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Tracking Mercury Biomagnification in Fish from the Gulf of Oman using Stable Isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$)

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Tracking Mercury Biomagnification in Fish from the Gulf of Oman using Stable Isotopes (13C/12C and 15N/14N)

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Abstract

Attempts to use stable isotope carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) ratios to construct trophic positions and track mercury biomagnification in zooplankton and 13 fish species from a coastal food web of the Gulf of Oman illustrated some potential differences in this environment compared to the aquatic ecosystem of the northern hemisphere. Due to the large difference in $\delta^{13}C$ values (3.4‰) between zooplankton planktivorous fish species (*S. crumenophthalmus*, *S. longiceps* and *R. kanagurta*), zooplankton would seem to not be the primary diet of these fish species as commonly described in literature.

Total mercury (T-Hg) concentrations of zooplankton were very low (range 0.010 to 0.037 $\mu g \cdot g^{-1}$, N = 27) with a mean methyl mercury (MeHg) of 0.001 $\mu g \cdot g^{-1}$ (range 1-19 %, N= 5). The lowest T-Hg (0.003 $\mu g \cdot g^{-1}$) was found in planktivore (*S. longiceps*) and the highest was 0.760 $\mu g \cdot g^{-1}$ in predator shark (*R. acutus*) with average MeHg for all fish of 72% (range: 33-100%, N= 150). Using ^{15}N as indicator of trophic position, neither total mercury (T-Hg) nor methyl mercury (MeHg) were found to biomagnify. Regression slopes were 0.08 and 0.05 for T-Hg and MeHg respectively as a function of $\delta^{15}N$. This indicates that biomagnification was lower in this tropical ocean compared to that found in freshwater and marine ecosystems of the arctic and temperate zones.

Methyl mercury levels in the fish species commonly consumed are low and intake calculations showed that individuals can safely consume fish.

Résumée

Les tentatives d'employer des rapports d'isotope stables du carbone ($\delta^{13}C$) et de l'azote ($\delta^{15}N$) pour construire des positions trophiques et tracer la biomagnification du mercure dans le zooplancton et 13 espèces de poisson dans l'enchaînement nutritif côtier du Golfe d'Oman a illustré quelques différences potentielles dans cet environnement comparé à l'écosystème aquatique de l'hémisphère nordique. En raison de la grande différence en valeurs $\delta^{13}C$ (3.4‰) entre l'espèce planktivorous de poissons (*S. crumenophthalmus*, *S. longiceps* et *R. kanagurta*) de zooplancton, zooplancton semblerait ne pas être le régime primaire des espèces planctonivores de poissons comme généralement décrit en littérature.

Les concentrations en mercure totale (T-Hg) du zooplancton étaient très basses (entre 0.010 au 0.037 $\mu g \cdot g^{-1}$, N = 27) avec un pour cent moyen en tant que mercure méthylique (MeHg) de 0.001 $\mu g \cdot g^{-1}$ (entre 1-19 %, N= 5). Le plus bas T-Hg (0.003 $\mu g \cdot g^{-1}$) a été trouvé dans le planctonivore (*S. longiceps*) et le plus haut était de 0.760 $\mu g \cdot g^{-1}$ dans le requin prédateur (*R. acutus*) avec MeHg moyen pour tous les poissons de 72% (entre : 33-100%, N = 150). En utilisant ^{15}N comme indicateur de la position trophique, ni le mercure de total (T-Hg) ni le mercure méthylique (MeHg) n'ont été trouvés biomagnifier. Les pentes de régression étaient 0.08 et 0.05 pour T-Hg et MeHg respectivement en fonction de $\delta^{15}N$. Ceci indique que le biomagnification était inférieur dans cet océan tropical comparé à cela trouvé dans des écosystèmes d'eau douce et marins des zones arctiques et tempérées.

Les niveaux de mercure méthyliques dans les espèces de poissons de consommation courante sont bas et les calculs de prise ont prouvé que les individus peuvent consommer des poissons sans risque.

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Figure 4.2: Calculated number of fish meals per day to stay below 0.23µg/kg-day, a daily WHO guideline for methyl mercury intake for selected fish species from the Gulf of Oman.

Rationale

Identification of inorganic and organic pollutants has become an important issue in the Arabian Gulf Region within the past few years. In the Gulf of Oman, most studies have been devoted to monitoring hydrocarbons and contaminants associated with oil pollution. There have been no previous investigations on mercury in biota from this gulf except the recent study of de Mora *et al.* (2004), who determined total mercury concentrations in two commercial fish and a bivalve species. Still, there has been no single report on the methyl mercury in fish species.

Most of Oman's population resides in coastal areas (mainly on the Gulf of Oman) and fish is a common diet item. Fish and shellfish contain high-quality protein and other essential nutrients (USFDA and USEPA, 2004) and they are also a dietary source of ω-3 fatty acids; eicosahexaenoic acid (EPA) and docosahexaenoic acid (DHA, exclusively limited to fish and shellfish) (Mahaffey, 2004a). However, fish and seafood consumption is the main if not the only exposure route of methyl mercury, a potential neurotoxin. Therefore, monitoring of mercury levels in seafood is essential for protecting pregnant women and young children, a population highly susceptible to methyl mercury toxicity. Determination of mercury levels in several fish species commonly encountered by fishermen from the Gulf of Oman is an important issue and it is the first main objective of this research.

The question intended to answer is: *is trophic position, as indicated by $\delta^{15}N$-values, significant enough as a predictive tool to account for mercury levels (T-Hg and MeHg) of fish species from the Gulf of Oman, a tropical marine ecosystem?* This question was based on the observation that mercury shows tendency to increase from

lower to higher trophic levels, a phenomenon referred to as Hg-biomagnification. Stable isotopes (mainly $^{15}N/^{14}N$) as more reliable method compared to dietary analysis have been utilized to demonstrate Hg-biomagnification in arctic and temperate marine ecosystems of the northern hemisphere (Jarman *et al.*, 1996; Atwell *et al.*, 1998). Recently, a few studies (Bowles *et al.*, 2001; Campbell *et al.*, 2004) have provided insights on Hg-biomagnification for fish from tropical freshwater lakes, however, studies are still lacking for tropical marine ecosystems. The second main objective is to categorize zooplankton and different fish species using $\delta^{15}N$-values and examine relationship between mercury levels and ^{15}N-signatures.

Chapter 1: Introduction

Heavy metals are metallic elements with high atomic weights. A few of them (e.g. iron, copper, zinc, arsenic and chromium) are regarded as essential for some biological functions in minute quantities, however, most are considered of environmental and human health concerns. This is due to their ubiquity in nature and tendency of some to bioaccumulate and induce toxicity in living tissues. Mercury, as an example, is a global pollutant because of its detrimental effects even on pristine ecosystems. Almost all mercury compounds are toxic, but methyl mercury is the most toxic.

1.1 Mercury in the environment

Mercury (Hg) is the only metal that forms a liquid at room temperature. Naturally, it exists in three valence states: elemental (Hg^0), monovalent (Hg^+) and the divalent (Hg^{2+}). It has been used and consumed in several applications. For instance, electrical apparatus, chlor-alkali industry, paints and industrial instruments have been the major applications. Other uses of mercury have been in other industries such as dental, agriculture, catalysis laboratory uses and pharmaceuticals (Clark, 2001). However, the uses of mercury in several of these applications have been phased out.

Since the discovery of methyl mercury involvement in the Minamata Bay disaster during the 1960s, the direct disposal of mercury-related effluents into aquatic bodies has been greatly reduced. However, environmental researchers are still detecting high mercury concentrations in fish and other biota from pristine ecosystems where no mercury disposal has been known to occur. Two main conclusions can be drawn from the previous statement. First, the atmospheric deposition is the primary source of mercury contamination to the aquatic ecosystems (Rolfhus and Fitzgerald, 1995; Downs *et at*,

1998; Bullock Jr, 2000). Second, mercury is a global pollutant causing detrimental environmental effects at remote locations (Pacyna and Pacyna, 2002).

The ubiquity of mercury in nature is due to natural releases and emissions. The major natural sources include erosion and geological weathering of mercury-enriched bedrocks (Gustin *et al.*, 2000; Gustin *et al.*, 2003), volcanic emissions (Ferrara *et al.*, 2000; Pirrone *et al.*, 2001; Nriagu and Becker, 2003; Pyle and Mather, 2003) and volatilization from the aquatic bodies and earth crust (Ferrara *et al.*, 2000; Pirrone *et al.*, 2001; Gårdfeldt *et al.*, 2003; Schlüter, 2000). Still, the situation is worsened further more due to anthropogenic disposals and discharges. Fossil fuel combustion, non-ferrous metal and iron production, cement production and waste disposal and incineration are the main anthropogenic sources (Pacyna and Pacyna, 2001; Pirrone *et al.*, 2001; Wilhelm, 2001; Pacyna and Pacyna, 2002; Lacerda, 2003).

1.2 Mercury speciation in the environmental compartments

Elemental mercury (Hg^0) or mercury vapor is the major species released from natural (Landis *et al.*, 2002; Ebinghaus *et al.*, 2002; Gustin *et al.*, 2000; Gustin *et al.*, 2003; Pyle and Mather, 2003) and anthropogenic sources (Pacyna and Pacyna, 2002; Pacyna *et al.*, 2001). This chemical form of mercury predominates in the atmosphere with a long (~1 year) residence time (Tokos *et al.*, 1998). Therefore, it is subject to long-range transport from points of production or emission to deposition sites, irrespective of geological barriers or political borders. Because of low solubility in water of 1.8·10-7 to 2.3·10-7 M (Lin and Pehkonen, 1998), the more likely fate of Hg^0 is oxidation to Hg^{2+} via reaction with air-borne particles containing atmospheric oxidants such as hydroxyl radicals, ozone, chlorine or bromine (Downs *et al.*, 1998; Burgan and Rhode, 2001).

Mercury bounded to air-bound particles circulates in the atmosphere until removal by wet precipitation or dry deposition.

The anthropogenic sources also emit relatively high amount of gaseous bivalent (Hg^{2+}) and particulate mercury (Capri, 1997; Pacyna *et al.*, 2001; Pacyna and Pacyna, 2001). These mercury species are more soluble than the elemental and are thought to be the dominant in precipitation and deposition. This is supported by the small organic (methyl mercury) proportion of the total mercury concentrations detected in the precipitation (Downs *et al.*, 1998; Mason *et al*, 1997; Holz *et al.*, 1999).

Organic mercury, particularly methyl mercury (CH_3Hg^+), constitutes the major form in biota. Methyl mercury, either in freshwater or marine aquatic ecosystems, is widely believed to be produced by sulfate-reducing bacteria (Gilmour and Henry, 1991; Gilmour and Henry, 1994; Regnell *et al.*, 1996; Kainz *et al.*, 2003; Loseto *et al.*, 2004). However, recent investigation showed that abiotic processes may also contribute to methyl mercury formation in ecosystems (Celo *et al*, 2004; Siciliano *et al.*, 2005).

1.3 Mercury in marine environment

Since they cover about 70 % of the earth surface, marine aquatic ecosystems are considered as ultimate sinks for deposited atmospheric mercury. It was found that the high oceanic mercury burden resulted from increasing anthropogenic emissions. As a consequence, several marine fish species tend to contain elevated methyl mercury concentrations of 1 to 10 million times higher than that found in the water they live in (USEPA, 2001).

1.3.1 Mercury in plankton

Besides bacteria, phytoplankton, at the base of almost all marine food webs, represent the primary producers. They constitute the diet of higher consumers like zooplanktons, crustaceans and planktivorous fish.

Mason *et al.* (1995) demonstrated that the major difference in absolute levels of mercury occurs between water and phytoplankton. Later, it was discovered that the passive uptake of uncharged, lipophilic chloride complexes is the principle accumulation route of both methyl- and inorganic mercury (Mason *et al.*, 1996). Once incorporated within the phytoplankton, methyl mercury tends to concentrate within the cytoplasm as indicated by Bloom (1992). On the other hand, inorganic mercury is sequestered in the algal membrane via bonding to thiols (-SH groups) (Downs *et al.*, 1998), due to high affinity of inorganic mercury ($Hg2^{+}$) to sulfur. Consequently, zooplankton assimilates methyl mercury in the phytoplankton cytoplasm 4-times more efficiently than inorganic mercury which is principally bound in the phytoplankton membranes (Mason *et al.*, 1996).

1.3.2 Mercury in fish

Mercury is detectable in tissues of almost all marine fish species. Well-known marine species such as marlin, shark, swordfish and tuna contain high mercury levels (Forsyth *et al.*, 2004), sometimes exceeding the international guidelines of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO), US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA), and Health Canada. Methyl mercury is the predominant form in fish regardless of inter- and intraspecies variability. Unlike other lipophilic contaminants, the

predominance of methyl mercury in fish tissues is not merely controlled by lipid solubility. It was shown that inorganic mercury complexes (e.g. $HgCl_2$) are as lipid soluble as their methyl mercury analogs (e.g. CH_3HgCl) (Mason *et al.*, 1996). Bloom (1992) suggested that high content of protein could be responsible for high methyl mercury content of tissues.

Several studies investigated mercury content and speciation in many marine bony fish species (Bloom, 1992; Francesconi and Lenanton, 1992; Andersen and Deplegde, 1997; Phillips *et al.*, 1997; Sydeman and Jarman, 1998; Joiris *et al.*, 1999; Al-Majed and Preston, 2000; Cossa and Gobeil, 2000; Joiris *et al.*, 2000; Kehrig *et al.*, 2002; Storelli *et al.*, 2002a; Baeyens *et al.*, 2003; Love *et al.*, 2003; Storelli *et al.*, 2003) and elasmobranches or cartilaginous fish species (Mol *et al.*, 2001; de Pinho *et al.*, 2002; Storelli *et al.*, 2002b). In most of these studies, the percentage of methyl mercury accounted between 70-100% of the total mercury. However, methyl mercury averages of $\leq 65\%$ were also reported (Al-Majed and Preston, 2000; Joiris *et al.*, 2000; Kehrig *et al.*, 2002; de Pinho *et al.*, 2002).

Mercury burden of aquatic organisms, including fish, is a function of abiotic and biotic factors. Newman and Unger (2003) defined these factors as qualities of the contaminant, the organism, and the environmental conditions under which the organism and contaminant are interacting. The abiotic factors are those characterizing non-living components of the ecosystems (e.g. water, sediment), while biotic factors are defining the organisms (e.g. size, longevity, growth rate, feeding habits and habitats).

Biological or biotic factors are important in determining mercury levels in marine fish because of their influences on physiological and biochemical qualities of the

organism. These, in turn, determine the organism's ability to minimize uptake, biotransform and eliminate the contaminant (Newman and Unger, 2003). It is well-established that size and longevity are the main biotic parameters governing mercury concentrations in fish tissues. Significant, positive correlations between total and/or methyl mercury concentration and body length were reported for many saltwater fish species (Francesconi and Lenanton, 1992; Andersen and Deplegde, 1997; Joiris *et al.*, 1999; Al-Majed and Preston, 2000; Joiris *et al.*, 2000; Kehrig *et al.*, 2002; de Pinho *et al.*, 2002). Few studies revealed no, weak or negative correlations (Francesconi and Lenanton, 1992; Mol *et al.*, 2001; Baeyens *et al.*, 2003). In many of these studies, the authors treated the length as a proxy for age. However, Phillips *et al.* (1997), using sagittal otoliths, found a moderate correlation between total mercury concentration and age of barred sand bass, *Paralabrax nebulifer*. Body weight, as another biotic parameter, showed positive correlation with mercury burden (Al-Majed and Preston, 2000; Storelli *et al.*, 2002; Kehrig *et al.*, 2002).

Ecological characteristics (e.g. feeding behavior) of the organism determine route of exposure and efficiency of the contaminant uptake (Newman and Unger, 2003). Feeding habits and habitats have been examined in relation to mercury contents in several marine species. Francesconi and Lenanton (1992) suggested that there is a relationship between total mercury concentrations and trophic positions. This observation was also noted by others (Joiris *et al.*, 2000; Storelli *et al.*, 2003). In similar-size individuals, de Pinho *et al.* (2002) observed that the picivorous shark species had an average total mercury concentration of 1.96 $\mu g \cdot g^{-1}$, while the invertivorous shark species contained an

average of 0.39 $\mu g \cdot g^{-1}$, indicating feeding habits were responsible for the significant difference in mercury burden.

For habitats, Monteiro *et al.* (1997) found that total Hg-concentrations increased 4-fold from epipelagic to mesopelagic fish species. This could be due to favorable methylation in the mesopelagic zone as indicated by elevated mercury levels of seabirds feeding primarily on mesopelagic fish compared to others preying on epipelagic species (Thompson *et al.*, 1998; Ochoa-acuña *et al.*, 2002). Other investigators (Al-Majed and Preston, 2000; Cossa and Gobeil, 2000; Storelli *et al.*, 2003) reported higher mercury concentrations in benthic and demersal fish species than that of pelagic species.

1.4 Toxicity of fish-borne methyl mercury

Human methyl mercury poisoning was recognized in Minamata, Japan during the 1950s and 1960s. More than 100 people died and several thousands were severely poisoned by MeHg which bioaccumulated in fish as a result of mercury discharge into the Minamata Bay from a local acetaldehyde plant (Kudo *et al.*, 1998). Nowadays, MeHg is an environmental neurotoxin and fish and other seafood consumption is the main route of exposure.

The central nervous system (CNS) is the target of MeHg and the symptoms associated with poisoning include paresthesia (numbness or tingling sensation in the extremities and circumorally), ataxia, and in severe cases, there is a rapid progression to visual and hearing loss (Clarkson *et al.*, 2003; Clarkson and Strain, 2003). Now, it is known that "Minamata Disease" is the end-point of acute MeHg exposure. On the other hand, neurotoxic effects of low-level (i.e. chronic) exposure have been recognized in different fish-consuming populations (Grandjean *et al.*, 1997; Lebel *et al.*, 1998; Renzoni

et al., 1997). Clarkson and Strain (2003) stated that the Minamata outbreak and a subsequent similar one in Niigata provided the first evidence that the fetal brain is especially sensitive to methyl mercury that is ingested by the mother. In consequence of that, long-term and frequent intake of high-mercury content fish would be associated with a risk in pregnant women, nursing mothers and young children.

Furthermore, there is a statistical connection between male subfertility and the level of Hg-hair level in relation to the consumption of contaminated fish (Dickman and Leung, 1998; Dickman *et al.*, 1999). There are also growing concerns about cardiovascular effects of methyl mercury (Sorensen *et al.*, 1999; Salonen *et al.*, 2000; Guallar *et al.*, 2002).

1.5 Stable isotopes and trophic structures

An isotope is one of two or more forms of an element (${}^{A}_{Z}Nu_{N}$) that have the same number of protons (Z), but different number of neutrons (N). For example, most oxygen (${}^{16}_{8}O_{8}$), in nature, has 8 protons and 8 neutrons giving a nuclide with 16 atomic mass unit, while about 0.2% of oxygen has ten neutrons (${}^{18}_{8}O_{10}$). Environmental isotopes are the naturally-occurring isotopes of elements found in abundance in our environment (Clark and Fritz, 1997). Carbon (C), hydrogen (H), nitrogen (N), sulfur (S) and oxygen (O) are principal elements in biological, ecological, geological and hydrological systems. Biologists and ecologists are mostly concerned with natural stable isotopes of C, N and S because they can serve as tracers of energy and nutrients between different compartments of a given system.

Natural abundance isotopes can be used to find patterns and mechanisms at the single organism level as well as to define trophic positions of the whole ecosystem (Lajtha and

Michener, 1994). Isotopes of carbon (${}^{12}_{6}C_{6}$ and ${}^{13}_{6}C_{7}$) and nitrogen (${}^{14}_{7}N_{7}$ and ${}^{15}_{7}N_{8}$) are heavily-utilized in ecological studies. Most carbon exists as ^{12}C (98.89%), while the natural abundance of ^{13}C is just 1.11% and the natural abundance of ^{14}N and ^{15}N is 99.63 and 0.37%, respectively (Clark and Fritz, 1997).

Measurement of the absolute abundance of the isotopes within a sample is not easily done and requires sophisticated mass spectrometers (Clark and Fritz, 1997). However, a simple approach is applied since the main interest is in comparing the variations in the isotope (e.g. heavy ^{13}C vs. light ^{12}C) rather than the actual abundance. Thus, the isotope mass spectrometer measures the ratio of the heavy and light isotope in the sample and compares this to a standard or reference (Lajtha and Michener, 1994). The isotopic concentrations are expressed in 'del' (δ) notation and have units of per mil (‰) according to the following equation:

$$\delta X = \left[\left(\frac{R_{sample}}{R_{reference}} \right) - 1 \right] \cdot 1000$$

Where; X is ^{13}C or ^{15}N

R is the corresponding ratio ^{13}C /^{12}C or ^{15}N /^{14}N

Based on the standard used, positive δ-values indicate an enrichment of the heavy isotope, but negative δ-values signify a decrease in heavy isotope content in the sample relative to the reference. For example, supposed in an isotopic analysis, if $\delta^{15}N$ equals + 10‰ this means that the sample has 10 per mil than the reference (i.e. the sample is enriched by 10‰ more ^{15}N than the reference). Pee Dee Belemnite (PDB) is the accepted reference for carbon with $^{13}C/^{12}C$ abundance ratio of 0.01237, while atmospheric air N_2

is the admitted standard for nitrogen isotope analysis with ^{15}N /^{14}N abundance ratio of 0.003677 (Clark and Fritz, 1997).

1.5.1 Trophic positions

Field observations and gut content examination have been employed for characterizing and defining trophic positions of organisms of a specific ecosystem. This would lead to a reasonable interpretation of trophic status of species, however much uncertainty remains because species in communities have complex feeding strategies that change with age, time and community compositions (Newman and Unger, 2003). The drawbacks of gut analysis include mis-identification or impossible identification of partially or completely digested prey items (Lajtha and Michener, 1994). Gut contents may not necessarily reflect the primary prey items assimilated in the diet. In addition, this type of analysis is time-consuming (DeNiro and Esptein, 1978), and the investigators are required to have adequate knowledge of organisms in that ecosystem (Lajtha and Michener, 1994).

A more accurate method of quantifying trophic status has recently emerged. Isotopic discrimination is the rate or extent to which participation in some biological or chemical processes depends on the mass of the isotope (Newman and Unger, 2003). Basically, it tends to reduce amount of lighter isotopes (e.g. ^{12}C and ^{14}N) in organisms relative to heavier ones (e.g. ^{13}C and ^{15}N). This process is known as trophic fractionation, enrichment or shift (denoted as $\Delta\delta$) and implies that heavier isotopic composition of consumer (predator) is relatively higher in magnitude than that of the diet (prey).

1.5.2 Carbon isotopic ratio ($\delta^{13}C$)

Phytoplankton have average carbon isotope signature of -21‰ in many oceans (Gearing *et al.*, 1984; Peterson and Howarth, 1987; Fry and Sherr, 1988). They are considered the sole source of fixed nitrogen (Fry and Sherr, 1988) for higher organisms such as zooplankton. The stable carbon isotope of phytoplankton depends on the concentration of dissolved carbon dioxide in seawater (Johnston and Kennedy, 1998). The carbon dioxide pool with $\delta^{13}C$-value of 0‰ represents the carbon source of for phytoplankton (Michener and Schell, 1994). Fractionation between dissolved carbon and phytoplankton has been attributed to several factors. These includes that isotopic composition of the dissolved inorganic carbon pool may vary with temperature and latitude, isotopic discrimination may be related to phytoplankton morphology and species composition, growth rates of phytoplankton can affect their carbon isotopic composition and biochemical discrimination by carboxylating enzyme involved in CO_2 fixation (Michener and Schell, 1994; Johnston and Kennedy, 1998). As a consequence, these factors would play combined or individually to affect the isotopic signatures displayed by higher organisms feeding on phytoplankton.

It has been observed that $\delta^{13}C$ shows a trophic enrichment of 0.5 to 1‰ between diet and consumer from laboratory-based studies (DeNiro and Esptein, 1978; Fry *et al.*, 1978; Tieszen *et al.*, 1983; McCutchan *et al.*, 2003) and field surveys (Gearing *et al.*, 1984; Fry, 1988; Rau *et al.*, 1983; Dittel *et al.*, 1997; Vander Zanden *et al.*, 2001; Hart and Lovvorn, 2002). This enrichment has been attributed to preferential loss of $^{12}CO_2$ during respiration, favored biochemical uptake of ^{13}C-compounds during digestion and metabolic fractionation during synthesis of different tissue types (DeNiro and Esptein,

1978; Rau *et al.*, 1983; Tieszen *et al.*, 1983; Michener and Schell, 1994). ^{13}C-trophic shift differs from one ecosystem to another. France and Peters (1997) found that it varies from 0.2 ‰ in freshwater, 0.5‰ in estuarine, 0.8‰ in coastal waters and 1.1‰ in the open ocean.

In marine ecosystems, $\delta^{13}C$ has been used to trace carbon flow in systems and differentiate between contributions of more than one primary producer (Vizzini and Mazzola, 2003; Parsons and Lee Chen, 1995; Kwak and Zędler, 1997; Darnaude *et al.*, 2004) and distinguish between feeding habits of some species (Abend and Smith, 1997; Beaudoin *et al.*, 1999). $\delta^{13}C$ is used for determination of food source. However, because of the small magnitude of transfer from one trophic level to the next, $\delta^{13}C$ has not been useful in characterizing trophic positions.

