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## **Mercury in fish: concentration versus fish-size and estimates intake dose.**

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**Mercury in fish: concentration versus fish-size and estimates intake dose.**

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6 **Mercury in fish: concentration versus fish-size and estimates of**  
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8 **mercury intake**  
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## Abstract

Total mercury concentrations were determined in different fish size classes of commercial importance such as, conger eel (*Conger conger*), starry ray (*Raja asterias*), forkbeard (*Phycis blennoides*), frostfish (*Lepidopus caudatus*), striped mullet (*Mullus barbatus*), red gurnard (*Aspitrigla cuculus*) and yellow gurnard (*Trigla lucerna*) in order to evaluate variations in consumer exposure to mercury as a function of fish consumption of a spectrum of different sizes. The highest mean levels of total mercury were detected in conger eel ( $0.80 \mu\text{g g}^{-1}$ ) and in starry ray ( $0.75 \mu\text{g g}^{-1}$ ). Forkbeard ( $0.67 \mu\text{g g}^{-1}$ ), frostfish ( $0.59 \mu\text{g g}^{-1}$ ) and striped mullet ( $0.55 \mu\text{g g}^{-1}$ ) showed slightly lower levels, while red gurnard ( $0.33 \mu\text{g g}^{-1}$ ) and yellow gurnard ( $0.22 \mu\text{g g}^{-1}$ ) exhibited the lowest concentrations. The results of the linear regression analysis showed significant relationship between mercury concentrations and fish size for all species. Consequently, dietary consumption of larger size specimens leads to an increase in the exposure level for consumers. Understanding by consumers of all factors leading to an increase of exposure to mercury is the first step to enable them to make decisions about eating fish.

**Keywords:** *total mercury, fish, risk assessment, PTWI*

## Introduction

Fish provide a healthy source of dietary protein, and are relatively low in cholesterol and high in omega-3 (n-3) fatty acids (National Research Council 2000). Several studies have shown that fish consumption reduces the risk of coronary heart disease, decreases mild hypertension, prevents certain cardiac arrhythmias, lowers the incidence of diabetes and appears to alleviate the symptoms of rheumatoid arthritis (Deckere *et al.* 1998, Billman *et al.* 1999, Rosenberg 2002). However, people who eat fish must balance the relative benefits from a low-fat source of protein against potential exposure to contaminants, above all mercury. Seafood consumption is, in fact, the main source of this toxin which accumulates in the human body and causes damage in many of its basic systems, particularly to the nervous system (Dey *et al.* 1999).

Literature data indicate that predator fish occupying high trophic positions and therefore have generally higher amount of mercury because this metal is particularly liable to biomagnify along marine food chains (Burger *et al.* 1992, Storelli *et al.* 1998, Brabo *et al.* 2000). Added to this other studies show differences in mercury concentrations between pelagic and benthic species (Romeo *et al.* 1999, Bustamante *et al.* 2003, Henry *et al.* 2004). Animals living in close association with sediments in which they bury and from where they mainly feed, are more exposed to eventually sediment-associated contamination than other fish. These remarks can have implications for human health.

Earlier studies have, in fact, shown a high human dietary exposure associated with the consumption of predatory fish and bottom-dwelling fish (Storelli *et al.* 2002, Storelli *et*

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6 *al.* 2005). Of critical concern is also the fish-size because generally mercury levels in  
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8 fish increase with body size. This issue has been discussed with supporting data in the  
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10 environmental health sciences literature (Dixon and Jones 1994, Storelli *et al.* 1998,  
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12 Joiris *et al.* 1999, Storelli and Marcotrigiano 2000, Storelli and Marcotrigiano 2001) is a  
13  
14 crucial point concerning consumer exposure. The positive relationship between fish-size  
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16 and mercury levels suggests that consumers that eat larger fish might have higher  
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18 exposure to mercury than those that eat smaller fish. In relation to this to address the  
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20 people who eat smaller size fish might arise an effective risk reduction. In this contest  
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22 we are particularly interested in examining variations in consumer exposure to mercury  
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24 as a function of consumption of different size spectra of fish. Such information will be  
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26 of value to those involved in risk communication. An understanding by consumers of all  
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28 factors leading to an increase of exposure to mercury is the first step to enable them to  
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30 make decisions about eating fish.  
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### 39 **Materials and methods**

