Quality assessment of *Zeus faber* (Peter’s fish) ovaries regularly commercialized for human consumption

Filippo Giarratana, Graziella Zino, Valerio D’Andrea, Antonio Panebianco, Alessandro Giuffrida
Department of Veterinary Science, University of Messina, Italy

Abstract

In the last few years, the consumption of fish eggs has increased rapidly, finding widespread use also in mass catering. This increase has involved also those of the Peter’s fish (*Zeus faber*). Females of this species, by their reproductive characteristics, have highly developed gonads in different periods of the year, making the raw material easy to find. The aim of the present study was to perform a quality assessment of *Zeus faber* ovaries regularly commercialized for human consumption. A total number of 34 samples, divided in fresh (11) and frozen (23), were processed for microbiological characterization, parasitological and histological evaluations. Fresh and frozen samples have significant (P<0.01) differences in total bacterial charge, with values of 4.75±0.5 Log CFU/g and 3.65±0.7 Log CFU/g respectively. The mean value of *Enterobacteriaceae* was 2.58±0.7 Log CFU/g in fresh products, while 52.17% (12) of frozen samples reported loads of <1 Log CFU/g. No *Salmonella* spp. and *Listeria monocytogenes* were found. *Aeromonas* spp. was detected in two frozen sample (with loads of 2.2 and <1 Log CFU/g) and in 5 fresh ovaries with value ranged from 1.70 to 3.48 Log CFU/g. *Vibrio* spp. was found in 4 (36.36%) and 3 (13.04%) of fresh and frozen samples respectively, with loads always <1 Log CFU/g. All 31 *Vibrio* strains isolated, were identified as *Vibrio alginolyticus* and 61.29% (19) of them was positive for the ToxRS factor and 6.45% (2) for ToxR. The 47.06% (16) of total samples showed infestations by larvae of *Anisakis* colonization that can be found frequently in ovaries (*Pekmezci et al.*, 2014; *Yardimci et al.*, 2011). No information on Peter’s fish roe microbiology are, instead, available. Furthermore, *Zeus faber* is often subjected to *Anisakidae* colonization that can be found frequently in ovaries (*Pekmezci et al.*, 2014; *Yardimci et al.*, 2014). For all these reasons, the aim of the present study was to perform a quality assessment of *Zeus faber* ovaries regularly commercialized for human consumption.

Introduction

The use and conservation of fish gonads as foodstuff dates back more than three thousand years ago in Sardinia as gift of the Phoenicians. Today, they are considered a valuable material and sometimes, as regards of some fishes, even difficult to find. Both female and male gonads are usually considered edible foods. Male gonads are constituted by fish testes and are commonly known as *latti* or *lattumi* (*Palese and Palese*, 1992). Female gonads have a wider distribution and are commercialized as fresh products but also salted, dried, smoked and marinated. Depending on the source, the local traditions and the preservation technologies, gonads are processed and then commercialized like *ovarian sack* (i.e. *Bottarga*) or as eggs, without the serous coating (caviar, salmon eggs, lumpfish roes, etc.) (*Giuffrida and Panebianco*, 2008). In the last few years, the consumption of fish eggs has increased rapidly, finding widespread use also in mass catering (EUROSTAT, 2017).

This increase has involved also those of the Peter’s fish (*Zeus faber*). Female of this species, thanks to their reproductive characteristics, show highly developed gonads in different periods of the year (*Fulton*, 1898). Moreover, this species is widespread all over Mediterranean areas, making the raw material easy to find. Several studies reported microbiological characterization of different fish roe product, revealing the presence, in some occasion, of food borne pathogens (*Altug and Bayrak* 2003; *Boĭko et al.*, 2004; *Oeleker et al.*, 2015; *Razavilar and Rezvani*, 2004; *Voidarou et al.*, 2011). No information on Peter’s fish roe microbiology are, instead, available. Furthermore, *Zeus faber* is often subjected to *Anisakidae* colonization that can be found frequently in ovaries (*Pekmezci et al.*, 2014; *Yardimci et al.*, 2014). For all these reasons, the aim of the present study was to perform a quality assessment of *Zeus faber* ovaries regularly commercialized for human consumption.

