# Status of mercury and other heavy metals in South African marine fish species

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# Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes three original papers published in peer-reviewed journals and books and five unpublished publications. The development and writing of the papers were the principle responsibility of myself and for each of the cases where this is not the case, a declaration is included in the dissertation indicating the nature and extent of the contribution of co-authors.

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# Notes

This thesis is presented in the format prescribed by the Department of Food Science, Stellenbosch University. The structure is in the form of one or more research chapters (papers prepared for publication) and is prefaced by an introduction chapter with the study objectives, followed by a literature review chapter and culminating with a chapter for elaborating a general discussion and conclusions. Language, style and referencing format used are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

**Results from this dissertation that have been published in the following journals:**

- Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E., & Hoffman, L. C. (2016). Heavy metal accumulation and toxicity in smoothhound (*Mustelus mustelus*) shark from Langebaan Lagoon, South Africa. *Food Chemistry*, **190**, 871–878.
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# Summary

Fish is an important food source in South Africa as it is globally, thus the importance of determining and monitoring its safety in terms of metal contaminants and consumer safety. Effective methodology for analysing total metal concentrations and toxic metal components together with representative sampling protocol for sampling individual fish and larger catches are therefore required for accurate assessment of meat safety.

Both inductively coupled plasma mass spectrometry (ICP-MS) and high pressure liquid chromatography coupled to ICP-MS was validated as effective methods for accurately determining concentrations of total metals and individual Hg species, respectively. Consequently, it was found that total mercury loads in fish meat consisted mainly of toxic methylmercury (MeHg) components with a minor addition of an inorganic Hg (iHg) component and ethylmercury concentrations being negligible.

This proportion of MeHg to iHg varied between muscle types in yellowfin tuna (*Thunnus albacares*), with higher iHg concentrations in dark muscle than in white muscle, whereas the toxic MeHg concentrations did not vary across the carcass. The MeHg to tHg relationship was caused to vary with variation in fish weight, being described by the following prediction model: $cMeHg = 0.073 + 1.365 \cdot ctHg - 0.008 \cdot w$; taking into account fish weight as covariate. For the eight other fish species studied [blacktail (*Diplodus sargus capensis*), hottentot (*Pachymetopon blochii*), yellowtail (*Seriola lalandi*), snoek (*Thyrsites atun*), blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), soupfin (*Galeorhinus galeus*) and smoothhound (*Mustelus mustelus*)], the relationships between MeHg and tHg were constant even with varying fish sizes as iHg components were considered an insignificant portion of tHg (ctHg = cMeHg). The tHg measurements could therefore be used as accurate indicators of MeHg concentrations without requiring addition speciation analyses.

Sampling from the cephalic region of the dorsal white muscle tissue proves representative of the entire edible portions (white muscle) of larger fish (tuna and sharks spp.) for determining both total metal concentrations and toxic Hg components. Where Hg concentrations are positively correlated to fish size (yellowfin tuna, yellowtail and soupfin), subsamples should include individuals representing the entire size range present per catch.

A summary of metal concentrations in all eight species studied indicate that Hg is the main metal of concern where a single portion of certain fish (yellowfin tuna, shortfin mako, soupfin and smoothhound shark) consumed per week could exceed regulatory limits for safe Hg intake, whereas other fish species (hottentot) could be consumed daily without concern of Hg toxicity.

Information provided by this study will prove useful to both the fishing and processing industry as well as to health authorities providing information for dietary exposure assessments.

# Opsomming

Vis word gesien as ‘n belangrike voedsel bron in Suid-Afrika sowel as globaal en die bepaling en monitering van visveiligheid in terme van metaal kontaminasie is dus van uiterse belang. Effektiewe metodes vir die analise van totale-metaal konsentrasies en protokol vir verteenwoordigende steekproefneming van enkele visse sowel as van groter vangste is dus belangrik vir die akkurate bepaling van voedselveiligheid in die visbedryf.

Beide ICP-MS en HPLC-ICP-MS is onderskeidelik gevalideer as effektiewe metodes vir die akkurate bepaling van totale-metaal konsentrasies en indiwiduele kwik (Hg) spesies. Gevolglik is daar gevind dat totale Hg ladings grootliks bestaan uit metiel-kwik (MeHg) met kleiner bydraes van anorganiese kwik (iHg). Die bydrae van etiel-kwik (EthHg) is egter nietig bevind.

Die proporsie MeHg tot iHg het verskil tussen spier-tipes in geelvin tuna (*Thunnus albacares*) met hoër iHg konsentrasies in die donker spier as in die ligte spier. Die konsentrasies van toksiese MeHg toon egter nie verskille tussen die twee spier tipes of regdeur die viskarkas nie. Die verhouding tussen MeHg en tHg in visse het wel verskille getoon soos die gewig van die visse verskil en kan beskryf word deur die volgende vergelyking: $cMeHg = 0.073 + 1.365 \cdot ctHg - 0.008 \cdot w$, waarin die gewig van die vis in ag geneem word. Vir al agt ander visspesies (kolstert (*Diplodus sargus capensis*), hottentot (*Pachymetopon blochii*), geelstert (*Seriola lalandi*), snoek (*Thyrsites atun*), blou haai (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), vaalhaai (*Galeorhinus galeus*) en spierhaai (*Mustelus mustelus*)) is die iHg komponent beskou as onbeduidend en dus is ‘n konstante verhouding gevind tussen MeHg en tHg onafhalklik van visgewig. Die metings van tHg kan gevolglik gebruik word as ‘n akkurate indikasie van die MeHg hoeveelhede teenwoordig sonder aparte metings van individuele Hg spesies.

Steekproefneming vanaf die voorste deel van die dorsale witspierweefsel blyk verteenwoordigend te wees van die totale eetbare weefsel (wit spiere) van groter visse (tuna en haai spp.) vir die bepaling van totale-metaal konsentrasies sowel as toksiese Hg komponente. Waar Hg konsentrasies positief gekorreleer is met visgrootte (geelfin tuna, geelstert en vaalhaai), moet visse van verskillende groottes geneem word as steekproefmonsters om verteenwoordigend te wees van alle grootte visse in die vangs.

‘n Opsomming van die metaal konsentrasies wat in al agt visse getoets is, dui daarop dat Hg die metaal is wat die grootste moontlike gevaar inhou in visvleis waar selfs enkele porsies van seker visse (geelfin tuna, shortfin mako, vaalhaai en spierhaai) die weeklikse limiet van Hg inname kan oorskry. Ander visspesies (hottentot) kan weer daagliks geëet word sonder gevaar van toksiese Hg inname.

Die huidige studie verskaf waardevolle inligting vir beide die visindustrie en gesondheidsowerhede waar dit kan bydra tot die bepaling van diëet-bloodstellingsperke.

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# CHAPTER 1:

## Introduction

The ocean is an important food source for the majority of the global human population. For inhabitants of coastal areas it is a considerable contributor to food security (Bell *et al*., 2009; Isaacs, 2013). Countries such as South Africa, which have extended coastlines (South Africa has approximately 3 000 km), have access to large ocean areas which can support commercial, recreational and subsistence fisheries of economic importance.

Fish is considered a good source of nutrients and contains high quality protein, minerals and essential omega-3 fatty acids (Kris-Etherton *et al*., 2002; Limin *et al.*, 2006) and is therefore recommended as part of a balanced healthy diet by South African and international health authorities (AHA, 1996; Schonfeldt *et al.*, 2013). Despite the benefits of fish consumption, fish may also accumulate toxic levels of metals from the environment (Smith & Sahyoun, 2005), which may consequently reduce or override benefits gained (Goyer, 1997). These metals may be naturally occurring in the marine environment, and natural levels increased though anthropogenic activity. Metals of particular concern in fish tissue are arsenic (As), mercury (Hg), lead (Pb) and cadmium (Cd). Human consumption of high quantities of these metals may lead to various health defects depending on the metal and the quantities and frequency consumed (Schroeder & Darrow, 1972; Goyer & Clarkson, 2001; Grandjean *et al.*, 2010; WHO, 2011; Guynup & Safina, 2012).

It is the responsibility of the food industry to ensure the food products it provides are safe for human consumption (Gardner, 1993; SCF, 2006), therefore, routine monitoring is needed to confirm metal concentrations do not exceed toxic limits (EC, 2007). As the metal content of fish tissue cannot be visually determined, the only way to determine the concentrations of metals in fish tissue is through chemical analysis of the tissue itself. Such analyses, however, requires extended periods of time, and therefore, in certain fish processing plants, fish and fish products can only be released onto the market four days after landing to allow for sufficient quality testing to be performed (Le Roux, 2009). The development of efficient analytical techniques is therefore required to allow for rapid and accurate determination of the safety of fish products. When analysis occurs, fish are generally subsampled at random in order to determine the safety of an entire batch/catch (EC, 2007). It is therefore important that subsamples are representative of the larger batch in terms of species, fishing area and fish size. However, detailed sampling protocol on which, how and where, in terms of the fish carcass and size, samples should be taken, is still lacking (EC, 2007).

Metal toxicity and accumulation in fish muscle may be influenced by several factors, both intrinsic and external. Firstly, the toxicity of certain metals (Hg and As) is dependent on their ambient chemical forms as the toxic character varies among organic and inorganic chemical forms (Boening, 2000). Methylmercury (MeHg) which is the organic form of Hg, is considered the most toxic Hg form and is also the major form

present in fish tissue varying from about 60 to 100% of total Hg (Kamps *et al.*, 1972; Walker, 1976; Joiris *et al.*, 1999; Storelli *et al.*, 2001). Secondly, metal accumulation in fish tissue may vary significantly among metals as well as within and among fish species. As prey consumption is one of the major pathways of metal intake in fish (Stewart *et al.*, 1997; De Gieter *et al*., 2002; Erasmus *et al.*, 2004), metal concentrations are expected to vary among fishes of different trophic levels (Campbell *et al*., 2006; Verdouw *et al.*, 2011), sampling locations (Carro *et al.*, 2012; Joubert, 2014) and developmental stages as prey composition and metal content of prey vary with respect to environmental pollution (Binning & Baird, 2001; Fatoki & Mathabatha, 2001). Several sites of major marine metal pollution have been identified around the South African coast with sources including industrial activity runoff, river mouths (where metal pollutants are carrying down stream from agricultural activity and industrial or urban developments) or harbours (where oil spills or pollution from shipping activity cause increased metal concentrations in marine water) (Cloete & Watling, 1981). Metal concentrations can also vary within species as they are often a function of fish size (Canli & Atli, 2003; Burger & Gochfeld, 2011; Verdouw *et al.*, 2011). Certain metals are biomagnified in larger fish (Kojadinovic *et al.*, 2007) whereas others decrease in concentration with decreasing metabolic rates of older fish (Canli & Atli, 2003). Lastly, the interactions between metals could affect the concentrations and toxicity of individual metals in fish muscle through either supportive or competitive relationships (Rahman *et al*., 2012; Carvalho *et al*., 2005), therefore high concentrations of a certain metals could indicate similarly high or inversely low concentrations of other individual metals.

In order to assess the actual metal toxicity of all fish consumed, the assessment of specific toxic metal species through speciation analysis is required (Lobinski, 1997) and sampling should be stratified across all fish species, size ranges and capture areas. Such comprehensive assessments may however become unaffordable and excessively time consuming for the fishing and processing industry and optimising the sampling strategy is required to streamline processing time and reduce analytical costs. A greater understanding of species specific contamination traits is therefore required. Specifically, the relationship of concentrations of individual metals in their various forms as well as groups of different metals with fish species, size, trophic level and sampling location (both anatomically and geographically) needs to be investigated in more detail. If subsampling is done efficiently, accurate results can be determined more effectively and wastage can be minimised. This understanding could also aid in the optimisation of fishing strategies to target fish expected to contain safe metal concentrations, further minimising wastage.

In addition to the responsibility of the food industry, consumers are equally responsible to ensure that the diet they consume is balanced and healthy. Frequent consumption of some fish considered safe for consumption, may lead to toxic metal effects in the human body. Health authorities have calculated provisional tolerable intakes (PTWI) per metal, through all sources of intake within a week without harmful effects to the human body (SCF, 2006). Consumers should plan their diet accordingly, limiting the consumption of fish with higher metal contents and if consumed regularly, fish with low metal concentrations

should be selected (OCEANA, 2008). The food industry and authorities can therefore help the consumer in making informed decisions by sensitising the public on the general metal contents of different fish species.

Public information on concentrations of metals and metal species in South African marine fish is scarcely available; therefore this study aims to add to current knowledge by:

1) Evaluating inductively coupled plasma mass spectrometry (ICP-MS) and high pressure liquid chromatography coupled with ICP-MS (HPLC-ICP-MS) methods to measure total metals and Hg species respectively, in order to assess the relationship between tHg and MeHg and evaluate the concentrations and interactions of mercury and 15 other common heavy metals;

2) Investigating the distribution of Hg species across the carcass of larger fish in order to determine an optimal sampling protocol for non-biased measurement of the concentrations of toxic Hg components in individual fish as well as large batches of fish;

3) Investigating the MeHg to total Hg relationship in fish muscle in order to develop effective methods to accurately determine the toxic Hg content of fish muscle; and

4) Determining the concentrations of 16 metals in South African marine fish in terms of species, trophic levels, sampling location and fish size in order to improve the safety of South African fish to the industry, government and consumers in terms of metal contaminants.

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# CHAPTER 2:

## Heavy metals in marine fish meat and consumer health: A review*

**ABSTRACT**

The numerous health benefits provided by fish consumption may be compromised by the presence of toxic metals and metalloids such as lead, cadmium, arsenic and mercury, which can have harmful effects on the human body if consumed in toxic quantities. The monitoring of metal concentrations in fish meat is therefore important to ensure compliance with food safety regulations and consequent consumer protection. The toxicity of these metals may be dependent on their chemical forms, which requires metal speciation processes for direct measurement of toxic metal species or the identification of prediction models in order to determine toxic metal forms from measured total metal concentrations. This review addresses various shortcomings in current knowledge and research on the accumulation of metal contaminants in commercially consumed marine fish globally and particularly in South Africa, affecting both the fishing industry as well as fish consumers.

**Keywords**: Heavy metals, Fish muscle, Consumer health, Maximum allowable limits, Provisional Tolerable Weekly Intake (PTWI), Metal speciation

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## 2.1 Introduction

Many populations globally depend on fish as part of their daily diet (FAO, 2012) as fish and seafood are healthy components of human nutrition providing many essential nutrients such as high-value proteins, various vitamins and minerals and polyunsaturated omega-3 fatty acids. In some communities, fish can be a primary food source that contributes substantially to food security (FAO, 2012). Fish and other marine organisms are, however, not independent of the environment in which they live. Both essential and harmful minerals and metals present in the environment can be absorbed into living organisms from the surrounding water, sediment and diet (Munoz-Olivas & Camara, 2001). Even though fish and seafood carry numerous health benefits, contaminants in this food group can also pose a significant threat to the health of consumers. Of the various environmental contaminants, metals and metalloids (will be discussed hereafter in combination as "metals") are amongst the most commonly accumulated toxins in fish and seafood which can lead to health defects when consumed in amounts exceeding safe consumption levels (Llobet *et al.*, 2003; Falcó *et al.*, 2006).

Metal contaminants are naturally present in the environment but can be increased through industrial activity and pollution (Erasmus *et al.*, 2004). The concentrations and uptake of these metals in marine organisms are subject to environmental and species-specific biological factors as well as the chemical and physical state of the metals (Erasmus *et al.*, 2004; Somero *et al.,* 1977; Canli & Atli, 2003). Canli and Atli (2003) have shown that different fish species accumulate metals at different rates and to different levels; that different metals accumulate differently within the same fish species; and also that one specific metal is accumulated at different levels in different tissues within one fish. Therefore it is imperative to consider these factors when determining the consumer safety of fish with regards to metal content (Somero *et al.,* 1977; Canli & Atli, 2003; Erasmus *et al.*, 2004).

It is important to note that not all metals are hazardous and toxic to fish and humans. They form part of a larger group of elements, some of which are essential to human health (Mertz, 1993). These can therefore be classified as essential, non-essential or toxic. Essential elements which play a specific role in body metabolism include iron (Fe), copper (Cu), zinc (Zn) and selenium (Se). Non-essential elements are elements that have no known specific function in the body, but are also not considered toxic in any significant amount and, lastly, toxic elements such as chromium (Cr), nickel (Ni), cadmium (Cd), mercury (Hg) and lead (Pb) are generally related to pollution and can have harmful effects on living organisms when exceeding certain concentrations. Some elements (e.g. Se) are essential in small quantities or up to certain concentrations above which they can have toxic effects. Schroeder and Darrow (1972) has also grouped metals into categories according to toxicity levels as follows: those that easily attain toxic levels (Pb, Ni, antimony (Sb), beryllium (Be), Cd and Hg) and those that can become toxic at extreme levels (barium (Ba), arsenic (As), germanium (Ge) and tungsten (W)). Several other metals are known to be inert and considered

non-toxic. Regulatory limits and main sources of essential, non-essential and toxic metals present in commonly consumed marine organisms are summarised in Table 2.1.

As individual metals have different degrees of toxicity, maximum allowable limits (MAL) and provisional tolerable weekly intake (PTWI) for metals in foodstuffs are determined specific to each metal for the protection of the consumer (Tressou *et al*., 2004). The MAL are specific to food products and provides a limit above which consumers are likely to be exposed to harmful contaminant levels, whereas PTWI represents "permissible human weekly exposure to metal contaminants unavoidably associated with the consumption of otherwise wholesome and nutritious foods" (Codex Standard, 1995). These limits can also be species-specific as metal accumulation is affected by different development and metabolic rates of different organisms. Individual countries or governing bodies can have specific MALs that differ from the general regulations as fish consumption patterns of specific population groups are taken into consideration (Table 2.1).

Although numerous foodstuffs may contain metal contaminants above regulatory limits, marine fish tend to have some of the highest levels where metals such as As, Cd, Hg and Pb predominate (Llobet *et al*., 2003; Falcó *et al.*, 2006). Due to frequent high concentrations of these four metals in marine fish and their potential harmful effects to consumers, they will be the metals of focus in the current review.

## 2.2 Toxic metals

### 2.2.1 Arsenic

Arsenic (As) is widely distributed in nature due to environmental sources (Goyer & Clarkson, 2001; WHO, 2011) and anthropogenic pollution which is largely due to smelting activities, glass manufacturing, manufacture and use of arsenic pesticides, herbicides, fungicides and wood preservatives (Goyer & Clarkson, 2001; Järup, 2003; Castro-González & Méndez-Armenta, 2008). Arsenic has a complex chemistry and can be present in several organic (trivalent and pentavalent arsenic) and inorganic (elemental, trivalent and pentavalent arsenic) forms which vary in their degree of toxicity. Inorganic As is seen as the most toxic form as it is stable and soluble and therefore absorbed by the digestive tract, abdominal cavity and muscles in the human body (WHO, 2011), whilst organic As does not accumulate in the human body due to rapid excretion (Goyer & Clarkson, 2001; WHO, 2011). Inorganic As is often found in high levels in drinking water whereas organic As is primarily found in fish and meat (Goyer & Clarkson, 2001; Castro-González & Méndez-Armenta, 2008).

**Table 2.1** Maximum allowable limits (MALs) with specifications for individual metals in fish by various regulatory bodies.

| Metal | MAL | Regulatory body | Specifications |
|---|---|---|---|
| Essential but toxic in excess amounts | | | |
| Sn | 50 mg·kg$^{-1}$ | DOH, 2004 | for all uncanned meat and meat products |
| Fe | - | - | - |
| Cu | 30 mg·kg$^{-1}$<br>20 mg·kg$^{-1}$<br>5 mg·kg$^{-1}$ | FAO, 1983<br>UK, Spain*<br>Turkey* | - |
| Cr | 0.1 mg·kg$^{-1}$ | Brazil Standard** | - |
| Zn | 30 mg·kg$^{-1}$<br>50 mg·kg$^{-1}$ | FAO, 1983<br>Turkey* | - |
| Se | 0.3 mg·kg$^{-1}$ | | - |
| Toxic | | | |
| As | 3.0 mg·kg$^{-1}$<br>2.0 mg·kg$^{-1}$ | DOH, 2004<br>Australia New Zealand Food Authority, 2011* | Fish and processed fish meat |
| Sb | 0.15 mg·L$^{-1}$ | DOH, 2004 | All liquid foodstuffs |
| Cd | 0.05 mg·kg$^{-1}$<br>0.1 mg·kg$^{-1}$ [a] | FAO. Heavy Metals Regulations<br>Legal Notice No 66/2003<br><br>Commission Regulation (EC) No 1881/2006 | [a] for the following species: bonito (*Sarda sarda*), wedge sole (*Dicologoglossa cuneata*), eel (*Anguilla Anguilla*), European anchovy (*Engraulis encrasicholus*), louvar/luvar (*Luvarus imperialis*), horse mackerel or scad (*Trachurus trachurus*), grey mullet (*Mugil labrosus labrosus*), common two-banded seabream (*Diplodus vulgaris*), European pilchard or sardine (*Sardina pilchardus*), mackerel (*Scomber* species), sardinops (*Sardinops* species), tuna (*Thunnus* species, *Euthynnus* species, *Katsuwonus pelamis*). |
| | 1.0 mg·kg$^{-1}$ | DOH, 2004 | Fish and processed fish |
| | 0.3 mg·kg$^{-1}$ | Commission Regulation (EC) No 1881/2006 | Muscle meat of swordfish |
| | 0.2 mg·kg$^{-1}$ [b]<br>0.3 mg·kg$^{-1}$ [bb] | Commission regulation (EC) No 629/2008 | [b]bullet tuna (*Auxis* spp)<br>[bb]anchovy (*Engraulis* spp) swordfish (*Xiphias gladius*) |

| | | | |
|---|---|---|---|
| Hg | 1 mg·kg$^{-1}$ [c,1]<br>0.5 mg·kg$^{-1}$ [ccc,1] | DOH, 2004 | [c]Predatory fish including swordfish<br>[ccc]All other fish and processed fish<br>[1]As methylmercury |
| | | FAO. Heavy Metals Regulations Legal Notice No 66/2003 | [c] Anglerfish( *Lophius*), Atlantic catfish *(Anarhichas lupus*), Bass *(Dicentrarchus labrak*), Blue ling *(Molva dipterygia*), Bonito *(Sarda spp*), Eel *(Anquilla spp*), Halibut *(Hippoglossus hippoglossus*), Little tuna (*Euthunnus spp*), Marlin *(Makaira spp*), Pike *(Esox lucius*), Plain bonito *(Orcynopsis unicolor*), Portuguese dogfish *(Centroscymnes coelolepis*), Rays *(Raja spp*), Redfish *(Sebastes marinus, S. mentella, S. uiviparus*), Sail fish *(Istiophoms platypterus*), Scabbard fish *(Lepidopus caudatus, Aphanopus carbo*), Shark (all species), Snake mackerel or butterfish (*Lepidocybium flavobrunneum, Ruvettus pretiosus, Gempylus serpens*), Sturgeon *(Acipenser spp*), Swordfish *(Xiphias gladius*), Tuna *(Thunnus spp*). |
| | | Commission regulation (EC) no 629/2008 [cc] | [cc] add: emperor, orange roughy, rosy soldierfish (*Hoplostethus species*), grenadier (*Coryphaenoides rupestris*), kingklip (*Genypterus capensis*), megrim (*Lepidorhombus species*), mullet (*Mullus species*), pink cusk eel (*Genypterus blacodes*), poor cod (*Tricopterus minutes*), seabream, pandora (*Pagellus species*).<br>[ccc] edible parts of the fishery products |
| Pb | 0.5 mg·kg$^{-1}$ | DOH, 2004 | Fish and processed fish |
| | 0.2 mg·kg$^{-1}$ [f]<br>0.4 mg·kg$^{-1}$ [ff] | FAO. Heavy Metals Regulations Legal Notice No 66/2003 | [f] edible parts of the fishery products<br>[ff] Wedge sole *(Dicologoglossa cuneata*), Eel *(Anguilla anguilla*), Spotted seabass *(Dicentrarchus punctatus*), Horse mackerel or Scad *(Trachurus trachurus*), grey mullet *(Mugil labrosus labrosus*), Common two-banded seabream *(Diplodus vulgaris*), Grunt *(Pomadasys benneti*), European pilchard or sardine *(Sardina pilchardus*) |
| | 0.3 mg·kg$^{-1}$ [d] | Commission regulation (EC) No 1881/2006 | Muscle meat of fish |

*(Rahman *et al*., 2012), **(Tarley *et al.*, 2001)

Seafood can contain several times the amount of As than other foods and is therefore the main source of dietary intake in humans (Ysart *et al.*, 2000; Llobet *et al*., 2003). Although high concentrations of As (up to 100 ppm) have been found in certain edible marine species (Table 2.2) (Edmonds *et al.,* 1977; Juresa &

Blanusa, 2003; WHO, 2011; Du *et al.*, 2012; Burger *et al.*, 2014), in most of these cases it is the total As concentrations that are measured instead of the toxic inorganic form (arsenite). Up to 90% of As in fish muscle is present in the non-toxic arsenobetain form (Zoorob *et al.*, 1998; Goyer & Clarkson, 2001). Nonetheless, total As concentration is the current standard whereby regulatory limits are set at 3.0 $mg \cdot kg^{-1}$ in fish and processed fish by the South African Department of Health (DOH, 2004). Measuring individual As species will produce more accurate results in terms of the true As toxicity in seafood as a PTWI of 15 $\mu g \cdot kg^{-1}$ body weight has been set for inorganic As (Falcó *et al.*, 2006). Early symptoms of As exposure in humans include abdominal pain, vomiting, diarrhoea, muscle weakness and skin flushing whereas chronic As toxicity has led to skin defects and cancer (Schroeder & Darrow, 1972; WHO, 2011).

### 2.2.2 Cadmium

Cadmium (Cd) is a metal contaminant which is introduced into the environment through both natural processes (volcanic emissions and weathering of rocks) and anthropogenic activities such as the smelting of other metals, burning of fossil fuels, incineration of waste materials and the use of certain fertilisers (EFSA, 2009). Cadmium is most commonly found as inorganic compounds in the +2 oxidation state and is mainly present as $CdCl_2^0$ and $CdCl^+$ complexes in seawater (Simpson, 1981). Cadmium can readily cross various biological membranes, and once inside living cells, has a high affinity to bind to ligands and form Cd complexes which can be more stable (EFSA, 2009). For example, in fish muscle most of the Cd present tends to bind to proteins (EFSA, 2009). Cadmium absorbed into the fish body is therefore eliminated at a very slow rate, causing bioaccumulation in the body. Cadmium can enter fish by passive diffusion across the gills or by entering the marine food chain at the plankton and microorganisms level and thereby entering fish through the diet (Erasmus *et al.*, 2004). As Cd is most readily taken up by aquatic organisms in its free form ($Cd^{2+}$), the high salinity in seawater which causes Cd to readily form complexes ($CdCl_2^0$ and $CdCl^+$) seems to reduce this bioaccumulation (Canli & Atli, 2003). Nonetheless, fish is still considered a major source of Cd (Castro-González & Méndez-Armenta, 2008), which has frequently been found to exceed maximum allowable limits in a number of commonly consumed fish species (Table 2.2).

Cadmium is highly toxic to humans and has a long biological half-life preventing the reduction of the accumulated body burden (Erasmus *et al.*, 2004; EFSA, 2009). Effects on human health include hypertension and cardiovascular function, neurological disorders, carcinogenic effects and skeletal weakness and defects (Schroeder & Darrow, 1972; Goyer & Clarkson, 2001). Cadmium exposure in humans is predominantly through food ingestion (Castro-González & Méndez-Armenta, 2008) where fish, meat and fruit can contain 1 to 50 $\mu g \cdot kg^{-1}$ Cd (Goyer & Clarkson, 2001). The European Commission has set a PTWI of 7 $\mu g \cdot kg^{-1}$ bw and MAL as seen in Table 2.1 (EC, 2006). The Food and Agriculture Organization of the United Nations presents species-specific maximum limits for Cd in fish from 0.05 $mg \cdot kg^{-1}$ fresh weight in fishery products to 1.0 $mg \cdot kg^{-1}$ fresh weight in bivalves and cephalopods (FAO, 2003). Within South Africa, the Department of Health's regulatory limit for Cd in fish and processed fish is 1.0 $mg \cdot kg^{-1}$ (DOH, 2004).

**Table 2.2** Summary of cases where fish and fish products contain Pb, Cd, As and Hg concentrations ($mg \cdot kg^{-1}$ ± Std dev) exceeding the respective maximum allowable limits (MAL) as per study region.

| Metal > MAL | Fish/fish products sampled | Metal concentrations | Country/Region | Reference |
|---|---|---|---|---|
| As | Flathead sole | 19.5 ± 1.01 | Aleutian islands | Burger *et al.*, 2014 |
| | Rock sole | 4.34 ± 0.70 | Aleutian islands | |
| | Horse mackerel | 6.85 ± 6.22 | Croatia | Juresa & Blanusa, 2003 |
| | Sardine | 8.08 ± 2.43 | Croatia | |
| | Hake | 10.03 ± 0.82 | Croatia | |
| | Hake | 23.30 ± 3.56 | Croatia | |
| | Red mullet | 59.91 ± 9.49 | Italy | Perugini *et al*., 2014 |
| | European hake | 38.70 ± 7.69 | Italy | |
| | Blue whiting | 35.30 ± 2.82 | Italy | |
| | Atlantic mackerel | 30.76 ± 9.95 | Italy | |
| Cd | Canned tuna | 0.06 | Jordan | Ababneh & Al-Momani, 2013 |
| | European conger eel | 0.11 ± 0.01 | Italy | Storelli & Barone, 2013 |
| | Blackbellied angler | 0.09 ± 0.02 | Italy | |
| | Rosefish | 0.10 ± 0.02 | Italy | |
| | Brown ray | 0.08 ± 0.04 | Italy | |
| | Red mullet | 0.08 ± 0.04 | Italy | |
| | European Pilchard | 0.045 ± 0.020 | Sicily | Copat *et al.*, 2012 |
| | Red mullet | 0.084 ± 0.069 | Sicily | |
| | Red mullet | 0.053 ± 0.027 | Italy | Pastorelli *et al.*, 2012 |
| | Salted Anchovies | 0.06 - 0.61 | Italy | Storelli *et al.*, 2011 |
| | Various species | 0.092 ± 0.267 | EU Member States, Iceland and Australia | EFSA, 2009 |
| | Louvar | 0.08 ± 0.01 | Spain | Herreros *et al.*, 2008 |
| | Albacore | 0.05 ± 0.03 | Mediterranean Sea | Storelli & Marcotrigiano, 2004 |
| | Grey mullet | 0.10 to 0.40 | Turkey | Filazi *et al.*, 2003 |
| | Atlantic Mackerel | 0.49 ± 0.01 | Nigeria | Ogundiran *et al.*, 2014 |
| | European Pilchard | 0.19 ± 0.0001 | Nigeria | |
| | Blue Whiting | 0.10 ± 0.06 | Italy | Perugini *et al.*, 2014 |
| | European Hake | 0.05 ± 0.04 | Italy | |
| | Red Mullet | 0.07 ± 0.05 | Italy | |
| Pb | Rudd | 4.31 | Bulgaria | Hristov & Kirin, 2014 |
| | Algae | *No value* | New Jersey, USA | Burger *et al.*, 2012 |
| | Salmon | 0.4 | Lithuania | Idzelis *et al.*, 2012 |
| | European Anchovy | 0.32 ± 0.22 | Sicily | Copat *et al.*, 2012 |
| | Canned Sardines | 2.15 ± 0.85 | Brazil | Tarley *et al.*, 2001 |
| | Rednose Labeo | 0.8 | South Africa | Jooste *et al.*, 2014 |
| | Atlantic Mackerel | 0.46 ± 0.02 | Nigeria | Ogundiran *et al.*, 2014 |

| | | | | |
|---|---|---|---|---|
| Hg | Shortfin mako | 2.65 ± 1.16 | New England | Teffer *et al.*, 2014 |
| | Common thresher | 0.88 ± 0.71 | New England | |
| | Albacore tuna | 0.46 ± 0.14 | New England | |
| | Yellowfin tuna | 0.30 ± 0.09 | New England | |
| | Dolphinfish | 0.21 ± 0.17 | New England | |
| | European conger eel | 1.14 ± 0.46 | Italy | Storelli & Barone, 2013 |
| | Rosefish | 1.04 ± 0.56 | Italy | |
| | Brown ray | 1.09 ± 0.39 | Italy | |
| | Blackbellied angler | 0.96 ± 0.32 | Italy | |
| | Red mullet | 0.43 ± 0.55 | Italy | |
| | Shortfin mako | 1.83 ± 0.17 | New Jersey | Burger & Gochfeld, 2011 |
| | Atlantic Bluefin tuna | 0.52 ± 0.03 | New Jersey | |
| | Striped bass | 0.39 ± 0.02 | New Jersey | |
| | Bluefish | 0.35 ± 0.02 | New Jersey | |
| | Swordfish | 0.93 ± 0.07 | Spain | Herreros *et al.*, 2008 |
| | Louvar | 0.99 ± 0.06 | Spain | |
| | Albacore | 1.56 ± 0.49 | Mediterranean Sea | Storelli & Marcotrigiano, 2004 |
| | Blue whiting | *No value* | Italy | Marcotrigiano & Storelli, 2003 |
| | Atlantic horse mackerel | *No value* | Italy | |
| | Bullet tuna | *No value* | Italy | |
| | European hake | *No value* | Italy | |
| | Spiny dogfish | 6.53 ± 2.19 | Italy | |
| | Small-spotted catshark | *No value* | Italy | |
| | Thornback ray | *No value* | Italy | |
| | Blackbellied angler | *No value* | Italy | |
| | Sandy ray | *No value* | Italy | |
| | Brown ray | *No value* | Italy | |
| | Mediterranean starry ray | *No value* | Italy | |
| | Silver scabbardfish | *No value* | Italy | |
| | Dogtooth tuna | 0.38 - 4.40 | Seychelles | Matthews, 1983 |
| | Bonito | 0.07 - 1.26 | Seychelles | |
| | Carangue balo | 0.03 - 1.51 | Seychelles | |
| | Becune | 0.26 - 1.58 | Seychelles | |
| | Kingfish | 0.06 - 1.46 | Seychelles | |
| | European Hake | 0.59 ± 0.14 | Italy | Perugini *et al.*, 2014 |

From a survey across 18 EU Member States, Iceland, Australia and three commercial organisations, 4.8% (n = 305), 8.2% (n = 102) and 2.0% (n = 7) of all samples from 3 respective categories of fish species had Cd levels exceeding the maximum limits in fish muscle according to FAO and EU regulations (EFSA, 2009). Even though Cd is a common contaminant in edible fish meat, how and where (muscle, bone, gills and organs) Cd

is accumulated in marine fishes is not homogenous (Heath, 1987) and therefore needs to be investigated in a wide variety of fish species in order to determine the true danger that Cd poses to the fish consumer.

### 2.2.3 Lead

Lead is one of the primary contaminants present in the environment (Schroeder & Darrow, 1972; Castro-González & Méndez-Armenta, 2008) and naturally occurs in rocks, soils and in the hydrosphere (Buljac *et al.*, 2014). However, Pb is also the most widely used metal and industrial Pb contributes a considerable quantity to that found in the natural environment (Harlavan *et al.,* 2010). Large amounts of lead tetraethyl can be completely converted to aerosols through the combustion of gasoline, subsequently contributing to atmospheric Pb (Reuer & Weiss, 2002; Von Storch *et al.,* 2003). The atmosphere in turn is the main source of Pb deposition in the marine environment, therefore acting as a Pb pathway from the terrestrial to the marine environment. Since it became evident that leaded petrol was the predominant source of atmospheric lead (Reuer & Weiss, 2002), regulations were adopted on the allowable gasoline lead content (Von Storch *et al.,* 2003). This reduction in anthropogenic lead pollution was evident in a reduction in seawater lead concentrations (Reuer & Weiss, 2002) forming a direct link from terrestrial sources to effects in the marine environment. Once in the marine environment, Pb is easily absorbed into the fish's bloodstream and accumulated in the body tissues, bones, gills, kidneys, liver and scales (Nussey *et al*., 2000). It can thus enter the human body through the diet and can accumulate, especially when seafood is consumed regularly.

The toxicity of Pb is dependent on its chemical form (Erasmus, 2004; Goyer & Clarkson, 2001) where the organolead compounds are more toxic than the inorganic Pb form (Munoz-Olivas & Camara, 2001). Lead is mostly found in its dissolved form in the ocean, of which a large proportion (50-70%) is organic compounds (Reuer & Weiss, 2002). As was shown by a series of studies by Sánchez-Marín *et al.* (2007; 2010; 2011) the bioavailability of Pb in the environment as organic compounds can be significantly increased by the presence of dissolved organic matter (DOM). The more methyl or ethyl carbon groups linked to the Pb molecule, the higher its toxic effect (Munoz-Olivas & Camara, 2001). The marine environment is therefore a significant source of toxic Pb exposure in fish and humans due to consumption (Table 2.2). In certain communities fish consumption is the main source of Pb exposure (Rubio *et al*., 2005) where excess exposure can result in neurological problems, haematological effects, renal failure, hypertension and cancer (Goyer & Clarkson, 2001; Munoz-Olivas & Camara, 2001). A PTWI of 50 µg/kg bw was first set by the JECFA, which was replaced in 1993 by a new PTWI of 25 µg/kg bw for all age groups (JECFA, 2011). At present, according to the South African Department of Health, the MAL for Pb in fresh and processed fish is 0.5 $mg \cdot kg^{-1}$ (DOH, 2004) with a MAL of 0.3 $mg \cdot kg^{-1}$ set by the European Commission (Table 2.1) (EC, 2006).

### 2.2.4 Mercury

Mercury (Hg) is a metal that is liquid at ambient temperature and pressure and can be present in several different chemical forms and compounds in the environment. It is the metal that presents the most concern with regards to fish and seafood consumption and human health (Marcotrigiano & Storelli, 2003) and will

thus be reviewed in more detail. Fish is considered the primary source of Hg in humans (Carrington & Bolger, 2002; Falcó *et al.*, 2006) and there are numerous (examples in Table 2.2) reports of high levels of Hg in fish muscle, exceeding the allowable maximum limits.

#### *2.2.4.1. Sources*

Mercury levels in the environment have increased markedly since the early 20$^{th}$ century due to both natural processes and human activity (Grandjean *et al.*, 2010). Natural Hg sources include forest fires and volcanic activity (Morel *et al.,* 1998), however, one to two thirds of the Hg present in the atmosphere and aquatic environment is from anthropogenic origin (Morel *et al.,* 1998; Boening, 2000). Mercury is used for the production of paint, electrical equipment, batteries and fungicides as well as in medicine, dentistry, wood pulping and the military sector (Boening, 2000). In addition, mining contributes significantly to Hg water pollution whilst the burning of fossil fuels and the smelting of Pb, Cu and Zn ores are major sources of atmospheric Hg pollution (Boening, 2000). Due to increasing awareness of Hg related health hazards, the use of Hg in many industries and consequently atmospheric Hg pollution has diminished in recent years (Grandjean *et al.*, 2010). However, current environmental Hg levels are still 10 times higher than in pre-industrial times (Grandjean *et al.*, 2010).

Due to anthropogenic input from various activities, seawater, sediments and biota near cities, harbours and industrial areas tend to have higher Hg concentrations compared to rural locations (Costa *et al.*, 2012). A number of marine based studies have corroborated such claims where black-mouthed dogfish, carp spp. and catfish, for example, had overall higher Hg concentration when sampled from industrialised and developed sites compared to those areas considered rural, less developed and/or clean (Storelli *et al.*, 2002; Horvat *et al.,* 2003; Ruelas-Inzunzu & Paez-Osuna, 2005). Rivers also carry metal contaminants from inland industrial and agricultural sources towards the ocean, affecting marine fish in estuaries and near river mouths (Oosthuizen & Ehrlich, 2001).