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42 In June-August 2005 during several trawl surveys, 100 conger eel (*Conger conger*,  
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44 length range: 32.0-85.0 cm; median: 59.3 cm), 253 red gurnard (*Aspitrigla cuculus*,  
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46 length range: 10.2-24.7 cm; median: 18.5 cm), 263 yellow gurnard (*Trigla lucerna*,  
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48 length range: 20.4-55.5 cm; median: 35.0 cm), 464 striped mullet (*Mullus barbatus*,  
49  
50 length range: 15.8-31.0 cm; median: 21.0 cm), 224 forkbeard (*Phycis blennoides*, length  
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52 range: 12.4-49.2 cm; median: 26.5 cm), 879 frostfish (*Lepidopus caudatus*, length  
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54 range: 110.0-144.0 cm; median: 128.0 cm) and 100 starry ray (*Raja asterias*, length  
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56 range: 23.0-58.8 cm; median: 48.5 cm) specimens were caught in the Adriatic Sea. For  
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6 each species, from the total number of specimens were formed pools within which  
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8 individual fish were collected as a function of their similar size length to investigate the  
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10 influence of size on mercury bioaccumulation.  
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15 From the organisms of each pool muscle tissue was removed and preserved at -25 °C  
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17 until analysis. The tissues were dissected with plastic materials that were washed with  
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19 HNO<sub>3</sub> and rinsed with distilled and deionized water, in order to avoid metal  
20  
21 contamination. For analyses of total Hg, homogenized samples of the tissue (2 g wet  
22  
23 weight) were digested to a transparent solution with 10 mL of the mixture H<sub>2</sub>SO<sub>4</sub>-HNO<sub>3</sub>  
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25 (1:1) under reflux. The resultant solutions were then diluted to a known volume with  
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27 deionized water (Official Journal of the European Communities 1990) and the total Hg  
28  
29 concentrations were measured by atomic absorption spectrophotometry (Analyst 800  
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31 Perkin Elmer) by the cold vapour technique, after reduction by SnCl<sub>2</sub> (FIMS-100,  
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33 Perkin Elmer). Acid washed glassware, analytical grade reagents and double distilled  
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35 deionized water were used in the tissue analysis.  
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44 In order to check on the purity of the chemical used, a number of chemicals blanks were  
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46 run; there was no evidence of any contamination in these blanks. Analytical quality  
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48 control was achieved using TORT-1 Lobster Hepatopancreas (National Research  
49  
50 Council of Canada). Replicate analyses (n=3) (Hg total 0.32±0.02 µg g<sup>-1</sup> dry weight)  
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52 were in the range of the certified material (Hg total 0.33±0.06 µg g<sup>-1</sup> dry weight). All  
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54 data were computed on a µg g<sup>-1</sup> wet weight basis. Mann-Whitney *U* test was used to test  
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56 significance of differences between data sets. The level of significance was set at P <  
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## 10 **Results and discussion**