1.5.3 Nitrogen isotopic ratio ($\delta^{15}N$)

Besides phosphorus, nitrogen is the most important nutrient controlling autotrophic production in marine ecosystem. Although dissolved organic nitrogen represents over 95% of the total nitrogen pool in surface ocean waters, inorganic nitrogen compounds, in particular nitrate and ammonium, are the significant nitogren supporting phytoplankton growth (Owens and Watts, 1998). Therefore, nitrogen isotope ratios in phytoplankton are affected by the abundance and various forms of inorganic nitrogenous compounds (Michener and Schell, 1994). Determination of $\delta^{15}N$ of utilized nitrogen form will ultimately determine $\delta^{15}N$ of phytoplankton. Fixed atmospheric nitrogen by autotrophic organisms gives them low $\delta^{15}N$-values, however phytoplankton utilizing upwelling nitrate (high in $\delta^{15}N$ which left during denitrification) have $\delta^{15}N$-signatures (Michener and Schell, 1994). In turn, zooplankton and planktivorous fish will display

higher $\delta^{15}N$-values. In marine environment, concentration of inorganic (and organic) forms of nitrogen shows vast spatial and temporal variations (Owens and Watts, 1998).

Unlike $\delta^{13}C$, $\delta^{15}N$ undergoes consistent enrichment with an average increase of 3.1‰ from the diet to consumer (Minawaga and Wada, 1984; Schoeninger and DeNiro, 1984; Cabana and Rasmussen, 1994; Dittel *et al.*, 1997; Vander Zanden *et al.*, 2001; Hart and Lovvorn, 2002; McCutchan *et al.*, 2003). Preferential excretion of ^{15}N-depleted nitrogenous wastes during metabolism (Minawaga and Wada, 1984) is the main process responsible for the observed ^{15}N-enrichment.

Because of clear and relatively large changes from one trophic level to the next, $\delta^{15}N$ can be used alone as an indicator of trophic position. Minagawa and Wada (1984) utilized it and could reveal trophic structure of diverse marine communities. Several studies (Pinnegar and Poluin, 2000; Kaehler *et al.*, 2000; Dunton, 2001; Gurney *et al.*, 2001; Davenport and Bax, 2002; Estrada et al., 2003; Fredriksen *et al.*, 2003) have demonstrated the usefulness of $\delta^{15}N$-application in marine food web characterization.

1.6 Mercury biomagnification

Because of the consistent enrichment per trophic transfer, $\delta^{15}N$ has been used to investigate whether mercury biomagnifies up food chains in freshwater and marine ecosystems. In freshwater lakes, for example, Kidd *et al.* (1995) described total mercury biomagnification in fish species from different Canadian lakes. Biomagnification power, as indicated by slopes of the regression equations between $\delta^{15}N$ and mercury, ranged between 0.17 to 0.48 suggesting mercury biomagnification (Kidd *et al.*, 1995). Power *et al.* (2002) found mercury to biomagify in the fish community of a Canadian sub-arctic

lake with biomagnification power of 0.19, while $\delta^{13}C$ signatures were inversely related to mercury levels.

Concerning mercury in marine ecosystems, several studies contended that Hg biomagnified in long, well-characterized food webs (Jarman *et al.*, 1996; Atwell *et al.*, 1998), but others did not show a correlation between $\delta^{15}N$-values and mercury concentrations (Camusso *et al.*, 1998). In marine birds, Thompson *et al.* (1998) found no correlation between feather $\delta^{15}N$ and mercury content. On the other hand, Bearhop *et al.* (2000a) and (2000b) indicated that $\delta^{15}N$ had influence on tissue Hg concentrations. Details of these studies and other (Yoshinaga *et al.*, 1992) are provided in table 1.1.

Concerning marine ecosystems, it is important to note that the investigations have been focused in subarctic and temperate zones of the northern hemisphere. In the tropics, Yoshinaga *et al.* (1998) explored relationships between $\delta^{15}N$ and 13 elements in animals mainly from terrestrial and freshwater ecosystems. It is worthwhile to point out that all studies (except Yoshinaga *et al.*, 1992) investigated only total mercury. Compared to other taxa, few fish species were included and appeared to occupy the same trophic position as indicated by $\delta^{15}N$-values. In addition, biological parameters (size and age) received no attention except Atwell *et al.* (1998) who showed no evidence of Hg-biomagnification with age in clams (*Mya truncata*) and ringed seals (*Ursus maritimus*). Thompson *et al.* (1998) suggested uncoupled association between marine bird feather $\delta^{15}N$ and Hg because it is well known that contaminants are often excreted through feathers (Gray, 2002). Therefore, higher concentrations of Hg in the feather would not necessarily have anything to do with accumulation by means of a food web (Gray, 2002). Bearhop *et al.* (2000a and 2000b) measured $\delta^{15}N$-signatures and Hg concentrations of

blood samples from great skua (*Catharacta skua*) chicks. Blood is a good indicator of recent exposure, but it seems not to be adequate to investigate Hg-accumulation over a relatively long time period.

1.7 Observations, hypotheses, predictions and objectives

Identification of inorganic and organic pollutants has become an important issue in the Gulf Region within the past few years. Marine environments represented by the Arabian Gulf, Gulf of Oman and Arabian Sea received high attention because of their importance as seawater source for clean desalinated freshwater, non-living sources (crude oil and natural gas), fisheries, and tourism. Several environmental studies were conducted after the 1991 Gulf War particularly in the Arabian Gulf due to massive oil spills (Fowler *et al.*, 1993; Michel *et al.*, 1993; Sauer *et al.*, 1993; Ehrhardt and burns, 1993; Krahn *et al.*, 1993; Al-Yaqoob *et al.*, 1993; Basaham *et al.*, 1993; Kureishy, 1993).

Oman overlooks 2092 km (1300 miles) of coastline mainly on the Gulf of Oman and Arabian Sea. The Gulf of Oman (Figure. 2.1) is defined by the hypothetical line between Ras Al-Hadd (Oman) and Ras Al-Fasteh (Iran) and it extends northwest till the Strait of Hormuz (Wilson, 2000), the entrance to the Arabian Gulf. The coastline can be viewed as two distinct zones. Between Ras Al-Hadd and Muscat, the 200-km coastline is rocky shore while the 320-Km sandy beach of Al-Batinah sweeps from Muscat northward to Al-Fujeirah, an Emirate of the United Arab Emirates (Wilson, 2000). The remaining 80 km rocky coastline is overlooked by Musandam (part of Oman) and Al-Fujeirah. The depth ranges between 100 m near the Strait of Hormuz to more than 3000 m in the open sea (Wilson, 2000).

Table 1.1: Previous mercury biomagnification studies using $\delta^{15}N$ in marine ecosystems

Study area	Investigated organisms	$\delta^{15}N$ of fish species	Study conclusion	References
The Gidra's Territory (New Papua Guinea)	Insects, crustaceans, shellfish, fish (3), reptiles, birds and mammals	11.8-12.9	Significant correlation between total, inorganic and organic mercury and $\delta^{15}N$	Yoshinaga *et al.*, 1992
Gulf of the Farallones (USA)	Invertebrates, fish (2), seabirds and marine mammals	13.3-14.7	Strong correlation of total mercury with $\delta^{15}N$	Jarman *et al.*, 1996
Lancaster Sound (Canada)	POM, invertebrates, fish (2), seabirds and marine mammals	14.6-16.4	Total mercury was correlated to mercury	Atwell *et al.*, 1998
Sacca del Canarin lagoon (Italy)	A macroalga, a bivalve, a gastropod and fish (3)	11.2-11.9	$\delta^{15}N$ and T-Hg were related macroalga, bivalve and gastropod, but not for fish	Camusso *et al*, 1998
Foula and St. Kilda (UK)	Seabirds	-	Mercury shows no correlation with $\delta^{15}N$	Thompson *et al*, 1998
Foula and St. Kilda (UK)	Seabirds	-	Significant correlation between $\delta^{15}N$ signatures and total mercury of blood samples	Bearhop *et al*, 2000a and Bearhop *et al*, 2000b

* Number of marine fish species included in each study is indicated in (parenthesis).

Although tropical, this part of the world is extremely arid with very low precipitation levels. The prevailing climate is hot with humid coastal areas (Wilson, 2000). The mean 24-hour temperatures are lowest in January (20°C) and rise to a peak of 35-43°C in summer. Maximum daytime summer temperatures may reach 50°C, whilst the minimum night-time temperatures in winter can drop to 6°C (Wilson, 2000). The maximum summer seawater temperature is 32°C (Wilson, 2000), but Coles (1997) found that the 24-hour fluctuation of seawater temperature in the Gulf of Oman can be up to 8°C. Over 90% of rainfall occurs in winter with much of the remainder falling during July and August (Wilson, 2000). It is important to note that most rain falls in heavy showers and storms and in some years up to 55% of the year's rain (~100 mm per year) may fall in a 24-hour period (Wilson, 2000). Therefore, terrestrial runoff into the Gulf of Oman is extremely limited taking into consideration that there is no permanent flow of rivers or streams from terrestrial Oman.

Sheppard *et al.* (1992) described the part of the Indian Ocean bordering Arabia as a rich and complex ecosystem of coral reefs and mangrove coastlines with deeper water communities. This part divides into several, large, semi-enclosed water bodies leading to often quite distinct habitats in close proximity to each other (Sheppard *et al.*, 1992). The Gulf of Oman supports high species diversity of phytoplankton approximately with 527 species dominated by dinoflagellates (Rao and Al-Yamani, 1998). Very high abundance of zooplankton (particularly copepods) is believed to be the best explanation for large fish stocks in the Gulf of Oman and Arabian Sea (Gjøsaeter, 1984). Concerning fish, a total of 1142 species have been identified from the Gulf of Oman and Arabian Sea

(Fouda *et al.*, 1998) with about 400 pelagic and 500 demersal species. Certainly, this Gulf shows high species diversity which is a typical feature of tropical marine ecosystems.

Several studies have been conducted in the Gulf of Oman to monitor chemical pollutants. However, they have intensively focused on oil and hydrocarbon contamination (El-Samra and El-Deeb, 1988; Burns *et al.*, 1982; Readman *et al.*, 1992; Fowler *et al.*, 1993; Emara *et al.*, 1990; Ahmed *et al.*, 1998a; Ahmed *et al.*, 1998b; Coles and Al-Riyami, 1996; Sharidah, 1999; Abu-Hilal and Khordagui, 1994; Sharidah, 2000). Few investigations were devoted to heavy metals (Burns *et al.*, 1982; Fowler *et al.*, 1984; Fowler *et al.*, 1993; de Mora *et al.*, 2004), organotin compounds (de Mora *et al.*, 2003) and radionuclides (Goddard and Jupp, 2001; Goddard *et al.*, 2003). There have been no previous studies on mercury in biota from the Gulf of Oman except the most recent one of de Mora *et al.* (2004). They only determined total mercury in two commercial fish species and a bivalve species.

To diversify the economy away from a reliance on oil, Oman has embarked two major chemical industrial projects on the Gulf of Oman and therefore mercury and other pollutant monitoring programs have become a necessity.

Regarding possible effect on isotopic signatures, little treated sewage is discharged to the sea from coastal cities because of re-cycling wastewater for agricultural and municipal irrigation (Wilson, 2000). Silver (Ag) is a good indicator of sewage pollution in the marine environment (Ravizza and Brothner, 1996), but de Mora *et al.* (2004) found very low sediment Ag concentrations (0.002- 0.010 $\mu g\ g^{-1}$ dry wt.) in the Gulf of Oman compared to that (~0.010-0.58 $\mu g\ g^{-1}$ dry wt.) of the Arabian Gulf. Therefore, sewage incorporation at the base of the food chain seems minimal.

I postulated that the isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) of investigated organisms would reveal variability in carbon and nitrogen sources. The overall hypothesis to be tested is that *$\delta^{15}N$ is will predict trophic positions intersepcifically (between zooplankton and fish) and intraspecifically (among fish species) and that trophic positions will be related to mercury biomagnification.* This could be subdivided into several hypotheses and qeutions that can be statistically examined:

I. *The investigated fish species show differences in isotopic signatures based on their feeding habit classification but not based on their habitat.*

Do tissue isotopic concentrations differ based on classifiying fish based on their habitats and feeding strategies?

II. *There are significant differences between mercury levels (T-Hg and MeHg) in the investigated organisms.*

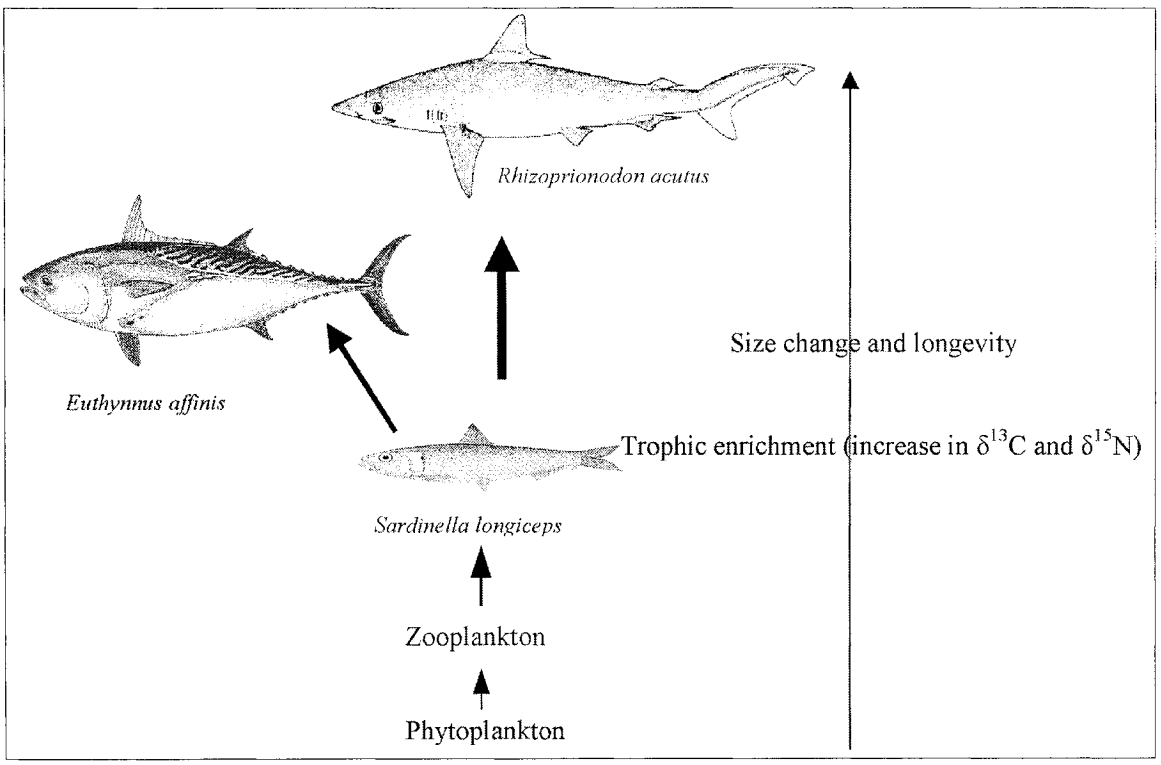
Are mercury concentrations (T-Hg and MeHg) of fish species similar?

III. *There is a linear relationship between mercury levels (T-Hg and MeHg) and $\delta^{15}N$-signatures.*

Is T-Hg or MeHg related to $\delta^{15}N$-values of the zooplankton and fish?

It is expected that $^{13}C/^{12}C$ will demonstrate whether there is variability in carbon sources available for these fish species. $^{15}N/^{14}N$ will differentiate fish species into distinct trophic positions. The most important prediction regarding mercury levels is that T-Hg and MeHg concentrations should be fairly low. Figure 1.1 shows the hypothetical Hg-biomagnification model for known prey-predator relationships of cetain fish species from the Gulf of Oman and included in the analysis.

Figure 1.1: The hypothetical Hg-biomagnification/accumulation model for known prey-predator relationships of *S. longiceps*, *E. affinis* and *R. acutus* from the Gulf of Oman. Thickness of the arrows indicates mercury level transfer.



The study was designed to achieve the following objectives;

- To determine mercury levels of several fish species from the Gulf of Oman and ascertain if they are within international guidelines.
- To categorize the investigated species into trophic positions based on their $\delta^{15}N$-signatures.
- To examine extent to which age, size parameters (length and weight) and trophic position ($\delta^{13}C$ and $\delta^{15}N$ values) affect mercury accumulation in fish species.
- To explore mercury biomagnification with the aid of $\delta^{15}N$-signatures as trophic position indicator.

Chapter 2: Materials and Methods

2.1 Study areas

On the coast of the Capital Area of Muscat, coastal waters of A'Seeb and Matrah were the sampling sites and they are about 40 km apart (Figure.2.1). A'Seeb is the highest populated city (223267 people, Census 2003) of the Capital Area and it supports a large community of fishermen. Matrah is the city of the Sultan Qaboos Port, the trading port of the northern Oman. Unlike sandy the beaches of A'Seeb, the coastal area of Matrah is rocky and bordered by high cliffs. There is no past direct point source of mercury known in the area. However, the three major industries situated along the coast between the two cities (oil refinery, water desalination and electricity production plant and the oil-tanker port).

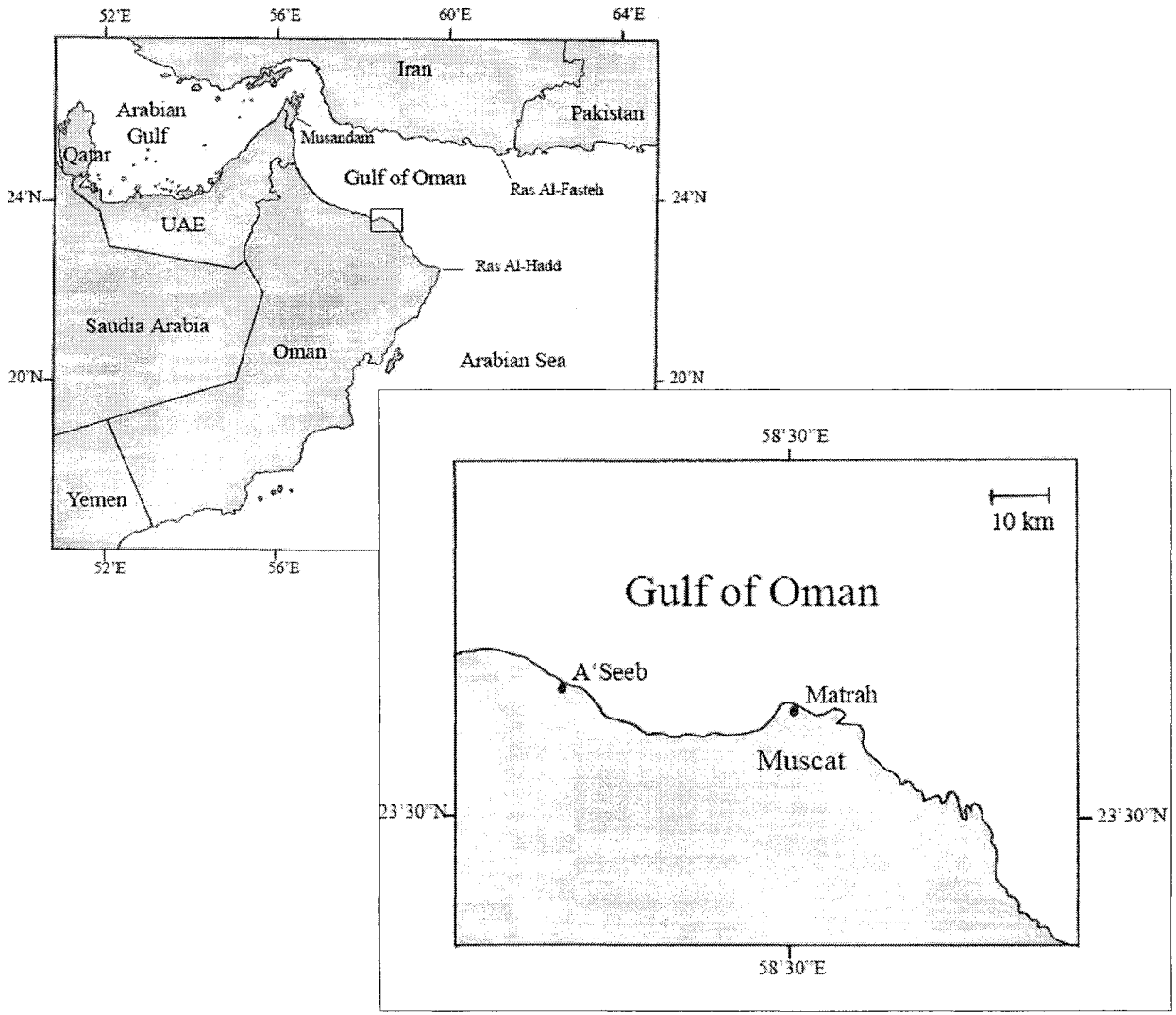
2.2 Sample Collection

Samples of zooplankton and different fish species were collected between the end of May and June, 2004. Some samples were obtained through joining staff of Department of Fisheries and Marine Sciences (DFMS), College of Agriculture and Marine Sciences at Sultan Qaboos University (SQU).

2.2.1 Zooplankton

Zooplankton collection was made using 250 μm-mesh net. The net was fastened to the back edge of the boat and towed horizontally while the boat was plowing slowly. After each collection, the samples were poured into 1-L plastic bottles with excess amount of seawater. In the laboratory, the excess seawater was poured out and zooplankton was transferred to 15 ml falcon tubes with little amount of seawater and kept frozen at -20 °C.

Figure 2.1: Map of the Gulf of Oman showing the sampling locations.



2.2.2 Fish species

Due to high fish diversity, 13 fish species (12 bony fish and 1 elasmobranch species) were selected to be included in the analysis (Table 2.1). Fish species were selected because they are commonly consumed by human. Some species like *Thunnus tonggol* is an important pelagic commercial fish (Wilson, 2000) and *Epinephelus epistictus* and relatives are highly valuable fish in the Gulf Region. Demersal fish species were collected by towing at depth range of 20-40 m. Fish species purchased from the artisanal fishermen were collected by different means. For example, jacks, mackerel, tunas and barracuda are usually caught using gill netting, trolling and handlines (Sheppard *et al*, 1992). Beach seining is well known in the Gulf of Oman for sardines (e.g. *Sardinella longiceps*) (Wilson, 2000).

Once collected, fish were put in cooler on ice and transported to the fish research laboratory at the DFMS. There, fish were identified to species level using Randle (1995). Then, total and fork length and weight were recorded for each individual fish (Table 2.2). Based on the fish size, 5-15 grams of boneless, skinless left side dorsal muscle were obtained using scalpel and forcep and placed in 15 ml polyethylene falcon tubes. Dissecting tools were thoroughly washed with 10% HNO_3 and deionized water before and after each fish. A new fish scalpel was used for each fish species. The samples were stored frozen at -20 °C.

The zooplankton and fish samples were transported in cooler filled with dry ice (solid CO_2) to the University of Ottawa.

Table 2.1: Biological characteristics of the investigated fish species from the Gulf of Oman.

Fish species	Family	Distribution	Habitat	Feeding habit
Sardinella longiceps	Clupeidae	tropical	Pelagic	Planktivorous
Selar crumenophthalmus	Carangidae	subtropical	Reef-associated	Planktivorous
Rastrelliger kanagurta	Scombridae	tropical	Reef-associated	Planktivorous
Argyrops spinifer	Sparidae	tropical	Demersal	Invertivorous
Carangoides malabaricus	Carangidae	tropical	Reef-associated	Opportunistic predator
Epinephelus epistictus	Serranidae	tropical	Demersal	Opportunistic predator
Euthynnus affinis	Scombridae	tropical	Pelagic	Opportunistic predator
Thunnus tonggol	Scombridae	tropical	Pelagic	Opportunistic predator
Rhizoprionodon acutus	Carcharhinidae	tropical	Benthopelagic	Opportunistic predator
Scomberoides tol	Carangidae	tropical	Reef-associated	Piscivorous
Sphyraena sp.	Sphyraenidae	tropical	Reef-associated	Piscivorous
Nemipterus randalli	Nemipteridae	tropical	Demersal	Unknown
Upeneus doriae	Mullidae	Subtropical	Demersal	Unknown

From Randle (1995) and Collette (1983).

Reef associated: living and feeding on/near coral reefs.
Demersal: living on/near the bottom.
Pelagic: free swimming in the seas.
Opportunistic predator: feeding on variety of prey items (particularly fish and crustaceans).
Planktivorous: feeding on plankton (Phyto- and zooplankton).
Invertivorous: feeding on benthic invertebrates.
Piscivorous: feeding on fish.

Table 2.2: Biometry (length and weight) of fish species collected from the Gulf of Oman.

