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Microbiological controls across the productive cycle of *Dicentrarchus labrax* L. and *Sparus aurata* L.: a study from the environment to the final product

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Abstract

The microbiological quality of water, sediments and seafood products of three Sicilian marine fish farms (Castellammare Gulf, Capo d'Orlando and Porto Palo) was investigated in order to draw a complete picture of the health conditions from the start (environment) to the end (seafood product) of the productive cycle. Before the beginning of fish farming, low concentrations of faecal contamination indicators (faecal coliforms, *Escherichia coli*, enterococci) were found in the water of each examined area. Due to the enhanced organic load released from cages, the set-up of fish farming significantly altered the distribution of faecal indicators and sometimes that of halophilic vibrios in the pelagic compartment. Significant differences in the density of heterotrophic bacteria were sometimes recorded at the sediment level. Despite this increase in microbial abundance, the microbiological conditions remained acceptable for the productive process. Pathogens (*Salmonella* spp., *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Staphylococcus aureus*) were mostly absent in seafood products. The study underlines that the achievement of good quality levels in aquaculture strongly depends on the conformity of the rearing environment to qualitative microbiological standards, highlighting the importance of sanitary controls along the different steps of the productive cycle.

Keywords: mariculture, microbiology, water, sediment, quality, sea bass, sea bream

Introduction

Aquaculture represents an important source of highly nutritional proteins for human feeding. The recent expansion of productive initiatives has resulted in an increasing interest in the assessment of their potential impact on aquatic ecosystems (Beveridge, Ross & Kelly 1994; Papoutsoglou, Costello, Stamou & Tziha 1996; La Rosa, Mirto, Marino, Alonzo, Maugeri & Mazzola 2001). The large biodeposition of pseudo-faeces, faeces and feed wastes from mariculture may potentially produce negative effects on the composition, structure and productivity of both pelagic and benthic communities. For aquaculture to be successful, activities must not cause unacceptable changes in water quality and not clash with other uses (tourism, industry and urban) of the coastal areas. This is an issue extensively reported in the studies aimed at evaluating the impact of fish farming on coastal zones. However, there is little information relating aquaculture to water quality degradation (Karakassis, Tsapakis & Pitta 2001).

The health risks associated with the consumption of low-quality seafood makes the assessment and control of food safety and quality another topic of global concern in aquaculture. Microbiological controls have to be addressed firstly to the environments designated to mariculture, to adequate fish-farm management practices and finally to educational programmes for the consumers (Liston 1990; Reilly & Kaferstein 1999). Application of preventive measures can result, in turn, in the improvement of production efficiency and reduction in costs.

The relationship linking the hygienic-sanitary quality of the seafood products to the quality of the rearing sites has been widely documented (Reilly & Kaferstein 1999; WHO 1999). Therefore the monitoring of productive areas to comply with microbiological standards has been recognized as a fundamental

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pre-requisite to obtain high-quality products (Caruso, Zaccone, Genovese & Crisafi 1998). The application of severe microbiological inspections along the different steps of the production line, from rearing to harvesting, processing and marketing of fish-farm products, is imposed by the Italian directives Decreto Legislativo 131/92 1992a; Decreto Legislativo 530/92 1992b; Decreto Legislativo 531/92 (1992c).

In the framework of a research contract funded by Centro Oceanologico Mediterraneo-Palermo, a multidisciplinary study has been carried out by the Istituto per l'Ambiente Marino Costiero-Messina on three Sicilian areas [Gulf of Castellammare (Northern Tyrrhenian Sea), Capo d'Orlando (Tyrrhenian coast, between Messina and Palermo) and Porto Palo (south-eastern side of the Sicily)] designated for mariculture, in order to verify the microbiological quality of the environment and of final products ready for distribution. The only available report on environmental health conditions in the studied areas concerned the Gulf of Castellammare, where a 3-year investigation (Caruso *et al*. 1998; Genovese, Caruso, La Ferla, Leonardi, Giacobbe, Maimone, Micale & Zaccone 1998) showed the presence of low levels of microbial contamination with respect to faecal indicator and potentially pathogenic vibrios. In contrast, for the other fish-farming areas included in this investigation, there is a lack of specific information about the microbiological conditions of the waters and on the extent of the potential hazard for human health consequent to their use as aquaculture sites.

Materials and methods

Water

Surface samples were collected from a total of eight stations, of which four were indicated as cage sites, and the others as control blanks, located at about 1 km from the fish farm. A total number of 48, 30 and 16 samples were drawn from Castellammare Gulf, Capo d'Orlando and Porto Palo respectively. One litre of each sample, aseptically collected with a Niskin bottle, was used to enumerate faecal coliforms (FCs) and enterococci (ENT), by membrane filtration of suitable volumes (10, 100 mL) of sample and incubation at 44.5 °C for 24 h and at 35 °C for 48 h into the selective culture media (m-FC added with 1.5% agar and Slanetz and Bartley medium (Oxoid, Hampshire, England) respectively). Colonies grown on m-FC agar were confirmed to be *Escherichia coli* by indole test (Decreto Presidente della Repubblica 470/82 1982).

