Salmo salar fish waste oil: Fatty acids composition and antibacterial activity

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ABSTRACT

Background and aims. Fish by-products are generally used to produce fishmeal or fertilizers, with fish oil as a by-product. Despite their importance, fish wastes are still poorly explored and characterized and more studies are needed to reveal their potentiality. The goal of the present study was to qualitatively characterize and investigate the antimicrobial effects of the fish oil extracted from Salmo salar waste samples and to evaluate the potential use of these compounds for treating pathogen infections.

Methods. Salmo salar waste samples were divided in two groups: heads and soft tissues. Fatty acids composition, and in particular the content in saturated (SAFAs), mono-unsaturated (MUFAs) and Polyunsaturated (PUFAs) fatty acids, was characterized through GC/MS Thermo Focus GC-DSQ II equipped with a ZB-5 fused silica capillary tubes column. The antimicrobial activity of the salmon waste oils was evaluated through the Minimum Inhibitory Concentration assay and the antibiotics contamination was determined by Liquid Chromatography with tandem Mass Spectrometry (LC-MS/MS) analysis. All experiments were done at least in triplicate.

Results. GC/MS analysis has shown the specific fatty acid composition of the salmon waste oils and their enrichment in MUFAs and PUFAs, with special reference to omega-3, -6, -7, -9 fatty acids. Furthermore, our study has highlighted the antimicrobial activity of the fish waste oil samples against two Gram+ and Gram- bacterial strains.

Conclusions. These data confirm that the fish waste is still quantitatively and qualitatively an important source of available biological properties that could be extracted and utilized representing an important strategy to counteract infective diseases in the context of the circular economy.

INTRODUCTION

Fish by-products are generally used to produce fishmeal or fertilizers, with fish oil as a by-product. Anyway, fish wastes are an enormous potential source of useful molecules, such as bioactive peptides, enzymes, antimicrobial components and polyunsaturated fatty...
Polyunsaturated fatty acids (PUFAs) are key constituents of the cell membranes. For this reason they are important regulators of the membrane fluidity, cell signalling, gene expression and cellular functions, and also represent important substrates for lipid mediators synthesis (Parolini, 2019). Low serum levels of ω-3 polyunsaturated fatty acids (PUFAs) have been associated to an increased incidence of cardiovascular disease and higher mortality rate. Furthermore, pre-clinical and clinical studies have shown that the addition of ω-3 PUFAs to daily diet can prevent and attenuate lipid accumulation, vascular inflammation and macrophage recruitment, which are among the major causes of the atherosclerosis plaque formation process (García-Hernández et al., 2013; Corsi, Momo Dongmo & Avallone, 2015; Yagi et al., 2017; Leshno et al., 2018). The American Institute of Medicine, Food and Nutrition Board recommended daily Adequate Intakes (AIs) of Omega-3s (Medicine, 2005) which, unfortunately, are often not met with the daily diet.

The role of PUFAs as pro- or anti-inflammatory molecules has been largely discussed (Fischer & Weber, 1983; Heidel et al., 1989; Hawkes, James & Cleland, 1992; Moreno, 2009; Simonetto et al., 2019). Lipid mediators derived from the ω-6 PUFA are involved in inflammation at different stages. For example, the ω-6 PUFA arachidonic acid is the precursor of prostaglandins, thromboxanes and pro-inflammatory leukotrienes. Instead, ω-3 PUFAs exhibit anti-inflammatory properties by competing with ω-6 PUFAs, altering the membrane phospholipid amount of arachidonic acid and reducing the production of pro-inflammatory eicosanoids. Furthermore, ω-3 PUFAs promote the resolution of inflammation through the synthesis of lipid mediators, including resolvins, protectins and maresins, which are known to be “specialized pro-resolving mediators” (SPMs) (Serhan, 2014).

PUFAs and free fatty acids (FFA) have also been studied for their antimicrobial activity which is characterized by a broad spectrum of activity and the lack of classical resistance mechanisms (Desbois & Smith, 2010; Desbois, 2012; Desbois & Lawlor, 2013). In a recent paper (Chanda et al., 2018), different hypothesis were proposed as PUFAs antimicrobial mode of action which include disruption of intercellular communication, interruption of ATP production, alteration of membrane properties, disruption of fatty acids synthesis, affecting the electron transport system and increasing the number of membrane pores (Zheng et al., 2005; Carballeira, 2008). In addition, as explained elsewhere (Desbois & Smith, 2010), FFAs can also impair Staphylococcus aureus skin colonization through stratum corneum acidification (Fluhr et al. 2001; Takigawa et al., 2005). FFAs may also impair the expression of virulence factors, which are necessary for the establishment of infection, and inhibit the cell-to-cell signalling, thus preventing initial bacterial adhesion and subsequent biofilm formation.

Marine organisms are a very important source of antimicrobial agents (Richards et al., 2001; Schillaci et al., 2010; Schillaci et al., 2013; Spinello et al., 2018; Vazzana et al., 2018; Niñez Acuña et al., 2018) and, among them, the PUFAs are normally present at high levels in Salmo salar (Linder, Fanni & Parmentier, 2005; Morais et al., 2009). Different studies...
have shown the antimicrobial activity of the salmon PUFAs against Gram-positive bacteria due to their specific components such as eicosapentaenoic acid (Desbois, Mearns-Spragg & Smith, 2009; Desbois & Lawlor, 2013), docosahexaenoic acid (Coonrod, 1987; Feldlaufer et al., 1993; Gladyshev et al., 2009), γ-linolenic acid (Asthana et al., 2006) and dihomoy-γ-linolenic acid (Feldlaufer et al., 1993).

It is well known that among fishes farmed in Europe, the Atlantic salmon (Salmo salar) is one of the most important aquaculture species. Norway, Scotland and Ireland are the three major producers of salmon, which, in Europe, represents an important resource in terms of production and economic value. Fish waste from salmon can reach up to 40% of the total weight of the animal and is mainly composed of bones, head and offal.

Even if the most important source of fatty acids is the adipose tissue interspersed in the muscular fibers, it is still possible to obtain valuable quantities of PUFAs from the fish waste due to their large amount. Furthermore, the possibility to use the fish leftovers and to produce, from them, high value products, represents an important step in the circular economy.

Thus, the aim of the present research was to characterize, by qualitative point of view, and to investigate, for the first time, the antimicrobial effects of the fish oil extracted from the market salmon waste through an unbiased approach regard fishing or farming source and condition. Furthermore the potential use of these compounds, for treating Gram + and Gram- bacterial infections, was evaluated through the determination of minimum inhibitory concentrations against S. aureus and Pseudomonas aeruginosa, two relevant pathogens cause of polymicrobial infections (Serra et al., 2015) and included in the global priority list of antibiotic-resistant bacteria from WHO/OMS (Tacconelli et al., 2017). In addition, we tried to understand if the antimicrobial activity could be due to any chemical contaminants present in the samples.