Fish species	English name	*Arabic name (Omani name)*	*N*	Length [mean ± SD] (cm)	Weight [mean± SD] (grams)
S. longiceps	Indian Oil Sardine	Uma	16	16.19 ± 0.46	37.00 ± 2.37
S. crumenophthalmus	Bigeye Scad	Sima	14	20.26 ± 2.92	92.86 ± 48.30
R. kanagurta	Indian Mackerel	Garfa	12	29.02 ± 0.95	294.17 ± 30.63
A. spinifer	King solider Bream	Kawfar	12	25.70 ± 2.30	363.25 ± 91.57
C. malabaricus	Malabar Jack	Sall	12	25.33 ± 3.29	217.50 ± 96.99
E. epistictus	Dotted Grouper	Hamour	12	42.51 ± 4.44	1193.75 ± 376.81
E. affinis	Kawakawa	Sadah	9	55.49 ± 6.42	2237.33 ± 648.80
T. tonggol	Longtail Tuna	Sahwa	10	73.49 ± 3.40	4212.80 ± 366.67
R. acutus	Milk Shark	Jarjur	7	79.34 ± 6.69	2413.14 ± 689.01
S. tol	Needlescale Queenfish	Seen	12	40.93 ± 2.49	400.67 ± 71.16
Sphyraena sp.	Barracuda	Kadd	11	59.18 ± 10.18	899.55 ± 373.91
N. randalli	Randhal's Threadfin Bream	Andaq	12	25.50 ± 2.53	108.58 ± 26.71
U. doriae	Gilded Goatfish	Sultan Ibrahim	11	27.05 ± 1.59	215.18 ± 37.61

2.2.3 Age determination

The age of fish species were determined using the methods of Secor *et al.* (1991). In brief, sagittal otoliths were removed from fish, cleaned in water, air-dried and then stored in small plastic vials. Then, they were embedded in epoxy resin and sectioned transversely. The sections were mounted on glass slide and examined under light microscope. Increments were counted and each one consisting of opaque and translucent zones represented a year.

2.3 Stable isotope analyses

Isotopic compositions ($^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios) of the zooplankton and fish samples were analyzed using an elemental analyzer, EA1110 (Carlo Erba instruments, Italy) connected to an isotope ratio mass spectrometer, Delta^{Plus} Advantage IRMS (ThermoFinnigan, Germany) at the G.G. Hatch Isotope Laboratories, Department of Earth Sciences, University of Ottawa.

2.3.1 Zooplankton

Zooplankton samples were lyophilized by a Freeze Dryer 3, model 75200 (Labconco Corporation, Missouri, USA) for 24 hours. The freezed-dried materials were ground into a fine powder using a porcelain mortar and pestle. The powedered samples were stored in closed scintillation vials which were kept in a desiccator. About 800-1000 μg of the powdered zooplankton samples were weighted into 3.5 x 5.0 mm tin capsules (Elemental Microanalysis Ltd., Iso-mass Scientific Inc., Alberta, Canada).

2.3.2 Fish

After thawing at room temperature, a small portion of 3.0-5.0 g of wet weight fish tissues were obtained using pre-cleaned scalpel and forcep and placed in aluminum dish (general purpose aluminum weighing dish, VWR International). The scalpel and forcep were dipped in diluted nitric acid and then rinsed thoroughly with deionized water after each sample preparation. The tissues were dried in oven (®Stabil-Therm Blue M Electric Company, Blue Island, Illinois, USA) at temperature range of 60.0 to 61.5 °C for 24 hours. The oven-dried samples were fine-powdered with a mortar and pestle. The powdered fish samples were stored in closed scintillation vials which were kept in a desiccator. About 700 µg of powdered fish tissues were packed in 3.5 x 5.0 mm tin capsules.

2.3.3 Stable isotope quatification and quatity control measurements

Measurement process of carbon and nitrogen isotopes involved conversion of the samples into gases and then quantification of the isotopic compositions. Simply, the tin capsules were introduced into the elemental analyzer (EA), where each individual sample was flash-combusted at ~ 1800 °C. The resulting gases were carried via high purity helium gas through oxidation and reduction tubes to purify and separate into carbon dioxide (CO_2) and nitrogen gas (N_2). Finally, the CO_2 and N_2 were transferred to the isotope ratio mass spectrometer (IRMS), where the heavy isotope concentrations were quantified relative to a standard reference material (PDB for carbon and atmospheric N_2 for nitrogen.

A batch of 32 samples was analyzed per run. Before the run, the linearity and "peak jump setting" were checked using three external standards. These are caffeine, glycine and methionine. Prior to the analysis, a blank consisting of an empty 3.5 x 5.0 mm tin capsule was assayed. Then, triplicate samples of the external standards were analyzed just after the blank and one sample of the standards was analyzed every 8 samples. They were used to normalize the data and to check whether there was any drifting throughout the run. The reason to use the caffeine, glycine and methionine is the difficulty to find a homogenous material with isotope signature for both C and N in appropriate ranges of the samples being analyzed. Periodically, the G.G. Hatch Laboratory checks these materials against international standards (ammonium sulfate, IAEA N-1 and IAEA N-2, sucrose, IAEA CH-6 and *L*-glutamic acid, USGS-41).

The 2-σ standard deviation of the internal standards (caffeine, glycine and methionine) was ± 0.20‰, confirming the reproducibility of the method employed to determine isotopic compositions of the samples.

2.4 Total mercury analysis

Total mercury (T-Hg) of the samples was determined by an automatic mercury analyzer, MERCURY SP-3D analyzer (Nippon Instruments Corporation, Japan). The samples required no chemical treatment prior to the analysis.

2.4.1 T-Hg in zooplankton

Using an acid-washed spatula, between 5.5 to 32.2 mg of freezed-dried zooplankton samples were placed on a layer of an additive (mixture of sodium carbonate and calcium hydroxide, EMD Chemicals Inc.) in a ceramic boat. Then, the sample was

covered with a layer of the same additive. A layer of aluminum oxide (Al_2O_3, EM Science) was placed on the sodium carbonate-calcium hydroxide layer. This aluminum oxide layer was covered by another layer of the latter one. After that, the boat was transferred manually into the ceramic thermal decomposition champer. Each sample took 15 min to be analyzed.

2.4.2 T-Hg in fish species

After thawing at room temperature, about 10-100 mg of wet weight tissue were obtained from each fish sample using new scalpel for each species. The dissecting tools were rinsed thoroughly with diluted acid and then deionized water between samples. The same procedures of zooplankton processing were applied for fish samples.

2.4.3 T-Hg quantification and quality control measures and assurance

MERCURY SP-3D was calibrated by a 0.05 ppm mercury standard solution with 0.001% L-cysteine as recommended by the instrument manual. Six standards (0.0, 0.5, 1.0, 2.0, 5.0 and 10 ppb) were consecutively injected to set the calibration curve. Each sample was analyzed in duplicate. A standard of 2.0 ppb was used to check reproducibility of the machine every 5 samples. Accuracy was also assessed every 5 samples using marine sediment (MESS-3, NRC) during zooplankton and some fish analyses and non-defated lobster hepatopancreas (LUTS-1, NRC) during mercury determination in fish species.

In principle, the analyzer completely heat-decomposes the sample into gas in which vaporized mercury is collected in the form of gold amalgam on the surface of trap or collector agent (gold-coated chromosorb) (Instruction Manual for MERCURY SP-3D). The gold-coated trap is then heated to liberate the mercury in the form of atomized

mercury which is transferred to an absorption cell that measures the quantity of mercury by the cold vapor atomic absorption (CVAA) method using wavelength of 253.7 nm (Instruction Manual for MERCURY SP-3D).

Table 2.3 lists the experimental and certified values of the reference materials used during total mercury analyses of zooplankton and fish species.

Table 2.3: Measured (± 1standard deviation) of total mercury levels found in certified reference compared to expected values.

Reference Materials	*N*	Experimental value ($\mu g\ g^{-1}$ dry wt.)	Certified value ($\mu g\ g^{-1}$ dry wt.)
LUTS-1*	37	0.116 ± 0.007	0.112 ± 0.015
MESS-3**	10	0.089 ± 0.005	0.091 ± 0.009

*Non defatted lobster hepatopancreas reference material for trace metals was dried to constant weight by heating at 105°C for 2 hours as described in LUTS-1 manual.

**Marine Sediment

2.5 Methyl mercury analysis

Determination of methyl mercury concentration was carried out by capillary gas chromatography coupled with atomic fluorescence spectrophotometry (GC-AFS) as described by Cai *et al.* (1997). Due to low levels of MeHg found in the preliminary sample analyses, only 5 composite samples were used for MeHg determination.

2.5.1 Sample preparation

About 0.3 g of composite freeze-dried zooplankton samples and between 0.05-0.1 g of wet weight fish samples were placed in 20-ml scintillation vials with PTFE caps. Then, 2.0 ml of deionized water and 2.0 ml of 6 N potassium hydroxide solution were added and the samples were shaken for 4 h at 300 rpm. After that, 2.0 ml of 6N hydrochloric acid were added and the pH was checked to be < 3.0 (high acidic medium). Four millimeters of acidic (5 %v/v H_2SO_4) potassium bromide/ 1.0 copper sulfate mixture were added. To extract methyl mercury into the organic phase, weighted 5.0 ml of methylene chloride were added and the vials were shaken overnight at 300 rpm. The next day, the samples were centrifuged for 10 min at 2500 rpm. Weighted 2.0 ml the methylene chloride were transferred to 7-ml glass tubes and 1.0 ml of 0.01 M sodium thiosulfate was added to each sample. The samples were shaken for 20 min, mixed on a vortex mixer and centrifiuged for 5 min at 5000 rpm. A volume of 0.4 ml of the aqueous top layer (sodium thiosulfate) was placed in polyethylene microcentrifuge vials and 0.3 ml of acidic potassium bromide/ 1.0 copper sulfate mixture and 0.5 ml of dichloromethane were added. Again, the vials were shaken for 15 min, mixed and centrifuged. Finally, the lower phase (dichloromethane containing the extracted MeHg) was extracted carefully and transferred through a small layer of anhydrous sodium sulfate (packed in a piptte tip) to 2-ml amber glass vial with a 200-µl glass insert.

Duplicate spiked samples were prepared every 5 samples. Blanks, standards and certified reference samples were treated in the similar way of the sample analytical preparation.

2.5.2 MeHg quantification and quality control measures and assurance

All glass ware was washed with hot water and soap followed by rinsing with 10% HNO_3 and then rinsed thoroughly with deionized water. On a monthly basis, a 10-ppm solution was prepared from 100 ppm methyl mercury chloride stock standard. The 10-ppm solution was subsequently diluted with deionized water to 200 and 5 ppb. On the day of the sample analyses, a five-point calibration curve (0.00, 1.25, 2.50, 3.75, 5.00 ppb) was made using the 5-ppb standard. The samples were analyzed in duplicate. One of these standards used to make calibration curve and a certified reference material (DORM-2, NRC) were run every five samples to evaluate reproducibility and accuracy of analysis method.

The autosampler injected 2 or 5 µl of blanks, standards, reference material samples and the samples into the gas chromatography, GC (PerkinElmer, Model 8410). The injector temperature was set at 250°C. A fused silica analytical column (dimensions of 15 m x 0.53-mm i.d, Megabore, and coated with a 1.5-µm film thickness of DB-1, J & W Scientific) connects the injector to pyrolyzer. The temperature of the pyrolysis oven was maintained at 800°C. The column temperature was held at 40°C for 30 seconds, programmed at 30°C min^{-1} to 85°C which was retained for 1 min, then programmed at 20°C min^{-1} to a final temperature of 200°C, and then held for 1 min. A split/splitless injector was used in the spiltless mode and maintained at 150°C. The carry gas flow was 4.0 ml min-1 of helium and the make-up gas flow was 60 ml min-1 of argon. The detection was carried achieved by atomic fluorescence spectrophotometry, AFS (Merlin Model 10.123, P.S. Analytica Ltd., Kent, UK).

The average recovery of the spiked samples (n = 54) was 92 ± 12 % (70-114%). The experimental value found for *DORM-2* was 4.15 ± 0.57 $mg \cdot kg^{-1}$, which represents 92% of the certified value of 4.47 ± 0.32 $mg \cdot kg^{-1}$.

2.6 Statistical analyses

Data were statistically analyzed using SPSS for Windows (Release 11.0.0, 2001). All statistics were reported at significance level of $p = .05$. Since data violated parametric assumptions, Kendall's tau (τ) was used to examine the relationship between size parameters (length and weight), age, $\delta^{13}C$, $\delta^{15}N$, T-Hg and MeHg for each species because of small data set. On the other hand, Spearman rank correlation (r_s) analysis was conducted to inspect correlations of $\delta^{13}C$ versus $\delta^{15}N$ and T-Hg versus MeHg for zooplankton and fish samples.

ANOVA counterpart nonparametric Kruskall-Wallis test (statistic denoted by *H*) was used to explore difference in stable isotopes and mercury concentrations between individual species, species grouped their habitats (demersal, pelagic and reef-assocaited) and species categorized by their feeding strategies (planktivorous, invertivorous, unknown, piscivorous and predatory species). Also, nonparametric *Post hoc* procedure, Mann-Whitney test (statistic denoted by *U*), was employed to follow up significant findings of the Kruskall-Wallis test. To reduce the type I error, a *Bonferroni correction* was applied and so all effects were reported at significance level of .05/number of Mann-Whitey tests or comparisons used (Field, 2005).

Linear regression models were first built on length, the factor known from previous research to be the best predictor of mercury concentrations in fish. Then, $\delta^{13}C$ and $\delta^{15}N$ were included. The models were assessed first based on the length and then

based on $\delta^{13}C$ and $\delta^{15}N$. The choice of length over weight was because to meet non-collinearity assumption required by the linear regression. The following four assumptions required by model I regression were checked; non-multilinearity, non-curvilinearity, independence of errors and normal distribution of variance of residuals. To achieve these assumptions, T-Hg and MeHg values were log-transformed.

Chapter 3: Results

Mean values of $\delta^{13}C$ and $\delta^{15}N$ for zooplankton and fish species collected from the Gulf of Oman are shown in table 3.1.

3.1 Carbon isotopic ratio

$\delta^{13}C$-values of zooplankton exhibited wide range of variability (-16.28 to -24.56 ‰). Although the maximum value (-16.28‰) was distinct from the rest, this sample was not a good representation of a typical zooplankton sample because it contained visible gelatinous parts of unidentified marine creatures, fish scales and green algae. Fish scales were removed from the sample prior to drying. Microscopic examination of the samples revealed that they were mainly composed of copepods. Few samples included shrimp larva and they tended to have higher $\delta^{13}C$-signatures than those containing copepods.

The isotopic carbon signatures of zooplankton were significantly depleted compared to that of fish species ($U = 56.00, p < .001$, r = -.60). It was found that the differences in mean $\delta^{13}C$-values between zooplankton and planktivorous fish species were 3.1‰ for *Selar crumenophthalmus*, 3.5‰ for *Sardinella longiceps* and 3.4‰ for *Rastrellinger kanagurta.*

The $\delta^{13}C$-values of fish species ranged between -18.59‰ in *Epinephelus epistictus*, and -11.31‰ in *Upeneus doriae*. For each fish species, the heavy isotopic carbon signatures were not related to length or weight. Correlations for all species are provided in table in appendix (Table A-1).

Among fish species, there was a significant difference between $^{13}C/^{12}C$ ratios ($H(12) = 102.54, p < .001$). They revealed that *C. malabaricus*, *Rhizoprionodon acutus* and *U.doriae* had the least negative similar $\delta^{13}C$-signatures. Two demersal species,

Argyrops spinifer and *Nemipterus randalli* had similar isotopic values, as did the two piscivorous fish, *Scomberoides tol* and *Sphyraena* sp. The most depleted signatures were found, as predicted, in the planktivorous species (*S. crumenophthalmus*, *S. longiceps* and *R. kanagurta*) and surprisingly in predatory fish species (*Euthynnus affinis*, *Thunnus tonggol* and *E. epistictus*). They all had similar δ^{13}C-values.

On habitat based classifications, Figure 3.1 shows mean δ^{13}C-signatures of reef-associated, demersal or deep-sea and pelagic fish species. The average for zooplankton was presented just for comparison. Demersal fish species exhibited higher δ^{13}C (mean = -15.72 ‰) than pelagic with mean of -16.47‰ ($U = 297.00$, $p < .001$, $r = -.54$). The carbon isotopic signatures of demersal fish were marginally different from the reef-associated species, having a mean of -16.06‰ ($U = 990.00$, $p = .013$, $r = -.24$). The marginal significance and small size effect ($r = -.24$) indicates that some species from the two habitats share the same carbon source. No significant difference was observed between pelagic and reef-associated species ($U = 757.00$, $p = .027$, $r = -.23$).

On feeding habit classification, there was a significant difference in δ^{13}C-values ($H(4) = 97.36$, $p < .001$) of planktivorous, invertivorous, unknown, piscivorous and predatory fish species (see table 3.2 for mean values). The planktivorous fish species had significantly depleted δ^{13}C-signatures compared to invertivorous ($U = 37.50$, $p < .001$, $r = -.60$), unknown ($U = 44.00$, $p < .001$, $r = -.74$), piscivorus ($U = 119.50$, $p < .001$, $r = -.62$) and opportunistic predators ($U = 550.00$, $p = .003$, $r = -.32$). The $^{13}C/^{12}C$ of invertivorous, unknown, piscivorous and predatory fish species were found to be similar except that of predatory species appeared slightly depleted to that of unknown species ($U = 256.00$, $p = .003$, $r = -.37$).

3.2 Nitrogen isotopic ratio

δ^{15}N-signatures of zooplankton ranged between 8.47 to 13.58‰. The nitrogen isotopic concentrations were significantly depleted in ^{15}N relative to the fish species (U = 10.00, $p < .001$, r = -.62).

For fish species, δ^{15}N-values spanned 5.94‰ from 12.54‰ in the opportunistic predator, *E. affinis* to 18.48‰ in piscivore, *Sphyraena* sp. and predator *C. malabaricus*. Significant difference existed between ^{15}N/^{14}N ratios of fish species ($H(12) = 118.88$, $p <$ .001). Opportunistic predators (*C.malabaricus*, *R. acutus* and *E. epistictus*) and pisivore *Sphyraena* sp. had the most enriched similar δ^{15}N-values. *A.spinifer*, *T.tonggol* and *U. doriae* had statistically similar δ^{15}N-signatures, as was *S.crumenophthalmus*, *N. randalli*, *E. affinis*, *S.tol*, *S. longiceps* and *R .kanagurta*.

Concerning size parameters, δ^{15}N-values were only correlated with length and weight of *E. affinis* (τ = .592, p = .028) and (τ = .556, p = .037). δ^{15}N-values showed a marginally significant correlation in *S. crumenophthalmus* (τ = .407, p = .043) and *S. tol* (τ = .443, p = .046).

Figure 3.3 illustrates average δ^{15}N-values for fish grouped by their habitats. The isotopic nitrogen signatures of demersal fish species were only significantly different from that of pelagic ones (U = 366.00, $p < .001$, r = -.46). Groups of demersal and reef-associated species (U = 1082.50, p = .058, r = -.18) and pelagic and reef-associated (U = 805.50, p = .059, r = -.19) had similar ^{15}N/^{14}N ratios. According to feeding habit categories, a significant difference was determined among the investigated species ($H(4)$ = 97.76 , $p < .001$). *Post hoc* comparisons revealed that the invertivorous, unknown,

piscivorous and predatory fish species had enriched ^{15}N-values to that of planktivorous species (see table 3.2).

3.2.1 $\delta^{15}N$ and trophic positions

Figure 3.4 demonstrates food web structure based on $\delta^{15}N$ and $\delta^{13}C$ of zooplankton and fish species. Obviously, trophic positioning of zooplankton is distinctly different from that of the fish species. Based on the assumption that zooplankton occupy trophic level 2 in the Gulf of Oman, trophic levels of the fish species could be calculated using this equation: $T.L = 2 + (^{15}N_{Fish\ species} - {}^{15}N_{Zooplankton})/ 3.4$, where 2 is the assumed trophic level of zooplankton and 3.4‰ is the average trophic enrichment ($\Delta\delta^{13}N$) recorded for transfer between zooplankton and their planktivorous predators (*S. crumenophthalmus*, *S. longiceps* and *R. kanagurta*). The calculated trophic positions of fish species ranged between 2.7-3.9 (Figure 3.3). The trend in species distribution was quite clear with planktivorous species occupying lower levels, invertivorous intermediate and predatory and piscivorous species at higher levels. *E. affinis* should be at higher trophic positions (i.e. high ^{15}N-signatures) according to the model (see Figure 1.1), however, because it had lower $\delta^{15}N$ value than it prey (*S. longiceps*), $\Delta\delta^{15}N$ was – 1.04‰. $\Delta\delta^{15}N$ of 2.65‰ found between *S. longiceps* and *R. acutus* concurs with the model.

3.3 Total mercury (T-Hg)

T-Hg concentrations of zooplankton samples varied from 0.010-0.037 $\mu g \cdot g^{-1}$ wet weight. T-Hg levels in fish species varied from 0.003 $\mu g \cdot g^{-1}$ in *S. crumenophthalmus* and *S. longiceps* (planktivorous) to 0.760 $\mu g \cdot g^{-1}$ in *R. acutus* (opportunistic predator). A Significant difference was observed in T-Hg concentrations in zooplankton and fish

species ($H(13) = 131.48$, $p < .001$). Non-parametric *Post hoc* multiple comparisons verified that zooplankton had T-Hg levels significantly higher than planktivores *S. longiceps* ($U = 0.00$, $p < .001$, $r = -.82$) and *R. kanagurta* ($U = 0.00$, $p < .001$, $r = -.79$). Althougth no statistically-significant difference existed between zooplankton and planktivore *S. crumenophthalmus*, predator *C. malabaricus* and demersal *U.doriae*, the mean mercury concentrations in these fish, in fact, were higher than that of zooplankton (Table 3.3).

Among fish, the smallest fish species *S. longiceps* had the lowest T-Hg concentrations, which were statistically different from all fish species. Although the difference in size parameters (length and weight, see table 2.2), *S. crumenophthalmus* and *R. kanagurta* showed similar T-Hg levels, nevertheless the former had similar concentrations to other fish species except *T. tonggol* ($U = 10.00$, $p < .001$, $r = -.72$), while *R. kanagurta* had lower concentrations than all. T-Hg of *R. acutus* demonstrated high variability with the two highest concentrations detected in this species (0.668 and 0.760 $\mu g \cdot g^{-1}$). In despite of that, its T-Hg burdens were not statistically different from that of other predatory species, *E. affinis* ($U = 19.00$, $p = .186$, $r = -.33$), *T. tonggol* ($U = 15.00$, $p = .051$, $r = -.47$) and *E. epistictus* ($U = 16.00$, $p = .028$, $r = -.50$). Species (*C. malabaricus*, *N. randalli*, *S. tol*, *Sphyraena* sp. and *U.doriae*) had similar tissue T-Hg levels.

Kruskal Wallis test uncovered a significant difference in T-Hg concentrations of zooplankton and fish species grouped by their feeding habit ($H(5) = 101.42$, $p < .001$) (see Figure 3.5). T-Hg levels of zooplankton were statistically higher than that of planktivorous species ($U = 224.00$, $p < .001$, $r = -.50$) and significantly lower than that of

other grouped-species. It was found that unknown and piscivorous species had similar T-Hg concentrations, as did invertivorous and predatory fish species.

3.3.1 T-Hg, age, size parameters and stable isotopes

For age, no signficant correlation was detected with T-Hg for *C. malabaricus* (τ = .119, p = .643), *S. crumenophthalmus* (τ = .109, p = .708) and *A. spinifer* (τ = .241, p = .309) although the age covered range of 3-11 years for *C. malabaricus*, 3-13 years for *S. crumenophthalmus* and 3-14 years for *A. spinifer*. Significant positive correlations between T-Hg and length/weight were observed for *C. malabaricus*, *S. crumenophthalmus*, *R. acutus* and *E. epistictus*. *U.doriae* showed positive correlation between T-Hg and length (τ = .500, p < .05), but not with weight (τ = .367, p =.118). Total mercury in the other species was not correlated to their size parameters (Table A-2 in Appendices).

Only a significant positive correlation was described between T-Hg and δ^{15}N-values for *U.doriae* (τ = .636, p < .05). Interestingly, T-Hg concentrations were statistically related to δ^{13}C-signatures of *A. spinifer* (τ = .576, p < .05) and *S. longiceps* (τ = .454, p < .05).

3.3.2 T-Hg accumulation

Figure 3.6 shows T-Hg concentrations of the investigated organisms organized based on their mean δ^{15}N-values. A weak significant linear relationship was determined between Log_{10}[T-Hg] and δ^{15}N-signatures for zooplankton and fish species (r^2 = .090, p < .001) (Figures 3.7). However, it is important to note that the assumptions required by linear regression were violated except non-multilinearity and non-curvilinearity. Therefore, an alternative approach was employed to overcome the problem. Data of *R.*

acutus were removed from the analyses because they represented outliers and severely violated the assumptions. Mercury accumulation was examined in subsets by grouping fish species based on habitat or feeding staregies. An outlier sample of *S. crumenophthalmus* in subset of planktivores and a sample of *U. doriae* of the group of demersal species were not included in the analysis.

Demersal fish species showed a significant linear relationship between tissue Log_{10}[T-Hg] and total length ($r^2 = .381, p < .001$). With inclusion of $\delta^{13}C$ and $\delta^{15}N$, the multiple regression model became non significant ($r^2 = .443, p = .113$). Total length of pelagic fish species accounted alone for 92% in Log_{10}[T-Hg] variability and this was further slightly improved by including $\delta^{13}C$ and $\delta^{15}N$-signatures (Multiple regression; $r^2 = .971, p < .001$). For reef-associated species, none of the predictors (length, $\delta^{13}C$ or $\delta^{15}N$) contributed significantly to explain variability in T-Hg except weak relationship by length ($r^2 = .158, p < .05$). Nevertheless, it violated the assumption of independence of errors.

