DOKUZ EYLÜL UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

AN ASSESSMENT OF MERCURY ACCUMULATION AND SPECIATION IN MARINE ORGANISMS FROM İZMİR BAY

by

Lütfi Tolga GÖNÜL

July, 2006

İZMİR

AN ASSESSMENT OF MERCURY ACCUMULATION AND SPECIATION IN MARINE ORGANISMS FROM İZMİR BAY

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by
Lütfi Tolga GÖNÜL

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M.Sc THESIS EXAMINATION RESULT FORM

We have read the thesis entitled **"AN ASSESSMENT OF MERCURY ACCUMULATION AND SPECIATION IN MARINE ORGANISMS FROM İZMİR BAY"** completed by **LÜTFİ TOLGA GÖNÜL** under supervision of **Prof. Dr. FİLİZ KÜÇÜKSEZGİN** and we certify that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science.

Prof. Dr. Filiz KÜÇÜKSEZGİN

Supervisor

Prof. Dr. Hatice PARLAK

(Jury Member)

Assoc. Prof. Dr. Ferah K. YILMAZ

(Jury Member)

Prof.Dr. Cahit HELVACI
Director
Graduate School of Natural and Applied Sciences

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Lütfi Tolga GÖNÜL
Chemist

AN ASSESSMENT OF MERCURY ACCUMULATION AND SPECIATION IN ORGANISMS FROM IZMIR BAY

ABSTRACT

Izmir Bay is impacted by organic matter, oil and heavy metals. In this study, total mercury (THg) and methylmercury (MeHg) concentrations and the MeHg to THg ratio (%MeHg) were investigated in 297 individuals of three different species from the Izmir Bay. Biota samples were collected by trawling at 4 sampling stations during *R/V K. Piri Reis* cruise in the Izmir Bay between February and September 2005.

The concentrations of total mercury (by cold vapour atomic absorption spectrophotometer) and methylmercury (by gas chromatography-electron capture detector) were detected in fish samples from the Izmir Bay. The levels of total mercury and methylmercury in fish tissue, ranging from 4.4 to 221.4 μg THg kg^{-1} wet wt. and 3.8-194.8 μg MeHg kg^{-1} wet wt., varied according to sampling point and season. Total and MeHg concentrations in fish differed between species and the mean levels of total mercury were 90.7 and 63.1 μg kg^{-1} wet wt. for annular sea bream (*Diplodus annularis*) and red mullet (*Mullus barbatus*), respectively. The percentages of methylated form to total mercury ranged from a minimum of 84 % in red mullet to a maximum of 98 % in annular sea bream. The positive relationship between specimen size and total mercury and methylmercury concentrations were found in red mullet. ANOVA were utilized to investigate effect of season, species and sampling area on variations in mercury concentrations. The significant differences between seasons for THg and MeHg were found in fish samples. The highest mercury concentrations were found in September, while the lowest were detected in February. None of the fish analysed was ≥ 0.500 μg g^{-1} THg, and exceed the WHO limit.

Keywords: Total mercury, Methylmercury, Fish, Red mullet, Annular sea bream, Izmir Bay

İZMİR KÖRFEZİNDEKİ ORGANİZMALARDA CİVA BİRİKİMİ VE TÜRLERİNİN DEĞERLENDİRİLMESİ

ÖZ

İzmir Körfezi organik madde, petrol ve ağır metaller tarafından etkilenmektedir. Bu çalışmada, İzmir Körfezi'ndeki 3 farklı balık türünde (*Mullus barbatus*, *Diplodus annularis*, *Solea vulgaris*) toplam civa, metilciva konsantrasyonları araştırılmış ve metil civanın total civaya oranları bulunmuştur. Biota örnekleri trolle Şubat-Eylül 2005 dönemleri arasında 4 örnekleme alanından *R/V K. Piri Reis* araştırma gemisinin seferleri sırasında toplanmıştır.

Örneklerde total civa konsantrasyonları soğuk buhar tekniğiyle atomik absorpsiyon spektrofotometresi, metil civa ise gaz kromatografisi cihazının "electron capture" detektörü kullanılarak saptanmıştır. Balıkta total ve metilciva konsantrasyonları 4.4-221.4 ve 3.7-194.8 (µg kg^{-1} yaş ağırlık) aralığında örnekleme noktası ve mevsime göre değişim göstermektedir. Total civa ve metilciva konsantrasyonları türler arasında farklılık göstermiştir ve ortalama total civa seviyeleri *Diplodus annularis* (Isparoz) 90.7, *Mullus barbatus* (Barbunya) da ise 63.1 µg kg^{-1} yaş ağırlık olarak saptanmıştır. Metil civanın total civaya oranı minimum seviyede *Mullus barbatus* (84%), maksimum olarak da *Diplodus annularis* (98%)'de bulunmuştur. *Mullus barbatus*'ta örnek boyu ve civa konsantrasyonları arasında pozitif bir ilişki olduğu ortaya çıkmıştır. Mevsim, tür ve örnekleme alanının civa konsantrasyonları üzerindeki etkilerini araştırmak için ANOVA kullanılmıştır. Total civa ve metilciva konsantrasyonlarının mevsimsel değişiminin anlamlı olduğu bulunmuştur. En yüksek civa konsantrasyonları eylül ayında saptanırken, en düşük konsantrasyonlar şubat ayında ölçülmüştür. Analiz edilen balıklardan hiçbirinin total civa miktarı WHO tarafından belirlenen 0.500 µg g^{-1} yaş ağırlık limitini aşmadığı görülmüştür.

Anahtar Kelimeler: Total Civa, Metilciva, Balık, Barbunya, Isparoz, İzmir Körfezi

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CHAPTER ONE
INTRODUCTION

1. Introduction

Contamination of marine organisms with toxic chemicals such as mercury is of ecological and health concern worldwide. The presence and behavior of mercury in aquatic systems is of great interest and importance since it is the only heavy metal which bioaccumulates and biomagnifies through all levels of the aquatic food chain.

Monomethylmercury (CH_3Hg^+) is the most toxic form and accounts for more than 95% of organic mercury in fish muscle tissues. The lipophilic nature of this organic compound facilitates its penetration across cell membranes. Moreover, methylmercury affinity for the sulphydryl groups of certain proteins and its long biological halflife, leads to its rapid accumulation in living organisms. The organic mercury, methylmercury (MeHg), in marine environment is from biogenic origin, mainly by anaerobic bacteria. This process is chiefly promoted by sulphate-reducing bacteria in the superficial layers of the bottom sediments. When the contaminated sediment is resuspended and transported, the MeHg formed may readily dissolve in the water column, and then could be taken up by the aquatic organisms and accumulated. MeHg readily enters the aquatic food chain and it may be biomagnified as it accumulates in higher trophic levels. It attains the highest concentration in higher consumer's fish tissues and marine mammals, at the top of the aquatic food chain. Carnivorous species and marine mammals can be considered good indicators of mercury bioaccumulation.

Izmir Bay is one of the great natural bay areas of the Mediterranean and compares well with similar coastal areas in the world. The natural beauty and the economic advantages it offers have been recognised early in human history and have been exploited ever since.

Few published data are present on total mercury concentrations in organisms from Izmir Bay (Demirkurt, Uysal & Parlak, 1990; Parlak and Demirkurt, 1990; Kucuksezgin, Uluturhan, Kontas & Altay, 2002, Kucuksezgin, Kontas, Altay, Uluturhan & Darilmaz, 2006), but no data are available on mercury accumulation and speciation in organisms from Izmir Bay. The aim of this study is to comparatively evaluate the total mercury (THg) and MeHg concentrations in three fish species, red mullet (*Mullus barbatus*), annular sea bream (*Diplodus annularis*) and common sole (*Solea vulgaris*), collected from different sites of Izmir Bay. The organisms were sampled during four seasons in 2005. They present some of the most abundant species in the bay, as well as those most frequently consumed by the local population.

CHAPTER TWO
CHARACTERISTICS OF IZMIR BAY

2.1 Study Area

Izmir Bay (western Turkey) is one of the great natural bays of the Mediterranean. The Bay divided into three zones: Inner Bay, Middle Bay, and Outer Bay according to the topographical, hydrological and ecological features of Izmir Bay (Figure 1). The main urban conurbation around the bay is the Izmir Metropolitan Municipality, covering 88000 hectares and population of close to 3 million inhabitants. Izmir is an important industrial, commercial and cultural city center. Industrial activities cover a large range of industries including food processing, tanneries, paint, chemicals, textile and petroleum refining. About 6.000 industrial establishments are registered with the Chamber of Industry in Izmir. However, many establishments are not registered. Various pollution sources and their contribution to the observed pollution levels are given in Table 2.1. As shown in table, the main sources of pollution in the bay are domestic and industrial effluents, which account for 50 % of the observed organic pollution.

Table 2.1 Pathways of pollutants in Izmir Bay (from UNEP, 1993)

Pollution arising from domestic and industrial wastes	50 %
Pollution due to flood water	15 %
Pollution due to transport of chemicals used in agriculture by surface and drainage waters	10 %
Pollution transported by rivers and streams	10 %
Pollution due to erosion	8 %
Pollution caused by ship traffic and bay activities	4 %
Others	3 %

The bay has a total surface area of over 500 km^2, water capacity of 11.5 billion m^3, a total length of 64 km and opens in the Aegean Sea. The hinterland is relatively fertile agricultural area watered by several rivers the largest of which is the Gediz which flows into the Outer bay. The climate of the Bay is relatively mild with a mean annual temperature of 17°C. A 13 m deep sill, the Yenikale Strait, separates the Middle Bay from the Inner Bay. The Gediz River, which flows to the Outer Bay, is the biggest river in the Izmir Bay. The Inner Bay is heavily polluted by nutrients and organic material (UNEP, 1993).

The Inner Bay extends from the head of the Bay to the Yenikale lighthouse. The water volume of the Inner Bay is 6x108 m^3, average water depth is about 7 m (depth changes between 0 m and 20 m). The Inner Bay is heavily polluted by organic material and nutrients; however metal concentrations were not high enough to indicate heavy metal pollution. The main source of pollution is streams that flow to the bay and organic materials were measured in the Inner Bay coastal stations that are located in the area of discharge points. The north part of the Inner Bay had been filled by alluvion of Gediz River just released in the west of Karsıyaka until the second half of nineteen century. Due to that, the Izmir Harbour had encountered a shoaling problem and the entrance of Gediz River had been moved toward the Outer Bay. The depth increase steeply from the inshore to the offshore that the ships must follow a shallow channel with a depth of 10 m between the old Gediz Delta and Narlıdere (UNEP, 1993).

The Middle Bay extends from Yenikale lighthouse to the Kokola point. The water volume of the Middle Bay 9x108 m^3, average water depth is about 16 m. Surface area of the Middle Bay is relatively 57 km^2. The Middle Bay is a passage zone with pollutant concentrations intermediate between Outer and Inner Bays, which are a clear indication of spreading pollution in the bay. Because of the shallow nature of the Inner and Middle Bays, water exchange and self-purification capacities are very limited.

The Outer Bay is extends from Kokola point to the mouth of the bay. The water volume of the Outer Bay 1x1010 m^3, average water depth is about 49 m. Surface area of the Outer Bay is relatively 417 km^2. Pollution in the Outer Bay is not significant; this part of the bay is relatively clean according to the most of the pollution indicators.

Gediz River is the second biggest river in the Aegean Region and main fresh water source of the Izmir Bay. It flows to the Outer Bay. Gediz River area is approximately 18000 km^2 and annual average flow is estimated to be 2.33x109 m^3.