Materials and Methods

Sample collection and macroscopic observations

A total number of 34 *Zeus faber* ovaries (11 fresh and 23 frozen samples) were collected from local market of Mazara del Vallo in a period from October to May. Fishes were caught in FAO area 37.1.2 and 37.2.2, by the trawl fleet of Mazara del Vallo (Sicily, Italy). Mazara del Vallo is widely considered to be the most important fishing center in Italy and contributes for more than 3/4 to the production and turnover of the national trawl fleet (ISTAT data).

Fresh ovaries were transported to our laboratory under refrigerated conditions (4°C) and processed within 24h from their arrival. Frozen samples were, instead, primarily thawed at 4°C for 24h. Each sample was carefully examined for the presumptive presence of parasites. All parasites were examined under stereoscopic microscope (Leica M 205C), and the belonging to the *Anisakis* genus was made according to guidelines proposed by *Murata et al.* (2011).

Microscopic analysis

Portions of each sample were fixed in buffered formalin (10%), embedded in paraffin, and sliced into 5-μm sections. Sections were stained using hematoxylin-eosin (HE) and trichrome Masson. Stained sections were examined using light microscopy (Leica DM 4000B).

Microbiological analysis

Each sample was processed for the count of: i) Aerobic mesophilic bacteria (AMB) according to UNI EN ISO 4833:2004; ii) *Enterobacteriaceae* according to UNI EN ISO 21528-2:2004; iii) *Vibrio* spp. on Thiosulphate Citrate Bile Salt Sucrose Agar (TCBS Oxoid, Italy), with 3% NaCl, incubated at 37°C for 24h;
iv) *Aeromonas* spp. in Glutamate Starch Phenol Red Agar (GSP) with Ampicillin Selective Supplement (Merck, Italy), incubated at 30°C for 24h.

The detection of the following parameter was also conducted: i) *Vibrio* spp. with a preliminary enrichment on Phosphate Buffer Saline (PBS) at 30°C for 24h, then spread on TCBS, with 3% NaCl and incubated at 37°C for 24h; ii) *Aeromonas* spp. with a preliminary enrichment on PBS at 30°C for 24h, then spread on in GSP with Ampicillin and incubated at 30°C for 24h; iii) *Listeria monocytogenes* detection according to UNI EN ISO 11290-1: 2005; iv) *Salmonella* spp. detection according to UNI EN ISO 6579: 2002.

**Vibrio identification: biochemical protocol**

Suspected alophilic vibrios were confirmed to genus level to according to biochemical protocol suggested by Ottaviani et al. (2003). Isolated colonies growth on TCBS, were tested for Gram stains, Ossidase test, vibriostatic factor O/129 (150 mg, Oxoid) and growth on Kliger Iron Agar (KIA, Biolife, Italy). All the strains that resulted belonging to Vibrio spp. were, then, confirmed by molecular methods.

**Vibrio identification: multiplex PCR assay**

For DNA extraction 1 mL of broth culture was centrifuged at 12,000 rpm for 5 min; the pellet was re-suspended in 1 mL of sterile distilled water, boiled for 5 min, and centrifuged again. The supernatant was stored at 20°C until use. DNA was quantified by means of a spectrophotometer (SmartSpec Plus, Bio-Rad, Milan, Italy). For Vibrio genus identification specific gene *rpoA* primers were employed, according to La Neve et al. (2006) and Dalmasso et al. (2009). Confirmed strains belonging to Vibrio genus were selected for further identification at species level. For *V. alginolyticus*, and *V. parahaemolyticus* identification, were employed specific primers encoding a collagenase gene portion, as suggested by Di Pinto et al. (2005). Primers for the screening of pathogenic factors toxR and toxRS were also added to the PCR mix as reported by Xie et al. (2005).

Multiplex PCR mix consisted in a total volume of 50 mL containing 5 mL of Buffer 10X, 200 mmol of each dNTPs, 1.5 mmol of MgCl2, 100 mmol of each primer, 1 mL of DNA template and 1U of Platinum Taq DNA Polymerase (Invitrogen).

**pH determination**

The pH of each sample was measured at room temperature with a pH meter HI90023CW (Hanna Instruments, Italy) equipped with a Mettler Toledo electrode (Mettler Toledo, Switzerland) and a pH electrode (Mettler Toledo, Switzerland).