Based on habitat-based categories, planktivorous fish demonstrated a significant correlation between Log_{10}[T-Hg] and stable isotope ratios ($\delta^{13}C$ or $\delta^{15}N$) (Multiple regression; $r^2 = .419, p < .001$), but for this the assumption of independence of errors and normal distribution of residuals were not met. For invertivorous fish, represented by only one species (*A. spinifer*, n = 12), Log_{10}[T-Hg] evidenced a marginal non-significant correlation with $\delta^{13}C$ and $\delta^{15}N$ (Multiple regression; $r^2 = .522, p = .058$). In spite of that, the contribution for explaining variation in T-Hg came from $\delta^{13}C$ ($\beta = .677, p = .031$), β is standardized value of b in the regression equation and indicates the number of standard deviations T-Hg will change as a result of one standard deviation change in $\delta^{13}C$. As

indicated by a non-statistically significant multiple regression model ($r^2 = .126, p = .459$), total length, $\delta^{13}C$ or $\delta^{15}N$ failed to account for variability in T-Hg concentrations of unknown species. For piscivorous fish species, the initial model based on length versus Log_{10}[T-Hg] was significant ($r^2 = .217, p < .05$), however, the second model based on $\delta^{13}C$, $\delta^{15}N$ and length was non-significant (Multiple regression; $r^2 = .535, p = .163$), indicating the lack of effect imposed by trophic position on the T-Hg burdens. A significant linear relationship was determined between T-Hg and total length (Log_{10}[T-Hg] = 0.014 Length – 1.686, $r^2 = .596, p < .001$) for predatory fish species.

3.4 Methyl mercury (MeHg)

Average MeHg concentrations of zooplankton and fish species are listed in table 3.3. Methyl mercury levels were significantly different between the investigated species ($H(13) = 111.99, p < .001$). Unlike T-Hg, MeHg of zooplankton was greatly lower than that of fish species. *Bonferroni corrected Post hoc* non-parametric comparisons revealed that *S. longiceps* and *R. kanagurta* had similar MeHg levels, as was *A. spinifer*, *E. affinis*, *R. acutus*, *Sphyraena* sp., *E. epistictus* and *U.doriae*. MeHg concentrations of *C. malabaricus*, *S. crumenophthalmus*, *N. randalli* and *S. tol* were not statistically different. Samples of the tuna species, *T. tonggol*, had similar MeHg to the former group except *Sphyraena* sp. ($U = 4.00, p < .001, r = -.78$), but not the the latter one.

It was observed that a statistically-significant difference existed in methyl mercury concentrations of fish of different feeding habits ($H(4) = 75.72, p < .001$) (Figure. 3.8). Similar to T-Hg, MeHg of planktivorous fish species were significantly lower than that of invertivorous ($U = 39.00, p < .001, r = -.60$), unknown ($U = 138.00, p < .001, r = -.57$), piscivorous ($U = 119.00, p < .001, r = -.62$) and predators ($U = 99.00, p$

$< .001, r = -.76$). Invertivorous, piscivorous and predatory fish species had significantly higher similar MeHg levels than that of unknown species.

Similar to T-Hg, MeHg levels of *C. malabaricus*, *S. crumenophthalmus* and *A. spinifer* were not related to age. Also, MeHg demonstrated significant correlation with length and weight of *C. malabaricus*, *S. crumenophthalmus*, *E. affinis* and *E. epistictus*. Methyl mercury of *R. acutus* was correlated to weight only ($\tau = .781, p < .05$).

Although not the case for T-Hg, MeHg levels of *S. crumenophthalmus* were negatively related to δ^{13}C-values ($\tau = -.398, p = .048$). Similar to T-Hg, positive correlations were recorded for *A. spinifer* ($\tau = .545, p < .05$) and *S. longiceps* ($\tau = .403, p < .05$). MeHg of *E. affinis* showed, contrary to T-Hg, positive correlation with δ^{15}N-values ($\tau = .556, p < .05$). Like T-Hg, significant correlation was observed between MeHg and δ^{15}N ($\tau = .527, p < .05$) for *U.doriae*.

The overall average MeHg fraction of T-Hg was 72%. Figure 3.9 shows a strong correlation ($r_s = .976$, $p < 0.001$) between methyl mercury versus total mercury for all fish species. Noticeable outliers are those of predatory species *E. affinis* (F5) and *R. acutus* (F8) which are values of T-Hg with ≤ 50 % MeHg (Figure 3.9).

3.4.1 MeHg accumulation

Figure 3.10 shows MeHg concentrations of zooplankton and fish species organized based on their mean δ^{15}N-values.Violating independence of error assumption only a linear relationship was found between MeHg and δ^{15}N ($r^2 = .228, p < .001$) (Figure 3.11).

There was a statistically significant linear relationship between length and methyl mercury (Log_{10}[MeHg] = 0.030 Length – 2.335, $r^2 = .402, p < .001$) for demersal fish

species. Concerning pelagic species, although $\delta^{13}C$ (β = -.081, p = .016) and $\delta^{15}N$ (β = -.154, p < .000) contributed significantly to the multiple regression model (r^2 = .971, p < .001), their contribution is minimal compared to that of length (β = 1.022, p < .001). As the case for T-Hg, no predictor took integral part in accounting for MeHg variability of reef-associated species.

In contrast to T-Hg, the assumptions of linear regression were completely met and together $\delta^{15}N$ and $\delta^{13}C$ explained about 48% of methyl mercury variations of planktivorous species. Similarly, a significant linear relationship was determined between Log_{10}[MeHg] and $\delta^{13}C$, $\delta^{15}N$ and length (r^2 = .575, p < .05) for the invertivorus fish species with $\delta^{13}C$ being the best predictor of MeHg concentrations (β = .688 , p = .023). Unknown fish species showed no significant correlation between MeHg and total length, $\delta^{15}N$ or $\delta^{13}C$. Length, on the other hand, accounted for about one-third of the variability in MeHg levels in piscivorous fish based on the initial model (r^2 = .278, p < .05). MeHg of oppurtunistic predators was best estimated, like T-Hg, by length (r^2 = .544, p < .001).

Table 3.1: Nitrogen and carbon isotopic compositions ($\delta^{15}N$ relative to N_2 in air and $\delta^{13}C$ relative to PDB) of zooplankton and fish species collected from the Gulf of Oman.

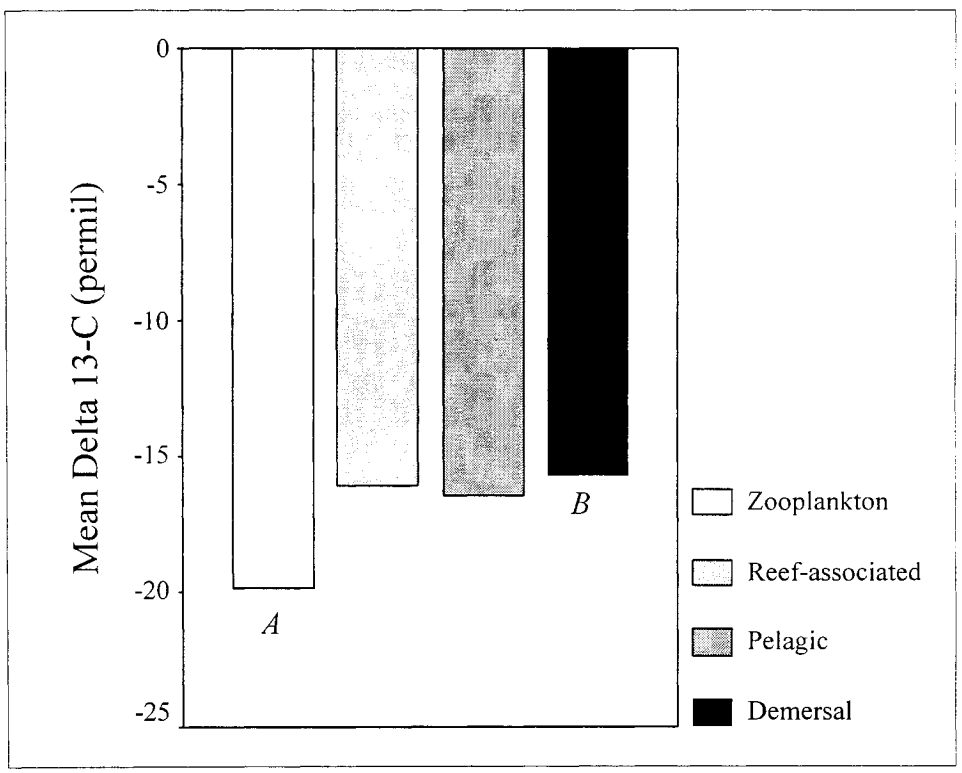
Species	*N*	$\delta^{15}N$ [mean ± SD] (‰)	$\delta^{13}C$ [mean± SD] (‰)
Zooplankton	27	11.16 ±1.30 *	-19.87 ±1.74*
Fish species:			
S. longiceps	16	14.74 ± 0.47 ^{A}	-16.35 ± 0.21 ^{A}
S. crumenophthalmus	14	14.26 ± 0.70 ^{A}	-16.77 ± 0.61 ^{A}
R. kanagurta	12	14.63 ± 0.46 ^{A}	-16.68 ± 0.28 ^{A}
A. spinifer	12	16.00 ± 0.21 ^{B}	-15.77 ± 0.51 ^{B}
C. malabaricus	12	17.03 ± 0.85 ^{C}	-14.94 ± 0.58 ^{C}
E. epistictus	12	17.05 ± 0.46 ^{C}	-16.49 ± 0.78 ^{A}
E. affinis	9	13.70 ± 0.68 ^{A}	-16.80 ± 0.61 ^{A}
T. tonggol	10	16.51 ± 0.87 ^{B}	-16.36 ± 0.65 ^{A}
R. acutus	7	17.39 ± 0.39 ^{C}	-15.15 ± 0.20 ^{C}
S. tol	12	14.78 ± 1.08 ^{A}	-16.20 ± 0.16 ^{D}
Sphyraena sp.	11	17.72 ± 0.48 ^{C}	-15.60 ± 0.57 ^{D}
N. randalli	12	15.25 ± 0.60 ^{A}	-16.05 ± 0.17 ^{B}
U. doriae	11	15.94 ± 0.71 ^{B}	-14.07 ± 1.24 ^{C}

Significance reported at *Bonferroni corrected*-level of .001.

* Isotopic concentrations of zooplankton were distinctly different from all fish species.

Different letters indicate different means.

Figure 3.1: Mean $\delta^{13}C$-values (‰ relative to PDB) of zooplankton and fish species grouped by their habitats.



Significance reported at *Bonferroni corrected*-level of .01.

Different letters indicate different means.

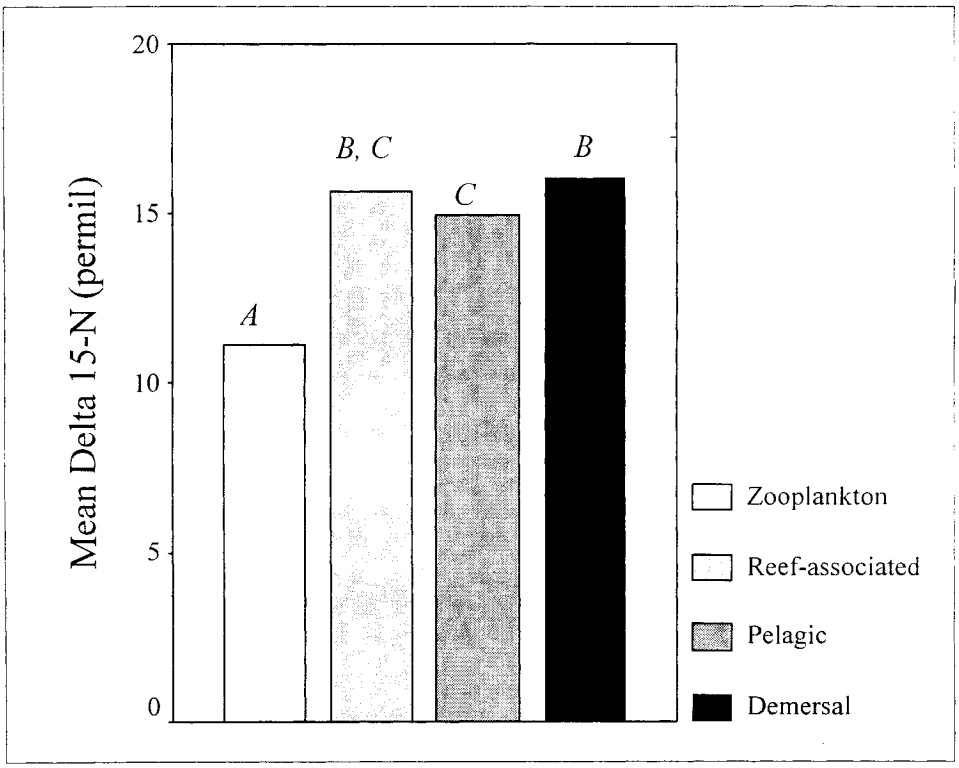
Table 3.2: Mean and range $\delta^{15}N$ (‰ relative to N_2 in air) and $\delta^{13}C$ (‰ relative to PDB) of fish species grouped by their feeding strategies.

Species	N	$\delta^{15}N$-mean ± SD	Range	$\delta^{13}C$-mean± SD	Range
Planktivores	41	14.56 ± 0.58 ^{A}	13.16 to 15.70	-16.59 ± 0.44 ^{A}	-18.18 to -16.03
Invertivores	12	16.00 ± 0.21 ^{B}	15.51 to 16.33	-15.77 ± 0.51 ^{B}	-16.40 to -14.84
Unknown	22	15.53 ± 0.68 ^{B}	14.33 to 17.16	-15.28 ± 0.98 ^{B}	-16.27 to -13.36
Piscivores	23	16.19 ± 1.72 ^{B}	13.28 to 18.48	-15.91 ± 0.51 ^{B}	-16.54 to -14.45
Predators	43	16.23 ± 1.50 ^{B}	12.54 to 18.48	-16.09 ± 0.98 ^{C}	-18.59 to -14.18

Significance reported at *Bonferroni corrected*-level of .01.

Different letters indicate different means.

Figure 3.2: Mean $\delta^{15}N$-values (‰ relative to N_2 in air) of zooplankton and fish species grouped by their habitats.



Significance reported at *Bonferroni corrected*-level of .01.

Different letters indicate different means.

Figure 3.3: Trophic structure (means and standard deviations of $\delta^{13}C$ and $\delta^{15}N$ signatures in permil or ‰) of zooplankton (n = 27 samples) and 13 fish species (n = 150 samples) from the Gulf of Oman.

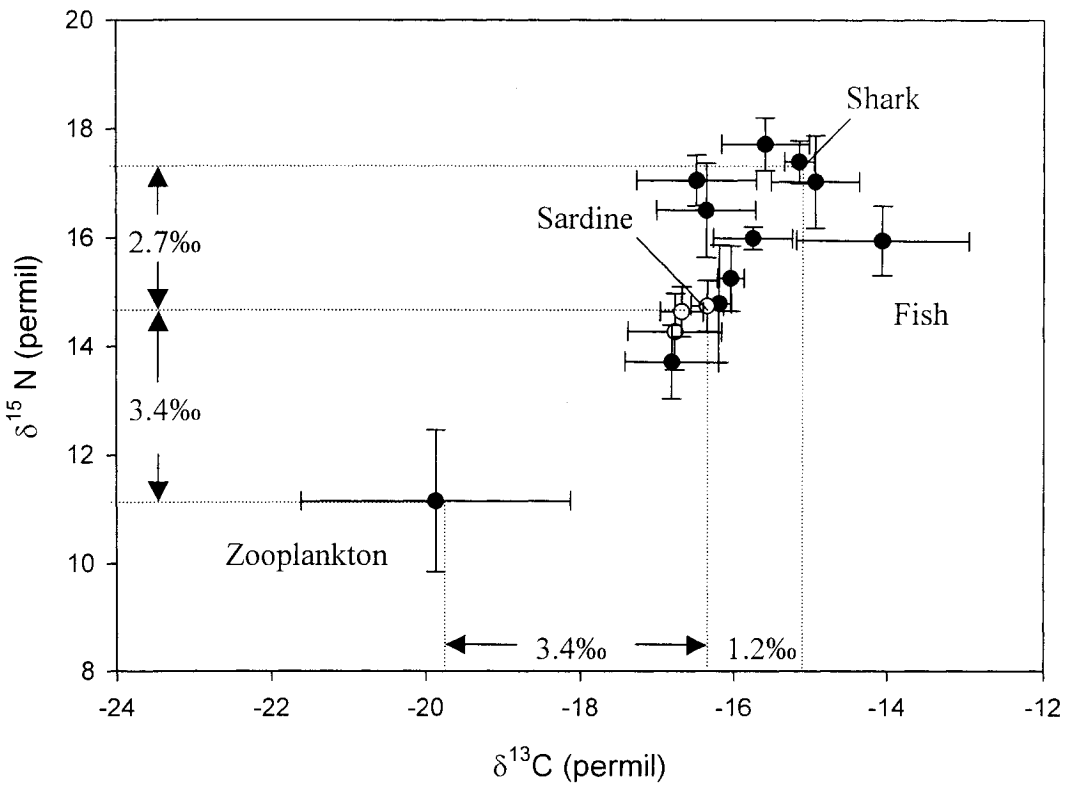

20
18
16
14
12
10
8
-24
-22
-20
-18
-16
-14
-12
δ15 N (permil)
δ13C (permil)
Shark
Sardine
Fish
Zooplankton
2.7‰
3.4‰
3.4‰
1.2‰

Figure 3.4: Trophic positions of the 13 fish species (n =150) from the Gulf of Oman based on isotopic value of $\delta^{15}N$ and assumption that zooplankton occupy the T.L of 2.

Species Key:

Zoo: Zooplankton
F1: *C. malabaricus*-predator
F2: *S. crumenophthalmus*-planktivore
F3: *A. spinifer*-invertivore
F4: *N. randalli*-unknown
F5: *E. affinis*-predator
F6: *S. tol*-piscivore
F7: *S. longiceps*-planktivore
F8: *R. acutus*-predator
F9: *T. tonggol*-predator
F10: *Sphyraena* sp.-piscivore
F11: *R. kanagurta*-planktivore
F12: *E. epistictus*-predator
F13: *U. doriae*-unknown

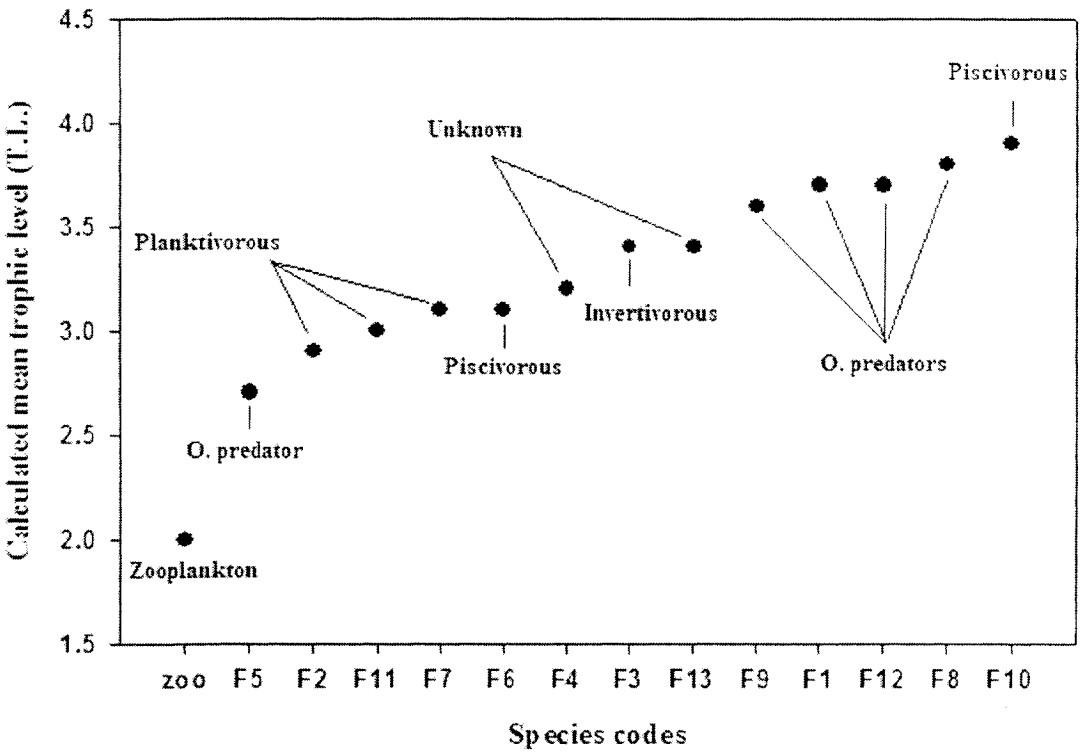

Calculated mean trophic level (T.L.)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Piscivorous
Unknown
Planktivorous
Invertivorous
Piscivorous
O. predators
O. predator
Zooplankton
zoo F5 F2 F11 F7 F6 F4 F3 F13 F9 F1 F12 F8 F10
Species codes

Table 3.3: Total mercury, methyl mercury concentrations (wet weight basis) and percentages of methyl mercury of total mercury of zooplankton and fish species collected from the Gulf of Oman.

Fish species	*N*	*T-Hg ($\mu g \cdot g^{-1}$)*	*MeHg ($\mu g \cdot g^{-1}$)*	*Mean (%) (range)*
Zooplankton[a]	27	0.022 ± 0.008		
	5*		0.001 ± 0.0006	10 (1-19)
Fish species:				
S. longiceps	16	0.004 ± 0.001 ^{A}	0.002 ± 0.001 ^{A}	70 (48-96)
S. crumenophthalmus	14	0.055 ± 0.066 ^{B}	0.043 ± 0.049 ^{B}	70 (34-96)
R. kanagurta	12	0.005 ± 0.001 ^{A}	0.004 ± 0.001 ^{A}	79 (53-92)
A. spinifer	12	0.090 ± 0.063 ^{C}	0.071 ± 0.050 ^{C}	79 (61-88)
C. malabaricus	12	0.044 ± 0.026 ^{B}	0.033 ± 0.023 ^{B}	73 (47-92)
E. epistictus	12	0.123 ± 0.052 ^{C}	0.106 ± 0.054 ^{C}	82 (43-97)
E. affinis	9	0.176 ± 0.099 ^{C}	0.101 ± 0.060 ^{C}	60 (37-100)
T. tonggol	10	0.154 ± 0.021 ^{C}	0.132 ± 0.024 ^{C}	86 (71-99)
R. acutus	7	0.351 ± 0.270 ^{C}	0.216 ± 0.124 ^{C}	71 (39-99)
S. tol	12	0.042 ± 0.010 ^{B}	0.022 ± 0.005 ^{B}	53 (33-68)
Sphyraena sp.	11	0.066 ± 0.042 ^{B}	0.053 ± 0.037 ^{C}	77 (36-96)
N. randalli	12	0.037 ± 0.011 ^{B}	0.025 ± 0.009 ^{B}	67 (54-89)
U. doriae	11	0.060 ± 0.087 ^{B}	0.051 ± 0.083 ^{C}	76 (56-99)

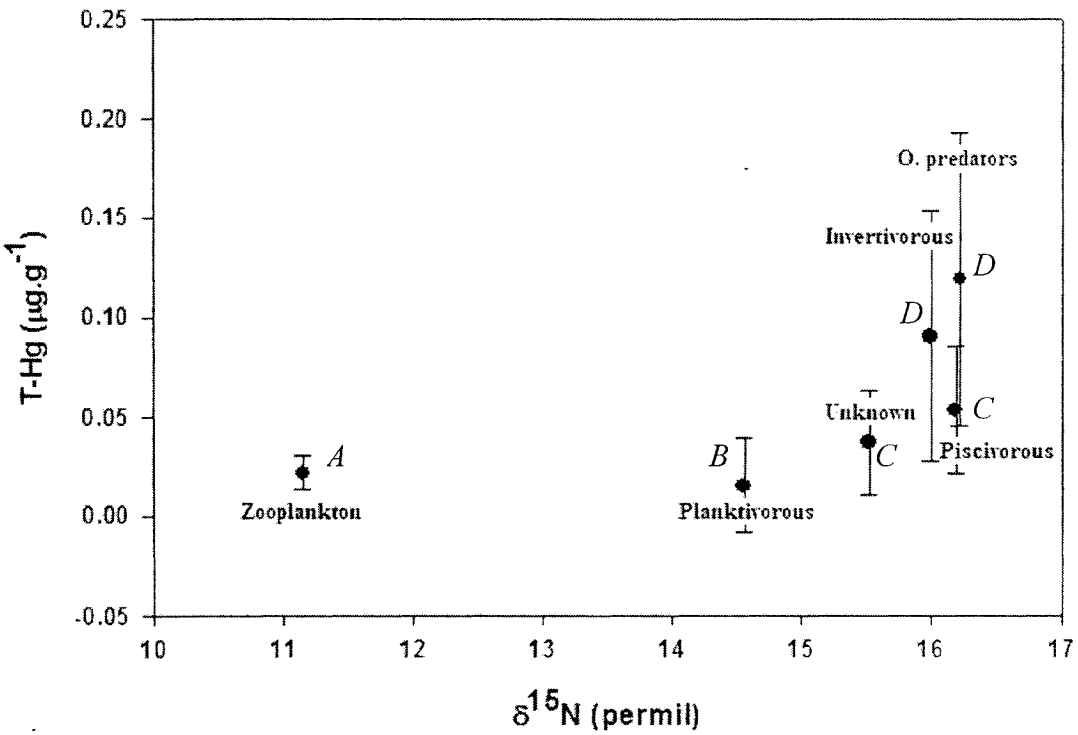
*Composite samples.

[a] Analyzed in dried samples and converted into wet weight based on 70% water content.

Significance reported at *Bonferroni corrected*-level of .001.

Different letters indicate different means.