The search for *Pseudomonas aeruginosa* and *Aeromonas hydrophila* strains was carried out by filtering 10 and 100 mL of sample on 0.45-μm Millipore membranes, and by incubating the filter on the respective media *P. aeruginosa* selective agar (Oxoid) and *A. hydrophila* medium (Oxoid) added with Ampicillin selective supplement (Oxoid) at 35 °C for 24–48 h. As an indicator of the trophic conditions of the environment, the viable heterotrophic aerobic flora able to grow at 20 °C and 35 °C was estimated on Marine agar (Difco, BD Franklin Lakes, NJ, USA) plates incubated for 7 days. The density of total halophilic vibrios growing at 20 °C was determined by membrane filtration and incubation of the filters on TCBS agar (Difco) plates added with 2% sodium chloride.

Sediment

From the same stations described above, at the same time as water sampling, sediment samples were collected using a Box corer; surface subsamples were taken with a sterile polypropylene container and maintained at +5 °C until assayed. The treatment of the sediments for the bacteriological analyses included the homogenization of 1 g of each sample in sterile seawater (1:10 w/v) and seeding into culture media of the supernatant obtained. The density of heterotrophic aerobic bacteria was determined by spreading into Marine agar plates (Difco) incubated at 20 °C for 7 days (Cavallo, Rizzi, Vozza & Stabili 1999). For the determination of *Clostridium perfringens*, suitable volumes (0.1 mL) of serial dilutions from the initial supernatant were poured into the selective medium, Perfringens selective base (Oxoid), added with supplements A and B (Oxoid), and allowed to resolidify. Plates were incubated at 35 °C for 24 and 48 h in AnaeroGen Compact System (Oxoid). Counts were expressed in terms of colony forming units (CFU) per gram of sediment (CFU g^{-1}), expressed in dry weight.

Fish

Five specimens of fish (*Dicentrarchus labrax* L. and *Sparus aurata* L.) achieving the commercial size were also sacrificed. Precautions were taken in aseptical surgery of each sample to avoid contamination of fillets with bacteria coming from other sites (skin and intestine). After homogenization for 15 min in sterile physiological saline solution of small amounts (from 2 to 5 g) of sample through Stomacher Lab Blender 80 (Seward Medical Uac House, London, England),

decimal dilutions in the same liquid medium were performed and inoculated in different media according to the parameter to be tested, as reported below.

Total bacterial counts were estimated after inoculation by spreading of Plate Count Agar (Oxoid) plates and incubation at 20 °C and 32 °C.

To estimate the *E. coli* density, the Petrifilm System (3M Microbiology Products, St Paul, MN, USA) was used (De W. Blackburn, Baylis & Petitt 1996); seeded films were further incubated at 35 °C for 24 h.

The qualitative search of *Salmonella* spp. was carried out by pre-enriching 25 g of sample in buffered alkaline peptone water at 35 °C for 18–24 h, and enriching in Rappaport Vassiliadis Broth (Oxoid) and Tetrationate Broth (Oxoid) added with an iodine solution (ICN Pharmaceuticals, Costa Mesa, CA, USA) incubated for 24–48 h at 42 °C and 35 °C respectively. Isolation was performed on plates of Salmonella-Shigella (SS, Oxoid) and Hektoen enteric agar (Oxoid) selective culture media. All the colonies presumed to be *Salmonella* spp. (lactose not fermenting and hydrogen sulphide producing) were biochemically characterized through inoculation in Triple Sugar Iron agar (Difco) tubes. Typical strains were further identified by using API 20E (Biomerieux, Marcy l'Etoile, France) strips incubated at 35 °C for 24 h.

To enumerate *Staphylococcus aureus*, plates of selective Baird Parker medium (Oxoid) containing egg-yolk tellurite emulsion were inoculated in duplicate with decimal dilutions of the sample homogenized, and incubated at 35 °C for 24–48 h; all the grey-black, tellurite-reducing colonies surrounded by a clear zone were tested for coagulase production by using the agglutination Staphylase (Oxoid) test.

Presence of strains belonging to the species *Vibrio parahaemolyticus* was detected by both the enrichment of 25 g of sample in alkaline peptone water (pH 8.6) incubated at 35 °C for 6–18 h and the inoculation of selective Salt Polymixin Broth (De Medici, Fenicia, Orefice & Stacchini 1996) incubated at 35 °C for 8 h. Isolation on TCBS agar (Difco) plates added with 2% NaCl at 35 °C yielded to presumed *V. parahaemolyticus* colonies, of which at least four per plate were tested for sensitivity to the vibriostatic agent O129 (2,4-diamino 6,7-di-isopropylpteridine, 150-μg diagnostic discs, Oxoid) in order to distinguish *Vibrio* from *Aeromonas* belonging strains.