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12 Among the different fish species examined the highest mean levels of total mercury  
13 were detected in conger eel ( $0.80 \mu\text{g g}^{-1}$ ) and in starry ray ( $0.75 \mu\text{g g}^{-1}$ ). Forkbeard ( $0.67$   
14  $\mu\text{g g}^{-1}$ ), frostfish ( $0.59 \mu\text{g g}^{-1}$ ) and striped mullet ( $0.55 \mu\text{g g}^{-1}$ ) showed levels slightly  
15 lower, while red gurnard ( $0.33 \mu\text{g g}^{-1}$ ) and yellow gurnard ( $0.22 \mu\text{g g}^{-1}$ ) exhibited the  
16 lowest concentrations (Table 1). Despite of mercury level variability, statistical  
17 comparisons did not reveal significant differences among various fish species, except  
18 among Triglidae and the other species ( $P < 0.04$ ).  
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32 It is known that differences in feeding habits generally assign the species to a trophic  
33 level and species belonging to higher trophic levels are considered to contain higher  
34 mercury concentrations (Wiener and Spry 1996; Watras *et al.* 1998, Snodgrass *et al.*  
35 2000). In our case the findings of statistical analysis led to suppose that all species  
36 analysed belonged to similar trophic levels. Published estimates of Mediterranean fish  
37 trophic levels confirmed this hypothesis being the species in question included in the  
38 same functional trophic group with the highest values corresponding to conger eel  
39 (Stergiou and Karpouzi 2002). In this picture Triglidae were an exception in that their  
40 trophic levels was the lowest. This latter information corroborated further the thesis that  
41 mercury levels in fish reflect its trophic level. Significant differences in mercury  
42 concentrations were, in fact, detected solely between Triglidae with the lowest trophic  
43 levels and the other species assigned to the highest.  
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8 Of primary importance in explaining mercury body burden of fish is also the habitat  
9 where they live. It is well known that benthic fish are species that tend to concentrate  
10 mercury to a higher degree than other organisms (Campbell 1994, Storelli *et al.* 2005),  
11 confirming the significant process of sedimentation and persistence of this metal in sea  
12 depths. For this reason the tested species were all benthic feeding fish but however,  
13 exhibiting different dietary preferences. It is clear from the above discussion that food  
14 habits as well as feeding location of fish, are factors of primary importance influencing  
15 mercury body load. However, independently from the complexity of interactions  
16 leading to different accumulation profiles among fish, a common point to all species in  
17 question was that metal concentration seemed to increase with size/age of fish  
18 suggesting that larger, older fish had higher mercury levels than smaller, younger fish.  
19 The linear regression analyses confirmed this trend being mercury concentrations  
20 positively and significantly correlated with fish length (striped mullet:  $R = 0.94$ ,  $P <$   
21  $0.001$ ; red gurnard:  $R = 0.92$ ,  $P < 0.001$ ; yellow gurnard:  $R = 0.96$ ,  $P < 0.001$ ; starry ray:  
22  $R = 0.88$ ,  $P < 0.001$ ; conger eel:  $R = 0.89$ ,  $P < 0.001$ ; forkbeard:  $R = 0.93$ ,  $P < 0.001$ ;  
23 frostfish:  $R = 0.82$ ,  $P < 0.001$ ).