MATERIALS AND METHODS
Salmon sample collection, storage and fish oils extraction
Ten animals were collected from small fish markets of Palermo (Italy) and utilized for the experiments. The animal size is the commercial size (about 2 years old and 3–5 Kg). Raised farm and feed composition are not available. Salmon wastes were transported at +4 °C to the laboratory for the analysis. Fish wastes were divided into two groups which comprise heads and soft tissues that were homogenized and stored at −20 °C until the time of analysis. Homogenate samples were diluted in distilled water (1:1) and heated at 90 °C for 1 h, to coagulate proteins and increase oil release from samples. This method, well established and utilized, was chosen for simplicity and laboratory cost effectiveness. After heat treatment, the homogenate was filtered to obtain the liquid fraction that was centrifuged at 10,000 g at 4 °C to separate the aqueous fraction from the liposoluble component corresponding to the fish oil.
**GC/MS analysis and identification of the components of *Salmo salar* PUFAs**

Fatty acids were analyzed using GC-MS method after extraction and hydrolysis of triacylglycerols. 0.1 g of oil samples were diluted in 1 ml of n-heptane and manually agitated for 10 s, followed by the addition of 0.1 ml of 2N KOH in MeOH solution and mixed in a vortex. When the solution turned clear, 500 µl of upper phase, containing fatty acid methyl esters was diluted with n-heptane to a final volume of 1 ml. For the separation and analysis of the fatty acid methyl esters, Thermo Scientific ISQ™ 9000 Quadrupole GC-MS System in EI (Electron Ionization) mode, working in full scan was used. The capillary column used was a ZB-WAX (30 m × 0.25 mm i.d., film thickness 0.25 µm, Phenomenex, Italy). The oven temperature was programmed so that column temperature started at 80 °C, increased at 15 °C/min to 250 °C and held for 8 min under isothermal conditions. Helium was used as the carrier gas at a flow rate of one mL/min. A sample of 1 µl was injected with a split ratio of 1:100. Mass spectroscopy conditions: The ion source temperature was 260 °C, the MS transfer line temperature was 265 °C and injector temperature was 250 °C. Ionization voltage was 70 eV and the mass range scanned was 35–550 m/z. Using Thermo Scientific Xcalibur Data system software for Windows peak areas were determined and identified by comparison of retention times with those of a FAMEs standard mix (Supelco 37 Component FAME Mix, CRM47885 Sigma-Aldrich) separated under the same chromatographic conditions. Triplicate analyses were prepared for each dried sample, and analysed FAMEs were expressed in percentage.

**Samples extraction and LC-MS/MS analysis**

Samples were subjected to extraction and antibiotics determination (Quinolones, Fluroquinoionolones, Penicillins, Tetracyclines, Macrolides, Sulfamidic and Sulfonamides) following the protocol reported in a previous paper (Cammilleri et al., 2019b). The analyses were performed on a Thermo Fischer UHPLC system (Thermo Fisher Scientific, California, USA) consisting of an ACCLELA 1250 quaternary pump and an ACCLELA autosampler. A Thermo Scientific Hypersil Gold reversed-phase UHPLC column (50 mm, 2.1 mm ID, 1.9 µm) was used for the chromatographic separation. The LC eluents were water (A) and acetonitrile (B), containing 0.1% (v/v) formic acid. The gradient started with 95% eluent A for 1.0 min, a linear variation to 10% A in 6.0 min; these conditions were maintained for 3.0 min. The system returned to 95% A in 0.5 min and was re-equilibrated for 5 min. The column temperature was 30 °C and the sample temperature was kept at 6 °C. The flow rate was 0.4 ml/min and the injection volume was 5 µl. A triple quadrupole TSQ Vantage (Thermo Fisher Scientific, California, USA) in positive electrospray ionization mode (ESI) mass spectrometer was used. The product ion scans of each analyte were performed by direct infusion (10 µl/min) of 1 mg l⁻¹ individual standard solutions with the built-in syringe pump through a T-junction, mixing with the blank column eluate (200 µl/min). The ESI parameters optimized were as follows: capillary voltage 4.5 kV; capillary temperature 310 °C; vaporizer temperature 150 °C; sheath and auxiliary gas pressure were fixed at 40 and 15 (arbitrary unit), respectively. The collision gas was argon at 1.5 mTorr and peak resolution of 0.7 FWHM was used on Q1 and Q3. The scan time for
each monitored transition was 0.02 s and the scan width was 0.02 m/z. Acquisition data were recorded and elaborated using Xcalibur™ version 2.1.0.1139 software from Thermo. The method was validated according to the parameters described by EU Commission Decision 2002/657/EC. All experiments were done at least in triplicate.

**Minimum inhibitory concentration determination**

Minimum inhibitory concentrations (MICs) of the fish oils from *Salmo salar*, head and muscle, were evaluated using an already described micromethod ([Schillaci et al., 2010; Schillaci et al., 2013; Schillaci et al., 2014]). A series of solutions were prepared with a range of concentrations from 50 to 0.75 % v/v (obtained by two-fold serial dilution). The serial dilutions were made in Mueller-Hinton Broth (MHB) in a 96-wells plate, starting from a stock solution of 1 mg/mL in NaCl 0.9% w/v. To each well, 10 µL of a bacterial suspension from a 24 h culture containing ∼10⁶ cfu/mL was added.

The plate was incubated at 37 °C for 24 h; after this time, the MICs were determined by a microplate reader (Glomax Multidetection System TM297 Promega, Milano Italy) as the lowest concentration of compound whose Optical Density (OD), read at 570 nm, was comparable with the negative control wells (broth only, without inoculum). Positive controls are instead the growth control, bacterial inoculum in the medium without any inhibitor. The antimicrobial properties were determined on Gram-positive bacterial reference strains *Staphylococcus aureus* (ATCC 25923; ATCC 6538) and on Gram-negative strains *Pseudomonas aeruginosa* (ATCC 15442; ATCC 9027). Each assay was performed in triplicates and repeated at least twice.

**RESULTS**

*Salmo salar* waste oils characterization by GC/MS analysis

The fish oil fatty acid compositions extracted from head and soft tissue were characterized, qualitatively, by GC/MS. The analysis, whose results are listed in Tables 1A and 1B, showed the salmon fatty acid composition and the percentage of the different unsaturated (UFAs) and saturated fatty (SFAs) acids both in the head and in the soft tissue respect to the total. In particular, the fish waste oil from the heads was composed of the 84% of UFAs and the 16% of SFAs while the fish waste oil from the soft tissue was composed of 83% of UFAs and the 17% of SFAs.

Among the unsaturated fatty acids, the GC/MS has highlighted the presence of the monounsaturated acids ω-9 oleic (C18:1 Δ⁹), which percentages were of 53.58% in the head oil and 39.47% in the soft tissue oil, ω-9 gondoic (C20:1 Δ¹¹), which percentages were of 5.75%, in the head oil and 4.02% in the soft tissue oil and ω-7 Palmitoleic (C16:1 Δ⁹), 3.24% in the head oil and 1.39% in the soft tissue oil. The polyunsaturated acids were mainly composed of ω-3 α-Linolenic (C18:3 Δ⁹,12,15), 5.91% in the head oil and 4.46% in the soft tissue oil and ω-6 Linoleic (C18:2 Δ⁹,12), 15.43% in the head oil and 14.56% in the soft tissue oil.