To determinate *Listeria monocytogenes*, serial dilutions of the sample homogenized were enriched by inoculation in triplicate tubes of the selective Fraser broth (Oxoid) in the most probable number (MPN) method, according to the procedure reported by the Ordinanza Ministeriale 7/12/93 (1993) for the quantitative determination of *L. monocytogenes*. After incubation at 35 °C for 24–48 h, the presumed positive cultures were further inoculated on Oxford agar (Oxoid) plates incubated at 35 °C for 24 h, to isolate the pathogen. Among the suspected colonies showing a dark pigmentation five were randomly selected and assayed for hemolysin production on Columbia agar base (Oxoid) added with 5% sheep erythrocytes (ICN Pharmaceuticals). Strains positive for Gram staining, beta hemolysis and catalase production, and negative for oxidase, were further subjected to biochemical identification using API Listeria (Biomérieux) strips.

In order to follow microbiological alterations occurring during refrigeration, the above-cited microbiological analyses were repeated at selected time intervals, namely at 1, 3, 9, 12, 15 days after sample conservation at +5 °C.

Results

Water

The mean values of bacteriological parameters recorded before and after the starting of mariculture experiment, at cage and control stations separately, are shown in Figs 1–3. In the Castellammare Gulf (Fig. 1a), after the introduction of fish into the cages (March, May and July 2001), an increase in the concentration of all faecal indicators, dependent on the release of faecal pellets, was recorded at cage stations only, where all faecal bacterial indicators were noticed at very low densities before fish farming (March 2000). Statistical analysis by ANOVA test confirmed that highly significant differences occurred in relation to time (before and after fish farming) and site (cage and control stations) for FCs ($F = 7.65$ and 7.81, $P < 0.05$ respectively), *E. coli* ($F = 7.75$ and 7.93, $P < 0.05$ respectively) and ENT ($F = 20.76$ and 25.48, $P < 0.01$ respectively).

Compared with the values obtained before the beginning of productive processes, the abundances of heterotrophic bacteria (Fig. 1b) also increased, although not significantly, both in the fraction growing at 20 °C (from 2.22×10^3 to 2.24×10^3 CFU mL^{-1}, at cages, and from 1.17×10^3 to 1.48×10^3 CFU mL^{-1} at control stations) as well as at 35 °C (from 1.51×10^3 to 1.59×10^3 CFU mL^{-1} at cage stations only).

Mariculture activity affected also the fraction of halophilic vibrios (Fig. 1c), although temporal and spatial variations in their mean values were not statistically significant. After fish rearing (July 2001)

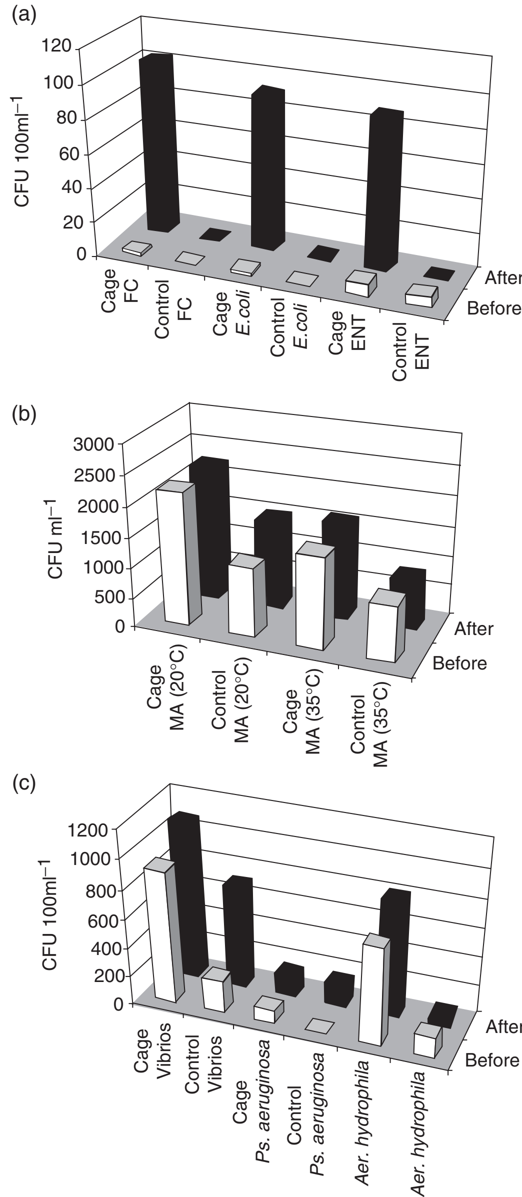

Figure 1 Water samples. Mean values of: (a) faecal pollution indicators (faecal coliforms, FCs; *Escherichia coli*, *E. coli*; enterococci, ENT), (b) heterotrophic bacteria (MA) and (c) presumptive vibrios (VPP) and bacteria potentially pathogenic to reared fish (*Aeromonas hydrophila*, *Pseudomonas aeruginosa*) found in Castellammare Gulf.