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48 Generally, the evaluation of the toxicological risk of the metal for humans is carried out  
49 through comparison of measured concentrations of a certain element in food with the  
50 levels imposed by law or following guidelines proposed by different international  
51 organizations. In the case of mercury, European legislation (Official Journal of the  
52 European Communities 2001) sets the maximum limit in edible parts of fish-products at  
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6 0.5  $\mu\text{g g}^{-1}$  wet wt, except for some species for which it is raised to 1.0  $\mu\text{g g}^{-1}$  wet wt.  
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8 Generally, these latter species are either high trophic level predators, that with their  
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10 considerable mercury load demonstrate the importance of biomagnification process  
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12 through food chain (Monteiro and Lopes 1990, Storelli and Marcotrigiano 2001), or  
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14 benthic organisms that spending a considerable time searching for food on the bottom of  
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16 the sediment are particularly prone to accumulate higher amounts of mercury (Romeo *et*  
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18 *al.* 1999, Kljakovic *et al.* 2002, Storelli *et al.* 2005). For fish analyzed in the present  
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20 study total mercury concentrations should not exceed 0.5  $\mu\text{g g}^{-1}$  wet wt, except for  
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22 conger eel, starry ray and frostfish for which the established value is 1.0  $\mu\text{g g}^{-1}$ . It is, in  
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24 fact, not by chance that these latter species had a more consistent mercury load in their  
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26 flesh respect to others. However, comparison of mercury mean concentrations detected  
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28 in the present study with the levels imposed by law revealed that almost all fish in  
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30 question (see conger eel, red and yellow gurnard, frostfish and starry ray) were suitable  
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32 for human consumption with the metal concentrations under the prescribed legal limits.  
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42 Only two species, striped mullet and forkbeard, had mercury mean concentrations  
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44 (striped mullet: 0.55  $\mu\text{g g}^{-1}$ ; forkbeard: 0.67  $\mu\text{g g}^{-1}$ ) slightly exceeding the standard of  
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46 0.5  $\mu\text{g g}^{-1}$  wet wt established by European legislation. With these results, it is likely to  
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48 conclude that the consumption of these fish by humans should be safe. However, this  
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50 assertion was not corroborated by the concentrations relative to each fish-size classes.  
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52 Within each species, the larger size specimens showed, in fact, concentrations  
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54 exceeding, to a more or less great extent, the maximum regulatory limits.  
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6 What was observed might be particularly relevant with respect to potential risk on  
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8 consumers' health. To test this hypothesis the metal intakes via dietary consumption of  
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10 these fish were calculated and compared with the Provisional Tolerable Weekly Intake  
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12 (PTWI) of  $5 \mu\text{g kg}^{-1}$  body weight, set by the Joint Expert Committee on Food Additives  
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14 (WHO 2003). The average weekly dietary intakes ( $0.52\text{-}1.90 \mu\text{g kg}^{-1}$  bw) calculated by  
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16 taking into account mean mercury concentrations in each species and a mean weekly  
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18 consumption of demersal fish of 142 g (FAO 2002), were below the fixed safe level  
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21 (Table 1).  
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27 However, the purpose here was not to evaluate the exposure to mercury due to sea  
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29 product consumption but rather to examine how varied the exposure according to  
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31 consumption of different sized fish. In this respect, as shown in table 1, weekly mercury  
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33 intakes were comprised in a wide range from  $0.02$  to  $3.34 \mu\text{g kg}^{-1}$  body weight. As the  
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35 figure indicated, mercury intake increased with consumption of larger size specimens  
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37 for all species. However, the consumption of certain species, even of large size, such as  
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39 yellow gurnard and red gurnard gave levels relatively small in comparison to PTWI  
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41 value ( $25.0\text{-}27.4\%$  of PTWI), whereas high exposure values, constituting from  $49.2\%$  to  
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43  $66.8\%$  of PTWI, were associated with consumption of largest specimens of the  
44  
45 remained species. In particular, the highest mercury intakes resulted from the  
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47 consumption of specimens of weight above 400 g of forkbeard ( $55.4\text{-}66.8\%$  of PTWI),  
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49 conger eel ( $51.0\text{-}62.4\%$  of PTWI) and starry ray ( $50.2\text{-}56.8\%$  of PTWI). These data  
50  
51 clearly demonstrate that people that eat larger fish have higher exposure to mercury than  
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53 those that eat smaller fish. This could be of concern for consumers, particularly if they  
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6 repeatedly ate the largest individuals of some species. It would be, in fact, sufficient a  
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8 weekly consumption of 250 g of largest specimens of these species to have a mercury  
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10 intake close or that surpass the reference limit.  
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15 Potential public health risks from dietary exposure to mercury continue to be the subject  
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17 of much research, regulation and debate. State and federal agencies can respond to  
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19 potential health risks from mercury in fish by issuing consumption advice. For example,  
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21 recently the US Food and Drug Administration (FDA 2002) has issued a series of  
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23 consumption advice notes based on mercury that suggested that pregnant women should  
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25 limit their fish consumption and avoid eating large predatory fish. However, the  
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27 question of risk from eating fish is complicated by the positive health benefits of  
28  
29 consuming fish. Because of this it becomes extremely important to reduce human  
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31 exposure to mercury from fish consumption. The data here obtained clearly show that  
32  
33 mercury levels vary in fish as a function of their trophic level and size. Consequently  
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35 this means that the consumers could substantially reduce their exposure to mercury by  
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37 eating selected species and fish of smaller size. However, such information is not  
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39 helpful if the general public is unaware of this possibility. Understanding by consumers  
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41 about the relationship between contaminant, fish-size, trophic level and mercury intake  
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43 is crucial to enable them to be able to make better decisions about eating fish. On this  
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45 basis educational programs to foster such an understanding and, thus, changes in the  
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47 fish species and size consumed would constitute an useful tool to reduce human  
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49 exposure to this neurotoxin.  
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Table 1. Total mercury concentrations ( $\mu\text{g g}^{-1}$  wet weight) and estimated weekly intake (EWI) of total mercury ( $\mu\text{g kg}^{-1}$  body weight) in dependence on consumption of differed sized fish.