The saturated fatty acids (SFA) represented the remaining part of the lipid fraction constituted by myristic acid (C14:0), 2.28% in the head oil and 2.56% in the soft tissue oil, palmitic acid (C16:0), 11.44% in the head oil and 9.57% in the soft tissue oil and stearic
Table 1  Salmo salar fatty acid characterization. Fatty acid composition of the oil extracted from wastes of Salmo salar head samples (A) and soft tissue samples (B). Values are reported as relative percentages and are means ± standard deviations. All experiments were done at least in triplicate.

| IUPAC  | NAME                  | ω-Group | R.T. | RELATIVE % ± D.S. |
|--------|-----------------------|---------|------|-------------------|
| (A)    |                       |         |      |                   |
| C14:0  | Myristic acid         | –       | 10.10| 2.28 ± 0.04       |
| C16:0  | Palmitic acid         | –       | 10.84| 11.44 ± 0.16      |
| C16:1 Δ9 | Palmitoleic acid  | ω-7     | 10.91| 3.24 ± 0.24       |
| C18:0  | Stearic acid          | –       | 11.52| 2.34 ± 0.12       |
| C18:1 Δ9 | Oleic acid          | ω-9     | 11.56| 53.58 ± 0.72      |
| C18:2 Δ9,12 | Linoleic acid | ω-6     | 11.70| 15.43 ± 0.37      |
| C18:3 Δ9,12,15 | α-Linolenic acid | ω-3    | 11.89| 5.91 ± 0.10       |
| C20:1 Δ11 | Gondoic acid       | ω-9     | 12.34| 5.75 ± 0.99       |
| (B)    |                       |         |      |                   |
| C14:0  | Myristic acid         | –       | 9.59 | 2.56 ± 0.29       |
| C14:1 Δ9 | Myristoleic acid  | –       | 9.74 | 0.03 ± 0.01       |
| C15:0  | Pentadecanoic acid   | –       | 10.05| 0.14 ± 0.12       |
| C16:0  | Palmitic acid         | –       | 10.33| 9.57 ± 0.38       |
| C16:1 Δ9 | Palmitoleic acid  | ω-7     | 10.49| 1.39 ± 1.59       |
| C16:2 Δ9,12 | 9,12-Hexadecadienoic acid | –   | 10.65| 0.13 ± 0.06       |
| C17:0  | Margaric acid         | –       | 10.73| 0.2 ± 0.03        |
| C17:1 Δ10 | cis-10- Heptadecenoic acid | ω-7 | 10.80| 0.16 ± 0.14       |
| C17:1 Δ8 | Heptadecenoic acid | ω-9     | 10.85| 0.13 ± 0.04       |
| C18:0  | Stearic acid          | –       | 10.95| 0.54 ± 0.6        |
| C18:1 Δ9 | Oleic acid          | ω-9     | 11.05| 39.47 ± 2.57      |
| C18:2 Δ9,12 | Linoleic acid       | ω-6     | 11.25| 14.56 ± 0.19      |
| C18:2 Δ12,15 | 12,15-Octadecanoic acid | ω-3 | 11.38| 0.09 ± 0.01       |
| C18:3 Δ6,9,12 | γ-Linolenic acid   | ω-6     | 11.45| 2.13 ± 3.49       |
| C18:3 Δ9,12,15 | α-Linolenic acid | ω-3    | 11.50| 4.46 ± 3.52       |
| C18:4 Δ6,9,12,15 | Stearidonic acid | ω-3    | 11.62| 0.59 ± 0.5        |
| C20:0  | Eicosanoic acid       | –       | 11.81| 2.25 ± 3.36       |
| C20:1 Δ11 | Gondoic acid       | ω-9     | 11.87| 4.02 ± 3.45       |
| C20:2 Δ8,11 | 8,11-Eicosenoic acid | –     | 12.01| 0.58 ± 0.91       |
| C20:2 Δ11,14 | cis-11,14-Eicosadienoic acid | ω-6 | 12.06| 1.27 ± 0.84       |
| C20:3 Δ8,11,14 | 8,11,14-Eicosatrienoic acid | ω-6 | 12.16| 0.39 ± 0.26       |
| C20:3 Δ11,14,17 | 11,14,17-Eicosatrienoic acid | ω-3 | 12.28| 0.82 ± 0.08       |
| C20:4 Δ8,11,14,17 | all-cis 8,11,14,17-Eicosatetraenoic acid | – | 12.36| 1.53 ± 0.80       |
| C20:5 Δ5,8,11,14,17 | Eicosapentaenoic acid (EPA) | ω-3 | 12.45| 1.90 ± 1.42       |
| C22:0  | Docosanoic acid       | –       | 12.63| 1.57 ± 2.64       |
| C22:1 Δ13 | Erucic acid         | ω-9     | 12.69| 3.33 ± 2.88       

(continued on next page)
Table 1 (continued)

| IUPAC                  | NAME                               | ω-Group | R.T. | RELATIVE % ± D.S. |
|------------------------|------------------------------------|---------|------|--------------------|
| C22:2 Δ\text{13,16}    | cis-13,1 6-Docosanoic acid         | ω-6     | 12,99| 0.26 ±0.12         |
| C22:5 Δ\text{4,7,10,14,16} | 4,7,10,13,16-Docosapentaenoic acid | ω-6     | 13,34| 0.45 ±0.61         |
| C22:5 Δ\text{7,10,13,16,19} | Clupanodonic acid (OPA)            | –       | 13,51| 2.03 ±1.17         |
| C22:6 Δ\text{4,7,10,13,16,19} | Docosahexaenoic acid (DHA)        | ω-3     | 13,65| 2.78 ±2.04         |
| C24:0                  | Tetracosanoic acid                 |         | 13,75| 0.12 ±0.10         |
| C24:1 Δ\text{15}       | Nervonic acid                      | ω-9     | 13,88| 0.29 ±0.24         |

Table 2  Salmon waste oils antibacterial activity. Antimicrobial activity (MIC) of the fish oil extracted from salmon head and soft tissue samples against two Gram+ and Gram- bacterial strains. Values are expressed as volume percentages (%v/v).

| Bacterial strains | Soft tissue | Head |
|-------------------|-------------|------|
| Gram-             | P. aeruginosa ATCC 9027 | 12.5 | 25 |
|                   | P. aeruginosa ATCC15442  | 12.5 | 25 |
| Gram+             | S. aureus ATCC 6538      | 12.5 | 25 |
|                   | S. aureus ATCC 25923     | 12.5 | 25 |

Acid (C18:0), 2.34% in the head oil and 0.54% in the soft tissue oil. Furthermore, eicosanoic acid (C20:0) and docosanoic acid (C22:0) were detected only in the soft tissue oils with the relative ratios of 2.25% and 1.57% respectively.

**Antimicrobial activity of the Salmo salar fish waste oils**

The antimicrobial activity of the oils extracted from the fish waste, both the head and the soft tissue, was evaluated through the determination of the MIC values against two reference strains of Gram-positive (S. aureus) and Gram-negative (P. aeruginosa) bacteria. Table 2 summarizes the results of the experiment in which the antimicrobial efficacy the fish waste oils has been determined. In fact, the fish oils extracted from the head and from the soft tissue were shown to inhibit the growth of the tested microorganisms at a concentration of 25% (v/v) and 12.5% (v/v) respectively.

**Antibiotics contaminants evaluation**

To assess the possibility of antibiotics contaminants in the fish waste oil samples, an LC-MS/MS was performed. The validation of the method produced satisfactory results in terms of linearity (r² > 0.996 for all the analytes examined), accuracy and precision of intra-day and inter-day analysis, with relative standard deviation (RSD) values within 10%. The trueness values obtained were in the range of 86–92%. The residues of antibiotics found in the samples examined are shown in Table 3.