#### *2.2.4.2. Chemistry and accumulation of mercury species*

Mercury consists and is present in the environment in several chemical forms, each displaying different characteristics (mobility and toxicity) (D'Itri, 1990). Elemental Hg ($Hg^0$) and mercuric ions ($Hg^{2+}$) are the predominant natural forms in the environment and generally do not accumulate in fish (Boening, 2000). Although not directly accumulated, elemental Hg is easily vapourised and transported through the atmosphere, providing circulation of Hg from land sources to the oceans (Boening, 2000) where it can be converted into other more soluble chemical forms (inorganic and organic Hg). The toxicity of these Hg compounds is dependent on their chemical form which affects their ability to be accumulated and excreted from the fish and human body (Harris *et al*., 2003; Clarkson *et al*., 2007). Organic Hg compounds are considered toxic as they are more stable and are more readily accumulated in fish tissue and in the human body whereas inorganic Hg compounds are considered non-toxic as they are accumulated in fish tissue in much lower concentrations and have a high rate of excretion from the human body and is therefore not

accumulated to quantities at which it becomes toxic and negatively affects the human body (Morel *et al.,* 1998; Boening, 2000). Inorganic Hg include compounds such as mercuric chloride ($HgCl_2$), mercurous chloride ($Hg_2Cl_2$), mercuric acetate ($HgC_4H_6O_4$) and mercuric sulphide (HgS) which is the most common form in nature, but is also insoluble (Peterson *et al*., 1973). Even though these inorganic forms are considered non-toxic and some of them insoluble, they can be methylated in the environment to form organic Hg compounds, such as methylmercury (MeHg) (Hempel *et al.*, 1995), which are considered toxic. This methylation occurs either by a photochemical reaction (photomethylation) or a process catalysed by microorganisms such as bacteria in the sediment (Storelli *et al*., 2002) or in the gills or gut of fish themselves (Boening, 2000). Sulphate reducing bacteria have proven to be responsible for the bulk of Hg methylation in natural waters (Morel *et al.,* 1998). Other organic Hg forms include dimethylmercury (DMHg) and ethylmercury (EthHg). Dimethylmercury is unreactive because its carbon-metal bonds are stable in water and it is therefore not absorbed into the food chain, except if partial demethylation of DMHg occurs, in which case it can then be absorbed as MeHg complexes (usually $CH_3HgCl$ and $CH_3HgOH$) (Morel *et al.,* 1998). EthHg is also considered a toxic organic form of Hg, but is not significantly absorbed and accumulated in fish tissue (Park *et al.*, 2011). Methylmercury is therefore the main chemical form absorbed into the food chain and also the most toxic. MeHg is passed easily across cell membranes as it is a stable organometallic compound, and has a high affinity for the sulfhydryl groups of amino acids (Järup , 2003; Storelli *et al*., 2002) and is therefore easily absorbed into and bioaccumulated up the marine food chain (Goyer & Clarkson, 2001; Chen *et al*., 2004). The average proportion of MeHg to total Hg increases from approximately 10% in the water column to 15% in phytoplankton, 30% in zooplankton and 95% in fish flesh (Watras & Bloom, 1992). MeHg generally accounts for 75 to 100% of the total Hg present in most fish species (Burger & Gochfeld, 2004). In the current review the term 'mercury' (Hg) will refer to total Hg (tHg) which is the sum of the inorganic Hg (iHg), MeHg, EthHg and any other Hg forms present.

Due to the significant role of diet in Hg accumulation (Hall *et al.*, 1997; Mason et al., 2000), fish at higher trophic levels are more likely to be exposed to and accumulate higher levels of Hg than those at lower trophic levels (Das *et al.*, 2000; Costa *et al.*, 2012). This process of Hg accumulation up the food chain is referred to as bioaccumulation (Ababouch *et al.*, 2004; Burger & Gochfeld, 2004). In addition, Hg can also be biomagnified within a single species with older/larger individuals having higher levels of accumulated Hg (Boush & Thieleke, 1983; Boening, 2000). Methylmercury has a longer half-life than inorganic Hg resulting in a strong correlation between the percentage of total Hg present as MeHg and the total Hg levels (Forsyth *et al.,* 2004), therefore, the percentage of total Hg present as MeHg tends to approach 100% with increasing total Hg burden and fish size/age (Forsyth *et al.,* 2004).

#### *2.2.4.3. Distribution*

Bioavailable Hg is primarily found in the muscular tissue of fish, hence its risk to consumer health as this part is most frequently consumed (Balshaw *et al.*, 2008). More specifically, Hg is known to be associated with the

protein fraction of the muscle as it binds to thiol group complexes (Harris *et al*., 2003; Nakao *et al.,* 2007; Balshaw *et al.*, 2008). The protein distribution (protein type and concentration) within a fish carcass and how it varies for example between white and dark muscle could therefore provide a link to the nature of Hg accumulation and distribution across the carcass.

Most large predatory fish such as tuna and shark have distinct muscle groups which are categorised as either white or dark muscle. These individual muscle groups have distinct functions (either fast, strong muscle movement or slow, continuous muscle movement) and distributions across the carcass (Altringham & Ellerby, 1999; Shadwick, 1999). The function and location of a muscle can affect the rate of development and composition of the muscle cells (Te Kronnié *et al*., 2000). Therefore, as metals are stored in muscle cells (Olsson *et al*., 1998), these differences in muscle cell development and composition could in turn influence the rate and degree of metal accumulation within the muscle. Several studies have found variation of Hg accumulation within carcasses of tuna fish (Ando *et al.*, 2008; Balshaw *et al.*, 2008; Lares *et al*., 2012), but reasons for Hg variation have not been clearly identified (Lares *et al*., 2012). Some authors suggest that lipid concentrations might have a diluting effect on accumulated Hg (Nakao *et al.,* 2007), however the Hg and lipid content relationship was insignificant in cultured Bluefin tuna and wild Albacore tuna (Morrissey *et al*., 2004; Nakao *et al.,* 2007). These studies measured total Hg concentrations and as far as authors are aware; no studies have been published on variation in the accumulation of individual Hg species and therefore variation in Hg toxicity across the fish carcass.

#### *2.2.4.4. Accumulation and effects in the human body*

Methylmercury is the main stable organic form of Hg that is taken up by the human body via seafood consumption. More than 95% of MeHg ingested is absorbed from the intestinal tract after consumption (Guynup & Safina, 2012) and is then distributed to all tissues and target organs via the bloodstream. MeHg readily crosses the blood-brain barrier (FSANZ, 2004; Clarkson *et al*., 2007), resulting in significant deposition (about 10% of the total Hg burden) in the brain region (Silbernagel *et al*., 2011). The accumulation of MeHg in the brain causes loss of cells in specific brain areas such as the cerebellum, visual cortex and other focal areas (FSANZ, 2004). Other main target organs include the pituitary gland, liver and kidney (Goyer & Clarkson, 2001; Clarkson *et al*., 2007). Methylmercury readily crosses the placental barrier subsequently affecting the neurological development in developing foetuses (FSANZ, 2004; Clarkson *et al*., 2007).

Symptoms of MeHg intoxication in humans include impaired vision and hearing, headaches, paraesthesia, movement difficulties and loss of coordination, fatigue, tremors and ataxia (Grandjean *et al.*, 2010). Low-level exposure of MeHg can adversely affect the cardiovascular system whereas chronic Hg exposure impacts the pituitary gland and the liver and leads to a compromise of the immune system (Guynup & Safina, 2012). Children exposed to Hg prenatally often show delays in the development of their speech and motor functions (Amin-zaki *et al*., 1978; Grandjean *et al.*, 2010). The onset of these various symptoms can take up to a few months from the time of Hg exposure or ingestion. This is especially dangerous to pregnant

women as doses of on fifth the toxic dose to adults could have adverse effects on the developing nervous system of a foetus or child (Grandjean *et al.*, 2010) and a high Hg intake can therefore affect the foetus before any signs of Hg poisoning are visible in the mother.

Rather than limiting fish intake, attention should be focused on determining which fish are safe for consumption and which should be avoided in regards to Hg levels as seafood is the main source of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kirk *et al.*, 2012) that have major beneficial effects on human health and neurocognitive development (especially DHA) (Smith & Sahyoun, 2005; Anonymous, 2010). It has been recommended that high-trophic-level predatory fish such as shark, swordfish, king mackerel, tilefish and albacore should be avoided or consumed in smaller quantities (FDA, 2001). Advisory committees such as the Food and Drug Administration have published recommendations for safe fish consumption (FDA, 2001) and should be consulted when consuming seafood.

Disposal of Hg from the body is a slow process and occurs mainly via the faecal route. MeHg is secreted in the bile from where a fraction is reabsorbed in the gallbladder and gastrointestinal tract (Clarkson *et al*., 2007). Some secretion may also occur across the intestinal membrane as intestinal flora in the gastrointestinal tract are capable of breaking the carbon-Hg bond, converting MeHg to iHg which is poorly absorbed and is then mostly excreted in the faeces (Clarkson *et al*., 2007). Mercury intake should therefore be limited and monitored in order to prevent toxic build-up of Hg which occurs when the amount of Hg absorbed exceeds that being excreted.

The maximum tolerable weekly intake for Hg, as recommended by the Expert Committee on Food Additives and Contaminants (JECFA) under the joint Food and Agriculture Organization (FAO) and the World Health Organization (WHO), as part of the international safety guidelines, is 1.6 $\mu g \cdot kg^{-1}$ (body weight) (EC, 2006) which replaces the previous PTWI of 3.3 $\mu g \cdot kg^{-1}$ (body weight) (JECFA, 2007). The first regulatory MALs for Hg in seafood were set as 0.5 $mg \cdot kg^{-1}$ fish meat, except for large predatory species, which were found to frequently exceed this limit and therefore only had to comply to a Hg MAL of 1 $mg \cdot kg^{-1}$ according to the European Commission (EC, 2006; Grandjean *et al.*, 2010). However, these limits were set for Hg and not MeHg and it is known that the latter is more toxic to humans. The South African Department of Health now requires this limit of 1 $mg \cdot kg^{-1}$ fish meat as specifically for MeHg (DOH, 2004).

#### *2.2.4.5. Mercury analysis*

In order to monitor the compliance of commercial fish meat to Hg maximum limit regulations, accurate and efficient analytic methods are required. A number of methods that include atomic florescence spectrometry (AFS), various forms of atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) have been developed for measuring total Hg in seafood and are currently widely used (Bloxham *et al*., 1996). South African regulations require Hg analysis done by cold vapour atomic absorption spectrometry (CVAAS) (Compulsory

Specification, 2003). However, in order to accurately monitor levels of toxic Hg in fish meat (Wan *et al.,* 1997; Rai *et al.*, 2002), metal speciation techniques should be used.

Metal speciation, is defined by Florence (1982) as "the determination of the concentrations of the individual physico-chemical forms of the element in a sample that together, constitute its total concentration" (p. 345). The analytical techniques used for Hg speciation are combinations of separation techniques such as gas or liquid chromatography and detection techniques such as CVAAS, inductively coupled plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (FAAS), electron-capture detection (ECD), cold vapour atomic fluorescence spectrometry (CVAFS) or atomic emission spectrometry (AES) (Van Loon & Barefoot, 1992; Emteborg *et al*., 1994; Caruso & Montes-Bayon, 2003). These separation and detection techniques can be used in different combinations with the choice of speciation technique depending on the performance priorities and requirements, as each technique has its own strengths and advantages (Leermakers *et al.*, 2005).

For these aforementioned speciation analyses, all analytical steps need to be properly planned from sample preparation to species separation and detection in order to ensure that all the Hg species in the samples analysed remain in their original form and none are lost or changed along the process. Although the total Hg content of a sample is stable and cannot be reduced through losses during processing steps, individual Hg species can be interconverted between organic and inorganic forms affecting measurement results of Hg toxicity (Qvarnstrom & Frech, 2002). Little is known about the stability of Hg species in biological samples during sample storage, but fresh samples are usually deep frozen or lyophilised in darkness for storage before analysis (Leermakers *et al.*, 2005). Sample preparation/digestion is a critical step as the analyte needs to be fully extracted from the sample matrix, but without losses, contamination or changes in species (Caruso & Montes-Bayon, 2003). Subsequent to metal extraction, the individual elemental species need to be separated as cleanly as possible prior to the detection process (Caruso & Montes-Bayon, 2003). Current metal speciation techniques are costly and time consuming, which is not beneficial for routine monitoring in the industry as analytical results on Hg toxicity of fish samples should be obtained before entire batches of fish are distributed onto the market (Wepener & Degger, 2012).

The fish and seafood industry is still in need of a time and cost effective, accurate way of determining levels of toxic MeHg for a true measurement of food safety for human consumption. The ratio of the total Hg burden present as MeHg can vary with more than 30% within one species and average ratios vary from approximately 50-100% (Forsyth *et al.,* 2004). It is therefore clear that a fixed conversion factor will not provide accurate estimates of toxic Hg concentrations in health assessments. There are, however, significant correlations between the percentage of total Hg present as MeHg and the total Hg concentrations as well as between total Hg concentrations and fish size/age (Forsyth *et al.,* 2004). Further research should therefore further explore these relationships for the possibility of setting up a model for calculating toxic MeHg levels from total Hg measurements.

## 2.3 Heavy metals in seafood

Hg accumulation in both marine and freshwater fish has been widely studied (summary in Table 2.3) as a result of an increased focus on Hg poisoning and its toxic effects due to a number of large scale human poisoning incidences (Harada, 1995; Grandjean *et al.*, 2010). These studies cover a wide variety of fish species and aquatic organisms, not all of which are commercially consumed, as some are merely used as biomonitors of environmental pollution.

Even though a large part of the current review has focused on Hg as a main contaminant, the possible hazardous effects of other metals should not be disregarded. The accumulation and effects of individual metals are not always independent of each other and correlations between various heavy metals have previously been identified for numerous fish species (Carvalho *et al*., 2005; Rahman *et al*., 2012). These correlations can be positive where certain metals facilitate absorption of other metals or negative where certain metals dominate and therefore decrease the uptake of other metals and minerals. The presence of Hg, for example, has been found to decrease the uptake of Cu and Zn in certain organisms (Erasmus *et al*., 2004) while Se has been shown to have a detoxifying effect on organic Hg in the liver of certain fish (Cravalho *et al.*, 2005; Branco *et al.*, 2007).

The effects of various external (marine environment) and internal (fish carcass parameters) factors can lead to variation in metal accumulation and inter-metal correlations within and among fish species, locations and seasons (Burger *et al.*, 2014). One such widely documented relationship is the size-age effect on metal concentration. In general Hg concentrations increase as fish size/age increases (especially in predatory fish) (Canli & Atli, 2003; Kraepiel *et al*., 2003; Erasmus *et al*., 2004; Endo *et al*., 2008; Campbell *et al.*, 2010). However, this trend is not apparent in all other metals (Storelli & Marcotrigiano, 2004). Rather, several metals (Cr, Cu, Fe, Cd, Ni, As and Pb) have negative correlations with fish size/age in a number of fish species (Widianarko *et al*., 2000; Canli & Atli, 2003; Erasmus *et al.*, 2004), which may be due to higher metabolic rates of younger individuals (Canli & Atli, 2003). Similarly, not all metals are bioaccumulated up the food chain as was previously described for Hg (Storelli *et al.*, 2002). Arsenic for example is found in higher concentrations in lower trophic level fish species (De Gieter *et al.*, 2002). Continuous research on individual metals and how they relate to each other in various fish species and various locations is therefore fundamental in understanding the overall food safety levels of fish meat with regards to metal contaminants.

**Table 2.3** A summary of studies on mercury and methylmercury in a variety of organs/muscles in fish from various continents or seas per trophic level.

| Trophic level | Continent/Sea | Species | Marine/ freshwater | Mercury levels (ppm) (mean ± SD) | | | | | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Liver | White meat | Red meat | Overall meat | MeHg | |
| Apex predators | Africa | Smoothhound (*Mustelus mustelus*) | Marine | - | - | - | 0.9 | - | Bosch *et al.*, 2013 |
| | Atlantic Islands | Blue Shark (*Prionace glauca*) | Marine | 0.03 - 0.96 | - | - | 0.22 - 1.30 | - | Branco *et al.*, 2007 |
| | Atlantic Islands | Blue Shark (*P. glauca*) | Marine | 0.15 - 2.20 | - | - | 0.68 - 2.50 | - | |
| | Atlantic Islands | Swordfish (*Xiphias gladius*) | Marine | 0.05 - 8.50 | - | - | 0.03 - 2.40 | - | |
| | Atlantic Islands | Swordfish (*X. gladius*) | Marine | 1.10 - 9.80 | - | - | 0.90 - 2.10 | - | |
| | Australia/NZ | Southern Bluefin Tuna (farmed) (*Thunnus maccoyii*) | Marine | - | 0.32 ± 0.03 | - | - | - | Balshaw *et al.*, 2008 |
| | Australia/NZ | Dog Shark (*Deania calcea*) | Marine | - | - | - | 7.2 ± 2.3 | - | Turoczy *et al.*, 2000 |
| | Australia/NZ | Dog Shark (*Centroscymnus crepidater*) | Marine | - | - | - | 4.3 ± 2.4 | - | |
| | Australia/NZ | Dog Shark (*Centroscymnus owstonii*) | Marine | - | - | - | 11.9 ± 1.1 | - | |
| | Australia/NZ | School Shark (*Galeorhinus australis*) | Marine | - | - | - | 0.9 | - | Walker, 1976 |
| | Australia/NZ | Gummy Shark (*Mustelus antarcticus*) | Marine | - | - | - | 0.37 | - | |
| | East Asia | Swordfish | Marine | - | - | - | 0.50 ± 0.01 | 0.41 ± 0.02 | Chiou *et al.*, 2001 |
| | East Asia | Dall's Porpoise | Marine | - | - | 1.26 ± 0.53 | - | - | Haraguchi & Sakata, 2003 |
| | East Asia | Baird's Beaked Whale | Marine | - | - | 1.64 ± 1.26 | - | - | |
| | East Asia | Pantropical Spotted Dolphin | Marine | - | - | 4.72 ± 0.39 | - | - | |
| | East Asia | Risso's Dolphin | Marine | - | - | 5.42 ± 4.68 | - | - | |
| | East Asia | Rough-toothed Dolphin | Marine | - | - | 6.00 | - | - | |
| | East Asia | Pilot Whale | Marine | - | - | 7.59 ± 6.12 | - | - | |
| | East Asia | Bottlenose Dolphin | Marine | - | - | 9.55 ± 6.01 | - | - | |
| | East Asia | Striped Dolphin | Marine | - | - | 15.0 ± 27.1 | - | - | |
| | East Asia | False Killer Whale | Marine | - | - | 46.9 ± 29.7 | - | - | |
| | East Asia | Tiger Shark (*Galeocerdo cuvier*) | Marine | 1.17 ± 3.14 | - | - | 0.78 ± 0.29 | - | Endo *et al.*, 2008 |
| | East Asia | Silvertip Shark (*Carcharhinus albimarginatus*) | Marine | 0.70 ± 0.42 | - | - | 1.80 ± 0.45 | - | |
| | Mediterranean | Tuna | Marine | - | - | - | 0.48 | - | Domingo, 2007 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Mediterranean | Swordfish | Marine | - | - | - | 1.93 | - | |
| Mediterranean (Adriatic Sea) | Blackmouth Dogfish (*Galeus melastomus*) | Marine | - | - | - | 2.66 ± 1.24 | 2.11 ± 0.96 | Storelli *et al.*, 2002 |
| Mediterranean (Ionian Sea) | Blackmouth Dogfish (*G. melastomus*) | Marine | - | - | - | 0.82 ± 0.62 | 0.74 ± 0.52 | |
| Mediterranean (Aegean Sea) | Blackmouth Dogfish (*G. melastomus*) | Marine | - | - | - | 2.14 ± 1.44 | 1.55 ± 1.23 | |
| Mediterranean | Small Spotted Shark (*Scyliorhinus canicula*) | Marine | - | - | - | 1.49 ± 0.61 | 1.23 ± 0.49 | |
| Mediterranean | Kitefin Shark (*Dalatias licha*) | Marine | - | - | - | 4.38 ± 1.07 | 3.81 ± 0.69 | |
| Mediterranean | Gulper Shark (*Centrophorus granulosus*) | Marine | - | - | - | 9.66 ± 0.69 | 0.09 ± 0.83 | |
| Mediterranean | Longnose Spurdog (*Squalus blainvillei*) | Marine | - | - | - | 4.53 ± 1.19 | 4.05 ± 1.29 | |
| Mediterranean | Velvet Belly (*Etmopterus spinax*) | Marine | - | - | - | 0.63 ± 0.29 | 0.58 ± 0.26 | |
| Mediterranean | Smoothhound (*M. mustelus*) | Marine | - | - | - | 0.31 ± 0.06 | 0.23 ± 0.05 | |
| Mediterranean | Sharpnose Sevengill (*Heptranchias perlo*) | Marine | - | - | - | 1.20 ± 0.17 | 1.20 ± 0.17 | |
| Mediterranean | Hammerhead (*Sphyrna zygaena*) | Marine | - | - | - | 18.29 ± 0.03 | 16.06±0.04 | |
| Pacific Islands | Yellowfin Tuna | Marine | - | - | - | 0.21 ± 0.11 | - | Kraepiel *et al.*, 2007 |
| South America | Scalloped Hammerhead (*Sphyrna lewini)* | Marine | - | - | - | 4.84 | - | Ruelas-Inzunzu & Paez-Osuna, 2005 |
| South America | Catfish (*Galeichthys peruvianus*) | Marine | - | - | - | 1.58 | - | |
| South America | Blue Shark (*P. glauca*) | Marine | - | - | - | 0.05 ± 0.03 | - | Lopez *et al.*, 2013 |
| South America | Mako Shortfin Shark (*Isurus oxyrinchus*) | Marine | - | - | - | 0.03 ± 0.02 | - | |
| South America | Brazillian Sharpnose Shark (*Rhizoprionodon lalandii*) | Marine | - | - | - | 0.20 ± 0.16 | - | Viana *et al.*, 2005 |
| USA | Swordfish | Marine | - | - | - | 1.07 | - | Carrington & Bolger, 2002 |
| USA | Shark | Marine | - | - | - | 0.96 | - | |
| USA | Bull Shark (*Carcharhinus leucas*) | Marine | - | - | - | 0.77 ± 0.32 | - | Adams & McMichael, 1999 |
| USA | Blacktip Shark (*Carcharhinus limbatus*) | Marine | - | - | - | 0.77 ± 0.71 | - | |
| USA | Atlantic Sharpnose Shark (*Rhizoprionodon terraenovae*) | Marine | - | - | - | 1.06 ± 0.63 | - | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | USA | Bonnethead Shark (*Sphyrna tiburo)* | Marine | - | - | - | 0.50 ± 0.36 | - | |
| | USA | Tuna (canned) | Marine | - | 0.41 ± 0.17 | - | - | - | Burger & Gochfeld, 2004 |
| | New England | Shortfin mako shark | Marine | - | - | - | 2.65 ± 1.16 | - | Teffer *et al.*, 2014 |
| | New England | Thresher shark | Marine | - | - | - | 0.87 ± 0.71 | - | |
| | New England | Albacore tuna | Marine | - | - | - | 0.45 ± 0.14 | - | |
| | New England | Yellowfin tuna | Marine | - | - | - | 0.32 ± 0.09 | - | |
| | New England | Dolphinfish | Marine | - | - | - | 0.20 ± 0.17 | - | |
| Mid-trophic level species | Africa (u'Mgeni River) | Sharp Toothed Catfish (*Clarias gariepinus*) | Freshwater | - | - | - | 0.4 | - | Oosthuizen & Ehrlich, 2001 |
| | Africa | Atlantic Mackerel | Marine | - | - | - | 0.116 ± 0.070 | - | Chahid *et al.*, 2014 |
| | Africa | Atlantic Bonito | Marine | - | - | - | 0.064 ± 0.180 | - | |
| | Africa | European Conger | Marine | - | - | - | 0.049 ± 0.002 | - | |
| | Africa (Inanda Dam) | Sharp Toothed Catfish (*C. gariepinus*) | Freshwater | - | - | - | 0.2 | - | Oosthuizen & Ehrlich, 2001 |
| | Africa (Nagle Dam) | Sharp Toothed Catfish (*C. gariepinus*) | Freshwater | - | - | - | 0.14 | - | |
| | Africa (Orange River) | Sharptooth catfish | Freshwater | - | - | - | 0.73 ± 0.02 | - | Pheiffer *et al.*, 2014 |
| | Africa (Vaal River) | Sharptooth catfish | Freshwater | - | - | - | 0.05 ± 0.01 | - | |
| | Australia/NZ | Orange Roughy (*Hoplostethus atlanticus*) | Marine | - | - | - | 0.5 | - | Van den Broek & Tracey, 1981 |
| | Mediteranean | Mackerel | Marine | - | - | - | 0.09 | - | Domingo, 2007 |
| | Mediteranean | Salmon | | - | - | - | 0.05 | - | |
| | Mediteranean | Hake | Marine | - | - | - | 0.19 | - | |
| | Mediteranean | Red Mullet | | - | - | - | 0.23 | - | |
| | Mediteranean | Sole | Marine | - | - | - | 0.08 | - | |
| | Adriatic Sea | Red mullet | Marine | - | - | - | 0.48 ± 0.09 | - | Perugini *et al.*, 2014 |
| | Adriatic Sea | European hake | Marine | - | - | - | 0.59 ± 0.14 | - | |
| | Adriatic Sea | Blue whiting | Marine | - | - | - | 0.38 ± 0.10 | - | |
| | Adriatic Sea | Atlantic mackerel | Marine | - | - | - | 0.36 ± 0.09 | - | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | USA | Pollock | Marine | - | - | - | 0.15 | - | Carrington & Bolger, 2002 |
| | Canada | Chinook salmon | Marine | - | - | - | 0.088 ± 0.077 | - | Laird & Chan, 2013 |
| | Canada | Sockeye salmon | Marine | - | - | - | 0.077 ± 0.028 | - | |
| | Alaska | Black rockfish | Marine | - | - | - | 0.145 ± 0.018 | - | Burger *et al.*, 2014 |
| | Alaska | Dolly Varden | Marine | - | - | - | 0.114 ± 0.013 | - | |
| | Alaska | Pacific Halibut | Marine | - | - | - | 0.148 ± 0.044 | - | |
| | Alaska | Great Sculpin | Marine | - | - | - | 0.294 ± 0.054 | - | |
| | Alaska | Pacific Cod | Marine | - | - | - | 0.173 ± 0.012 | - | |
| | Alaska | Rock greenling | Marine | - | - | - | 0.099 ± 0.014 | - | |
| | Alaska | Yellow Irish Lord | Marine | - | - | - | 0.272 ± 0.029 | - | |
| | Alaska | Pink Salmon | Marine | - | - | - | 0.042 ± 0.005 | - | |
| | Alaska | Flathead Sole | Marine | - | - | - | 0.276 ± 0.012 | - | |
| | Alaska | Rock Sole | Marine | - | - | - | 0.095 ± 0.023 | - | |
| Lower-trophic level species | Africa | Cape Silverside (*Atherina breviceps)** | Estuarine | - | - | - | 0.5 - 5.3 | - | Hutching & Clarke, 2010 |
| | Africa | Cape Silverside (*A.breviceps)** | Estuarine | - | - | - | 0.3 | - | |
| | Africa | Barehead Goby (*Caffrogobius nudiceps*)* | Marine | - | - | - | 0.3 - 1.0 | - | |
| | Africa | Barehead Goby (*C. nudiceps*)* | Marine | - | - | - | 0.4 - 0.9 | - | |
| | Africa | Common Carp (*Cyprinius carpio*)* | Freshwater | - | - | - | 0.3 - 0.8 | - | |
| | Africa | Round Herring (*Gilchristella aestuaria)** | Estuarine | - | - | - | 0.2 - 0.8 | - | |
| | Africa | Round Herring (*G. aestuaria)** | Estuarine | - | - | - | 0.3 - 0.4 | - | |
| | Africa | Mullet (*Liza richardsonii*)* | Estuarine | - | - | - | 0.3 - 0.8 | - | |
| | Africa | Mullet (*L. richardsonii*)* | Estuarine | - | - | - | 0.3 - 0.6 | - | |
| | Africa | Tilapia (*Oreochromis mossambicus*)* | Estuarine | - | - | - | 0.3 - 0.5 | - | |
| | Africa | Tilapia (*Tilapia sparrmanii*)* | Estuarine | - | - | - | 0.4 - 1.0 | - | |
| | Africa | Common Carp (*Cyprinius carpio*) | Freshwater | - | - | - | 0.35 | - | Oosthuizen & Ehrlich, 2001 |
| | Africa | Common Carp (*C. carpio*) | Freshwater | - | - | - | 0.11 | - | |
| | Africa | Common Carp (*C. carpio*) | Freshwater | - | - | - | 0.37 | - | |
| | Africa | Pacific Thread Herring (*Opisthonema libertate*) | Marine | - | - | - | - | - | Ruelas-Inzunzu & Paez-Osuna, 2005 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Africa | European Pilchard | Marine | - | - | - | 0.084 ± 0.080 | - | Chahid *et al.*, 2014 |
| Africa | Rubberlip Grunt | Marine | - | - | - | 0.059 ± 0.020 | - | |
| Africa | Atlantic Horse Mackerel | Marine | - | - | - | 0.034 ± 0.030 | - | |
| Africa | Bogue | Marine | - | - | - | 0.194 ± 0.008 | - | |
| Africa | *Trisopterus capelanus* | Marine | - | - | - | 0.097 ± 0.020 | - | |
| Mediteranean | Sardine | Marine | - | - | - | 0.08 | - | Domingo, 2007 |
| Mediteranean | Anchovy | Marine | - | - | - | 0.08 | - | |
| USA | Flatfish | Marine | - | - | - | 0.09 | - | Carrington & Bolger, 2002 |

*Hutchings K, Clark BM, unpublished

In Africa, where malnutrition is a major underlying cause of death, assurance of food safety of the continent's natural resources is of utmost importance. Fish meat is one of these natural food sources. The average contribution of fish protein to the total animal protein supply in Africa is 19.4% which is exceeded in many countries with high per capita fish consumption, especially in coastal West-Africa (FAO, 2012). Research on metal contaminants in marine fish around the African continent is however very limited (Table 2.4) and further research is needed to ensure that fish which is commonly known as a healthy food source, does not carry unknown hazards to consumer health.

South Africa's extensive coast line (close to 3 000 km) and diverse ocean systems have facilitated the development of a major fishing industry where approximately 80% of fish is exported globally and the remainder is further processed and/or consumed locally. However a number of factors (over-fishing, global climate change, habitat destruction and pollution) have and continue to pose problems for the national fishing industry. Hutchings and Clark (unpublished) identified a number of South African estuarine systems (Diep and Berg Estuaries) with sediment and biota trace metal levels exceeding the recommended safe levels for natural environments (Taljaard, 2006). This is largely due to anthropogenic activity such as waste water treatment works, storm water and industrial waste water, which can in turn have a significant effect on the consumption safety of South African fishes. Few studies have been done on metals in South African freshwater fish from contaminated rivers and dams (Table 2.4). However, even though commercial fish are being monitored on an on-going basis (Compulsory Specification, 2003), the lack of reported information on heavy metals and especially Hg in South African marine fish is of great concern with regards to consumer health and industry economics as the fishing industry has no guidelines as to which fish or which areas to avoid to minimise catches of fish containing Hg levels above allowable limits.

## 2.4 Recommendations for future research

Research on heavy metal concentrations in commonly consumed fish species is still needed, especially in Africa, yet such research is essential in order to understand true toxicity and eventual effects on the consumer. However, the majority of published studies to date predominantly focus on only the few most toxic metals (Hg, As, Cd, Pb) in fish meat; their concentrations and comparisons to various allowable limits. As has been stated earlier, there are numerous factors which can affect the levels of heavy metals detected in a fish and therefore to understand how and why this can vary is essential. Some studies have described mercury speciation and total metals present in toxic form (Hg and As) as obtained from fish tissue; however, fish monitoring programs continue to only measure total As and Hg assuming that 100% of total Hg is present in its toxic MeHg form. This is largely due to unwanted extra costs and time which metal speciation techniques would add to routine monitoring (Wepener & Degger, 2012). Therefore, the identification of a toxicity predictive model could allow for a more accurate and time- and cost-effective method of monitoring true toxicity in fish samples.

**Table 2.4** Summary of African research done on metals in various marine and freshwater fish and organisms.

| Metals analysed | Samples studied | Country/Region | Reference |
|---|---|---|---|
| **Marine:** | | | |
| Zn, Cd, Cu, Pb, Mn, Ni, Co, Bi | Mussel: *Choromytilus meridionalis* | Saldanha Bay, South Africa | Watling & Watling, 1976 |
| Pb, Cd, Cu, Zn | Phytoplankton, zooplankton, shrimps | North-West Africa | Bruegmann, 1978 |
| Cd Cu, Zn, Hg | Round sardinella, chub mackerel, Atlantic horse mackerel, painted comber, golden grouper, Niger hind, West African goatfish | Mauritanian coast | Roméo *et al.*, 1999 |
| Al, Cr, Mn, Fe, Co, Cu, Zn, As, Cd, Pb | Shortnose Spurdog, smoothhound shark | Southeastern Coast of South Africa | Erasmus *et al.*, 2004 |
| Al, Cr, Cu, Fe, Mn, Pb, Zn | Groovy mullet | Mhlathuze Estuary, South Africa | Mzimela *et al.*, 2006 |
| Cu, Cd, Pb, Hg | Various marine fish | Egypt | Khorshed, 2009 |
| As, Cd, Hg | Manta Ray | Ghana | Essumang, 2010 |
| Cd, Cu, Cr, Fe, Mn, Ni, Pb, Zn | Harder, estuarine round herring, Tilapia, silverside, crabs, polychaete worms, insect larvae | Diep River Estuary, South Africa | Hutching & Clarke, 2010 |
| Hg | Basa, calamari, shrimp, mussels, sardines, salmon (fresh and tinned), sole, fishfingers, red snapper, monktail, silver, snoek, tinned tuna, butterfish, angelfish, yellowtail, kingklip, dorado, fresh tuna, rockcod | Supermarkets and seafood restaurants in Gauteng, South Africa | Maritz, 2010 |
| Si, Ti, Al, Fe, Mn, Mg, Ca, Na, K, P, Cd, V, Cr, Ni, Cu, Zn, Sr, Zr, Ba, Pb, U | *Dentex* spp., *Galeoides decadactylus, Chloroscombrus chrysurus, Trichiurus lepturus*, Mussel spp. | Togo | Gnandi *et al.*, 2011 |
| Hg | Smooth hound shark | Langebaan, South Africa | Bosch *et al.*, 2013 |

| Metals analysed | Samples studied | Country/Region | Reference |
|---|---|---|---|
| Fe, Pb, Ni, Cd, Zn, Cu | *Scomber scombrus*<br>*Sardina pilchardus*<br>Jack mackerel<br>*Gadus macrocephalus* | South Western Nigeria | Ogundiran *et al.*, 2014 |
| As, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Zn | *Drepane africana,*<br>*Cynoglossus senegalensis,*<br>*Pomadasys peroteti* | Ghana | Bandowe *et al.*, 2014 |
| Status of marine pollution research in SA | | | Wepener & Degger, 2012 |
| **Freshwater (South Africa):** | | | |
| Fe, Mn, Zn, Cu, Ni, Pb | Southern mouthbrooder | Transvaal (Gauteng), South Africa | De Wet *et al.*, 1994 |
| Fe, Zn, Pb, Ni, Cu, Cd, Mn | Tigerfish | Olifants River, South Africa | Du Preez & Steyn, 2000 |
| Cr, Mn, Ni, Pb | Moggel | Witbank dam, South Africa | Nussey *et al.*, 2000 |
| Hg | Sharp toothed catfish, wide mouthed bass | KwaZulu-Natal, South Africa | Oosthuizen & Ehrlich, 2001 |
| Al, Sb, As, Ba, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Ag, Sr, Sn, V, Zn, | Rednose laboe | Olifants River, South Africa | Jooste *et al.*, 2014 |
| Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Ag, Pt, Au, Cd, Hg, Pb, U | Sharptooth catfish | Vaal River, South Africa | Pheiffer *et al.*, 2014 |
| Al, Sb, As, Ba, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Ag, Sr, Sn, V, Zn | Sharptoot catfish | Olifants River, South Africa | Jooste *et al.*, 2015 |

Standardised sampling strategies are necessary to allow cross study and species comparisons. To date no standard protocol for sampling fish anatomical sections exists (Wepener & Degger, 2012); however, different muscles have different functions and can absorb and utilise nutrients and pollutants differently. Therefore, research on cross-carcass metal accumulation, especially between different muscle types (dark and white) of large predatory fish is recommended.

Heavy metal concentrations are species, location and trophic level dependent which can result in considerable variation making comparison and meaningful interpretation difficult. Therefore, more research is required to cover each of these aspects. Research on: 1) trophic level disparities can aid the understanding as to how metals accumulate within the food chain, while 2) spatial scale studies (between and within species) may provide links between environmental pollution and the effects on fish contamination and consequently food safety and consumer health.

Monitoring of metals in seafood is compulsory according to South African legislation (Compulsory Specification, 2003); however the results are not publically available and therefore generally remain

unpublished. In addition, very limited research has been published on the human effects of metal contamination through fish and seafood consumption on the African continent. This lack of knowledge and information transfer has led to large knowledge deficits for scientists, consumers and industry as a whole. It is therefore suggested that all data collected be made publically available within a predetermined time from collection.

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# CHAPTER 3:

## Heavy metal accumulation and toxicity in smoothhound shark (*Mustelus mustelus*) from Langebaan Lagoon, South Africa*

**ABSTRACT**

Together with several health benefits, fish meat could introduce toxins to consumers in the form of heavy metal contaminants. High levels of mercury (Hg), especially, are frequently detected in certain predatory fish species. *Mustelus mustelus* fillets were analysed for 16 metals and three individual Hg species (inorganic Hg, ethylmercury, methylmercury) with inductively coupled plasma mass spectrometry (ICP-MS) and HPLC–ICP-MS respectively. Eleven of the 30 sharks had total Hg levels above the maximum allowable limit with toxic methylmercury found as the dominant mercury species with a strong correlation ($r = 0.97$; $p < 0.001$) to total mercury concentrations. Limited correlations between metals and shark size parameters were observed; therefore metal accumulation in *M. mustelus* is mostly independent of size/age. Average values for arsenic ($28.31 \pm 18.79$ mg·kg$^{-1}$) exceed regulatory maximum limits and Hg ($0.96 \pm 0.69$ mg·kg$^{-1}$) is close to the maximum limit with all other metals well below maximum limits.

**Keywords:** Mercury, Methylmercury, Mercury speciation, Heavy metals, Arsenic, ICP-MS, HPLC-ICP-MS, *Mustelus mustelus*, Shark meat, Regulatory limits

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## 3.1 Introduction

Fish and seafood are considered to be a highly nutritious food source providing high value proteins and several essential fatty acids (Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) necessary for normal human development and continued health (Domingo *et al.*, 2006). Despite the numerous health benefits of a diet high in fish and seafood, frequent consumption of specific seafood may lead to an increased exposure to chemical and metal contaminants which can pose a risk to human health (Domingo *et al.*, 2006) as numerous studies have shown that seafood consumption in general is a major contributor to the uptake and accumulation of heavy metals and other contaminants in the human body (Castro-González & Méndez-Armenta, 2008). Heavy metals of high concern such as mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) have been found to accumulate in fish meat in toxic quantities (Llobet *et al.*, 2003). Coastal communities that rely on fish as their main source of nutrition therefore stand an increased risk of consuming and bioaccumulating toxic quantities of contaminants (Costa *et al*., 2012) which in turn can lead to serious health issues such as development and birth defects and detrimental effects on the nervous system and its functions (Grandjean *et al.*, 2010).

Mercury in particular has received growing attention in recent years as it is seen as one of the most toxic metals in the environment (Storelli *et al.*, 2002). Mercury is naturally present in the environment but can also be artificially introduced through waste from industrial activities (Castro-González & Méndez-Armenta, 2008). Although industrial Hg pollution has been significantly decreased due to a greater awareness of its toxicity and danger to human health (Harada, 1995), the presence of toxic metals persist in the environment due to accumulation in water and sediment (Castro-González & Méndez-Armenta, 2008).