Figure 3.5: Mean total mercury concentrations (± 1 SD) in zooplankton (n = 27) and muscle tissue of fish species (Planktivorous n = 42, unknown n = 23, piscivorous = 23, invertivorous = 12, opportunistic predators n = 43) collected from the Gulf of Oman. Zooplankton and fish species are arranged based on their mean δ^{15}N-signatures (‰) of assigned trophic position.



Significance reported at *Bonferroni corrected*-level of .003.

Different letters indicate different means.

Figure 3.6: Mean total mercury concentrations (± 1 SD) of zooplankton (n = 27) and the 13 fish species (n =150) from the Gulf of Oman. The organisms were organized based on their mean δ^{15}N-values (± SD).

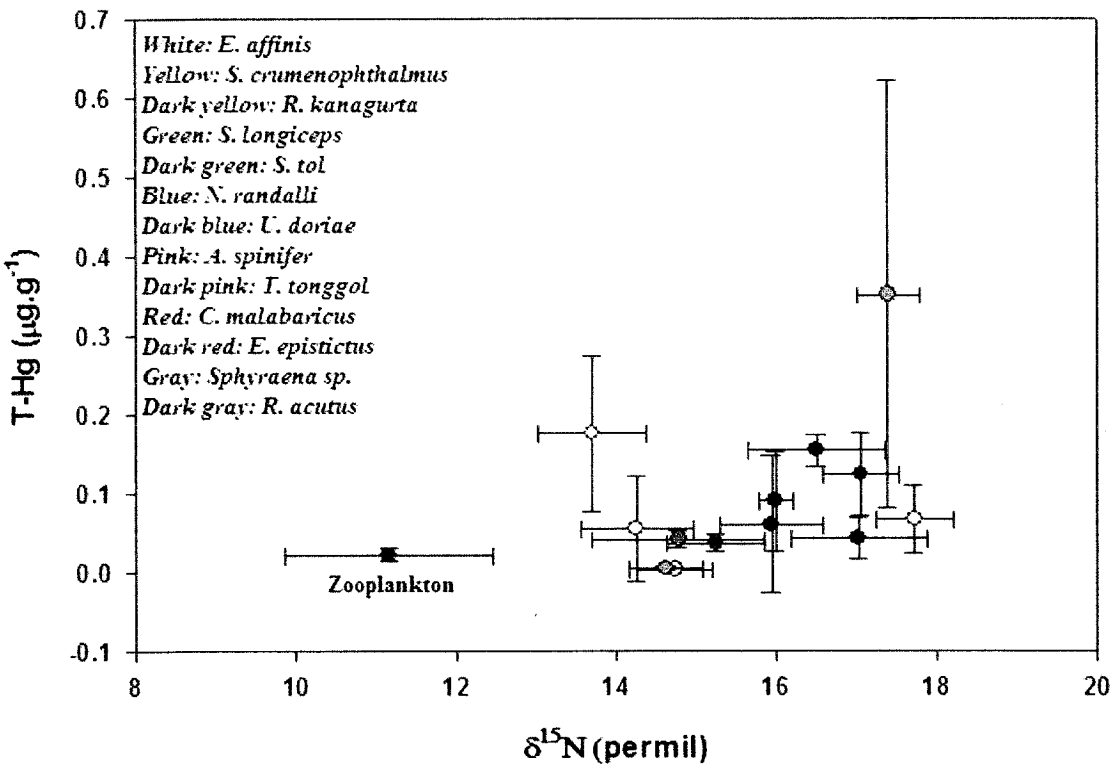

White: E. affinis
Yellow: S. crumenophthalmus
Dark yellow: R. kanagurta
Green: S. longiceps
Dark green: S. tol
Blue: N. randalli
Dark blue: U. doriae
Pink: A. spinifer
Dark pink: T. tonggol
Red: C. malabaricus
Dark red: E. epistictus
Gray: Sphyraena sp.
Dark gray: R. acutus
Zooplankton
T-Hg (μg.g-1)
δ15N (permil)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
8
10
12
14
16
18
20

Figure 3.7: Relationship between Log_{10}[T-Hg] and $\delta^{15}N$ of zooplankton and fish species collected from the Gulf of Oman. Regression equation: Log_{10}[T-Hg] = 0.075 ($\delta^{15}N$) – 2.628, $r^2 = .090$, $p < .001$.

Species Key:

Zoo: Zooplankton
F1: *C. malabaricus*-predator
F2: *S. crumenophthalmus*-planktivore
F3: *A. spinifer*-invertivore
F4: *N. randalli*-unknown
F5: *E. affinis*-predator
F6: *S. tol*-piscivore
F7: *S. longiceps*-planktivore
F8: *R. acutus*-predator
F9: *T. tonggol*-predator
F10: *Sphyraena* sp.-piscivore
F11: *R. kanagurta*-planktivore
F12: *E. epistictus*-predator
F13: *U. doriae*-unknown

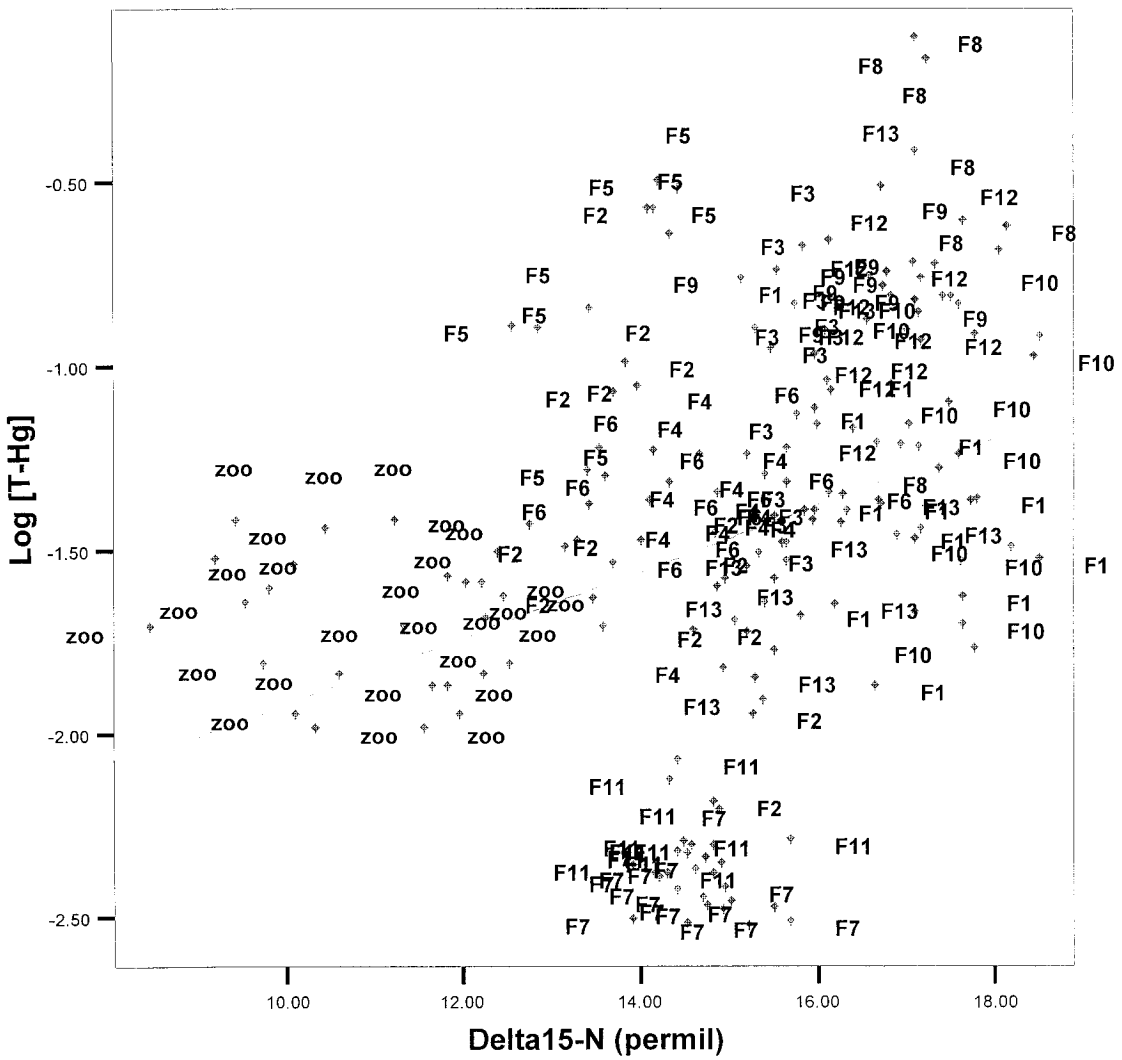
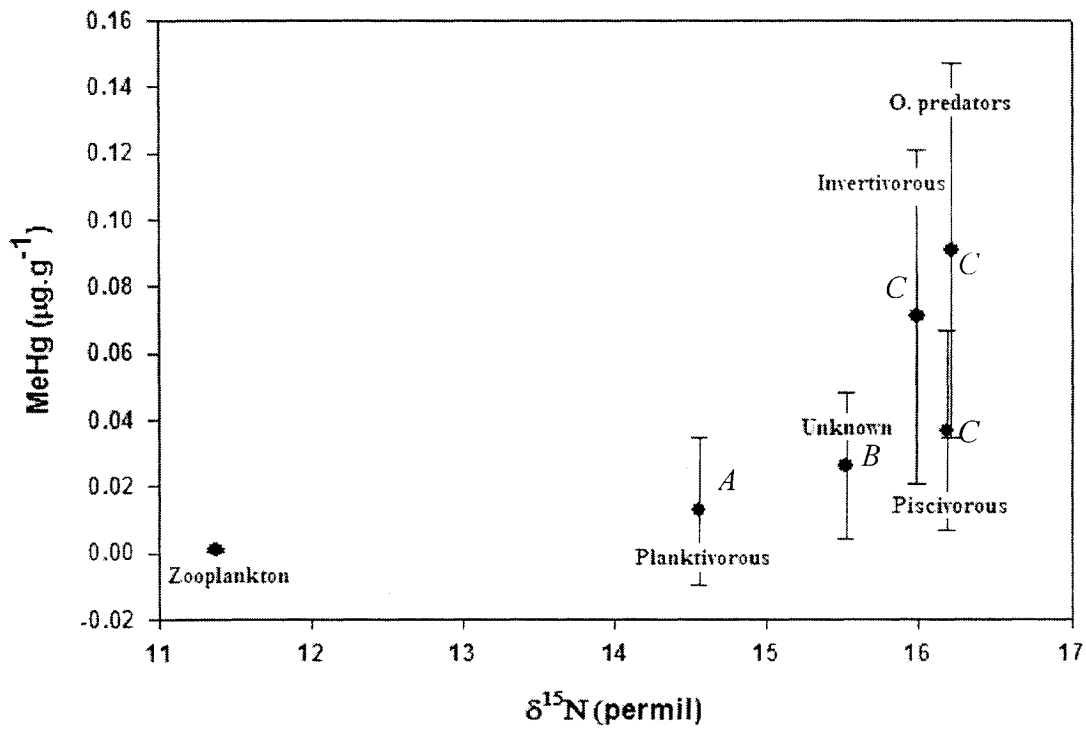

Log [T-Hg]
-0.50
-1.00
-1.50
-2.00
-2.50
10.00
12.00
14.00
16.00
18.00
Delta15-N (permil)
zoo
F1
F2
F3
F4
F5
F6
F7
F8
F9
F10
F11
F12
F13

Figure 3.8: Mean (± 1 SD) methyl mercury concentrations in zooplankton (n = 5) and muscle tissue of fish species (Planktivorous n = 42, unknown n = 23, picivorous = 23, invertivorous = 12, opportunistic predators n = 43) collected from the Gulf of Oman. Zooplankton and fish species are arranged based on their mean δ^{15}N-signatures (‰) of assigned trophic position.



Significance reported at *Bonferroni corrected*-level of .01.

Different letters indicate different means.

Figure 3.9: Relationship between total mercury and methyl mercury concentrations ($\mu g \cdot g^{-1}$) for all fish species (n = 150) from the Gulf of Oman.

Species Key:

F5: *E. affinis*-predator
F8: *R. acutus*-predator

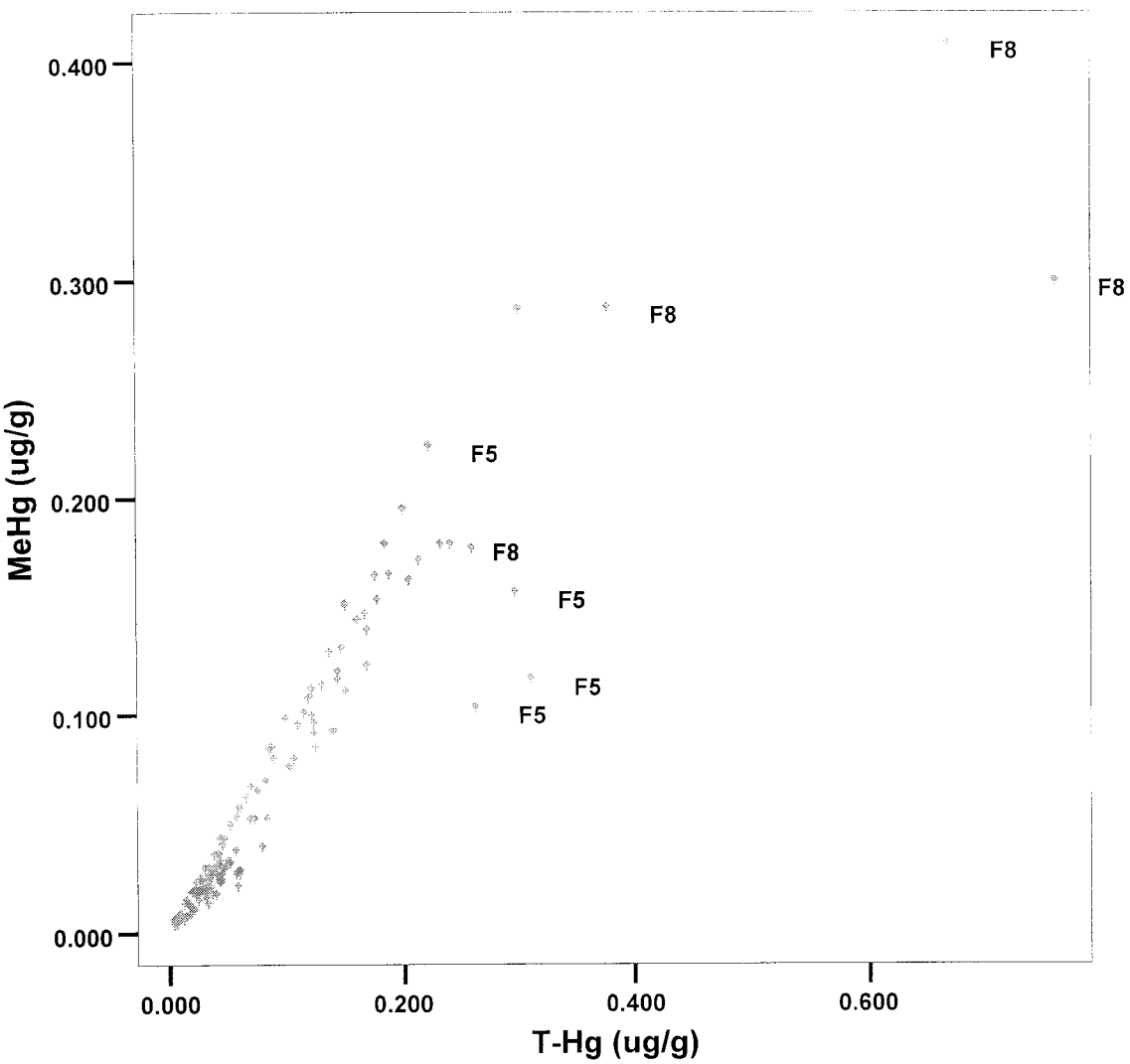

0.400
0.300
0.200
0.100
0.000
MeHg (ug/g)
F8
F8
F8
F5
F8
F5
F5
F5
0.000
0.200
0.400
0.600
T-Hg (ug/g)

Figure 3.10: Mean methyl mercury concentrations (± 1 SD) of zooplankton (n = 5) and the 13 fish species (n =150) from the Gulf of Oman. The organisms were organized based on their mean δ^{15}N-values (± 1 SD).

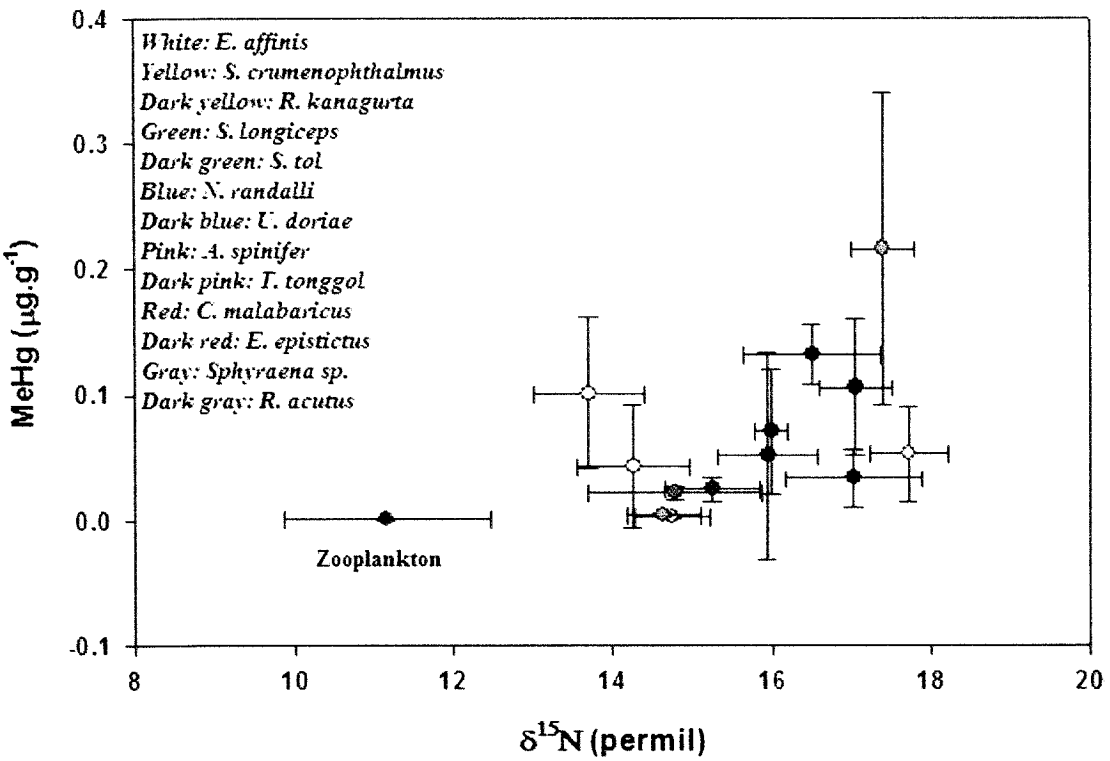
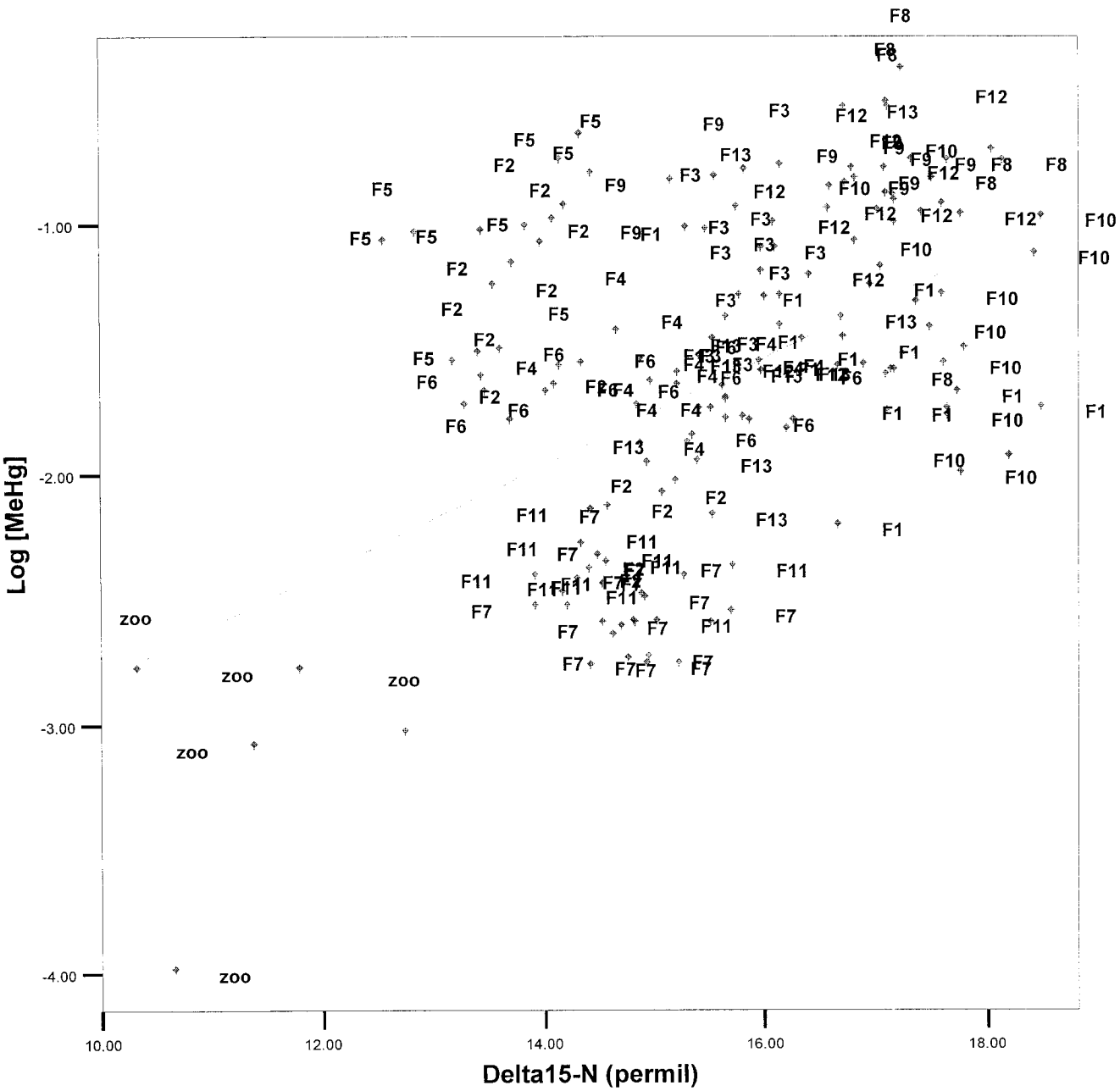

White: E. affinis
Yellow: S. crumenophthalmus
Dark yellow: R. kanagurta
Green: S. longiceps
Dark green: S. tol
Blue: N. randalli
Dark blue: U. doriae
Pink: A. spinifer
Dark pink: T. tonggol
Red: C. malabaricus
Dark red: E. epistictus
Gray: Sphyraena sp.
Dark gray: R. acutus
Zooplankton
MeHg (μg.g-1)
δ15N (permil)
0.4
0.3
0.2
0.1
0.0
-0.1
8
10
12
14
16
18
20

Figure 3.11: Relationship between Log_{10}[MeHg] and $\delta^{15}N$ of zooplankton and fish species collected from the Gulf of Oman. Regression equation: Log_{10}[MeHg] = 0.045 ($\delta^{15}N$) – 2.359, $r^2 = .228$, $p < .001$.

Species Key:

Zoo: Zooplankton
F1: *C. malabaricus*-predator
F2: *S. crumenophthalmus*-planktivore
F3: *A. spinifer*-invertivore
F4: *N. randalli*-unknown
F5: *E. affinis*-predator
F6: *S. tol*-piscivore
F7: *S. longiceps*-planktivore
F8: *R. acutus*-predator
F9: *T. tonggol*-predator
F10: *Sphyraena* sp.-piscivore
F11: *R. kanagurta*-planktivore
F12: *E. epistictus*-predator
F13: *U. doriae*-unknown


Log [MeHg]
-1.00
-2.00
-3.00
-4.00
10.00
12.00
14.00
16.00
18.00
Delta15-N (permil)
zoo

Chapter 4: Discussion

The $\delta^{13}C$ and $\delta^{15}N$ values for zooplankton and fish species from the Gulf of Oman used to determine the food web structure of this area. They were found useful in distinguishing zooplankton with distinct isotopic signals from that of fish species. However, it is important to point out that the interpretation of carbon isotope data for food web analyses has proven complex.

4.1 Isotopic compositions of zooplankton

Zooplankton (250μm) samples were constituted principally of copepods. This observation concurs with finding of Gjøsaeter (1984) for the Arabian Sea and the Gulf of Oman and similar to that reported by Michel (1986 in Al-Majed and Preston, 2000) for Kuwait Bay, a northern embayment of the Arabian Gulf. Calanoid copepods are the most dominant and diverse group of zooplankton for the region with more than 300 species found in the Arabian Sea (Weikert, 1987 in Sheppard *et al.*, 1992). Such huge diversity is common in tropical ecosystems.

The average $\delta^{13}C$-value of zooplankton (-19.89 ± 1.74‰ relative to PDB) was similar to values reported for copepods (-20.80‰) and euphausiids (-19.80 ± 0.40‰) for northwestern Atlantic (Fry, 1988), euphausiids (-20.10 ± 0.30‰) in the Gulf of Farallones (Jarman *et al.*, 1996) and calanoid copepods (-20.70 ± 1.90‰) from a western Mediterranean lagoon (Vizzini and Mazzola, 2003). The range of $\delta^{13}C$-signatures, in this study, was also similar to that of a subarctic (Parson and Lee Chen, 1995) and arctic (Hobson *et al.*, 2002) marine ecosystems, but higher than subantarctic (Kaehler *et al.*, 2000) and North Amercian Arctic (Fisk *et al.*, 2003) systems.