2.2 The Economics of the Bay

The Aegean region around Izmir produces 15% of Turkey's agricultural output with 30% of the land in the region used for agriculture. The harbor's storage capacity is 200.000 tons with 25% of Turkey's export and 55% of its import passing through the port. Tourism is also an important activity with between 100.000 and 300.000 tourist annually either visiting or passing through Izmir. The saltpan of over 800 hectares produces over 1 million tons of salt annually. In addition defense activities are also present in the Bay.

Urbanization, industrial activities and agriculture impact heavily on the environment in particular on the water quality of the Bay and shore areas. Domestic and industrial wastes, urban and agricultural runoff, discharges from ships, sediments and contaminated waters of rivers and streams have cumulatively had a significant adverse effect on the water quality and natural values of the Inner Bay. The Bay is heavily polluted with organic material, hydrocarbons, heavy metals, nutrients and pathogenic organisms; eutrophication of the Inner Bay is a serious problem throughout the year and red tide events are becoming more frequent (UNEP, 1993).

CHAPTER THREE
MERCURY

3.1 Definitions and Properties of Mercury

Mercury, a silver-white metal that is liquid at room temperature and is highly volatile, can exist in three oxidation states: elemental mercury (Hg^0), mercurous ion (Hg_2^{2+}), and mercuric ion (Hg^{2+}). It can be part of both inorganic and organic compounds (USEPA 1980; Clarkson, Hamada & Amin-Zaki, 1984; Table 3.1).

Table 3.1 Some Properties of Mercury and Its Compounds

Property	Elemental Mercury	Mercurous Chloride	Mercuric Chloride	Methyl mercury Chloride
Empirical formula	Hg	Hg_2Cl_2	$HgCl_2$	CH_3HgCl
Molecular weight	200.59	472.09	271.52	251.09
Chlorine, %	0	15.02	26.12	14.12
Mercury, %	100	84.98	73.88	79.89
Melting Point, °C	-38.87	400-500	277	170
Density, g/ml	13.534	7.15	5.4	4.063
Solubility, mg/l				
In water	0.056	2.0	74.070	1016
In benzene	2.387	insol.	5000	6535

All mercury compounds interfere with thiol metabolism, causing inhibition or inactivation of proteins containing thiol ligands and ultimately leading to mitotic disturbances (Das, Sharma & Talukder, 1982; Elhassani, 1983). The mercuric species is the most toxic inorganic chemical form, but all three forms of inorganic mercury may have a common molecular mechanism of damage in which Hg^{2+} is the toxic species (Clarkson & Marsh, 1982; Figure 3.1.). Chemical speciation is probably the most important variable influencing ecotoxicology of mercury, but mercury speciation is difficult, especially in natural environments (Boudou & Ribeyre, 1983).

Mercury compounds in an aqueous solution are chemically complex. Depending on pH, alkalinity, redox potential and other variables, a wide variety of chemical species can be formed, each having different electrical charges and solubilities. For example, $HgCl_2$ in solution can speciate into $Hg(OH)_2$, Hg^{2+}, $HgCl^{+}$, $Hg(OH)^{-}$, $HgCl_3^{-}$, and $HgCl_4^{2-}$; anionic forms predominate in saline environments (Boudou & Ribeyre, 1983). Some properties and compounds of mercury are given in Table 3.1.

3.2 Sources and Inputs to the Sea

Natural inputs of mercury to the sea are from the weathering of mercury-bearing rocks and degassing of the earth's crust, particularly through volcanic activity. Forest fires and the biological formation of elemental mercury are additional natural sources. Estimates of natural inputs, especially which from degassing vary widely. Formerly, they were thought to dwarf inputs resulting from human activities, but currently they are estimated to be of the same order, at amounting to 50-75 per cent of a total input of 6000-7500 t.

Following the discovery, in the early 1960s of the dangers to human health of mercury in the marine environment, there has been a steady reduction of man-made inputs, partly through the imposition of strict controls on discharges of wastes containing mercury and partly through elimination of the use of mercury and mercurial compounds.

Cinnabar mine tailings dumped in the sea is among the mercury inputs to the sea. Cinnabar has low solubility, but under oxic conditions it is converted to divalent and elemental mercury, most of which is then converted to the more toxic methyl mercury. Fish that are used as food by the local population are contaminated for several kilometers from the site.

Mercury used on a large scale to extract gold and silver in the Amazon basin results in a 100 t $year^{-1}$ loss of mercury to the environment: 55 per cent to the

atmosphere, 45 per cent to the river. Inhabitants of fishing villages downstream have up to 149.2 ppb in their blood, 99 per cent of it methyl mercury (Clark, 1997).

Although it has been possible to curtail direct inputs of mercury to the sea from industrial activities, it has proved more difficult to reduce discharges to the atmosphere. These are important because they are subsequently deposited by fallout over a wide area. Estimates of inputs to the atmosphere from the combustion of fossil fuel, municipal waste, sewage sludge, and smelting vary between 1000 and 6000 t $year^{-1}$. Systems to reduce emissions of particulates and sulphur dioxide in flue gases are not particularly effective at removing mercury (Clark, 1997).

3.3 Production and Uses

3.3.1 Production

In 1989, the primary world production was well over 5,700 tonnes. In 1996, the total world production was estimated to be 3,260 tones in 8 countries although overestimation (by more than 1,000 tonnes) for the former USSR was probably done (World Metal Statistics, 1997). Reduction of the 1997 production in Europe to about 400 tonnes per year has certainly lowered the world production to less than 2,000 tonnes. It has to be noted that mercury recovery from various applications can represent a non negligible source of mercury use.

3.3.2 Uses

Mercury is applied in various industrial processes and products: dental clinics, measuring and control equipment (e.g. thermometers, blood pressure measuring equipment etc), batteries, lamps, and the chlor alkali industry (Table 3.2). In the chloralkali industry mercury is used as cathode in the electrolysis process to produce chlorine gas and caustic soda from a sodium chloride solution in closed cells.

Table 3.2 Usage of mercury in Europe (1985-1992) (OECD Monograph N^0 98, 1994 (a) in tonnes/year) and estimates for 1996

Application	Mercury content	Quantity	Estimated quantities for 1996 (c)
Dental clinics	50% per amalgam filling	68.9	68.9
Laboratories		31.5 (b)	31.5
Measuring and control equipment		31.7	(31.7) or lower
Batteries		99.4	5-8
-cylindrical	0.025 – 0.15		
-button cell-mercury oxide	30%		
-alkaline	0.5 – 1%		
-silver oxide	1%		
-zinc-air	1%		
Lamps & switches	5-15 mg/fluorescent lamp	12.2	> 12.2
-fluorescent lamps			
-energy saving lamps			
Pesticides as seed dressing		13.1	0
Chlor-alkali industry		152.4	120
Other		<44.4 (b)	(73) (d)
TOTAL		<453.6	345

(a)10 countries considered (DK, S, N, NL, FIN, GB, F, D CH, B)
(b)Mainly in Germany
(c)Estimation based on informal data from ALMADEN (E) and Euro Chlor Mercury balance for 1996
(d)Extrapolated value from total and known uses

3.4 Mercury Cycling

3.4.1 Mercury in the Atmosphere

At a global scale, the atmosphere is the environmental compartment with the largest influence on mercury transportation and fluxes. This has been demonstrated by recent modeling studies (Petersen, Iverfeldt & Munthe, 1995; Iverfeldt, Munthe & Hulberg, 1996). The residence time of elemental mercury in the atmosphere is estimated to be between 1.1 and 1.4 year, which allows not only long-range transportation but also a relatively homogeneous concentration in the atmosphere around the world. This was measured to be of about 2 ng/m^3 (Slemr, 1996).

As there is no reliable data available on the vertical distribution of mercury in the atmosphere, it is assumed a homogeneous distribution in a realistic atmospheric layer of about 10 km height. In this case the total amount of mercury in the atmosphere is calculated to be about 10000 t (Slemr, 1996). The elemental mercury could be oxidised and then hydrolysed into the atmosphere. In those forms the mercury can be easily re-deposited either through wet or dry processes. The re-deposition is of course more important in the regions where the concentrations in oxidants and in particulate materials are higher, i.e. mainly in the atmosphere over continental areas. The re-deposition fluxes to land are 2 to 4 times higher than those to oceanic areas.

Inputs of mercury into the atmosphere

The main sources of emissions of mercury to the atmosphere were characterised by the Expert Panel of Atmospheric Mercury Processes (1994) and defined as follows:

•Anthropogenic mercury emissions refer to the mobilisation and release of geologically bound mercury by man activities (e.g. coal combustion) with mass transfer of mercury to the atmosphere.

•Natural mercury emissions refer to the mobilisation and releases of geologically bound mercury by natural biotic and abiotic processes, with mass transfer of mercury to the atmosphere (e.g. volcanoes).

•Re-emission of mercury is the mass transfer of mercury to the atmosphere by biotic and abiotic processes from a pool of mercury that was deposited to earth's surface after initial mobilisation by either anthropogenic or natural activities.

The two last pathways are considered as mercury emission from natural surfaces and they represent a large uncontrolled area of emissions sources. The total amount of mercury in the atmosphere is thus built from a mix of anthropogenic, natural and re-emission sources. In the frame of the realistic scenario proposed here, the inputs into the atmosphere could be estimated as follows**:**

Real natural sources of atmospheric mercury coming from the terrestrial compartment

•Erosion and degassing from mineralised soils is estimated to be of about 700 t/y on the basis of flux measurements, 500 t/y coming from the mercuriferous belts (Lindqvist et al., 1991).

•Volcanic eruptions and geothermal activities may significantly contribute to the natural emissions of mercury. The best global estimate of mercury released by volcanoes is about 830 t/y, 95% of it coming from active erupting volcanoes (Varekamp & Buseck, 1986).

•Evasion of mercury from the earth's subsurface crust occurs through faults and fractures in bedrock. This phenomenon display extreme spatial and temporal variation, but a conservative estimate of 1000 t/y is considered here. This does not take into account mercury evasion from earth's crust directly to the deep ocean (Wilken, Walsh, & Falter, 1999).

Anthropogenic emissions: A total emission to the atmosphere from anthropogenic sources for the Western world is about 870 t/y (Ebinghaus, Turner, Lacerda, Vasiliev & Salomons, 1999). This estimate represents approximately 15% of the total global anthropogenic emissions. This leads to a total anthropogenic emission into the atmosphere of about 5600 t/y. However 30% of this amount is quickly redeposited at a local or regional scale, leaving about 4000 t/y globally distributed in the atmosphere.

Exchange processes, which can act as sources or sinks of atmospheric mercury. These exchanges affect the total amount of mercury and are occurring both at the air-water and the air-soil interfaces. Before going into detail of these exchanges, the aquatic and terrestrial compartments situations will be shortly reviewed.

3.4.2 Mercury in the Aquatic Environment

In the aquatic environment, under naturally occurring conditions of pH and temperature, mercury may also become methylated by biological or chemical processes, or both (Beijer & Jernelov, 1979; USEPA 1980; Ramamoorthy & Blumhagen, 1984; Zillioux, Porcella & Benoit, 1993; Figure 3.1.), although a biological methylation is limited (Callister & Winfrey, 1986). Methylmercury is the most hazardous mercury species due to its high stability, its lipid solubility, and its possession of ionic properties that lead to a high ability to penetrate membranes in living organisms (Beijer & Jernelov, 1979; Hamasaki, Nagase, Yoshioka, & Sato, 1995). In general, essentially all mercury in freshwater fish tissues is in the form of methylmercury; however, methylmercury accounts for less than 1% of the total mercury pool in a lake (Regnell, 1990).