The toxicity of heavy metals such as mercury is often dependant on their chemical state or species such as the elemental, organic or inorganic species (Hempel *et al.*, 1995). However, species identification is rarely considered in fish meat analysis, which can result in inaccurate misrepresentation of toxicity. Mercury is introduced into the environment in an inorganic form, but is converted to organic forms (methyl- and ethylmercury) by bacteria within the environment (Park *et al*., 2011,). Methylmercury (MeHg) is considered the most toxic form of Hg (Hempel *et al.*, 1995) and is also the most prominent Hg species in fish, accounting for 75 - 100% of the total mercury (tHg) levels present (Burger & Gochfeld, 2004). Methylmercury is a stable chemical form of Hg (Hempel *et al*., 1995), binding to thiol complexes in proteins (Spry & Wiener, 1991; Clarkson *et al.*, 2007) and is therefore only excreted in very small amounts, facilitating accumulation in fish and human tissue over time (Clarkson *et al.*, 2007). Due to the bioaccumulation of Hg, levels are biomagnified up the food chain where large predatory fish such as tuna, shark and swordfish contain the highest levels (Burger & Gochfeld, 2004; Domingo *et al*., 2006).

Monitoring of Hg and MeHg levels is becoming increasingly important particularly in commercial marine species due to their associated negative health effects. The current maximum allowable limit for Hg in predatory fish species (tuna, swordfish, shark) is 1 $mg \cdot kg^{-1}$ fresh weight for tHg (FAO, 2003) with regulations

by the South African Department of Health specifying this same limit (1.0 $mg \cdot kg^{-1}$) as for MeHg (DOH, 2004) as the main toxic component of tHg. A provisional tolerable weekly intake (PTWI) of 1.6 $\mu g \cdot kg^{-1}$ body weight is also specified for MeHg (JECFA, 2002). Methylmercury is, however, rarely measured in fish species as tHg measurements are normally considered sufficient for monitoring Hg toxicity. This could however result in a misrepresentation of the true Hg toxicity in fish if the MeHg to tHg ratio is not sufficiently investigated.

The concentrations and therefore toxicity of Hg and Hg species can be identified using various laboratory techniques (Caruso & Montes-Bayon, 2003). However, due to the variation in detection limits and species detection between methods the continued development of these techniques is required. The cold vapour atomic absorption spectroscopy (CVAAS) technique has been the most commonly used method for measuring total Hg and other heavy metals. Other alternative methods for measuring total metal concentrations have also been developed, such as inductively coupled plasma mass spectrometry (ICP-MS), which can have a higher detection sensitivity (Chen *et al.*, 2015) than CVAAS (0.003 $mg \cdot kg^{-1}$ compared to 0.01 $mg \cdot kg^{-1}$). Although these methods are sufficient for the detection of total metals, an additional separation phase is required for the detection of the individual metal species. Separation techniques such as liquid chromatography (LC), gas chromatography (GC) or capillary electrophoresis (CE) are often coupled to detection techniques such as CVAAS, inductively coupled plasma mass spectrometry (ICP-MS), flame atomic absorption spectrometry (FAAS), electron-capture detection (ECD), cold vapour atomic fluorescence spectrometry (CVAFS) or atomic emission spectrometry (AES) in various combinations. The combination of separation and detection techniques depends on the required specifications such as speed, sensitivity, cost and the substance analysed (Caruso & Montes-Bayon, 2003).

According to literature, ICP-MS is one of the detection techniques which is currently most commonly used (Clémens *et al.*, 2012) and linked to several separation techniques (mostly variations of LC and GC) (Balarama Krishna *et al.*, 2010; Chen *et al.*, 2015; Deng *et al.*, 2015). However, several other speciation methods such as a rapid flow injection catalytic cold vapour atomic absorption spectrometric (FI-CCV-AAS) method and liquid chromatography coupled to on-line UV irradiation and cold vapour atomic fluorescence spectroscopy (LC-UV-CV-AFS) are also currently being used to accurately determine concentrations of Hg species (Zhang & Adeloju, 2012; Zmozinski *et al.*, 2014). No standardised method is yet specified for monitoring of Hg species concentrations in fish and seafood, however, certified reference materials (CRM) with known total Hg and MeHg concentrations are available to check validity of whichever speciation method used.

In this study, inductively coupled plasma mass spectrometry (ICP-MS) was used for the assessment of total mercury and other metals. The ICP-MS system by itself is sufficient for total metal analyses, but could also be used for metal speciation analyses through a simple coupling with a high pressure liquid chromatography (HPLC) separation apparatus (Caruso & Montes-Bayon, 2003). This study proposes to use HPLC-ICP-MS for the assessment of individual toxic and non-toxic Hg species in *Mustelus mustelus* (smoothhound) samples.

*Mustelus mustelus* is a commercial shark species commonly caught off the Southern African coastline and has been targeted in South Africa since the late 1980's (Smale & Compagno, 1997; Bosch *et al.*, 2013). It is a major export species predominantly to Australia where it is commonly used in the 'fish and chips' and minced fish products trade (Da Silva & Bürgener, 2007). Due to the long life expectancy of sharks (Smale & Compagno, 1997) they can potentially accumulate high levels of heavy metals. Therefore, the high commercial importance and longevity of *M. mustelus* make it a model species for Hg and MeHg assessment and monitoring in South Africa.

The aim of the present study was to: determine the levels of MeHg, ethylmercury (EthHg), inorganic mercury (iHg) and total mercury (tHg); assess the relationship between tHg and MeHg; and evaluate the concentrations and interactions of mercury and 15 other common heavy metals from a single population of *M. mustelus* caught in the Langebaan lagoon on the South African West Coast.

## 3.2 Materials and methods

### 3.2.1 Sampling

*Mustelus mustelus* were caught by rod and line in the Langebaan lagoon, Western Cape, South Africa (DAFF ethics clearance number: 2009V17CA). The overall catch consisted of males and females from juvenile to fully grown individuals (minimum total length = 570 mm, maximum total length = 1650 mm). A representative subsample of 30 sharks was randomly selected from the total catch (n = 63) for total heavy metal analysis and culled by percussion stunning and bleeding on board the vessel.

Live weight and length were recorded after which the heads of the sharks were cut off behind the last gill slit and the tails were removed at the precaudal pit. Carcasses were filleted and belly flaps removed. One muscle sample per shark was cut from the cephalic region (up to the first dorsal fin) of the dorsal right muscle. This entire meat sample was homogenised and a 10 g sample taken for analysis. Homogenised tissue was stored in vacuum sealed polyethylene bags at -20 °C. Total metal concentrations of 16 metals were measured in all 30 samples while a subsample of 18 was used for Hg speciation (MeHg, EthHg and iHg).

### 3.2.2 Analytical

#### *3.2.2.1. Total mercury*

ICP-MS was used to measure the concentrations of 16 metals (aluminium (Al), manganese (Mn), cobalt (Co), nickel (Ni), molybdenum (Mo), tin (Sn), iron (Fe), copper (Cu), chromium (Cr), zinc (Zn), selenium (Se), arsenic (As), antimony (Sb), cadmium (Cd), mercury (Hg) and lead (Pb)) in *M. mustelus* muscle. Approximately 0.3 g of the homogenised meat samples (n = 30) were digested in 2 ml HCl and 8 ml $HNO_3$ (Merck Suprapur® acids) in a Mars 240/50 microwave digester (produced by CEM) at 160 °C and for 20 min. After cooling, the solutions were diluted to 50 ml with deionised water in sample bottles cleaned with 5% $HNO_3$. The digested samples

were then analysed on an Agilent 7700 ICP-MS, with Hg measured in no-gas mode using the unique HMI function of the instrument, which provides robust conditions and online dilution with argon gas. The instrument was tuned to optimise sensitivity and minimise oxides to < 1% and calibrated with NIST-traceable standards (Inorganic Ventures, 300 Technology Drive, Christiansburg, VA 24073, USA), with quality control checks performed to verify accuracy of results. Results are given as $mg \cdot kg^{-1}$ meat sample. The method detection limits for all individual metals are as in Table 3.1.

**Table 3.1** Lowest detection limits for individual metals as measured on ICP-MS.

| Metal | Lowest detection limit ($mg \cdot kg^{-1}$) |
|---|---|
| Al | 0.555 |
| Cr | 0.030 |
| Mn | 0.020 |
| Fe | 0.068 |
| Co | 0.001 |
| Ni | 0.018 |
| Cu | 0.006 |
| Zn | 0.050 |
| As | 0.006 |
| Se | 0.242 |
| Mo | 0.006 |
| Cd | 0.003 |
| Sn | 0.006 |
| Sb | 0.003 |
| Hg | 0.003 |
| Pb | 0.002 |

#### *3.2.2.2. Mercury speciation*

Two certified reference materials (CRMs) from the Institute for Reference Materials and Measurements (IRMM) in Belgium were used as standards for validation of the Hg speciation method. These CRMs (BCR®-463 and ERM®-CE464) are both made from tuna muscle with known tHg and MeHg concentrations of 2.85 ± 0.16 $\mu g \cdot g^{-1}$ and 3.04 ± 0.16 $\mu g \cdot g^{-1}$, respectively, for BCR®-463, and 5.24 ± 0.10 $\mu g \cdot g^{-1}$ and 5.50 ± 0.17 $\mu g \cdot g^{-1}$, respectively, for ERM®-CE464.

##### *3.2.2.2.1. Instrumentation and equipment*

An Agilent 7700 ICP-MS connected to an 1100 HPLC was used to measure iHg, MeHg and EthHg. The instrument was tuned to optimise sensitivity and minimise oxides to < 1%, with Hg analysed in no-gas mode. The Hg species were separated on a C-18 column (2.1 x 50 mm) with particle size of 5 µm (ZORBAX Eclipse XDB) and mobile phase of L-cysteine solution (0.1% $w \cdot v^{-1}$ L-cysteine + 0.1% $w \cdot v^{-1}$ L–cysteine·HCl·$H_2O$) + 2% methanol at 1 $ml \cdot min^{-1}$. Calibration standards of the individual species (iHg, MeHg and EthHg) were run at

the beginning of the analysis, with control standards every 8–10 samples. The MassHunter workstation software was used for the setup and control of the coupled HPLC–ICP-MS system. All glassware used for sample preparation was soaked in 15% $HNO_3$ for 24 h and rinsed with deionised water before every use to avoid sample contamination.

#### *3.2.2.2.2. Sample preparation*

The extraction process used is based on a study by Hight and Cheng (2006), with a subsample of 18 samples prepared for mercury speciation. Of the homogenised tissue, 0.5 g was extracted with L-cysteine hydrochloride monohydrate (L-cysteine·HCl·$H_2O$) solution in a waterbath at 60 °C for two hours. The extraction was filtered through a syringe filter (0.2 µm with a 0.8 µm prefilter, Acrodisc) and one to two millilitres of the filtrate collected in a glass autosampler vial and kept in the dark to be analysed immediately after extraction. The same procedure, measuring 0.52 g BCR®-463 and 0.28 g ERM®-CE464 lyophilised material, was used to prepare CRMs for measurement of total Hg and MeHg. Both BCR®-463 and ERM®-CE464 extracts were diluted to a 10x dilution before analysis to avoid carry-over from high concentrations.

#### *3.2.2.2.3. Analyses*

The HPLC–ICP-MS system was calibrated with inorganic Hg (iHg), MeHg and EthHg standards at five different concentrations from 1 µg·$L^{-1}$ to 20 µg·$L^{-1}$ to set up calibration curves for the measurement of Hg species in meat samples. The chromatographic peaks for iHg, MeHg and EthHg were eluted as follows: iHg at 95 s, MeHg at 155 s and EthHg at 340 s (Fig. 3.1) with detection limits at 0.03 µg·$L^{-1}$, 0.03 µg·$L^{-1}$ and 0.05 µg·$L^{-1}$ for iHg, MeHg and EthHg respectively. Detection limits were reported as 2x background equivalent concentration, calculated by the Agilent ICP-MS Mass Hunter data processing software. All results were read as µg·$L^{-1}$ and converted to mg·$kg^{-1}$ wet weight for meat samples and mg·$kg^{-1}$ dry mass for CRMs.

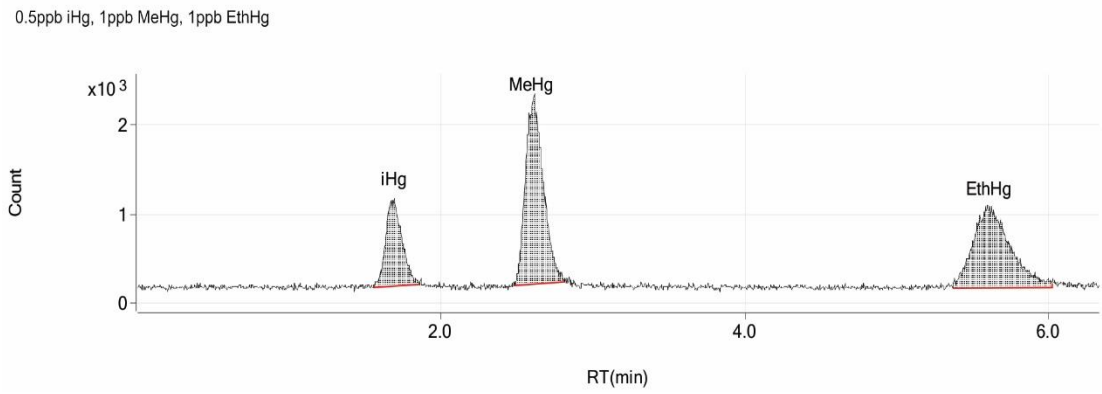


**Figure 3.1** Chromatographic peaks showing retention times for individual Hg species.

To test whether speciation results were accurate and true and whether contamination could occur during sample preparation and analysis, the following trials were run. Firstly duplicate samples from CRMs and all reagents and blanks used (including water, L-cysteine·HCl·H2O solution and 20% methanol solution used for dilutions), were analysed for total Hg on the ICP-MS to ensure all reagents were Hg free. The tHg

values for BCR®-463 measured by ICP-MS were within 0.001% of the certified values. Total Hg levels in water and methanol solution samples used as solvents and dilutants were below 0.01 $mg \cdot kg^{-1}$ and therefore considered clean. Average total Hg levels of 0.033 $mg \cdot kg^{-1}$ were detected in L-cysteine·HCl·H2O solution. To minimise quantitation errors from minor Hg contamination, standards and extracts were prepared with the same batch of L-cysteine·HCl·H2O solution.

Four sample blanks were prepared and run on the HPLC–ICP-MS. No measurable Hg species were detected in any of the blanks which indicated no Hg contamination from glassware or other sources during the extraction process. Additionally, the efficiency of the extraction method and quantity of Hg lost (if any) during the process were measured. Again two reference material samples (BCR®-463 and ERM®-CE464) were prepared and measured on the HPLC–ICP-MS to compare the speciation results with the CRM certified values. MeHg and Hg measurements were within 10% and 1% of the certified MeHg and Hg values, respectively. One sample of each CRM was further run with each sample batch and samples were reanalysed if MeHg measurements for CRMs were not within 10% of the certified values.

Two different meat samples fortified separately with 10 $\mu g \cdot L^{-1}$ iHg and 10 $\mu g \cdot L^{-1}$ MeHg prior to extraction were used to measure the recovery percentage after Hg extraction (Table 3.2). The average recovery percentage of iHg (99 ± 0.7) was higher than for MeHg (80 ± 1.4). For all samples analysed, the total of the individual Hg species measured (iHg, MeHg and EthHg where detected) by speciation, when compared to the total Hg concentrations measured with ICP-MS, gave an average recovery of 96%, indicating that all Hg species present were extracted and detected by HPLC–ICP-MS.

**Table 3.2** Recovery of iHg and MeHg in fish muscle samples with HPLC-ICP-MS speciation.

| Fortification added ($\mu g \cdot L^{-1}$) | | Unfortified sample ($\mu g \cdot L^{-1}$) | | Fortified sample ($\mu g \cdot L^{-1}$) | | Recovery (%) | |
|---|---|---|---|---|---|---|---|
| iHg | MeHg | iHg | MeHg | iHg | MeHg | iHg | MeHg |
| 10 | - | 0.209 | 6.049 | 10.039 | - | 98 | - |
| - | 10 | 0.209 | 6.049 | - | 12.622 | | 79 |
| 10 | - | 0.243 | 6.545 | 10.243 | - | 99 | - |
| - | 10 | 0.243 | 6.545 | - | 13.139 | - | 81 |

One meat sample was prepared and analysed in 5 replicates to measure consistency of the results and repeatability of the method. All replicates had MeHg measurements within 10% of the average value for the 5 replicates with a standard deviation of 0.023 $mg \cdot kg^{-1}$. Duplicate samples run at the beginning and at the end of the batch had similar values within 10% difference from each other showing consistency of measurements over time. Measurements were therefore accurate within 10% error.

### 3.2.3 Statistics

Data were statistically analysed using STATISTICA 12.5. Because normality of data was rejected, Spearman's correlations were conducted to assess the relationships between the 16 heavy metals; total mercury and the

three individual mercury species; as well as the relationship between body parameters (total length and weight) and the heavy metals (n = 16) and mercury species. The correlation coefficient (r) was calculated together with p-values to determine the significance and strength of each correlation. The significance level was set at 0.05.

## 3.3 Results and discussion

### 3.3.1 Mercury and mercury species

The average tHg concentration measured (n = 30) was 0.96 mg·kg$^{-1}$ (± 0.69) and 11 of the 30 sharks analysed had tHg concentrations above the maximum allowable limit (1.0 mg·kg$^{-1}$) for tHg in shark (FAO, 2003; DOH, 2004). Subsamples taken from commercial fish batches for routine analyses consist of individual fish or portions of fish (EC No 333/2007). However, subsampling may not lead to a true representation of the mercury level of the whole batch as was observed in the present study, where only 36.7% of the sharks had levels above the maximum limit, but the average tHg concentration of the sampled sharks is almost equal to the maximum allowable limit (MAL). However, the whole batch is declared as not suitable for human consumption if the average tHg concentration of the subsample contains tHg levels above the legal maximum limit (EC No 333/2007). Consequently wastage and loss of revenue can occur due to unrepresentative subsampling and more stringent sampling protocols are therefore required. In addition, although total mercury can give an indication of the toxicity of a fish, not all mercury species are toxic (Hempel *et al*., 1995) and therefore the total mercury concentration may ill-represent total fish toxicity. The separation and quantification of toxic mercury species such as MeHg may give a more representative indication of fish toxicity.

The Food and Agriculture Organisation of the United Nations (FAO, 2003) provides legislation for MALs in edible part of fishery products as follows: 1.0 mg·kg$^{-1}$ for mean total mercury content in predatory fish. This same limit is applied by the Foodstuffs Cosmetics and Disinfectant Act, 1972 (Act 54 of 1972) for the average MeHg content of predatory fish (DOH, 2004). When comparing MeHg measurements with this limit, only 3 out of 18 sharks (16.7%) are considered above the maximum allowable limit compared to the 36.7% when using tHg measurements to test compliance with the maximum allowable limit. It is therefore important to determine the MeHg concentration of fish samples in order to avoid their inaccurate classification as unsuitable for human consumption and the consequent wastage of discarded stock.

A provisional tolerable weekly intake (PTWI) for MeHg of 1.6 lg/kg body weight has been recommended (JECFA, 2002). When calculated for an average adult (70 kg body weight), none of the 18 sharks analysed had MeHg concentrations over 30% of the PTWI (mean = 9.5%; max = 28.1%; min = 1.0%) per 150 g portion. A single portion of *M. mustelus* meat per week is therefore well within regulatory guidelines with regards to mercury consumption as long as other meals consumed are also within safe limits.

Although other toxic mercury species identified (EthHg) were present, their concentrations were very low, whereas MeHg accounted for the majority (average 81.9%) of the total mercury present (Fig. 3.2). Similar results were found for numerous other fish species (Branco *et al.*, 2007; Campbell *et al.*, 2010; Spry & Wiener, 1991; Storelli *et al*., 2002), indicating that this is a common phenomenon in fish tissue. Methylmercury's rapid absorption into fish tissue and its persistence within the muscle may be due to its high permeability across cell membranes and its affinity to sulfhydryl groups of amino acids (Storelli *et al.*, 2002). These results suggest that although MeHg is the largest component of tHg in *M. mustelus*, the levels present do not pose a significant health risk when consuming not more than 3 portions (150 g each) per week.

The low levels of iHg (min: 0.00 $mg \cdot kg^{-1}$, max: 0.211 $mg \cdot kg^{-1}$) present in the muscle are indicative of the low uptake and rapid elimination rate (Spry & Wiener, 1991) resulting in only residual concentrations retained within the muscle. The negligible amounts of EthHg detected (min: 0.00 $mg \cdot kg^{-1}$, max: 0.0003 $mg \cdot kg^{-1}$) may be due to de-alkylation and conversion to iHg in the presence of heat during the extraction phase (Hight & Cheng, 2006). However, given the small quantities of iHg detected, such conversion is not likely in the current study. It is therefore suggested that the EthHg measured is the true EthHg concentration present in the shark muscle and confirms that EthHg is not accumulated in fish muscle to any great extent and does therefore not contribute to Hg toxicity of *M. mustelus* meat.

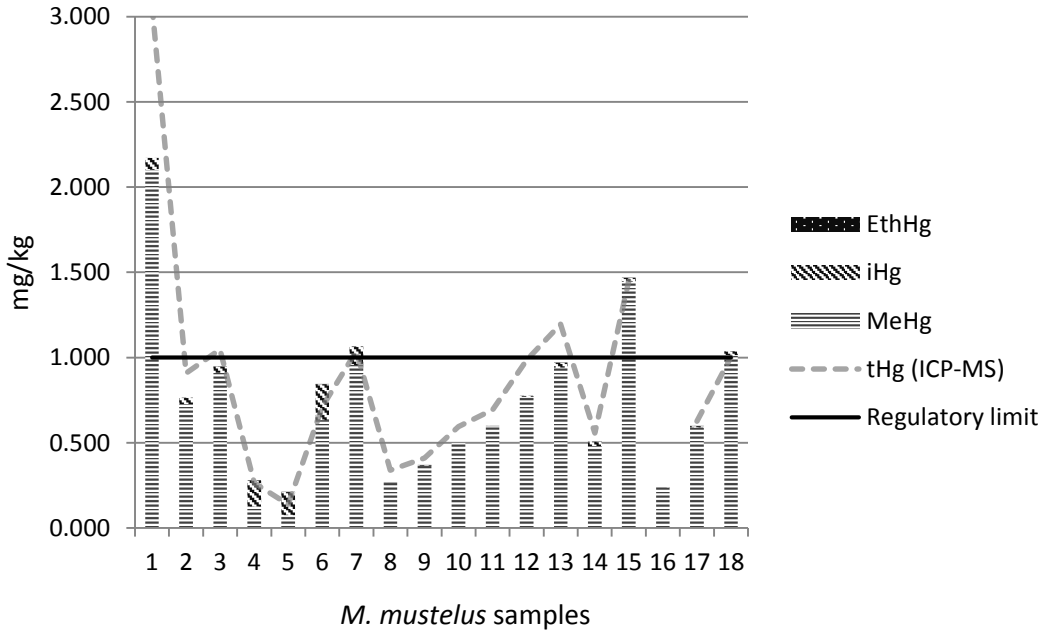


**Figure 3.2** Individual Hg species (EthHg, iHg, MeHg) proportions of tHg concentration per sample. *No tHg was obtained for sample nr 16.

Less is known about the accumulation and transport of EthHg in the fish and human body than of MeHg, but it is assumed that the EthHg pathway is similar to that explained for MeHg binding to low molecular weight thiol complexes (Clarkson *et al.*, 2007). Nonetheless, EthHg does not seem to be significantly absorbed and

accumulated in fish tissue (Park *et al.*, 2011), which may be due to limited presence of EthHg in the environment.

### 3.3.2 Total metals

An overview of the total metal concentrations within a fish population can give an indication of environmental contamination and potential effects on consumer health. The maximum allowable regulatory limits for metals can vary between metals and fish species (Table 3.3) (FAO, 2003), and are predominantly determined by the species' position in the food chain, life span and frequency of consumption. The concentration of the 16 heavy metals measured varied between and among each of the heavy metals. All results are presented in Table 3.4. In decreasing order, As, Zn, Fe and Al were the most predominant heavy metals detected in *M. mustelus* (Table 3.3). However, it is worth noting that toxicity varies between metals and those of high concentrations are not necessarily most toxic (FAO, 2003; DOH, 2004). A number of heavy metals (Cd, Pb, Hg, As) present in minor or trace quantities can have deleterious effects when consumed even in small quantities (FAO, 2003; DOH, 2004; EC, 2008). Arsenic was the only metal with average concentrations (As: 28.31 $mg \cdot kg^{-1}$; n = 30) above the maximum allowable limit of 3 $mg \cdot kg^{-1}$ (DOH, 2004) with all 30 sharks containing toxic levels of As. Concentrations for As varied considerably between the *M. mustelus* samples (As: 7.81–92.32 $mg \cdot kg^{-1}$) which demonstrates the difficulty in extrapolating results to whole populations or a specific region. Similar results have been recorded where As concentrations ranged from 0.4 to 118 $mg \cdot kg^{-1}$ in various marine fish (De Gieter *et al*., 2002; WHO, 2011), however, reasons for this variation within one species are still unclear.

Fish diet is the primary source of As accumulation (De Gieter *et al*., 2002). In contrast to Hg, however, As is not biomagnified up the food chain. It has been found that fish species feeding on benthic organisms and smaller fish have higher levels of accumulated As than those feeding on larger fish (De Gieter *et al*., 2002). This could explain the toxic levels of As in all *M. mustelus* sharks sampled as these are primary benthic feeders (Smale & Compagno, 1997).

Fish is one of the major sources of human dietary exposure to As (WHO, 2011). Similarly to Hg, the toxicity of As is dependent on the presence and concentration of specific As species (De Gieter *et al*., 2002). In terms of human consumption, inorganic As is considered the most toxic form which can accumulate in the skin, bone, liver, kidney and muscle; whereas elemental and organic As, which is the most abundant form (up to 95%) in fish meat and other foods (De Gieter *et al*., 2002; WHO, 2011), are completely eliminated by the kidneys soon after ingestion (WHO, 2011) and are therefore not considered toxic. These measurements of total As are therefore not truly representative of the toxicity of the samples and future research on As speciation is necessary to determine a more accurate measure of toxicity (De Gieter *et al*., 2002).

**Table 3.3** Summary of the average and standard deviation levels of 16 essential, nontoxic and toxic metals in *M. mustelus* fillets ($mg \cdot kg^{-1}$ wet weight). Regulatory maximum limits are included for comparison. Numbers denote species specific maximum limits.

| Metal | Average ± std. dev. (n = 30) | Maximum value | Regulatory maximum limit | Regulatory limit reference |
|---|---|---|---|---|
| Essential | | | | |
| Al | 1.34 ± 0.519 | 2.86 | | |
| Mn | 0.09 ± 0.030 | 0.20 | | |
| Co | 0.003 ± 0.002 | 0.01 | | |
| Ni | 0.28 ± 0.449 | 2.29 | | |
| Mo | 0.04 ± 0.058 | 0.31 | | |
| Essential but toxic in excess amounts | | | | |
| Sn | 0.10 ± 0.058 | 0.28 | 50 $mg \cdot kg^{-1}$ [1] | DOH, 2004 |
| Fe | 3.54 ± 1.542 | 9.07 | - | - |
| Cu | 0.31 ± 0.082 | 0.53 | 5 $mg \cdot day^{-1}$ | SCF, 2006 |
| Cr | 0.09 ± 0.221 | 1.25 | 0.25 $mg \cdot day^{-1}$ | SCF, 2006 |
| Zn | 4.38 ± 0.409 | 5.23 | 25 $mg \cdot day^{-1}$ | SCF, 2006 |
| Se | 0.70 ± 0.444 | 1.56 | 0.3 $mg \cdot day^{-1}$ | SCF, 2006 |
| Toxic | | | | |
| As | 28.31 ± 18.790 | 92.32 | 3.00 $mg \cdot kg^{-1}$ | DOH, 2004 |
| Sb | 0.02 ± 0.075 | 0.41 | 0.15 $mg \cdot kg^{-1}$ | DOH, 2004 |
| Cd | 0.04 ± 0.023 | 0.09 | 0.05 $mg \cdot kg^{-1}$ [2] | FAO, 2003; DOH, 2004; EC No 629/2008 |
| Hg | 0.96 ± 0.692 | 3.78 | 1 $mg \cdot kg^{-1}$ [3] | DOH, 2004 |
| | | | 0.5 $mg \cdot kg^{-1}$ [3] | FAO, 2003; DOH, 2004 |
| Pb | 0.04 ± 0.056 | 0.32 | 0.2 $mg \cdot kg^{-1}$ [4] | FAO, 2003 |
| | | | 0.5 $mg \cdot kg^{-1}$ | DOH, 2004 |
| | | | 0.3 $mg \cdot kg^{-1}$ | EC No 629/2008 |

[1] for all uncanned meat and meat products

[2] 0.1 $mg \cdot kg^{-1}$ for the following species: bonito (*Sarda sarda*), wedge sole (*Dicologoglossa cuneata*), eel (*Anguilla Anguilla*), European anchovy (*Engraulis encrasicholus*), louvar/luvar (*Luvarus imperialis*), horse mackerel or scad (*Trachurus trachurus*), grey mullet (*Mugil labrosus labrosus*), common two-banded seabream (*Diplodus vulgaris*), European pilchard or sardine (*Sardina pilchardus*), mackerel (*Scomber* species), sardinops (*Sardinops* species), tuna (*Thunnus* species, *Euthynnus* species, *Katsuwonus pelamis*).

0.2 $mg \cdot kg^{-1}$ for bullet tuna (*Auxis* species)

0.3 $mg \cdot kg^{-1}$ for anchovy (*Engraulis* species) and swordfish (*Xiphias gladius*)

[3] as total mercury (FAO, 2003) and as methylmercury (DOH, 2004). 1 $mg \cdot kg^{-1}$ for predatory fish including swordfish, shark, tuna

[4] 0.4 $mg \cdot kg^{-1}$ for the following species: Wedge sole (*Docologoglossa cuneata*), eel (*Anguilla Anguilla*), spotted seabass (*Dicentrarchus punctatus*), horse mackerel or scad (*Trachurus trachurus*), grey mullet (*Mugil labrosus labrosus*), common two-banded seabream (*Diplodus vulgaris*), grunt (*Pomadasys benneti*), European pilchard or sardine (*Sardina pilchardus*).

**Table 3.4** Concentrations in mg·kg$^{-1}$ for 16 metals and 3 individual Hg species.

| sample nr | Al | Cr | Mn | Fe | Co | Ni | Cu | Zn | As | Se | Mo | Cd | Sn | Sb | Pb | Hg | iHg | MeHg | EthHg |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 1.505 | 0.042 | 0.101 | 4.045 | 0.007 | 2.288 | 0.409 | 4.528 | 26.305 | 0.574 | 0.308 | 0.008 | 0.284 | 0.025 | 0.025 | 1.307 | | | |
| 4 | 1.028 | 0.042 | 0.082 | 3.333 | 0.003 | 1.106 | 0.375 | 4.638 | 12.023 | 0.228 | 0.111 | 0.013 | 0.152 | 0.007 | 0.022 | 0.965 | | | |
| 5 | 1.296 | 0.052 | 0.070 | 2.655 | 0.003 | 0.465 | 0.347 | 4.460 | 16.327 | 0.292 | <0.01 | 0.007 | <0.06 | <0.002 | 0.025 | 3.778 | 0.069 | 2.101 | 0.000 |
| 6 | 1.007 | 0.036 | 0.078 | 3.697 | 0.002 | 0.206 | 0.420 | 4.717 | 54.488 | 0.575 | 0.082 | 0.004 | 0.152 | 0.007 | 0.023 | 1.037 | 0.037 | 0.727 | 0.000 |
| 9 | 0.964 | 0.118 | 0.102 | 3.548 | 0.003 | 0.441 | 0.352 | 4.475 | 19.134 | 0.426 | 0.065 | 0.015 | 0.131 | 0.005 | 0.042 | 1.204 | 0.035 | 0.912 | 0.000 |
| 16 | 2.432 | 0.134 | 0.107 | 3.376 | 0.003 | 0.429 | 0.481 | 4.595 | 11.948 | 0.251 | 0.055 | 0.059 | 0.117 | 0.006 | 0.050 | 0.307 | 0.153 | 0.127 | 0.000 |
| 17 | 2.259 | 1.247 | 0.202 | 9.065 | 0.012 | 0.780 | 0.526 | 4.722 | 8.922 | 0.492 | 0.076 | 0.088 | 0.115 | 0.008 | 0.319 | 0.168 | 0.135 | 0.075 | 0.000 |
| 19 | 1.190 | 0.054 | 0.089 | 2.685 | <0.002 | 0.044 | 0.328 | 4.797 | 20.591 | 0.676 | 0.054 | 0.057 | 0.108 | 0.004 | 0.033 | 0.396 | | | |
| 20 | 0.840 | 0.034 | 0.059 | 3.535 | 0.002 | 0.408 | 0.325 | 4.915 | 7.811 | 0.264 | 0.036 | 0.038 | 0.093 | 0.004 | 0.025 | 0.569 | | | |
| 22 | 1.250 | 0.018 | 0.114 | 3.066 | 0.002 | 0.241 | 0.313 | 5.231 | 32.068 | 1.385 | 0.028 | 0.078 | 0.098 | 0.005 | 0.022 | 0.440 | | | |
| 23 | 1.492 | 0.027 | 0.077 | 4.050 | 0.005 | 0.078 | 0.295 | 3.411 | 22.369 | 1.423 | 0.011 | 0.053 | 0.093 | 0.410 | 0.022 | 0.852 | 0.211 | 0.632 | 0.000 |
| 31 | 1.753 | 0.022 | 0.085 | 2.918 | 0.003 | 0.055 | 0.191 | 4.028 | 39.503 | 1.016 | 0.016 | 0.070 | 0.073 | 0.007 | 0.037 | 1.261 | 0.108 | 0.956 | 0.000 |
| 36 | 1.073 | 0.013 | 0.063 | 2.265 | 0.002 | 0.065 | 0.237 | 3.997 | 15.192 | 0.372 | 0.011 | 0.050 | 0.059 | 0.071 | 0.022 | 0.388 | 0.000 | 0.267 | 0.000 |
| 38 | 1.367 | 0.030 | 0.066 | 2.244 | 0.002 | 0.091 | 0.224 | 3.996 | 30.039 | 0.197 | <0.01 | 0.045 | 0.059 | 0.003 | 0.047 | 0.638 | | | |
| 42 | 1.839 | 0.017 | 0.106 | 3.873 | 0.002 | 0.169 | 0.299 | 3.527 | 20.988 | 0.292 | <0.01 | 0.075 | 0.280 | 0.006 | 0.043 | 0.512 | 0.004 | 0.365 | 0.000 |
| 43 | 1.253 | 0.029 | 0.068 | 2.286 | 0.002 | 0.063 | 0.228 | 4.321 | 40.371 | 0.286 | <0.01 | 0.044 | 0.055 | 0.004 | 0.022 | 0.714 | 0.000 | 0.499 | 0.000 |
| 45 | 0.714 | 0.031 | 0.075 | 2.477 | 0.004 | 0.135 | 0.221 | 4.275 | 12.064 | 0.362 | <0.01 | 0.062 | 0.076 | 0.003 | 0.065 | 0.844 | | | |
| 46 | 1.543 | 0.031 | 0.068 | 2.346 | 0.003 | 0.160 | 0.273 | 4.583 | 12.553 | 0.274 | <0.01 | 0.046 | 0.064 | 0.003 | 0.023 | 0.833 | 0.000 | 0.597 | 0.000 |
| 47 | 0.866 | 0.028 | 0.063 | 3.030 | 0.002 | 0.077 | 0.267 | 4.289 | 47.645 | 1.515 | <0.01 | 0.040 | 0.081 | 0.003 | 0.020 | 1.000 | | | |
| 48 | 2.104 | 0.044 | 0.077 | 4.091 | 0.003 | 0.103 | 0.326 | 4.601 | 60.785 | 1.270 | <0.01 | 0.030 | 0.093 | 0.035 | 0.106 | 1.229 | 0.006 | 0.771 | 0.000 |
| 49 | 1.398 | 0.083 | 0.102 | 4.376 | 0.002 | 0.120 | 0.396 | 4.709 | 26.490 | 1.557 | <0.01 | 0.027 | 0.055 | 0.044 | 0.024 | 1.416 | 0.029 | 0.941 | 0.000 |
| 50 | 0.945 | 0.044 | 0.086 | 4.484 | <0.002 | 0.058 | 0.354 | 4.121 | 24.880 | 1.017 | <0.01 | 0.027 | <0.06 | 0.003 | 0.017 | 2.116 | | | |
| 53 | 0.758 | 0.025 | 0.090 | 2.323 | 0.002 | 0.075 | 0.250 | 4.397 | 46.712 | 0.677 | <0.01 | 0.020 | <0.06 | <0.002 | 0.026 | 0.682 | 0.024 | 0.481 | 0.000 |
| 54 | 0.952 | 0.041 | 0.091 | 2.614 | <0.002 | 0.063 | 0.227 | 4.451 | 43.429 | 1.240 | <0.01 | 0.020 | <0.06 | 0.008 | 0.019 | 0.675 | | | |
| 56 | 0.885 | 0.022 | 0.086 | 3.415 | <0.002 | 0.128 | 0.281 | 3.768 | 92.323 | 1.128 | <0.01 | 0.027 | <0.06 | 0.003 | 0.033 | 1.006 | | | |
| 58 | 1.064 | 0.072 | 0.084 | 3.626 | 0.002 | 0.097 | 0.255 | 4.284 | 39.944 | 1.109 | <0.01 | 0.027 | <0.06 | <0.002 | 0.021 | 1.752 | 0.023 | 1.444 | 0.000 |
| 60 | 1.035 | 0.055 | 0.091 | 2.875 | 0.001 | 0.156 | 0.295 | 4.413 | 19.756 | 0.739 | <0.01 | 0.017 | <0.06 | <0.002 | 0.019 | 0.424 | | | |
| 62 | 2.825 | 0.069 | 0.170 | 8.222 | <0.002 | 0.080 | 0.369 | 4.929 | 17.558 | 0.462 | 0.014 | 0.040 | <0.06 | <0.002 | 0.036 | 0.361 | 0.000 | 0.237 | 0.000 |
| 63 | 1.546 | 0.021 | 0.084 | 2.529 | <0.002 | 0.043 | 0.217 | 3.903 | 14.096 | 0.396 | <0.01 | 0.022 | <0.06 | 0.006 | 0.021 | 0.742 | 0.008 | 0.589 | 0.000 |
| 64 | 1.055 | 0.077 | 0.074 | 3.029 | 0.003 | 0.054 | 0.235 | 4.443 | 12.898 | 0.410 | 0.015 | 0.019 | <0.06 | 0.003 | 0.020 | 1.205 | 0.022 | 1.014 | 0.000 |
| Av | 1.341 | 0.085 | 0.090 | 3.536 | 0.003 | 0.276 | 0.311 | 4.384 | 28.307 | 0.697 | 0.063 | 0.038 | 0.112 | 0.027 | 0.961 | 0.041 | 0.048 | 0.708 | |

### 3.3.3 Relationships between accumulated heavy metals

Correlations between various heavy metals have previously been identified for a number of fish species (Carvalho *et al.*, 2005; Rahman *et al*., 2012). These accumulation relationships between metals could have negative correlations where metals compete for binding sites, or positive correlations where metals accumulate together and influence one another. It has for example been shown that Hg and Se concentrations in the muscle and/or liver can be positively correlated as Se has a detoxifying effect on Hg (Carvalho *et al*., 2005; Branco *et al*., 2007). Measuring the concentration of either one of the metals could, therefore, give an indication of the concentration of the other corresponding metal. No such correlations ($p > 0.05$) were, however, observed in fish muscle in the present study and metal concentrations are therefore independent of each other and should all be measured individually.

Relationships between metal concentrations and body parameters are common in certain fish species including sharks (Canli & Atli, 2003; Kraepiel *et al.*, 2003; Erasmus *et al.*, 2004; Endo *et al.*, 2008; Campbell *et al*., 2010). In *M. mustelus* in Langebaan lagoon, Mn and Se had weak negative correlations with both fish length (Mn: $r = -0.44$, $p < 0.05$; Se: $r = -0.46$, $p < 0.05$) and weight (Mn: $r = -0.47$, $p < 0.01$; Se: $r = -0.48$, $p < 0.05$), whereas Fe had a weak negative correlation with fish weight ($r = -0.41$, $p < 0.05$). Similar negative correlations between heavy metals (Cr, Cu, Fe, Cd, Ni, As and Pb) and fish size have been observed in other studies (Widianarko *et al.*, 2000; Canli & Atli, 2003; Erasmus e*t al*., 2004) which may be due to size dependent variability in metabolic activity. Metabolic activity decreases with fish growth resulting in higher metal accumulation in younger fish with faster metabolic rates (Canli & Atli, 2003).