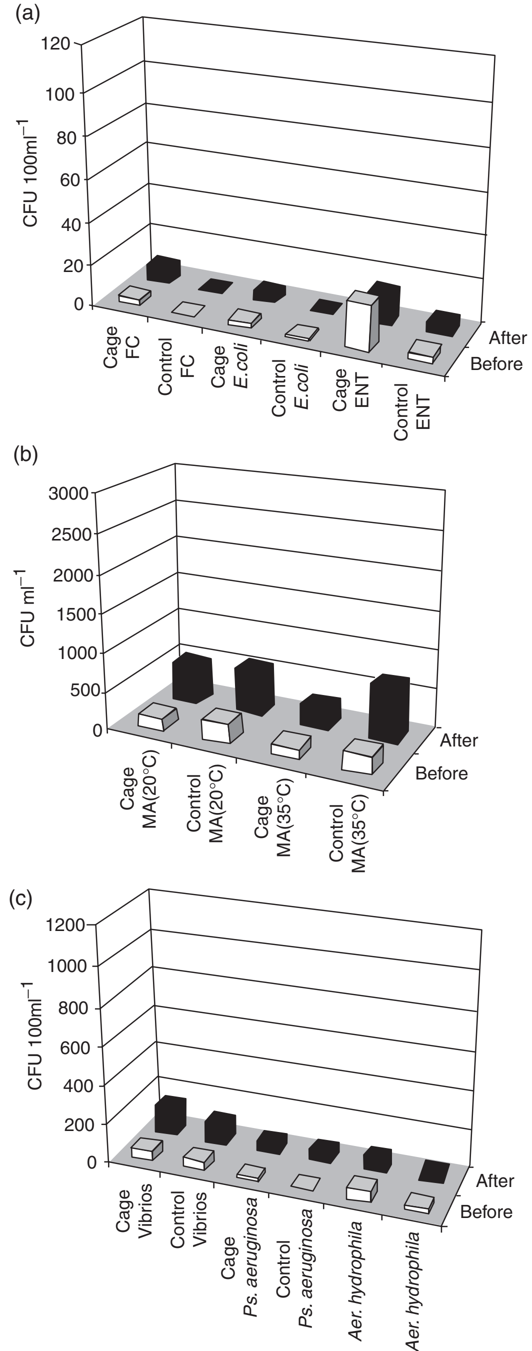

Figure 2 Water samples. Mean values of: (a) faecal pollution indicators (faecal coliforms, FCs; *Escherichia coli*, *E. coli*; enterococci, ENT), (b) heterotrophic bacteria (MA) and (c) presumptive vibrios (VPP) and bacteria potentially pathogenic to reared fish (*Aeromonas hydrophila*, *Pseudomonas aeruginosa*) found in Capo d'Orlando.

their initial mean concentrations (9.19×10^2 and 2.26×10^2 CFU 100 mL^{-1} at cage and control stations, respectively, before fish farming) had increased, reaching 1.08×10^3 and 7.06×10^2 CFU 100 mL^{-1} at cage and control stations respectively. *Vibrio* genus represented a considerable fraction within the bacterial heterotrophic flora, ranging on average from 65.74 to 98.38% and from 33.50 to 86.87% before and after cage installation respectively. Microorganisms presumed to be *P. aeruginosa* and *A. hydrophila*