All mercury discharged into rivers, bays or estuaries as elemental (metallic) mercury, inorganic divalent mercury, phenylmercury or alkoxyalkyl mercury can be converted into methylmercury compounds by natural processes. The mercury methylation in ecosystems depends on mercury loadings, microbial activity, nutrient content, pH and redox condition, suspended sediment load, sedimentation rates, and other variables (NAS 1978; Compeau & Bartha, 1984; Berman & Bartha, 1986; Callister & Winfrey, 1986; Jackson, 1986).

The finding that certain microorganisms are able to convert inorganic and organic forms of mercury into the highly toxic methylmercury or dimethylmercury has made it clear that any form of mercury is highly hazardous to the environment (USEPA, 1980; 1985). The synthesis of methylmercury by bacteria from inorganic mercury compounds present in the water or in the sediments is the major source of this molecule in aquatic environments (Boudou & Ribeyre, 1983). This process can occur under both aerobic and anaerobic conditions (Beijer & Jernelov, 1979; Clarkson, Hamada, Amin-Zaki, 1984). Transformation of inorganic mercury to an organic form by bacteria alters its biochemical reactivity and hence its fate (Figure 3.1).

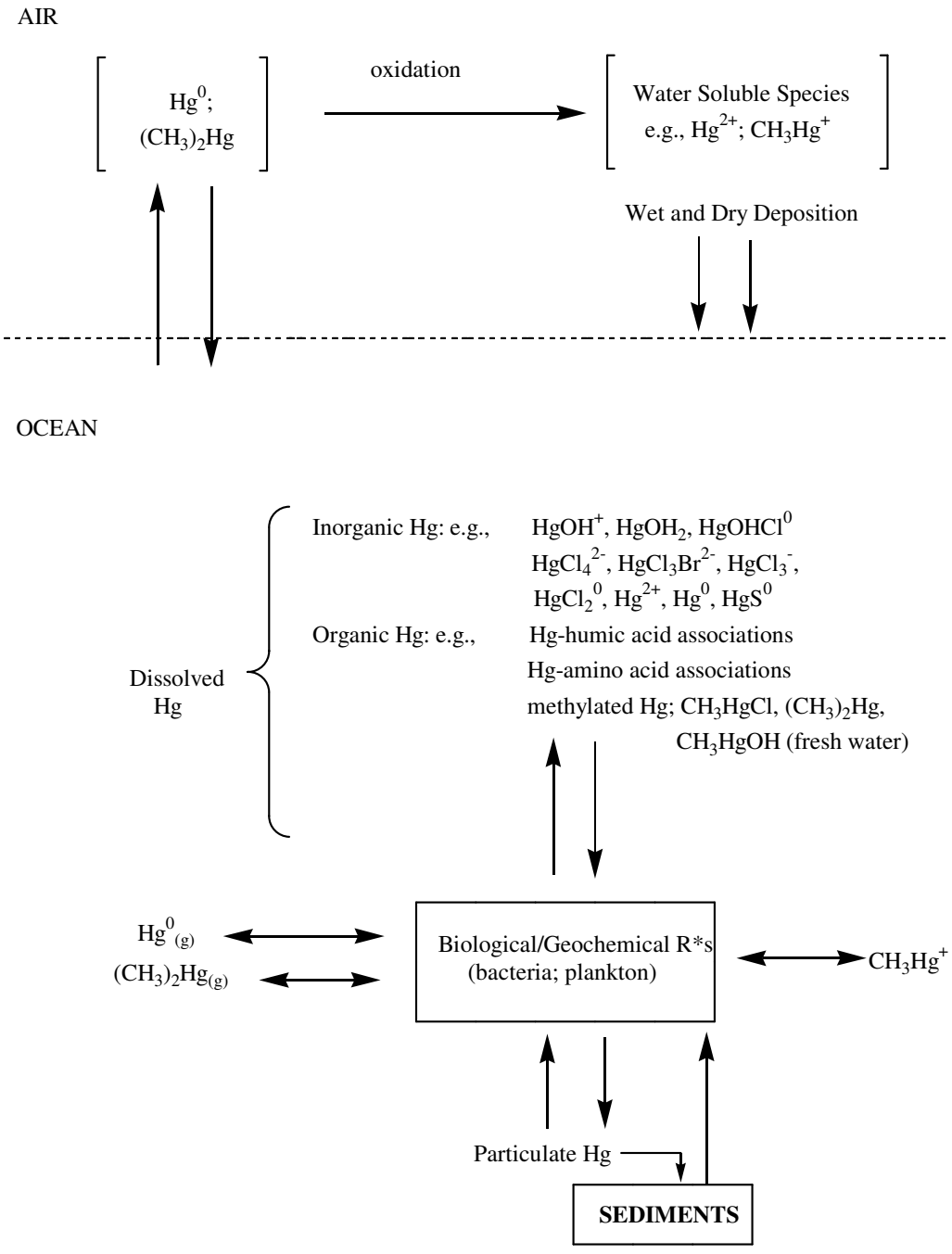

Figure 3.1 Physicochemical view of the biogeochemical cycling of Hg with emphasis on the water-air interface (Fitzgerald & Clarkson, 1991)

Methylmercury is decomposed by bacteria in two phases. First, hydrolytic enzymes cleave the C–Hg bond, releasing the methyl group. Second, a reductase enzyme converts the ionic mercury to the elemental form, which is then free to diffuse from the aquatic environment into the vapor phase. These demethylating microbes appear to be widespread in the environment; they have been isolated from water, sediments and soils and from the gastrointestinal tract of mammals, including humans (Clarkson, Hamada, & Amin-Zaki, 1984). Some strains of microorganisms contain mercuric reductase, which transforms inorganic mercury to elemental mercury, and organomercurial lyase, which degrades organomercurials to elemental mercury (Baldi, Semplici & Filippelli, 1991).

Humic substances can reduce inorganic divalent mercury (Hg^{2+}) to elemental mercury (Hg^0). In aquatic environments, Hg^0 was highest under anoxic conditions, in the absence of chloride, and at pH 4.5. Under these conditions, about 25% of 400 µg Hg^{2+}/L was reduced to Hg^0 in 50 h. Production of Hg^0 was reduced in the presence of europium ions and by methylated carboxyl groups in the humic substances (Allard & Arsenie, 1991). Mercury is efficiently transferred through wetlands and forests in a more reactive form relative to other land-use patterns, resulting in an increased uptake by organisms inhabiting these rivers or downstream impoundments and drainage lakes (Hurley et al., 1995). The behavior and accumulation of mercury in forest soils of Guyana, South America, is related to the penetration of humic substances and the progressive adsorption onto iron oxy-hydroxides in the mineral horizons; flooding of these soils may lead to a release of 20% of the mercury initially present (Roulet & Lucotte, 1995).

3.5 Mercury in Fish

Most species of fish in oceanic waters contain 150 µg kg^{-1} (0.15 ppm) mercury in muscles, although much higher levels can be found in fish from contaminated waters. Large hake (*Merluccius spp.*) from the Tyrrhenian Sea off the coast of Tuscany, where there is a natural input from mercury-bearing ores, may contain up to 3.2 ppm.

Some species, notably tunny (*Thunnus spp*.) swordfish (*Xiphias gladius*), and marlin (*Makaira indica*), naturally contain high concentrations of mercury. Concentrations of 1 ppm in the muscle are common, and may be as high as 4.9 ppm. These fish are large carnivores at the end of food chains and their diets, therefore, contain high levels of mercury resulting from bioaccumulation and biomagnification. Food is not their only source of mercury; they are very active fish with a high metabolic rate and they swim continuously with their mouths open, so producing a forced flow of water across the gills. These results in a large uptake of oxygen, but also of metals (including mercury) dissolved in the water. Much of this mercury is in the form of methylmercury and since the fish cannot excrete it is concentration increases with the age of the fish. Large, old specimens have high body burdens of mercury and warnings are given about the danger of eating the flesh of very large specimens of these species (Clark, 1997).

3.6 Human Health and Mercury Poisoning

3.6.1 Human Health

The toxicity of mercury to humans has been known for centuries. Mad hatters in Victorian got their name from the convulsions and loss of neuromuscular coordination symptomatic of chronic poisoning from the mercury used in the treatment of felt in hat manufacture. Inorganic mercury can be readily excreted, and, while it is dangerous to those exposed to it occupationally, it is not a hazard for the general public. Organic mercury, such as methylmercury, on the other hand, cannot be excreted easily and so may accumulate to toxic concentrations as a result of intermittent exposure over several years. It is a cumulative poison and since it can cross the barrier from blood vessels in the brain into the nervous tissue (the blood-brain barrier), it causes progressive and irreversible brain damage.

As far as is known, human exposure to methylmercury occurs only through the consumption of contaminated fish and sea food. This was brought to light by an

outbreak of methylmercury poisoning in the small Japanase coastal town of Minimata (Minimata disease). Part of the population was dependent on fishing for a livelihood. The only industry was a factory that began producing vinyl chloride and acetaldehyde in 1952, both processes involving the use of mercury catalysts, large quantities of which were lost in washing the product and were discharged into the bay. The illness first appeared in 1953 and affected only fishermen and their families, but it was not diagnosed as metal poisoning derived from fish and seafood taken from Minimata Bay until 1956. In all, 2000 cases were recognized; of these, 43 died during the epidemic and over 700 of the survivors were left with severe permanent disabilities.

Fishing in part of the bay was banned at the start of 1957 and the epidemic was halted, but it was not until 1959 that it was shown that mercury was the toxic element involved, and 1960 that the source was the factory effluent, which was discharged directly into the bay. In addition to methylmercury produced by methylating bacteria from the inorganic mercury included in the waste water from the factory, 5 per cent of the mercury in the discharge was in the form of methylmercury. During the investigations in 1959, sediments near the outfall were found to contain 200 ppm mercury, bivalves in intertidal areas contained 10-39 ppm (dry wt), and fish in the bay contained 10-55 ppm (dry wt) mercury, most of it methylated (Clark, 1997).

3.6.2 Public Health Standards

Following the Minimata disaster there was a greater appreciation of the risk of mercury poisoning eating contaminated seafood. The World Health Organization (WHO, 1990) recommended a maximum tolerable consumption of mercury in food of 0.2 mg of methylmercury or 0.3 mg of total mercury per week.

Any standard must take into account how much fish is eaten, as well as the concentration of mercury in it. In Japan, for example, a far wider range and quantity of sea food is eaten in Britain, where the average per capita consumption of fish is

only 20 g per day. Most countries have set statutory limits between 0.5 and 1.0 ppm fresh weight of total mercury, often with the higher limit applying only to species such as shark, swordfish, and tuna which are known to accumulate high levels of mercury. The reason for this exception is that a substantial proportion of the catch would be excluded from the market if the more stringent limit applied to it. In some countries (Canada, Sweden, South Australia) the more relaxed limit of 1.0 ppm for species that naturally have high mercury content is coupled with advice to the public to limit its consumption of these species (Clark, 1997).

3.6.3 Mercury Poisoning

Mercury and methylmercury are referred to as nerve toxicants or neurotoxicants. They affect the central nervous system, causing a number of serious disorders. The impact will depend on the level of exposure specific to each individual, but studies have demonstrated early symptoms of health alteration from regular exposure to very small amounts of methylmercury.

In order to understand the health effects of mercury, it is important to distinguish between “acute” exposure (exposure to high levels of mercury for a short period of time) and “chronic” exposure (exposure to small amounts of mercury for an extended period of time). Usually, acute exposure occurs through accidental contact with high amounts of mercury following isolated incidents, as was the case in the mass poisonings that occurred in Japan and Iraq. Chronic exposure occurs through frequent and continued ingestion of a food source (such as fish) contaminated with methylmercury. Ongoing exposure to low levels of mercury and its compounds in the environment is less well understood than acute toxicity, but has recently received increased attention from medical researchers. This is now one of the most critical areas of mercury health research, since many people are exposed to methylmercury levels that are higher than background levels — mainly through fish consumption — but not high enough to cause obvious signs of poisoning.