Positive correlations between metal concentration and fish size have also been found for some metals (Mn, Zn and Hg) in certain shark species (*Galeocerdo cuvier, Squalus megalops* and *M. mustelus*) (Erasmus *et al*., 2004; Endo *et al*., 2008). Mercury bioaccumulation in fish muscle with size has been well documented (Van den Broek & Tracey, 1981; Boening, 2000; Storelli *et al*., 2002; Kraepiel *et al*., 2003; Campbell *et al*., 2010) where certain Hg species such as MeHg readily binds to thiol groups of proteins whose content increases with fish age (Branco *et al*., 2007) and combined with a slow rate of elimination results in an increase over time and subsequently with fish size (Spry & Wiener, 1991). No such correlations ($p > 0.05$) were however apparent in the current study. The absence of such a relationship may be due to species specific metabolic activities, prey preference and/or local environmental conditions of *M. mustelus* in this study.

Mercury uptake through prey is the major route of Hg absorption into the fish body (Spry & Wiener, 1991). *Mustelus mustelus* broaden their prey niche (19 - 44 prey taxa) and prey size as they grow and develop and gradually shift prey type from crustaceans and polychaetes to cephalopods (Smale & Compagno, 1997). This could therefore contribute to the increased Hg concentrations in larger sharks as their prey constitute a larger variety of higher trophic level species already containing higher Hg concentrations than lower trophic level crustaceans and organisms (Smale & Compagno, 1997).

*Mustelus mustelus* can live up to 25 years giving it ample time to bioaccumulate Hg in its flesh with a slow rate of Hg elimination. The unexpected lack of tHg level correlation ($P > 0.05$) with shark size in this study could possibly be accounted for by the environment and its effect on the prey composition of this specific *M. mustelus* population. Previous studies have shown that *M. Mustelus* in the Langebaan lagoon, contrary to what has been reported for *M. mustelus* in general (Smale & Compagno, 1997), feed mainly on inshore crustaceans throughout their lifespan (Smale & Compagno, 1997) with no significant difference seen in the stomach contents between larger and smaller sharks (Bosch *et al.*, 2013). This could be due to a lack of availability in prey diversity in the Langebaan lagoon. The constant nature of these sharks' diet could therefore be a major cause explaining the lack of correlation between tHg levels and fish size, but further research on environmental conditions and effects on Hg accumulation in this specific population could provide better explanation of this finding.

Other cases, such as the study by Canli & Atli (2003), found no significant correlations between various metals and fish size in muscle tissue of certain species, which is also the case for most metals in *M. mustelus* muscle, confirming that correlations between metals and fish size are metal and species specific. Shark size therefore does not give an indication of most of the individual metal concentrations.

A strong correlation ($r = 0.97$; $p < 0.001$) was observed between MeHg and tHg indicating tHg can give a good indication of the MeHg levels present in *M. mustelus* muscle. However, given that MeHg is the toxic form of Hg, MeHg measurement is still relevant in the absence of a correction formula to accurately predict MeHg concentrations from tHg measurements.

No significant correlations ($p > 0.05$) were observed between tHg and the other two species (iHg and EthHg); between individual Hg species (MeHg, iHg and EthHg); or between Hg species and body parameters (total length and weight). All individual Hg species therefore accumulate at their own rate. Where the concentration readings of individual iHg and EthHg concentrations in *M. mustelus* meat are required, measurement of these individual Hg species must be done, as no other Hg species or parameter would give an indication of their concentrations.

## 3.4 Conclusion

Limited information is available on the accumulation and current levels of several important heavy metals such as Hg and particularly methylmercury (MeHg) in commercial fish meat in South Africa. Methods for measuring MeHg are currently restricted due to the limited availability and high cost of the analytical equipment and methods. This study has confirmed the feasibility of using accurate and effective analytical methods for determining mercury speciation (HPLC-ICP-MS), total mercury and other heavy metals (ICP-MS).

The fact that 36.7% of this subsample of the *M. mustelus* population measured had tHg levels above maximum regulatory limits means that any single *M. mustelus* caught from the Langebaan lagoon is likely to contain toxic levels of Hg not suitable for human consumption. Fishing *M. mustelus* for human consumption

should therefore be limited from this area. As *M. mustelus* in other areas are known to shift their diet to higher trophic level species, it is likely that the bioaccumulation of Hg is even more severe. Further research is required on other *M. mustelus* populations in order to investigate the degree to which Hg is accumulated to toxic levels in *M. mustelus* in South Africa.

In terms of food safety, MeHg is the major Hg species of concern and importance and should therefore be more carefully monitored to determine true toxicity of fish consumed. Alternatively, ways of accurately predicting MeHg levels from tHg concentrations should be investigated as there is a strong positive correlation between these two components which could allow this prediction.

Even though mercury is one of the major heavy metals of concern in seafood, it is not the only heavy metal of concern in this *M. mustelus* population as As has also been found in levels exceeding the maximum guideline levels for human consumption. Further detailed studies on speciation and analysis of this heavy metal are therefore needed in order to more accurately estimate or determine the safety of commercial fish meat in South Africa with regards to total heavy metal contaminants and toxicity.

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# CHAPTER 4:

## Mercury accumulation in yellowfin tuna (*Thunnus albacares*) with regards to muscle type, muscle position and fish size*

**ABSTRACT**

The concentrations and relationships between individual mercury species and total mercury were investigated in different muscle parts and sizes of yellowfin tuna (*Thunnus albacares*). Fourteen yellowfin tuna caught in the South Atlantic off the coast of South Africa had an average total Hg (tHg) concentration of 0.77 mg·kg$^{-1}$ wet weight. No differences were detected ($P > 0.05$) in tHg, methylHg (MeHg) or inorganic Hg (iHg) accumulation among the four white muscle portions across the carcass, but both tHg and iHg were found in higher concentrations ($p < 0.001$) in dark muscle than white muscle. Positive linear correlations with fish weight were found for both tHg ($r = 0.79$, $p < 0.001$) and MeHg ($r = 0.75$, $p < 0.001$) concentrations. A prediction model was formulated to calculate toxic MeHg concentrations from measured tHg concentrations and fish weight ($cMeHg = 0.073 + 1.365 \cdot ctHg - 0.008 \cdot w$). As sampling sites and subsampling methods could affect toxicity measurements, we provide recommendations for sampling guidelines.

**Keywords:** Fish muscle, mercury speciation, HPLC-ICP-MS, Yellowfin tuna, cross-carcass variation, size effects

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## 4.1 Introduction

Fish meat is widely consumed and considered a main source of nutrition in many coastal communities. It contributes to a healthy diet by providing high-value amino acids and nutrients (vitamins and minerals) and is an excellent source of essential omega-3 fatty acids associated with many health benefits (Domingo *et al.*, 2006). Although highly nutritious, high consumption of some fish meat can have significant adverse effects on human health due to the bioaccumulation of heavy metals in fish muscles from the surrounding aquatic environment (Järup, 2003; Castro-González & Méndez-Armenta, 2008).

The accumulation of heavy metals in fish can occur due to metals being naturally present in the aquatic environment, but can also be exacerbated by anthropogenic activities such as industrial activity and pollution (Järup, 2003). However, not all metals are hazardous as some are essential elements in biological systems, only becoming toxic when present at high concentrations (Schroeder & Darrow, 1972; Munos-Olivas & Camara, 2001). Amongst the metals that accumulated in fish and seafood, mercury (Hg) is one of the most abundant toxic metals (Carvalho *et al.*, 2005; Chen *et al.*, 2012). The total Hg (tHg) content of fish can consist of a combination of several Hg species (MethylHg, EthylHg and inorganic Hg) (Morel *et al.*, 1998). The toxicity of these individual Hg species differs; the organic mercury species (MethylHg and EthylHg) are considered toxic and inorganic Hg (iHg) is considered non-toxic as it is not as easily absorbed into living organisms compared to the organic forms and is very slow to cross the blood-brain barrier and therefore does not display toxic effects in fish or humans (Guynup & Safina, 2012). MethylHg (MeHg) is considered the most toxic form and it is also the most abundant Hg species (75 - 100% of tHg) in fish meat (Burger & Gochfeld, 2004). Measuring the various Hg species can therefore improve the determination of true fish toxicity and subsequently inform regulatory bodies on fish safety.

Current FAO legislation (FAO, 2003) has stipulated a maximum tHg limit of 0.5 $mg \cdot kg^{-1}$ for fish and seafood with the exception of predatory fish (shark, tuna and swordfish) which has a limit of 1 $mg \cdot kg^{-1}$. Regulations by the South African Department of Health specify these same limits (0.5 and 1.0 $mg \cdot kg^{-1}$) as for MeHg (DOH, 2004) as the main toxic component of tHg. Mercury levels are monitored according to Commission Regulation (EC) No 333/2007 as enforced by the National Regulator for Compulsory Specifications (NRCS). As it is normally assumed that almost 100% of tHg is present as MeHg, commercial fish samples are only tested for tHg and not specifically for MeHg. The actual levels of toxic Hg in commercial fish remain unknown. Routine monitoring of MeHg concentrations in addition to the current tHg analysis procedure would require Hg speciation and thus additional analyses with associated equipment and costs. An accurate model to predict MeHg content from tHg measurements would therefore greatly benefit the fishing industry.

The Commission Regulation (EC) No 333/2007 describes sampling for routine Hg analysis. However, this regulation lacks detail as to which fish sizes and carcass sites need to be sampled. In large pelagic fish species such as tuna, chemical composition of the various muscles and anatomical sections can vary (Balshaw

*et al.*, 2008), as these fish have two very distinct muscle types (dark and white muscle), which have different functions (dark = slow, continuous movement; white = fast, sudden movement) and compositions (Te Kronnié, 2000). Mercury is accumulated in the protein fraction of the muscle as it binds to thiol groups (Harris *et al.*, 2003; Nakao *et al.*, 2007). The presence of such different muscle types with varying protein compositions can therefore result in variation in heavy metal accumulation and concentration across the fish carcass. Balshaw *et al.* (2008) found such variation in Hg levels between different commercial cuts of farmed Southern Bluefin tuna (*Thunnus maccoyii*). Sampling and measuring fish muscle at various carcass positions can therefore shed light on the extent of intra fish variability in heavy metal concentrations and can aid method standardisation for fish subsampling.

A positive relationship between fish size/age and tHg and MeHg concentrations has been identified for numerous fish species investigated (Walker, 1976; Van den Broek & Tracey, 1981; Andersen & Depledge, 1997; Storelli *et al.*, 2002a; Kraepiel *et al.*, 2003; Campbell *et al.*, 2010). Within a single fish population, Hg concentrations can vary widely (Bosch, 2012) from well below the maximum allowable limit in smaller sized fish to levels substantially above the limit in larger, older fish. Detailed, species-specific research is needed to determine the fish weight above which Hg limits are likely to be exceeded, as this limit might depend on metabolism and growth rates specific to each fish species or sub population. This threshold weight could be used to introduce weight specific catch limits to avoid wastage of fish likely to be considered not suitable for consumption and advise more accurate subsampling for routine analyses avoiding biased results from misrepresented fish sizes.

Yellowfin tuna is a commercially important fish with a large size range and is widely consumed due to its high quality meat. Several studies have reported on the total Hg and Hg species content in yellowfin tuna (Menasveta & Siriyong, 1977; Kraepiel *et al.*, 2003; Ruelas-Inzunza *et al.*, 2011, Ferriss & Essington, 2011; Teffer *et al.*, 2014; Schmidt *et al.*, 2015), however limited knowledge still exists on the extent of individual mercury species and the relationships and variations between the accumulation of these individual species and total mercury in different muscle parts and different sizes of yellowfin tuna. Therefore, the overarching aim of this study was to investigate the accumulation of total mercury and individual mercury species (methylmercury, ethylmercury, inorganic mercury) in South African yellowfin tuna (*Thunnus albacares*) meat and determine variation caused by carcass position, muscle type and fish size. A subsample was used to test for correlations between total Hg measurements and Hg speciation results that could be used in prediction models.

## 4.2 Materials and methods

### 4.2.1 Sampling

Fourteen yellowfin tuna fish were caught off the Atlantic coast of South Africa (S34°29' E17°54'and S34°35' E17°58') and ranged in size from 29.0 to 50.8 kg. Six muscle subsamples per fish were used for chemical analysis. Ceramic knives were used for cutting meat samples to minimise sample contamination. Three samples were taken anteriorly in the dorsal (A), mid (B) and ventral (C) axial muscles, located at the start of the first dorsal fin, and three samples were taken at the dorsal (D), mid (E) and ventral (F) axial muscles at the start of the second dorsal fin (Fig. 4.1). The middle samples (B, E) consisted of dark muscle and the dorsal and ventral samples (A, D, C, F) consisted of white muscle. All meat samples were homogenised prior to further analysis.

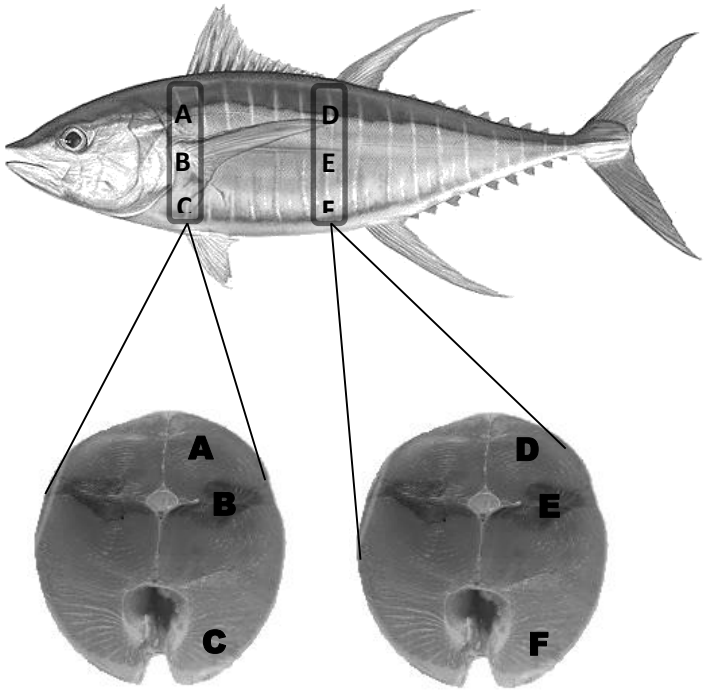


**Figure 4.1** Tranverse section of a tuna carcass indicating the positions of the white (A, C, D and F) and dark (B and E) muscle. Letters A, B, C, D, E and F indicate sampling locations.

### 4.2.2 Analyses

#### *4.2.2.1. Total mercury – ICP-MS*

Total Hg was measured by means of inductively coupled plasma mass spectrometry (ICP-MS). Approximately 0.3 g of the homogenised meat sample and standard (certified reference material: BCR®-463) were used for sample digestion. This was done in 2 ml HCl and 8 ml $HNO_3$ (Merck Suprapur® acids) in a Mars 240/50 microwave digester (produced by CEM) at 160°C and 800 psi for 20 min after which the solution was diluted to 50 ml with deionised water in a sample bottle cleaned with 5% $HNO_3$. The digested samples were then

analysed on an Agilent 7700 ICP-MS, with Hg measured in no-gas mode, under robust conditions and online dilution with argon gas provided by the unique HMI function of the instrument. The instrument was tuned to optimise sensitivity and minimise oxides to < 1%. It was subsequently calibrated using the NIST-traceable standards, with quality control checks performed to verify accuracy of results. A rinse program was set up to ensure efficient wash-out of Hg in the expected concentration range of the samples. Samples with unexpectedly high concentrations are diluted and re-analysed, as well as samples that followed the initial high concentration sample. Results are given as $mg \cdot kg^{-1}$ meat sample with the detection limit of tHg at 0.003 $mg \cdot kg^{-1}$.

#### *4.2.2.2. Mercury speciation – HPLC-ICP-MS*

##### *4.2.2.2.1. Instrumentation, standards and reagents*

An Agilent 7700 ICP-MS connected to an Agilent 1100 HPLC was used to measure inorganic, methyl- and ethylmercury in prepared samples. The system specifications are given in Table 4.1. The MassHunter workstation software was used for the setup and control of the coupled HPLC-ICP-MS system. The instrument was tuned to optimise sensitivity and minimise oxides to < 1%, with Hg analysed in no-gas mode. The Hg species were separated in a mobile phase of 98% L-cysteine solution (0.1% $w \cdot v^{-1}$ L-cysteine + 0.1% $w \cdot v^{-1}$ L-cysteine·HCl·$H_2O$) + 2% methanol at 1 $ml \cdot min^{-1}$. Mercury(II) chloride (ACS reagent, ≥ 99.5%, Sigma-Aldrich), methylmercury(II) chloride (PESTANAL®, analytical standard, Sigma-Aldrich) and ethyl mercuric chloride (Supelco analytical standard, Sigma-Aldrich) were used to prepare stock solutions of 2 000 000 $\mu g \cdot L^{-1}$ iHg, 1 000 000 $\mu g \cdot L^{-1}$ MeHg and 40 000 $\mu g \cdot L^{-1}$ EthHg respectively. Calibration standards for the individual species (iHg, MeHg and EtHg) prepared by appropriate dilution of stock solutions in 0.1% $w \cdot v^{-1}$ L-cysteine hydrochloride monohydrate (L-cysteine·HCl·$H_2O$) solution to concentrations of 1 $\mu g \cdot L^{-1}$ to 20 $\mu g \cdot L^{-1}$ were run at the beginning of each analytical batch, with control standards every 8 – 10 samples. Detection limits for individual species on the ICP-MS were 0.030 $\mu g \cdot L^{-1}$, 0.030 $\mu g \cdot L^{-1}$ and 0.050 $\mu g \cdot L^{-1}$ for iHg, MeHg and EtHg, respectively. Two certified reference materials (CRMs) (BCR®-463 and ERM®-CE464) from the Institute for Reference Materials and Measurements (IRMM) in Belgium were included in every sample batch for accuracy evaluation. Deionised water was used for all solutions and standards. All glassware used was soaked in 15% $HNO_3$ for 24 hours and rinsed with deionised water before every use to avoid sample contamination.

##### *4.2.2.2.2. Sample preparation and Hg speciation*

Using the mercury extraction process for extraction of iHg, MeHg and EthHg components based on the method described by Hight & Cheng (2006), 0.5 g of homogenised tissue was extracted with L-cysteine·HCl·$H_2O$ solution in a water-bath at 60 °C for two hours. The extract was filtered through a syringe filter (0.2 µm with a 0.8 µm prefilter) and one to two millilitres of the filtrate collected in a glass auto-sampler vial and kept in the dark, to be analysed on the same day as soon as possible after extraction. The same procedure was used to prepare and extract the lyophilised CRMs with certified values for total Hg and MeHg.

Per sample, 20 µL was injected manually into the HPLC-ICP-MS on-line system and the injector rinsed with mobile phase solution in-between every injection. No carry-over was detected for any of the Hg species as monitored between single Hg species standards analysed. Samples were reanalysed if individual MeHg measurements for CRMs were more than 10% from the certified values. Average MeHg levels for BCR®-463 (2.97 ± 0.322) and ERM®-CE464 (5.45 ± 0.356) were within specification according to certified values for MeHg (2.83 ± 0.16 mg Hg·kg$^{-1}$ for BCR®-463 and 5.12 ± 0.17 mg Hg·kg$^{-1}$ for ERM®-CE464). The total of the Hg species concentrations determined by speciation had an average recovery of 104% when compared to the total Hg concentrations measured by the ICP-MS method for total metals.

**Table 4.1** HPLC-ICP-MS instrument parameters.

| Agilent 7700 ICP-MS Instrument parameters: | |
|---|---|
| RF power | 1550 W |
| Sampling depth | 8 mm |
| Carrier gas flow | 1.06 L·min$^{-1}$ |
| Make-up gas flow | 0.18 L·min$^{-1}$ |
| Agilent 1100 HPLC parameters: | |
| Column | ZORBAX Eclipse XDB C-18; 2.1 mm id x 50 mm, 5 µm |
| Flow rate | 1 ml·min$^{-1}$ |
| Injection volume | 20 µl |
| Mobile phase | 2% methanol + 98% (0.1% w·v$^{-1}$ L-cysteine + 0.1% w·v$^{-1}$ L-cysteine·HCl·$H_2O$) |

### 4.2.3 Statistics

STATISTICA 12.5 was used for data analysis. Preliminary tests (normality) were conducted and true outliers were removed (n = 1) prior to analysis. All data conformed to the necessary assumptions. A mixed model repeated measures ANOVA was conducted to determine the variation of Hg concentrations between various fish carcass sites. To determine relationships between tHg, and Hg species and fish weight (before evisceration), Spearman correlations were reported in order to compensate for outliers and a simple regression analysis was conducted. A multiple regression analysis was done to investigate a prediction equation for MeHg concentrations. Results were reported at a 95% confidence level.

## 4.3 Results

Overall, tHg values ranged from 0.45 to 1.52 mg·kg$^{-1}$ wet weight with an average concentration of 0.77 mg·kg$^{-1}$ where the average was calculated from six anatomical sites of 14 tuna (n = 84) and 28.6% of the samples analysed were above the maximum allowable limit (1.0 mg·kg$^{-1}$). MeHg values ranged from 0.23 to 1.24 mg·kg$^{-1}$ and iHg was present at much lower values (0.003 mg·kg$^{-1}$ to 0.41 mg·kg$^{-1}$). EtHg values were all below the detection limit (0.005 mg·kg$^{-1}$) of the analytical method and were therefore considered insignificant and are not analysed or discussed further.

### 4.3.1 Cross-carcass Hg (tHg, iHg and MeHg) variation

Both tHg and iHg concentrations varied between sampling sites, where sites B and E (dark muscle sites) had higher ($p < 0.001$) concentrations compared to the rest of the carcass sites (white muscle) (Tables 4.2 and 4.3) with one exception (tHg in site B was statistically similar ($p > 0.05$) to site D). In addition, variability within the dark muscle sites was observed where site E had consistently higher concentrations than site B for both iHg ($p < 0.05$) and tHg ($p < 0.001$).

Limited inter-carcass variation in MeHg concentrations was apparent where the only difference ($P < 0.05$) observed was between the dark muscle portions (site E > site B). Therefore MeHg concentration did not vary significantly ($p > 0.05$) between the muscle types (white vs dark).

Overall it was noted that no variation ($p > 0.05$) in iHg, MeHg or tHg was found within the white muscle portions (A, C, D, F).

**Table 4.2** Average concentration (± standard deviation) of iHg, MeHg and tHg ($mg \cdot kg^{-1}$ wet weight) for each carcass sampling site: A to F (n = 14 tuna). Superscript letters indicate significant ($p < 0.05$) differences between sampling sites for each Hg species.

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| iHg | $0.06^{c} \pm 0.045$ | $0.13^{b} \pm 0.093$ | $0.07^{c} \pm 0.043$ | $0.07^{c} \pm 0.049$ | $0.17^{a} \pm 0.086$ | $0.06^{c} \pm 0.040$ |
| MeHg | $0.64^{ab} \pm 0.192$ | $0.64^{b} \pm 0.226$ | $0.65^{ab} \pm 0.212$ | $0.66^{ab} \pm 0.231$ | $0.67^{a} \pm 0.226$ | $0.66^{ab} \pm 0.197$ |
| tHg | $0.73^{c} \pm 0.213$ | $0.84^{b} \pm 0.249$ | $0.72^{c} \pm 0.229$ | $0.73^{bc} \pm 0.219$ | $0.88^{a} \pm 0.302$ | $0.73^{c} \pm 0.221$ |

**Table 4.3** Comparison of average concentrations (± standard deviation) of iHg, MeHg and tHg ($mg \cdot kg^{-1}$ wet weight) in dark muscle (data from 2 sampling sites per carcass combined) and white muscle (data from 4 sampling sites per carcass combined) (n = 14 tuna).

| | Dark muscle | White muscle | P-value |
|---|---|---|---|
| iHg | 0.16 ± 0.075 | 0.07 ± 0.035 | $P < 0.001$ |
| MeHg | 0.66 ± 0.235 | 0.65 ± 0.209 | $P > 0.05$ |
| tHg | 0.87 ± 0.286 | 0.73 ± 0.216 | $P < 0.001$ |

### 4.3.2 Regression analyses

#### *4.3.2.1. Relationship between fish size and tHg, MeHg and iHg concentrations*

Strong positive linear correlations were found between the average tHg concentration of each fish (six carcass sites) and fish weight (n = 14) ($r = 0.79$, $p < 0.001$) (Fig. 4.2) and similar results were found between the average MeHg concentrations per fish (six carcass sites) and fish weight (n = 14) ($r = 0.75$, $p < 0.001$).

However, iHg was not significantly correlated with weight (n = 14) (r = 0.08, p > 0.05). From these regressions, yellowfin tuna above 70kg fresh weight are likely to exceed the tHg maximum limit (Fig. 4.2).

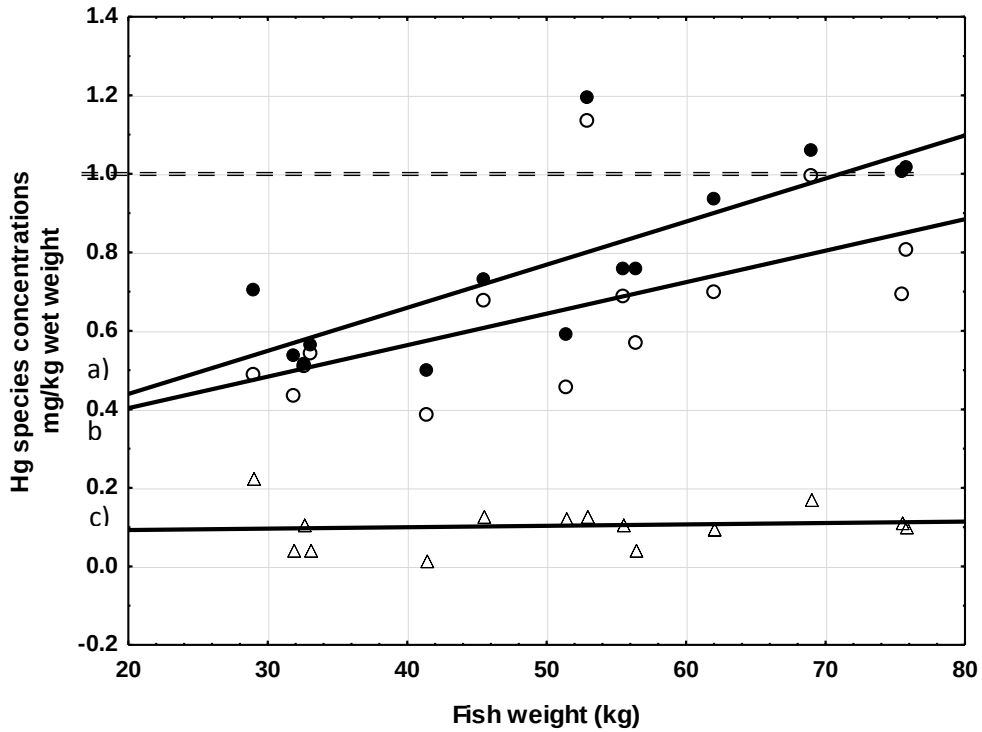


**Figure 4.2** Correlations between fish weight w (n = 14) and average concentrations of a) tHg = 0.22 + 0.01w (●), b) MeHg = 0.24+0.01w (○) and c) iHg = 0.09+0.0004w (Δ); where individual Hg concentrations are given as mg·kg$^{-1}$ wet weight and the fish weight (w) is in kg. The horizontal dotted line indicates the maximum allowable limit of tHg in tuna meat.

#### *4.3.2.2. Relationship between MeHg and tHg concentration*

Methylmercury had a strong positive linear correlation with tHg (r = 0.77, p < 0.001) when all 6 portions from all of the 14 sampled fish were included (n = 84). A simple regression analysis showed that when tHg measurements are used to predict the MeHg content in fish, the results have a root mean square error of calibration (RMSEC) of 0.133 mg·kg$^{-1}$, which is more than 10% error of the maximum allowable limit of 1.0 mg·kg$^{-1}$. This large RMSEC could be caused by the slight variation of both MeHg and iHg concentrations within the dark muscle (Table 4.2) and the iHg variation between dark and white muscle of the tuna (Table 4.3), which can all cause variation within the tHg measurements. Fish weight could also affect the MeHg to tHg relationship, as MeHg is increasingly accumulated with increasing fish weight whereas iHg was shown to be independent of fish weight and the MeHg proportion of tHg would therefore increase with fish weight. Therefore to minimise the effect of muscle type and to incorporate fish weight, the regression was reanalysed using only data from white muscle portions (average per fish) and including both tHg and fish weight as variables in a multiple regression analyses. This resulted in the following prediction model: $cMeHg = 0.073 + 1.365 \cdot ctHg - 0.008 \cdot w$ (Fig. 4.3) where tHg is the measured total mercury concentration (mg·kg$^{-1}$), w is fish weight (kg) and cMeHg is the predicted/calculated concentration for MeHg. A relatively low RMSEC of 0.06 mg·kg$^{-1}$ (r = 0.95) indicates that this is a more accurate prediction of the true MeHg values.

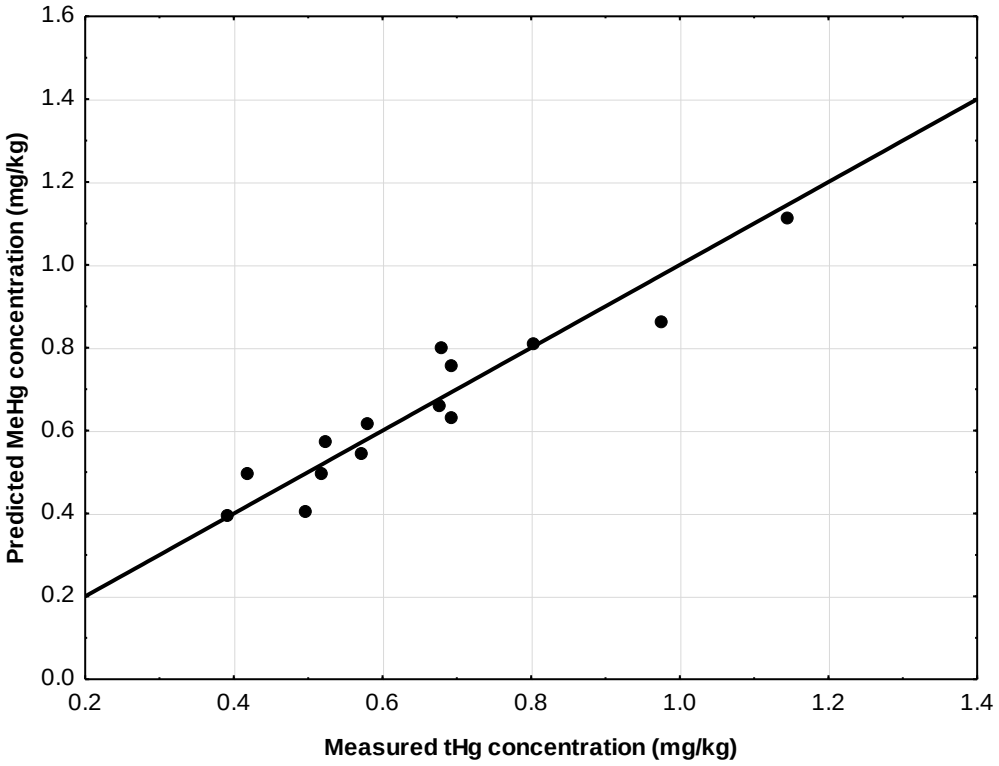


**Figure 4.3** Scatterplot of predicted MeHg against measured tHg concentrations (mg·kg$^{-1}$ wet weight). Regression equation: $\boldsymbol{cMeHg = 0.073 + 1.365 \cdot ctHg - 0.008 \cdot w}$ (r = 0.95).

## 4.4 Discussion

The average tHg value (0.77 mg·kg$^{-1}$) of the subsample is below the maximum allowable limit of 1.0 mg·kg$^{-1}$ (FAO, 2003; DOH, 2004). However, four fish had an average tHg concentration exceeding this limit. Therefore, almost 29% of the tuna fish sampled would be considered unsafe for human consumption. Due to bioaccumulation of Hg up the food chain, higher trophic level fish often have tHg levels close to or exceeding the maximum limit (Peterson *et al.*, 1973; Storelli, Giacominelli-Stuffler & Marcotrigiano, 2002b). Total Hg however includes both toxic and non-toxic species and is therefore not necessarily representative of meat toxicity.

MeHg is the most toxic and most abundant Hg species (Clarkson *et al.*, 2007) often assumed to constitute 100% of the tHg present (Walker, 1976; Spry & Wiener, 1991; Andersen & Depledge, 1997; Storelli *et al.*, 2002a; Campbell *et al.*, 2010). The maximum allowable limit for MeHg (DOH, 2004) is the same as for tHg (1.0 mg·kg$^{-1}$) (FAO, 2003). However, if we consider the measured MeHg values with regards to the maximum limit, we find that only 14% of the tuna fish measured would be considered unsuitable for human consumption (compared to the 29% when tHg is used). Current research on sampling and measuring protocol with regards to specific mercury species (Schmidt *et al.*, 2013) and compliance to maximum allowable limits can therefore add to current knowledge and specifications in order to acquire more accurate measurements and reports of the toxicity of fish meat (Branch, 2001; Van Dael, 2001). Therefore, improved understanding

and knowledge regarding how MeHg accumulates and the MeHg:tHg ratios in fish meat could potentially reduce unnecessary wastage of fish due to the inaccuracy of toxic classification.

### 4.4.1 Cross-carcass Hg variation

The inter and intra muscle type (dark and white) variability in Hg accumulation in yellowfin tuna suggests that potential biases can exist when subsampling fish for measuring toxicity as iHg concentrations are higher in dark muscle than in white muscle whereas MeHg is equally accumulated in both white and dark muscle. Sampling from dark muscle will therefore result in higher tHg readings and Hg toxicity of the fish carcass could therefore be overestimated. Systematic differences in Hg among different muscle types need to be taken into account in the sampling protocol in order to obtain representative and accurate monitoring of Hg toxicity in fish.

Other studies (Ando *et al*., 2008; Balshaw *et al.*, 2008; Lares *et al.*, 2012) have also found inter and intra muscle type variation in Hg concentrations in some tuna species (*Thunnus oriantalis* and *Thunnus macccoyii*). Lares *et al.* (2012) found higher Hg concentrations in the caudal peduncle muscle tissue (CPMT) than in the rest of the body regions as was similarly found in the present study with higher tHg concentrations in the posterior sample (site E) of the dark axial muscle. Both Ando *et al.* (2008) and Lares *et al.* (2012) found lower Hg concentrations in the front of the abdomen (white muscle) compared to the rest of the white muscle regions in the fish body. This variation in Hg concentration could be caused by a dilution effect of the higher fat content of this portion of the carcass (Balshaw *et al*., 2008). No significant differences were, however, found within the white muscle between different body regions in yellowfin tuna in the current study.

Apart from the possible effect of lipid content of muscle, the Hg variation observed within the dark muscle and between dark and white muscle may be due to differences in muscle function and therefore differing muscle fibre development and composition (Shadwick *et al.*, 1999). Te Kronnié (2000) found that in zebrafish (*Danio rerio*) larvae, the white muscles used for fast movement were the first to develop with relatively late maturation of the lateral layer of dark (slow) muscle. Stickland (1983) also found differences in the rates of muscle cell growth and increase between dark and white muscle in rainbow trout (*Salmo gairdneri*). Te Kronnié (2000) also found that muscle activity had an effect on the rate of muscle fibre development. Larger migratory fish such as tuna are known for continuous strong swimming driven by the dark muscle with virtually all of the thrust produced at the tail blade (Shadwick *et al*., 1999). This higher activity in the caudal region could possibly explain a higher rate of dark muscle fibre development in this region of the fish. As Hg is continuously accumulated in fish by binding to protein sites (Menasveta & Siriyong, 1977; Harris *et al.*, 2003; Nakao *et al.*, 2007), it could be expected that Hg accumulation is affected by the rates and regions of muscle development. This relationship between Hg accumulation and muscle development, however, needs further investigation in order to prove such an assumption.

Previous studies have concluded that CPMT of tuna is an appropriate region for subsampling for routine toxicity measurement as it would represent the highest Hg concentration within the carcass (Ando *et al*., 2008; Lares *et al.*, 2012). This conclusion is however based on investigations of tHg concentrations and not individual Hg species. From the Hg speciation results in the current study, it is apparent that the higher tHg concentration in the caudal dark muscle compared to that in the white muscle of the rest of the carcass would be due to higher non-toxic iHg concentrations while toxic dark muscle MeHg concentrations are in fact not different from concentrations in white muscle regions. Therefore sampling from the white muscle regions for tHg measurements would render more representative results of the true Hg toxicity of the entire edible muscle portion. Previous studies found that sampling from the front abdominal white muscle in certain tuna species (*Thunnus orientalis*) could result in under-representation of the Hg content in the rest of the carcass (Ando *et al*., 2008; Lares *et al.*, 2012). It would therefore be suggested to sample from any of the other white muscle portions, even though this is not supported by results from the current study.

### 4.4.2 Relationship between Hg and fish size

The differences in accumulation patterns of individual Hg components (iHg and MeHg) could be explained by their pathways of absorption and accumulation in the fish body. Both iHg and MeHg is readily absorbed by fish from their diet and the surrounding environment, but the majority of iHg is rapidly eliminated from the fish body whereas MeHg is largely absorbed into fish tissue where it binds to thiol groups and is continually accumulated (Spry & Wiener, 1991). Toxic Hg levels therefore increase with increasing fish age and therefore fish size. This finding is supported by Andersen & Depledge (1997) on edible crab muscle, where a positive correlation between MeHg concentration and carapace length was found whereas iHg concentrations were low and independent of crab size.

A positive correlation between tHg and fish size has been found in numerous fish and marine species from various trophic levels, but especially in top predator species including yellowfin tuna, bigeye tuna and several shark species (Walker 1976; Menasveta & Siriyong, 1977; Van den Broek & Tracey, 1981, Andersen & Depledge, 1997; Storelli *et al.*, 2002a; Kraepiel *et al*., 2003; Campbell *et al*., 2010). Fish size could therefore be one of many factors which could give an indication towards estimated Hg levels in individual fish as larger individuals would be more likely to contain Hg levels close to or exceeding the maximum limit (1.0 $mg \cdot kg^{-1}$). Results from the current study show that 70 kg is the weight limit above which yellowfin tuna are likely to contain tHg levels exceeding the regulatory limit (Fig. 4.2) and avoiding catches of fish above this size would reduce unnecessary wastage of having to discard fish not suitable for consumption.

### 4.4.3 Relationship between tHg and MeHg

No prediction model for MeHg has previously been formulated that we are currently aware of. A prediction model as formulated in this study can allow for an accurate prediction of the true toxic Hg levels in tuna meat without additional speciation techniques which would require additional equipment and funds. In addition

to tHg values, which are routinely measured by the fishing industry, fish weight is the only other information needed to predict MeHg levels.

As this study only includes 14 tuna, the model presented here should be validated with larger sample sizes. The approach presented here should also be investigated for other fish species. The accumulation of individual and total Hg species in fish muscle, and therefore the correlation between them, could vary between fish species, as muscle type and metabolism vary between species and these factors play a role in Hg accumulation (Walker, 1976).

## 4.5 Conclusion

The cross-carcass analysis of Hg species (methylmercury, ethylmercury and inorganic mercury) and total mercury (tHg) in yellowfin tuna showed that toxic methylmercury (MeHg) concentrations vary only within dark muscle but concentrations do not vary significantly between white and dark muscle, neither does it vary within the white muscle across the carcass. Routine tHg analyses for measuring the toxicity levels of Hg in fish meat can therefore be sampled from any white meat portion for a representative result of Hg toxicity per fish. Sampling from dark meat could result in higher tHg levels caused by higher levels of non-toxic inorganic mercury (iHg), giving a false indication of the Hg toxicity of the flesh. For representative sampling from a batch of fish, samples should be measured from fish of all represented size categories as MeHg concentrations were found to increase with increasing fish size and concentrations of toxic Hg could therefore be higher in larger fish. Due to this increasing MeHg accumulation with increasing fish size, catches of yellowfin tuna above 70 kg should be avoided for consumption as these fish have higher risks of containing toxic levels of Hg. The low RMSEC values for the prediction of MeHg based on tHg and fish weight indicates that with further research, MeHg concentrations could be accurately calculated from tHg measurements without extra costs of additional analytical methods for MeHg measurements.