**Results and Discussion**

**Microbiological results**

Table 1 shows mean values for each microbiological parameter. The mean values for AMC and Enterobacteriaceae were 3.94±0.5 Log CFU/g and 1.30±1.2 Log CFU/g respectively. Fresh and frozen samples have significant (P<0.01) differences in AMC, with mean values of 4.75±0.5 log CFU/g and 3.65±0.7 log CFU/g respectively. In fresh ovaries, AMC oscillated from 3.65 to 5.17 Log CFU/g, while, in frozen samples ranged from 1.74 to 4.60 Log CFU/g. Also for Enterobacteriaceae loads, significant differences (P<0.01) were observed between fresh and frozen products. In fresh products Enterobacteriaceae charges ranged from 1.17 to 3.43 Log CFU/g, while 52.17% (12) of frozen samples reported Enterobacteriaceae loads <1 Log CFU/g.

Considering bacteriological hygiene indicators (AMC and Enterobacteriaceae), *Zeus faber* ovaries presented an overall satisfactory condition even as fresh product stored at refrigeration temperatures (Altug and Bayrak 2003; Boiko et al., 2004; Oelecker et al., 2015; Razavilar and Rezvani, 2004; Vaidouar et al., 2011).

The only microbiological concern may be related to pathogen detection. No *Salmonella* spp. and *Listeria monocytogenes* were found. Otherwise, *Aeromonas* spp. and *Vibrio* spp. were detected in several samples of *Zeus faber* ovaries. *Aeromonas* strains have a significant incidence in marine environment all over Europe and were frequently detected in fishery product, including fish eggs (Davies et al., 2001; Hänninen et al., 1997; Hansen and Olafsen, 1989; Muscolino et al., 2014). Bacteria belonging to *Aeromonas* genus may be, also, responsible of gastro-enteric diseases outbreaks in humans, thanks to the production of enterotoxins, cytotoxins and haemolysins (Deodhar et al., 1991; Kirov, 1993). For all these reasons, they could represent a microbiological hazard for the consumer.

In our study, *Aeromonas* spp. was found in seven samples: two in frozen and five in fresh samples. The two positive frozen samples, were characterized by loads of 2.2 Log CFU/g and <100 CFU/g respectively. In fresh ovaries, instead, *Aeromonas* spp was reported in 45.45% (5) of samples with loads ranging from 2.0 to 3.0 Log CFU/g. However, *Aeromonas* spp. mean loads revealed no significative difference (P>0.01) between fresh and frozen samples (Table 1). Considering its psychrophilic behavior, its tolerance and survival at frozen temperatures as well as its stress resistant variants, this study confirms *Aeromonas* spp. as potential microbiological hazard in *Zeus faber* ovaries intended for consumption (Castro-Escarpuli et al., 2003; Giuffrida et al., 2010).

*Vibrio* spp. was detected in the 36.36% (4) and 13.04% (3) of fresh and frozen samples respectively, but always with loads <1 Log CFU/g.

A total number of 31 suspected *Vibrio* spp. strains (14 from fresh and 17 from frozen samples) were isolated from TCBS with 3% NaCl. All suspected strains were confirmed as belonging to *Vibrio* spp., resulted positive for biochemical test and for molecular identification at genus level (*rpoA* +). Their presence in frozen samples, confirmed the remarkable resistance of halophilic Vibrio at freezing temperatures (Bang and Drake, 2002; Johnston and Brown, 2002). Multiplex PCR analysis

**Table 1. Mean values ± standard deviation expressed in Log CFU/g, of microbiological loads.**

|                  | AMB | Enterobacteriaceae | Aeromonas spp. |
|------------------|-----|---------------------|---------------|
| **Fresh**        | 4.75±0.5 (11/11) | 2.58±0.7 (11/11)  | 1.09±1.3 (5/11) |
| **Frozen**       | 3.65±0.7 (23/23) | 0.58±0.7 (11/23)  | 0.09±0.4 (1/23)  |
| **Total**        | 3.94±0.8 (34/34) | 1.30±1.2 (22/34)  | 0.42±0.9 (634)  |

*aDifferent letters represent significative differences (P<0.01), (positive samples/total samples).*

[Italian Journal of Food Safety 2018; 7:6997] [page 29]
revealed that all 31 strains isolated were *V. alginolyticus*.