3.6.3.1 Acute Toxicity of Mercury

Exposure to high levels of mercury or its compounds can cause permanent brain damage, central nervous system disorders, memory loss, heart disease, kidney failure, liver damage, loss of vision, loss of sensation and tremors. It is also a suspected "endocrine disruptor," meaning it damages the reproductive and hormonal development and growth of fetuses and infants. Some studies suggest that mercury may be linked to neurological diseases, such as Alzheimer's and Parkinson's, but the evidence of this is far from conclusive (Pendergrass, Haley, Vimy, Winfield & Lorscheider, 1997, Hock et al., 1998 and Clarkson, 1997). A recent study by medical researchers identified the process of mercury damage to the brain. Mercury tends to concentrate in major organs, such as the brain and kidney. When in the brain, mercury attacks and dissolves the neurons in certain parts of the brain, leading to various nervous system disorders and organ damage. Metallic mercury, in the form of liquid or vapour, can be poisonous when ingested through water, food or air. It is especially hazardous when mercury vapours are inhaled. Individuals exposed to mercury spills in the workplace, home or school may be exposed to dangerous levels of mercury.

Each year, there are incidences of mercury spills around the world that send children to hospitals and, in some cases, cause fatal poisonings. Until mercury use is curtailed, these accidents will continue to take place, posing a risk to anyone who is exposed. There are rare forms of mercury that are highly toxic, even through contact with the skin. One tiny drop of dimethylmercury on the skin is enough to kill a human.

3.6.3.2 Chronic Exposure to Mercury

Children and developing fetuses are most at risk to low-level methylmercury exposure, particularly if a family regularly consumes fish that have high levels of methylmercury in them. The health effects of low-level exposure include

neurological damage, reproductive system damage, behavioural problems and learning disabilities. Some recent medical research indicates that there may be no "safe level" of methylmercury in a child's body. Medical researchers have also noted that mercury is one of the only natural elements with no known biological benefit at any concentration in humans. A small portion of the population is particularly sensitive to mercury in their bodies — in essence they are allergic to it. For these people, even very small amounts of mercury entering their bodies, such as the mercury in dental fillings, present a health problem, often causing symptoms resembling multiple sclerosis (Ganser & Kirschner, 1985, Siblerud, 1994, Windebank, 1986). Health warnings from governments, medical and scientific organizations have increased as a result of new research indicating that health effects occur at lower mercury levels than previously thought (International Labor Organization, 1983).

CHAPTER FOUR
MATERIAL AND METHODS

4.1 Sampling

Sampling took place in February, April, August and September 2005, at four locations shown in Figure 4.1 during *R/V* K. Piri Reis Cruise in the Izmir Bay. A total of 297 individuals of *Mullus Barbatus* (N=186), *Diplodus annularis* (N=108), *Solea vulgaris* (N=3) were collected by trawling from Izmir Bay in 2005 (Foca-Gediz, Uzunada, Gulbahce, Guzelbahce).

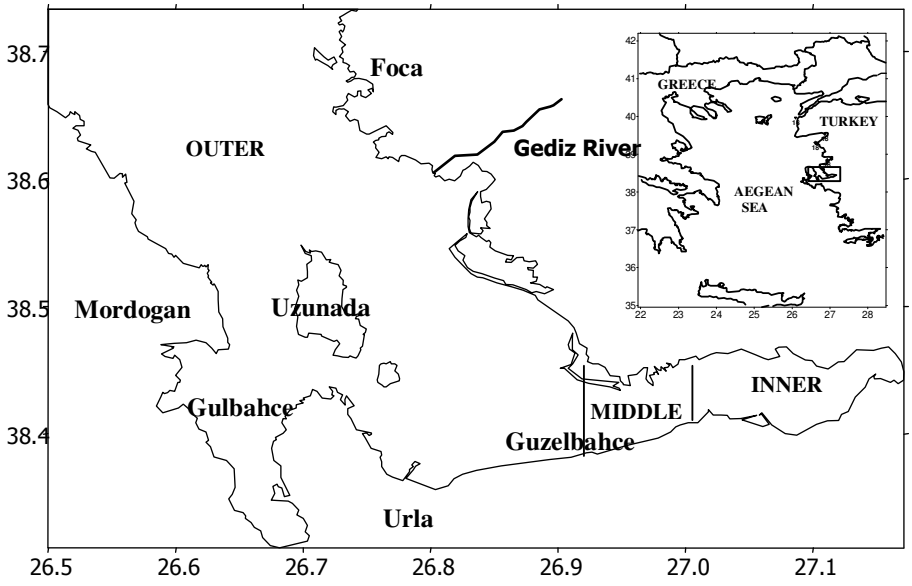

Figure 4.1 Sampling areas in the Izmir Bay

The three species are characteristic of Izmir Bay and occur along the coast with abundance. Details of individual species were given in Table 4.1. Weight and length were measured on every fish and the sex was determined. Following this muscle

tissue of the samples was removed in the field using surgical sheets, acid-washed lancet, wrapped in aluminum foil and preserved at -20˚C until analysis.

Table 4.1 Details of individual species in the Izmir Bay

Species	No of Fish	Length (mm)	Habitat	Feeding habits	Reproduction
Mullus barbatus	155	92-185	Muddy bottom (100-300 m)	Carnivorous	April-August
Diplodus annularis	150	100-168	Littoral and sandy bottoms (0-90 m)	Carnivorous	February-April
Solea vulgaris	3	220-290	Sandy, muddy bottoms (0-200 m)	Carnivorous	January-April

4.2 Analysis of Total Mercury in Fish

Muscle tissue was placed in plastic bags and stored at below -20°C until analysis. In the laboratory, approximately 0.5 g of fish muscle was digested with 5:1 HNO_3:$HClO_4$ in microwave digestion system. The resultant solutions were then diluted to the desired volume with double distilled water and the total Hg concentrations were measured in VARIAN atomic absorption spectrophotometry (Spectra AA-300 Plus) by cold vapour technique, after reducing the Hg^{2+} to Hg^0 with $SnCl_2$ (stannous chloride), the volatile Hg^0 is bubbled into the closed system of the VGA-76 Vapour Generation Accessory analyser (wavelength: 253.7 nm) (UNEP, 1982).

The detection limit for mercury was 0.05 µg/l. Accuracy of atomic absorption spectrophotometer and validity of the processes tested with a reference material (homogenate muscle sample, IAEA-407, from the International Laboratory of Marine Radioactivity, IAEA, Monaco). The values obtained for the analysis of three replicates of this sample (certified ; observed values $\pm$ standard deviation) were as follows: Hg (certified 0.222±0.024 mg kg^{-1}dry weight; found 0.214±0.0015 mg kg^{-1} dry weight).

4.3 Determination of Methylmercury (MeHg) in Fish

Measurement of MeHg was conducted using the UNEP/FAO/IAEA/IOC (1992) method. This method is based on liberating the organic form of mercury (R-Hg) from the dried biological matrices by homogenization of the sample in an acid medium. In order to extract all the organic Hg, freeze-dried samples were treated with KBr and $CuSO_4$ in sulphuric acid, which facilitates the liberation of CH_3-Hg bound to thiol groups; the bromine forms a stable compound in a sulphuric medium with CH_3-Hg. The R-Hg-Br was extracted with toluene. This extract was then treated with cysteine solution which selectively complexes the organo-mercury compounds to form water-soluble derivatives. Any co-extracted interfering materials within the toluene phase were thus removed. The complexed organomercury compounds within the aqueous phase were liberated in an acid medium and were back extracted into toluene. The resulting solution can then be subjected to gas chromatographic separation for quantification of the individual organo-Hg compounds (Figure 4.2).

Final measurement of the methylmercury content was performed using a Chrompack CP 9000 Gas Chromatography, equipped with a splitless capillary injection system and ^{63}Ni electron capture detector (ECD). The capillary column was used a 50m x 0.25mm CP-Sil 8CB (0.25 μm film thickness, WCOT fused silica). The injector and detector temperatures were same and maintained at 160°C. The oven temperature was set to 130°C. Nitrogen was used both as the carrier (2.50 ml min^{-1}) and make-up (32.5 ml min^{-1}).

The accuracy of the methylmercury analyses was tested by analysis of a certified reference material (homogenate muscle sample, IAEA 436, from the International Laboratory of Marine Radioactivity. IAEA, Monaco). Replicate analyses (n=5) (MeHg 3.57±0.35 mg kg^{-1} dry weight) were in the range of the certified material (MeHg 3.68±0.42 mg kg^{-1} dry weight). All data were computed on a mg g^{-1} wet weight basis.

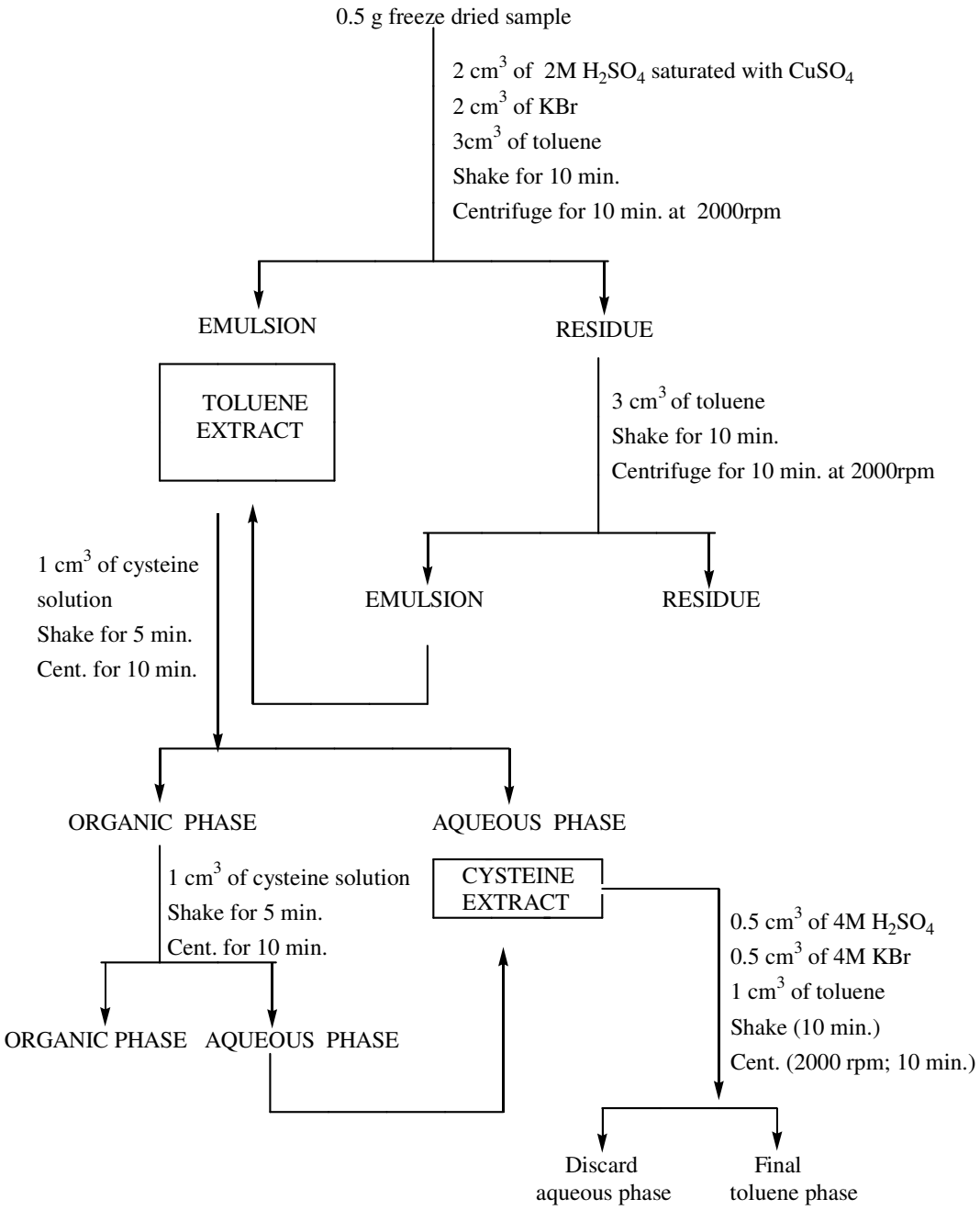

Figure 4.2 Schematic flow diagram of extraction procedures for methylmercury detection (from UNEP/FAO/IAEA/IOC, 1992)

4.4 Statistical Data Analyses

Statistical analysis was performed using STATISTICA for Windows, Release 6.0, Copyright StatSoft, Inc.1995. Pearson's Product-Moment Correlation test used to check for significant relationships between mercury and species length. In all case, the level of significant was set at $p< 0.05$. One-way analysis of variance (ANOVA) were utilised to investigate effect of species, sampling area and season on variations in total mercury and methylmercury concentrations in fish from Izmir Bay. Post hoc Tukey HSD test were applied to determine statistically significant differences ($P< 0.05$) following ANOVA.

