Mercury accumulation in Mediterranean Fish and Cephalopods Species of Sicilian coasts: correlation between pollution and the presence of Anisakis parasites

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ABSTRACT
The aim of this study was to investigate mercury accumulation in some species, caught in Mediterranean Sea, in the period between May and December 2015, and to compare it to the presence of Anisakis parasites. The samples were examined by direct mercury analyzer (DMA-80) for their Hg levels. The metal concentration was compared to the presence or the absence of Anisakis parasites. Significant differences in Hg concentration in analysed samples were observed. The low-infested fishes contained 1–6 larvae of parasites whereas the high-infested one had 7–83 larvae.

1. Introduction
The increasing environmental pollution is caused by the growing urbanisation and industrialisation (AtaAkcil et al. 2015; Bua et al. 2016; Vadala et al. 2016).

Pollution typically refers to chemicals or other substances in concentrations greater than would occur under natural conditions. Heavy metals, because of their toxicity persistence and bioaccumulation, have deteriorated the aquatic ecosystems (Hosono et al. 2011).
Several indexes were applied to assess the contamination degree and the adverse ecological effects in seafood (Nollet 2012). The World Health Organization (WHO) considers mercury (Hg) among the top 10 chemicals of ‘major public health concern’ (World Health Organization 2013). Mercury and health Fact sheet No. 361. People may be exposed to inorganic Hg through their occupation or to organic Hg, such as methylmercury (CH₃Hg⁺), predominantly through the consumption of seafood (World Health Organization 1990; Committee on Toxicological Effects of Methylmercury, National Research Council of the United States, National Academies of Science 2000; Storelli et al. 2005; Lo Turco et al. 2012; Salvo et al. 2014; Sheehan et al. 2014; Bastam et al. 2015; Di Bella et al. 2015; Salvo et al. 2015). Methylmercury is formed by aquatic sedimentary micro-organisms from elemental and mercuric Hg and it is rapidly taken up by living organisms and enters in the food chain through plankton filter-feeding bottom invertebrates (Compeau and Bartha 1985; Gilmour et al. 1992). Metallic Hg undergoes a number of chemical changes when it is released into the environment. In aqueous solution, a balance between the Hg, Hg^{2+} and Hg^{2+} states is reached. Hg^{2+} ions are able to form many stable complexes with biological compounds. Mercurous Hg is rather unstable and in the presence of biological material, it tends to dissociate forming a metallic Hg atom and a Hg^{2+} ion. Generally in an aerobic aquatic system, methylation is the most important transformation (Harris et al. 2003). Biomagnification is the passage of a xenobiotic chemical from food to an organism, resulting in a higher concentration within the organism than source (Gray 2002).

The accumulation of Hg in fish is related to age, size and habitat use, and to the possible parasitic infestation of fish. Fish intestinal nematodes accumulate heavy metals at high concentration (Sures et al. 1999). Wild freshwater and marine fishes are subjected to infection by different species of parasites (Cipriani et al. 2015). This aspect, in addition to their capacity to accumulate heavy metals, suggests that parasites may be useful indicators of the biologically available metals in aquatic ecosystems (Evans et al. 2001; Dural et al. 2011). Acute methylmercury toxicity in fish tissue (6.0 mg/kg wet weight) may lead to neurological disease, including reduced swimming activity, loss of equilibrium and possibly death (Smith & Weis, 1995; Giarratana et al. 2014). National agencies have used limits for Hg concentrations in fish, the US Food and Drug Administration (FDA) has set an action level of 1 μg g⁻¹ wet wt for the concentration of total Hg in fish; in Japan, maximum permitted limit suitable for human consumption for the Japanese is 0.4 μg g⁻¹ wet wt; in Europe, the limit value for total Hg is set at 0.5 μg g⁻¹ wet wt, except for some species, in which it is raised to 1 μg g⁻¹. However, total Hg concentrations above the regulatory limits have been observed in species living on or close to the sea bed (Storelli & Marcotrigiano 2001; Storelli et al. 2002).

The content of heavy metals in fish neutralizes their beneficial effects as demonstrated by several studies (Rahman et al. 2012; Bastam et al. 2015). Consequently, the knowledge of their concentrations is an important tool towards both environmental management and human consumption.

In Sicily, Syracuse petrochemical industry is the cause of a high Hg concentration in fishes of the east Sicilian coasts (Ferrantelli et al. 2014; Shreadah et al. 2015). In order to safeguard public health, the Hg concentration of Mediterranean fish and cephalopod species, collected in the western Sicilian coasts, was evaluated. The aim of this study was to investigate mercury accumulation in some species, caught in Mediterranean Sea and to compare it with the presence of Anisakis parasites.
2. Results and discussion

2.1. Hg levels in fish species

In the Table 1, the size and the general characteristics of fish species are reported.