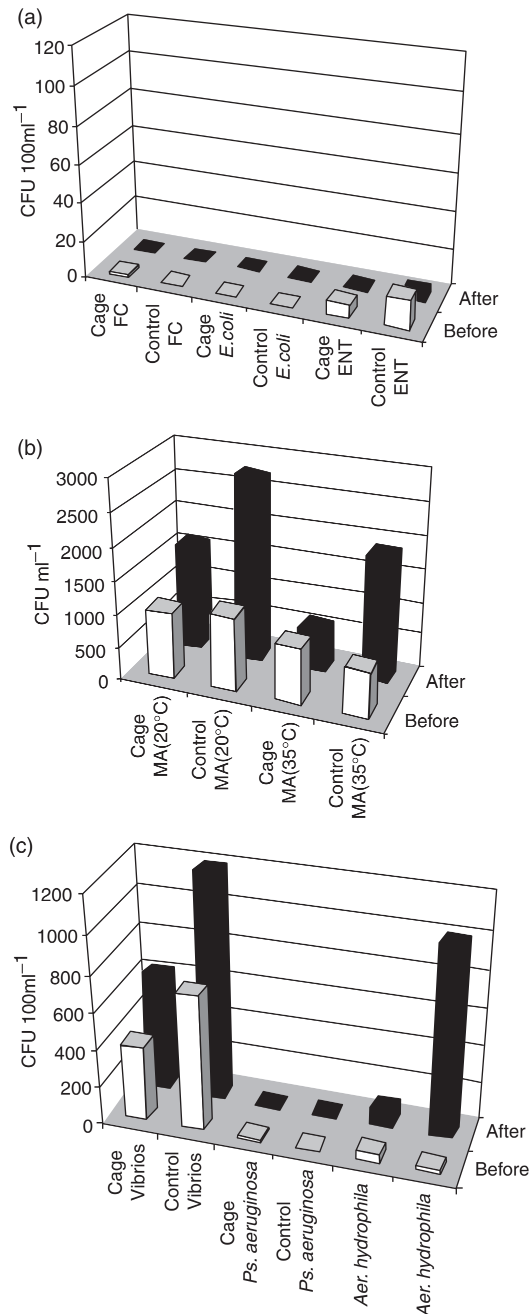

Figure 3 Water samples. Mean values of: (a) faecal pollution indicators (faecal coliforms, FCs; *Escherichia coli*, *E. coli*; enterococci, ENT), (b) heterotrophic bacteria (MA) and (c) presumptive vibrios (VPP) and bacteria potentially pathogenic to reared fish (*Aeromonas hydrophila*, *Pseudomonas aeruginosa*) found in Porto Palo.

(Fig. 1c) were present throughout the study period with a mean density of about 10^2 CFU 100 mL^{-1}.

In the waters of Capo d'Orlando, following fish introduction, both FCs and *E. coli* (Fig. 2a) displayed only a slight increase with respect to that recorded in Castellammare Gulf, but differences between cage and control stations were statistically significant ($F = 5.39$, $P < 0.05$). Differences between stations were detected by ANOVA values before mariculture starting only ($F = 11.14$, $P < 0.01$).

After mariculture experiments, mean concentrations of heterotrophic bacteria (Fig. 2b), one order of magnitude lower than those of Castellammare area, increased not only at cage (from 1.6×10^2 to 4.91×10^2 CFU mL^{-1} and from 1.36×10^2 to 2.61×10^2 CFU mL^{-1}, for the fraction growing at 20 °C and 35 °C respectively) but also at control stations (from 2.66×10^2 to 5.74×10^2 CFU mL^{-1} and from 2.26×10^2 to 6.86×10^2 CFU mL^{-1}).

The halophilic vibrios (Fig. 2c), initially occurring in low concentrations (5.3×10^1 and 4.8×10^1 CFU 100 mL^{-1} at cage and control stations respectively), exceeded 10^2 CFU 100 mL^{-1} at both sites after cage installation. The low values recorded for trophic-related bacterial parameters (heterotrophic bacteria and vibrios) suggested the low availability of organic matter in this area with respect to the former; in these waters vibrios represented a moderately abundant fraction of the total heterotrophic flora, ranging on average from 11.40 to 50.69%, before fish farming, and from 17.59 to 75.07% after fish farming. ANOVA results showed that 'site' (cages versus controls $F = 9.84$, $P < 0.05$) and 'time' (before versus after, $F = 19.11$, $P < 0.01$) variables accounted for variance in the distribution of halophilic vibrios at cage stations. Like heterotrophic bacteria, temporal differences were also extended to control stations ($F = 8.40$, $P < 0.05$).

Presumed *P. aeruginosa* strains were recovered in concentrations lower than those observed in Castellammare waters, without any significant temporal variation (Fig. 2c). *A. hydrophila* displayed significant variations in relation to the site sampled ($F = 23.89$, $P < 0.01$).

The waters of Porto Palo were characterized in both samplings (before, July 2000, and after fish farming, July 2001) by mostly negative values of FCs and *E. coli*, while ENT did not exceed 15 CFU 100 mL^{-1} (Fig. 3a). After fish farming, mean densities of heterotrophic bacteria growing at 20 °C increased both at cage and control stations (from 1.01×10^3 to 1.59×10^3 CFU mL^{-1} and from 1.13 to 2.76×10^3 CFU mL^{-1}, respectively, Fig. 3b). Also halophilic vibrios (Fig. 3c) almost doubled in number, reaching mean values of 6.25×10^2 and 1.23×10^3 CFU 100 mL^{-1} at cage and control stations

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respectively. They constituted 3.60–96.91% and 1.15–100% of the total heterotrophic flora, respectively, before and after fish farming. After mariculture beginning, the abundance of *P. aeruginosa* decreased (Fig. 3c), with significant temporal differences at control sites ($F = 12.31$, $P < 0.01$). At cages, the concentration of *A. hydrophila* was low and similar to that recorded in Capo d'Orlando (Fig. 3c).