CHAPTER FIVE
RESULTS AND DISCUSSION

5.1 Concentrations of Total Mercury and Methylmercury

The concentrations of total mercury and methylmercury for each organism are summarized in Table 5.1. The concentrations are expressed as µg kg^{-1} wet weight. In this section, results are discussed according to fish species, location and season. Other variables including, ratio of MeHg to THg (MeHg %), length (mm) and weight (g) are also considered. The observed minimum and maximum mercury levels of different seasons and stations were represented in Table 5.2.

A variety of biotic and abiotic factors can affect the efficiency with which marine animals, accumulate metals and metalloids in their tissues (Reinfelder, Fisher, Luoma, Nichols & Wang, 1998). Many parameters may effect mercury accumulation; such are specimen size, sexual maturity, and seasonal sensitivity, feeding habit, trophic level, water quality and levels of environmental contamination (Kehrig, Malm & Moreira, 1998).

Among the different species examined the highest levels of total mercury, ranging from 23.1 to 221.4 µg/kg wet weight (mean 90.7µg/kg wet weight), were determined in *Diplodus annularis*. *Solea vulgaris* showed levels slightly lower between 35.7 and 157 µg/kg wet weight (mean 86.9 µg/kg wet weight), while in *Mullus barbatus* total mercury concentrations ranged from 4.4 to 157.9 µg/kg (mean 63.1 µg/kg wet weight) (Table 5.1).

The lowest total mercury level (4.4µg/kg wet weight) was found in *Mullus barbatus* taken from Gulbahce in February while the highest concentration (221.4µg/kg wet weight) was detected in *Diplodus annularis* from Gulbahce in August.

Table 5.1 Total Hg (THg) and methyl Hg (MeHg) levels (μg kg^{-1} wet wt.) and the ratios of MeHg to THg in biota

Sampling Location	Organism	Length (mm)	Weight (g)	Sex	N	THg	MeHg	MeHg %
Feb. 2005								
Foca-Gediz	*Mullus barbatus*	122-136	26-42	F	12	10.96	10.08	92
	Mullus barbatus	156-170	60-74	F	12	23.5	22.3	95
	Diplodus annularis	110-119	24-34	M	11	30.1	26.45	88
Gulbahce	*Mullus barbatus*	121-129	24-32	F	20	13.96	13	93
	Mullus barbatus	92-116	12-24	M	20	4.4	3.8	86
Uzunada	*Mullus barbatus*	110-128	24-40	F	17	20.3	17.85	88
	Diplodus annularis	150-168	64-104	F	5	23.1	21.9	95
	Diplodus annularis	153-165	66-88	M	5	31.8	28.6	90
April 2005								
Gulbahce	*Mullus barbatus*	97-115	12-22	M	13	61.13	51.34	84
	Diplodus annularis	110-120	28-42	M	10	92.6	82.4	89
Uzunada	*Mullus barbatus*	149-165	56-84	F	8	77.9	70.9	91
	Diplodus annularis	120-137	38-58	M	10	46.1	42.9	93
Guzelbahce	*Mullus barbatus*	111-120	22-30	F	10	49.5	45.6	92
	Diplodus annularis	116-129	30-42	M	10	45.2	41.2	91
August 2005								
Foca-Gediz	*Mullus barbatus*	140-158	42-72	F	19	103.1	96.9	94
	Diplodus annularis	100-120	20-36	M	13	132	112.3	85
	Solea vulgaris	220-290	142-206		3	112.5	106.9	95
Gulbahce	*Mullus barbatus*	142-158	48-66	F	16	82.2	78.5	95
	Diplodus annularis	105-122	24-38	M	18	221.4	194.82	88
Uzunada	*Mullus barbatus*	152-169	54-82	F	7	75.6	71.85	95
Sep. 2005								
Uzunada	*Mullus barbatus*	121-146	28-48	M	17	139.5	121.4	87
	Mullus barbatus	163-185	70-110	F	15	157.9	148.3	94
	Diplodus annularis	125-140	36-58	M	14	127.5	116	91
	Diplodus annularis	120-138	36-60	F	12	157.2	154.03	98

Organic Hg (mainly methylmercury) varied between 21.9 and 194.8 µg/kg wet weight (mean 82.1 µg/kg wet weight) in *Diplodus annularis*, between 3.8 and 148.3 µg/kg wet weight (mean 57.83 µg/kg wet weight) in *Mullus barbatus* and between 33.9 and 149 µg/kg wet weight (mean 82.53 µg/kg wet weight) in *Solea vulgaris* (Table 5.2). The average of methylmercury for all fish (N=297) was 70±52.3 µg kg^{-1}. Methylmercury concentrations varied from 3.8-194.8 µg kg^{-1} wet wt in all species. The highest (194.8 µg kg^{-1}) and the lowest (3.8µg kg^{-1}) methylmercury concentration were found at Gulbahce.

Table 5.2 Mean and range of Total Hg (THg) and methyl Hg (MeHg) levels (µg kg^{-1} wet wt.) and the ratios of MeHg to THg in biota

Sampling Area	Length Mean	Length Min-Max	THg Mean	THg Min-Max	MeHg Mean	MeHg Min-Max	InorgHg Mean	InorgHg Min-Max
Foca-Gediz								
M. barbatus	147	122-170	45.8	11.0-103	43.1	10.1-96.9	2.8	0.88-6.2
D. annularis	110.7	100-120	81.0	30.1-132	69.4	26.4-112	11.6	3.7-19.6
S. vulgaris	263	220-290	86.9	35.7-157	82.5	33.9-149	4.4	1.8-8.0
Gulbahce								
M. barbatus	121.0	92-158	40.4	4.4-82.2	36.6	3.8-78.5	3.8	0.63-9.8
D. annularis	114	110-120	157	92.6-221	139	82.4-195	18.4	10.2-26.6
Uzunada								
M. barbatus	144.2	110-185	94.2	20.3-158	86.1	17.8-148	8.2	2.5-18.1
D. annularis	136.5	120-168	77.1	23.1-157	72.7	21.9-154	4.5	1.2-11.5
Guzelbahce								
M. barbatus	115.7	111-120	49.5	49.5	45.6	45.6	3.9	3.9
D. annularis	123	116-129	45.2	45.2	41.2	41.2	4.0	4.0

The percentage of MeHg was high and constant in muscle. The mean percentages of methylmercury to total mercury for all fish samples were 91±3.7 % with a range of 84 to 98 %, indicating that organic mercury was the predominant form of mercury in the muscle tissue of fish.

The influence of environmental and biological variables (e.g., body size, age, acclimation temperature, water quality, and pH) on the accumulation of Hg in fish has been reported in the literature. In general, larger, older fish in low alkaline, low pH lakes tend to accumulate the highest concentrations of Hg. Mercury body burden can vary widely among fish species within a lake, and this difference is often attributed to the trophic level of the species, with top predators such as largemouth bass (Micropterus salmoides) accumulating more Hg than forage species.

5.2 The Distribution of Hg According to Habitat/Feeding Habits

The results may be categorized according to the habitats occupied by the sampled species and their feeding habits that are identified in Table 4.1. There is an obvious difference between the contaminant distributions in *Mullus barbatus* and *Diplodus annularis* (Table 5.1). The suggested reason for this difference relates to differences in feeding habits and also differences in exposure with *Diplodus annularis* being more exposed to atmospheric inputs and photochemical production of MeHg. *Diplodus annularis* is carnivorous marine fish, feeds on worms, crustaceans, molluscs, echinoderms and hydrozoans and inhabits chiefly *Zostera* seagrass beds but is also found on *Posidonia* beds and sandy bottoms, rarely on rocky bottoms with depth range 0-90 m. The carnivorous fish, *Diplodus annularis* showed higher THg (221.4 µg kg^{-1}) and MeHg (194.8 µg kg^{-1}) concentrations in the bay. *Mullus barbatus* feeds on small benthic crustaceans, worms and molluscs and is found on gravel, sand and mud bottoms of the continental shelf with depth range from 100-300 m. The lower mercury levels were observed for *Mullus barbatus*. The habitat and feeding habits probably constitute the main causes of the variations in mercury concentrations among the species studied here.

Various workers have addressed measurements of mercury in fish from different region of the world and some of these data are summarized in Table 5.3. The findings of this study are not similar to other studies especially for the difference of the mean Hg content between carnivorous and small benthic invertebrates. (Nakagawa, Yumita & Hiromoto, 1997; Joiris, Laroussi Moatemri & Holsbeek, 1997). Carnivorous fish can be used as a good indicator for monitoring of mercury pollution (Vigh, Mastala & Balogh, 1996) while other species with other feeding habits should also be monitored for human health aspects.

Table 5.3 Levels of Total Hg and Methyl Hg in fish from the different regions of the world (as µg/kg wet wt)

Area	∑Hg (mean, min-max)	MeHg (mean, min-max)	MeHg %	References
Black Sea	332 (90-720)	216 (60-540)	65	Joiris *et al.* (2001)
Brazilian estuary	199 (63.0-556.1)	195 (65.0-561)	98	Kehrig *et al*. (2002)
Bangladesh				
R. kanagurta	64.0 (24-124)	48.0 (17-120)	77	Joiris *et al.* (2000)
T. thalassinus	120 (57-370)	101 (34-340)	83	
Arabian Gulf	105-631	-	7-100	ROPME (1988)
Ionian Sea	12150 (8500-21070)	14000 (7450-19570)		Storelli *et al.* (2003)
Brazilian Amazon	-	240 (30-900)	96	Kehrig *et al.* (1998)
Mediterranean Sea				
C. monstrosa	3104 (1300-5160)	2670 (1140-4560)	83.6	Storelli *et al.* (2002)
T. nobiliana	2420 (1650-3590)	1900 (1150-2760)	81	
M. aquila	830 (670-1010)	630 (400-840)	71.6	
Izmir Bay (Aegean)				
Mullus barbatus	63.1 (4.4-157.9)	57.8 (3.8-148.3)	91.0	This study
Diplodus annularis	90.7 (23.1-221.4)	82.1 (21.9-194.8)	90.8	

A geographic comparison about total Hg and methylmercury in the examined species is difficult because to our knowledge there are no studies on mercury and its speciation in *Mullus barbatus* and *Diplodus annularis* from the Aegean Sea. High levels of mercury in deep-water organisms are not unexpected findings, but there is part of a general picture that suggests that deep sea fauna, especially those occupying a high trophic level, may be especially prone to accumulate this element. On the other hand, it is generally known that in sea water concentrations of a number of trace metals, including mercury, tend to increase with water depth (Leonzio, Bacci, Focardi & Renzoni, 1981).