Differences in Hg concentrations were found among analysed samples. In Figure 1, the averages of Hg content, present in the analysed samples, are reported.

A non-parametric analysis of variance by the Kruskal Wallis test was performed. Hg levels were correlated with fish species. The results of the tests gave: $R^2 = 37.139$, $df = 7$ and $p$-value <0.05. The box plots reported show significant differences among the different species (Figure 2).

The differences in Hg concentration of the various fish and cephalopods species could be a result of human impact and traffic increase.

Significant differences in Hg levels ($p$ value <0.05) were found among the different species (Figure 1). After the analysis of variance performed by the Kruskal Wallis test, significant differences among the different groups of samples were observed. The difference in Hg levels between *Sardina pilchardus* and *Conger conger* were probably due to the difference in size, weight and lifestyle; the difference observed between *Merluccius merluccius* and

| Fish species       | No. of organism | Size wet wt | General characteristics |
|--------------------|-----------------|-------------|-------------------------|
|                    | (sample)        | Length (cm) | Weight (g)              | Demersal | Pelagic | Benthic |
| Conger conger      | 6               | 35–50       | 450–863                 | X        |
| Loligo vulgaris    | 7               | 23–30       | 302–483                 |          |
| Merluccius merluccius | 15            | 13–17       | 400–560                 | X        |
| Mullus barbatus    | 17              | 10–18       | 263–358                 | X        |
| Trachurus trachurus| 9               | 18–25       | 352–626                 | X        |
| Todarodes sagittatus| 8              | 24–38       | 152–328                 | X        |
| Sardina pilchardus | 12              | 12–14       | 56–102                  | X        |
| Scomber scombrus   | 6               | 18–30       | 136–395                 | X        |

Figure 1. The mean concentrations of mercury in examined species. Figure 2 dual cells detector – air calculation
Trachurus trachurus, that have a similar size, may be due to the different lifestyle; between S. pilchardus and T. trachurus, both pelagic fishes, the variation may be due to the different size and weight; the diversity observed between Mullus barbatus and T. trachurus that are benthonic and pelagic fishes, respectively, may be due to different weight and lifestyle; between Scomber scombrus and T. trachurus, although these two samples had the same length, weight and lifestyle, and both are pelagic fish, the different concentration may be due to the different degree of infestation.

In our work, we detect differences in Hg concentrations between the benthic, pelagic and demersal species. Variations in the concentration of Hg in fish tissues are connected with the diversity in feeding habits and behavior of the different species.

2.2. Influence of Anisakis infestation in Hg level in fishes

In this study, 41 out of 80 fish samples were found to be infected by parasites of Anisakidae family. The total number of parasites (range), the prevalence (%), the mean intensity and mean abundance values of samples were 444 (0–83), 51.3%, 10.83 ± 6, and 5.55 ± 3.5, respectively. Moreover, the percentage of Anisakis larvae infestation in each examined species was evaluated. The Figure 3 shows the prevalence of infestation in the different species: C. conger 100%, Loligo vulgaris 0%, M. merluccius 66.7%, M. barbatus 41%, S. pilchardus 25%, S. scombrus 50%, Todarodes sagittatus 50% and T. trachurus 100%.

The samples analysed were divided into three groups: non-infested, low infested and high infested. The low infested contained 1–6 larvae of parasites, the high infested contained 7–83 larvae. The three groups of samples were subjected to non-parametric analysis of variance, the Kruskal Wallis methods were used. This test gave the following values: $R^2 = 2305$; df = 2; p-value <0.05.

Figure S1(A) shows a significant differences between the non-infested and high-infested groups, and also between the group of samples with low infestation and those with high infestation. No significant differences were found between low-infested and non-infested samples. Also among benthic, pelagic and demersal species, there were no significant differences in Hg concentrations.
The differences, found among the three different pack grouped according to different levels of infestation, were significant ($p$ value $<0.05$) (Figure S1(A) and (B)). The analysis of variance, conducted by Kruskal Wallis test, showed significant differences among the groups. The difference in significance between the non-infested and high-infested samples and between the group of samples with low and those with high infestation may be due to the gradual increase in Hg concentration. Every obtained data were evaluated according to the European regulation limits (Reg. UE 1881/2006).

3. Experimental

See Supplementary materials for: sampling area and storage, chemical analyses, Anisakis family assessment, instrumental conditions and calibration curves.