δ^{15}N-signals of zooplankton in this study (8.47-13.58‰) were higher than those determined for copepods and euphausiids (7.00 and 7.9± 0.50‰, respectively) in northwestern Atlantic (Fry, 1988), zooplankton (5.4 – 8.6 ‰) from tropical coastal water (Parsons and Lee Chen, 1995), copepods and euphausiids (< 0 and 3‰, respectively) (Kaehler *et al.*, 2000), copepod, *Calanus hyperboreus* (9.00 ± 0.20‰) from Canadian Arctic (Tittlemier *et al.*, 2002), zooplankton (range: 7.00-9.00‰) of polar water of Barents Sea (Hop *et al.*, 2002), and zooplankton (9.80‰) from Beaufort-Chukchi Seas (Hoekstra *et al.*, 2003). On the other hand, the nitrogen isotopic signatures of zooplankton were similar to δ^{15}N-values of zooplankton samples of a subarctic strait (Parsons and Lee Chen, 1995), *Mysis oculata* and *Sagitta* sp. (Tittlemier *et al.*, 2002), and copepods of north American Arctic (Fisk *et al.*, 2003).

The observed zooplankton δ^{13}C and δ^{15}N enrichments of the Gulf of Oman could be explained by the fact that there are no permanent rivers or streams flowing into the gulf and terrestrial runoff due to heavy flash rains is restricted to a few days a year (Wilson, 2000). Therefore, incorporation of terrestrial carbon and nitrogen, with low δ^{13}C and δ^{15}N signals, at the base of this coastal marine food web seems minimal if not nil. The zooplankton samples were collected 1.5- 3 km off-shore, and more enriched δ^{15}N isotopic values were expected as highlighted by Parson and Lee Chen (1995). The trend was also observed by Bode *et al.* (2003) in plankton samples (size range of 20 to 1000 μm), of coastal stations from an upwelling ecosystem of Galicia (Spain), which displayed greater δ^{13}C and δ^{15}N-values than those from off-shore stations. Variability exhibited by δ^{13}C and δ^{15}N-signals suggests probable omnivory/carnivory among zooplankton species (i.e. copepods). Hobson *et al.* (2002) found that herbivorous copepods (*C. hyperboreus*)

had average $\delta^{15}N$ of 7.9‰, while carnivorous copepods (*E. glacialis*) had average of 11.8‰. The average reported here (11.16‰) is close to the latter value while the lowest value found for zooplankton from the Gulf of Oman was 8.47‰.

4.2 Isotopic carbon compositions of fish species

The least negative value of $\delta^{13}C$ (-11.31‰) was found in the largest of demersal fish *U.doriae*, suggesting that this individual utilized a different carbon source from the rest of individuals of the same species. The most negative value (-18.59‰) of predatory fish *E. epistictus* should be the signal of the smallest individual, but it was not the case. This fish was medium sized (41.4 cm) individual and the range of the species was 33.5 to 48.8 cm. This could be due to ontogenetic changes in diet and this individual probably fed at lower trophic levels compared the other individuals of the same species. Therefore, it had carbon isotope composition depleted in ^{13}C. No gut content analysis was done for any of the fish species. However, table 4.1 presents feeding behavior observations and stomach content for the fish species from Oman waters and different tropical systems. The variable diets account for inter- and intraspecific $\delta^{13}C$-variations.

The $\delta^{13}C$-values of each fish species were consistent except signatures of *U.doriae* which showed higher variability (coefficient of variation, C.V. = 8.8%) compared to the rest of the species. There have been no previous investigations on isotopic compositions of the fish species analyzed in this study and therefore inter-study comparisons could not be done. However, similar range in $\delta^{13}C$ (-18.60 to -13.4‰) was reported by Thimdee *et al.* (2004) for different fish species from the tropical system of Khung Krabaen (Thailand). Yellowfin tuna (*Thunnus albacares*) had muscle $\delta^{13}C$ of -16.10‰ (Rau *et al.*, 1983), almost the same value (-16.34‰) reported here for its closely

related species, longtail tuna (*T.tonggol*) from the Gulf of Oman. Also, the range of -18.59 to -15.94‰ of *E.epistictus* was similar to -17.90 to -15.90‰ documented for the closely related species *E.margintus* (Reñones *et al.*, 2002) from a western Mediterranean littoral ecosystem.

The absence of relationship between carbon signatures and size parameters (length and weight) for each fish species (except a marginal negative correlation between $\delta^{13}C$ and weight for *C.malabricus*) indicated that fish species utilize food from primary producers with the same carbon signatures.

Demersal fish species (*A.spinifer*, *N.randalli*, *E.epistictus* and *U.doriae*) had ^{13}C-enriched values relative to reef-associated (*C.malabricus*, *S.crumenopthalmus*, *S.tol*, *Sphyraena* sp. and *R.kanagurta*) and pelagic fish species (*E. affinis*, *S.longiceps*, *T. tonggol*). Such observations were noticed by Gearing *et al.* (1984) for a temperate estuary, Kaehler *et al.* (2000) for a subantarctic ecosystem and Fisk *et al.* (2003) for North American Arctic. This ^{13}C-enrichment observed for the demersal fish species could be, as suggested by Hobson *et al.* (2002), due to use of cycled as well as ungrazed carbon available for benthic organisms, which make up the diets for these fish.

Based on feeding-strategy categories, tissues of planktivorous fish (*S.crumenopthalmus*, *S.longiceps* and *R.kanagurta*) were ^{13}C-depleted compared to invertivorous (*A.spinifer*), unknown (*N.randalli* and *U.doriae*) and piscivorous fish species (*S.tol*, *Sphyraena* sp.). The slight ^{13}C-depletion of planktivores relative to predatory fish species (*C.malabricus*, *E. affinis*, *T. tonggol* and *E.epistictus*) was due, as stated above, to similar diet compositions.

Table 4.1: Observed diet compositions of fish species

Fish species	Observed diet compositions	References
S. longiceps	Phytoplankton, zooplankton and small crustaceans	Randall (1995) and Pet *et al.* (1997)
S. crumenophthalmus	Zooplankton	Randall (1995)
R. kanagurta	zooplankton	Randall (1995)
A. spinifer	Benthic invertebrates (mainly mollusks)	Bauchot and Smith (1984)
C. malabaricus	Crustaceans, small squids and fish	Randall *et al.* (1990)
E. epistictus	Fish and crustaceans	Randall (1995)
E. affinis	Small fish (clupeids and antherinids), squids and crustaceans and zooplankton	Collette (1983)
T. tonggol	Fish, cephalopods and crustaceans (stomatopod larvae and prawns)	Collette (1983)
R. acutus	Benthic and schooling fish (clupeids), cephalopods, crustaceans and gastropods	Randall (1995)
S. tol	Fish	Randall (1995)
Sphyraena sp.	Fish	Randall (1995)

In addition, no difference in ^{13}C-signals of invertivorous, unknown, piscivorous and predatory fish strengthens the conclusion of similar prey items available for the examined fish species. Fry (1988) reported lower ranges of -22.10 to -18.40‰ for planktivorous, -18.00 to -15.80‰ for opportunistic generalists and -17.60 to -16.30‰ for piscivorous than those reported in this study.

4.3 Isotopic nitrogen compositions of fish species

The highest $\delta^{15}N$-values were recorded for opportunistic predators and piscivore, *Sphyraena* sp. The invertivorous-demersal species (*A.spinifer*) had mean $\delta^{15}N$-value of 16.00‰ which is almost identical to 15.94‰ of *U.doriae*, another demersal fish species but nothing is known about its biology including feeding habit. The nitrogen isotopic signals suggest that it feeds at the same level of *A.spinifer* and could be inferred that *U.doriae* survives on the same diet compositions of *A.spinifer*. On average, the lowest $\delta^{15}N$-values were found in the predatory fish, *E.affinis* and planktivorous fish species. The lower $\delta^{15}N$-signatures (average: 13.70‰) of *E.affinis* than that for planktivorous fish species could be attributed to the feeding habit of *E.affinis* which is a highly opportunistic predator (Collette, 1983), and the reported variability in $\delta^{15}N$-values did reflect the feeding nature of this species. A lower $\delta^{15}N$-range (6.8 – 14.6‰) was determined by Thimdee *et al.* (2004), probably due to different nitrogen sources at the base of the food chain.

Of fish species, the significant correlation between $\delta^{15}N$-signatures and length and weight of *Euthynnus affinis* suggests a shift in feeding habit from one prey items to another over time. A similar gradual increase of trophic position was described for *Epinephelus margintus* (Reñones *et al.*, 2002). The other fish species exhibited no trophic

change as they grow in size and probably the lack of correlations could be related to limited individual sizes included in the sample for each species (particularly *N.randall*, *R.kanagurta*, *S.longiceps* and *U.doriae*). Otherwise, size-independent $\delta^{15}N$-values reinforced the usefulness of the nitrogen stable isotopes as an indicator of trophic position.

Like $\delta^{13}C$, isotopic nitrogen compositions of demersal fish species were higher than the pelagic fish species. The same trend was observed by Kaehler *et al.* (2000) and Fisk *et al.* (2003) for benthic organisms compared to pelagic ones. Similarities between reef-associated and pelagic fish species and reef-associated and demersal species implied that reef-associated fish feed on pelagic and benthic prey. Feeding-habit categories (invertivorous, unknown, piscivorous and predatory fish) had similar $^{15}N/^{14}N$ ratios which were enriched on average by 1.43‰ to that of planktivorous fish. $\delta^{15}N$-signatures showed an increasing trend from planktivores, through invertivores and piscivores, consecutively to predatory fish species. Fry (1988) found that $\delta^{15}N$-signals increased from lower trophic level to higher trophic levels.

4.4 Trophic structures

In the samples from coastal water of the Capital Area of Muscat, the trophic fractionation ($\Delta\delta$) of $\delta^{13}C$ between zooplankton and their planktivorous consumers (*S.crumenopthalmus*, *R.kanagurta* and *S.longiceps*) averaged 3.4‰. This represents a huge enrichment between prey and predator compared to the more frequently observed 0.50-1.00‰ increase in $\delta^{13}C$-values between trophic levels (Gearing et al., 1984; Fry, 1988; Rau *et al.*, 1983; Dittel *et al.*, 1997; Vander Zanden *et al.*, 2001; Hart and Lovvorn, 2002). This implies that the zooplankton were not the main diet of these fish species.

Further sampling is required but it would seem that the direct feeding on zooplankton is not possible.

Great caution should be taken with the previous conclusion of disconnection between zooplankton and planktivorous fish species in term of energy transfer as indicated by carbon isotope data. Limited temporal sampling of zooplankton would result in carbon isotope estimates that may not have been representative of isotope signatures in the zooplankton community over a longer time period. In a western Mediterranean lagoon, Vizzini and Mazzola (2003) showed that carbon signatures in zooplankton, particularly copepods, can vary greatly through time reflecting variations in phytoplankton signatures. At the base of the food web, these variations do not manifest themselves to the same extent in fish because of longer life-spans and longer tissue turn – over times (Vizzini and Mazzola, 2002). Gut content analyses could improve the conclusion since it could provide evidence if the zooplankton were among items consumed by the fish. No gut content analysis was carried to verify the interpretation of the carbon isotope data. Because lipid is depleted in ^{13}C isotope, lipid could result in more negative $\delta^{13}C$-values than they should be if the lipid were removed. Copepods species have high content of lipids than many other zooplankton species (Arts, 1999).

Clupeids (e.g. *S. longiceps*) are small schooling fish consumed by Scombrides (e.g. *E.affinis*) (Collette, 1983). The $\Delta\delta^{13}C$ contradicts with our initial model between *S. longiceps* and *E.affinis* (Figure 1.1) and the explanation for this discrepancy is that the sampled *E.affinis* fed on other prey items rather than *S. longiceps*. However, shark (*R. acutus*) also feed on *S. longiceps* (Collette, 1983) and their $\delta^{13}C$-values concurred with the model with $\Delta\delta^{13}C$ of 1.20‰, similar to values reported in literature.

For $\delta^{15}N$, the main trophic shift ($\Delta\delta$) between zooplankton and planktivores was 3.4‰, which is identical or close to average value reported by others (Minawaga and Wada, 1984; Schoeninger and DeNiro, 1984; Cabana and Rasmussen, 1994; Dittel *et al.*, 1997; Vander Zanden *et al.*, 2001; Hart and Lovvorn, 2002; McCutchan *et al.*, 2003). In addition, a close value of $\Delta\delta^{15}N$ (2.65‰) found between prey, *S. longiceps* and predator, *R. acutus*. Because of the gap of huge $\delta^{13}C$ transfer between zooplankton and planktivorous fish, assuming that zooplankton occupy trophic level 2 appeared doubtful. It seems that there would be additional trophic links between zooplankton and planktivorous fish. Therefore, further work is required to elucidate isotopic compositions of primary producers (algae or phytoplankton) as well as benthic invertebrates.

4.5 T-Hg and MeHg of zooplankton

T-Hg concentrations of zooplankton reported in this study were higher than that (0.003-0.010 $\mu g \cdot g^{-1}$) determined by Al-Majed and Preston (2000) for samples from Kuwait Bay and 0.005-0.021 $\mu g \cdot g^{-1}$ for zooplankton from Monterey Bay, California (Knauer and Martin, 1972). It is important to note that Al-Majed and Preston (2000) and Knauer and Martin (1972) used 334 μm- and 366 μm-mesh size nets which are larger than 250 μm net used in this study. Zooplankton from the Gulf of Oman were collected using surface tows 1.5 to 3 from shore. Total mercury range of zooplankton was well below that documented for polluted water bodies such as a Norwegian fjord (0.156-7.563 $\mu g \cdot g^{-1}$) (Skei *et al.*, 1976) and Bombay harbor (0.103- 0.139 $\mu g \cdot g^{-1}$) (Zindge and Desai, 1981).

The MeHg proportion of T-Hg varied from 1 to 19%. However, MeHg is almost never near 1% and this is suspected to very low amount of composite zooplankton

samples included in the analysis. In general zooplankton contains 20-30% methyl mercury of T-Hg. Al-Majed and Preston (2002) found proportion of < 25% MeHg in zooplankton samples from Kuwait Bay.

4.6 T-Hg and MeHg of fish species

Planktivorous fish species (*R.kanagurta* and *S.longiceps*) contained T-Hg concentrations lower than that of zooplankton. However, the fraction of methyl mercury of T-Hg ranged in these species from 48-96%, higher than 1-19% of zooplankton. Higher mercury concentrations (mainly T-Hg) for zooplankton and higher MeHg percentage of T-Hg of planktivorous fish suggests low contribution of water-borne organic mercury in zooplankton and fish species. It could be inferred that direct uptake of MeHg from water is unlikely or at least minimal compared to that received via diets. Food was found to be the dominant pathway of methyl mercury uptake in fish and that MeHg accumulation at the base of food chain is the ultimate determinant of mercury concentration in fish (Mason *et al.*, 1996; Hall *et al.*, 1997; Lawson and Mason, 1998).

Some fish species (e.g. *S.crumenophthalmus*, *N. randalli*, *E. affinis*, *S.tol*, *S. longiceps* and *R .kanagurta*) had similar δ^{15}N-signatures, but their mercury burden was different. This implies that factors (e.g. longevity and size) other than trophic position measured by δ^{15}N-signals contributed more to determination of mercury. Table 4.2 lists T-Hg and MeHg concentrations for the same or closely related species from different tropical marine ecosystems. This study revealed that the mercury concentrations of fish species from the Gulf of Oman were fairly low compared to other tropical regions. However, average T-Hg of *R. acutus* from the Gulf of Oman was higher than that of Powell *et al.* (1981), and Powell and Powell (2001) because larger individuals were

analyzed in this study. Al-Majed and Preston (2000) found elevated T-Hg and MeHg in *E.coioides* and were related to past discharges from a salt and chlorine plant. Adams and Onorato (2005) related elevated T-Hg concentrations of red drum (*Sciaenops ocellatus*) from eastern Florida bay to drainage from the Everglades, urban and agricultural runoff. Lack of such runoff into the coastal water of Capital Area of Muscat could account for low level of mercury detected in fish species. The three industries (oil refinery, water desalination and electricity production plant and the oil-tanker port) could be potential point sources of mercury release. However, there have been no previous reports on eleveated mercury levels in the vicinity or the surrounding waters of these industries.

The mean T-Hg and MeHg concentrations increased from lower trophic levels to higher trophic level, but they were associated with high variability at each trophic level. For example, piscivorous fish species (*S.tol* and *Sphyraena* sp.) had similar T-Hg concentrations to that of unknown species (*N.randalli* and *U.doriae*), as did invertivorous fish (*A. spinifer*) and predatory fish (*C. malabricus*, *E. affinis*, *T. tonggol* and *E. epistictus*). Similar MeHg concentrations of invertivorous, piscivorous and predatory fish species were higher than that of unknown fish species. Atwell *et al.* (1998) found that T-Hg levels showed trend of increasing variability with increasing trophic position that were more pronounced for vertebrates compared to invertebrates. Higher trophic level organisms are more likely to be migratory and have larger foraging ranges than low trophic level organisms (Atwell *et al.*, 1998). Interspecific differences in movement patterns could account for the variability in mercury exposure. Planktivorous fish like *S. longiceps* showed very little variability in mercury burden compared to the migratory tuna species *E. affinis* (Collette, 1983), which had highly variable mercury burden.

Also, variability in mercury burdens of fish species could be attributed to lipid contents of muscle tissues (e.g. Francesconi and Lenanton, 1992). However, muscle lipid contents of fish tend to be small (e.g. 0.58 – 1.89% in several bony and cartilaginous fish species analyzed by Rau *et al.* 1983 and 0.5 – 13.0% reported by Bloom, 1992). Bloom (1992) stated that there is no discernable trend of greater MeHg levels in fish of higher fat content.

The fraction of methyl mercury in fish species varied considerably and appeared lower than the percentages frequently reported for methyl mercury (>85%). The lowest fraction (33%) was found in piscivore (*S. tol*). Al-Majed and Preston (2000) found mean % MeHg of 40.1 in *Hilsha ilisha*. Others (Joiris *et al.*, 2000; Kehrig *et al.*, 2002; de Pinho *et al.*, 2002) also reported lower MeHg proportions of T-Hg for different fish species.

Table 4.2: T-Hg concentrations for the same or closely-related species from different tropical marine ecosystems

Fish species	*T-Hg ($\mu g \cdot g^{-1}$)wet wt.*	*Reference*
C. malabaricus	0.044	This study
C. fulvoguttatus	0.059	Denton and Burdon-Jones (1986)
	0.060	Rayment *et al.* (2000)
S. crumenophthalmus	0.055	This study
	0.020	Bayen *et al.* (2005)
A. spinifer	0.090	This study
	0.168	Jaffar *et al.* (1995)
E. affinis	0.176	This study
	0.065-1.260	Matthwes (1983)
	0.024-0.062	Denton and Burdon-Jones (1986)
S. tol	0.042	This study
	< 0.025	Chen (2002)
S. longiceps	0.004	This study
	0.034-0.158	Tariq *et al.* (1991)
S.aurita	0.027	Roméo *et al.* (1999)
R. acutus	0.351	This study
	0.020-2.000	Powell *et al.* (1981)
	0.122	Powell and Powell (2001)
T. tonggol	0.154	This study
T. alalunga	0.840-1.000	Storelli *et al.* (2002a)
T. thynnus	0.160	Storelli *et al.* (2002a)
Sphyraena sp.	0.066	This study
S. forsteri	0.260- 1.580	Matthwes (1983)
S. putnamae	< 0.025	Chen (2002)
R. kanagurta	0.005	This study
	0.064	Joiris *et al.* (2000)
E. epistictus	0.123	This study
E. tauvina	0.180-1.290	Al-Hashimi and Al-Zorba (1991)
	0.107	Ahmad and Al-Ghais (1996)
E. coioides	1.177	Al-Majed and Preston (2000)

4.7 T-Hg and MeHg accumulation

Mercury burden of fish is affected by several variables including age, size, trophic position and degree of exposure (e.g. movement patterns). T-Hg and MeHg concentrations in the planktivore *S.crumenophthalmus*, in the invertivore *A.spinifer* and predatory *C. malabricus*, were not related to their age although the age ranged between 3-14 years. In more than 80 individuals of barred sand bass (*Paralabrax nebulifer*), Philip *et al.* (1997) described a moderate correlation (r^2 = .34) between age and Log_{10}[T-Hg]. The lack of relationship could be due to mechanisms for getting rid of mercury. For example, fish have low metabolic rates and therefore require little food and grow very fast so that the assimilated mercury is diluted by biomass accumulation (Hopkins *et al.*, 2003).

The positive relationships between T-Hg and MeHg and size length/weight for *C. malabaricus*, *S. crumenophthalmus*, *R. acutus*, *E. epistictus* and *U.doriae* indicated that T-Hg levels increased as they grow in size, a trend frequently observed for fish (Francesconi and Lenanton, 1992; Andersen and Deplegde, 1997; Joiris *et al.*, 1999; Al-Majed and Preston, 2000; Joiris *et al.*, 2000; Kehrig *et al.*, 2002; de Pinho *et al.*, 2002). Limited size of individuals for *S. longiceps* and *R. kanagurta* masked the relationship between mercury burden and size parameters. However, the rest of fish species with good size range did not show correlation and this is probably due to the fact that some fish species have short live spans with fast growth rates and the mercury burden is diluted by rapid muscle build up (Hopkins *et al.*, 2003).

For a demersal fish *U.doriae*, there was no statistically significant correlation between $\delta^{15}N$ and size parameters but a stronger correlation between T-Hg and $\delta^{15}N$ (τ =

.636, $p < .05$) than that with length ($\tau = .500, p < .05$), this may imply that mercury burden of *U.doriae* is more likely to be controlled by trophic position that of size parameters. Despite lack of correlation with size (due to limited size of individuals in case of *S. longiceps*), T-Hg and MeHg were correlated to δ^{13}C-signals of demersal fish *A. spinifer* and pelagic fish *S. longiceps*. At present, no explanation can be drawn for such observations. Power *et al.* (2002) found that T-Hg concentrations were inversely related to δ^{13}C-signatures.

Weak linear relationships between δ^{15}N-signatures and T-Hg and MeHg concentrations of zooplankton and fish species from the Gulf of Oman were described by regression equations; $Log_{10}[T\text{-}Hg] = 0.08(\delta^{15}N) - 2.63$ and $Log_{10}[MeHg] = 0.05(\delta^{15}N) - 2.34$. The slopes of the regressions (0.08 and 0.05) which are biomagnification power as described by Atwell *et al.* (1998) are substantially smaller than 0.17 to 0.48 found by Kidd *et al.* (1995) for six lakes in northwestern Ontario, 0.32 determined by Jarman *et al.* (1996) in the Gulf of Farallones, 0.20 found from marine food web of Lancaster Sound (Atwell *et al.*, 1998) and 0.19 determined by Power et al. (2002) for Stewart Lake, a subarctic lake. It could be inferred that T-Hg and MeHg do not seem to biomagnify in this tropical marine ecosystem.

For demersal fish species (*A. spinifer*, *N. randalli*, *E. epistictus* and *U. doriae*), total length was found to explain 38 and 40% of variability in T-Hg and MeHg concentrations, respectively. On the other hand, trophic position as indicated appeared to be an insignificant predictor of mercury burden in these fish. T-Hg of pelagic fish species (*E. affinis*, *S. longiceps* and *T. tonggol*) was almost completely predicted by length (r^2 = .92) as was MeHg (r^2 = .94). None of predictors (length, δ^{13}C and δ^{15}N) contributed to

explain variability of T-Hg and MeHg of reef-associated species (*C. malabaricus*, *S. crumenophthalmus*, *S. tol*, *Sphyraena* sp. and *R. kanagurta*).

T-Hg and MeHg concentrations of fish species were related to the feeding habits by including stable isotopes in multiple regression models. Both $\delta^{13}C$ and $\delta^{15}N$ contributed equally to account for variability in T-Hg and MeHg for planktivorous species (*S. crumenophthalmus*, *S. longiceps* and *R. kanagurta*). The amount of variance in mercury explained by $\delta^{13}C$ and $\delta^{15}N$ increased from 0.42 for T-Hg to 0.51 for MeHg concentrations, proposing that trophic effect is relatively more pronounced on MeHg transfer than T-Hg. It is important to note that the negative *standardized* β-values for both $\delta^{13}C$ and $\delta^{15}N$ indicate that T-Hg and MeHg would decrease as planktivorous fish exhibit higher isotopic signatures. T-Hg and MeHg of invertivore (*A. spinifer*) were depicted by regression equations: $Log_{10}[T\text{-}Hg] = 0.43(\delta^{13}C) - 1.52$, $r^2 = .52$ and $Log_{10}[MeHg] = 0.43(\delta^{13}C) - 0.47$, $r^2 = .58$. Identical slopes for both equations (0.43) implied that T-Hg and MeHg accumulated equally in tissues of *A. spinifer*.

Mercury levels in fish species grouped as unknown (*N.randalli* and *U. doriae*) were not accounted for by length, $\delta^{13}C$ or $\delta^{15}N$. Both species are demersal but they differed in their feeding habits as indicated by their $\delta^{15}N$-values. *U. doriae* showed a positive correlation between $\delta^{15}N$ and T-Hg and MeHg while *N.randalli* did not. This showed that grouping them into one trophic position is doubted.