5.3 Mercury Concentrations and International Limits

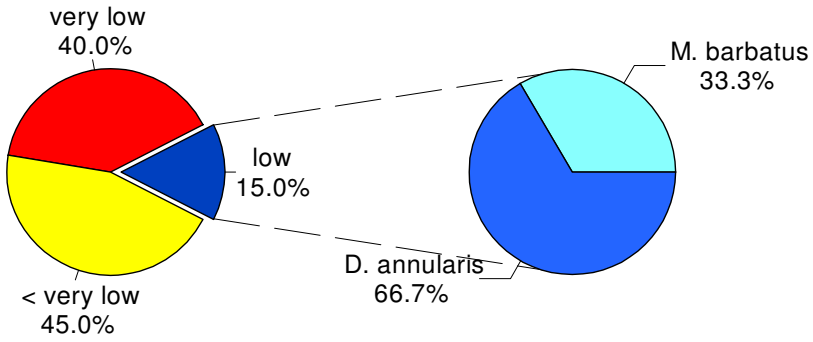

Figure 5.1 The distribution of THg in fishes according to concentration categories (Chvojka *et al.*,1990)

It is important to have an overall picture of the obtained THg in the analysed species from a public health perspective. So as to achieve this it was first necessary to convert concentrations to a wet weight values using dry weight/wet weight ratio (0.36±0.03 for *Mullus barbatus*, 0.26±0.02 for *Diplodus annularis*). It is then possible to apply the categorization of Chvojka, Williams & Fredrickson (1990) who described THg in the wet weight of fish from 0.05 to 0.15 µg g^{-1} as very low, from

0.15 to 0.25 µg g^{-1} as low, from 0.25 to 0.35 µg g^{-1} as medium, from 0.35 to 0.45 µg g^{-1} as high and total Hg (THg) above 0.45 µg g^{-1} as very high. The results from Izmir Bay are shown in Figure 5.1.

It is clear that there are not even any fishes in the "medium" category and that *Mullus barbatus* and *Diplodus annularis* represent significantly lower health threats. A simpler grouping utilizing the WHO permissible limits (WHO, 1990) can be also used where 0.5 µg g^{-1} is the limit for THg and 0.3 µg g^{-1} for MeHg. The results for THg indicate that none of the fish analyzed was ≥ 0.500 µg g^{-1} THg, and did not exceed the WHO limit. For MeHg, the results indicate that none was ≥ 0.300 µg g^{-1} and did not exceed the WHO limit.

5.4 Public Health Implications

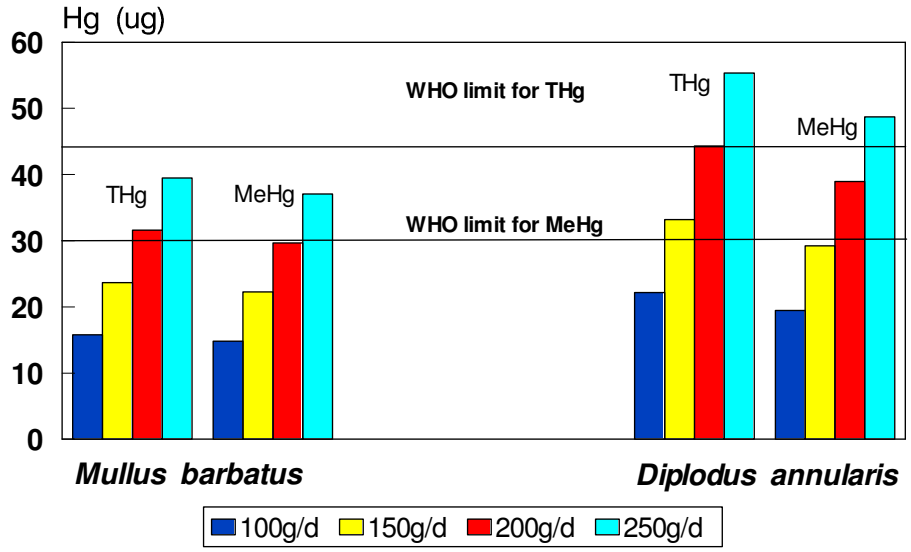

Figure 5.2 The estimated human intake of Thg and MeHg (µg) from *M. barbatus* and *D. annularis* according to quantities of fish consumed

To assess the potential health impact, the 43 µg day^{-1} maximum intake limit for THg (WHO, 1989) and the 30 µg day^{-1} maximum intake limit for MeHg of a 70 kg person was used as a guideline (USEPA, 1984). Using the observed concentrations in

the fish species and a range of fish consumption (100, 150, 200 and 250 g d^{-1}) the results shown in Figure 5.2 were obtained (Note: Among the species, samples that showed maximum concentrations were considered). From this it can be seen that at consumption rate of 250 g d^{-1} or more the WHO limit was exceeded for THg in *Diplodus annularis* while it was not exceeded for THg in *Mullus barbatus* at a consumption rate of 250 g d^{-1}. For MeHg, at a consumption rate of 200 g d^{-1} the EPA limit was approached and exceeded at a rate of 250 g d^{-1} for *Mullus barbatus* while at a consumption rate of 150 g d^{-1} it was approached and exceeded at a consumption rate of 200 g d^{-1} or more for *Diplodus annularis*.

5.5 Statistical Analyses

A general examination of the statistical summary (Figure 5.3-5.4) shows distribution of total mercury and methylmercury in *Mullus barbatus* and *Diplodus annularis* within different seasons. The summary statistics of total mercury and methylmercury contents in seasons indicate that dots represent mean values (as µg/kg wet weight); lower and upper box edges represent mean±SE; outlying bars are mean±1.96 SE. According to results *Mullus barbatus* accumulated higher levels of total mercury and methylmercury in September in comparison with other months while total mercury and methylmercury levels in August are higher than other months in *Diplodus annularis*. Interestingly, the standard error for Hg mean concentrations were very large in August.

Figure 5.5 and 5.6 shows the means, related standard errors and approximate confidence intervals of mercury concentrations for different sampling areas in Izmir Bay. THg and MeHg concentrations in *Mullus barbatus* are increased at Uzunada while Hg levels decreased at Gulbahce. On the other hand, *Diplodus annularis* demonstrates maximum levels at Gulbahce and minimum levels at Foca-Gediz. Mercury concentrations at Uzunada were similar to Foca-Gediz sampling area. The Hg levels were lower in *Mullus barbatus* than recorded in *Diplodus annularis* and these data are summarised in Figure 5.7.

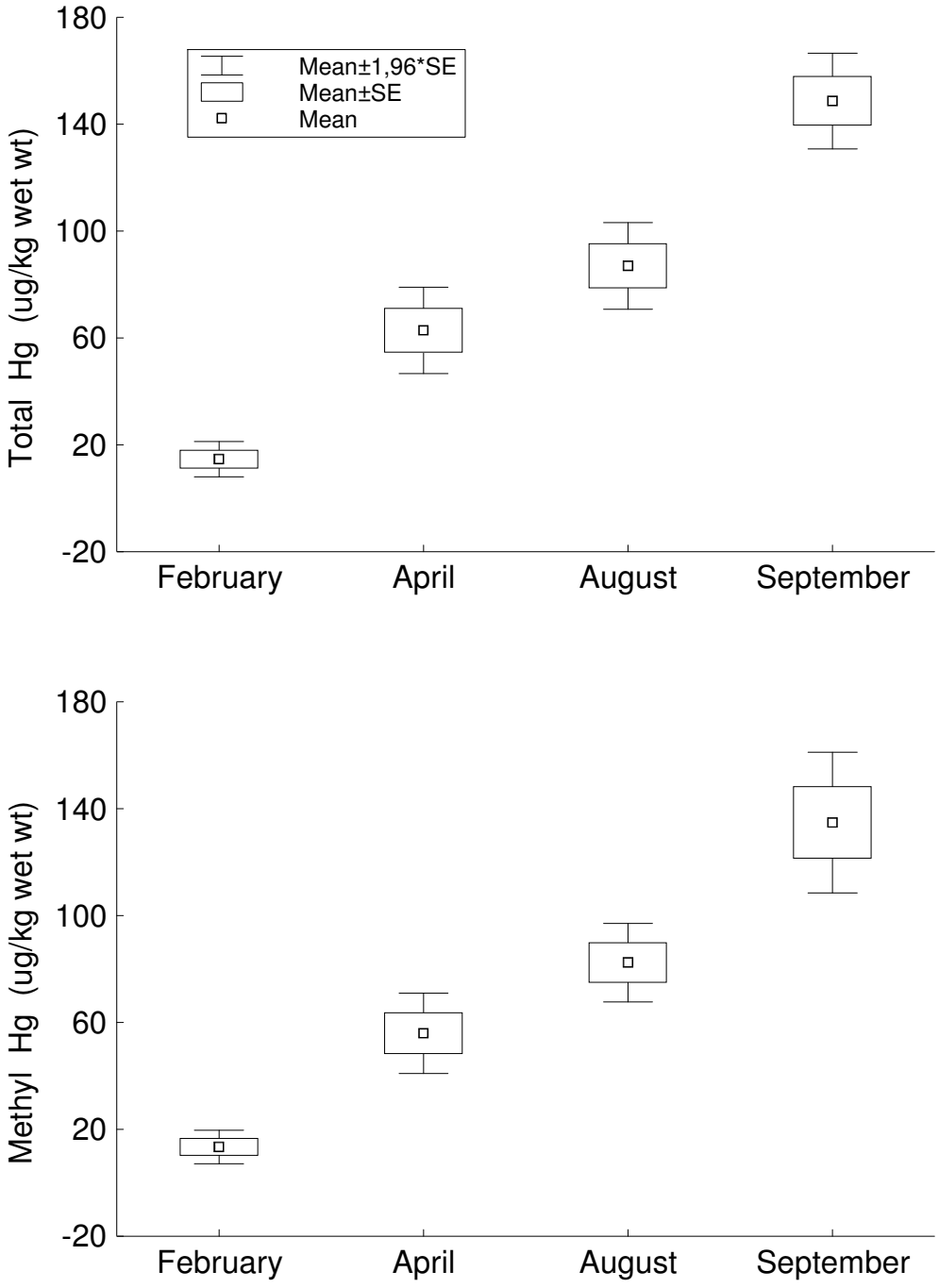

Figure 5.3 Means, mean±standard error and confidence intervals (1.96σ) of Total and Methyl Mercury in *Mullus barbatus* according to sampling periods