Sediment

Before mariculture, heterotrophic bacteria in the sediments (Fig. 4a) showed mean concentrations variable from 9.43×10^3 (control)–1.54×10^4 (cage) CFU g^{-1} found in Castellammare Gulf to 3.53×10^3 (control)–1.85×10^3 (cage) CFU g^{-1} in Capo d'Orlando and to 3.65×10^3 (control)–3.0×10^3 (cage) CFU g^{-1} in Porto Palo. With the beginning of mariculture activity, their concentrations increased by about four times under the cages (cages versus controls, $F = 28.57$, $P < 0.01$) in Castellammare Gulf, while no significant increases were recorded in Capo d'Orlando (on average, from 1.85×10^3 to 6.03×10^3 CFU g^{-1}). As previously observed in waters, in this area bacterial densities were initially one order of magnitude lower than that recorded in Castellammare Gulf, reflecting a lower trophic content.

Microorganisms identified to be *C. perfringens* (Fig. 4b), which grow well in environments with low Eh redox values, occurred at higher densities in Castellammare area (mean 5.5×10^1, at control, 1.38×10^2 CFU g^{-1}, at cage) and showed a significant decrease ($F = 9.56$, $P < 0.05$) after fish farming. In Capo d'Orlando and Porto Palo they were detected in lower concentrations, ranging in the same order of magnitude (0.4×10^1, cage – 3.6×10^1 CFU g^{-1}, control; 0, control – 3.4×10^1 CFU g^{-1}, cage respectively).

Increases recorded in Porto Palo in the counts of heterotrophic bacteria (from 3.0×10^3 to 4.1×10^3 CFU g^{-1}) and *C. perfringens* (from 3.4×10^1 to 2.14×10^2 CFU g^{-1}) were not statistically significant.

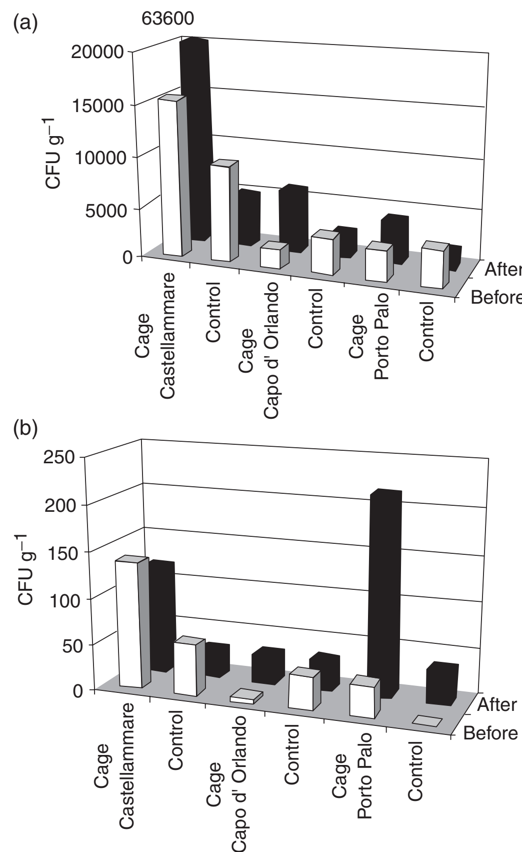

Figure 4 Sediment samples. Mean values of indicators of: (a) trophic conditions (heterotrophic bacteria) and (b) remote pollution (*Clostridium perfringens*) found in the three mariculture sites.

Fish

Results of quantitative determinations of the microbial flora present in the samples of food examined are shown in Table 1. They refer to two different species (*D. labrax* L. and *S. aurata* L.) and to two sites only, Castellammare Gulf and Porto Palo, as productive processes were stopped in the third site (Capo d'Orlando). In sea bass (*D. labrax*) reared in Castellammare, the total microbial flora was in the order of 10^3 CFU g^{-1} during the first day of sampling, while lower densities (10^2 CFU g^{-1}) were found in the specimens reared at Porto Palo both at 20 °C and 35 °C. Values obtained suggested that the microbiological quality levels of the sea bass specimens reared at Castellammare were acceptable within the ninth day from sampling. In contrast, in the specimens of sea bass reared at Porto Palo, the deterioration of the microbiological quality had occurred earlier, by the third day. Bacterial concentrations increased to 10^8 and 10^9 CFU g^{-1} after 12 days of storage.