Total length was the only significant predictor of T-Hg ($r^2 = .22$) and MeHg ($r^2 = .28$) for piscivorous fish species (*S.tol* and *sphyraena* sp.) and of T-Hg ($r^2 = .60$) and MeHg ($r^2 = .54$) for predatory fish species. Matthews (1983) observed that low weighted individuals of the piscivore (*sphyraena forsteri*) contained high T-Hg levels which do not

increase with weight at a rate which might be expected. Length effect on mercury burden was observed to be more pronounced in predatory fish species.

4.8 Tolerable daily intake

Fish consumption represents the dominant pathway of methyl mercury exposure (Goyer and Clarkson, 2001). However, MeHg intake depends on the species of fish consumed as well as the quantity of fish eaten (Mahaffey, 2004b; USFDA and USEPA, 2004). Although average T-Hg concentrations were well below WHO/Health Canada guidelines for all fish species from the Gulf of Oman (Figure 4.1), two samples (0.668 and 0.760 $\mu g \cdot g^{-1}$) of shark (*R. acutus*) exceeded the 0.5 $\mu g \cdot g^{-1}$ wet weight guideline which is intended to regulate fresh and processed fish suitable for human consumption. Using average methyl mercury concentrations for fish species, number of fish meals were per day was calculated based on an average daily fish meal of 50, 100 and 150 grams for child of 30-kg , child bearing-age women of 60-kg and average adult body weight of 70 kg. The number of fish meals is presented in Figure 4.2 for the following species: *A. spinifer*, *E. affinis R. acutus*, *T. tonggol* and *E. epistictus*. High T-Hg and MeHg concentration were found in these species. The bars represent the allowable meals per day to stay below 0.23 μg/kg-day, a daily WHO guideline for methyl mercury. This guideline is similar to the Health Canada guideline of 0.20 μg MeHg/kg-day. Consumption of most of the fish species would not result in exceeding the daily guideline, however, restrictions should be applied for children and women of bearing-age. Children and women of child bearing-age should not exceed the calculated meals per day (Figure 4.2) of bream (*A. spinifer*), kawakawa (*E. affinis*), grouper (*E. epistictus*), shark (*R. acutus*) and longtail tuna (*T. tonggol*) in order to minimize exposure to methyl mercury.

Figure 4.1: Mean total mercury concentrations of fish species collected from the Gulf of Oman were all below 0.5 $\mu g \cdot g^{-1}$, a WHO guideline for mercury in fish for human consumption.

Species Key:

Zoo: Zooplankton
F1: *C. malabaricus*-predator
F2: *S. crumenophthalmus*-planktivore
F3: *A. spinifer*-invertivore
F4: *N. randalli*-unknown
F5: *E. affinis*-predator
F6: *S. tol*-piscivore
F7: *S. longiceps*-planktivore
F8: *R. acutus*-predator
F9: *T. tonggol*-predator
F10: *Sphyraena* sp.-piscivore
F11: *R. kanagurta*-planktivore
F12: *E. epistictus*-predator
F13: *U. doriae*-unknown

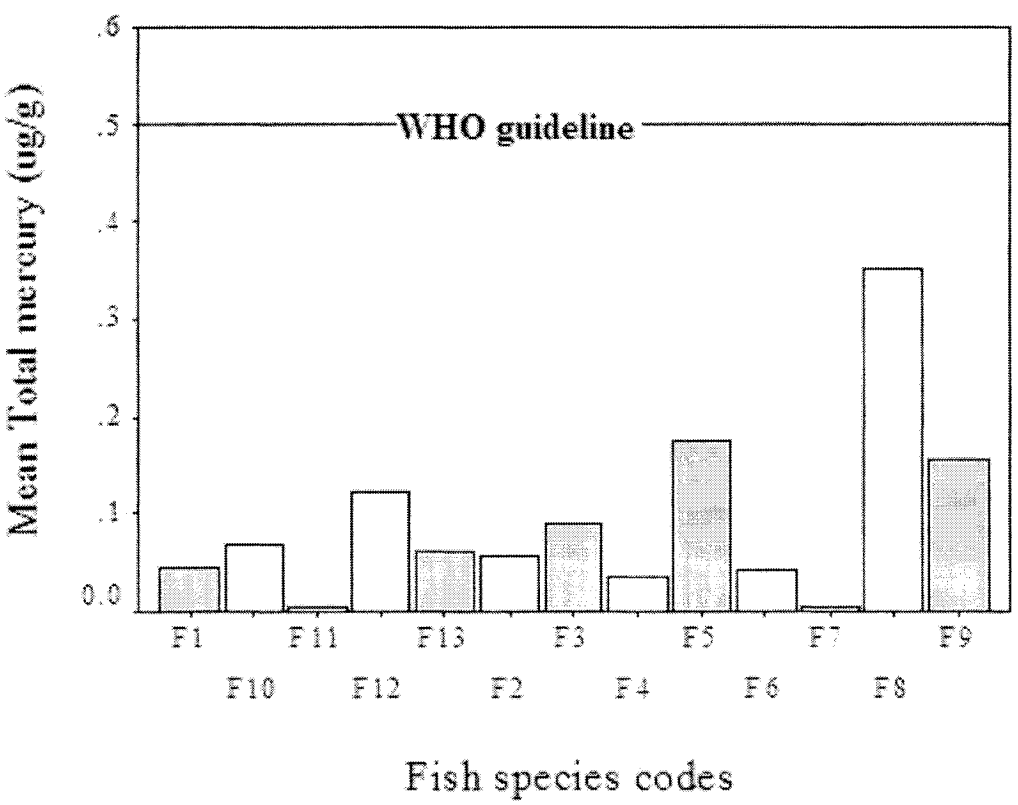
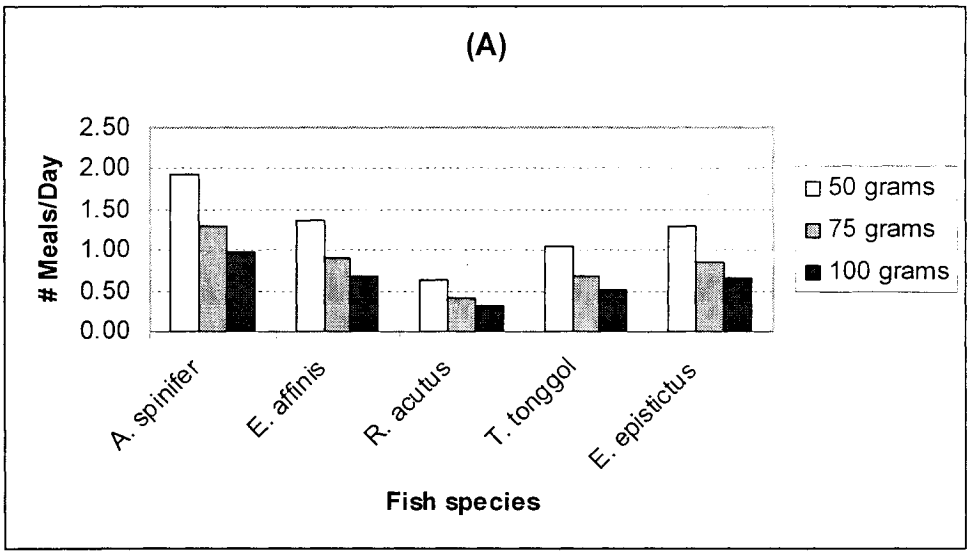
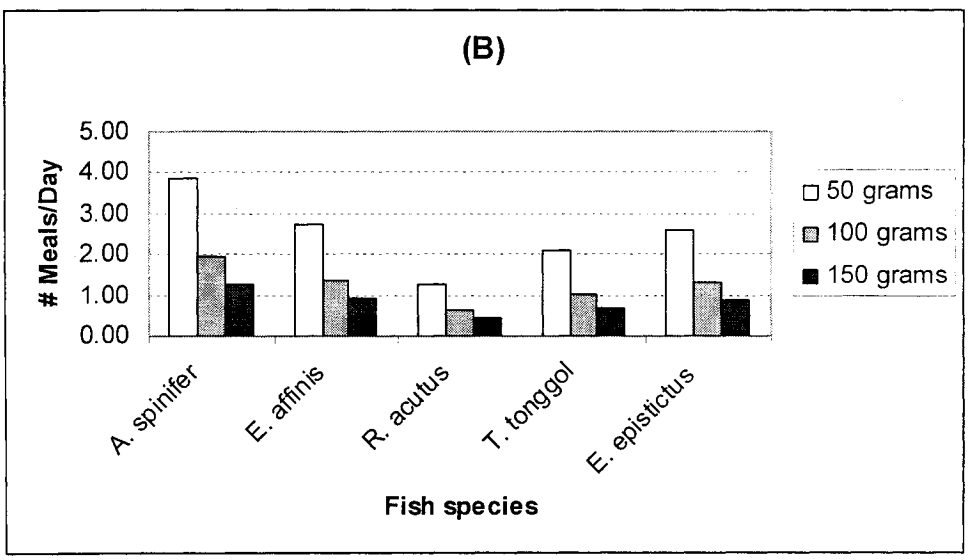
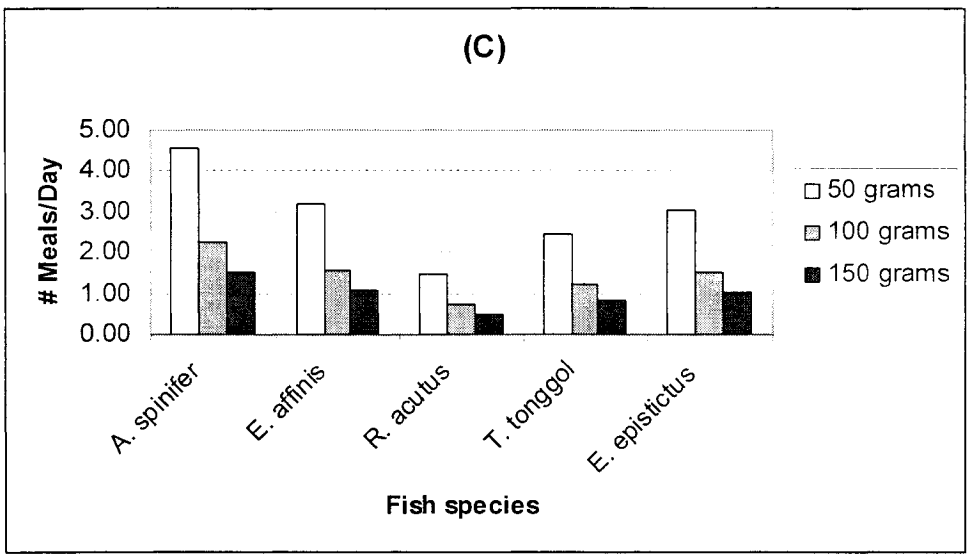

Mean Total mercury (ug/g)
.6
.5
.4
.3
.2
.1
0.0
WHO guideline
F1
F10
F11
F12
F13
F2
F3
F4
F5
F6
F7
F8
F9
Fish species codes

Figure 4.2: Calculated number of fish meals per day to stay below 0.23μg/kg-day, a daily WHO guideline for methyl mercury intake for selected fish species (*A. spinifer*, *E. affinis* *R. acutus*, *T. tonggol* and *E. epistictus*) from the Gulf of Oman. (A) for child of 30-kg body weight, (B) for child bearing-age woman of 60-kg body weight and (C) for adult of 70-kg body weight.


(A)
Meals/Day
2.50
2.00
1.50
1.00
0.50
0.00
A. spinifer
E. affinis
R. acutus
T. tonggol
E. epistictus
Fish species
50 grams
75 grams
100 grams


(B)
Meals/Day
5.00
4.00
3.00
2.00
1.00
0.00
A. spinifer
E. affinis
R. acutus
T. tonggol
E. epistictus
Fish species
50 grams
100 grams
150 grams


(C)
5.00
4.00
3.00
2.00
1.00
0.00
Meals/Day
50 grams
100 grams
150 grams
A. spinifer
E. affinis
R. acutus
T. tonggol
E. epistictus
Fish species

The health risks from overexposure to methyl mercury through consumption of fish are recognized. However, in Oman, the mercury levels in the species commonly consumed are low and intake calculations showed that individuals can safely consume fish when the type of fish is properly selected. Thus, the risk is low whereas the benefits are numerous and cannot be overlooked. Fish are excellent sources of high-quality protein and low in saturated fat (CFIA, 2002). Fish intake is associated with a reduction in the risk of coronary heart diseases (Kris-Etherton *et al.*, 2002). The fisheries are considered the most important non-oil source of income for Oman (FAO, 2001). The fishing industry still uses traditional methods of harvesting fish and other seafood. More than 26,000 artisanal fishermen are directly employed in the fisheries sector (Zaibet, 2001) with fish landings of 118,877 metric tons (86% of total fish landings) in 2003 (Ministry of National Economy, 2004). These figures highlight the economic importance (e.g. source of income and employment opportunities) and social benefits (e.g. promoting cooperation and social cohesion as indicated by Wheatley and Wheatley, 2000) of the traditional fishing activities.

4.9 Conclusion

$\delta^{13}C$ and $\delta^{15}N$ values for zooplankton and fish species from the coastal waters of the Capital Area of Muscat on the Gulf of Oman used to determine the food web structure of this area. They were found useful in distinguishing zooplankton with distinct isotopic signals from that of fish species. Because of observed large trophic enrichment ($\Delta\delta^{13}C$) of 3.4‰, zooplankton would seem to not be the main diet of the planktivorous fish species (*S. crumenophthalmus*, *S. longiceps* and *R. kanagurta*). Trophic shifts ($\Delta\delta^{15}N$) of 3.4 and 2.7 ‰ between zooplankton and planktivores and *S. longiceps* and *R. acutus*,

respectively, were found between prey-predator relationships. These values are similar to those reported by others.

Total mercury (T-Hg) concentrations of zooplankton were very low (range: 0.010 to 0.037 $\mu g \cdot g^{-1}$, N= 27) with mean methyl mercury (MeHg) levels of 0.001 $\mu g \cdot g^{-1}$ (percentage of T-Hg range: 1-19%, N = 5). Total and methyl mercury levels of fish species varied considerably. The lowest T-Hg (0.003 $\mu g \cdot g^{-1}$) was found in a planktivore (*S. longiceps*) and the highest was 0.760 $\mu g \cdot g^{-1}$ in a predator shark (*R. acutus*). The fraction of methyl mercury in fish species (average: 72%) appeared lower than the percentages frequently reported for methyl mercury (>85%).

Slopes of regression equations between mercury levels and $\delta^{15}N$ values (0.08 and 0.05 for T-Hg and MeHg, respectively, as biomagnification power) indicated that mercury biomgnification was lower in this tropical water body compared to that found in freshwater and marine ecosystems of the arctic and temperate zones of the northern hemisphere. T-Hg and MeHg levels were negatively related to both $\delta^{13}C$ and $\delta^{15}N$ in planktivorous species and positively to $\delta^{13}C$ in invertivorus species (*A. spinifer*). Mercury concentrations accumulated with length in fish species grouped as demersal (*A. spinifer*, *N. randalli*, *E. epistictus* and *U. doriae*) and pelagic (*E. affinis*, *S. longiceps* and *T. tonggol*), piscivorous (*S. tol* and *Sphyraena* sp.) and opportunistic predators (*C.malabricus*, *E. affinis*, *T. tonggol* and *E.epistictus*).

Methyl mercury concentrations in fish species commonly consumed are low and intake calculations showed that the humans can safely consume fish when the type of fish is properly selected.

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Appendix A: Correlation Coefficients (Kendall's tau, τ)

Table A-1: Correlations between size parameters (length and weight) and isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$).

Fish species	N	$\delta^{13}C$ vs. length	$\delta^{13}C$ vs. weight	$\delta^{15}N$ vs. length	$\delta^{15}N$ vs. weight	$\delta^{15}N$ vs. $\delta^{13}C$
		τ	τ	τ	τ	τ
		p-value	*p*-value	*p*-value	*p*-value	*p*-value
C. malabaricus	12	-.424	-.455*	-.121	-.091	.333
		.055	<.05	.583	.681	.131
S. crumenophthalmus	14	-.389	-.376	-.300	-.265	.407*
		.055	.062	.138	.188	<.05
A. spinifer	12	.091	.182	.015	.046	.137
		.681	.411	.945	.837	.536
N. randalli	12	-.047	.236	-.338	-.277	.047
		.835	.297	.130	.215	.835
E. affinis	9	-.141	-.167	.592*	.556*	-.056
		.600	.532	<.05	<.05	.835
S. tol	12	.308	.137	.321	.212	.443*
		.168	.536	.149	.337	.046
S. longiceps	16	-.276	-.279	.137	.143	-.269
		.146	.152	.468	.489	.149
R. acutus	7	-.524	-.333	-.333	-.333	-.143
		.099	.293	.293	.293	.652
T. tonggol	10	.405	.449	-.111	.200	-.180
		.106	.072	.655	.421	.472
Sphyraena sp.	11	-.110	-.220	-.404	-.367	.019
		.636	.349	.086	.118	.938
R. kanagurta	12	-.125	-.260	.188	.229	-.030
		.579	.243	.405	.303	.891
E. epistictus	12	.242	.242	.030	-.030	.424
		.273	.273	.891	.891	.055
U. doriae	11	-.056	-.164	.463	.273	.273
		.814	.484	.050	.243	.243

Table A-2: Correlations between size parameters (length and weight) and mercury concentrations (T-Hg and MeHg).

Fish species	N	T-Hg vs. length	T-Hg vs. weight	MeHg vs. length	MeHg vs. weight
		τ	τ	τ	τ
		p-value	p-value	p-value	p-value
C. malabaricus	12	.515*	.606*	.485*	.576*
		<.05	<.05	<.05	<.05
S. crumenophthalmus	14	.878*	.840*	.883*	.833
		<.001	<.001	<.001	<.001
A. spinifer	12	.030	.121	.061	.152
		.891	.583	.784	.439
N. randalli	12	.123	.215	.168	.351
		.582	.335	.450	.114
E. affinis	9	.873*	.889*	.648	.667
		<.01	<.01	.016	.012
S. tol	12	.229	.091	.123	-.137
		.303	.681	.582	.536
S. longiceps	16	-.120	-.347	-.137	-.151
		.526	.072	.468	.433
R. acutus	7	.714*	.905*	.586	.781*
		<.05	<.01	.068	<.05
T. tonggol	10	-.067	.067	.022	-.111
		.788	.788	.929	.655
Sphyraena sp.	11	.236	.127	.127	.091
		.312	.586	.586	.697
R. kanagurta	12	.219	.351	.063	.168
		.332	.114	.782	.450
E. epistictus	12	.576*	.576*	.576*	.576*
		<.05	<.05	<.05	<.05
U. doriae	11	.500*	.345	.389	.236
		<.05	.139	.100	.312

Table A-3: Correlations between mercury concentrations (T-Hg and MeHg) and isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$).

Fish species	N	T-Hg vs. $\delta^{13}C$	T-Hg vs. $\delta^{15}N$	MeHg vs. $\delta^{13}C$	MeHg vs. $\delta^{15}N$	MeHg vs. T-Hg
		τ	τ	τ	τ	τ
		p-value	*p*-value	*p*-value	*p*-value	*p*-value
C. malabaricus	12	-.364	-.182	-.394	-.273	.848*
		.100	.411	.075	.217	<.001
S. crumenophthalmus	14	-.297	-.363	-.398*	-.331	.906*
		.139	.071	.048	.100	<.001
A. spinifer	12	.576*	.076	.545*	.046	.970*
		<.01	.731	<.05	.837	<.001
N. randalli	12	-.158	-.123	.031	-.046	.687*
		.487	.582	.890	.837	<.01
E. affinis	9	-.056	.444	-.278	.556*	.667*
		.835	.095	.297	<.05	<.05
S. tol	12	.290	.212	.000	-.076	.534*
		.192	.337	1.000	.731	<.05
S. longiceps	16	.454*	-.233	.403*	-.267	.300
		<.05	.207	<.05	.150	.105
R. acutus	7	-.238	-.238	-.293	-.098	.878*
		.453	.453	.362	.761	<.01
T. tonggol	10	-.180	.156	-.360	-.022	.644*
		.472	.531	.151	.929	<.01
Sphyraena sp.	11	.220	-.073	.183	-.183	.818*
		.349	.755	.435	.435	<.001
R. kanagurta	12	-.091	.030	-.303	-.303	.545*
		.681	.891	.170	.170	<.05
E. epistictus	12	.424	.152	.424	.152	1.000*
		.055	.493	.055	.493	<.001
U. doriae	11	.273	.636*	.309	.527*	.891*
		.243	<.01	.186	<.05	<.001

Appendix B: Regression models for T-Hg.

Table B-1: Zooplankton and fish species (except *R. acutus*).

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.628			
	$\delta^{15}N$	0.076	0.300	<.001	.090
2	Constant	-3.351			
	$\delta^{15}N$	0.093	0.368	<.01	
	$\delta^{13}C$	-0.028	-0.095	.365	.094

Table B-2: Demersal fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.070			
	Total length	0.026	0.617	<.001	.381
2	Constant	-4.446			
	Total length	0.015	0.355	<.05	
	$\delta^{13}C$	-0.033	-0.095	.463	
	$\delta^{15}N$	0.137	0.337	<.05	.443

Table B-3: Pelagic fish species.

Model		b_0	β	***Sig.***	r^2
1	Constant	-2.894			
	Total length	0.031	0.960	<.001	.921
2	Constant	-2.981			
	Total length	0.034	1.037	<.001	
	$\delta^{13}C$	-0.129	-0.078	<.05	
	$\delta^{15}N$	0.143	-0.213	<.001	.971

Table B-4: Reef-associated fish species.

Model		b_0	β	***Sig.***	r^2
1	Constant	-1.999			
	Total length	0.012	0.397	<.000	.158
2	Constant	-3.824			
	Total length	0.034	0.379	<.01	
	$\delta^{13}C$	-0.136	0.295	.107	
	$\delta^{15}N$	0.105	0.082	.682	.216

Table B-5: Planktivorous fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.260			
	Total length	0.007	0.083	.519	.007
2	Constant	-5.061			
	Total length	-0.011	-0.128	.351	
	$\delta^{13}C$	-0.455	-0.440	<.01	
	$\delta^{15}N$	-0.301	-0.386	<.01	.419

Table B-6: Invertivorous fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-1.717			
	Total length	0.022	0.158	.623	.025
2	Constant	1.517			
	Total length	-0.004	-0.025	.924	
	$\delta^{13}C$	0.427	0.677	<.05	
	$\delta^{15}N$	0.260	0.170	.514	.522

Table B-7: Unknown fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.077			
	Total length	0.022	0.205	.373	.042
2	Constant	-5.035			
	Total length	0.031	0.279	.249	
	$\delta^{13}C$	-0.073	-0.291	.289	
	$\delta^{15}N$	0.105	0.296	.278	.126

Table B-8: Piscivorous fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-1.801			
	Total length	0.094	0.465	<.05	.217
2	Constant	2.288			
	Total length	0.010	0.502	.059	
	$\delta^{13}C$	0.212	0.454	.063	
	$\delta^{15}N$	-0.047	-0.339	.244	.353

Table B-9: Opportunistic predatory fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-1.686			
	Total length	0.014	0.772	<.001	.596
2	Constant	-2.503			
	Total length	0.012	0.656	<.001	
	$\delta^{13}C$	-0.066	-0.195	.117	
	$\delta^{15}N$	-0.088	-0.040	.715	.628

Appendix C: Regression models for MeHg.

Table C-1: Zooplankton and fish species (except *R. acutus*).

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.359			
	$\delta^{15}N$	0.048	0.478	<.001	.228
2	Constant	-2.063			
	$\delta^{13}C$	0.095	0.505	<.05	
	$\delta^{15}N$	0.065	0.955	<.001	.255

Table C-2: Demersal fish species.

Model		b_0	β	***Sig.***	r^2
1	Constant	-2.335			
	Total length	0.030	0.634	<.001	.402
2	Constant	-4.446			
	Total length	0.019	0.400	<.05	
	$\delta^{13}C$	-0.013	-0.032	.797	
	$\delta^{15}N$	0.152	0.331	<.05	.461

Table C-3: Pelagic fish species.

Model		b_0	β	***Sig.***	r^2
1	Constant	-3.093			
	Total length	0.032	0.969	<.001	.940
2	Constant	-3.824			
	Total length	0.034	1.022	<.001	
	$\delta^{13}C$	-0.136	-0.081	<.05	
	$\delta^{15}N$	0.105	-0.154	<.001	.971

Table C-4: Reef-associated fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.168			
	Total length	0.012	0.375	<.001	.141
2	Constant	-3.824			
	Total length	0.011	0.348	<.05	
	$\delta^{13}C$	-0.126	0.229	.218	
	$\delta^{15}N$	-0.011	-0.037	.855	.181

Table C-5: Planktivorous fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.589			
	Total length	0.015	0.171	.286	.029
2	Constant	-4.740			
	Total length	-0.004	-0.047	.710	
	$\delta^{13}C$	-0.485	-0.438	<.01	
	$\delta^{15}N$	-0.377	-0.451	<.01	.510

Table C-6: Invertivorous fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-1.966			
	Total length	0.028	0.197	.539	.039
2	Constant	0.471			
	Total length	0.016	0.011	.964	
	$\delta^{13}C$	0.436	0.688	<.05	
	$\delta^{15}N$	0.319	0.208	.402	.575

Table C-7: Unknown fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.448			
	Total length	0.030	0.252	.270	.064
2	Constant	-5.142			
	Total length	0.037	0.308	.205	
	$\delta^{13}C$	-0.045	-0.164	.544	
	$\delta^{15}N$	0.118	0.301	.269	.131

Table C-8: Piscivorous fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-2.215			
	Total length	0.013	0.527	<.05	.278
2	Constant	2.503			
	Total length	0.012	0.471	.065	
	$\delta^{13}C$	0.258	0.437	.063	
	$\delta^{15}N$	-0.033	-0.190	.492	.402

Table C-9: Opportunistic predatory fish species.