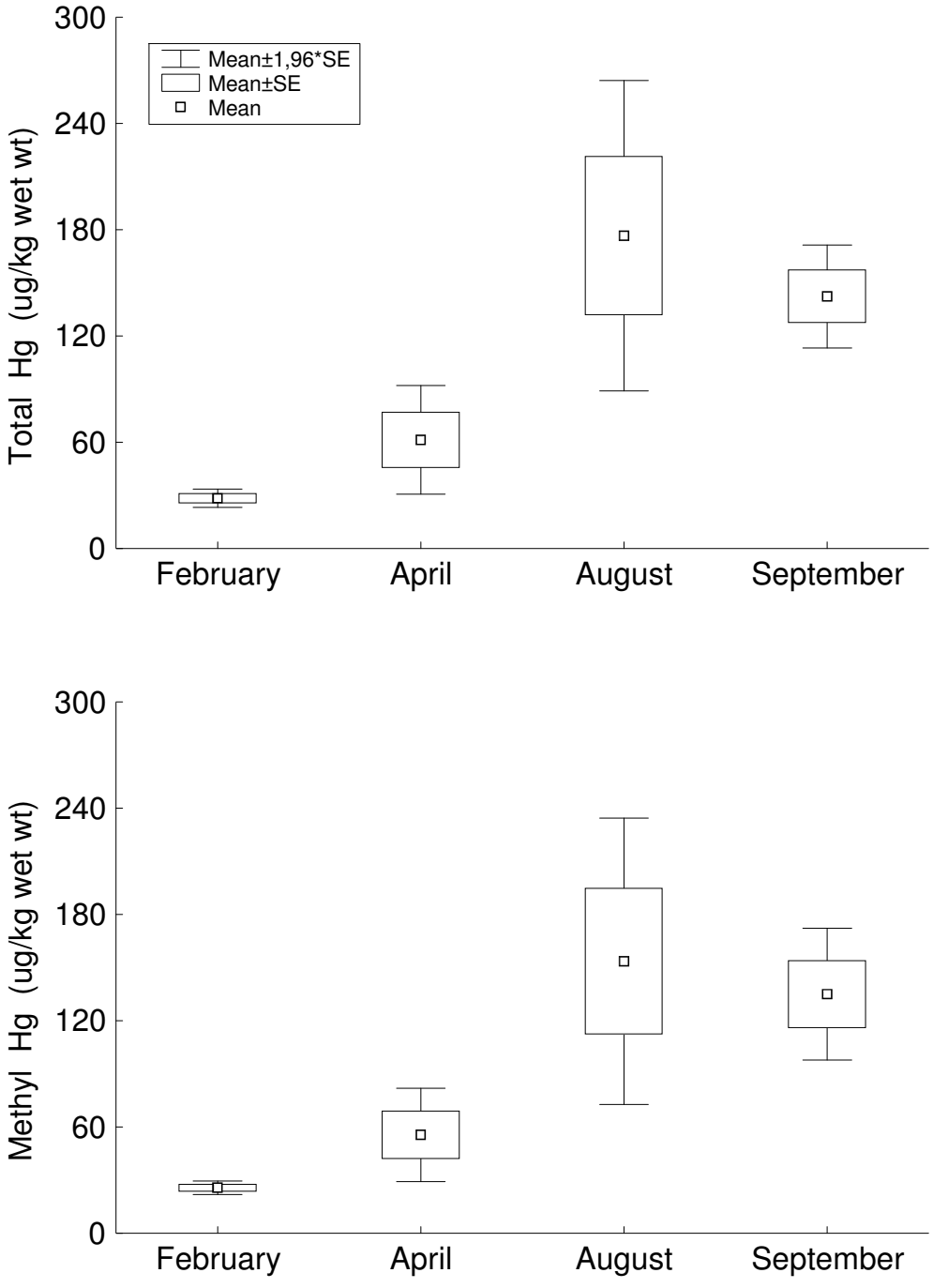

Figure 5.4 Means, mean±standard error and confidence intervals (1.96σ) of Total and Methyl Mercury in *Diplodus annularis* according to sampling periods

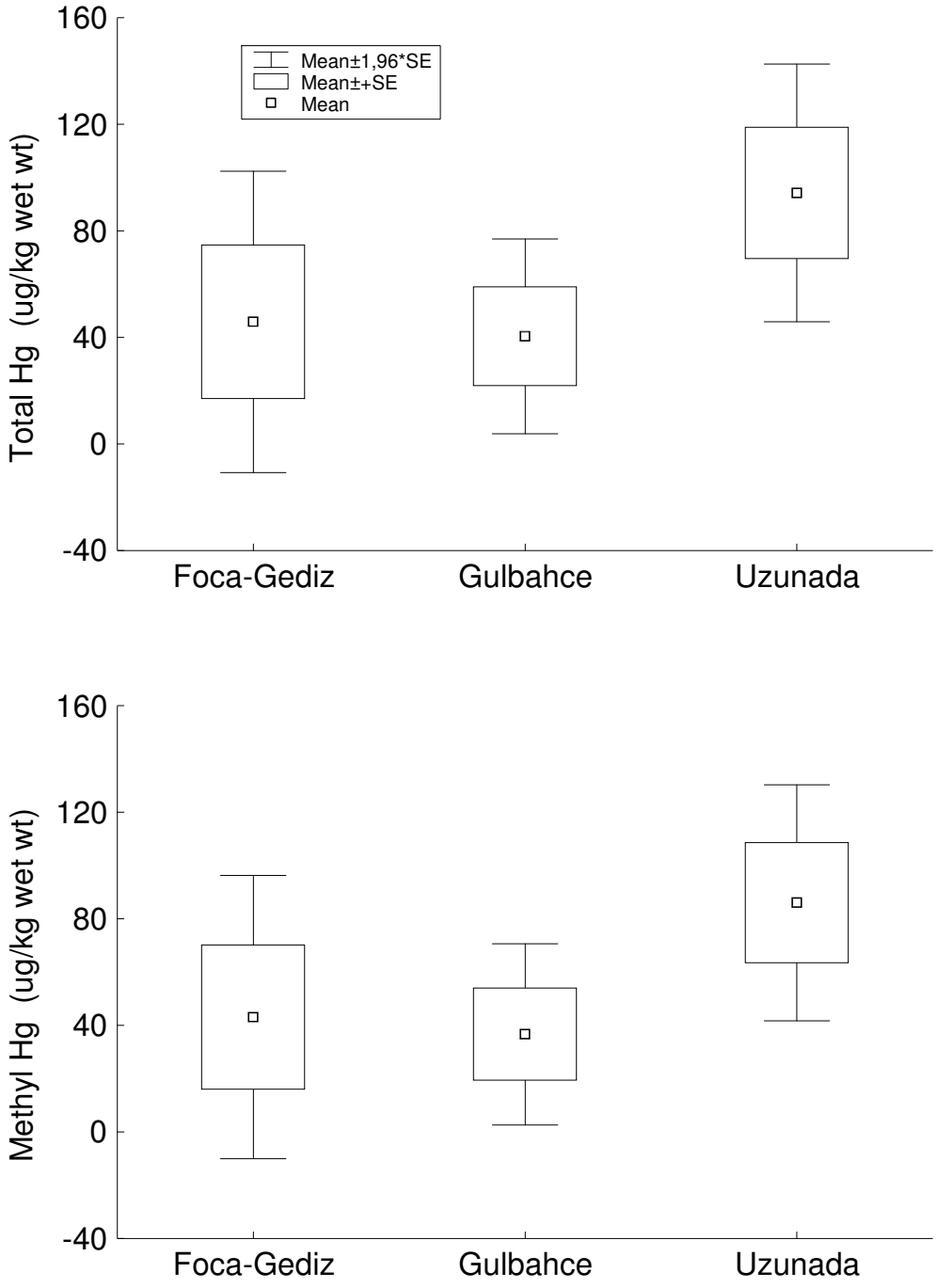

Figure 5.5 Means, mean±standard error and confidence intervals (1.96σ) of Total and Methyl Mercury in *Mullus barbatus* according to sampling area

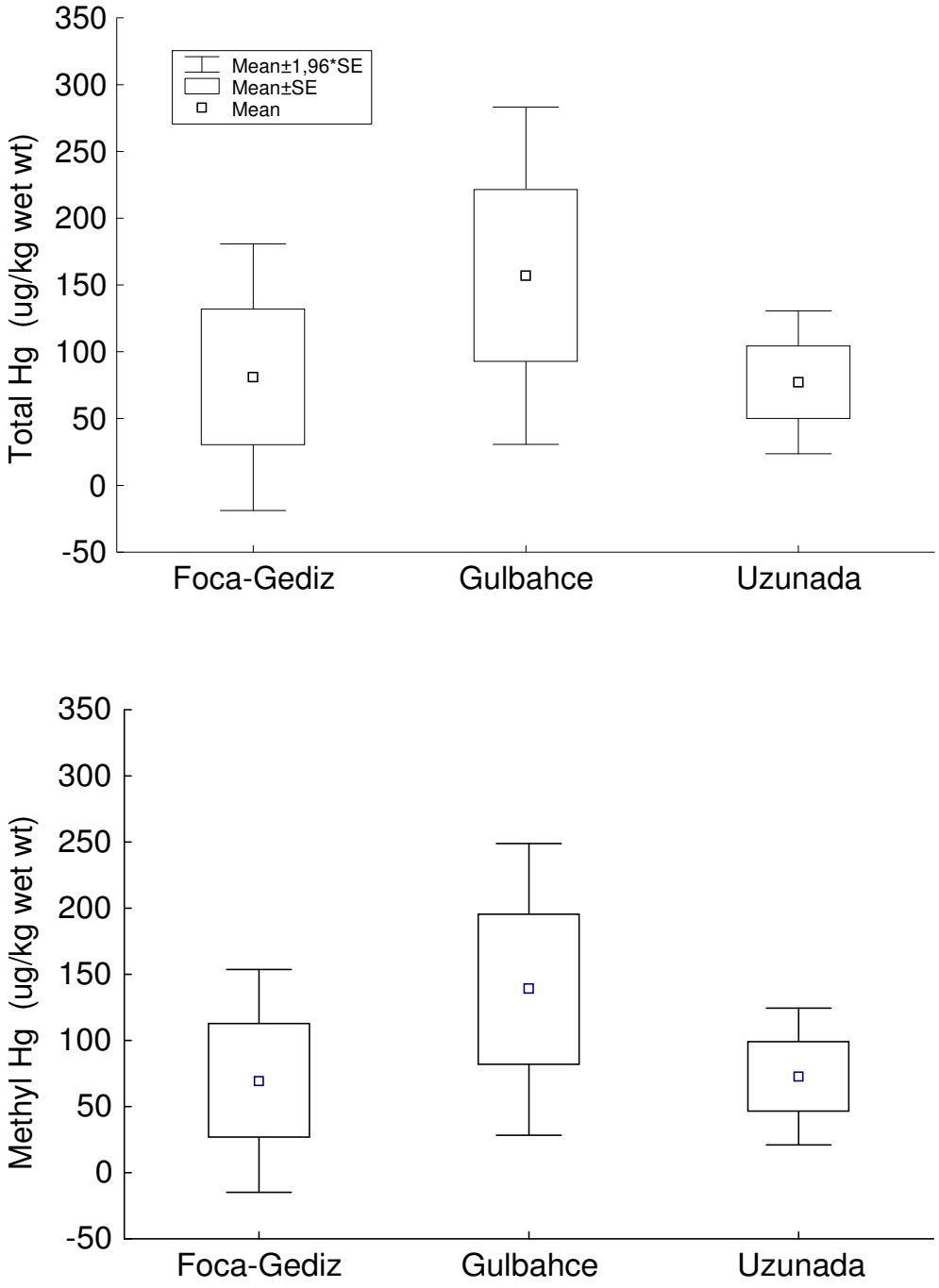

Figure 5.6 Means, mean±standard error and confidence intervals (1.96σ) of Total and Methyl Mercury in *Diplodus annularis* according to sampling area