| Pools    | Striped mullet |      | Red gurnard |      | Yellow gurnard |      | Starry ray |      | Conger eel |      | Forkbeard |      | Frostfish |      |
|----------|----------------|------|-------------|------|----------------|------|------------|------|------------|------|-----------|------|-----------|------|
|          | [Hg]           | EWI  | [Hg]        | EWI  | [Hg]           | EWI  | [Hg]       | EWI  | [Hg]       | EWI  | [Hg]      | EWI  | [Hg]      | EWI  |
| 1        | 0.16           | 0.38 | 0.07        | 0.17 | 0.01           | 0.02 | 0.15       | 0.35 | 0.26       | 0.61 | 0.30      | 0.71 | 0.16      | 0.38 |
| 2        | 0.18           | 0.43 | 0.12        | 0.28 | 0.09           | 0.21 | 0.65       | 1.54 | 0.29       | 0.69 | 0.30      | 0.71 | 0.47      | 1.11 |
| 3        | 0.39           | 0.92 | 0.40        | 0.95 | 0.08           | 0.19 | 0.30       | 0.71 | 0.55       | 1.30 | 0.42      | 0.99 | 0.39      | 0.92 |
| 4        | 0.58           | 1.37 | 0.33        | 0.78 | 0.10           | 0.24 | 0.81       | 1.92 | 0.42       | 0.99 | 0.49      | 1.16 | 0.32      | 0.76 |
| 5        | 0.63           | 1.49 | 0.42        | 0.99 | 0.08           | 0.19 | 0.60       | 1.42 | 0.43       | 1.02 | 0.60      | 1.42 | 0.48      | 1.14 |
| 6        | 1.04           | 2.46 | 0.39        | 0.92 | 0.25           | 0.59 | 0.78       | 2.55 | 1.08       | 2.55 | 0.48      | 1.14 | 0.27      | 0.64 |
| 7        | 0.88           | 2.08 | 0.58        | 1.37 | 0.15           | 0.35 | 1.08       | 2.51 | 1.32       | 3.12 | 0.64      | 1.51 | 0.68      | 1.61 |
| 8        |                |      |             |      | 0.37           | 0.88 | 1.20       | 2.84 | 1.30       | 3.07 | 0.73      | 1.73 | 1.15      | 2.72 |
| 9        |                |      |             |      | 0.35           | 0.83 | 1.19       | 2.81 | 1.08       | 2.55 | 1.17      | 2.77 | 0.80      | 1.89 |
| 10       |                |      |             |      | 0.41           | 0.97 |            |      | 1.29       | 3.05 | 0.87      | 2.06 | 0.99      | 2.34 |
| 11       |                |      |             |      | 0.53           | 1.25 |            |      |            |      | 1.41      | 3.34 | 0.82      | 1.94 |
| Min      | 0.16           | 0.38 | 0.07        | 0.17 | 0.01           | 0.02 | 0.15       | 0.35 | 0.26       | 0.61 | 0.30      | 0.71 | 0.16      | 0.38 |
| Max      | 1.04           | 2.46 | 0.58        | 1.37 | 0.53           | 1.25 | 1.20       | 2.84 | 1.32       | 3.12 | 1.41      | 3.34 | 1.15      | 2.72 |
| Mean     | 0.55           | 1.30 | 0.33        | 0.78 | 0.22           | 0.52 | 0.75       | 1.85 | 0.80       | 1.90 | 0.67      | 1.59 | 0.59      | 1.40 |
| Median   | 0.58           | 1.37 | 0.39        | 0.92 | 0.15           | 0.35 | 0.78       | 1.92 | 0.82       | 1.93 | 0.60      | 1.42 | 0.48      | 1.14 |
| St. Dev. | 0.33           | 0.79 | 0.18        | 0.42 | 0.17           | 0.40 | 0.37       | 0.91 | 0.45       | 1.06 | 0.35      | 0.84 | 0.32      | 0.75 |