Among 53 antibiotics tested, both types of sample examined showed the simultaneous presence of 4 antibiotics classes, Quinolonics, β-lactams, Macrolides and Sulfonamides while Fluoroquinolons and Sulfamidics were highlighted only in the head oil sample.
Table 3  Antibiotics contaminants determination in salmon waste oil. LC-MS/MS antibiotics determination in the fish oil extracted from salmon head and soft tissue samples. Values are expressed as g/Kg. N/D, not detected.

| Functional groups | Antibiotic       | Head oil (µg/Kg) | Soft tissue oil (µg/Kg) |
|-------------------|------------------|------------------|------------------------|
| Sulfonamide       | Sulfaguanidine   | 6.48             | 6.54                   |
|                   | Sulfamerazine    | N/D              | 0.72                   |
|                   | Ofloxacin        | 2.32             | N/D                    |
| Fluoroquinolon    | Ciprofloxacin    | 3.85             | N/D                    |
|                   | Lomefloxacin     | 1.83             | N/D                    |
|                   | Enrofloxacin     | N/D              | 0.17                   |
| Sulfamidic        | Sulfachinossalin | 2.19             | N/D                    |
| Quinolon          | Nalidixic Acid   | 2.04             | 1.97                   |
|                   | Oleandomycin     | 4.58             | 4.83                   |
| Macrolides        | Tylosina         | 4.48             | N/D                    |
|                   | Penicillin G     | 9.29             | 31.29                  |
| β-lactam          | Penicillin V     | N/D              | 50.73                  |
|                   | Oxacillin        | N/D              | 34.34                  |
|                   | Nafcillin        | 21.31            | 151.04                 |

No tetracycline residues were found. β-lactams were found to be present at the highest concentrations.

DISCUSSION

Despite its potential value, fish waste actually represents a significant cost for fish industries and markets. This huge mass is normally discarded but could represent an important source of bioactive compounds for pharmaceutical, cosmetic, nutrition and biotechnological applications. Molecules such as proteins (i.e., enzymes and collagen), lipids, protein hydrolysates, astaxanthin, chitin (Caruso, 2015) can be extracted and utilized. Fish oil, enriched in Polyunsaturated Fatty Acids (PUFAs), is still another important source of high quality bioactive molecules that could be extracted from the fish waste.

Salmo salar fish waste can represent up to 30–50% of the total weight of the animal (Torrissen et al., 2011; He, Franco & Zhang, 2011; Opheim et al., 2015; Dinh et al., 2018). It is well known that the salmon oil has important features in terms of PUFAs and is a rich source of omega-3 fats. In our study we have confirmed the presence of PUFAs in the fish waste both in the oil extracted from head and soft tissues and we have shown how these PUFAs are characterized by the presence of omega-3 and omega-6 fatty acids (Tables 1A and 1B). The most abundant omega-3 found in the fish waste oil was the linolenic acid, which represent 5.91% in head oil and 4.46% in soft tissue oil. The α-Linolenic acid is really important in the human diet due to its role as substrate for the synthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which confer unique biophysical properties to cell membranes and are necessary for tissue functions (Burdge & Calder, 2006). Linoleic acid was found to be the most abundant omega-6 fatty acid both in head oil (15.43% ± 0.47%) and in soft tissue oil (14.56% ± 0.19%). This fatty acid is known for its important
physiological role, as a constituent of acylglycosyl ceramides, in maintaining the integrity of the water permeability barrier of the skin (Sanders, 2016). The omega-6 linoleic acid is also the precursor of the arachidonic acid, which, in turn, is the major precursor of the eicosanoids, such as prostaglandins, thromboxanes, prostacyclins, leukotrienes and anandamides, which control a large number of physiological processes. Furthermore, arachidonic acid regulates the membrane fluidity, modulates the function of specific membrane proteins involved in cellular signalling and act maintaining cell and organelle integrity and vascular permeability (Tallima & El Ridi, 2018). Arachidonic acid also affects neuronal excitability and synaptic transmission through acting on voltage-gated ion channels and the accumulation of unesterified arachidonic acid compromises cell survival via induction of apoptosis. Omega-9 oleic acid was the most abundant fatty acid found both in the head oil (53.58% ± 0.72) and in soft tissue oil (39.47% ± 2.57%). This molecule, that is a monounsaturated fatty acid, has been shown to exert many biological functions such as regulation of plasma lipid and lipoprotein concentrations, modification of coagulation properties, improvement of glucose homeostasis, and attenuation of inflammation and oxidative stress (Lopez et al., 2010). Furthermore oleic acid has been shown to inhibit tumour cell proliferation in a dose- and time-dependent manner, inducing cell cycle G0/G1 arrest, increasing the apoptosis and the expression of p53 and cleaved caspase-3, and decreasing the expression of CyclinD1 and Bcl-2 (Jiang et al., 2017).

Fatty acids also exert an important role against microorganism infections. Pathogenic agents can cause infections in different ways, such as through the production of virulence factors and biofilms formation, (Beceiro, Tomás & Bou, 2013; Schroeder, Brooks & Brooks, 2017). In vitro and in vivo studies have demonstrated that omega-3 fatty acids, and in particular linolenic acid and its derivatives, used alone or in combination with conventional antibiotics, possess antimicrobial properties (Chanda et al., 2018). Omega-6, -7, -9 fatty acids, such as γ-linolenic, linoleic, arachidonic, palmitoleic and oleic acids, their ethyl esters and methyl esters, were also shown to be effective against various microorganisms (Huang, George & Ebersole, 2010). The mechanism of action that would explain the antimicrobial properties of fatty acids could be the alteration of cell membrane hydrophobicity, charge and integrity, which result in electron leakage and subsequent cell death (Desbois & Smith, 2010; Lopez-Romero et al., 2015; Calo et al., 2015). Furthermore fatty acids can contribute to bacterial death through cell lysis, inhibition of enzyme activity, impairment of nutrient uptake and the generation of lethal oxidation products (Desbois & Smith, 2010).

In our research, the antimicrobial properties of the salmon head and soft tissue waste oils were tested against two important Gram positive and Gram negative pathogens and the results showed a MIC of 25% and 12.5% (v/v) respectively (Table 2). These data seem to indicate that the fish oil extracted from the waste is still active against tested microorganisms and can act independently from the bacterial wall type. Furthermore, the antimicrobial activity of the waste oil extracted from the salmon soft tissue seems to be more efficient than head oil against the two bacterial strains utilized. This difference could be probably due to the different fatty acid composition of the two tissue sources (Tables 1A and 1B). In fact, comparing the fatty acids composition in the two samples, SUFAs, MUFAs and PUFAs...
were highest in the oil extracted from soft tissue. These data seem to be in accordance with similar results obtained in other fishes (Li, Sinclair & Li, 2011; Hong et al., 2015).

Our research was performed through an unbiased approach regard fishing or farming source and condition. However, it is legitimate to ask whether farming conditions, and in particular fish diet, could alter the fish oil composition. Recently studies have shown that alternative salmon feeds can increase the salmon oil concentration but not, at least completely, its composition (Ruyter et al., 2019; Bruni et al., 2020).