Model		b_0	β	*Sig.*	r^2
1	Constant	-1.864			
	Total length	0.015	0.737	<.001	.544
2	Constant	-3.454			
	Total length	0.013	0.666	<.001	
	$\delta^{13}C$	-0.076	-0.200	.132	
	$\delta^{15}N$	-0.027	0.111	.347	.572

Appendix D: All Data.

Table D-1: Date of collection, composition, $\delta^{13}C$, $\delta^{15}N$, and total mercury concentrations of zooplankton species from the Gulf of Oman.

ID	Date	Location	Microscopic examination	$\delta^{13}C$	$\delta^{15}N$	Total mercury (ug/g)	
ZOO1	29-5-04	Matrah	copepods	-19.19	10.07	0.035	0.037
ZOO2	29-5-04	Matrah	copepods, shrimp larva	-19.26	10.59	0.015	0.014
ZOO3	29-5-04	Matrah	copepods,	-19.06	11.95	0.012	0.011
ZOO4	29-5-04	Matrah	copepods, shrimp larva	-19.52	8.47	0.025	0.013
ZOO5	29-5-04	Matrah	Copepods	-19.71	11.64	0.026	0.019
ZOO6	29-5-04	Matrah	copepods	-19.26	11.81	0.016	0.010
ZOO7	29-5-04	Matrah	copepods	-19.22	11.82	0.025	0.027
ZOO8	29-5-04	Matrah	copepods	-19.68	12.24	0.012	0.015
ZOO9	29-5-04	Matrah	copepods	-19.50	12.38	0.015	0.026
ZOO10	13-6-04	A'Seeb	copepods, green algae, large diatoms	-19.30	9.73	0.024	0.036
ZOO11	13-6-04	A'Seeb	copepods	-19.59	9.19	0.016	0.015
ZOO12	13-6-04	A'Seeb	copepods, shrimp larva	-23.61	12.03	0.023	0.035
ZOO13	13-6-04	A'Seeb	copepods	-24.56	12.21	0.023	0.027
ZOO14	13-6-04	A'Seeb	copepods	-19.60	10.42	0.022	0.027
ZOO15	13-6-04	A'Seeb	copepods	-19.57	9.52	0.034	0.036
ZOO16	13-6-04	A'Seeb	copepods	-18.94	11.32	0.021	0.024
ZOO17	13-6-04	A'Seeb	copepods	-23.53	11.21	0.019	0.019
ZOO18	14-6-04	A'Seeb	copepods	-19.13	12.45	0.035	0.037
ZOO19	14-6-04	A'Seeb	copepods	-19.28	9.79	0.015	0.016
ZOO20	14-6-04	A'Seeb	copepods, large diatoms	-19.93	9.41	0.025	0.021
ZOO21	14-6-04	A'Seeb	copepods	-19.25	13.58	0.024	0.024
ZOO22	14-6-04	A'Seeb	copepods	-22.85	12.52	0.034	0.030
ZOO23	14-6-04	A'Seeb	copepods	-19.44	10.08	0.019	0.020
ZOO24	14-6-04	A'Seeb	copepods	-19.36	12.22	0.012	0.011
ZOO25	14-6-04	A'Seeb	copepods	-19.41	11.54	0.013	0.014
ZOO26	14-6-04	A'Seeb	copepods, shrimp larva	-16.28	10.32	0.010	0.010
ZOO27	14-6-04	A'Seeb	copepods, shrimp larva	-18.44	12.74	0.010	0.011

Table D-2: Date of collection, scientific names, total length, weight, age, $\delta^{13}C$, $\delta^{15}N$, total mercury and methyl mercury concentrations of fish species from the Gulf of Oman.

ID	Date	Location	Fish species	T.Length (cm)	Weight (grams)	Age (years)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	T-Hg ($\mu g \cdot g^{-1}$)		MeHg ($\mu g \cdot g^{-1}$)	
F1	30-5-04	Matrah	*C. malabaricus*	23.7	184	11	-15.22	16.70	0.042	0.039	0.036	0.032
F2	30-5-04	Matrah	*C. malabaricus*	27.8	270	3	-14.53	17.23	0.044	0.037	0.030	0.028
F3	30-5-04	Matrah	*C. malabaricus*	21.9	140	7-6	-14.33	16.88	0.034	0.034	0.021	0.032
F4	30-5-04	Matrah	*C. malabaricus*	31.1	395	9	-15.99	15.46	0.111	0.106	0.086	0.100
F5	30-5-04	Matrah	*C. malabaricus*	25.3	186	4	-14.72	16.94	0.063	0.056	0.055	0.055
F6	30-5-04	Matrah	*C. malabaricus*	22.8	152	5	-14.18	17.73	0.038	0.046	0.017	0.024
F7	30-5-04	Matrah	*C. malabaricus*	23.0	160	4	-15.27	17.63	0.021	0.025	0.018	0.017
F8	30-5-04	Matrah	*C. malabaricus*	24.1	170	undefined	-14.69	18.48	0.028	0.031	0.019	0.017
F9	30-5-04	Matrah	*C. malabaricus*	31.5	418	4	-15.56	15.76	0.068	0.075	0.052	0.049
F10	30-5-04	Matrah	*C. malabaricus*	22.4	137	undefined	-15.19	16.65	0.013	0.013	0.006	0.006
F11	30-5-04	Matrah	*C. malabaricus*	26.8	243	5-4	-15.40	17.78	0.036	0.048	0.031	0.031
F12	30-5-04	Matrah	*C. malabaricus*	23.5	155	4	-14.25	17.10	0.020	0.022	0.018	0.017
F13	30-5-04	Matrah	*S. crumenophthalmus*	20.3	98	4-3	-16.18	14.00	0.032	0.033	0.022	0.020
F14	30-5-04	Matrah	*S. crumenophthalmus*	21.9	120	3	-16.74	13.54	0.056	0.061	0.060	0.050
F15	30-5-04	Matrah	*S. crumenophthalmus*	20.3	97	10-9	-17.11	13.46	0.024	0.022	0.020	0.021
F16	30-5-04	Matrah	*S. crumenophthalmus*	22.7	135	undefined	-18.18	13.96	0.080	0.090	0.082	0.082
F17	30-5-04	Matrah	*S. crumenophthalmus*	26.7	208	7	-16.43	14.13	0.253	0.265	0.170	0.179
F18	30-5-04	Matrah	*S. crumenophthalmus*	20.1	94	6	-17.18	13.16	0.030	0.033	0.027	0.028
F19	30-5-04	Matrah	*S. crumenophthalmus*	22.9	127	14-13	-16.57	13.82	0.101	0.098	0.093	0.098

F20	30-5-04	Matrah	*S. crumenophthalmus*	22.6	127	undefined	-17.68	13.70	0.083	0.081	0.067	0.069
F21	30-5-04	Matrah	*S. crumenophthalmus*	15.5	34	8	-16.09	14.81	0.006	0.006	0.003	0.002
F22	30-5-04	Matrah	*S. crumenophthalmus*	17.5	47	-	-16.07	15.26	0.010	0.012	0.004	0.004
F23	30-5-04	Matrah	*S. crumenophthalmus*	17.6	45	-	-16.51	14.58	0.019	0.018	0.007	0.007
F24	30-5-04	Matrah	*S. crumenophthalmus*	18.0	49	6	-16.62	15.06	0.020	0.019	0.009	0.007
F25	30-5-04	Matrah	*S. crumenophthalmus*	19.6	69	-	-17.00	15.19	0.017	0.019	0.010	0.009
F26	30-5-04	Matrah	*S. crumenophthalmus*	18.0	50	-	-16.39	14.95	0.028	0.023	0.025	0.020
F27	29-5-04	Matrah	*A. spinifer*	23.5	305	14	-14.84	16.09	0.086	0.090	0.072	0.083
F28	29-5-04	Matrah	*A. spinifer*	28.4	493	12	-15.24	16.13	0.209	0.216	0.162	0.176
F29	29-5-04	Matrah	*A. spinifer*	24.9	310	13	-15.90	15.96	0.073	0.076	0.071	0.055
F30	29-5-04	Matrah	*A. spinifer*	25.0	329	9	-16.09	16.33	0.040	0.039	0.028	0.039
F31	29-5-04	Matrah	*A. spinifer*	24.2	301	7	-15.76	16.14	0.085	0.081	0.051	0.050
F32	29-5-04	Matrah	*A. spinifer*	23.0	288	9	-15.77	15.97	0.109	0.101	0.068	0.088
F33	29-5-04	Matrah	*A. spinifer*	29.3	449	12	-15.99	15.80	0.020	0.020	0.014	0.018
F34	29-5-04	Matrah	*A. spinifer*	25.5	338	11	-15.80	16.07	0.118	0.123	0.088	0.108
F35	29-5-04	Matrah	*A. spinifer*	23.1	261	10	-16.40	15.51	0.026	0.025	0.015	0.020
F36	29-5-04	Matrah	*A. spinifer*	26.9	388	12-11	-16.06	16.13	0.043	0.045	0.038	0.038
F37	29-5-04	Matrah	*A. spinifer*	29.4	560	3	-14.94	15.82	0.198	0.211	0.165	0.154
F38	29-5-04	Matrah	*A. spinifer*	25.2	337	-	-16.39	15.99	0.066	0.068	0.050	0.050
F39	29-5-04	Matrah	*N. randalli*	28.1	142	-	-16.16	15.65	0.042	0.050	0.042	0.040
F40	29-5-04	Matrah	*N. randalli*	22.4	99	6	-15.97	15.95	0.036	0.038	0.026	0.029
F41	29-5-04	Matrah	*N. randalli*	22.4	70	-	-16.05	16.25	0.035	0.037	0.015	0.017
F42	29-5-04	Matrah	*N. randalli*	24.7	90	-	-16.16	15.96	0.039	0.040	0.025	0.025
F43	29-5-04	Matrah	*N. randalli*	26.0	105	-	-15.92	15.52	0.038	0.038	0.033	0.035
F44	29-5-04	Matrah	*N. randalli*	21.8	91	11-10	-15.89	14.86	0.043	0.045	0.030	0.025
F45	29-5-04	Matrah	*N. randalli*	24.9	110	-	-15.79	14.86	0.025	0.024	0.012	0.013
F46	29-5-04	Matrah	*N. randalli*	24.6	91	-	-16.27	14.93	0.014	0.016	0.011	0.011
F47	29-5-04	Matrah	*N. randalli*	26.5	123	-	-16.20	14.65	0.054	0.058	0.038	0.034

F48	29-5-04	Matrah	*N. randalli*	28.4	138	-	-16.16	14.33	0.048	0.045	0.028	0.027
F49	29-5-04	Matrah	*N. randalli*	29.8	159	14	-15.79	15.20	0.027	0.029	0.024	0.020
F50	29-5-04	Matrah	*N. randalli*	26.4	85	ND	-16.22	14.83	0.032	0.036	0.023	0.013
F51	01-6-04	A'Seeb	*E. affinis*	57.0	2497	ND	-17.97	13.43	0.138	0.141	0.089	0.093
F52	01-6-04	A'Seeb	*E. affinis*	60.0	2802	ND	-17.14	14.31	0.225	0.218	0.220	0.223
F53	01-6-04	A'Seeb	*E. affinis*	63.0	2880	ND	-17.06	14.42	0.289	0.302	0.148	0.160
F54	01-6-04	A'Seeb	*E. affinis*	63.2	2964	ND	-16.22	14.18	0.309	0.309	0.119	0.110
F55	01-6-04	A'Seeb	*E. affinis*	51.5	1909	ND	-17.23	12.54	0.122	0.126	0.091	0.074
F56	01-6-04	A'Seeb	*E. affinis*	46.0	1279	ND	-16.52	13.40	0.050	0.052	0.032	0.027
F57	01-6-04	A'Seeb	*E. affinis*	48.0	1568	ND	-16.11	12.83	0.120	0.125	0.099	0.080
F58	01-6-04	A'Seeb	*E. affinis*	59.2	2637	ND	-16.80	14.07	0.256	0.266	0.109	0.093
F59	01-6-04	A'Seeb	*E. affinis*	51.5	1600	ND	-16.19	14.13	0.059	0.056	0.029	0.023
F60	01-6-04	A'Seeb	*S. tol*	40.0	369	ND	-16.18	13.43	0.040	0.041	0.027	0.020
F61	01-6-04	A'Seeb	*S. tol*	37.5	350	ND	-16.54	13.28	0.032	0.033	0.019	0.018
F62	01-6-04	A'Seeb	*S. tol*	43.5	490	ND	-16.00	14.09	0.040	0.044	0.024	0.020
F63	01-6-04	A'Seeb	*S. tol*	39.8	342	ND	-16.33	13.59	0.047	0.050	0.032	0.030
F64	01-6-04	A'Seeb	*S. tol*	40.7	387	ND	-16.21	13.68	0.028	0.029	0.018	0.014
F65	01-6-04	A'Seeb	*S. tol*	43.5	428	ND	-16.07	15.86	0.039	0.040	0.017	0.015
F66	01-6-04	A'Seeb	*S. tol*	38.4	330	ND	-16.09	15.40	0.048	0.050	0.030	0.028
F67	01-6-04	A'Seeb	*S. tol*	43.7	441	ND	-16.32	15.20	0.053	0.059	0.027	0.023
F68	01-6-04	A'Seeb	*S. tol*	41.5	381	ND	-15.98	15.64	0.057	0.058	0.012	0.027
F69	01-6-04	A'Seeb	*S. tol*	39.5	397	ND	-16.33	15.34	0.029	0.031	0.015	0.013
F70	01-6-04	A'Seeb	*S. tol*	38.1	327	ND	-16.16	15.61	0.032	0.033	0.021	0.022
F71	01-6-04	A'Seeb	*S. tol*	45.0	566	ND	-16.15	16.28	0.042	0.045	0.026	0.023
F72	01-6-04	A'Seeb	*S. longiceps*	17.0	43	ND	-16.37	14.82	0.004	0.004	0.002	0.002
F73	01-6-04	A'Seeb	*S. longiceps*	15.6	36	ND	-16.09	15.51	0.003	0.003	0.002	0.002
F74	01-6-04	A'Seeb	*S. longiceps*	15.4	36	ND	-16.28	14.70	0.003	0.004	0.002	0.002
F75	01-6-04	A'Seeb	*S. longiceps*	16.4	35	ND	-16.48	14.94	0.004	0.004	0.001	0.002
F76	01-6-04	A'Seeb	*S. longiceps*	16.5	35	ND	-16.36	14.75	0.003	0.003	0.000	0.003
F77	01-6-04	A'Seeb	*S. longiceps*	16.2	36	ND	-16.21	14.91	0.004	0.004	0.004	0.002
F78	01-6-04	A'Seeb	*S. longiceps*	15.7	35	ND	-16.05	14.20	0.004	0.004	0.003	0.002
F79	01-6-04	A'Seeb	*S. longiceps*	15.6	36	ND	-16.19	14.62	0.004	0.004	0.002	0.002

F80	01-6-04	A'Seeb	*S. longiceps*	16.5	37	ND	-16.03	14.30	0.004	0.004	0.004	0.004
F81	01-6-04	A'Seeb	*S. longiceps*	16.3	38	ND	-16.37	13.91	0.003	0.003	0.003	0.003
F82	01-6-04	A'Seeb	*S. longiceps*	16.2	38	ND	-16.56	14.42	0.003	0.004	0.002	0.002
F83	01-6-04	A'Seeb	*S. longiceps*	16.1	36	ND	-16.58	14.53	0.003	0.003	0.003	0.002
F84	01-6-04	A'Seeb	*S. longiceps*	15.9	34	ND	-16.21	14.40	0.004	0.005	0.004	0.004
F85	01-6-04	A'Seeb	*S. longiceps*	16.9	41	ND	-16.57	15.21	0.003	0.003	0.002	0.002
F86	01-6-04	A'Seeb	*S. longiceps*	16.5	37	ND	-16.73	14.93	0.003	0.003	0.001	0.003
F87	01-6-04	A'Seeb	*S. longiceps*	16.3	39	ND	-16.51	15.69	0.003	0.003	0.003	0.003
F88	06-6-04	A'Seeb	*R. acutus*	76.5	2217	ND	-15.16	18.13	0.222	0.242	0.173	0.179
F89	06-6-04	A'Seeb	*R. acutus*	66.5	1165	ND	-14.78	17.09	0.032	0.034	0.023	0.026
F90	06-6-04	A'Seeb	*R. acutus*	76.6	2009	ND	-15.22	17.50	0.143	0.156	0.149	0.147
F91	06-6-04	A'Seeb	*R. acutus*	83.6	2774	ND	-15.25	17.22	0.651	0.684	0.406	0.407
F92	06-6-04	A'Seeb	*R. acutus*	84.5	3275	ND	-15.01	17.08	0.733	0.786	0.288	0.306
F93	06-6-04	A'Seeb	*R. acutus*	82.7	2626	ND	-15.29	17.63	0.230	0.250	0.179	0.173
F94	06-6-04	A'Seeb	*R. acutus*	85.0	2826	ND	-15.35	17.10	0.377	0.374	0.288	0.283
F95	06-6-04	A'Seeb	*T. tonggol*	73.0	3963	ND	-16.82	17.14	0.134	0.137	0.131	0.123
F96	06-6-04	A'Seeb	*T. tonggol*	78.4	4273	ND	-15.82	15.14	0.165	0.169	0.141	0.148
F97	06-6-04	A'Seeb	*T. tonggol*	70.3	4208	ND	-16.22	17.16	0.174	0.164	0.121	0.120
F98	06-6-04	A'Seeb	*T. tonggol*	76.5	4492	ND	-15.23	15.28	0.129	0.115	0.092	0.097
F99	06-6-04	A'Seeb	*T. tonggol*	75.6	4236	ND	-16.10	16.72	0.155	0.164	0.130	0.152
F100	06-6-04	A'Seeb	*T. tonggol*	70.5	3800	ND	-16.28	16.56	0.125	0.132	0.113	0.109
F101	06-6-04	A'Seeb	*T. tonggol*	78.0	4994	ND	-16.40	17.31	0.185	0.183	0.179	0.173
F102	06-6-04	A'Seeb	*T. tonggol*	72.5	4288	ND	-16.22	17.41	0.149	0.150	0.110	0.107
F103	06-6-04	A'Seeb	*T. tonggol*	71.1	4189	ND	-17.60	16.81	0.147	0.154	0.148	0.149
F104	06-6-04	A'Seeb	*T. tonggol*	69.0	3685	ND	-16.93	15.54	0.176	0.177	0.137	0.163
F105	07-6-04	A'Seeb	*Sphyraena* sp.	68.5	1254	ND	-15.27	17.59	0.139	0.147	0.119	0.116
F106	07-6-04	A'Seeb	*Sphyraena* sp.	63.9	1024	ND	-15.69	18.42	0.096	0.109	0.076	0.072
F107	07-6-04	A'Seeb	*Sphyraena* sp.	58.5	805	ND	-15.65	18.18	0.032	0.031	0.011	0.012
F108	07-6-04	A'Seeb	*Sphyraena* sp.	45.0	464	ND	-15.32	18.48	0.118	0.116	0.104	0.105
F109	07-6-04	A'Seeb	*Sphyraena* sp.	74.5	1313	ND	-15.24	17.48	0.077	0.078	0.037	0.038
F110	07-6-04	A'Seeb	*Sphyraena* sp.	70.1	1537	ND	-16.40	17.36	0.052	0.050	0.050	0.045
F111	07-6-04	A'Seeb	*Sphyraena* sp.	68.0	1173	ND	-15.86	16.81	0.085	0.088	0.084	0.081

F112	07-6-04	A'Seeb	*Sphyraena* sp.	50.4	596	ND	-16.14	17.76	0.016	0.017	0.010	0.010
F113	07-6-04	A'Seeb	*Sphyraena* sp.	50.5	565	ND	-14.45	17.59	0.052	0.060	0.047	0.055
F114	07-6-04	A'Seeb	*Sphyraena* sp.	51.5	598	ND	-16.30	17.63	0.019	0.020	0.017	0.017
F115	07-6-04	A'Seeb	*Sphyraena* sp.	50.1	566	ND	-15.24	17.61	0.028	0.030	0.026	0.028
F116	07-6-04	A'Seeb	*R. kanagurta*	29.0	294	ND	-16.63	14.33	0.007	0.008	0.005	0.005
F117	07-6-04	A'Seeb	*R. kanagurta*	30.0	325	ND	-16.57	14.42	0.008	0.008	0.007	0.007
F118	07-6-04	A'Seeb	*R. kanagurta*	28.0	271	ND	-16.20	14.52	0.004	0.005	0.004	0.003
F119	07-6-04	A'Seeb	*R. kanagurta*	28.5	271	ND	-16.79	13.92	0.004	0.004	0.004	0.004
F120	07-6-04	A'Seeb	*R. kanagurta*	28.7	276	ND	-16.32	14.16	0.004	0.004	0.003	0.003
F121	07-6-04	A'Seeb	*R. kanagurta*	28.2	257	ND	-16.45	15.02	0.003	0.003	0.003	0.002
F122	07-6-04	A'Seeb	*R. kanagurta*	30.2	333	ND	-16.86	14.82	0.004	0.005	0.004	0.004
F123	07-6-04	A'Seeb	*R. kanagurta*	28.0	270	ND	-17.05	14.48	0.005	0.005	0.004	0.005
F124	07-6-04	A'Seeb	*R. kanagurta*	27.7	274	ND	-16.95	14.56	0.004	0.005	0.004	0.004
F125	07-6-04	A'Seeb	*R. kanagurta*	30.0	337	ND	-16.99	14.73	0.004	0.005	0.003	0.004
F126	07-6-04	A'Seeb	*R. kanagurta*	30.0	340	ND	-16.89	15.70	0.005	0.005	0.004	0.004
F127	07-6-04	A'Seeb	*R. kanagurta*	29.9	282	ND	-16.50	14.88	0.006	0.006	0.002	0.004
F128	09-6-04	Matrah	*E. epistictus*	42.5	1113	ND	-16.13	17.76	0.119	0.117	0.109	0.103
F129	09-6-04	Matrah	*E. epistictus*	48.8	1784	ND	-16.14	17.08	0.147	0.145	0.129	0.129
F130	09-6-04	Matrah	*E. epistictus*	46.5	1766	ND	-16.16	16.77	0.172	0.177	0.157	0.165
F131	09-6-04	Matrah	*E. epistictus*	47.2	1592	ND	-16.59	17.06	0.184	0.189	0.152	0.173
F132	09-6-04	Matrah	*E. epistictus*	43.5	1263	ND	-16.01	16.58	0.166	0.172	0.140	0.133
F133	09-6-04	Matrah	*E. epistictus*	42.4	1032	ND	-15.78	18.03	0.192	0.207	0.186	0.199
F134	09-6-04	Matrah	*E. epistictus*	46.7	1388	ND	-15.94	17.00	0.116	0.125	0.105	0.115
F135	09-6-04	Matrah	*E. epistictus*	39.7	978	ND	-16.15	17.16	0.116	0.111	0.095	0.102
F136	09-6-04	Matrah	*E. epistictus*	41.4	1081	ND	-18.59	16.39	0.070	0.061	0.061	0.060
F137	09-6-04	Matrah	*E. epistictus*	40.6	930	ND	-16.60	17.03	0.069	0.065	0.064	0.066
F138	09-6-04	Matrah	*E. epistictus*	37.3	829	ND	-17.37	16.66	0.059	0.061	0.026	0.027
F139	09-6-04	Matrah	*E. epistictus*	33.5	569	ND	-16.43	17.13	0.059	0.059	0.025	0.025
F140	09-6-04	Matrah	*U. doriae*	26.9	213	ND	-14.43	15.64	0.028	0.029	0.016	0.016
F141	09-6-04	Matrah	*U. doriae*	25.6	180	ND	-15.14	15.40	0.022	0.022	0.017	0.019
F142	09-6-04	Matrah	*U. doriae*	28.1	251	ND	-15.55	15.52	0.016	0.016	0.006	0.007
F143	09-6-04	Matrah	*U. doriae*	29.1	242	ND	-11.31	16.70	0.302	0.295	0.296	0.273

F144	09-6-04	Matrah	*U. doriae*	25.2	162	ND	-13.90	15.38	0.012	0.012	0.012	0.010
F145	09-6-04	Matrah	*U. doriae*	28.8	264	ND	-14.07	15.73	0.145	0.140	0.111	0.116
F146	09-6-04	Matrah	*U. doriae*	24.8	172	ND	-13.36	17.16	0.034	0.037	0.024	0.026
F147	09-6-04	Matrah	*U. doriae*	28.2	243	ND	-14.90	16.69	0.043	0.040	0.041	0.041
F148	09-6-04	Matrah	*U. doriae*	27.6	214	ND	-13.94	15.65	0.032	0.033	0.019	0.020
F149	09-6-04	Matrah	*U. doriae*	25.2	174	ND	-14.12	15.29	0.014	0.014	0.013	0.013
F150	09-6-04	Matrah	*U. doriae*	28.1	252	ND	-14.08	16.20	0.022	0.022	0.014	0.015