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# CHAPTER 5:

## Mercury and mercury species in South African marine fish

**ABSTRACT**

The relationship between total mercury (tHg) and its individual inorganic and organic forms was assessed in blacktail (*Diplodus sargus capensis*), hottentot (*Pachymetopon blochii*), yellowtail (*Seriola lalandi*), snoek (*Thyrsites atun*), blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), soupfin (*Galeorhinus galeus*) and smoothhound (*Mustelus mustelus*). Inorganic mercury (iHg) proportions of tHg in all species were negligible and toxic methylmercury (MeHg) was the predominant Hg form present. The percentages of MeHg with respect to tHg varied overall (data from all eight fish species combined) from 36 to 100%. However, Spearman's correlations revealed consistent, strong (r = 1.0), positive correlations (Y = x) between MeHg and tHg for all fish species studied with low root mean square errors of calculation (RMSEC) of 0.009 $mg \cdot kg^{-1}$ (blacktail), 0.007 $mg \cdot kg^{-1}$ (hottentot), 0.002 $mg \cdot kg^{-1}$ (yellowtail), 0.001 $mg \cdot kg^{-1}$ (snoek), 0.008 $mg \cdot kg^{-1}$ (blue shark), 0.004 $mg \cdot kg^{-1}$ (shortfin mako), 0.007 $mg \cdot kg^{-1}$ (soupfin), 0.061 $mg \cdot kg^{-1}$ (smoothhound). Both MeHg and tHg were positively correlated to fish length in blacktail, yellowtail and soupfin shark, but this did not affect the MeHg:tHg relationship. Total Hg measurements as currently used by industry are therefore sufficient to determine toxic mercury (Hg) levels in fish muscle for these eight marine species.

**Keywords**: Fish muscle, Mercury speciation, HPLC-ICP-MS, Methylmercury, Inorganic mercury, Prediction model

## 5.1 Introduction

It is the responsibility of the food industry to provide product that is safe for human consumption. Fish meat is seen as an important contributor to a healthy diet as it provides a good source of essential omega-3 fatty acids (Kris-Etherton *et al.*, 2002; Schonfeldt *et al.*, 2013). However, the safety of fish meat can be compromised by the presence of metal contaminants in the meat (Domingo *et al.*, 2006). Once over a specified concentration, these metals are considered toxic and can have harmful effects on human health when consumed (Grandjean *et al.*, 2010). One such metal of concern is mercury (Hg), which when ingested in high quantities, can have adverse effects on the well-being of individuals and even large human populations (Harada, 1995).

Mercury can be present in several chemical forms (organic and inorganic) in the marine environment which vary in toxicity (D'Itri, 1990). The total mercury (tHg) levels in fish meat consist mainly of two forms: inorganic mercury and organic mercury. Organic mercury is mainly present as methylmercury (MeHg). Inorganic mercury (iHg) is considered non-toxic as it is not accumulated in the human body because of a high natural excretion rate. Methylmercury on the other hand is the main toxic form, as it is not excreted to any great extent and therefore accumulates in the human body and can affect vital organs such as the brain (Morel *et al.,* 1998, Boening, 2000).

Food safety authorities have established maximum allowable limits (MAL) for Hg concentrations in fish meat in order to protect fish and seafood consumers from toxic levels of Hg (Du Preez *et al.*, 2006). The general MAL specified by several authorities is 0.5 $mg \cdot kg^{-1}$ for MeHg in fish muscle, with the exception of large predatory fish for which the MAL is set at 1.0 mg MeHg/kg (DOH, 2004; FAO, 2003; EC, 2008). As MeHg concentrations are often strongly correlated to tHg concentrations (frequently comprising more than 90% of tHg), measuring tHg is currently considered adequate for assessing MeHg concentrations in fish samples (JECFA, 2007). Even though MeHg is the predominant form of Hg in fish muscle, variation in the MeHg percentage in fish muscle exists, varying between 60 to 100% among fish species (Kamps *et al.*, 1972; Walker, 1976; Joiris *et al.*, 1999; Storelli *et al.*, 2001). Hg toxicity in fish muscle may therefore be overestimated if MeHg is estimated from tHg. Such overestimations can lead to unnecessary losses for the fishing industry as fish considered unsuitable or hazardous for consumption are discarded.

Currently, several methods are used to measure tHg concentrations in fish samples. These consist of an extraction of the Hg components from the biological sample, followed by a detection technique such as atomic fluorescence spectrometry (AFS), various forms of atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS) (Bloxham *et al.*, 1996). Determining MeHg concentrations requires a speciation technique which can be similar to the previous methods, but includes a step such as liquid chromatography (LC) or gas chromatography (GC) to separate tHg into individual Hg components prior to the detection technique.

An earlier study (Bosch *et al.*, 2016a; Chapter 4) identified a prediction model which could be used to calculate MeHg concentrations from tHg measurements in yellowfin tuna taking into account fish weight. Therefore, this study further aims to investigate MeHg and iHg concentrations as proportions of tHg in order to better define the MeHg:tHg relationship as a function of fish size. This study also intends to determine whether tHg measurements can be accurately used to identify toxic (MeHg) Hg concentrations of fish muscle.

## 5.2 Materials and methods

Fish were sampled at various sites around the South African coastline as described in chapters 7, 8 and 9 of this dissertation with size parameters per species presented in Table 5.1. Fish species included blacktail (*Diplodus sargus capensis*), hottentot (*Pachymetopon blochii*), yellowtail (*Seriola lalandi*), snoek (*Thyrsites atun*), blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), soupfin (*Galeorhinus galeus*) and smoothhound (*Mustelus mustelus*).

**Table 5.1** Size range (weight and length) and average total length with the number of specimens (n) sampled per species.

| Common name | Species | n | Weight range (g) | Total length range (cm) | Average total length (cm) |
|---|---|---|---|---|---|
| hottentot | *P. blochii* | 58 | 305 - 900 | 25.5 - 36.8 | 30.7 |
| blacktail | *D. sargus capensis* | 76 | 107 - 1272 | 18.8 - 53.8 | 30.1 |
| yellowtail | *S. lalandi* | 37 | 2510 - 15600 | 67.5 - 137.0 | 93.3 |
| snoek | *T. atun* | 20 | 3000 - 4850 | 94.0 - 112.5 | 100.7 |
| blue shark | *P. glauca* | 10 | - | 108.0 - 187.8 | 129.7 |
| shortfin mako | *I. oxyrinchus* | 10 | - | 166.0 - 210.0 | 193.6 |
| soupfin | *G. galeus* | 12 | - | 93.6 - 151.0 | 118.2 |
| smoothhound | *M. mustelus* | 30 | - | 60.1 - 165.2 | 118.4 |

Duplicates of all samples were separately prepared and analysed on ICP-MS and HPLC-ICP-MS systems to determine tHg and individual Hg species respectively as described in Chapter 3 of this dissertation.

Data were analysed with STATISTICA 12.5. To determine the relationships between tHg, MeHg and iHg and fish length, Spearman's correlations were reported in order to compensate for data that did not follow a normal distribution and simple regression analyses were conducted to determine the relationship between MeHg and tHg in terms of predicted versus observed values.

## 5.3 Results

The percentage (mean and range) of MeHg present in the tHg measured for each fish species is summarised in Table 5.2. Large percentage variations were seen in three (blacktail, hottentot and smoothhound) of the eight species assessed.

**Table 5.2** A summary of the percentages of tHg present as MeHg in muscle tissue of 8 fish species (n = number of samples per species).

| Fish species | n | MeHg range (%) | Mean MeHg (%) |
|---|---|---|---|
| *D. sargus capensis* | 76 | 63 - 100 | 95 |
| *P. blochii* | 58 | 74 - 100 | 92 |
| *S. lalandi* | 36 | 91 - 100 | 99 |
| *T. atun* | 20 | 97 - 100 | 98 |
| *P. glauca* | 10 | 94 - 100 | 99 |
| *I. oxyrinchus* | 10 | 98 - 100 | 99 |
| *G. galeus* | 12 | 97 - 100 | 99 |
| *M. mustelus* | 30 | 36 - 100 | 90 |

Correlation results between fish length and three individual mercury concentrations (tHg, MeHg and iHg) are presented in Table 5.3. Both tHg and MeHg had similar positive correlations ($p < 0.01$) with fish length for blacktail, yellowtail and soupfin shark. In yellowtail muscle, iHg was also positively correlated ($p < 0.01$) with fish length, but with a weaker correlation ($r = 0.48$) than MeHg ($r = 0.82$) and tHg ($r = 0.81$).

**Table 5.3** Spearman's correlation coefficients and p-values for correlations of individual Hg species and tHg with fish total length (cm) in eight fish species. Bold values indicate significant ($p < 0.01$) correlations.

| | *D. sargus capensis* | *P. blochii* | *S. lalandi* | *T. atun* | *P. glauca* | *I. oxyrin-chus* | *G. galeus* | *M. mustelus* |
|---|---|---|---|---|---|---|---|---|
| iHg | r = -0.09<br>p = 0.43 | r = -0.24<br>p = 0.07 | **r = 0.48**<br>**p < 0.01** | r = 0.47<br>p = 0.04 | r = 0.54<br>p = 0.24 | r = 0.29<br>p = 0.42 | r = 0.21<br>p = 0.54 | r = -0.33<br>p = 0.18 |
| MeHg | **r = 0.41**<br>**p < 0.01** | r = 0.19<br>p = 0.16 | **r = 0.81**<br>**p < 0.01** | r = 0.14<br>p = 0.55 | r = 0.43<br>p = 0.22 | r = 0.23<br>p = 0.53 | **r = 0.87**<br>**p < 0.01** | r = 0.45<br>p = 0.06 |
| tHg | **r = 0.39**<br>**p < 0.01** | r = 0.16<br>p = 0.24 | **r = 0.82**<br>**p < 0.01** | r = 0.15<br>p = 0.53 | r = 0.49<br>p = 0.15 | r = 0.23<br>p = 0.53 | **r = 0.87**<br>**p < 0.01** | r = 0.38<br>p = 0.12 |

Upon examination of relationships between tHg and each mercury species (iHg and MeHg); MeHg concentrations were positively correlated ($p < 0.001$) with and equal to ($y = x$) tHg concentrations with Spearman's correlation coefficients equal to or close to 1 for all fish species (Fig. 5.1 – 5.8). The root mean square error of calculation (RMSEC) for predicting MeHg concentrations from tHg measurements were very low for all species (Fig. 5.1 – 5.8) with the highest RMSEC found for smoothhound (0.06 $mg \cdot kg^{-1}$), which was still less than 10 % of the mean tHg concentration in this species.

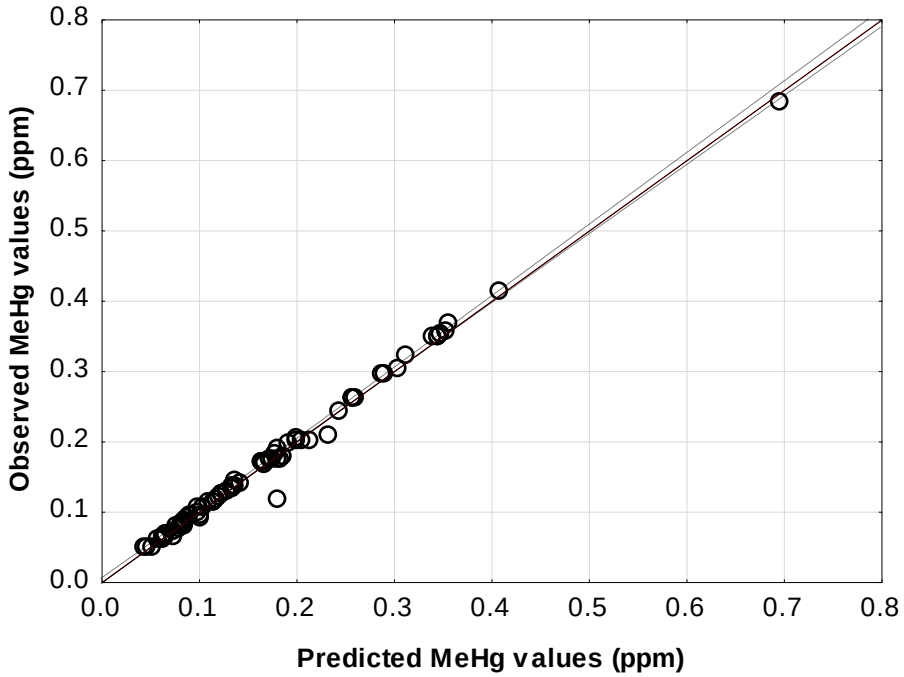


**Figure 5.3** Regression analysis representing the predicted versus observed values for MeHg (ppm) in blacktail (*D. sargus capensis*) (n = 76) when predicting MeHg concentrations from tHg measurements (Y = x; r = 1.00; RMSEC = 0.009 ppm). The dashed lines indicate the 95% confidence interval for the fit.

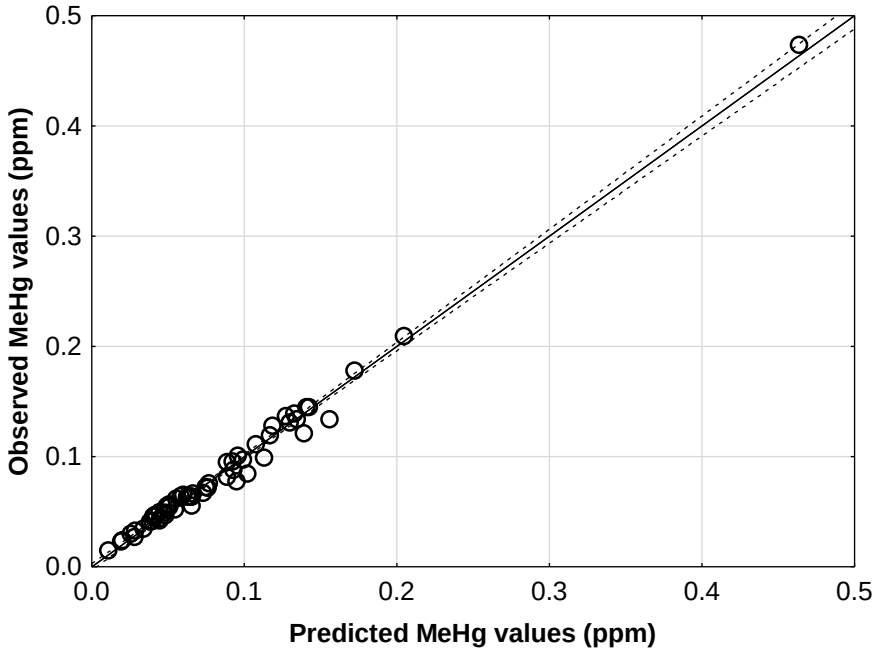


**Figure 5.4** Regression analysis representing the predicted versus observed values for MeHg (ppm) in hottentot (*P. blochii*) (n = 58) when predicting MeHg concentrations from tHg measurements (Y = x; r = 0.99; RMSEC = 0.007 ppm). The dashed lines indicate the 95% confidence interval for the fit.

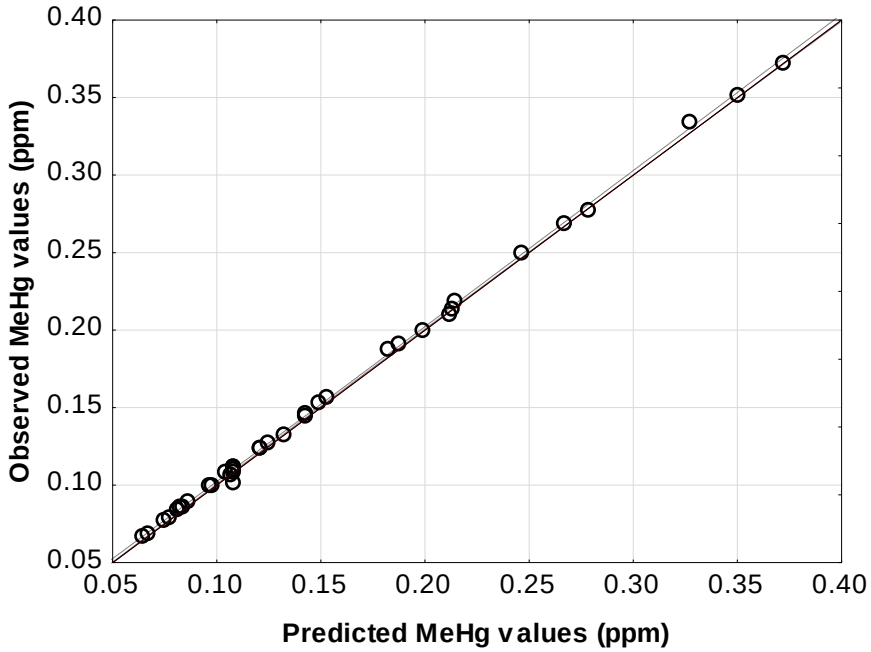


**Figure 5.5** Regression analysis representing the predicted versus observed values for MeHg (ppm) in yellowtail (*S. lalandi*) (n = 37) when predicting MeHg concentrations from tHg measurements (Y = x; r = 1.00; RMSEC = 0.002 ppm). The dashed lines indicate the 95% confidence interval for the fit.

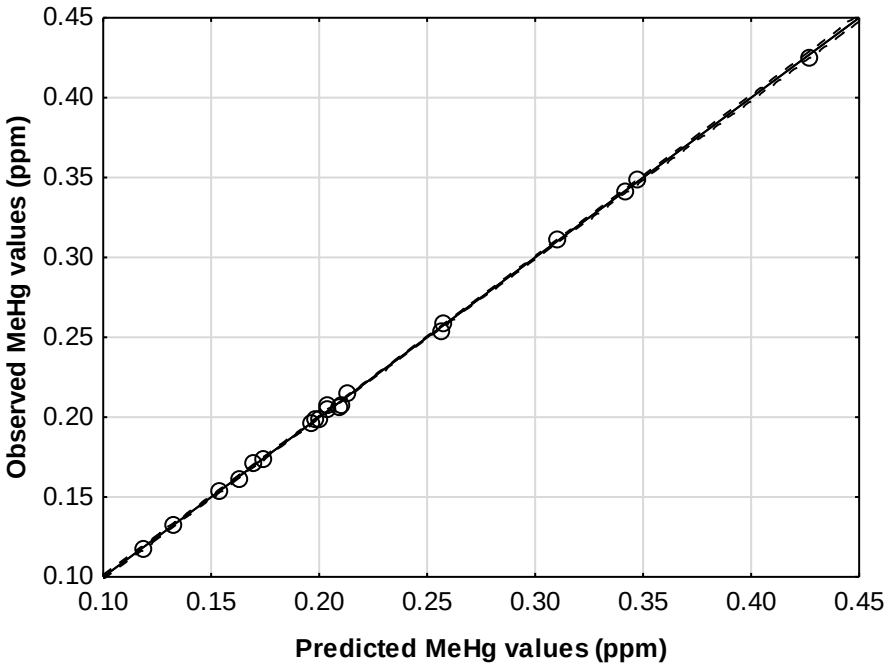


**Figure 5.6** Regression analysis representing the predicted versus observed values for MeHg (ppm) in snoek (*T. atun*) (n = 20) when predicting MeHg concentrations from tHg measurements (Y = x; r = 1.00; RMSEC = 0.001 ppm). The dashed lines indicate the 95% confidence interval for the fit.

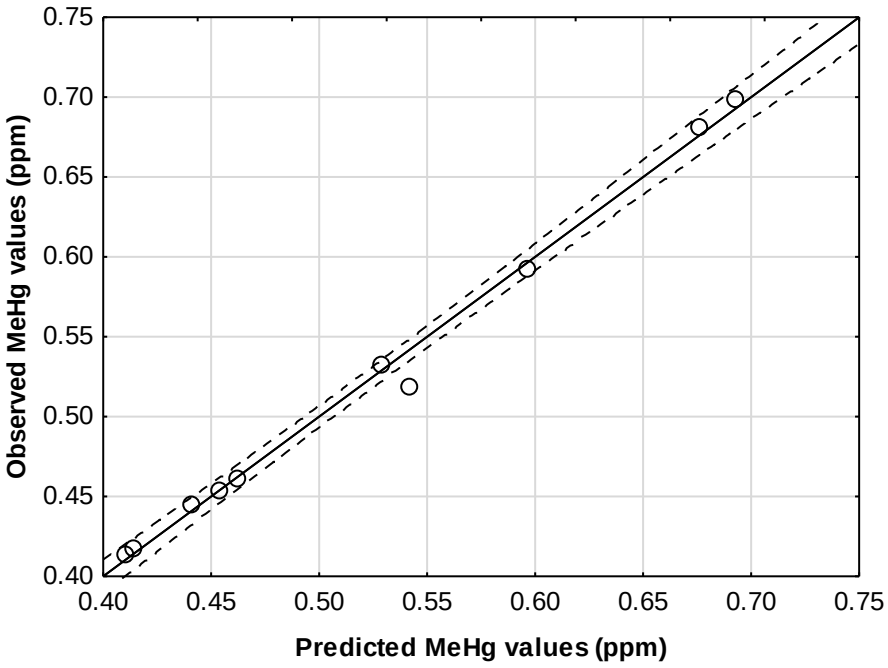


**Figure 5.7** Regression analysis representing the predicted versus observed values for MeHg (ppm) in blue shark (*P. glauca*) (n = 10) when predicting MeHg concentrations from tHg (Y = x; r = 1.00; RMSEC = 0.008 ppm). The dashed lines indicate the 95% confidence interval for the fit.

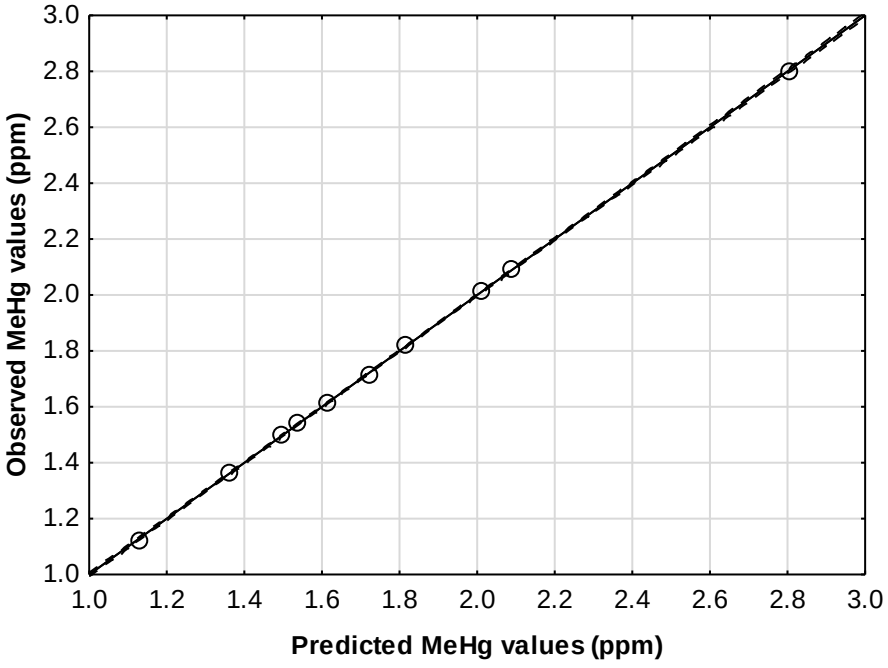


**Figure 5.8** Regression analysis representing the predicted versus observed values for MeHg (ppm) in shortfin mako (*I. oxyrinchus*) (n = 10) when predicting MeHg concentrations from tHg measurements (Y = x; r = 1.00; RMSEC = 0.004 ppm). The dashed lines indicate the 95% confidence interval for the fit.

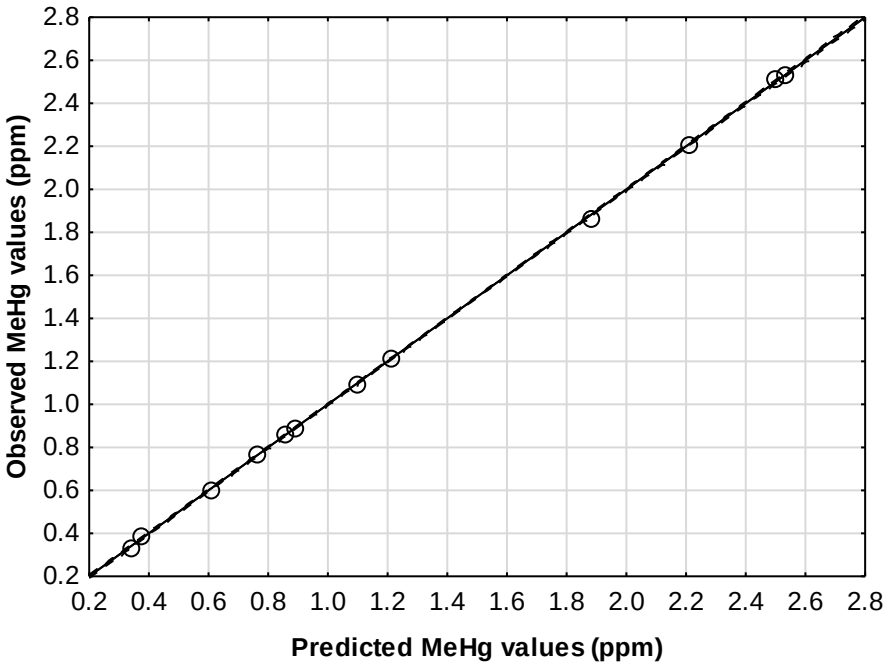


**Figure 5.9** Regression analysis representing the predicted versus observed values for MeHg (ppm) in soupfin (*G. galeus*) (n = 12) when predicting MeHg concentrations from tHg measurements (Y = x; r = 1.00; RMSEC = 0.007 ppm). The dashed lines indicate the 95% confidence interval for the fit.

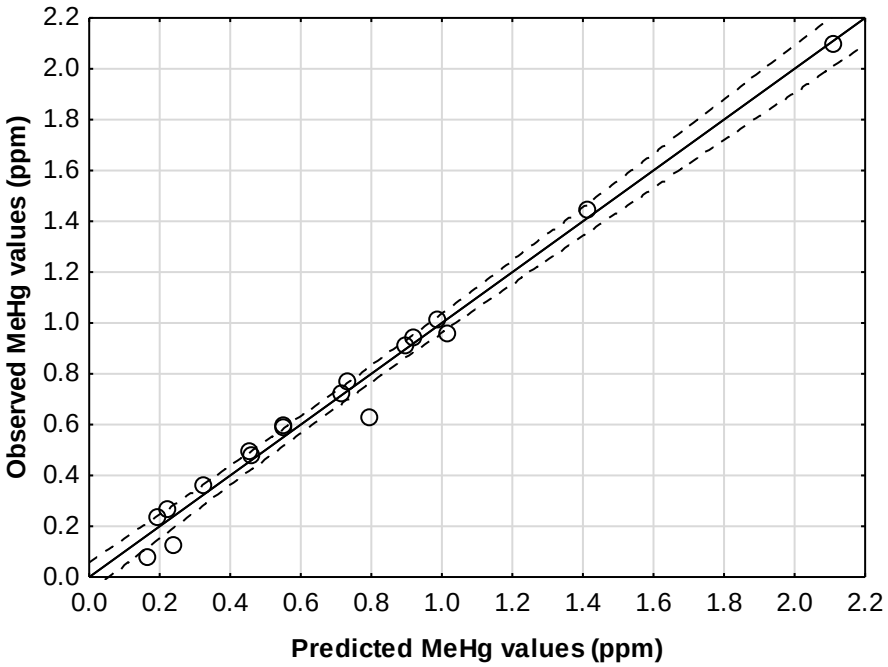


**Figure 5.10** Regression analysis representing the predicted versus observed values for MeHg (ppm) in smoothhound (*M. mustelus*) (n = 30) when predicting MeHg concentrations from tHg measurements (Y = x) with r = 0.99 and a RMSEC of 0.061 ppm. The dashed lines indicate the 95% confidence interval for the fit.

## 5.4 Discussion

The tHg load in fish tissue consists mainly of two Hg species, iHg and MeHg, which, when measured individually via Hg speciation, should add up to the tHg measurement of each sample (Bosch *et al.*, 2016b). Therefore if the proportions of the individual Hg species to tHg are known, only one measurement of either tHg or one of the Hg species (iHg or MeHg) should be needed in order to determine the concentrations of the other component. The concentrations of iHg are generally not related to fish size whereas MeHg concentrations are increasingly accumulated with increasing fish size (Andersen & Depledge, 1997; Bosch *et al.*, 2016a), resulting in the percentage of tHg present as MeHg to increase proportionally with increasing fish size (Forsyth *et al.*, 2004; Bosch *et al*., 2016a). In the current study, five (hottentot, snoek, blue shark, shortfin mako and smoothhound) out of the eight fish species showed no significant correlations between either Hg species and fish length. The absence of significant correlations in hottentot and snoek could be due to limited size ranges sampled for both these species (hottentot: 25.5 - 36.8 cm total length; snoek: 94.0 – 112.5 cm total length) compared to the size ranges sampled for blacktail (18.8 - 53.8 cm total length) and yellowtail (67.5 - 137.0 cm total length) for which significant correlations ($p < 0.01$) were found between fish length and both MeHg and tHg concentrations. In the fish species (blacktail, yellowtail and soupfin) where MeHg was positively correlated with fish length, correlations of iHg with fish length were either insignificant or much weaker that MeHg correlations. An increase in the percentage of tHg present as MeHg with increasing fish length is therefore expected in blacktail, yellowtail and soupfin (Forsyth, 2004; Bosch *et al.,* 2016a) and this relationship was further investigated.

The high mean percentages of MeHg with respect to tHg per fish species (90 - 99%) suggest that Hg is present predominantly as MeHg. Similar findings in previous studies showed that MeHg percentages varied from around 60 to 100% in various fish species (Kamps *et al.*, 1972; Walker, 1976; Joiris *et al.*, 1999; Storelli *et al.*, 2001). Fish consumers are therefore exposed to Hg mainly in its toxic form (MeHg) and measuring tHg concentrations in fish muscle may give an indication of toxic MeHg present in the muscle. However, high variation was seen in individual MeHg percentages in blacktail (63 – 100%), hottentot (74 – 100%) and smoothhound shark (36 – 100%) indicating that a direct estimation of toxic MeHg concentrations from tHg measurements may result in inaccurate results for specific species. Love *et al.* (2003) similarly found that certain fish species (*Genypterus blacodes*, *Galeorhinus australis, Chrysophrys auratus, Squalus acanthias, Caranx geogianus*) had lower proportions of MeHg to tHg than other species (*Thyrsites atun, Parapercis colias, Polyprion oxygenios, Rexea solandri*) sampled from the same area. In order to further investigate this relationship between MeHg and tHg and possible causes (such as fish length) of variation between and within species, correlation equations between MeHg and tHg were calculated per fish species.

Despite the variations in the percentages of tHg present as MeHg, constantly strong positive correlations between MeHg and tHg measurements were found throughout the eight fish species studied showing that MeHg was the predominant form of Hg present in every species examined with a negligible

contribution of iHg. The variations seen in MeHg percentages are likely due to slight outliers, as relatively low errors in calculation (0.01 $mg \cdot kg^{-1}$) could cause large differences in percentage as measurements are low (MeHg: 0.1 - 2.1 $mg \cdot kg^{-1}$; tHg: 0.2 - 2.2 $mg \cdot kg^{-1}$). The MeHg:tHg proportion was therefore not effected by fish size in any of the fish species studied. Previous studies (Bosch *et al.*, 2016a; Chapter 4) have found that fish size should be considered in models to accurately calculate MeHg concentrations from tHg measurements. However, measurements for tHg in fish muscle of blacktail, hottentot, yellowtail, snoek, blue shark, shortfin mako, soupfin and smoothhound can accurately be used as measurements of MeHg when monitoring toxic Hg concentrations for the purpose of determining food safety.

## 5.5 Conclusion

Strong positive linear correlations between MeHg and tHg measurements indicate that there was a strong, consistent relationship between these two components for all fish species studied. Total Hg was present almost entirely as MeHg with the contribution of an iHg proportion to tHg measurements considered negligible. This relationship was consistent for all fish species and fish of all sizes. This study confirms that the current measurement of tHg in fish muscle in the fishing industry is sufficiently accurate as a method for determining toxic quantities of Hg in terms of MeHg in blacktail, hottentot, yellowtail, snoek, and four shark species obtained off the South African coast which would spare the industry the costs of additional metal speciation equipment and methods.

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# CHAPTER 6:

## Heavy metal accumulation and toxicity in yellowfin tuna (*Thunnus albacares*)

**ABSTRACT**

The total concentrations of 16 metals in yellowfin tuna muscle were measured in order to assess influencing factors such as muscle type, muscle position, fish size and metal interactions as well as to determine safety of yellowfin muscle in terms of human consumption. Metal concentrations were found to vary between dark and white muscle types with Mn, Fe, Co, Cu, As, Se, Cd and Hg having higher ($p < 0.05$) concentrations in dark muscle than in white muscle. Intermuscular variation was seen mostly within the dark muscle where metals (Mn, Fe, Co, Cu, As, Se and Cd) were more highly concentrated ($p < 0.05$) towards the posterior portions of the fish. White muscles displayed uniformity in terms of metal concentrations except for a select few differences ($p < 0.05$) (Fe, Cu, Cd and As) between dorsal and ventral muscles. Hg, Cd and Pb are all strongly associated with each other with Hg and Cd increasing and Pb proportionately decreasing with increasing fish weight. Even though average concentrations of all metals were within regulatory guidelines, individual samples or specimens were found to exceed As and Hg maximum allowable limits and as Hg is positively correlated to fish weight, it is recommended that yellowfin tuna of 70 kg and larger be avoided for human consumption.

**Keywords**: Fish muscle, Heavy metals, Mercury, ICP-MS, Yellowfin tuna, Cross-carcass variation, Consumer health

## 6.1 Introduction

Heavy metals, ranging from negligible amounts to significant concentrations, may be present in fish tissue. Several of these metals are considered essential to the human body as trace elements but some may become toxic when present in concentrations above what is necessary for their biological functions (Fraga, 2005). Other metals have no known biological function and are introduced to the environment as toxins mainly through anthropogenic activities (Shroeder & Darrow, 1972). Metals that are frequently accumulated to toxic quantities in fish tissue include mercury (Hg), cadmium (Cd), lead (Pb) and arsenic (As) (Llobet *et al*., 2003; Falcó *et al.*, 2006).

Toxic metals can have varying effects on the human body depending on their biochemical characteristics, their concentrations and interactions with other metals (Goyer, 1997). Individual metals may also display different accumulation characteristics depending on both internal (chemical form) and external factors (environmental conditions, fish species, fish body parameters and the presence of other metals) (Canli & Atli, 2003; Erasmus *et al*., 2004). As certain individual metals occur in several chemical forms which can differ in solubility and bioavailability, the concentrations of individual chemical forms affect the ultimate total metal concentration in fish tissue. Individual metal concentrations can be correlated to those of other metals present (Carvalho *et al.*, 2005; Rahman *et al.*, 2012). These correlations can be either positive, where one or more metals may cause the accumulation of other metals, or negative, where the accumulation of certain metals is restricted by others. The uptake of Cu and Zn, for example, has been found to be limited by the presence of Hg in certain organisms (Erasmus *et al*., 2004). In addition, metal concentrations can be either positively or negatively correlated with fish age/size. Mercury is generally positively correlated with fish age/size, especially in predatory fish species (Canli & Atli, 2003; Kraepiel *et al.*, 2003; Erasmus *et al.*, 2004; Endo *et al*., 2008; Campbell *et al.*, 2010). However, negative relationships have been reported for several other metals (Cr, Cu, Fe, Cd, Ni, As and Pb) with fish age/size in certain fish species (Widianarko *et al*., 2000; Canli & Atli, 2003; Erasmus e*t al*., 2004).

Fish is generally promoted as a healthy source of nutrition and moderate consumption of tuna meat has been associated with lowered risks of heart disease (Mozaffarian *et al.*, 2003). Yellowfin tuna (*Thunnus albacares*) is one of the major species of the total South African large pelagic longline catches in both the Atlantic and Indian Oceans, consumed locally as well as being exported (DAFF, 2005). Several studies have described the Hg content in yellowfin tuna (Boush & Thieleke, 1983; Kraepiel *et al.*, 2003; Forsyth *et al.*, 2004; Ferris *et al.*, 2011; Ordiano-Flores *et al.*, 2011; Ruelas-Inzunza *et al.*, 2011), but limited published information is available on other metals for this fish species and few studies have investigated heavy metal concentrations in Southern African tuna.

The distinct muscle types in tuna (dark and white muscle) have specific functions and compositions which may affect the rate and degree of metal accumulation and consequent variation in measurable metal concentrations across the fish carcass (Ando *et al.,* 2008; Balshaw *et al.*, 2008; Lares *et al.*, 2012) as was

shown for Hg in Chapter 4 (Bosch *et al.*, 2016). This could result in certain muscle parts or fish cuts having higher metal loads and toxic effects on consumer health. As yellowfin tuna are large fish, the meat is often portioned into smaller commercial cuts (Balshaw *et al.*, 2008) and cross-carcass metal accumulation needs to be investigated to inform food safety of tuna meat.

The aim of this study was to investigate the concentrations and variation of Hg and 15 other heavy metals within the anatomy of yellowfin tuna, as well as to assess the relationships between individual heavy metal concentrations and between heavy metal concentration and tuna size and weight.

## 6.2 Materials and methods

### 6.2.1 Sampling

Fourteen yellowfin tuna (29.0 - 50.8 kg) were sampled off the South African south-west coast (S34°29' E17°54' and S34°35' E17°58'). Six muscle samples were obtained from each fish as described in the materials and methods section of Chapter 4 and depicted in Figure 6.1. All samples were taken using a ceramic knife to minimise metal contamination. Samples were homogenised, vacuum sealed in clean polyethylene bags and stored at -20 °C for further analyses.

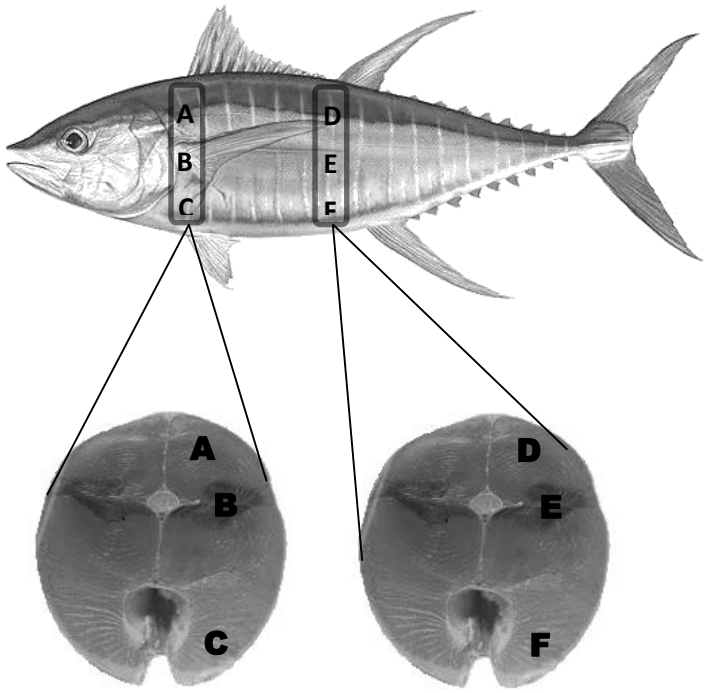


**Figure 6.1** Tranverse section of a tuna carcass indicating the positions of the white (A, C, D and F) and dark (B and E) muscle. Letters A, B, C, D, E and F indicate sampling locations.