4. Conclusions

The results provide information about the accumulation of Hg in fish in comparison with the presence of Anisakis. Differences in bioaccumulation may be due to length, weight, lifestyle and grade of infestation (Fazio et al. 2014). The presence of the parasite in fish is an important factor for Hg accumulation (Pascual and Abollo 2003). Even if parasites do not accumulate organic pollutants, they are able to alter the uptake of chemicals of their hosts, including metals (Evans et al. 2001). The study and knowledge of the presence of parasites in fish are fundamental to know both the health and ecological problems of fishes. (Sures 2004). The developmental stage of the parasites and the amount of time that they stay inside a particular host are other factors that influence metal accumulation. The application of certain parasites such as bioindicator organisms could provide a new tool for the assessment of aquatic system pollution (Ferrantelli et al. 2015; Naccari et al. 2015).

Supplementary material

Supplementary material relating to this article is available online, including Figure S1 and Table S1.

Figure 3. Prevalence of Anisakis infestation in the different species.
Disclosure statement
No potential conflict of interest was reported by the authors.

References
AtaAkcil CE, Erust C, Ozdemiroglu S, Fonti V, Beolchini F. 2015. A review of approaches and techniques used in aquatic contaminated sediments: metal removal and stabilization by chemical and biotechnological processes. J Clean Prod. 86:24–36.
Bastam KD, Afkhami M, Mohammadizadeh M, Ehsanpour M, Chambari S, Aghaei S, Esmaeilzadeh M, Neyestani MR, Lagzaeef B, Baniamam M. 2015. Bioaccumulation and ecological risk assessment of heavy metals in the sediments and mullet Liza klunzingeri in the northern part of the Persian Gulf. Mar Pollut Bull. 94:329–334.
Bua GD, Annuario G, Albergamo A, Cicero N, Dugo G. 2016. Heavy metals in aromatic spices by inductively coupled plasma-mass spectrometry. Food Addit Contam Part B. doi:10.1080/19393210.2016.1175516.
Cipriani P, Smaldone G, Accera V, D'Angelo L, Anastasio A, Bellisario B, Palma G, Nascetti G, Mattiucci S. 2015. Genetic identification and distribution of the parasitic larvae of Anisakis pegreffii and Anisakis simplex (s. s.) in European hake Merluccius merluccius from the Tyrrhenian Sea and Spanish Atlantic coast: implications for food safety. Int J Food Microbiol. 198:1–8.
Committee on Toxicological Effects of Methylmercury, National Research Council of the United States, National Academies of Science. 2000. Toxicological effects of methylmercury. Washington, DC: National Academies Press.
Compeau GC, Bartha R. 1985. Sulfate-reducing bacteria: principal methylators of Hg in anoxic estuarine sediment. Appl Environ Microbiol. 50:498–502.
Di Bella G, Potortì AG, lo Turco V, Bua GD, Licata P, Cicero N, Dugo G. 2015. Trace elements in Thunnus Thynnus from Mediterranean sea: benefit-risk assessment for consumer. Food Addit Contam Part B. 8:175–181.
Dural M, Genc E, Sangun MK, Güner Ö. 2011. Accumulation of some heavy metals in Hysterothylacium aduncum (Nematoda) and its host sea bream, Sparus aurata (Sparidae) from North-Eastern Mediterranean Sea (Iskenderun Bay). Environ Monit Assess. 174:147–155.
Evans DW, Irwin SWB, Fitzpatrick S. 2001. The effect of digenean (Platyhelminthes) infections on heavy metal concentrations in Littorina littorea. J Mar Biol Assoc uK. 81:349–350.
Fazio F, Piccione G, Tribulato K, Ferrantelli V, Giangrosso G, Arfuso F, Faggio C. 2014. Bioaccumulation of heavy metals in blood and tissue of striped mullet in two Italian lakes. J Aquat Anim Health. 26:278–284.
Ferrantelli V, Cicero A, Costa A, Alongi A, Palumbo P, Graci S, Giangrosso G. 2014. Anisakidae in fishing products sold in Sicily. Ital J Food Saf. 3:47–48.
Ferrantelli V, Costa A, Graci S, Buscemi MD, Giangrosso G, Porcarello C, Palumbo S, Cammilleri G. 2015. Anisakis nematodes as possible markers to trace fish products. Ital J Food Saf. 4:49–53.
Giarratana F, Muscolino D, Beninati C, Giaffrida A, Panebianco A. 2014. Activity of Thymus vulgaris essential oil against Anisakis larvae. Exp Parasitol. 142:7–10.
Gilmour CC, Henry EA, Mitchell R. 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ Sci Technol. 26:2281–2287.
Gray JS. 2002. Biomagnification in marine systems: the perspective of an ecologist. Mar Pollut Bull. 45:46–52.
Harris HH, Pickering IU, Graham NG. 2003. The chemical form of mercury in Fish. Science. 301:1203–1203.
Hosono T, Su CC, Delinom R, Umezawa Y, Toyota T, Kaneko S, Taniguchi M. 2011. Decline in heavy metal contamination in marine sediments in Jakarta Bay, Indonesia due to increasing environmental regulations. Estuar Coast Shelf Sci. 92:297–306.
Lo Turco V, Di Bella G, Furci P, Cicero N, Pollinico G, Dugo G. 2012. Heavy metals content by ICP-OES in Sarda sarda, Sardinella aurita and Lepidopus caudatus from Straits of Messina (Sicily, Italy). Nat Prod Res. 27:518–523.
Naccari C, Cicero N, Ferrantelli V, Giangrosso G, Vella A, Macaluso A, Naccari F, Dugo G. 2015. Toxic metals in pelagic, benthic and demersal fish species from Mediterranean FAO zone 37. Bull Environ Contam Toxicol. 95:567–573.