This bacteria is often found in Italian marine coastal environments and sea products (Dumontet et al., 2000; Panebianco et al., 2011; Narracci et al., 2013; Ziino et al., 2010; Ziino et al., 2014). It is also, widely spread all over the world, and usually has the highest prevalence in *Vibrio* communities (Schets et al., 2010; Jones et al., 2013; Narracci et al., 2013; Ziino et al., 2010; Ziino et al., 2014). It is also, widely spread all over the world, and usually has the highest prevalence in *Vibrio* communities (Schets et al., 2010; Jones et al., 2013). *V. alginolyticus* pathogenic potential in humans is well recognized, causing various infections and inflammation patterns (Schmidt et al., 1979). It raised great importance as emergent foodborne pathogen, responsible for several cases of gastrointestinal linked to the consumption of fish products (Mustapha et al., 2013). Moreover, in immune-compromised subjects, serious extra-intestinal infections, such as septic shock, have been reported as consequence of consumption of seafood contaminated by *V. alginolyticus* (Dong-Young et al., 2008). ToxRS and ToxR pathogenic factors encode trans-membrane proteins that mediate the regulation of the virulence gene expression in several pathogenic *Vibrio* strains, including *V. alginolyticus* (Das et al., 2016). These factors are frequently investigated for a better characterization of bacterial pathogenic potential (Fu et al., 2016). Moreover, ToxR is widely proposed for the specie identification and phylogenetic analyses (Montieri et al., 2010). Considering all the 31 *V. alginolyticus* strains, 61.5% carried the ToxRS factor and in lower percentage (6.5%) the ToxR gene. In particular, among the 14 strains isolated from fresh samples, 12 (85.7%) were ToxR positive and 2 (14.3%) carried the ToxRS pathogenic factor. Otherwise, among the 17 strains isolated from frozen samples, 7 (41.2%) were ToxR positive but no ToxRS positive strains were found. It was asserted that ToxR may assist *V. alginolyticus* in host target cells adhesion, which is crucial step for the initiation of the infection (Chang et al., 2002). As reported by previous studies, fish roe represent an ideal setting for *Vibrio* colonization (Voidarou et al., 2011). Our results highlight the role of *Zeus faber* ovaries as source of *V. alginolyticus* pathogenic strains, which could be potentially implicated in food-borne disease episodes.

**pH results**

The pH mean values was 6.32±0.2 for fresh ovaries and 6.22±0.3 for frozen products with no significant differences (P>0.01) between the two kind of samples.

**Macroscopic and microscopic analysis results**

The 47.05% (16) of total samples revealed parasitic infestation by presumptive nematode, confirmed by stereomicroscopic observation as *Anisakis* larvae type I. Specifically, larvae were found in 16 ovaries: 45.45% (5) and 11 (47.82%) of fresh and frozen samples respectively. Various levels of infestation were observed: from isolated larvae to a massive infestation, in which nematodes were bunch-shaped aggregated (Figure 1).

The histological investigation showed the presence of various patterns, ascribable to different gonadic development stages (Abou-Seedo et al., 2003; Macrì et al., 2011). Specifically, in some analyzed ovaries, a primary stage of oocyte growth (Previtellogenic stage) was observed (Figure 2), while in other samples a secondary stage of oocyte maturation (Vitellogenic stage) was predominant (Figure 3), with no relation to season or area of fishing.

In samples infested by *Anisakis* larvae, parasites were found encysted on the serosa, and were not observed tissue and cellular reactions (Figure 4). Only in two cases, however, it was possible to observe parasites inside the gonads, not surrounded by any inflammatory reaction (Figure 5). Our results confirm the parasitological risk due to the consumption of *Zeus faber* ovaries. *Anisakis* larvae detection deserve great attention, as the nematode have been also observed in the depth of the gonads. The lack of connective capsule and inflammatory response around the parasites, are consequences of larvae tissue mobilization.

Figure 1. Massive infestation of *Anisakis* larvae type I in fresh sample of *Zeus faber* ovaries.

Figure 2. Previtellogenic stage: a primary stage of oocyte growth in fresh sample of *Zeus faber* ovaries.
searching for favorable conditions after the fish death. In these cases, *Anisakis* could be unnoticed to a superficial inspection, especially if a moderate infestation occurs. However, it would still represent a risk for the consumer as thermal shock is not sufficient to avoid possible allergic reactions in sensitive subjects, caused by allergens resistant to high temperatures and freezing conditions (Audicana and Kennedy, 2008; Speciale *et al.*, 2017).