### 6.2.2 Analyses

Total metal analyses were done by microwave digestion and ICP-MS for 16 metals (aluminium (Al), manganese (Mn), cobalt (Co), nickel (Ni), molybdenum (Mo), tin (Sn), iron (Fe), copper (Cu), chromium (Cr),

zinc (Zn), selenium (Se), arsenic (As), antimony (Sb), cadmium (Cd), mercury (Hg) and lead (Pb)) as described in the materials and methods section in Chapter 3 and 4. The limits of detection of the ICP-MS are tabulated in Chapter 3 (Table 6.1).

### 6.2.3 Statistical analyses

Statistical analyses were done with STATISTICA 12.5. Outliers were visually identified and removed prior to analysis. Analysis of variance (ANOVA) was performed to test for differences in metal concentrations in tuna muscle between the six carcass sites. Data were not normally distributed, and log transformations did not improve normality of the data. Therefore, bootstrap analyses were done incorporating a bonferroni correction. Where significant differences were identified, post hoc comparisons established where these differences lay. Principal component analysis was done and Spearman's correlations were calculated to investigate correlations between individual metal concentrations (combining data from all 6 sample sites) and between metal concentrations and fish weight (average values per tuna).

## 6.3 Results

The two most abundant metals measured were Fe and zinc. Antimony and Mo were not detected in any fish samples. Tin and Ni were only detected in 10 samples (out of 84) in low concentrations. These four of the 16 metals (Sb, Mo, Sn and Ni) were excluded from the ANOVA when investigating cross-carcass accumulation as most of the measurements was below quantifiable levels in most samples.

### 6.3.1 Inter- and intramuscular variation in metal accumulation

Ten of the 12 metals included in the ANOVA had significant variation among the six body sites (Table 6.1). Several common trends in variation are apparent among these metals; Mn, Fe, Co, Cu, As, Se, Cd and Hg had higher concentrations ($p < 0.05$) in Sites B and E than Sites A, C, D and F. In addition, all of these metals, except for Hg, had higher concentrations ($p < 0.05$) in Site E than Site B. These metals therefore tend to be more highly accumulated in dark muscle (B and E) and more specifically towards the posterior part of the dark muscle. One exception to this trend is found for Al, where Site E had lower concentrations ($p < 0.05$) than both Sites A and B. Only four metals (Cr, Zn, Hg and Pb) had no significant variation between Sites B and E and were therefore uniformly accumulated within the dark muscle of the fish.

No significant variation in Al, Cr, Co, Zn, Se, Hg and Pb concentration was observed among the white meat portions (A, C, D and F) of the yellowfin analysed. In addition, Cr and Pb were uniformly concentrated across all muscle portions (dark and light meat). Where significant variation did occur (Fe, Cu, Cd, As and Mn) within the white muscle, it was generally restricted between the dorsal and ventral sections. Average Fe and Cu concentrations were lower ($p < 0.05$) in the ventral muscles (F) than the dorsal muscles (D) in the posterior carcass sites whereas average Cd concentrations were lower ($p < 0.05$) in the dorsal muscle than the ventral

muscle in the anterior carcass sites and average As concentrations were lower ($p < 0.05$) in both the anterior and posterior dorsal muscle sites than the corresponding ventral sites. Mn is the only metal which showed variation ($p < 0.05$) within the ventral white muscle (C > F).

**Table 6.1** Concentration means ($mg \cdot kg^{-1}$) ± standard deviation (n = 14) for individual metals per carcass site (A, B, C, D, E and F) in yellowfin tuna (*Thunnus albacares*) muscle.

| Metal | Site A | Site B | Site C | Site D | Site E | Site F |
|---|---|---|---|---|---|---|
| Al | $0.83^{a} \pm 0.504$ | $1.18^{a} \pm 1.398$ | $0.65^{a} \pm 0.475$ | $1.13^{ab} \pm 1.857$ | $0.55^{b} \pm 0.384$ | $0.70^{ab} \pm 0.493$ |
| Cr | $0.09^{a} \pm 0.125$ | $0.13^{a} \pm 0.204$ | $0.05^{a} \pm 0.077$ | $0.04^{a} \pm 0.036$ | $0.05^{a} \pm 0.075$ | $0.05^{a} \pm 0.078$ |
| Mn | $0.05^{cd} \pm 0.026$ | $0.17^{b} \pm 0.087$ | $0.06^{c} \pm 0.018$ | $0.05^{cd} \pm 0.017$ | $0.29^{a} \pm 0.077$ | $0.04^{d} \pm 0.011$ |
| Fe | $7.12^{cd} \pm 4.362$ | $55.84^{b} \pm 40.441$ | $5.74^{cd} \pm 1.574$ | $6.62^{c} \pm 2.112$ | $108.94^{a} \pm 29.405$ | $5.53^{d} \pm 1.430$ |
| Co | $0.004^{bc} \pm 0.0066$ | $0.014^{b} \pm 0.0112$ | $0.001^{c} \pm 0.0009$ | $0.002^{c} \pm 0.0017$ | $0.023^{a} \pm 0.0058$ | $0.001^{c} \pm 0.001$ |
| Cu | $0.34^{cd} \pm 0.092$ | $1.84^{b} \pm 1.283$ | $0.36^{cd} \pm 0.196$ | $0.50^{c} \pm 0.414$ | $4.00^{a} \pm 0.896$ | $0.28^{d} \pm 0.045$ |
| Zn | $6.41^{b} \pm 3.470$ | $9.59^{ab} \pm 4.603$ | $6.50^{b} \pm 1.876$ | $7.42^{ab} \pm 6.940$ | $12.36^{a} \pm 3.499$ | $8.27^{b} \pm 6.667$ |
| As | $0.93^{de} \pm 0.261$ | $1.79^{b} \pm 1.004$ | $1.16^{c} \pm 0.260$ | $0.91^{e} \pm 0.254$ | $3.36^{a} \pm 1.580$ | $1.07^{cd} \pm 0.301$ |
| Se | $0.48^{c} \pm 0.145$ | $3.46^{b} \pm 2.592$ | $0.54^{c} \pm 0.248$ | $0.46^{c} \pm 0.330$ | $6.12^{a} \pm 2.103$ | $0.41^{c} \pm 0.156$ |
| Cd | $0.01^{d} \pm 0.006$ | $0.03^{b} \pm 0.024$ | $0.01^{c} \pm 0.009$ | $0.01^{cd} \pm 0.007$ | $0.04^{a} \pm 0.033$ | $0.01^{cd} \pm 0.008$ |
| Hg | $0.73^{b} \pm 0.220$ | $0.85^{a} \pm 0.264$ | $0.72^{b} \pm 0.228$ | $0.73^{b} \pm 0.221$ | $0.87^{a} \pm 0.309$ | $0.72^{b} \pm 0.211$ |
| Pb | $0.02^{a} \pm 0.024$ | $0.005^{a} \pm 0.0083$ | $0.009^{a} \pm 0.0152$ | $0.005^{a} \pm 0.0080$ | $0.007^{a} \pm 0.010$ | $0.011^{a} \pm 0.020$ |

Identical superscript letters indicate non-significant ($p > 0.05$) differences between sites per metal.

### 6.3.2 Inter-metal correlations

Several strong positive correlations were observed between concentrations of individual metals accumulated in yellowfin tuna muscle. Table 6.2 shows the values for all significant correlations ($p < 0.05$, $p < 0.01$) between metals. From Table 6.2 it is evident that the 16 metals could be separated into two main groups as the metals are associated with each other by correlation. Group 1: significant positive correlations are observed among metals Cr, Mn, Fe, Co, Ni, Cu and As. Group 2: significant positive as well as negative correlations are observed among metals Cd, Hg and Pb. Aluminium and Se are not included in either of these two groups as they are each associated with an equal number of metals from both groups: Al with Cr from Group 1 and Pb from Group 2, and Se with Fe and Cu from Group 1 and Cd and Hg from Group 2.

These observations are confirmed by the PCA plot (Fig. 6.1) which gives a representation of the relationships between metal concentrations. The first principal component, which accounts for 40% of the variance, was related to Cr, Al, Co, Mn, Fe, Cu, As and Se. Several of these metals were strongly positively correlated with each other (Spearman correlation coefficients also shown in Table 6.2). Principal component 2 accounts for 24% of the variance and was associated with Pb, Cd and Hg with a strong positive correlation between Cd and Hg ($p < 0.01$; $r = 0.91$) and Pb being negatively correlated to both Cd

**Table 6.2** Pearson correlation coefficients (two-tailed significance denoted by asterisks) for concentrations of 13 metals in yellowfin tuna muscle. Shaded areas represent metals grouped together by inter-correlations.

| Metal | Al | Cr | Mn | Fe | Co | Ni | Cu | Zn | As | Se | Cd | Hg | Pb |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Al | 1.00 | 0.62* | - | - | - | - | - | - | - | - | - | - | 0.65* |
| Cr | - | 1.00 | 0.61* | 0.59* | - | 0.57* | - | - | - | - | - | - | - |
| Mn | - | - | 1.00 | 0.85** | 0.66* | - | 0.72** | - | - | - | - | - | - |
| Fe | - | - | - | 1.00 | 0.72** | - | 0.79** | - | 0.64* | 0.64* | - | - | - |
| Co | - | - | - | - | 1.00 | - | 0.70** | - | 0.56* | - | - | - | - |
| Ni | - | - | - | - | - | 1.00 | - | - | - | - | - | - | - |
| Cu | - | - | - | - | - | - | 1.00 | - | - | 0.70** | - | - | - |
| Zn | - | - | - | - | - | - | - | 1.00 | - | - | - | - | - |
| As | - | - | - | - | - | - | - | - | 1.00 | - | - | - | - |
| Se | - | - | - | - | - | - | - | - | - | 1.00 | 0.72** | 0.65* | - |
| Cd | - | - | - | - | - | - | - | - | - | - | 1.00 | 0.91** | -0.56* |
| Hg | - | - | - | - | - | - | - | - | - | - | - | 1.00 | -0.65* |
| Pb | - | - | - | - | - | - | - | - | - | - | - | - | 1.00 |

* P < 0.05
** P < 0.01

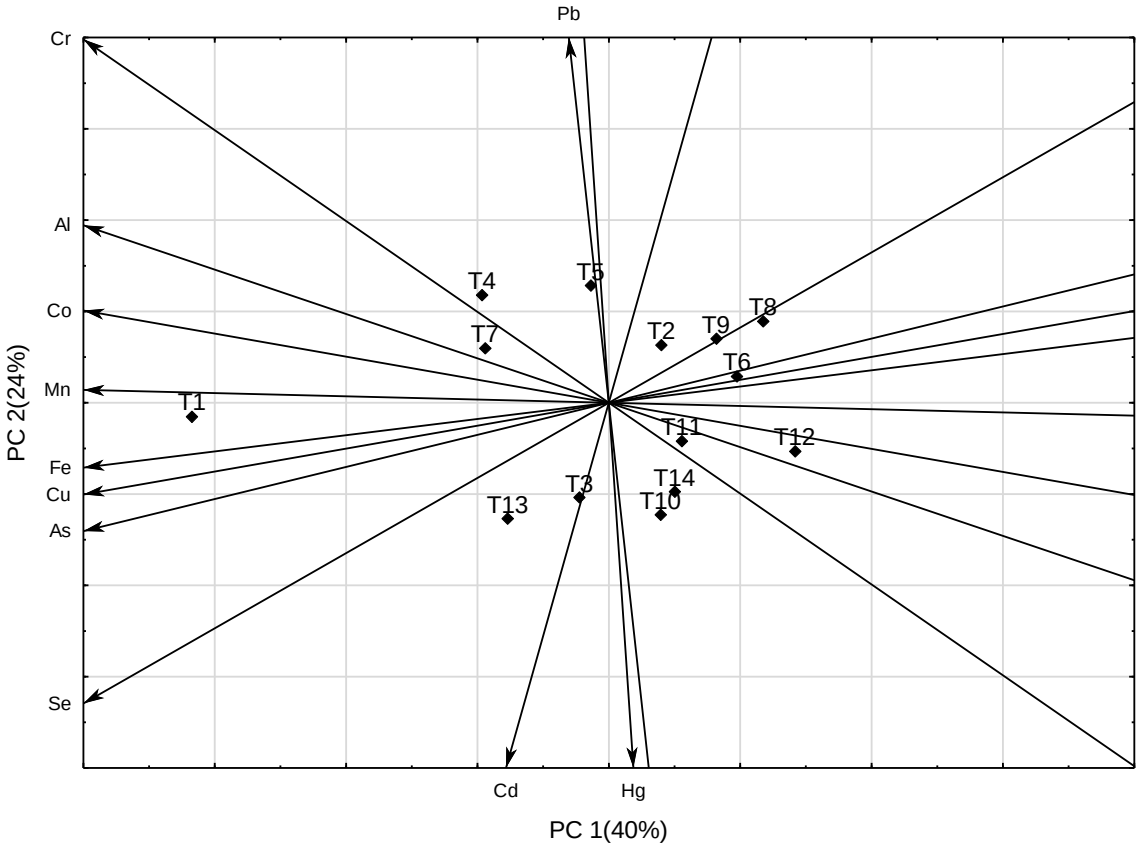


**Figure 6.2** Principal component analysis (PCA) of metal accumulation in yellowfin tuna. Individual tuna shown as black dots (T1-T14).

($p < 0.05$; $r = -0.56$) and Hg ($p < 0.05$; $r = -0.65$). From the Spearman correlation analysis it is seen that all metals associated with principal component 1 were also significantly correlated to fish weight (Hg: $p < 0.001$, $r = 0.83$; Cd: $p < 0.001$, $r = 0.75$; Pb: $p < 0.05$, $r = -0.69$). A positive correlation was also found between Se and fish weight ($p < 0.05$, $r = 0.64$).

## 6.4 Discussion

The levels at which metals become toxic are metal specific. Individual metal concentrations in a fish sample therefore need to be assessed and compared with regulatory toxic upper limits to determine which metals are closest to toxic levels and could lead to consumer health concerns. Regulatory maximum allowable limits (MAL) may vary as specified by various regulatory bodies or for various countries/regions as fish consumption varies between populations (Food and Nutrition Board, 2001) (Table 6.3).

**Table 6.3** Summary of the mean metal concentrations in yellowfin tuna measured in the current study as well as maximum allowable limits (MAL) or upper limits (UL) for tuna meat (or other closely related foodstuffs regulation if specifications for fish meat are lacking) by various regulatory bodies.

| Metal | Mean conc. $mg \cdot kg^{-1}$ (n = 14 x 6) | Max. conc. $mg \cdot kg^{-1}$ (number of samples exceeding MAL) | South African regulation[1] | USA regulation[2] | EU regulation[3] |
|---|---|---|---|---|---|
| Fe | 31.34 | 152.10 | - | 45 $mg \cdot day^{-1}$ | - |
| Zn | 13.88 | 26.48 | - | 40 $mg \cdot day^{-1}$ | - |
| Se | 1.89 | 10.59 | - | 0.4 $mg \cdot day^{-1}$ | - |
| As | 1.53 | 6.86 (7) | 3 $mg \cdot kg^{-1}$ | UL not established | - |
| Cu | 1.21 | 5.23 | - | 10 $mg \cdot day^{-1}$ | - |
| Al | 0.84 | 7.33 | - | - | - |
| Hg | 0.77 | 1.52 (16) | 1.0 $mg \cdot kg^{-1}$ | - | 1.0 $mg \cdot kg^{-1}$ |
| Mn | 0.11 | 0.41 | - | 11 $mg \cdot day^{-1}$ | - |
| Cr | 0.07 | 0.46 | - | UL not established | - |
| Ni | 0.03 | 0.20 | - | 1 $mg \cdot day^{-1}$ | - |
| Cd | 0.03 | 0.10 | 1.0 $mg \cdot kg^{-1}$ | - | 0.1 $mg \cdot kg^{-1}$ |
| Sn | 0.01 | 0.15 | 50 $mg \cdot kg^{-1}$ (in uncanned meat and meat products) | - | - |
| Pb | 0.009 | 0.075 | 0.5 $mg \cdot kg^{-1}$ | - | 0.3 $mg \cdot kg^{-1}$ |
| Co | 0.008 | 0.044 | - | - | - |
| Mo | 0.006 | 0.006 | - | 2 $mg \cdot day^{-1}$ | - |
| Sb | 0.003 | 0.003 | 0.15 $mg \cdot kg^{-1}$ (in all liquid foodstuffs) | - | - |

[1]Department of Health, 2004; [2]Food and Nutrition Board, 2000; 2001; [3]Commission Regulation (EC), 2001; 2006; 2008

Iron and Zn had the highest mean concentrations (n = 14 tuna x 6 carcass sites), similar to what was found in a study by Uysal *et al*. (2008) in tissue (muscle, gill, skin, intestine and liver) of six common marine fish off the Turkish coast. Even though this might be a common trend, neither South African, nor European Union regulations have established MALs for these metals in fish products because cases of toxic Fe and Zn exposure through fish consumption have not yet been reported. USA regulations specify that Fe and Zn intake should not exceed 45 $mg \cdot day^{-1}$ and 40 $mg \cdot day^{-1}$ respectively from any source of intake. However, Fe deficiency in humans is generally a more serious concern than overexposure (Food and Nutrition Board, 2001). An average 150 g portion of yellowfin tuna meat from the current study would barely exceed 10.5% and 5% of this MAL for Fe and Zn respectively, but would provide almost 60% of the recommended daily intake (RDI) for Fe (8 $mg \cdot day^{-1}$) (Food and Nutrition Board, 2001) and could therefore be considered a healthy source of Fe. Iron has an essential function for many enzymes and proteins in the human body such as in haemoglobin which is necessary for carrying oxygen in the blood through the body. However, an excessively high Fe concentration can decrease absorption of Zn (EC, 2003), which is considered an essential metal at low concentrations. Zinc is of very low toxicity at low or moderate levels (Dural *et al.*, 2007), and levels in the current yellowfin tuna samples would therefore not contribute to health concerns, but prolonged exposure to Zn can lead to Fe and Cu deficiencies as well as symptoms such as nausea, vomiting, fever, headache, tiredness, and abdominal pain (Dural *et al.*, 2007).

All average (of six sample sites per tuna) metal concentrations were well within the corresponding safety limit regulations; however, some individual samples measured exceeded these limits (Table 6.3). For As and Cd (according to EU regulation only), all samples that were very close to or exceeding the upper limit were restricted to the posterior sample of the dark muscle and to entire dark muscle for Se. However, for five of the 14 tuna (36%), Hg levels close to or above maximum allowable limits were detected across the entire carcass. Mercury levels in yellowfin and other tuna species in the range of 0.03 – 2.12 $mg \cdot kg^{-1}$ (Ferriss & Essington, 2011; Ordiano-Flores *et al.*, 2011; Forsyth *et al.*, 2014) have been reported, with several measurements above the maximum allowable limit (1.0 $mg \cdot kg^{-1}$ total Hg), suggesting that yellowfin tuna contains high levels of Hg compared to other fish species (Al-Busaidi *et al.*, 2011).

### 6.4.1 Inter- and intramuscular variation in metal accumulation

Differences in function and composition between white and dark muscle in fish could account for the differences in metal accumulation observed between the two yellowfin muscle types. Whereas white muscle is used mainly during short-term vigorous movement with anaerobic metabolic activity, dark muscle is responsible for sustained swimming motion with oxidative metabolic activity (Hamoir & Gerardin-Otthiers, 1980; Ashoka *et al.*, 2011). Dark muscle therefore has a high content of myoglobin and haemoglobin, which are oxygen-binding proteins which contain Fe as part of the haem group structures, contributing to the dark muscle colour (Ashoka *et al.*, 2011). This accounts for the significantly higher Fe measurements in the dark muscle compared to white muscle found in this study.

Aside from Fe, seven other metals (Mn, Co, Cu, As, Se, Cd and Hg) had higher concentrations in the dark yellowfin tuna muscle compared to the white muscle. This agrees with other studies that found metals such as Fe, Cu, Zn and Cd to be more highly accumulated in dark muscle than in white muscle of ling (*Genypterus blacodes*) (Ashoka *et al.*, 2011). Ashoka *et al*. (2011) ascribes the increased accumulation of essential metals in dark muscle to the metals' biological functions in enzymes and proteins, which have shown notable differences between white and dark muscle. Dark muscle has been found to have higher concentrations of enzymes involved in oxidative metabolism similar to that of the liver, which could explain its increased metal accumulation (Ashoka *et al.*, 2011).

The increase of metal accumulation towards the tail end of the dark muscle could result from differences in muscle fibre development within the fish. As migratory fish, tuna are known for continuous strong swimming which is driven by the dark muscle with virtually all of the thrust produced at the tail blade (Shadwick *et al*., 1999). Te Kronnié (2000) found the rate of muscle fibre development to be correlated to muscle activity. Therefore, higher activity in the posterior/caudal region of tuna could cause an increased rate of muscle fibre development in this region. Love (1958, 1968) has also observed that muscle cells and myomeres decrease in size towards the tail end of the fish with connective tissue between muscle cells increasing (Ashoka *et al.*, 2011). This could be a possible reason for the higher concentration of metals accumulated in the dark muscle tissue towards the tail. Ashoka *et al*. (2011) found similar results for increasing concentrations of several metals (Fe, Cu, Zn and Cd) towards the tail end of ling fillets, whereas other metals (Mn, As, Se) had a decrease in concentration toward the tail region, as was similarly found for Fe in the ventral lateral yellowfin muscle in the current study.

### 6.4.2 Inter-metal correlations

Many of the variables are positively correlated with one another (Fig. 6.1), even though some of these associations are weak. Even though several individual significant positive correlations are seen among metals in Group 1 (Cr, Mg, Fe, Co, Ni, Cu and As), not all of these metals are equally correlated with each other. From these results, it is therefore not possible to say whether accumulation of all of these metals in Group 1 is facilitated or promoted by the same influencing factors or whether one or more of these metals facilitate accumulation of others. Further research, which specifically investigates the accumulation processes of individual metals and the different factors which affect this accumulation, is necessary in order to make any such conclusions. However, measurement of one or more of these metals could be used as an indication of the concentrations of other metals in the same group.

The concentrations of Hg, Cd and Pb in Group 2 are all strongly associated with each other, and Hg and Cd increases and Pb proportionately decreases with increasing fish weight. Hg, Cd and Pb are therefore the only metals where levels could possibly be predicted by measurable body parameters (fish weight). From the current results, smaller yellowfin tuna (below 70 kg body weight) would be considered safe for consumption with regards to overall metal toxicity as maximum Pb concentrations are still well below the

maximum limit (0.5 mg·kg$^{-1}$) (Table 6.3), whereas larger individuals (above 70 kg body weight) are more likely to have Cd and/or Hg levels close to or exceeding maximum allowable limits (Fig. 6.2) as discussed for Hg in Chapter 3.

Similar correlations between concentrations of Hg, Cd and Pb were found in salted anchovies (Storelli *et al.*, 2011), whereas in canned tuna muscle Ashraf (2006) found negative correlations between Hg and both Pb and Cd. No relationship between Hg, Cd and Pb was found in 10 marine fish species in Oman (Al-Busaidi *et al.*, 2011). In a study on demersal fish from the Mediterranean Sea, Storelli and Barone (2013) found that Hg had a strong positive relationship with fish size, whereas both Cd and Pb were not affected by fish size. Therefore, even though Hg, Cd and Pb are often correlated, inter-metal correlations as well as correlations between metals and fish size are clearly species and area specific (Canli & Atli, 2003).

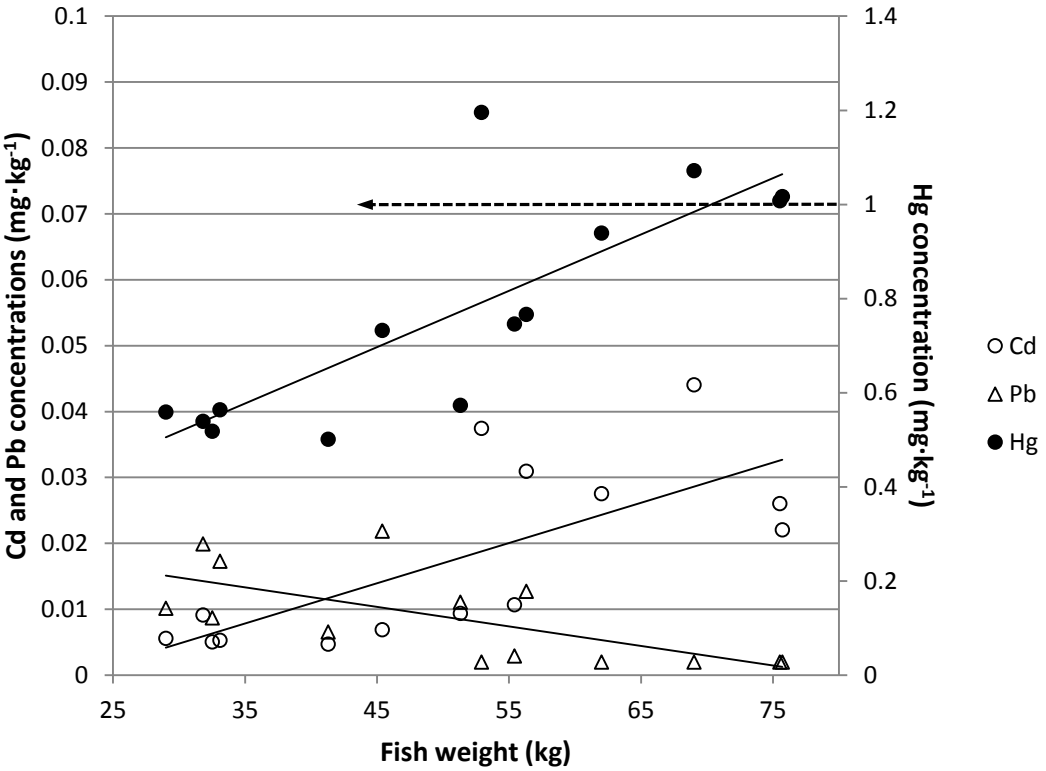


**Figure 6.2** Correlations between fish weight (kg) and average concentrations of Cd (○), Pb (Δ) and Hg (●) per fish. Dashed line indicates maximum allowable limit for Hg.

## 6.5 Conclusion

Yellowfin tuna caught off the South African coast can be considered a safe source of essential metals such as Fe and Zn when consuming both white and dark muscle. Variation in metal accumulation is evident across the carcass; therefore sampling from a single carcass position for monitoring of toxic metal levels in yellowfin tuna would misrepresent the overall carcass muscle. Toxic levels for some metals (Se, As, Cd) have been detected in individual samples of dark muscle, whereas white muscle can be considered safe with regards to

most metals. Restricting the consumption of yellowfin dark muscle may protect consumers from toxic metal concentrations without unnecessarily discarding white muscle portions which could be considered safe for consumption. Mercury has, however, been detected in toxic levels in both white and dark muscle samples even though the overall average Hg level (n=6x14) is below the maximum allowable limit. Implementing the suggested size limit of 70kg, for commercial yellowfin tuna could limit consumer exposure to toxic Hg levels as Hg levels increase with fish weight.

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# CHAPTER 7:

## Heavy metal concentration and toxicity in blacktail (*Diplodus sargus capensis*) and hottentot (*Pachymetopon blochii*) along the South African coastline

**ABSTRACT**

The concentrations of 16 metals were measured in blacktail (*Diplodus sargus capensis*) and hottentot (*Pachymetopon blochii*) fish flesh sampled from several locations along the South African coast to assess the inter-spatial and interspecific variations in metal accumulation. Interspecific variation was evident with higher mean concentrations of Al, Fe, Co, Zn, As, Se, Hg and Pb in blacktail than hottentot. Spatial variation is metal specific and metal concentrations in blacktail and hottentot are not clearly correlated to location, however, As is found in higher concentrations in fish along the South-east coast than the West coast. Both blacktail and hottentot can be considered safe for human consumption with regards to metal toxicity, as As (blacktail: 1.82 ± 1.22 mg·kg$^{-1}$; hottentot: 0.77 ± 0.22 mg·kg$^{-1}$), Cd (blacktail: 0.005 ± 0.012 mg·kg$^{-1}$; hottentot: 0.005 ± 0.005 mg·kg$^{-1}$), Hg (blacktail: 0.19 ± 0.12 mg·kg$^{-1}$; hottentot: 0.10 ± 0.07 mg·kg$^{-1}$) and Pb (blacktail: 0.03 ± 0.03 mg·kg$^{-1}$; hottentot: 0.005 ± 0.010 mg·kg$^{-1}$) levels are within safety guideline limits.

**Keywords**: Fish muscle, Heavy metals, Mercury, ICP-MS, blacktail, hottentot, Consumer health

## 7.1 Introduction

Industrial and agricultural advances are imperative to the nutritional and overall development of third world countries in a continent such as Africa (Mellor, 1986). However, these developments could also negatively affect the environment by increasing the amount of bioavailable metal contaminants in the environment through pollution from industrial and agricultural activity and even informal settlements (Cloete & Watling, 1981). High levels of metals such as lead (Pb), mercury (Hg), cadmium (Cd), aluminium (Al) and copper (Cu) have been detected in South African rivers and estuaries all along the coast, clearly showing how industrial activity and urbanisation close to rivers affect the metal content of the aquatic environment (Binning & Baird, 2001; Learner *et al.*, 2009; Hutchings & Clark, 2010; Jooste *et al.*, 2014; Olaniran *et al.*, 2014). Such metal contaminants can be carried downstream (Oosthuizen & Ehrlich, 2001), eventually reaching the marine system. Previous studies have measured water metal concentrations in South African harbours indicating how pollution from point sources around harbours enters the marine environment (Fatoki & Mathabatha, 2001) and from there the marine food chain (Clarkson & Magos, 2006). Given the importance of the marine ecosystem in supplying food globally and locally, the content of pollutants in consumed marine species needs to be investigated (Donoghue & Marshall, 2003).

Blacktail (*Diplodus sargus capensis*) and hottentot (*Pachymetopon blochii*) are resident fish species in South African waters, often caught in harbours or rocky shores near harbours (Coetzee, 1986), and may therefore be influenced by metal pollution in water and sediments near the coast. Hottentot is found in cool/temperate water and is therefore concentrated on the west coast of South Africa, whereas blacktail is more commonly found on the eastern coast from Cape Point to southern Mozambique (Kerwath & Winker, 2013; Mann & Dunlop, 2013). Both species are important angling species caught in South Africa, with blacktail currently ranked as the third most important shore angling species (Mann & Dunlop, 2013) and hottentot among the most important target species for the traditional linefishery on the West coast. They are both resident omnivores with very similar diets, blacktail feeding on benthic prey consisting mostly of small crustaceans, algae and polychaetes (Mann & Buxton, 1992) including smaller proportions of bivalves, crabs, ascidians and sea urchins (Coetzee, 1986) and hottentot feeding on algae, polychaetes, amphipods, crabs, shrimp, hydrozoa, sea urchins, molluscs, redbait and occasionally fish (Kerwath & Winker, 2013). Larger long-lived predatory fish (shark, tuna and swordfish) can reach elevated levels of certain metals such as mercury due to a life-long bioaccumulation (Ordiano-Flores *et al.*, 2011, Bosch *et al.*, 2016a, b). Even though blacktail and hottentot are not high up in the food chain, both species have been recorded to live up to 21 years and weigh up to 2.7 kg (Kerwath & Winker, 2013; Mann & Dunlop, 2013), which may allow for significant metal accumulation in such species (Pastor *et al.*, 1994; Yilmaz, 2005; Ferreira *et al.*, 2008).

Despite the commercial and recreational importance of blacktail and hottentot within South Africa (Smale, 1992; Penney *et al*., 1999), little information is available on the safety of these species in terms of metal toxicity. Contaminant monitoring is needed in order to protect the South African fish consumer,

whether fish are obtained from a commercial source or caught directly. The United States has implemented monitoring strategies which involve an initial screening of water bodies in order to get an overview of contamination levels and to identify harvesting sites where edible fish may provide potential concern to consumer health (USEPA, 2000). In order to obtain a representative overview, samples need to be taken from various locations along the coast (USEPA, 2000) as other marine organisms along the South African coast have shown clear variation in accumulated metal contents between different locations and could be linked to areas of higher and lower metal contamination (Carro *et al.*, 2012; Joubert, 2014).

The aim of this study was to measure the concentration of 16 individual metals in hottentot and blacktail from 10 sites along the South African coastline (Hondeklip Bay, Lamberts Bay, Saldanha Bay, Dassen Island, Hout Bay, False Bay, Witsand, Blombos, Mossel Bay, Port Elizabeth and Durban) in areas considered minimally and heavily polluted (industrial, agricultural and urban pollution) in order to get an overview of metal contamination in two of South Africa's important lower trophic level fish species and to investigate whether these species could serve as possible indicators of environmental pollution.

## 7.2 Materials and methods

### 7.2.1 Sampling

Hottentot (*P. blochii*) were sampled mainly on the west coast from Hondeklip Bay (n = 10), Lamberts Bay (n = 10), Saldanha Bay (n = 7), Dassen Island (n = 10), Hout Bay (n = 10) and Kalk Bay (n = 10) (in False Bay). Blacktail (*D. sargus capensis*) were sampled mainly from the east and south coasts at Durban (n = 16), Port Elizabeth (n = 8), Mossel Bay (n = 15), Blombos (n = 5), Witsand (including 2 locations: in the Breede river (n = 4) and from the Witsand beach about 2km from the river mouth (n = 6)), Muizenberg (in False Bay) (n = 14) and Saldanha Bay (n = 10) (Fig. 7.1). Therefore an overlap in sampling location for the two species occurred at two locations, namely, Saldanha Bay and False Bay (Kalk Bay and Muizenberg).

All sampling sites are common fishing areas, either commercially or recreationally. Seven of the sampling sites (Saldanha Bay, Hout Bay, False Bay (Kalk Bay & Muizenberg), Mossel Bay, Port Elizabeth and Durban) had been previously identified by the Committee of Marine Pollution in South Africa (Cloete & Watling, 1981) as major sites of pollution needing frequent monitoring. Hondeklip Bay, Lamberts Bay and Blombos are considered non-polluted areas as they are free from major industrial activity and although Witsand itself is free from industrial activity and pollution, it is situated at the mouth of the Breede River which runs through large agricultural areas, possibly carrying effluent contaminants towards the river mouth.

Overall 134 samples were collected and analysed. Fish were caught by rod and line or spearfished by recreational fisher persons and researchers, frozen whole (-18 °C) and transported to research facilities at Stellenbosch University. Samples were thawed at 4 °C for 12 to 24 hours and lengths and weights recorded before filleting and homogenising the individual meat samples. The entire fillets were homogenised in order

to obtain a representative sample for analysis. Ceramic knives were used for processing and flesh that was pierced by the spear was trimmed and discarded to minimise metal contamination of samples. Homogenised tissue samples were vacuum sealed in clean, labelled polyethylene bags and stored at -20 °C until analysed.

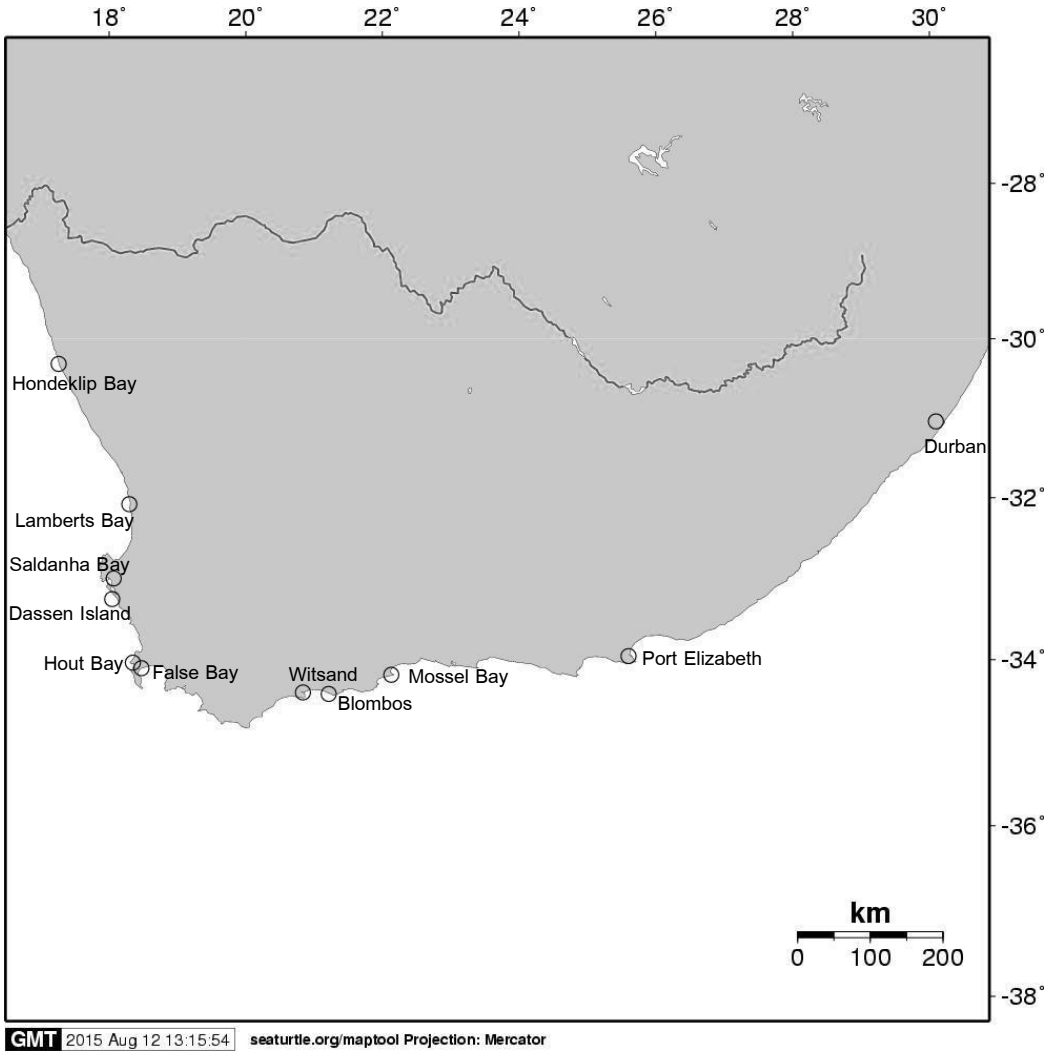


**Figure 7.1** Locations of sampling sites along the South African coastline.

### 7.2.2 Analytical method

The concentrations of 16 metals (aluminium (Al), manganese (Mn), cobalt (Co), nickel (Ni), molybdenum (Mo), tin (Sn), iron (Fe), copper (Cu), chromium (Cr), zinc (Zn), selenium (Se), arsenic (As), antimony (Sb), cadmium (Cd), mercury (Hg) and lead (Pb)) were analysed through inductively coupled plasma mass spectrometry (ICP-MS) for each meat sample (n = 134) as described in Chapters 3 and 4.

### 7.2.3 Statistical analysis

STATISTICA 12.5 was used for statistical analysis. All data were log-transformed to conform to assumptions of normality. Levene's test was used to test for homogeneity of variance between groups and where the null hypothesis was rejected weighted means were used when performing parametric analysis together with Games-Howell post-hoc test. Differences in the mean metal concentrations with regards to location were

tested by analysis of covariance (ANCOVA) at 95% confidence level with fish weight included as covariate. Principal component analysis was done and Spearman's correlations were calculated to investigate correlations between individual metal concentrations (data from both species pooled) and between metal concentrations and fish weight.

## 7.3 Results

Mean concentrations per metal for each species are given in Table 7.1. Molybdenum, Sn and Sb were detected in low concentrations in only a select few (n = 8 to 14) samples and were therefore considered insignificant. The ranking order of metal concentrations in muscle samples were Zn > Fe > Al > As > Cu > Se > Mn > Hg > Ni > Cr > Pb > Cd > Co and Zn > Fe > Al > As > Se > Cu > Hg > Mn > Ni > Cr > Pb > Co > Cd for hottentot (n = 58) and blacktail (n = 76), respectively.