Almost the totality of the Salmon sold in Italian fish markets is produced through aquaculture methods. The importance of food safety is crucial for fish farm and the potential hazards include dangerous chemicals and microorganisms. The former could be accumulated by the fish, especially in their fat tissues, from the aquaculture environment, the feed and residues from veterinary prophylaxis, the latter are represented by parasites, viruses and bacteria that may be harmful for humans (Fairgrieve & Rust, 2003; Estévez et al., 2018; Ben Hamed et al., 2018; Gjessing et al., 2019; Feist et al., 2019). In addition seafood produced through aquaculture can be contaminated by different types of toxic substances from natural and/or anthropogenic origins due to both indirect and direct pollution from continental human activities (Freny & Bordet, 2002; Chiesa et al., 2019; Quiones et al., 2019; Heldal et al., 2019). Aquaculture fish management practices can represent an important stressing factor for fishes, in particular for salmon, and could result in high mortalities leading to significant economic loss for producers (Wilson et al., 2009; Sudheesh et al., 2012; Overton et al., 2019). In fact, stressors like handling, stripping of brood stock, antimicrobial treatments, vaccination, temperature, crowding, starvation and transport can result in an increase of a number of diseases evaluable through the measure of the levels of cytokines, heat shock proteins (HSP), corticosteroid hormones, immunoglobulin and immune cells levels, haematological parameters (Gabriel & Akinrotimi, 2011; Cordero et al., 2016; Rehman et al., 2017; Parisi et al., 2017; Chiaramonte et al., 2019; Cammilleri et al., 2019a; Inguglia et al., 2020; Vazzana et al., 2020). Moreover, salmon, which are usually farmed in crowded conditions, are easily targeted by infective pathologies (Poppe, Barnes & Midtlyng, 2002; Håstein, 2004; Bang-Jensen, Gu & Sindre, 2019). Over the past years, fish farms, and in particular that of salmon, have increased their productivity in parallel with the growth of the use of substances used to prevent and treat microbial and bacterial disease, such as antibiotics (Miranda, Godoy & Lee, 2018). We must say that other solutions are being tried, such as vaccines and immunostimulants (Eslamloo et al., 2017; Meza et al., 2019; Xue et al., 2019; Chalmers et al., 2020), in order to limit the use of antibiotics (Gravningen, Sorum & Horsberg, 2019) but they still remain a largely utilized solution especially in Non-European countries. Specific data about antibiotics in the aquaculture industry are not easy to report due to the different current laws of the involved countries. For this reason data are often unavailable or unattainable (Heuer et al., 2009; Romero, Gloria & Navarrete, 2012; Miranda, Godoy & Lee, 2018). However, based on available data, the qualities and quantities of the used antibiotics are variable. For example, among the world countries with the highest rates of aquaculture antibiotic use (Van Boeckel et al., 2015) there are Chile and Vietnam. In Norway, the antibiotics amount used in aquaculture, decreased enormously in the last years but still present, is of 1g/ton of farmed Salmon while
in Vietnam the utilized amount is of 700 g/ton of farmed shrimp (Smith, 2008; Bang-Jensen, Gu & Sindre, 2019).

Considering this information, we performed the LC-MS/MS analysis to exclude the involvement of aquaculture contaminants in the biological antibacterial activity of the fish waste oil (Table 3). The analysis has shown the presence of traces of molecules that, anyway, are considerably under the maximum residue limits indicated by European law (REGULATION, 2009; Commission Regulation (EU), 2009). Furthermore, the small drug amounts highlighted by the experiment would not seem explain the MIC values observed. However, we cannot completely exclude the hypothesis of a contribute of this molecules to the antibacterial properties of the fish waste oil and further analysis are needed to totally exclude this possibility.

CONCLUSIONS

The present research have shown, through GC-MS analysis, the specific composition of the fish waste oil extracted from different discarded parts of the *Salmo salar* present in Italian fish markets. The analysis has also highlighted the oil enrichment in polyunsaturated fatty acids and, among them, in omega-6, -7 and -9 fatty acids. In addition, the MIC experiments have revealed the antibacterial activity of the extracted Salmon waste oil.

These data confirm that the fish waste is still quantitatively and qualitatively an important source of available biological properties that could be extracted and utilized representing an important strategy to counteract infective diseases in the context of the circular economy.

ADDITIONAL INFORMATION AND DECLARATIONS

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**Competing Interests**

The authors declare there are no competing interests.

**Author Contributions**

- Luigi Inguglia conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Marco Chiaramonte conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
• Vita Di Stefano, Gaetano Cammilleri and Licia Pantano performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
• Domenico Schillaci, Manuela Mauro, performed the experiments, prepared figures and/or tables, and Mirella Vazzana, Vincenzo Ferrantelli and Rosalia Nicolosi approved the final draft.
• Vincenzo Arizza conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability
The following information was supplied regarding data availability:
Raw data are available in the Supplementary Files.

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REFERENCES

Commission Regulation (EU). 2009. Commission regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. 2010.

Asthana RK, Srivastava A, Kayastha AM, Nath G, Singh SP. 2006. Antibacterial potential of γ-linolenic acid from Fischerella sp. colonizing Neem tree bark. World Journal of Microbiology and Biotechnology 22:443–448 DOI 10.1007/s11274-005-9054-8.

Bang-Jensen B, Gu J, Sindre H. 2019. The Health Situation in Norwegian Aquaculture 2018 Report 6b - 2019.

Beceiro A, Tomás M, Bou G. 2013. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world? Clinical Microbiology Reviews 26:185–230 DOI 10.1128/CMR.00059-12.

Ben Hamed S, Tavares Ranzani-Paiva MJ, Tachibana L, de Carla Dias D, Ishikawa CM, Esteban MA. 2018. Fish pathogen bacteria: adhesion, parameters influencing virulence and interaction with host cells. Fish and Shellfish Immunology 80:550–562 DOI 10.1016/j.fsi.2018.06.053.

Bruni L, Belghit I, Lock E, Secci G, Taiti C, Parisi G. 2020. Total replacement of dietary fish meal with black soldier fly (Hermetia illucens) larvae does not impair physical, chemical or volatile composition of farmed Atlantic salmon (Salmo salar L.). Journal of the Science of Food and Agriculture 100:1038–1047 DOI 10.1002/jsfa.10108.

Burdge GC, Calder PC. 2006. Dietary α-linolenic acid and health-related outcomes: a metabolic perspective. Nutrition Research Reviews 19:26–52 DOI 10.1079/NRR2005113.
Calo JR, Crandall PG, O’Bryan CA, Ricke SC. 2015. Essential oils as antimicrobials in food systems —a review. *Food Control* 54:111–119 DOI 10.1016/J.FOODCONT.2014.12.040.

Cammilleri G, Galluzzo P, Pulvirenti A, Giangrosso IE, Lo Dico GM, Montana G, Lampiasi N, Mobilia MA, Lastra A, Vazzana M, Vella A, Placa PLa, Macaluso A, Ferrantelli V. 2019a. Toxic mineral elements in Mytilus galloprovincialis from Sicilian coasts (Southern Italy). *Natural Product Research* 34:1–6 DOI 10.1080/14786419.2019.1610963.

Cammilleri G, Pulvirenti A, Vella A, Macaluso A, Dico GLo, Giaconne V, Giordano V, Vinciguerra M, Cicero N, Cicero A, Giangrosso G, Vullo S, Ferrantelli V. 2019b. Tetracycline residues in bovine muscle and liver samples from sicily (Southern Italy) by LC-MS/MS method: a six-year study. *Molecules* 24:Article 695 DOI 10.3390/molecules2404695.

Carballeira NM. 2008. New advances in fatty acids as antimalarial, antimycobacterial and antifungal agents. *Progress in Lipid Research* 47:50–61 DOI 10.1016/j.plipres.2007.10.002.