In frozen samples, other microscopic findings are the damage related to the presence of intra and extra-cytoplasmic ice crystals. During the thawing process, ice particles damaged the oocyte cell wall, causing the loss of cellular content. In particular, oocytes appeared coerced, with irregular contours, fragmented and well spaced. The cytoplasm lost its characteristic granular appearance, becoming uniformly acidophilus and, sometimes, voluminous intracytoplasmic vacuoles were observed (Figure 5). As reported in previous studies microscopic analysis resulted a valid means to discriminate fresh products from those thawed (Meistro *et al.*, 2016; Muscolino *et al.*, 2012).

**Conclusions**

In conclusion, *Zeus faber* fish ovaries showed more than satisfactory hygiene conditions. The only microbiological concern was related to the presence of potentially pathogenic strains of *V. alginolyticus* and *Aeromonas* spp. However, microbiological hazard can be reduced by adequate cooking and the prevention of cross-contamination. In relation to the presence of *Anisakis* larvae, only an appropriate consumer information seems the most important measure to prevent allergic manifestations, beside an efficient thermal treatment or similar strategies in order to inactivate nematodes (Anastasio *et al.*, 2015; Giarratana *et al.*, 2012; Giarratana *et al.*, 2014; Giarratana *et al.*, 2015; Giarratana *et al.*, 2017a; Giarratana *et al.*, 2017b; Valero *et al.*, 2015).

**References**

Abou-Seedo F, Dadzie S, Al-Kanaan KA, 2003. Histology of ovarian development and maturity stages in the yellowfin seabream Acanthopagrus latus (Teleostei: Sparidae) (Hottuyn, 1782)

---

Figure 3. Vitellogenic stage: a secondary stage of oocyte maturation in fresh sample of *Zeus faber* ovaries.

Figure 4. Presence of Anisakis larvae type I encysted on the serosa, without tissue and cellular reactions, in fresh sample of *Zeus faber* ovaries.