**Table 7.1** Comparison of mean metal concentrations ($mg \cdot kg^{-1}$ ± std dev) between species (data from all sampling sites pooled).

| Metal | hottentot | blacktail | p-value |
|---|---|---|---|
| Al | 1.14 ± 1.342 | 1.89 ± 1.996 | < 0.01 |
| Cr | 0.02 ± 0.037 | 0.04 ± 0.067 | > 0.05 |
| Mn | 0.14 ± 0.053 | 0.14 ± 0.214 | > 0.05 |
| Fe | 2.85 ± 1.024 | 3.66 ± 1.407 | < 0.001 |
| Co | 0.002 ± 0.0014 | 0.009 ± 0.0089 | < 0.0001 |
| Ni | 0.06 ± 0.103 | 0.04 ± 0.088 | > 0.05 |
| Cu | 0.26 ± 0.162 | 0.27 ± 0.120 | > 0.05 |
| Zn | 3.35 ± 0.529 | 3.70 ± 0.702 | < 0.05 |
| As | 0.77 ± 0.218 | 1.82 ± 1.223 | < 0.0001 |
| Se | 0.21 ± 0.081 | 0.28 ± 0.131 | < 0.001 |
| Cd | 0.005 ± 0.0048 | 0.005 ± 0.0115 | > 0.05 |
| Hg | 0.10 ± 0.070 | 0.19 ± 0.116 | < 0.0001 |
| Pb | 0.005 ± 0.0097 | 0.03 ± 0.031 | < 0.0001 |
| Mo | > 90% under LOD* | > 90% under LOD | - |
| Sb | > 90% under LOD | > 90% under LOD | - |
| Sn | > 90% under LOD | > 90% under LOD | - |

*LOD = Level of detection

The size parameters of each sampling group are shown in Table 7.2. Fish weight (g) and total length (cm) were strongly correlated (r > 0.90), therefore only fish weight was used as indication of fish size during the statistical analyses. Results show that there were significant correlations (p < 0.01) between fish weight and metal concentrations for several of the metals, with concentrations of Cr, Mn, Co, Zn, Cd and Pb being negatively correlated (r = -0.54, -0.39, -0.50, -0.43, -0.43, -0.31, respectively) and Al and Hg positively correlated (r = 0.41 and 0.40, respectively) to fish weight in blacktail whereas only two metals (Cr and Fe) were positively correlated (r = 0.39 and 0.43, respectively) to fish weight in hottentot. Principle component

analysis reveals that there were limited inter-metal correlations where only 20% and 14% of variation was accounted for by principal component 1 and 2, respectively.

**Table 7.2** Size parameters of sample groups per sampling location.

| Species | Location | n | Weight range in g (average) | Total length range in cm (average) |
|---|---|---|---|---|
| hottentot | Hondeklip Bay | 10 | 451.92 - 696.35 (587.71) | 30.00 - 32.50 (31.38) |
| hottentot | Lamberts Bay | 10 | 371.36 - 591.70 (450.85) | 26.90 - 32.00 (28.67) |
| hottentot | Saldanha Bay | 7 | 304.93 - 753.49 (469.80) | 25.50 - 34.00 (29.07) |
| blacktail | Saldanha Bay | 10 | 397.13 - 672.27 (550.43) | 29.00 - 34.50 (32.08) |
| hottentot | Dassen Island | 10 | 680.00 - 900.00 (748.00) | 30.10 - 34.20 (32.37) |
| hottentot | Hout Bay | 10 | 405.88 - 840.08 (590.59) | 29.00 - 36.80 (32.71) |
| hottentot | Kalk Bay | 11 | 369.61 - 675.60 (488.36) | 26.20 - 33.20 (29.60) |
| blacktail | Muizenberg | 14 | 354.00 - 1272.00 (759.93) | 27.00 - 40.70 (34.34) |
| blacktail | Blombos | 5 | 490.29 - 617.65 (552.96) | 30.40 - 33.50 (31.68) |
| blacktail | Breede River mouth | 4 | 203.59 - 656.00 (368.15) | 23.20 - 32.70 (26.68) |
| blacktail | Witsand beach | 6 | 280.53 - 831.00 (620.95) | 24.10 - 36.10 (32.12) |
| blacktail | Mossel Bay | 15 | 274.63 - 867.99 (431.48) | 25.50 - 36.00 (28.70) |
| blacktail | Port Elizabeth | 8 | 233.73 - 869.32 (464.71) | 26.20 - 53.80 (32.59) |
| blacktail | Durban | 14 | 106.95 - 391.72 (218.15) | 18.80 - 27.20 (22.98) |
| hottentot | Total | 58 | 304.93 - 900.00 (559.17) | 25.50 - 36.80 (30.70) |
| blacktail | Total | 76 | 106.95 - 1272.00 (491.45) | 18.80 - 53.80 (30.08) |

### 7.3.1 Interspecific variation

Blacktail has overall (data from all sampling sites pooled) significantly higher ($p < 0.05$) metal concentrations than hottentot for most metals (Al, Fe, Co, Zn, As, Se, Hg and Pb) whilst the remainder (Cr, Mn, Ni, Cu and Cd) displayed no significant differences (Table 7.1). In overlapping sampling areas (Saldanha Bay and False Bay), blacktail had significantly ($p < 0.05$) higher concentrations than hottentot for Fe, Cu, Zn, Hg, and Pb in Saldanha Bay and Al, Hg and Pb in False Bay (Table 7.3). Ni was the only metal which showed higher concentration ($p < 0.05$) in hottentot than in blacktail (False Bay). The remaining six metals showed no significant differences ($p > 0.05$) between species.

### 7.3.2 Spatial variation

From the mean metal concentrations per sampling site shown in Table 7.3, it is clear that metal concentrations are not identical in all sampling sites. However, there are several sites with similar concentrations for each individual metal, although these sites of similar metal accumulations vary between metals. Due to variation within sites, no single sampling site has higher or lower metal concentrations than all other sites. Compared to other sites, blacktail from Durban has some of the higher metal accumulations for Cr, Fe, Co, Zn, Cd, Hg and Pb, but lower concentrations for Al, As and Se and concentrations that do not

**Table 7.3** Weighted means of metal concentrations (mg·kg$^{-1}$) in *D. sargus capensis* (B) and *Pachymetopon blochii* (H) per sampling group with standard deviation below in parentheses. HO = Hondeklip Bay; LB = Lamberts Bay; SB = Saldanha Bay; DI = Dassen Island; HB = Hout Bay; KB = Kalk Bay; MZ = Muizenberg; BB = Blombos; BR = Breede River mouth; W = Witsand beach; MS = Mossel Bay; PE = Port Elizabeth; D = Durban. Identical superscript letters indicate non-significant ($p > 0.05$) differences between sites per metal.

| | H: HO | H: LB | H: SB | B: SB | H: DI | H: HB | H: KB | B: MZ | B: BB | B: BR | B: W | B: MB | B: PE | B: D |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Al | $0.75^{a}$ (1.264) | $1.62^{b}$ (2.357) | $0.15^{a}$ (0.104) | $1.82^{a}$ (2.012) | $1.02^{a}$ (0.745) | $1.20^{a}$ (0.872) | $1.75^{a}$ (1.068) | $4.27^{a}$ (0.674) | $1.51^{a}$ (0.973) | $2.25^{ab}$ (2.394) | $2.82^{ab}$ (3.163) | $0.78^{b}$ (0.782) | $0.45^{a}$ (0.464) | $1.19^{b}$ (1.913) |
| Cr | $0.008^{b}$ (0.0056) | $0.02^{ab}$ (0.026) | $0.005^{b}$ (0.0063) | $0.04^{ab}$ (0.061) | $0.07^{ab}$ (0.063) | $0.008^{b}$ (0.0030) | $0.01^{b}$ (0.017) | $0.01^{b}$ (0.0056) | $0.03^{ab}$ (0.047) | $0.09^{ab}$ (0.087) | $0.04^{ab}$ (0.045) | $0.03^{ab}$ (0.041) | $0.09^{ab}$ (0.168) | $0.05^{a}$ (0.015) |
| Mn | $0.15^{a}$ (0.025) | $0.16^{a}$ (0.048) | $0.14^{ab}$ (0.036) | $0.13^{abc}$ (0.098) | $0.15^{abc}$ (0.061) | $0.12^{abc}$ (0.080) | $0.10^{abc}$ (0.033) | $0.08^{bc}$ (0.036) | $0.10^{abc}$ (0.055) | $0.72^{abc}$ (0.723) | $0.10^{abc}$ (0.027) | $0.07^{c}$ (0.026) | $0.10^{abc}$ (0.061) | $0.16^{abc}$ (0.121) |
| Fe | $2.62^{gc}$ (0.827) | $2.62^{ge}$ (1.020) | $1.88^{g}$ (0.574) | $4.67^{a}$ (0.841) | $4.00^{ab}$ (0.904) | $2.56^{gd}$ (0.588) | $3.12^{bcdef}$ (0.962) | $3.42^{b}$ (0.421) | $3.43^{abcde}$ (0.597) | $3.82^{abc}$ (1.560) | $3.55^{abcd}$ (0.916) | $3.47^{b}$ (0.854) | $2.69^{gf}$ (2.034) | $4.04^{ab}$ (2.255) |
| Co | $0.003^{b}$ (0.002) | $0.002^{b}$ (0.0008) | $0.002^{b}$ (0.002) | $0.01^{ab}$ (0.010) | $0.002^{b}$ (0.001) | $0.003^{b}$ (0.001) | $0.002^{b}$ (0.0002) | $0.003^{b}$ (0.002) | $0.003^{b}$ (0.0005) | $0.02^{ab}$ (0.010) | $0.005^{b}$ (0.003) | $0.004^{b}$ (0.002) | $0.01^{ab}$ (0.010) | $0.02^{a}$ (0.009) |
| Ni | $0.10^{ab}$ (0.176) | $0.10^{ab}$ (0.165) | $0.007^{b}$ (0.007) | $0.02^{ab}$ (0.028) | $0.05^{ab}$ (0.033) | $0.03^{ab}$ (0.019) | $0.06^{a}$ (0.030) | $0.01^{a}$ (0.010) | $0.03^{ab}$ (0.023) | $0.06^{ab}$ (0.062) | $0.17^{ab}$ (0.269) | $0.03^{ab}$ (0.031) | $0.06^{ab}$ (0.089) | $0.02^{ab}$ (0.022) |
| Cu | $0.23^{abc}$ (0.097) | $0.33^{abc}$ (0.249) | $0.16^{c}$ (0.045) | $0.30^{a}$ (0.037) | $0.29^{ab}$ (0.075) | $0.19^{c}$ (0.027) | $0.34^{abc}$ (0.228) | $0.26^{ab}$ (0.047) | $0.22^{bc}$ (0.010) | $0.27^{abc}$ (0.175) | $0.25^{abc}$ (0.040) | $0.29^{abc}$ (0.192) | $0.30^{abc}$ (0.212) | $0.25^{abc}$ (0.069) |
| Zn | $3.34^{bcd}$ (0.325) | $3.89^{a}$ (0.371) | $2.70^{e}$ (0.290) | $3.99^{a}$ (0.703) | $3.18^{cd}$ (0.422) | $3.43^{bc}$ (0.577) | $3.37^{bcd}$ (0.488) | $3.01^{de}$ (0.398) | $3.88^{ab}$ (0.567) | $4.25^{a}$ (0.651) | $3.73^{ab}$ (0.351) | $3.64^{ab}$ (0.445) | $3.73^{ab}$ (0.977) | $4.00^{a}$ (0.760) |
| As | $0.59^{d}$ (0.203) | $1.02^{ce}$ (0.149) | $0.73^{cd}$ (0.202) | $0.92^{cde}$ (0.648) | $0.88^{cde}$ (0.158) | $0.64^{d}$ (0.103) | $0.74^{cd}$ (0.182) | $1.31^{be}$ (0.499) | $2.73^{abcd}$ (1.373) | $1.11^{abcd}$ (0.377) | $1.86^{abe}$ (0.560) | $2.76^{a}$ (1.480) | $2.94^{ab}$ (1.071) | $1.18^{cde}$ (0.881) |
| Se | $0.21^{b}$ (0.065) | $0.27^{ab}$ (0.087) | $0.27^{ab}$ (0.053) | $0.37^{a}$ (0.089) | $0.16^{b}$ (0.066) | $0.23^{b}$ (0.042) | $0.15^{b}$ (0.083) | $0.26^{ab}$ (0.066) | $0.17^{b}$ (0.060) | $0.47^{ab}$ (0.257) | $0.43^{ab}$ (0.191) | $0.27^{ab}$ (0.123) | $0.21^{b}$ (0.026) | $0.23^{b}$ (0.064) |
| Cd | $0.005^{b}$ (0.003) | $0.007^{ab}$ (0.009) | $0.001^{b}$ (0.002) | $0.004^{b}$ (0.006) | $0.005^{b}$ (0.003) | $0.005^{b}$ (0.002) | $0.006^{ab}$ (0.004) | $0.001^{b}$ (0.0006) | $0.002^{b}$ (0.0003) | $0.002^{b}$ (0.0006) | $0.002^{b}$ (0.000) | $0.005^{b}$ (0.004) | $0.008^{ab}$ (0.007) | $0.01^{a}$ (0.025) |
| Hg | $0.07^{ce}$ (0.011) | $0.05^{de}$ (0.011) | $0.03^{d}$ (0.016) | $0.26^{ab}$ (0.107) | $0.11^{cf}$ (0.036) | $0.12^{bc}$ (0.039) | $0.17^{abce}$ (0.113) | $0.22^{a}$ (0.082) | $0.12^{bc}$ (0.023) | $0.22^{abcd}$ (0.160) | $0.18^{abcd}$ (0.086) | $0.19^{abce}$ (0.169) | $0.21^{abcd}$ (0.131) | $0.15^{abf}$ (0.063) |
| Pb | $0.003^{de}$ (0.003) | $0.01^{bde}$ (0.021) | $0.0003^{e}$ (0.0000) | $0.03^{abc}$ (0.020) | $0.007^{dc}$ (0.003) | $0.003^{de}$ (0.006) | $0.001^{e}$ (0.0007) | $0.02^{b}$ (0.009) | $0.009^{bde}$ (0.015) | $0.01^{bde}$ (0.009) | $0.005^{dec}$ (0.004) | $0.01^{bde}$ (0.018) | $0.02^{bde}$ (0.020) | $0.07^{a}$ (0.043) |

differ significantly from either the highest or lowest concentrations for Mn, Ni and Cu. Blacktail from Saldanha Bay has some of the highest concentrations for Fe, Cu, Zn, Se, Hg and Pb and of the lower concentrations for As and Cd and concentrations for Al, Cr, Mn, Co and Ni that do not differ significantly from either the sites of the highest or the lowest concentrations. For the same area, hottenhot had the lowest concentration of Al, Cr, Fe, Co, Ni, Cu, Zn, Cd, Hg and Pb. On the contrary, no significant ($p > 0.05$) differences in metal accumulation in blacktail were observed among three spatially close sites; one which is a private nature reserve and considered an unpolluted site (Blombos) and the other two (Breede River and Witsand beach next to river mouth) which may be polluted from inland agricultural runoff carried downstream by the Breede River.

## 7.4 Discussion

Human exposure to toxic levels of metals is dependent on the frequencies, quantities and types of fish consumed. As these may differ among communities and cultures (WHO/FAO, 2011a,b), regulatory limits for metals in fish may vary as per regulatory body (Food and Nutrition Board, 2001). In this study metal concentrations will be compared to South African regulatory limits as well as EU and USA regulation (Table 7.4). As in the case of exports, fish safety should be evaluated according to the regulations of the country in which the fish will be consumed.

**Table 7.4** Summary of the mean metal concentrations ($mg \cdot kg^{-1} \pm$ std dev) measured in blacktail and hottentot around the South African coastline as well as specific regulatory maximum allowable limits (MAL) and upper limits (UL) as set by South African, United States and European Union legislation for metals in fish meat. The number of individuals exceeding the regulatory limits is given in parentheses.

| Metal | hottentot (n = 58) | blacktail (n = 76) | South African regulation[1] | USA regulation[2] | EU regulation[3] |
|---|---|---|---|---|---|
| Zn | 3.35 ± 0.529 | 3.70 ± 0.702 | - | 40 $mg \cdot day^{-1}$ | - |
| Fe | 2.85 ± 1.024 | 3.66 ± 1.407 | - | 45 $mg \cdot day^{-1}$ | - |
| Al | 1.14 ± 1.342 | 1.89 ± 1.996 | - | - | - |
| As | 0.77 ± 0.218 | 1.82 ± 1.223 (13) | 3.0 $mg \cdot kg^{-1}$ | UL not established | - |
| Cu | 0.26 ± 0.162 | 0.27 ± 0.120 | - | 10 $mg \cdot day^{-1}$ | - |
| Se | 0.21 ± 0.081 | 0.28 ± 0.131 | - | 0.4 $mg \cdot day^{-1}$ | - |
| Mn | 0.14 ± 0.053 | 0.14 ± 0.214 | - | 11 $mg \cdot day^{-1}$ | - |
| Hg | 0.10 ± 0.070 | 0.19 ± 0.116 (1) | 0.5 $mg \cdot kg^{-1}$ | - | 0.5 $mg \cdot kg^{-1}$ |
| Ni | 0.06 ± 0.103 | 0.04 ± 0.088 | - | 1 $mg \cdot day^{-1}$ | - |
| Cr | 0.02 ± 0.037 | 0.04 ± 0.067 | - | UL not established | - |
| Pb | 0.005 ± 0.010 | 0.03 ± 0.031 | 0.5 $mg \cdot kg^{-1}$ | - | 0.3 $mg \cdot kg^{-1}$ |
| Co | 0.002 ± 0.001 | 0.009 ± 0.009 | - | - | - |
| Cd | 0.005 ± 0.005 | 0.005 ± 0.012 | 1.0 $mg \cdot kg^{-1}$ | - | 0.1 $mg \cdot kg^{-1}$ |

[1]Department of Health, 2004; [2]Food and Nutrition Board, 2000; 2001; [3]Commission Regulation (EC), 2001; 2006; 2008

Toxicity levels of metals are metal specific (Goyer, 1997); therefore high concentrations of certain metals in fish meat may not present any danger to consumer health while other metals with lower concentrations may be extremely harmful when consumed. Zinc and Fe are the two most abundant metals in both blacktail and hottentot as was similarly presented for other fish species (Kojadinovic *et al*., 2007; Uysal *et al*., 2008). However, both these metals are well below their upper limits (UL) for safe daily consumption as set in the USA regulations (Food and Nutrition Board, 2001). For the majority of the 16 metals assessed in this study, no maximum allowable limits (MAL) have been set for fish as they are not known to contribute substantially to toxic metal intake through fish meat. Four metals that have been reported to contribute to toxic intake of metals through fish consumption are As, Cd, Hg and Pb (Llobet *et al.*, 2003; Falcó *et al.*, 2006). Mean concentration ranges for Cd, Pb and As in finfish and marine fish were reported by the WHO/FAO on data submitted from, amongst others, countries such as Australia, China, Japan, USA, Chile, Lebanon, Korea, Brazil, France, New Zealand and Singapore (WHO/FAO, 2011a,b), whilst no record could be found of South Africa's submission. Current means for Cd, Pb and As in blacktail (0.005, 0.03 and 1.82 $mg \cdot kg^{-1}$, respectively) and hottentot (0.005, 0.005 and 0.77 $mg \cdot kg^{-1}$, respectively) fall within these mean concentration ranges reported as none detected (ND) - 0.008 $mg \cdot kg^{-1}$ for Cd, < LOD - 0.22 $mg \cdot kg^{-1}$ for Pb and 0.10 - 62 $mg \cdot kg^{-1}$ for As (WHO/FAO, 2011a,b). MALs have been set by the South African Department of Health (DOH) as 3.0 $mg \cdot kg^{-1}$ and 1.0 $mg \cdot kg^{-1}$ for As and Cd, respectively and 0.5 $mg \cdot kg^{-1}$ for both Hg and Pb (DOH, 2004). Average concentrations per metal for As, Cd, Hg and Pb were all below these limits. However, a number of individual blacktail exceeded the regulatory safety limits of As and Hg (Table 7.4). The toxicity of both Hg and As, however, depends on the chemical form in which these metals are present and whereas total Hg consists mainly of the toxic MeHg form (Burger & Gochfeld, 2004; Bosch *et al*., 2016a), the biggest proportion of total As in fish meat is usually in its non-toxic organic form (Goyer & Clarkson, 2001; Castro-González & Méndez-Armenta, 2008). Therefore even though total As concentrations for 17% of blacktail sampled might exceed the MAL for toxic As in fish meat, a large proportion of the concentrations measured may be non-toxic to human health. Further improvement of data on the specific As species in fish and methods for direct measurement of toxic inorganic As is needed in order to determine the true health hazard of As in fish meat (WHO/FAO, 2011). As only 1% of all blacktail sampled exceeded the MAL, it can be concluded that blacktail and hottentot caught along the South African coastline are safe for human consumption.

### 7.4.1 Interspecific variation

Overall, metals appear to have similar accumulation trends in blacktail and hottentot as the ranking orders for metal accumulation are similar for the two fish species with minor variations. However, differences are seen in the metal quantities accumulated within the two species as blacktail displayed significantly higher concentrations than hottentot in several of the metals assessed. Similarly, differences in quantities and

trends of metal accumulation were found among species sampled from the same area (Pastor *et al.*, 1994). As prey consumption is one of the major ways of contaminant uptake (Stewart *et al.*, 1997; De Gieter *et al*., 2002; Erasmus *et al.*, 2004), it has been assumed that prey contamination is one of the major causes of interspecific variation among fish, with higher trophic level fish accumulating higher levels of metals such as mercury than lower trophic level species (Campbell *et al*., 2006; Verdouw *et al.*, 2011). Differences in metal concentrations in fish tissue between cultured and wild *Diplodus sargus* have even been attributed to differences in prey/feed metal concentrations, pointing to the importance of prey as dominant pathway of metal accumulation in these fish species (Ferreira *et al.*, 2008). However, several studies (Trudel & Rasmussen, 2006; Burger & Gochfeld, 2011; Teffer *et al*., 2014) have suggested that prey contamination and trophic differences may not be sufficient to explain such variation. Rather, other factors such as fish size, age (Adams & Onorato, 2005; Verdouw *et al.*, 2011), growth rate, energy expenditure and consumption rates (Trudel & Rasmussen, 2006) or a combination of these may cause interspecific variation of metal concentrations in fish muscle. This seems to be confirmed by the present results as significant interspecific variation is seen between blacktail and hottentot even where the effect of location has been removed as in the overlapping sampling sites (False Bay and Saldanha Bay), allowance has been made for size effect and considering the fact that blacktail and hottentot are of the same trophic nature with similar diets (Coetzee, 1986; Mann & Buxton, 1992; Kerwath & Winker, 2013; Mann & Dunlop, 2013). When regarding fish size effects on metal accumulation we find this to be species specific as Cr was increasingly accumulated with fish growth in hottentot whereas Cr concentrations decreased in larger individuals of blacktail. Lead was found to decrease and Hg increase with increasing fish size, as was similarly seen in yellowfin tuna muscle (Chapter 3), but only in blacktail. Even though fish size did affect metal concentrations, variations in concentrations within individual metals were not large. It is therefore assumed that other factors such as fish activity, growth rate and metabolism add to the variation between species. Blacktail seems to have a faster initial growth rate as 50% maturity is reached slightly earlier (3 years) as opposed to hottentot (4 to 5 years) at approximately the same size (blacktail: 211 mm fork length; hottentot: 220 mm fork length) (Kerwath & Winker, 2013; Mann & Dunlop, 2013), which could be a cause for interspecific variation. The mechanisms of metal accumulation with fish growth, activity and metabolism should be further investigated in order to more clearly define and predict metal accumulation between different fish species.

### 7.4.2 Spatial variation

In 1981, the South African Committee of Marine Pollution published a report on a marine pollution survey which led to the identification of several sites of major pollution (Saldanha Bay, Hout Bay, Strand, Mossel Bay, Port Elizabeth and Durban) around the South African coastline (Cloete & Watling, 1981). Each of these sites had specific sources or causes of pollution, some more than others. Saldanha Bay had several sources of pollution including fish factory effluent, debris from the ore jetty and oil pollution from oil off-loading procedures at the jetty. As a major fishing harbour, Hout Bay is mainly subject to organic pollution from fish

processing factories and sewage from the expanding urban area. However, the industrial area in the bay may also be a source of metal pollution. False Bay received, specifically from the Strand area, effluent from factories producing explosives, paints, fertiliser and chemicals for the mining, manufacturing and agricultural sectors, all possible sources of metals that can be harmful to marine life and, through seafood, to humans (Goyer & Clarkson, 2001; Jarup, 2003; Castro-González & Méndez-Armenta, 2008). Even though some of these activities, such as the production of explosives, paint and chemicals have been phased out in this area (AECI, 2015), the effects of such contamination can persist in sediments. Mossel Bay is the largest urban and industrial centre between Cape Town and Port Elizabeth and has 3 important rivers which enter the sea in close vicinity (Cloete & Watling, 1981). Port Elizabeth is one of the most important harbours on the South African east coast where shipping traffic can cause oil pollution. The larger Algoa Bay is a sink for industrial and domestic effluent from the extended developments of Port Elizabeth, Uitenhage and Despatch and both the Sunday and Swarkops Rivers carry agricultural pollutants into the bay, which can contribute to higher metal concentrations on the Port Elizabeth coast and the surrounding bay. Watling and Watling (1983), however, found that the marine environments around Mossel Bay and Port Elizabeth still appeared fairly unpolluted with metals by the time the pollution monitoring plan was in place (Cloete & Watling, 1981). Durban is a large city and port with various sources of pollution including several outlets of industrial and urban pollution and polluted rivers, such as the Umgeni River and the Umdloti River both with high metal contents (Olaniran *et al*., 2014), entering the sea in and around Durban. Several other studies have confirmed that industrial, urban and agricultural developments throughout the country and along its coast have led to coastal areas of high metal pollution (Binning & Baird, 2001; Fatoki & Mathabatha, 2001). A marine pollution monitoring program was implemented to assess the effect of the pollution on marine biota in these polluted areas, but was run for only a short-term (3 years) (Cloete & Watling, 1981).

The current results did prove statistically significant variations in metal concentrations between sampling sites, however, these differences were not large. Results show no clear distinction between polluted and non-polluted sites and their effects on metals accumulated in blacktail and hottentot indicating that spatial distribution is metal specific. Similar findings were obtained for metal accumulation in guppies in urban streams in Indonesia (Widianarko *et al*., 2000) and several marine species in the western Mediterranean (Pastor *et al.*, 1994). Durban and Saldanha Bay both displayed more metals at higher concentrations (Durban: Cr, Fe, Co, Zn, Cd, Hg and Pb; Saldanha Bay: Fe, Cu, Zn, Se, Hg and Pb) than at lower concentrations (Durban: Al, As and Se; Saldanha Bay: As and Cd). These two areas differ considerably in size and type of populations and industrial activities, as the amount of industrial and municipal effluent from Durban is much higher than in Saldanha Bay and the main source of metal pollution in Saldanha Bay seems to be the ore jetty and activity around it (Cloete & Watling, 1981; Erasmus *et al*., 2004). Even so, these two areas appear to have similarities in metal accumulation in blacktail for several metals. Metal concentrations in blacktail and hottentot therefore do not appear to be strongly correlated to environmental pollution levels. Similar results were observed by Verdouw *et al*. (2011) in several estuarine fish species where Hg levels were

not always linked to sediment levels, and it is therefore suggested that other factors such as fish biology and life history could better explain the variation within metal accumulation (Verdouw *et al*., 2011).

Mean total As concentrations in blacktail muscle were below the MAL (3.0 $mg \cdot kg^{-1}$), however, individual samples from Blombos, Mossel Bay, Port Elizabeth and one individual from Durban (Amanzimtoti south of Durban) measured total As concentrations exceeding the MAL for toxic As in fish. The percentage individuals per sampling site exceeding the MAL were 40% (n = 5) for Blombos, 33% (n = 15) for Mossel Bay, 63% (n = 8) for Port Elizabeth and 6% (n = 16) for Durban. This indicates that frequent blacktail consumption from the South African east coast should be limited in order to avoid consumption of toxic levels of As. However, as previously explained (section 4, paragraph 2), further research on As speciation is required in order to accurately determine the true toxicity of As in fish muscle (WHO/FAO, 2011).

Mercury concentrations in both blacktail and hottentot does not vary significantly among sampling sites, with all concentrations measured being well below the MAL (0.5 $mg \cdot kg^{-1}$), except for one individual blacktail from Mossel Bay (0.72 $mg \cdot kg^{-1}$). The consumption of toxic levels of Hg through South African blacktail and hottentot is therefore unlikely even in areas of major pollution if moderate fish consumption is followed.

## 7.5 Conclusion

Metal accumulation in fish muscle is species specific and South African blacktail accumulates higher concentrations of several metals than hottentot, including the three metals As, Hg and Pb, which could be accumulated to toxic quantities in fish meat. However, both blacktail and hottentot can be considered safe for human consumption with regards to metal toxicity, as Hg and Pb levels are within current safety guideline limits. More investigations on individual As species present is needed in order to prove the safety of blacktail and hottentot consumption with regards to As poisoning. Total metal concentrations do not distinguish between traditionally classified polluted and non-polluted marine areas, as spatial metal distribution is metal-specific and individual metals display limited variation between sites within species. The data accumulated from this investigation is the first for these two species in South Africa and will significantly contribute to the knowledge of metal concentrations with regards to consumer safety in marine fish.

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# CHAPTER 8:

## Heavy metal concentrations and toxicity in South African snoek (*Thyrsites atun*) and yellowtail (*Seriola lalandi*)

**ABSTRACT**

The concentrations of 16 metals were assessed in South African snoek (*Thyrsites atun*) and yellowtail (*Seriola lalandi*) sampled from three areas on the West and South-east coasts to compare metal concentrations to regulatory guidelines in order to assess consumer safety. Significant variations were displayed between species and among sampling locations, with variations being metal specific. Arsenic had higher concentrations in general on the South-east coast whereas Cu had higher concentrations in fish on the West coast and Cu was negatively correlated, whereas Zn, As and Hg were positively correlated to yellowtail fish size (both length and weight). No significant correlations were observed between metal concentrations and snoek size. Concentrations for As (0.61 ± 0.21 mg·kg$^{-1}$; 0.98 ± 0.43 mg·kg$^{-1}$), Cd (0.008 ± 0.004 mg·kg$^{-1}$; 0.004 ± 0.003 mg·kg$^{-1}$), Hg (0.27 ± 0.12 mg·kg$^{-1}$; 0.16 ± 0.09 mg·kg$^{-1}$) and Pb (0.009 ± 0.005 mg·kg$^{-1}$; 0.005 ± 0.007 mg·kg$^{-1}$) for snoek and yellowtail respectively were all within regulatory guidelines and therefore two meals (150 g) on average per week of snoek and larger yellowtail (12 – 15 kg) are safe for human (adult) consumption whereas even higher quantities of smaller yellowtail could be safely consumed.

**Keywords**: Fish muscle, Heavy metals, Mercury, ICP-MS, snoek, yellowtail, Consumer health

## 8.1 Introduction

Snoek (*Thyrsites atun*) and yellowtail (*Seriola lalandi*) are the two most commonly caught fish species in the South African linefishery (Sassi, 2015, Griffiths, 2000). Snoek has been part of the commercial fishing sector since the 1800s (Isaacs, 2013), played an important role in the development of the linefishery in the Western Cape and remains the premier commercial linefish, comprising more than 50% of the linefish landed in the Western Cape (Griffiths, 2000; Isaacs, 2013). The importance of yellowtail as a commercial linefish in South Africa increased in the 1930's after the discovery of offshore banks off Struisbaai (Griffiths, 2000). Snoek is a seasonal fishery and is mainly caught in winter (Griffiths, 2000), whereas unpredictable seasonal migration of yellowtail along the South African coast causes variability in catch dates and fishing sites (Sassi, 2015).

Both species are medium sized pelagic predators and although both exhibit a number of similarities in life history traits, habitat and prey composition; differences also exist. Snoek are considered a migratory species and travel large distances largely due to seasonal prey availability (Griffiths, 2002; 2003), but are restricted to the cool/temperate Benguela oceanic system which runs along the West African coast, distributed predominantly from northern Namibia to the southern tip of Africa (Cape Agulhus) (Griffiths, 2003). Yellowtail are nomadic species moving between offshore reefs mainly concentrated on the Agulhas Bank on the South coast and around the Cape, but also inhabits sections of the West coast between Dassen Island and Hondeklip Bay (Kerwath & Wilke, 2012a,b).

Yellowtail and snoek diets are similar yet variable; both feed predominantly on teleosts (> 90%) such as sardine, anchovy (snoek) and horse mackerel (yellowtail) with a minor contribution of crustaceans (crab) (Kerwath & Wilke, 2012a,b). Yellowtail are more opportunistic feeders than snoek and the proportion of crustaceans in their diet therefore varies and increases according to prey abundance and availability (Dunn, 2014). South African yellowtail and snoek are both fast growing species maturing at around 3 years of age (yellowtail: 615 mm fork length (FL); snoek: 730 mm total length (TL)) (Kerwath & Wilke, 2012a,b; Dunn, 2014). Higher maximum ages (9 - 21 years) have been recorded for yellowtail than for snoek (10 years) (Kerwath & Wilke, 2012a,b; Dunn, 2014).

Snoek contributes greatly to food security in South Africa as it is high in essential omega-3 fatty acids and provides a large portion of the required dietary protein in poorer and working class households in the Western Cape (Isaacs, 2013). Yellowtail is also commonly consumed throughout South Africa and is considered a good nutritional source particularly containing high levels of essential fatty acids (O'Neill *et al.*, 2015). However, health benefits provided by these nutritional elements could be compromised through the presence of toxic metals accumulated in these fish. Varying concentrations of several metals have been measured in both yellowtail and snoek muscle across the globe (Van den Broek *et al.*, 1981; Love *et al*., 2003; Ruelas-Inzunza & Páez-Osuna, 2005; Ruelas-Inzunza & Páez-Osuna, 2007, Chung *et al.*, 2008; Padula *et al.*, 2012). Concentrations of certain metals, such as mercury (Hg), increases up the food chain and over time (Boening, 2000; Costa *et al.*, 2012); therefore the predatory nature and longevity of both yellowtail and snoek

can lead to high concentrations of these heavy metals in fish muscle. It is therefore of great importance to the consumer safety aspect of the fishing industry to investigate the levels of possibly toxic metals in these commonly consumed fish.

Therefore the aim of this study was to present an overview of 16 heavy metals in both snoek and yellowtail by determining: presence/absence, concentration, interspecific and spatial variability whilst also comparing observed heavy metal concentration values with current legal maximum limits. The proposed study may provide valuable consumer safety information of interest to a number of industry stakeholders as well as consumers.

## 8.2 Materials and methods

### 8.2.1 Sampling

Snoek (n = 20) were collected by commercial handline fisher persons at Dassen Island located on the West coast of South Africa. Yellowtail samples (n = 37) were collected by both commercial line and recreational fisher persons from various locations including Dassen Island on the West coast (n = 11) and Struisbaai (n = 16) and Port Elizabeth (n = 10) on the South-east coast. A large size range of yellowtail (675 – 1370 mm TL) and smaller size range of snoek (940 – 1125 mm TL) (Table 8.1) were sampled.

Upon collection, fish were received whole and frozen. Prior to laboratory analysis fish were thawed at 4 °C for ± 24 hours (depending on the size of the fish). Weight and length (fork length and total length) of individual fish were recorded prior to gutting and filleting. A ceramic knife was used for filleting and the removal of a subsample for heavy metal analysis in order to minimise metal contamination of the meat samples during processing. The subsample was removed from the anterior section (between the head and the first dorsal fin) of the dorsal muscle from the left fillet. All meat samples retained for analysis were homogenised individually and stored in sealed polyethylene bags at -20 °C until further analysis.

**Table 8.1** Size ranges (weight and length) and number of yellowtail and snoek (n) sampled from 4 locations around the West and South-east coasts of South Africa.

| Location | n | Weight range (kg) | Total length range (mm) |
|---|---|---|---|
| yellowtail | | | |
| Dassen Island | 11 | 2.51 - 4.27 | 675 - 820 |
| Struisbaai | 16 | 2.70 – 8.70 | 700 – 1121 |
| Port Elizabeth | 10 | 12.30 - 15.60 | 1165 - 1370 |
| snoek | | | |
| Dassen Island | 20 | 3.00 – 4.85 | 940 - 1125 |

### 8.2.2 Analysis

The concentrations of 16 metals: aluminium (Al), manganese (Mn), cobalt (Co), nickel (Ni), molybdenum (Mo), tin (Sn), iron (Fe), copper (Cu), chromium (Cr), zinc (Zn), selenium (Se), arsenic (As), antimony (Sb),

cadmium (Cd), mercury (Hg) and lead (Pb) were analysed for each meat sample (n = 57) through inductively coupled plasma mass spectrometry (ICP-MS) as described in Chapters 3 and 4 of this dissertation.

### 8.2.3 Statistical Analysis

Data were analysed with STATISTICA 12.5. Where data did not fit a normal distribution, data were log-transformed to conform to assumptions of normality. Data were subjected to Levene's test for homogeneity of variance and one-way analysis of variance (ANOVA) at 95 % confidence level with fish weight included as covariate to assess the variation between fish species and sampling locations. Where null hypothesis for Levene's test was rejected, weighted means were used when performing parametric analysis together with Games-Howell post hoc test. Spearman's correlation coefficients were calculated to determine correlations between metals and fish weights and lengths.

## 8.3 Results

The ranking order of mean metal concentrations in yellowtail from all sampling locations grouped together (n = 36) (Fe > Zn > Al > As > Cu > Se > Hg > Mn > Ni > Cr > Pb > Cd > Co) is similar to that of snoek (Zn > Al > Fe > As > Se > Cu > Mn > Hg > Cr > Ni > Pb > Cd > Co). The metals of highest average concentrations in both snoek and yellowtail were Fe, Zn and Al with Fe being the highest in yellowtail and Zn the highest in snoek. Most measurements for Mo, Sn and Sb were below the limit of detection (LOD) and therefore considered insignificant.

Mean metal concentrations of snoek and yellowtail from different sampling areas with statistical differences between species and sampling groups are summarised in Table 8.2. Inter- and intraspecific differences in metal concentrations varied for individual metals. Mean concentrations of Co, Ni, Zn, Se and Cd in yellowtail did not differ significantly (P > 0.05) between three of the sampling locations: Port Elizabeth, Struisbaai (South-east coast) and Dassen Island (West coast). However, yellowtail sampled from Port Elizabeth had significantly higher concentration of Al, Cr, Hg and Pb and lower concentrations of Mn and Cu than fish sampled from Struisbaai. Upon examination of regional differences (South-east versus West coast) only limited variation (Cu and As) was observed where Cu was significantly higher in West coast yellowtail and As was significantly lower. As snoek were sampled from just one site (Dassen Island) spatial variability could not be assessed.