Chalmers L, Migaud H, Adams A, Vera LM, McStay E, North B, Mitchell C, Taylor JF. 2020. Response of triploid Atlantic salmon (Salmo salar) to commercial vaccines. *Fish and Shellfish Immunology* 97:624–636 DOI 10.1016/j.fsi.2019.12.070.

Chanda W, Joseph TP, Guo X, Wang W, Liu M, Vuai MS, Padhiaar AA, Zhong M. 2018. Effectiveness of omega-3 polyunsaturated fatty acids against microbial pathogens. *Journal of Zhejiang University. Science. B* 19:253–262 DOI 10.1631/JZUS.B1700063.

Caruso G. 2015. Fishery wastes and by-products: A resource to be valorised. *Journal of FisheriesSciences.com* 9(4):80–83.

Chiaramonte M, Inguglia L, Vazzana M, Deidun A, Arizza V. 2019. Stress and immune response to bacterial LPS in the sea urchin Paracentrotus lividus (Lamarck, 1816). *Fish and Shellfish Immunology* 92:384–394 DOI 10.1016/j.fsi.2019.06.017.

Chiesa LM, Nobile M, Ceriani F, Malandra R, Arioli F, Panseri S. 2019. Risk characterisation from the presence of environmental contaminants and antibiotic residues in wild and farmed salmon from different FAO zones. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment* 36:152–162 DOI 10.1080/19440049.2018.1563723.

Coonrod JD. 1987. Rôle of surfactant free fatty acids in antimicrobial defenses. *European Journal of Respiratory Diseases Supplement* 153:209–214.

Cordero H, Mauro M, Cuesta A, Cammarata M, Esteban MÁ. 2016. In vitro cytokine profile revealed differences from dorsal and ventral skin susceptibility to pathogen-probiotic interaction in gilthead seabream. *Fish & Shellfish Immunology* 56:188–191 DOI 10.1016/J.FSII.2016.07.018.

Corsil, Momo Dongmo B, Avallone R. 2015. Supplementation of omega 3 fatty acids improves oxidative stress in activated BV2 microglial cell line. *International Journal of Food Sciences and Nutrition* 66:293–299 DOI 10.3109/09637486.2014.986073.
Desbois AP. 2012. Potential applications of antimicrobial fatty acids in medicine, agriculture and other industries. Recent Patents on Anti-Infective Drug Discovery 7:111–122 DOI 10.2174/157489112801619728.

Desbois AP, Lawlor KC. 2013. Antibacterial activity of long-chain polyunsaturated fatty acids against Propionibacterium acnes and Staphylococcus aureus. Marine Drugs 11:4544–4557 DOI 10.3390/md1114544.

Desbois AP, Mearns-Spragg A, Smith VJ. 2009. A fatty acid from the diatom phaeodactylum tricornutum is antibacterial against diverse bacteria including multi-resistant Staphylococcus aureus (MRSA). Marine Biotechnology 11:45–52 DOI 10.1007/s10126-008-9118-5.

Desbois AP, Smith VJ. 2010. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. Applied Microbiology and Biotechnology 85:1629–1642 DOI 10.1007/s00253-009-2355-3.

Dinh T, Vo L, Pham KT, Ha DQ. 2018. Recovery of proteolysate from salmon by-product: investigation of antioxidant activity, optimization of hydrolysis, determination of iron-binding activity and identification of bioactive peptides. The International Journal of Engineering and Science (IJES) 7:23–42 DOI 10.9790/1813-0709041830.

Eslamloo K, Xue X, Hall JR, Smith NC, Caballero-Solares A, Parrish CC, Taylor RG, Rise ML. 2017. Transcriptome profiling of antiviral immune and dietary fatty acid dependent responses of Atlantic salmon macrophage-like cells. BMC Genomics 18:706 DOI 10.1186/s12864-017-4099-2.

Estévez RA, Mostazo MGC, Rodriguez E, Espinoza JC, Kuznar J, Jónsson ZO, Guomundsson GH, Maier VH. 2018. Inducers of salmon innate immunity: an in vitro and in vivo approach. Fish and Shellfish Immunology 72:247–258 DOI 10.1016/j.fsi.2017.10.058.

Fairgrieve WT, Rust MB. 2003. Interactions of Atlantic salmon in the Pacific northwest. Fisheries Research 62:329–338 DOI 10.1016/S0165-7836(03)00067-5.

Feist SW, Thrush MA, Dunn P, Bateman K, Peeler EJ. 2019. The aquatic animal pandemic crisis. Revue Scientifique et Technique (International Office of Epizootics) 38:437–457 DOI 10.20506/rst.38.2.2997.

Feldlaufer MF, Knox DA, Lusby WR, Shimamuni H. 1993. Antimicrobial activity of fatty acids against Bacillus larvae, the causative agent of American foulbrood disease. Apidologie 24:95–99 DOI 10.1051/apido:19930202.

Fischer S, Weber PC. 1983. Thromboxane A3 (TXA3) is formed in human platelets after dietary eicosapentaenoic acid (C20:5 ω3). Biochemical and Biophysical Research Communications 116:1091–1099 DOI 10.1016/S0006-291X(83)80254-X.

Fluhr JW, Kao J, Jain M, Ahn SK, Feingold KR, Elias PM PM. 2001. Generation of free fatty acids from phospholipids regulates stratum corneum acidification and integrity. Journal of Investigative Dermatology 117(1):4451 DOI 10.1046/j.0022-202x.2001.01399.x.

Fremy J-M, Bordet F. 2002. Evaluation of consequences on human health related to the occurrence of contaminants in seafood. Revue Medecine Veterinaire 153:735–740.
Gabriel U, Akinrotimi O. 2011. Management of stress in fish for sustainable aquaculture development. Researcher 3(4):28–38.

García-Hernández VM, Gallar M, Sánchez-Soriano J, Micol V, Roche E, García-García E. 2013. Effect of omega-3 dietary supplements with different oxidation levels in the lipidic profile of women: a randomized controlled trial. International Journal of Food Sciences and Nutrition 64:993–1000 DOI 10.3109/09637486.2013.812619.

Ghaly A, Ramakrishnan VV, Brooks MS, Budge SM. 2013. Fish processing wastes as a potential source of proteins, amino acids and oils: a critical review. amino acids and oils: a critical review. Journal of Microbial and Biochemical Technology 5:107–129 DOI 10.4172/1948-5948.1000110.

Gjessing MC, Steinum T, Olsen AB, Lie KI, Tavornpanich S, Colquhoun DJ, Gjevre AG. 2019. Histopathological investigation of complex gill disease in sea farmed Atlantic salmon. PLOS ONE 14(10):e0222926 DOI 10.1371/journal.pone.0222926.

Gladyshev MI, Sushchik NN, Makhutova ON, Kalachova GS. 2009. Content of essential polyunsaturated fatty acids in three canned fish species. International Journal of Food Sciences and Nutrition 60:224–230 DOI 10.1080/09637480701664761.

Gravningen K, Sorum H, Horsberg TE. 2019. The future of therapeutic agents in aquaculture. Revue Scientifique et Technique (International Office of Epizootics) 38:641–651 DOI 10.20506/rst.38.2.3010.

Håstein T. 2004. Animal welfare issues relating to aquaculture. In: Proceedings of the global conference on animal welfare: an OIE initiative. Paris, France, 219–231.