Nollet LM. 2012. Handbook of meat, poultry and seafood quality. 2nd ed. Wiley, New York, NY.

Pascual S, Abollo E. 2003. Accumulation of heavy metals in the whaleworm Anisakis simplex s.l (Nematoda: Anisakidae). J Mar Biol Assoc UK. 83:905–906.

Rahman MS, Molla AH, Saha N, Rahman A. 2012. Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka. Bangladesh Food Chem. 134:1847–1854.

Salvo A, Potorti AG, Cicero N, Bruno M, Lo Turco V, Di Bella G, Dugo G. 2014. Statistical characterization of heavy metal contents in Paracentrotus lividus from Mediterranean Sea. Nat Prod Res. 28:718–726.

Salvo A, Cicero N, Vadalà R, Mottese AF, Bua GD, Mallamace D, Giannetto C, Dugo G. 2015. Toxic and essential metals determination in commercial seafood: Paracentrotus lividus by ICP-MS. Nat Prod Res. 28:1–8.

Sheehan MC, Burke TA, Navas-Acien A, Breysse PN, Mc Gready J, Fox MA. 2014. Global methylmercury exposure from seafood consumption and risk of developmental neurotoxicity: a systematic review. Bull WHO. 92:254–269F.

Shreadah MA, Abdel Fattah LM, Fahmy MA. 2015. Heavy metals in some fish species and bivalves from the Mediterranean coast of Egypt. J Environ Prot. 06:1–9.

Smith GM, Weis JS. 1995. Predator-prey relationships in mummichogs (Fundulus heteroclitus (L.): effects of living in a polluted environment. J Exp Mar Biol Ecol. 209:75–87.

Storelli MM, Marcotrigiano GO. 2001. Total mercury levels in muscle tissue of swordfish (Xiphias gladius) and bluefin tuna (Thunnus thynnus) from the Mediterranean sea. J Food Prot. 64:1058–1061.

Storelli MM, Giacominielli Stuffer R, Marcotrigiano GO. 2002. Total and methylmercury residues in tuna-fish from Mediterranean sea. Food Addit Contam. 19:715–720.

Storelli MM, Storelli A, Giacominielli-Stuffer R, Marcotrigiano GO. 2005. Mercury speciation in the muscle of two commercially important fish, hake (Merluccius merluccius) and striped mullet (Mullus barbatus) from the Mediterranean sea: estimated weekly intake. Food Chem. 89:295–300.

Sures B. 2004. Environmental parasitology: relevancy of parasites in monitoring environmental pollution. Trends Parasitol. 20:170–177.

Sures B, Knopf K, Würtz J, Hirt J. 1999. Richness and diversity of parasite communities in European eels Anguilla anguilla of the River Rhine, Germany, with special reference to helminth parasites. Parasitology. 119:323–330.

Vadalà R, Mottese AF, Bua GD, Salvo A, Mallamace D, Corsaro C, Vasi S, Alfa M, Cicero N, Dugo G. 2016. Statistical analysis of mineral concentration for the geographic identification of garlic samples from Sicily (Italy), Tunisia and Spain foods. Foods. doi:10.3390/foods5010020.

World Health Organization. 1990. Environmental health criteria document 101: methylmercury. Geneva: International Program for Chemical Safety.

World Health Organization. 2013. Mercury and health (fact sheet No. 361). Geneva. Available from: http://www.who.int/mediacentre/factsheets/fs361/en/ [accessed 2015 Oct 11].