Figure 5. Presence of Anisakis larvae type I inside the gonads, not surrounded by any inflammatory reaction, in frozen sample of *Zeus faber* ovaries. Aspect of structural damage in frozen samples: oocytes coerced, with irregular contours, fragmented and well spaced. The cytoplasm lost its characteristic granular appearance and are evident intracytoplasmic vacuoles.
reared in cages. Kuwait J Sci Engin 30.
Altug G, Bayrak Y. 2003. Microbiological
canavarian from Russia and Iran.
Food Microbiol 20:83-6.
Anastasio A, Smaldone G, Cacace D,
Marrone R, Lo Voi A, Santoro M,
Cringoli G, Pozio E, 2015. Inactivation of
Anakisaksis pegreffii larvae in anchovies (Engraulis encrasiaculus) by
salting and quality assessment of
finished product. Food Control 64:115-9.
Audicana MT, Kennedy MW, 2008.
Anikakis simplex: from obscure infec-
tious worm to inducere of immune
hypersensitivity. Clin Microbiol Rev
21:360-79.
Bang W, Drake MA, 2002. Resistance of
cold-and starvation-stressed Vibrio vul-
nificus to heat and freeze-thaw expo-
sure. J Food Protect 65:975-80.
Boikov AV, Pogorelova NP, Zhuravleva LA,
Lartseva LV, 1993. Microbial coloniza-
tion of the caviar of the sturgeon fishes.
Gigiena i sanitariia 11:30-1.
Castro-Escarpuh G, Figueras MJ,
Aguiar-Arrella G, Soler L,
Fernandez-Rondon E, Aparicio GO,
Chacon MR, 2003. Characterisation of
Aeromonas spp. isolated from frozen
fish used for human consumption in
Mexico. Int J Food Microbiol 84:41-9.
Chang C, Qing-bai W, Zhu-Hong L, Jing-
ing Z, Xiao J, Hong-yan S, Chao-quin H,
2012. Characterization of role of the
toxR gene in the physiology and patho-
genicity of Vibrio alginolyticus.
Antonie Van Leeuwenhoek 101:281-8.
Dalmaso A, La Neve F, Suffredini E, Croci
L, Serracca L, Bottero MT, Civera T,
2009. Development of a PCR Assay
Targeting the rpoA Gene for the
Screening of Vibrio Genus. Food
Analytical Methods 2:317-24.
Das SC, Kumar A, Kaushik P, Kumari S,
2016. Pathogenic and pandemic Vibrio
parahaemolyticus detection in fish and
shellfish isolates. Indian J Geo-Marine
Sci 45:1195-8.
Davies AR, Capell C, Jehanno D, Nychas
GJ, Kirby RM, 2001. Incidence of food-
borne pathogens on European fish.
Food Control 12:67-71.
Deodhar LP, Saraswathi K, Varudkar A,
1991. Aeromonas spp. and their associ-
ation with human diarrheal disease. J
Clin Microbiol 29:853-6.
Di Pinto A, Ciccacese G, Tantillo G,
Catalano D, Forte VT, 2005. A collage-
nase-targeted multiplex PCR assay for
identification of Vibrio alginolyticus,
Vibrio cholerae, and Vibrio para-
haemolyticus. J Food Protect 68:150-3.
Dong-Young L, Soo-Youn M, Sang-Oh L,
Hee-Young V, Hee-Joo L, Mi Suk L,
2008. Septic Shock due to Vibrio algi-
noyctis in a Cirrhotic Patient: The
First Case in Korea. Yonsei Med J 49:329-32.
Dumontet S, Krovacek K, Svenson SB,
Pasquale V, Baloda SB, Figliuolo G,
2000. Prevalence and diversity of
Aeromonas and Vibrio spp. in coastal
waters of Southern Italy. Comparative
Immunol Microbiol Infect Dis 23:53-
72.
EUROSTAT 2017. Production of fish eggs
for human consumption from aquacul-
ture (from 2008 onwards). EU open
data platform. Available at:
data.europa.eu/cdu/pdp/data/datas
et/lexxxNEN7uHv2yXTYlaog
Fu K, Li J, Wang Y, Liu J, Yan H, Shi L,
Zhou L, 2016. An Innovative Method
for Rapid Identification and Detection
of Vibrio alginolyticus in Different
Infection Models. Front Microbiol 7:651.
Fulton TW, 1898. The ovaries and ovarian
eggs of the angler or frog-fish (Lophius
piscatorius) and of the John dory (Zeus
fabor). Ann Rep Fish Board Scotland
16:125-34.
Giarratana F, Giuffrida A, Gallo F, Ziino G,
Panebianco A, 2012. Study of the
Resistance Variability of Anisakis
Larvae to Some Technological
Stressors. In Veterinary Science.
Springer Berlin Heidelberg, pp. 155-
159.
Giarratana F, Muscolino D, Beninati C,
Giuffrida A, Panebianco A, 2014.
Activity of Thymus vulgaris essential
oil against Anisakis larvae. Experiment
Parasitol 142: 7-10.
Giarratana F, Muscolino D, Patania A,
Beniani C, Ziino G, Giuffrida A, 2015.
Activity of R (+)
limonene against Anisakis larvae. Ital J
Food Saf 4:5499.
Giarratana F, Muscolino D, Ziino G,
Giuffrida A, Marotta SM, Lo Presti V,
Chiofalo V, Panebianco A, 2017a.
Activity of Tagetes minuta Linnaeus
(Asteraceae) essential oil against L3
Anisakis larvae type 1. Asian Pacific J
Trop Medicine 10:461-5.
Giarratana F, Muscolino D, Ziino G,
Lo Presti V, Chiofalo V, Giuffrida A,
Panebianco A, 2017a. Activity of
Catmint (Nepeta cataria) essential oil
against Anisakis larvae. Trop Biomed
34:22-31.
Giarratana F, Panebianco F, Muscolino D,
Beninati C, Ziino G, Giuffrida A, 2015.
Effect of allyl isothiocyanate against
Anisakis larvae during the anchovy
marinating process. J Food Protect
78:767-71.
Giuffrida A, Panebianco A, 2008. Igiene e
tecnologie dei prodotti della pesca fre-
schi e trasformati,. In: Colavita G, ed.
Igiene e tecnologia dei prodotti di origi-
ne animale. Le Point Vetérinarie Italie,
Milano, Italy, pp 274-276.
Giuffrida A, Ziino G, Valenti D, Donato G,
Panebianco A, 2007. Application of an
interspecific competition model to pre-
dict the growth of Aeromonas hydrophi-
la on fish surfaces during refrigered
storage. Archiv Lebensmittelhyg
58:136-41.
Hanninen ML, Oivanen P, Hirvelä-Koski V,
1997. Aeromonas species in fish, fish-
eggs, shrimp and freshwater. Int J Food
Microbiol 34:17-26.
Hansen G H, Olafsen JA, 1989. Bacterial
colonization of cod (Gadus morhua L.)
and halibut (Hippoglossus hippoglos-
sus) eggs in marine aquaculture. App
Environment Microbiol 55:1435-46.
ISO 11290-1:1996. Microbiology of food
and animal feeding stuffs. Horizontal
method for the detection and enumera-
tion of Listeria monocytogenes. Part 1:
Detection method. International
Standardization Organization Geneva,
Switzerland.
ISO 21528-2:2004. Microbiology of the
food chain — Horizontal methods for the
detection and enumeration of
Enterobacteriaceae Part 2: Colony-
count method. International
Standardization Organization Geneva,
Switzerland.
ISO 4833-1:2013. Microbiology of the
food chain. Horizontal method for the
enumeration of microorganisms. Part 1:
Colony count at 30 degrees C by the
pour plate technique. International
Standardization Organization Geneva,
Switzerland.
ISO 6579:2002. Microbiology of food and
animal feeding stuffs. Horizontal
method for the detection of Salmonella
spp. International Standardization
Organization Geneva, Switzerland.
Johnston MD, Brown MH, 2002. An inves-
tigation into the changed physiological
state of Vibrio bacteria as a survival
mechanism in response to cold temper-
atures and studies on their sensitivity to
heating and freezing. J App Microbiol
92:1066-77.
Jones EH, Feldman KA, Palmer A, Butler
E, Blythe D, Mitchell CS, 2013. Vibrio
infections and surveillance in
Maryland, 2002-2008. Public Health
Rep 128:537-45.
Kirov SM, 1993. The public health signifi-
cance of Aeromonas spp. in foods. Int J
Food Microbiol 20:179-98.
La Neve F, Pedone F, Nuvoloni R,
D’Ascenzi C, Dalmasso A, Civera T, 2006. Identificazione di Vibrioni di interesse sanitario in orate di allevamento mediante metodiche biomolecolari. LX Convegno Nazionale della Società Italiana delle Scienze Veterinarie, 432.