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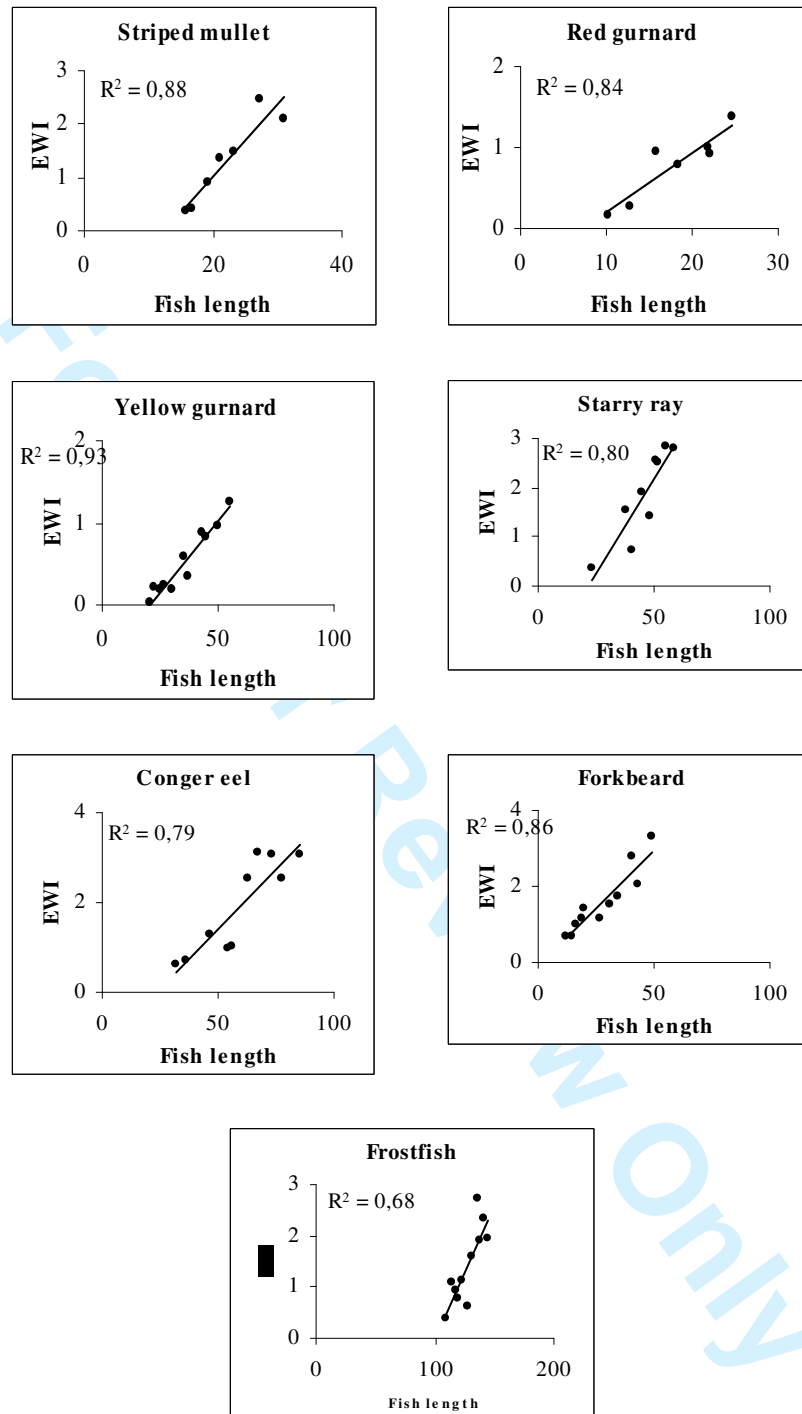


Figure 1. Estimated weekly mercury intake ( $\mu\text{g kg}^{-1}$  body wt) versus fish length (cm).