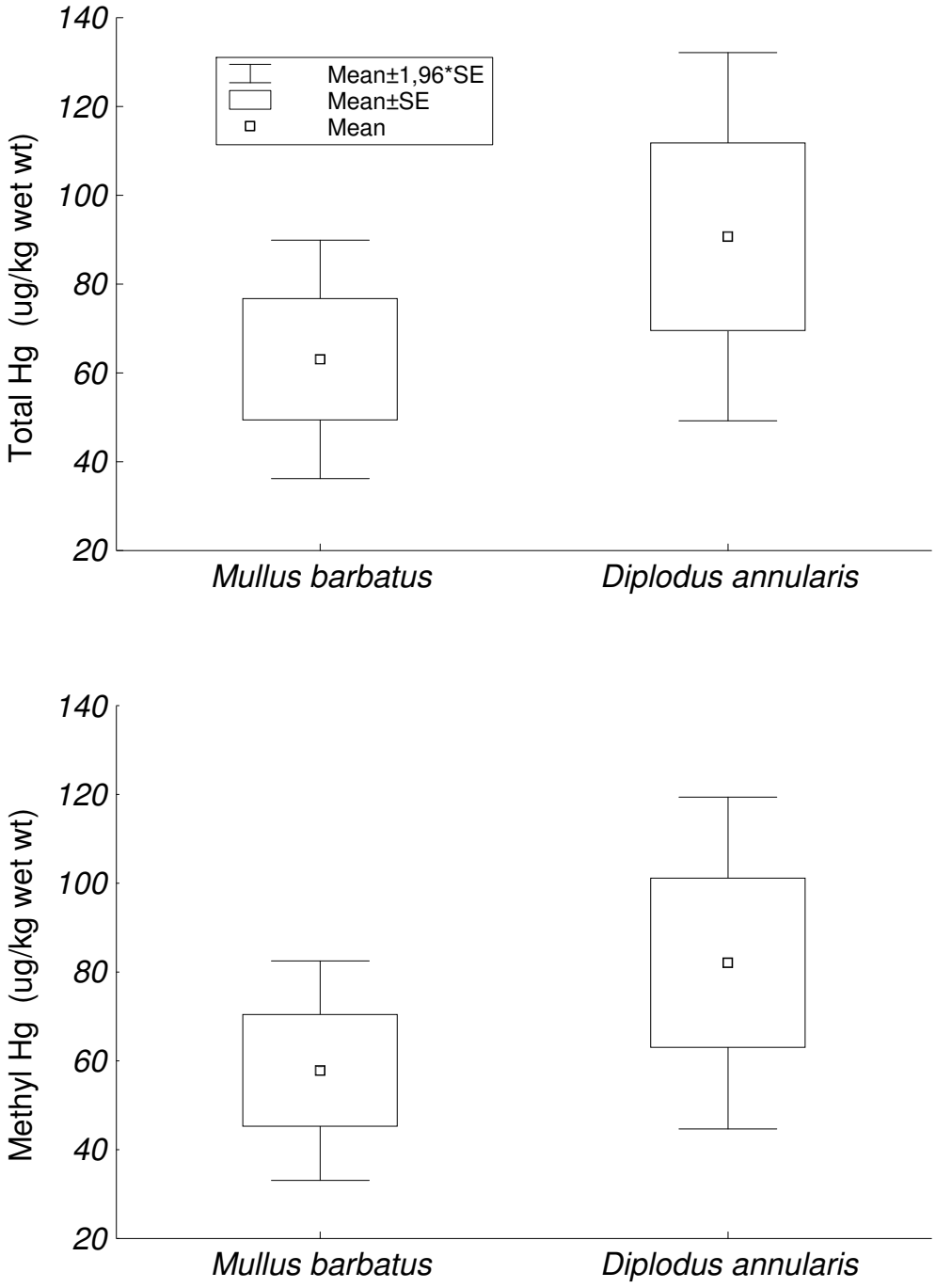

Figure 5.7 Means, mean±standard error and confidence intervals (1.96σ) of Total and Methyl Mercury in different species

5.5.1 Correlations between Mercury Concentrations and Length in Fish

There was a strong and statistically significant ($p<0.01$) correlation between T-Hg and MeHg concentrations (Figure 5.8). A similar correlation has recently been described by Kannan *et al*. (1998).

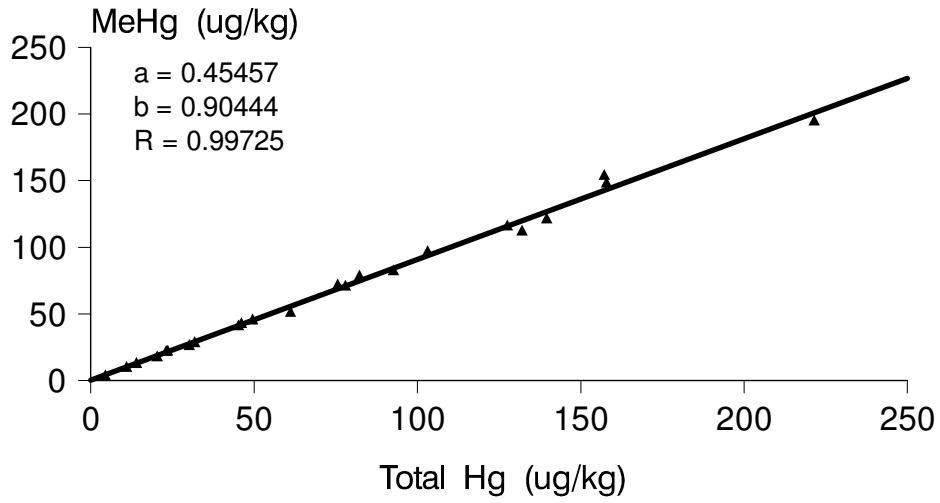

Figure 5.8 The relationship between THg and MeHg concentrations for both species

The relationships between fork lengths and mercury concentrations were significant ($R= 0.57$, $n=186$, $a= -114.4$, $b= 1.29$, $p<0.05$ for THg, $R= 0.61$, $n=186$, $a= -111.7$, $b= 1.25$, $p<0.05$ for MeHg) in *Mullus barbatus*. There are no significant correlations between fork lengths and mercury concentrations in *Diplodus annularis.* There were significant correlations between both THg and MeHg concentrations and length, reflecting an accumulation with age as described commonly in the literature (Lange, Royals & Connor, 1994; Joiris, Ali, Holsbeek, Bossicart & Tapia, 1995a; Holsbeek, Das & Joiris, 1997) for *Mullus barbatus*. This result represents the fact that weight is in effect a proxy for age and hence exposure. The levels of mercury in

fish increase with body size, so larger, older fish have generally higher concentrations than smaller, younger fish.

Hg accumulation observed in *Mullus barbatus* was a kind of accumulation that organic Hg concentrations increased with length, while inorganic Hg concentrations remained at a constant low level (Table 5.2), so that the relative methyl Hg concentrations increased with length. This pattern is generally regarded as normal in those fish species exhibiting a high relative organic Hg concentration. Some fish species may be capable of the demethylation of MeHg, albeit over long time periods. The same positive correlations were found earlier by several authors.

5.5.2 One-way ANOVA

The results of one-way ANOVA between stations and seasons during sampling periods are given in Table 5.4.

Table 5.4 Values of one-way analysis of variance for all sampling periods

Variable	Season			Sampling area		
	Df	**F**	**p level**	**df**	**F**	**p level**
Mullus barbatus						
THg	3	69.2899	***	3	1.1219	ns
MeHg	3	56.38798	***	3	1.0826	ns
Diplodus annularis						
THg	3	11.53691	**	3	0.8440	ns
MeHg	3	10.49812	**	3	0.7384	ns

*p<0.05;**p<0.01; ***<0.001, ns: non significant.

Concentrations in muscle tissue of *Mullus barbatus* differed significantly among seasons for THg ($p<0.05$, df=3, F=69.2899) and MeHg ($P<0.05$, df=3, F=56.38798). THg and MeHg are showed with a Post hoc Tukey HSD analyses that levels in April differed significantly from February and September. THg and MeHg concentrations

are different in September and February from other months in muscle tissue of *Mullus barbatus*.

Concentrations in muscle tissue of *Diplodus annularis* differed significantly among season for THg ($p<0.05$, df=3, F=11.53691) and MeHg ($P<0.05$, df=3, F=10.49812). THg and MeHg are showed with a Post hoc Tukey HSD analyses that levels in September differed significantly from February. August is different from February and April. Lastly, April is different from August in muscle tissue of *Diplodus annularis*.

The significant differences between sampling areas for THg ($p<0.05$, df=3, F=1.1219) and MeHg ($p<0.05$, df=3, F=1.0826) were not found in muscle tissue of *Mullus barbatus*. In the same way concentrations in muscle tissue of *Diplodus annularis* did not differ among sampling areas for THg ($p<0.05$, df=3, F=0.8440) and MeHg ($p<0.05$, df=3, F=0.7384). Hg is showed with a Post hoc Tukey HSD analysis that there is no difference between sampling areas.

The one-way ANOVA test showed that non-significant differences for THg ($p<0.05$, df=1, F=1.299) and MeHg ($p<0.05$, df=1, F=1.211) *Mullus barbatus* and *Diplodus annularis*. Such variability is in accordance with the process of mercury uptake in fish and the interaction of biotic parameters such as size, sex, longevity, growth rate, feeding habits and trophic position. Therefore, the knowledge of the physiology and ecology of the organisms is necessary in order to understand the meaning of the data on pollutant concentrations. Generally, the differences in contaminant burdens between the different species are related to the physiological differences between different species or the quantity and type of food taken by different populations of the same species. Variations in concentration within species may come about through the migration of fish species from unpolluted areas to relatively more polluted areas.

CHAPTER SIX
CONCLUSIONS

6. Conclusions

This study first documented the speciation and distribution of mercury in organisms from Izmir Bay. Results obtained with the present study provided useful information in order to assess mercury accumulation and speciation in selected organisms of the Izmir Bay.

The lowest total mercury level (4.4 µg kg^{-1} wet weight) was found in *Mullus barbatus* taken from Gulbahce in February while the highest concentration (221.4 µg kg^{-1} wet weight) was detected in *Diplodus annularis* from Gulbahce in August. This distribution pattern reflects the fact that *Diplodus annularis,* which is carnivorous fish, can be used as a good indicator for monitoring of mercury pollution while other species with other feeding habits should also be monitored for human health aspects.

Of the total 297 individuals analyzed, mercury is mostly in the organic form in all species (>84 % MeHg). The result of this research demonstrated that the percentage of MeHg was high and constant in muscle tissue of *Mullus barbatus*, *Diplodus annularis* and *Solea vulgaris*. The mean percentages of methylmercury to total mercury for all fish samples were 91±3.7 % with a range of 84 to 98 %, indicating that organic mercury was the predominant form of mercury in the muscle tissue of fish.

The results show that THg and MeHg for both species seem to be more concentrated (127.5-157.9 µg THg kg^{-1} wet wt) and (116-154.03 µg MeHg kg^{-1} wet wt) at Uzunada in September. The highest mercury concentrations were found in September, while the lowest were detected in February. Moreover, mercury levels in February for *Diplodus annularis* and *Mullus barbatus* were three to seven times lower in comparison with the other seasons.

Total mercury and methylmercury concentrations in *Mullus barbatus* were positively correlated with length, reflecting an accumulation of Hg with time (considering length as directly depending on age). However, there were no significant correlations between fork lengths and mercury concentrations in muscle tissue of *Diplodus annularis*.

None of the fish analysed was ≥.0.500 μg g^{-1} THg, and exceed the WHO limit. The maximum Hg content was 221.4 μg ∑Hg kg^{-1} wet weight, corresponding to 194.82 μg MeHg kg^{-1} wet weight. For a person eating 200-250 g of *Diplodus annularis* daily, the maximum amount of MeHg ingested is of 39-49 μg daily (273-343 μg weekly), a value of the same order of magnitude as the 210 μg permissible tolerable weekly intake (PTWI) for methylmercury proposed by United States Environmental Protection Agency (USEPA, 1984). Our results indicated that *Diplodus annularis* exceeded this limit.

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