Macrì F, Rapisarda G, Marino G, De Majo M, Aiudi G, 2011. Use of Laparoscopy for the Evaluation of the Reproductive Status of Tench (Tinca tinca). Reprod Domestic Anim 46:130-3.

Meistro S, Pezzolato M, Muscolino D, Giarratana F, Baioni E, Panebianco A, Bozzetta E, 2016. Histology as a valid tool to differentiate fresh from frozen-thawed marinated fish. J Food Protect 79:1457-9.

Montieri S, Suffredini E, Ciccozzi M, Croci L, 2010. Phylogenetic and evolutionary analysis of Vibrio parahaemolyticus and Vibrio alginolyticus isolates based on toxR gene sequence. New Microbiol 33:359-72.

Murata R, Suzuki J, Damasus K, Kai A, 2011. Morphological and molecular characterization of Anisakis larvae (Nematoda: Anisakidae) in Beryx splendens from Japanese waters. Parasi tol Int 60:193-8.

Pekmezci GZ, Onuk EE, Bolukbas CS, Yardimci B, Gurler AT, Acici M, Umur S, 2014. Molecular identification of Anisakis species (Nematoda: Anisakidae) from marine fishes collected in Mediterranean Sea. Ankara Universitesi Veteriner Fakultesi Dergisi 61:233-6.

Ziino G, Donato G, Giarratana F, Panebianco A, 2014. Bacteriological analysis to Area noae (Linné, 1758) in the Nord of Italy: the first record of halophilic Vibrio. Cahiers Biol Marine 55:389-97.

Zhao G, Nibali V, Panebianco A, 2010. Bacteriological investigation on “Mauro” sold in Catania. Vet Res Comm 34:157-61.