In the specimens of sea bream (*S. aurata*) reared in Castellammare Gulf, total bacteria counts were higher than in sea bass of the same site, displaying on the first sampling day densities equal to 10^4 and 10^3 CFU g^{-1} of food at 20 °C and 35 °C respectively. During sample storage at +5 °C, bacterial concentrations increased progressively, reaching peaks of 10^8 CFU g^{-1} of food, both at 20 °C and 35 °C, after 15 days of refrigeration. Deterioration in quality levels occurred mainly between the third and the ninth day of storage.

Table 1 Periodical microbiological seafood controls performed on sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) specimens of Castellammare Gulf and Porto Palo

Days	a	b	c	d	e	f	g
Castellammare Gulf							
D. labrax							
1	2.22E+03	2.00E+03	0	0	0	0	0
3	3.20E+03	1.00E+03	0	0	0	0	0
9	3.86E+07	4.03E+07	0	0	0	0	0
12	1.61E+08	1.97E+08	0	0	0	0	0
15	1.85E+08	2.01E+08	0	0	0	0	0
S. aurata							
1	1.63E+04	1.17E+03	0	0	0	0	0
3	1.01E+05	1.57E+04	0	0	0	0	0
9	7.85E+07	6.69E+07	0	0	0	0	0
12	4.55E+07	1.07E+08	0	0	0	0	0
15	1.65E+08	1.17E+08	0	0	0	0	0
Porto Palo							
D. labrax							
1	6.40E+02	4.00E+02	0	0	1	0	0
3	2.61E+06	2.85E+07	0	0	0	0	0
9	4.60E+06	6.30E+06	0	0	0	0	0
12	2.86E+08	1.31E+08	0	0	0	0	0
15	1.19E+09	5.65E+08	0	2	7	0	0

Total bacterial counts, as estimated on Plate Count Agar at 20 °C (a) and 32 °C (b), and results of the search for *Escherichia coli* (c), *Staphylococcus aureus* (d), *Listeria monocytogenes* (e), *Salmonella* spp. (f) and *Vibrio parahaemolyticus* (g) are reported.

No strains of *E. coli*, *V. parahaemolyticus* and *L. monocytogenes* were isolated from the sea bass and bream specimens analysed. In fish samples collected from Castellammare, no *S. aureus* and *Salmonella* spp. strains were isolated.

In the samples coming from Porto Palo rearings, negative results were obtained with respect to *Salmonella*, while two strains identified as *S. aureus* were isolated after 15 days of storage. During the first sampling, two strains confirmed to be *L. monocytogenes* were also isolated, and the recovery of a higher number (seven strains) of this microorganism was recorded after 15 days of refrigeration only.

The low levels of saprophytes recorded and the non-recovery of bacteria such as Enterobacteriaceae and *S. aureus* suggested that samples examined did not represent potential risks for consumers with respect to current regulations in microbiology of fishery products. These indicate the guideline values of 10^2 CFU g^{-1} for *S. aureus*, 11 g^{-1} for *L. monocytogenes* and the absence of *Salmonella* strains.

Discussion

The values of the microbial variables obtained before the set-up of fish farming showed that the selected areas were in compliance with the microbiological water quality standards for productive purposes. Mariculture activity was responsible for a significant increase in faecal pollution indicators (FCs, *E. coli* and ENT) in the waters; despite this, the samples examined showed bacterial counts that fell within the threshold levels (*E. coli:* 2 CFU 100 mL^{-1}) recommended by Decreto Legislativo 131/92 (1992a) for the water considered as 'acceptable' for shellfish culture. The low densities of *P. aeruginosa* found in the water also confirmed that fish farming caused limited alterations in environmental quality.

The impact of fish rearing, even if not significant, was sometimes extended not only to cage stations, but also to the control ones; this suggested a wide impact of mariculture activity over the area or the presence of local organic inputs in sites different from those directly concerned with the cage.

The viable bacterial concentrations found in the waters examined varied in the same range as reported in oligotrophic ecosystems (10^2–10^3 CFU mL^{-1}); these low bacterial densities depended also on the geographic position of sampled sites, far from the organic inputs drawn from coastal zone. In particular, *Pseudomonas* spp., common both in seawater and sediments of temperate or tropical zones (Palleroni 1984; Stolp 1988; Cavallo *et al.* 1999), are charac-

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terized by a wide metabolic versatility and tolerance to a wide variety of physical conditions, thus being able to colonize ecological niches where nutrients are limited. Some strains belonging to this genus are opportunistic pathogens causing disease in susceptible (immunocompromised) fish (Austin, Garges & Conrad 1979).