Hawkes JS, James MJ, Cleland LG. 1992. Biological activity of prostaglandin E3 with regard to oedema formation in mice. Agents and Actions 35:85–87 DOI 10.1007/BF01990956.

He S, Franco C, Zhang W. 2011. Characterisation of processing wastes of Atlantic Salmon (Salmo salar) and Yellowtail Kingfish (Seriola lalandi) harvested in Australia. International Journal of Food Science and Technology 46:1898–1904 DOI 10.1111/j.1365-2621.2011.02699.x.

Heidel JR, Taylor SM, Laegreid WW, Silflow RM, Liggitt HD, Leid RW. 1989. In vivo chemotaxis of bovine neutrophils induced by 5-lipoxygenase metabolites of arachidonic and eicosapentaenoic acid. The American journal of pathology 134:671–676.

Heldal HE, Volynkin A, Komperød M, Hannisdal R, Skjerdal H, Rudjord AL. 2019. Natural and anthropogenic radionuclides in Norwegian farmed Atlantic salmon (Salmo salar). Journal of Environmental Radioactivity 205–206:42–47 DOI 10.1016/j.jenvrad.2019.05.002.

Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FJ. 2009. Human health consequences of use of antimicrobial agents in aquaculture. Clinical Infectious Diseases 49(8):1248–1253 DOI 10.1086/605667.

Hong H, Fan H, Wang H, Lu H, Luo Y, Shen H. 2015. Seasonal variations of fatty acid profile in different tissues of farmed bighead carp (Aristichthys nobilis). Journal of Food Science and Technology 52:903–911 DOI 10.1007/S13197-013-1129-1.
Huang CB, George B, Ebersole JL. 2010. Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. *Archives of Oral Biology* **55**:555–560 DOI 10.1016/j.archoralbio.2010.05.009.

Inguglia L, Chiaramonte M, Arizza V, Turiák L, Vékey K, Drahos L, Pitonzo R, Avellone G, Di Stefano V. 2020. Changes in the proteome of sea urchin Paracentrotus lividus coelomocytes in response to LPS injection into the body cavity. *PLOS ONE* **15**(2):e0228893 DOI 10.1371/journal.pone.0228893.

Jiang L, Wang W, He Q, Wu Y, Lu Z, Sun J, Liu Z, Shao Y, Wang A. 2017. Oleic acid induces apoptosis and autophagy in the treatment of Tongue Squamous cell carcinomas. *Scientific Reports* **7**:11277 DOI 10.1038/s41598-017-11842-5.

Leshno M, Goldbourt U, Pinchuk I, Lichtenberg D. 2018. The cardiovascular benefits of indiscriminate supplementation of omega-3 fatty acids; meta-analysis and decision-making approach. *International Journal of Food Sciences and Nutrition* **69**:549–556 DOI 10.1080/09637486.2017.1402868.

Li G, Sinclair AJ, Li D. 2011. Comparison of lipid content and fatty acid composition in the edible meat of wild and cultured freshwater and marine fish and shrimps from China. *Journal of Agricultural and Food Chemistry* **59**:1871–1881 DOI 10.1021/jf104154q.

Linder M, Fanni J, Parmentier M. 2005. Proteolytic extraction of salmon oil and PUFA concentration by lipases. *Marine Biotechnology* **7**:70–76 DOI 10.1007/s10126-004-0149-2.

Lopez S, Bermudez B, Pacheco YM, Ortega A, Varela LM, Abia R, Muriana FJG. 2010. Oleic acid: the main component of olive oil on postprandial metabolic processes. *Olives and Olive Oil in Health and Disease Prevention* 1385–1393 DOI 10.1016/B978-0-12-374420-3.00154-6.

Lopez-Romero JC, González-Ríos H, Borges A, Simões M. 2015. Antibacterial effects and mode of action of selected essential oils components against escherichia coli and staphylococcus aureus. *Evidence-Based Complementary and Alternative Medicine* **2015**:Article 795435 DOI 10.1155/2015/795435.

Medicine I of. 2005. *Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids*. Washington, D.C.: The National Academies Press DOI 10.17226/10490.

Meza K, Inami M, Dalum AS, Lund H, Bjelland AM, Sørum H, Løvoll M. 2019. Comparative evaluation of experimental challenge by intraperitoneal injection and co-habitation of Atlantic salmon (Salmo salar L) after vaccination against Piscirickettsia salmonis (EM90-like). *Journal of Fish Diseases* **42**:1713–1730 DOI 10.1111/jfd.13091.

Miranda CD, Godoy FA, Lee MR. 2018. Current status of the use of antibiotics and the antimicrobial resistance in the chilean salmon farms. *Frontiers in Microbiology* **9**:Article 1284 DOI 10.3389/fmicb.2018.01284.

Morais S, Monroig O, Zheng X, Leaver MJ, Tocher DR. 2009. Highly unsaturated fatty acid synthesis in atlantic salmon: characterization of ELOVL5- and ELOVL2-like elongases. *Marine Biotechnology* **11**:627–639 DOI 10.1007/s10126-009-9179-0.
Moreno JJ. 2009. Differential effects of arachidonic and eicosapentaenoic acid-derived eicosanoids on polymorphonuclear transmigration across endothelial cell cultures. *Journal of Pharmacology and Experimental Therapeutics* **331**:1111–1117 DOI 10.1124/jpet.109.157891.

Núñez Acuña G, Gallardo-Escárate C, Fields DM, Shema S, Skiftesvik AB, Ormazábal I, Browman HI. 2018. The Atlantic salmon (Salmo salar) antimicrobial peptide cathelicidin-2 is a molecular host-associated cue for the salmon louse (Lepeophtheirus salmonis). *Scientific Reports* **8**:13738 DOI 10.1038/s41598-018-31885-6.

Opheim M, Śliżyte R, Sterten H, Provan F, Larssen E, Kjos NP. 2015. Hydrolysis of Atlantic salmon (Salmo salar) rest raw materials - Effect of raw material and processing on composition, nutritional value, and potential bioactive peptides in the hydrolysates. *Process Biochemistry* **50**:1247–1257 DOI 10.1016/j.procbio.2015.04.017.

Overton K, Dempster T, Oppedal F, Kristiansen TS, Gismervik K, Stien LH. 2019. Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. *Reviews in Aquaculture* **11**:1398–1417 DOI 10.1111/raq.12299.

Parisi MG, Mauro M, Sarà G, Cammarata M. 2017. Temperature increases, hypoxia, and changes in food availability affect immunological biomarkers in the marine mussel *Mytilus galloprovincialis*. *Journal of Comparative Physiology B* **187**:1117–1126 DOI 10.1007/s00360-017-1089-2.

Parolini C. 2019. Effects of Fish n-3 PUFAs on intestinal microbiota and immune system. *Marine Drugs* **17**(6):374 DOI 10.3390/md17060374.

Poppe TT, Barnes A, Midtlyng PJ. 2002. Welfare and ethics in fish farming. *Bulletin of the European Association of Fish Pathologists* **22**(2):148–151.

Quiñones RA, Fuentes M, Montes RM, Soto D, León-Muñoz J. 2019. Environmental issues in Chilean salmon farming: a review. *Reviews in Aquaculture* **11**:375–402 DOI 10.1111/raq.12337.

REGULATION (EC). 2009. REGULATION (EC) No 470/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL laying down Community procedures for the establishment of residue limits of pharmacologically active substances in foodstuffs of animal origin, repealing Council Regulation (EEC) No. 2009..

