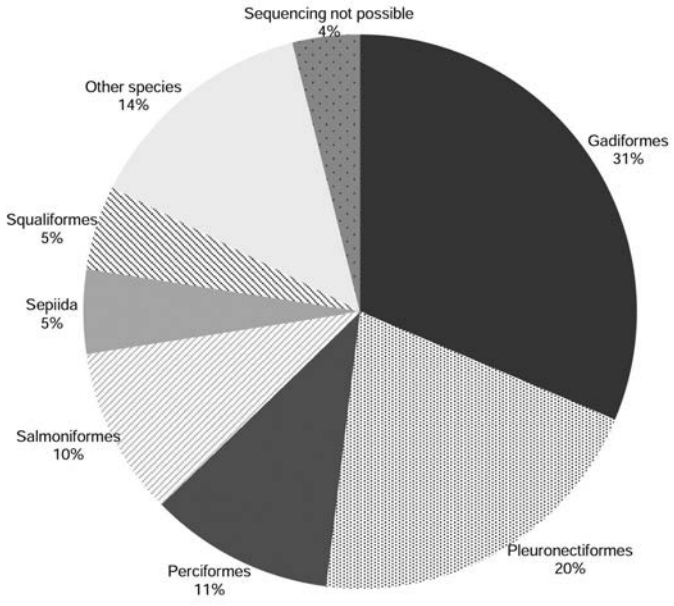


**Figure 2. Sample size for species identification.**

controlled. Concerning pathogens, no *Listeria monocytogenes* have been found on food contact and non-contact surfaces indicating that no cross contamination occurred even if three samples tested positive for *Listeria monocytogenes*.

In food catering services, especially if dedicated to peoples at high health risk, it is essential to maintain high hygiene standards starting from raw materials. It's therefore necessary to ensure the authenticity and the origin of seafood, particularly for those products which are visually not recognizable after processing and freezing. The results of this investigation show that a considerable portion (75%) of analyzed samples revealed a correct species declaration, and most cases of mislabelling were example of species with a low market value sold as others more expansive. Major frauds concerned codfish and groupers; one labelled grouper was identified as *Scarus* spp. at molecular level, a species with a very low commercial value with respect to grouper. In accordance to our findings, Filonzi *et al*. (2010) reported the Mediterranean grouper among the major substituted species.

## Conclusions

The results of the microbiological raw fish and fish products, served by mass catering, can be defined as quite satisfactory, given that the majority of samples complied with the reference standards. Anyway the unsatisfactory results of aerobic mesophilic bacteria on environmental samples indicate inadequate sanitation procedures or a recontamination.

The results of species identification reveal the need to improve controls on raw fish, in order to avoid frauds which can damage the consumers not only economically but also from a nutritional perspective. Thus, food business operators have to maintain a high level of attention, especially when providing meals to vulnerable populations.

The results of this survey can provide valuable information for the design of monitoring and surveillance programs for the control of quality of seafood and fish products.

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