Besides the species of *Vibrio* that are saprophytes and usual inhabitants of the marine environment (Montanari, Pruzzo, Pane & Colwell 1999), some species are confirmed pathogens, being involved in fish (*V. alginolyticus and V. anguillarum*) and human (*V. cholerae* non-O1, *V. parahaemolyticus*, *V. vulnificus*) diseases (De Paola, Hopkins, Peeler, Wentz & McPhearson 1990). The frequent occurrence of potentially pathogenic species in marine environments (Barbieri, Falzano, Fiorentini, Pianetti, Baffone, Fabbri, Matarrese, Casiere, Katouli, Kühn, Möllby, Bruscolini & Donelli 1999; Ripabelli, Sammarco, Grasso, Fanelli, Caprioli & Luzzi 1999) whose abundance does not correlate with FC concentrations (Caruso *et al*. 1998) support the need to include the qualitative analysis of these bacteria in monitoring programmes of aquaculture sites, to achieve a more complete evaluation of microbiological quality.

Microbiological controls in aquaculture have to be addressed also to some motile *Aeromonas* species, such as *A. hydrophila*, that in recent years have been involved in the transmission of diseases to reared fish (Bartoli, Monetti & Licciardi 1997). In contrast, risks for human health coming from *Aeromonas* spp. in farmed fish are considered low (WHO 1999).

With respect to the benthic environment, the significant increase in heterotrophic bacteria detected in the sediments of most of fish-farming sites, supported their potential as a sensitive biomarker of organic enrichment. The stimulating effect of organic loads over the heterotrophic fraction of bacterioplankton has also been shown by La Rosa, Mirto, Favaloro, Savona, Sarà, Danovaro & Mazzola (2002) in the sediments of a fish-farming coastal area of the Tyrrhenian Sea. In Castellammare Gulf, the higher increase in heterotrophic bacterial counts with respect to that found in water suggested that the benthic environment suffered the major impact of the modified environmental conditions, in consequence of a heavier organic enrichment of the ecosystem. In comparison with the other areas examined, the higher numbers of *C. perfringens* detected in Castellammare was indicative of a remote pollution. In Capo d'Orlando sediments, the lack of significant differences due to fish farming in the abundance of both heterotrophic bacteria and *C. perfringens* led us to conclude that the mariculture experience had not favoured bacterial growth in this area, which kept a low trophism condition.

As far as the quality of fish-farming products is concerned, it is known that the greatest number of seafood-associated illnesses are associated with consumption of raw fish harvested in waters contaminated with raw or poorly treated human wastes (Ahmed 1991; Hackney & Pierson 1994); the qualitative profile of microflora associated to reared fish undergoes normal changes during storage. Fish spoilage may be slowed down through maintenance at low temperature, on ice or in refrigerating containers, but good practice procedures from the moment fish are captured up to their final marketing represent the best tool for food preservation. In the samples of fish examined, total bacterial density increased with time, probably as a consequence of the remarkable growth rate that vibrios are known to exhibit in raw fish, even kept at low temperatures. Values recorded in our investigation, however, fell within the International Commission on Microbial Specifications for Foods (ICMSF 1986) standard values for fresh fish products (5×10^5–10^7 CFU g^{-1}). So we considered the quality of aquaculture products within 6 days of storage at 5 °C acceptable. After the ninth day of refrigeration, total bacterial concentrations increased, with evident organoleptic (taste and flavour) changes. For the enumeration of *E. coli*, the Petrifilm System, already accepted by the Association of Official Analytical Chemists (AOAC 1995) for food analysis, confirmed to be a reliable alternative to traditional standard techniques.

The negative result obtained in the first sampling with the search for *L. monocytogenes* and *V. parahaemolyticus* strains confirmed the good microbiological standards of farmed products and the limited distribution of such a species in coastal Sicilian waters, as previously reported by Scoglio, Di Pietro, Mauro, Picerno, Laganà & Delia (2000).

Conclusions

Our study underlines the importance of environmental monitoring as a fundamental step in aquaculture, providing assurance of good quality of seafood products. In Italy the problem of the safety of seafood products has only recently received adequate attention and, in particular, the need to define quality standards for national fish production has become

more and more urgent (Giulini, Mietti, Rambaldi, Priore & Serra 1998). Contamination of aquaculture products by pathogens may be due to the indigenous bacterial flora of the environment (*A. hydrophila*, *V. parahaemolyticus*, *Vibrio vulnificus*, *L. monocytogenes*) or to bacteria from allochthonous inputs (enteric bacteria introduced into the sea from animal and human wastes) (Huss 1994). Hazards may also arise from the bacterial contamination of food as a result of poor hygienic standards during handling and processing (WHO 1999).

The low contamination found in our seafood samples reflect the global quality of water fish farms; therefore, our study confirms that good fish-farm management practices may control many of the potential hazards at the production level and consequently reduce the risks of outbreaks of human and fish diseases.

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