Rehman S, Gora AH, Ahmad I, Rasool SI. 2017. Stress in aquaculture hatcheries: source, impact and mitigation. *International Journal of Current Microbiology and Applied Sciences* **6**:3030–3045 DOI 10.20546/ijcmas.2017.610.357.

Richards RC, O’Neil DB, Thibault P, Ewart KV. 2001. Histone H1: an antimicrobial protein of atlantic salmon (Salmo salar). *Biochemical and Biophysical Research Communications* **284**:549–555 DOI 10.1006/BBRC.2001.5020.

Romero J, Gloria C, Navarrete P. 2012. Antibiotics in aquaculture –use, abuse and alternatives. In: *Health and environment in aquaculture*. London: InTech DOI 10.5772/28157.

Ruyter B, Sissener NH, Ostbye TK, Simon CJ, Krasnov A, Bou M, Sanden M, Nichols PD, Lutfi E, Berge GM. 2019. N-3 Canola oil effectively replaces fish oil as a new safe dietary source of DHA in feed for juvenile Atlantic salmon. *British Journal of Nutrition* **122**:1329–1345 DOI 10.1017/S0007114519002356.
Sanders TAB (ed.) 2016. The role of fats in human diet. In: Functional dietary lipids. Amsterdam: Elsevier, 1–20 DOI 10.1016/B978-1-78242-247-1.00001-6.

Schillaci D, Arizza V, Parrinello N, Di Stefano V, Fanara S, Muccilli V, Cunsolo V, Haagensen JJA, Molin S. 2010. Antimicrobial and antistaphylococcal biofilm activity from the sea urchin Paracentrotus lividus. Journal of Applied Microbiology 108:17–24 DOI 10.1111/j.1365-2672.2009.04394.

Schillaci D, Cusimano M, Cunsolo V, Saletti R, Russo D, Vazzana M, Vitale M, Arizza V. 2013. Immune mediators of sea-cucumber Holothuria tubulosa (Echinodermata) as source of novel antimicrobial and anti-staphylococcal biofilm agents. AMB Express 3:Article 35 DOI 10.1186/2191-0855-3-35.

Schillaci D, Cusimano MG, Spinello A, Barone G, Russo D, Vitale M, Parrinello D, Arizza V. 2014. Paracentrin 1, a synthetic antimicrobial peptide from the sea-urchin Paracentrotus lividus, interferes with staphylococcal and Pseudomonas aeruginosa biofilm formation. AMB Express 4:Article 78 DOI 10.1186/s13568-014-0078-z.

Schroeder M, Brooks BD, Brooks AE. 2017. The complex relationship between virulence and antibiotic resistance. Gene 8(1):39 DOI 10.13390/genes8010039.

Serhan CN. 2014. Pro-resolving lipid mediators are leads for resolution physiology. Nature 510:92–101 DOI 10.1038/nature13479.

Serra R, Grande R, Butrico L, Rossi A, Settimio UF, Caroleo B, Amato B, Gallelli L, De Franciscis S. 2015. Chronic wound infections: the role of Pseudomonas aeruginosa and Staphylococcus aureus. Expert Review of Anti-Infective Therapy 13:605–613 DOI 10.1586/14787210.2015.1023291.

Simonetto M, Infante M, Sacco RL, Rundek T, Della-Morte D. 2019. A novel anti-inflammatory role of omega-3 PUFAs in prevention and treatment of atherosclerosis and vascular cognitive impairment and dementia. Nutrients 11:Article 2279 DOI 10.3390/nu11102279.

Smith P. 2008. Antimicrobial resistance in aquaculture. Revue Scientifique et Technique (International Office of Epizootics) 27:243–264 DOI 10.20506/rst.27.1.1799.

Spinello A, Cusimano MG, Schillaci D, Inguglia L, Barone G, Arizza V. 2018. Antimicrobial and antibiofilm activity of a recombinant fragment of β-Thymosin of Sea Urchin Paracentrotus lividus. Marine Drugs 16(10):366 DOI 10.3390/md16100366.

Sudheesh PS, Al-Ghabshi A, Al-Mazrooei N, Al-Habsi S. 2012. Comparative pathogenomics of bacteria causing infectious diseases in fish. International Journal of Evolutionary Biology 2012:Article 457264 DOI 10.1155/2012/457264.

Tacconelli E, Carrara E, Savoldi A, Kattula D, Burkert F. 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Health Organization. Available at https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1.

Takigawa H, Nakagawa H, Kuzukawa M, Mori H, Imokawa G. 2005. Deficient production of hexadecenoic acid in the skin is associated in part with the vulnerability of atopic dermatitis patients to colonization by Staphylococcus aureus. Dermatology 211(3):240–248 DOI 10.1159/000087018.
Tallima H, El Ridi R. 2018. Arachidonic acid: physiological roles and potential health benefits - a review. *Journal of Advanced Research* 11:33–41 DOI 10.1016/j.jare.2017.11.004.

Torrissen O, Olsen RE, Toresen R, Hemre GI, Tacon AGJ, Asche F, Hardy RW, Lall S. 2011. Atlantic Salmon (Salmo salar): The Super-Chicken of the Sea? *Reviews in Fisheries Science* 19:257–278 DOI 10.1080/10641262.2011.597890.

Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R. 2015. Global trends in antimicrobial use in food animals. *Proceedings of the National Academy of Sciences of the United States of America* 112:5649–5654 DOI 10.1073/pnas.1503141112.

Vazzana M, Celi M, Chiaramonte M, Inguglia L, Russo D, Ferrantelli V, Battaglia D, Arizza V. 2018. Cytotoxic activity of Holothuria tubulosa (Echinodermata) coelomocytes. *Fish and Shellfish Immunology* 72:334–341 DOI 10.1016/j.fsi.2017.11.021.

Vazzana M, Mauro M, Ceraulo M, Dioguardi M, Papale E, Mazzola S, Arizza V, Beltrame F, Inguglia L, Buscaino G. 2020. Underwater high frequency noise: Biological responses in sea urchin Arbacia lixula (Linnaeus, 1758). *Comparative Biochemistry and Physiology -Part A: Molecular and Integrative Physiology* 242:Article 110650 DOI 10.1016/j.cbpa.2020.110650.

Wilson A, Magill S, Black KD. 2009. Review of environmental impact assessment and monitoring in salmon aquaculture. n FAO. Environmental impact assessment and monitoring in aquaculture. FAO Fisheries and Aquaculture Technical Paper. No. 527. Rome, FAO.. 455–535.

Xue X, Woldemariam NT, Caballero-Solares A, Umashuthan N, Fast MD, Taylor RG, Rise ML, Andreassen R. 2019. Dietary immunostimulant CpG modulates MicroRNA biomarkers associated with immune responses in atlantic salmon (Salmo salar). *Cell* 8:Article 1592 DOI 10.3390/cells8121592.

Yagi S, Fukuda D, Aihara K, Akaike M, Shimabukuro M, Sata M. 2017. n-3 polyunsaturated fatty acids: promising nutrients for preventing cardiovascular disease. *Journal of Atherosclerosis and Thrombosis* 24:999–1010 DOI 10.5551/jat.RV17013.

Zheng CJ, Yoo JS, Lee TG, Cho HY, Kim YH, Kim WG. 2005. Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids. *FEBS Letters* 579:5157–5162 DOI 10.1016/j.febslet.2005.08.028.