**Table 8.2** Summary of the average metal concentration ($mg \cdot kg^{-1}$ ± std. dev.) in snoek (n = 1 site) and yellowtail (n = 3 sites) sampled along the West and South-east coast of South Africa. Identical superscript letters indicate non-significant (p > 0.05) differences between the four sampling groups.

| Metal | snoek | yellowtail Yzerfontein | yellowtail Struisbaai | yellowtail Port Elizabeth |
|---|---|---|---|---|
| Al | $3.61^{b}$ ± 9.369 | $0.97^{b}$ ± 1.692 | $0.17^{b}$ ± 0.135 | $4.51^{a}$ ± 0.794 |

| | | | | |
|---|---|---|---|---|
| Cr | $0.03^{a} \pm 0.039$ | $0.006^{b} \pm 0.002$ | $0.004^{c} \pm 0.004$ | $0.01^{b} \pm 0.005$ |
| Mn | $0.29^{a} \pm 0.134$ | $0.09^{c} \pm 0.071$ | $0.10^{b} \pm 0.021$ | $0.08^{c} \pm 0.017$ |
| Fe | $2.80^{c} \pm 0.873$ | $6.15^{a} \pm 1.302$ | $5.29^{ab} \pm 1.600$ | $4.81^{b} \pm 1.418$ |
| Co | $0.008^{a} \pm 0.006$ | $0.003^{b} \pm 0.001$ | $0.003^{b} \pm 0.001$ | $0.003^{b} \pm 0.001$ |
| Ni | $0.02^{a} \pm 0.027$ | $0.01^{a} \pm 0.016$ | $0.01^{a} \pm 0.019$ | $0.01^{a} \pm 0.007$ |
| Cu | $0.29^{d} \pm 0.047$ | $0.59^{a} \pm 0.121$ | $0.46^{b} \pm 0.108$ | $0.36^{c} \pm 0.025$ |
| Zn | $4.62^{a} \pm 0.699$ | $3.87^{ab} \pm 0.543$ | $3.96^{ab} \pm 0.450$ | $4.27^{b} \pm 0.284$ |
| As | $0.61^{b} \pm 0.210$ | $0.51^{b} \pm 0.094$ | $1.21^{a} \pm 0.440$ | $1.12^{a} \pm 0.182$ |
| Se | $0.41^{a} \pm 0.048$ | $0.41^{ab} \pm 0.060$ | $0.39^{ab} \pm 0.035$ | $0.38^{b} \pm 0.024$ |
| Cd | $0.008^{a} \pm 0.004$ | $0.006^{ab} \pm 0.002$ | $0.004^{b} \pm 0.003$ | $0.003^{b} \pm 0.002$ |
| Hg | $0.27^{a} \pm 0.121$ | $0.10^{b} \pm 0.023$ | $0.11^{b} \pm 0.023$ | $0.29^{a} \pm 0.070$ |
| Pb | $0.009^{a} \pm 0.006$ | $0.006^{b} \pm 0.005$ | $0.002^{c} \pm 0.008$ | $0.009^{ab} \pm 0.004$ |

Significant interspecific variation was found in snoek and yellowtail from Dassen Island (West coast) for 7 of the 16 heavy metals examined where snoek had significantly higher concentrations of Cr, Mn, Co, Hg and Pb and lower concentrations of Fe and Cu compared to yellowtail. Compared to all yellowtail (West and South-east coast), snoek had higher mean concentration of Cr, Mn and Co and lower mean concentrations of Fe and Cu. Nickel was the only metal which showed no statistical differences ($p > 0.05$) in concentrations between the two fish species irrespective of sampling locations.

In yellowtail (all sample groups pooled), Cu concentrations were negatively correlated to both length (TL) ($p < 0.01$; $r = -0.64$) and weight ($p < 0.01$; $r = -0.61$). On the contrary, significant ($p < 0.01$) positive correlations were seen for Zn, As and Hg with fish length ($r = 0.63$, 0.53 and 0.71, respectively) and weight ($r = 0.62$, 0.53 and 0.72, respectively). No significant ($p > 0.01$) correlations were observed between fish size and any of the 13 detectable heavy metal concentrations in snoek.

## 8.4 Discussion

Interspecific and spatial variations in metal concentrations in yellowtail and snoek were metal-specific with no consistent trends observed between the two species or spatially (3 sites sampled in yellowtail). Several factors may play a role in affecting metal accumulation in fish muscle, such as prey composition, location, fish size and species (Burger *et al*., 2014; Stewart *et al*., 1997). Prey consumption is considered the leading source of metal intake in fish (Stewart *et al.*, 1997; De Gieter *et al*., 2002; Erasmus *et al.*, 2004) and therefore plays an important role in overall metal concentrations. However, even though snoek and yellowtail feed on similar diets on the west coast (Kerwath & Wilke, 2012a,b; Dunn, 2014), several metals (Cr, Mn, Fe, Co, Cu, Hg and Pb) displayed significantly different concentrations between the two species from this area. This indicates that factors other than differences in prey may contribute to variation in metal accumulations between species, as was found by Storelli *et al.* (2001) who found that growth rate plays a more significant role than prey consumption in variation of Hg concentrations between different shark species.

Differences in location and the environment from which the fish are sampled could also cause variation in metal accumulation within and between fish species (Burger *et al*., 2014) as different sources of pollution may cause variation in the amounts and types of metals present in the environment (Carvalho *et al.*, 2005; Rahman *et al.*, 2014). Despite the amount of metals present, the amount taken up into biota can be directly affected by environmental conditions such as levels of dissolved organic matter present or water pH, influencing the bioavailability and uptake (Spry & Wiener, 1991; Sánchez-Marín *et al*., 2007). The Western and South-eastern coasts of South Africa consist of two separate marine systems: the Benguela system on the West coast and the Agulhas current on the South-east coast (Hutchings *et al.,* 2009) giving reason for variation between these two areas. In addition, South African yellowtail appears to be two separate stocks inhabiting these two marine systems and it can therefore be assumed that yellowtail from Port Elizabeth and Struisbaai are of the same stock and yellowtail from Dassen Island (West coast) form part of a separate stock (Swart *et al.*, unpublished). Despite these differences, Cu and As were the only two metals measuring significantly different concentrations in fish from the West coast compared to both sampling locations on the South-east coast (Struisbaai and Port Elizabeth). Differences in location and stock therefore do not appear to play a major role in causing variation in metal accumulation in South African yellowtail and snoek. Arsenic measured significantly ($p < 0.05$) higher concentrations in fish on the South-east coast than on the West coast irrespective of species. Similar results were found in the previous chapter for As in blacktail, which could be due to increased amounts of agricultural pollution on the East coast leading to higher concentrations of As in the marine environment (Watling & Watling, 1983; Fatoki & Mathabatha, 2001; Castro-González & Méndez-Armenta, 2008). Therefore, even though As levels are well within safety limits in both snoek and yellowtail studied, other fish and seafood likely to contain higher levels of As, such as organisms lower down the food chain (De Gieter *et al.*, 2002), should be monitored for As toxicity along the South African South-east coast. For the rest of the metals, no clear distinction is seen in concentrations between the two separate marine systems.

Both positive and negative correlations between fish size and heavy metal concentrations have been reported for numerous marine and freshwater fish species (Canli & Atli, 2003; Burger & Gochfeld, 2011; Verdouw *et al.*, 2011). However, such correlations can vary between species (Verdouw *et al*., 2011) and can be restricted to certain metals (Canli & Atli, 2003; Burger & Gochfeld, 2011) as metals differ in their characteristics and methods of accumulation (Carvalho *et al.*, 2005). This variation between species and metals was evident in the current study where Cu was negatively correlated and Zn, As and Hg positively correlated to both fish length and weight in yellowtail. Of these three metals that were positively correlated to yellowtail size, Hg had the strongest positive correlation with fish size, being biomagnified in yellowtail tissue as is commonly found in other fish species (Walker, 1976; Menasveta & Siriyong, 1977; Van den Broek & Tracey, 1981; Boush & Thieleke, 1983; Boening, 2000; Storelli *et al.*, 2002; Kraepiel *et al*., 2003; Campbell *et al*., 2010; Bosch *et al.*, 2016), leading to higher Hg concentrations in larger/older fish. The lack of correlations between metal concentrations and snoek size is similar to what was found in *T. atun* from New

Zealand (Van den Broek *et al.*, 1981), but may be due to a restricted size range in snoek samples compared to the size range in yellowtail samples.

To determine whether any of the metals assessed present possible health hazards to consumers, current concentrations should be compared to metal-specific maximum regulatory guidelines. Fe, Zn and Al measured the highest concentrations in yellowtail and snoek, but are not the most toxic metals as all concentrations for these metals were far below the maximum daily intake set by the USA regulation (Food and Nutrition Board, 2001). Maximum allowable limits (MALs) have been set specifically for four metals (Hg, As, Pb and Cd) which have been found to commonly accumulate to toxic levels in fish muscle. All four these metals were well below specified levels in both snoek and yellowtail, with average Pb and Cd concentrations reaching less than 5% of the MALs of both South African and EU legislation (Table 8.3). The provisional tolerable weekly intake (PTWI) for Hg, as recommended by the Expert Committee on Food Additives and Contaminants (JECFA) under the joint Food and Agriculture Organization (FAO) and the World Health Organization (WHO), as part of the international safety guidelines, is 1.6 $\mu g \cdot kg^{-1}$ (JECFA, 2007). Therefore, on average the consumption of 150 g of snoek twice a week by an adult (70 kg body weight) is considered within safety limits. However, such regular consumption is not recommended for children under 12 kg where regular to moderate intake of Hg could result in negative health effects (Grandjean *et al.*, 2010). Although, on average, snoek is safe for human consumption, exceptions can be found as two individual snoek samples (10% of all snoek sampled) had Hg concentrations exceeding the MAL (0.5 $mg \cdot kg^{-1}$) indicating that snoek has the ability to accumulate Hg to toxic concentrations. Further studies should be done on other populations off the South African coast in order to confirm the above recommendations for snoek consumption as snoek is a species commonly consumed throughout South Africa especially by coastal communities (Isaacs, 2013) and this study only sampled from one location.

Differences in the rate of Hg accumulation between snoek and yellowtail species were evident as snoek had similar mean Hg concentrations than yellowtail that were on average more than 3 times its size, but had significantly higher Hg concentrations when compared to yellowtail of similar size. This is explained by the biomagnification of Hg in larger sized yellowtail. The same consumption guidelines for snoek would therefore apply to large yellowtail (12 - 16 kg) whereas smaller sized yellowtail would provide smaller quantities of Hg allowing more frequent consumption without negatively effecting consumer health.

**Table 8.3** Summary of the mean ($mg \cdot kg^{-1}$ ± Std. dev.) and maximum metal concentrations ($mg \cdot kg^{-1}$) (number of samples exceeding the MAL) in snoek and yellowtail assessed. Maximum allowable limits (MAL) or upper limits (UL) for fish flesh by various regulatory bodies were included for facilitate comparisons between measured concentration and regulatory limits.

| | Mean concentration | | Maximum concentration | | South African regulation[1] | USA regulation[2] | EU regulation[3] |
|---|---|---|---|---|---|---|---|
| | snoek (n = 20) | yellowtail (n = 36) | snoek | yellowtail | | | |
| Fe | 2.80 ± 0.873 | 5.41 ± 1.521 | 5.26 | 9.84 | - | 45 $mg \cdot day^{-1}$ | - |
| Zn | 4.62 ± 0.699 | 4.02 ± 0.462 | 6.05 | 5.19 | - | 40 $mg \cdot day^{-1}$ | - |
| Al | 3.61 ± 9.369 | 1.58 ± 2.084 | 41.63 | 5.69 | - | - | - |
| As | 0.61 ± 0.210 | 0.98 ± 0.431 | 1.23 | 1.75 | 3.0 $mg \cdot kg^{-1}$ | UL not established | - |
| Cu | 0.29 ± 0.047 | 0.47 ± 0.130 | 0.43 | 0.79 | - | 10 $mg \cdot day^{-1}$ | - |
| Se | 0.41 ± 0.048 | 0.39 ± 0.042 | 0.51 | 0.48 | - | 0.4 $mg \cdot day^{-1}$ | - |
| Hg | 0.27 ± 0.121 | 0.16 ± 0.093 | 0.64 (2) | 0.40 | 0.5 $mg \cdot kg^{-1}$ | - | 0.5 $mg \cdot kg^{-1}$ |
| Mn | 0.29 ± 0.134 | 0.09 ± 0.042 | 0.63 | 0.30 | - | 11 $mg \cdot day^{-1}$ | - |
| Ni | 0.02 ± 0.027 | 0.01 ± 0.016 | 0.12 | 0.08 | - | 1 $mg \cdot day^{-1}$ | - |
| Cr | 0.03 ± 0.039 | 0.006 ± 0.005 | 0.19 | 0.02 | - | UL not established | - |
| Pb | 0.009 ± 0.005 | 0.005 ± 0.007 | 0.02 | 0.03 | 0.5 $mg \cdot kg^{-1}$ | - | 0.3 $mg \cdot kg^{-1}$ |
| Cd | 0.008 ± 0.004 | 0.004 ± 0.003 | 0.02 | 0.01 | 1.0 $mg \cdot kg^{-1}$ | - | 0.1 $mg \cdot kg^{-1}$ |
| Co | 0.008 ± 0.006 | 0.003 ± 0.001 | 0.02 | 0.006 | - | - | - |

[1]Department of Health, 2004; [2]Food and Nutrition Board, 2000; 2001; [3]Commission Regulation (EC), 2001; 2006; 2008

## 8.5 Conclusion

Concentrations of 16 metals assessed in South African yellowtail and snoek displayed significant variation between species and among sampling locations. This variation appears to be caused by a combination of factors rather than being ascribed to one single influencing factor such as prey composition, location or fish size. Metal specific accumulation patterns are evident as no consistent accumulation trends are apparent between sampling location or fish species. Limited correlations were found between metal concentrations and fish size. Even though all metals were within regulatory limits, As and Hg concentrations were positively correlated to fish size, therefore larger yellowtail (12-16 kg) and snoek should be limited to 2 meals on average per week for adults. Although the measured As levels in snoek and yellowtail are not considered sufficiently high to have hazardous effects on human health, results do indicate that As pollution along the South-east coast is higher than the West coast and therefore monitoring is suggested in fish species more vulnerable to toxic As accumulation.

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# CHAPTER 9:

## Heavy metal concentrations in four South African shark species

**ABSTRACT**

The concentrations of 16 metals in muscular tissue of blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), soupfin (*Galeorhinus galeus*) and smoothhound (*Mustelus mustelus*) were assessed as functions of gender, fish length and species. Limited significant differences between genders were found while mercury (Hg) and selenium (Se) were the only metals correlated to shark length in soupfin (Hg: r = 0.88), shortfin mako (Se: r = -0.85) and smoothhound (Se: r = -0.46). Mercury concentrations exceeded levels for safe human consumption in shortfin mako, soupfin (specimens larger than 120 cm total length) and smoothhound, with highest concentrations in shortfin mako. Total arsenic (As) concentrations exceeded the maximum allowable limit (3.0 $mg \cdot kg^{-1}$) in blue shark (7.54 ± 1.61 $mg \cdot kg^{-1}$), soupfin (18.41 ± 4.10 $mg \cdot kg^{-1}$) and smoothhound (29.50 ± 19.32 $mg \cdot kg^{-1}$), but speciation analysis is required to determine the true As toxicity of these three shark species.

**Keywords**: Fish muscle, Heavy metals, Mercury, ICP-MS, Blue shark, Shortfin mako, Soupfin, Smoothhound, Consumer health

## 9.1 Introduction

Sharks play an important role in the marine food web due to their position as top predators (Cortés, 1999). As elsewhere (Da Silva & Bürgener, 2007), they are targeted and caught as by-catch by several South African fisheries (Da Silva *et al.*, 2015). Even though there is little acknowledged consumption of shark meat in South Africa, sharks form a substantial portion of the South African fishing industry (Kroese & Sauer, 1998), mainly as exports (Da Silva & Bürgener, 2007). The principle South African export market is Australia where the shark meat is used in the fish-and-chips trade and an increasing demand has resulted in an increase in shark catches around the South African coast (Da Silva & Bürgener, 2007). However, the safety of shark meat consumption is of concern as sharks are specifically vulnerable to the accumulation of toxic concentrations of metals such as Hg. The high bioaccumulation and biomagnification in shark tissue is largely due to their longevity and higher trophic positions (Boening, 2000; Costa *et al.*, 2012).

Since the Minamata Bay outbreak in Japan in the 1950s, where the consumption of fish with high levels of Hg caused a series of health defects and even deaths in infants (Harada, 1995), the accumulation and levels of Hg as well as several other metals (especially in shark and other predatory fish) have been investigated (Turoczy *et al*., 2000; Erasmus *et al.*, 2004; Branco *et al*., 2007; Endo *et al*., 2008; Storelli *et al*., 2011; Lopez *et al.*, 2013). Only a few studies have investigated the safety of South African shark meat in terms of toxic metal content (Erasmus *et al.*, 2004; Bosch *et al.*, 2013); therefore a major knowledge gap currently exists within the South African context.

Metals are mostly absorbed into the fish tissue via their diet, where the exposure to metals through a variety of prey items from lower trophic levels results in increased absorption and accumulation in sharks (Stewart *et al.*, 1997; De Gieter *et al*., 2002; Erasmus *et al.*, 2004). Metabolic processes that cause the breakdown of food and food components in the fish body are therefore responsible for the release and uptake of metals into the fish tissue. Metabolic rates can vary among shark species due to variations in their biological profiles and characteristics such as habitat and movement, diet, growth rate and trophic level count (Table 9.1), which can influence the rate and degree of metal uptake in respective species (Teffer *et al.*, 2014). Some of the main targeted shark species in South Africa include smoothhound (*Mustelus mustelus*), soupfin (*Galeorhinus galeus*) shortfin mako (*Isurus oxyrinchus*) and blue shark (*Prionace glauca*) (Da Silva & Bürgener, 2007; Da Silva *et al.*, 2015). The current understanding of the biological profiles of these four shark species is summarised in Table 9.1.

This study aims to improve knowledge on the current state of metals accumulated in South African commercial shark species by assessing the concentrations of 16 metals in four shark species caught off the South African coast to determine the effects of species and fish size on metal concentrations and to compare current metal levels with regulatory guidelines in terms of consumer safety.

**Table 9.1** Species profiles of four South African commercial shark species (after Mann *et al.,* 2013).

| | Blue shark | Shortfin mako shark | Soupfin shark | Smoothhound |
|---|---|---|---|---|
| Movement | Migratory | Migratory | Migratory | Resident |
| Diet | Cephalopods & small pelagic fish | Pelagic cephalopods, pelagic teleosts, crustaceans & small elasmobranchs | Mainly demersal & pelagic fish with crustaceans, cephalopods, worms & echinoderms | Mainly crustaceans & invertebrates |
| Trophic level (Cortes, 1999)* | 4.1 | 4.3 | 4.2 | 3.8 |
| Length @ 50% maturity | 190 cm FL (female)<br>183 cm FL (male) | 253-275 cm FL (female)<br>185 – 199 cm FL (male) | 110 cm TL | 120-140 cm TL (female)<br>95-130 cm TL (male) |
| Age @ 50% maturity | 5.5-6 years (female)<br>4.9-7 years (male) | 14-21 years (female)<br>7-9 years (male) | 6.04 years | 10-12 years (female)<br>7-9 years (male) |
| Max age recorded | 16 years | 28-38 years (female)<br>21-34 years (male) | 33 years (SA),<br>70 years (Australia) | 24 years |
| Max weight recorded | 198 kg | 553.8 kg | 33 kg | 31 kg |
| Max length recorded | 383 cm TL | 411 cm TL | 190 cm TL | 173.2 cm TL |

*Estimated trophic levels based on diet compositions obtained from quantitative studies.
FL = fork length
TL = total length

## 9.2 Materials and methods

### 9.2.1 Sampling

Four species of shark were sampled off the South African coast. Smoothhound (n = 30) were caught by rod and line in the Langebaan lagoon, Western Cape, South Africa (DAFF ethics clearance number: 2009V17CA). Blue shark (n = 10) were collected from 2 tuna line-fisher persons caught in the South West Atlantic off Cape Point. Shortfin mako (n = 10) and soupfin (n = 12) sharks were collected by longline research vessels off the coast of St. Francis Bay and Port Elizabeth, respectively. Whole sharks were kept on ice or frozen until dissection, when sharks were defrosted, measured and weighed and biological data recorded. A sample of 400 g to 1 kg was taken from the anterior dorsal muscles between the head and the first dorsal fin using a ceramic knife in order to minimise metal contamination of sample tissue. Muscle tissue was homogenised and stored in clean polyethylene bags at -20 °C.

### 9.2.2 Analysis

The concentrations of 16 metals (aluminium (Al), manganese (Mn), cobalt (Co), nickel (Ni), molybdenum (Mo), tin (Sn), iron (Fe), copper (Cu), chromium (Cr), zinc (Zn), selenium (Se), arsenic (As), antimony (Sb), cadmium (Cd), mercury (Hg) and lead (Pb)) were analysed through inductively coupled plasma mass spectrometry (ICP-MS) for each meat sample (n = 62) as described in Chapter 3 and 4 of this dissertation.

### 9.2.3 Statistical Analysis

Data were analysed with STATISTICA 12.5. Where data did not fit a normal distribution, data were log-transformed to approach a normal distribution. Data were subjected to Levene's test for homogeneity of variance and analysis of covariance (ANCOVA) at 95 % confidence level to test for variation of metal concentrations between shark species and between genders. Where the null hypothesis for Levene's test was rejected, weighted means were used instead of LS-means together with Games-Howell post hoc test. Data from both genders are pooled for the assessment of variation among species and analysis of correlations between metal concentrations and fish size. Spearman's correlation coefficients were calculated on the original data to test for correlations between metal concentrations and shark length as well as among individual metals.

## 9.3 Results

Table 9.2 shows the sample sizes (n), locations, size ranges and gender distributions of all sharks sampled. Shortfin makos were the largest sharks on average. Smoothhound samples consisted of considerately more female than male sharks. Data for some individual metals (Cr, Mn, Co, Ni, Mo, Sn, Sb and Pb) did not follow

normal distributions, even after log transformations. Results for these metals should therefore be interpreted with caution.

**Table 9.2** Sample size (n), location, size range and gender distribution of all four shark species sampled.

| Common name | Species | n | Location | Size range | Gender |
|---|---|---|---|---|---|
| Blue shark | *P. glauca* | 10 | Cape Point | 108.0 - 187.8 cm | Male: 3<br>Female: 7 |
| Shortfin mako | *I. oxyrinchus* | 10 | Cape St. Francis | 166.0 - 210.0 cm | Male: 4<br>Female: 5 |
| Soupfin | *G. galeus* | 12 | Port Elizabeth | 93.6 - 151.0 cm | Male: 7<br>Female: 5 |
| Smoothhound | *M. mustelus* | 30 | Langebaan lagoon | 60.1 - 165.2 cm | Male: 6<br>Female: 24 |

A summary of the mean concentrations of each metal per shark species including statistical results is presented in Table 9.3. More than 50% of all shark muscle samples had Mo, Sn and Sb concentrations below the limits of detection (LOD) and, where detectable (mostly in smoothhound), concentrations were low. All individual metals except for Cr displayed significant variation in concentrations among the four shark species. Smoothhound shark had the highest concentrations for eight metals measured (As, Zn, Al, Se, Cu, Ni, Cd and Pb). For Zn, Se, Ni and Cd, smoothhound shark had significantly higher concentrations than all other shark species, whereas As concentrations in smoothhound did not differ significantly from soupfin, but were higher than those in both blue shark and shortfin mako. Concentrations of Al in smoothhound did not differ significantly from either blue shark or shortfin mako, but were significantly higher than Al concentrations in soupfin. Concentrations for Cu and Pb in smoothhound did not differ significantly from shortfin mako, but were significantly higher than concentrations in both blue shark and soupfin. Iron and Hg concentrations in shortfin mako were significantly higher than all other shark species and Mn in soupfin did not differ significantly from shortfin mako, but was significantly higher than in blue shark and smoothhound.

Inter-sex variation was observed for individual metals in three of the shark species. Concentrations of Mn in shortfin mako and Fe in blue shark were significantly ($p < 0.05$) higher in females than in males, whereas Pb was lower ($p < 0.05$) in female smoothhound than in males.

Shark size (total length) was found to affect only a select few metals in certain shark species. Total length was negatively correlated to Se concentration in shortfin mako shark ($p < 0.01$; $r = -0.85$) and smoothhound shark ($p < 0.05$; $r = -0.46$) and positively correlated to Hg concentrations in soupfin ($p < 0.01$; $r = 0.88$).

The ranking orders of metal concentrations were similar for the four shark species with minor variations (Table 9.3). For three of the shark species (blue, soupfin and smoothhound) the highest mean

metal concentrations in descending order were As, Zn and Fe. These three metals were similarly the most abundant metals in shortfin mako, but in the following descending order: Fe, Zn and As.

**Table 9.3** Mean metal concentrations ($mg \cdot kg^{-1}$ ± std dev) per metal per shark specie. Identical superscript letters indicate non-significant ($p > 0.05$) differences between sites per metal.

| | Blue shark (n = 10) | Shortfin mako (n = 10) | Soupfin (n = 12) | Smoothhound (n = 30) |
|---|---|---|---|---|
| As | $7.54^{b} \pm 1.613$ | $1.62^{c} \pm 1.257$ | $18.41^{a} \pm 4.096$ | $29.50^{a} \pm 19.321$ |
| Zn | $3.52^{b} \pm 0.795$ | $2.89^{b} \pm 0.500$ | $2.82^{b} \pm 0.155$ | $4.35^{a} \pm 0.411$ |
| Fe | $2.39^{c} \pm 0.904$ | $4.24^{a} \pm 1.138$ | $2.36^{c} \pm 0.447$ | $3.14^{b} \pm 0.677$ |
| Al | $2.27^{ab} \pm 2.118$ | $1.28^{ab} \pm 1.336$ | $0.72^{b} \pm 0.311$ | $1.25^{a} \pm 0.416$ |
| Hg | $0.52^{c} \pm 0.198$ | $1.74^{a} \pm 0.516$ | $0.96^{b} \pm 0.581$ | $1.00^{b} \pm 0.701$ |
| Se | $0.26^{c} \pm 0.064$ | $0.29^{bc} \pm 0.073$ | $0.37^{b} \pm 0.129$ | $0.72^{a} \pm 0.464$ |
| Cu | $0.24^{b} \pm 0.056$ | $0.25^{ab} \pm 0.024$ | $0.24^{b} \pm 0.096$ | $0.30^{a} \pm 0.070$ |
| Mn | $0.08^{b} \pm 0.010$ | $0.09^{ab} \pm 0.027$ | $0.10^{a} \pm 0.013$ | $0.08^{b} \pm 0.015$ |
| Cr | 0.01 ± 0.011 | 0.009 ± 0.007 | 0.01 ± 0.014 | 0.05 ± 0.030 |
| Ni | $0.01^{c} \pm 0.007$ | $0.02^{b} \pm 0.006$ | $0.01^{bc} \pm 0.008$ | $0.19^{a} \pm 0.224$ |
| Cd | $0.009^{b} \pm 0.005$ | $0.005^{b} \pm 0.003$ | $0.004^{b} \pm 0.002$ | $0.04^{a} \pm 0.021$ |
| Pb | $0.004^{b} \pm 0.004$ | $0.01^{ab} \pm 0.006$ | $0.001^{b} \pm 0.0000$ | $0.03^{a} \pm 0.019$ |
| Co | $0.003^{a} \pm 0.002$ | $0.001^{b} \pm 0.0007$ | $0.002^{ab} \pm 0.002$ | $0.002^{ab} \pm 0.001$ |

Dark shaded cells indicate significantly ($p < 0.05$) highest mean concentrations
Light shaded cells indicate non-significantly ($p > 0.05$) highest mean concentrations

## 9.4 Discussion

Top predatory fish are generally active fish of large size with high metabolic and food consumption rates and have longer life-spans. As prey consumption is one of the major routes of metal uptake in fish (Hall *et al.*, 1997; Mason *et al.*, 2000), top predators such as tuna, shark and swordfish have an increased rate of exposure to metals (Kojadinovic *et al.*, 2007). However, this large group of predatory fish consists of many orders and species, all of which have different characteristics (growth rate, metabolism, size, age, prey preference, habitat, etc.) and can therefore differ significantly in their mechanism and rate of metal uptake. Metal accumulation has previously been found to be affected by species, fish size, gender and location, especially in larger predatory fish (Branco *et al*., 2007; Kojadinovic *et al*., 2007; Endo *et al.,* 2008). Similar to data in this study, Erasmus *et al.* (2004) found that the data from two shark species studied at different locations were not normally distributed due to these various factors affecting metal accumulation and consequently the measurable metal concentrations in fish tissue. Variation in size ranges between shark species, uneven sampling of males and females and differences in metal accumulation between shark sizes, species and gender might therefore be reasons for the skewed distribution of the current data for several metals.

Gender had a limited effect on metal accumulation in general in the four shark species studied with individual differences in blue shark, shortfin mako and smoothhound. Although Bosch *et al.* (2013) examined

the same smoothhound population, and found higher Hg concentrations in males than females of similar length categories, such variation was not observed in the current study. However, the effect of inter-sex fish length difference was not considered in the current study as in the previous study by Bosch *et al.* (2013). Similar to the current results, other studies have found gender effect on metal accumulation to be limited in several large pelagic as well as freshwater species, with only a select few cases where gender effect was metal, species and tissue specific (Nussey *et al.*, 2000; Kojadinovic *et al.*, 2007; Lopez *et al.*, 2013).

Similar to gender, shark length had limited effects on metal accumulation in this study. Even though several metals such as Cd, Hg, Se and Zn have been commonly observed to be positively correlated with fish length in pelagic fish (tuna, swordfish and dolphin) (Kojadinovic *et al.*, 2007) and specifically in several shark species (Walker, 1976; Lyle, 1986; Marcovecchi *et al*., 1991; Lacerda *et al.*, 2000; Turoczy *et al.*, 2000; Branco *et al.*, 2007; Endo *et al.*, 2008), no consistent correlations were seen among the current shark species studied; rather, such correlations are metal- and species-specific. This specificity in correlations has been seen in other studies where only limited metals displayed significant correlations between muscle concentration and size (weight and length) (Erasmus *et al.*, 2004) and where the same metal can have either positive, negative or no correlation with fish size depending on the shark species (Eustace, 1974; Endo *et al.*, 2008). The strong positive correlation between Hg concentration and soupfin shark length agrees with previous findings where Hg is increasingly accumulated in sharks and predatory fish (Adams and McMichael, 1999; Storelli *et al.*, 2002). However, metal accumulation in blue shark was found to be independent of shark length, contrary to the findings of Branco *et al*. (2007) who found positive correlations between both Hg and Se and shark length in blue shark from the Atlantic Ocean. This lack in observed correlation could be a result of the small size range sampled. The absence of correlation between length and Hg concentration in both blue and shortfin mako shark, which is contrary to what was expected, may also be due to the absence of sharks from higher size ranges (Table 9.2 and 9.3) as Adams and McMichael (1999) have found the relationship between Hg and fish length in small (young) bull sharks to be less clear. The negative correlations between Se and shark length in both shortfin mako and smoothhound are also contradictory to the positive correlations found between this metal and fish size in other studies (Kojadinovic *et al.*, 2007; Branco *et al.*, 2008). Lyle (1986) has, however, confirmed this relationship between Se and shark length (assessed in 18 shark species) to be inconsistent.

Increasing accumulation of metals with fish growth can be explained by the absence or slow rate of excretion of specific metals from the fish body leading to an accumulative effect (Kojadinovic *et al.*, 2007); whereas negative correlations between metal concentrations and fish length could be attributed to a higher body metabolism in younger fish (Canli & Atli, 2003) causing higher rates of metal intake and accumulation than in older fish. Fish can also display a constant balance between metal uptake and excretion where the metabolism between younger and older fish remains unchanged within species, so that neither positive nor negative correlations are observed between metal concentrations and fish length (Bosch *et al.,* 2013). For the lack of significant correlations between metal concentrations and fish size, either of two explanations

may be assumed: 1) sampling groups do not include sufficient individuals across the size range or 2) the four shark species studied do not undergo major changes in their metabolism during their life-spans. Both these aspects warrant further research.

The general metal accumulation patterns in all four shark species followed similar trends consistent with findings in previous studies. The accumulation and concentration of Mo, Sn and Sb is considered minimal in many shark species as levels rarely reach detectable levels (Hamilton & Wiedmeyer, 1990; Guérin *et al*., 2011; Hosseini *et al.,* 2013). Although Fu *et al.* (2011) found that Sb is not bioaccumulated in fish tissue, two freshwater fish species (*Labeo rosae* and *Clarias gariepinus*) from the highly polluted Olifants River in South Africa revealed Sb levels exceeding the maximum allowable limits for human consumption (Jooste *et al*., 2014, 2015). It appears that even though Sb is not bioaccumulated, it may be absorbed to significant amounts in fish in close proximity to Sb pollution sources (Jooste *et al*., 2015). When comparing average concentrations of individual metals, the high levels of Zn and Fe were comparable with other studies which focused on pelagic and migratory fish (Kojadinovic *et al.,* 2007; Uysal *et al*., 2008). Upon direct species to species comparison, Zn and Fe concentrations were similar to those previously measured (Appendix I) with just one exception; Fe concentrations in South African smoothhound measured by Erasmus *et al.* (2004) were exceedingly higher than the current and other studies. Even though Zn and Fe are of the top accumulated metals in all four shark species, these metal concentrations are considered low in terms of maximum allowable limits (MALs) (Table 9.4) and recommended daily intake (8 $mg \cdot day^{-1}$ for Fe) set by the USA Food and Nutrition Board (2001).

In terms of metals that are more likely to accumulate to toxic levels in fish tissue (As, Hg, Cd and Pb); As and Hg are accumulated at higher levels than Cd and Pb in all four shark species, which is in agreement with previous chapters of this dissertation and other studies on several marine fish species (Kojadinovic *et al.,* 2007). Levels for both Cd and Pb were considered low in all shark species studied as all measured concentrations were well below the MAL for both metals and therefore not considered a hazard to consumer health. Both As and Hg concentrations measured in this study are within the range of concentrations previously measured in similar shark species (Appendix I). Arsenic concentrations, however, displayed significant variation within and among shark species reviewed (Appendix I), which was similarly observed in the current study. In blue shark, soupfin and smoothhound, all individual samples measured As levels equal to or above the MAL (3.0 $mg \cdot kg^{-1}$), whereas only one individual shortfin mako sample had As concentrations exceeding this limit (Table 9.4). When considering that the major proportion of As in fish tissue is in its non-toxic form (Goyer & Clarkson, 2001; Castro-González & Méndez-Armenta, 2008), it can

**Table 9.4** A summary of the mean metal concentrations (mg·kg$^{-1}$ ± std dev)(number of samples exceeding the South African maximum allowable limit) in four shark species with maximum allowable limits (MAL) or upper limits (UL) for shark meat by various regulatory bodies. The numbers of shark samples exceeding the MALs are shown in parentheses.

| | Blue shark (n = 10) | Shortfin mako (n = 10) | Soupfin (n = 12) | Smoothhound (n =30) | South African regulation[1] (MAL) | USA regulation[2] (UL) | EU regulation[3] (MAL) |
|---|---|---|---|---|---|---|---|
| As | 7.54 ± 1.613 (10) | 1.62 ± 1.257 (1) | 18.41 ± 4.096 (12) | 29.50 ± 19.321 (30) | 3.0 mg·kg$^{-1}$ | UL not established | - |
| Zn | 3.52 ± 0.795 | 2.89 ± 0.500 | 2.82 ± 0.155 | 4.35 ± 0.411 | - | 40 mg·day$^{-1}$ | - |
| Fe | 2.39 ± 0.904 | 4.24 ± 1.138 | 2.36 ± 0.447 | 3.14 ± 0.677 | - | 45 mg·day$^{-1}$ | - |
| Al | 2.27 ± 2.118 | 1.28 ± 1.336 | 0.72 ± 0.311 | 1.25 ± 0.416 | - | - | - |
| Hg | 0.52 ± 0.198 | 1.74 ± 0.516 (10) | 0.96 ± 0.581 (4) | 1.00 ± 0.701 (12) | 1.0 mg·kg$^{-1}$ | - | 1.0 mg·kg$^{-1}$ |
| Se | 0.26 ± 0.064 | 0.29 ± 0.073 | 0.37 ± 0.129 | 0.72 ± 0.464 | - | 0.4 mg·day$^{-1}$ | - |
| Cu | 0.24 ± 0.056 | 0.25 ± 0.024 | 0.24 ± 0.096 | 0.30 ± 0.070 | - | 10 mg·day$^{-1}$ | - |
| Mn | 0.08 ± 0.010 | 0.09 ± 0.027 | 0.11 ± 0.013 | 0.08 ± 0.015 | - | 11 mg·day$^{-1}$ | - |
| Sn | 0.03 ± 0.058 | 0.003 ± 0.001 | 0.01 ± 0.026 | 0.07 ± 0.065 | | - | - |
| Cr | 0.01 ± 0.011 | 0.009 ± 0.007 | 0.01 ± 0.014 | 0.05 ± 0.030 | - | UL not established | - |
| Ni | 0.01 ± 0.007 | 0.02 ± 0.006 | 0.01 ± 0.008 | 0.19 ± 0.224 | - | 1 mg·day$^{-1}$ | - |
| Cd | 0.009 ± 0.005 | 0.005 ± 0.003 | 0.004 ± 0.002 | 0.04 ± 0.021 | 1.0 mg·kg$^{-1}$ | - | 0.1 mg·kg$^{-1}$ |
| Pb | 0.004 ± 0.004 | 0.01 ± 0.006 | 0.001 ± 0.000 | 0.03 ± 0.019 | 0.5 mg·kg$^{-1}$ | - | 0.3 mg·kg$^{-1}$ |
| Co | 0.003 ± 0.002 | 0.001 ± 0.0007 | 0.002 ± 0.002 | 0.002 ± 0.001 | - | - | - |
| Mo | 0.003 ± 0.001 | 0.003 ± 0.000 | 0.003 ± 0.000 | 0.02 ± 0.029 | - | 2 mg·day$^{-1}$ | - |
| Sb | 0.001 ± 0.0001 | 0.001 ± 0.0000 | 0.001 ± 0.0008 | 0.024 ± 0.0787 | | - | - |

[1]Department of Health, 2004; [2]Food and Nutrition Board, 2000; 2001; [3]Commission Regulation (EC), 2001; 2006; 2008

be assumed that the actual levels of toxic As were lower than those measured in the current study. However in order to determine the true toxicity of As in shark meat, speciation analyses are required to measure specific toxic As chemical forms. Average Hg concentrations were equal to or above the MAL (1.0 $mg \cdot kg^{-1}$) for shortfin mako, soupfin and smoothhound shark, whereas all individual blue shark samples had Hg concentrations within safe regulatory limits (Table 9.4). In terms of Hg toxicity, shortfin mako shark had the highest measured levels overall and far exceeded levels considered safe for human consumption. Shortfin mako is larger in size and occupies a higher trophic level than blue shark, smoothhound and soupfin (Table 9.1). Teffer *et al*. (2014) has found that Hg concentrations are higher in shortfin mako muscle than in larger sized thresher shark due to their consumption of higher trophic level prey. The high mercury levels in shortfin mako muscle may therefore rather be attributed to their high trophic position (Table 9.1) than to their size (Table 9.2).

Due to the high levels of Hg detected in shortfin mako, soupfin and smoothhound, human consumption may pose adverse health effects and limited intake is suggested. Overall 100% of shortfin mako, 33% of soupfin and 40% of smoothhound samples assessed exceeded safe limits for Hg and therefore close monitoring prior to market release is required. Due to the positive linear relationship ($R^2 = 0.87$) between Hg concentration and shark length (Fig. 9.1), Hg concentrations can be considered a function of shark size/age where an increase in Hg concentrations is observed after maturity is reached (Francis & Mulligan, 1998; Da Silva & McCord, 2012). Current results show that individuals smaller than 120 cm (TL) are within safe Hg levels (Fig. 9.1) whereas mature soupfin should be avoided for consumption. As a precautionary measure, the South African shark industry does not receive and process sharks above 12 kg in order to minimise the risk of human Hg exposure through fish consumption (Da Silva & Bürgener, 2007). This weight limit should, however, be re-evaluated per shark species as metal accumulation is species specific and maximum weight limits could therefore vary considerably among species. Even though blue shark is not popularly marketed as it is considered a low quality meat, this species can be considered safe for human consumption with regards to Hg levels (Da Silva & Bürgener, 2007).

## 9.5 Conclusion

Sharks are generally long-lived species occupying high trophic positions and can therefore accumulate increasing quantities of metals in their tissue, which constitute a risk of toxic metal exposure to consumers. The accumulation of metals in shark tissue varied in select cases with gender, fish size and species which may be due to differences in activity, prey consumption and metabolic rates, however, limited correlations were seen between metal concentrations and both gender and total length (TL) of blue shark, shortfin mako, soupfin and smoothhound. Therefore fish characteristics that are easily determined visually, such as fish size and gender will give no indication of toxic metal concentrations within the fish muscle with the exception of soupfin where larger sharks contain higher Hg concentrations and individuals above 120 cm TL are more likely

to contain Hg levels that are unsafe for human consumption. It is therefore recommended to target smaller soupfin sharks for human consumption. Mercury concentrations exceeded levels for safe human consumption in shortfin mako, soupfin and smoothhound, but with shortfin mako having the highest concentrations and therefore being the most dangerous in terms of Hg toxicity. Blue shark is considered safe for human consumption in terms of Hg toxicity. However, As concentrations exceeded safe regulatory limits (3.0 $mg \cdot kg^{-1}$) in blue shark, soupfin and smoothhound, but as the largest proportion of As in fish tissue is considered non-toxic, speciation analyses is required to determine the true toxicity levels of these three shark species**.**

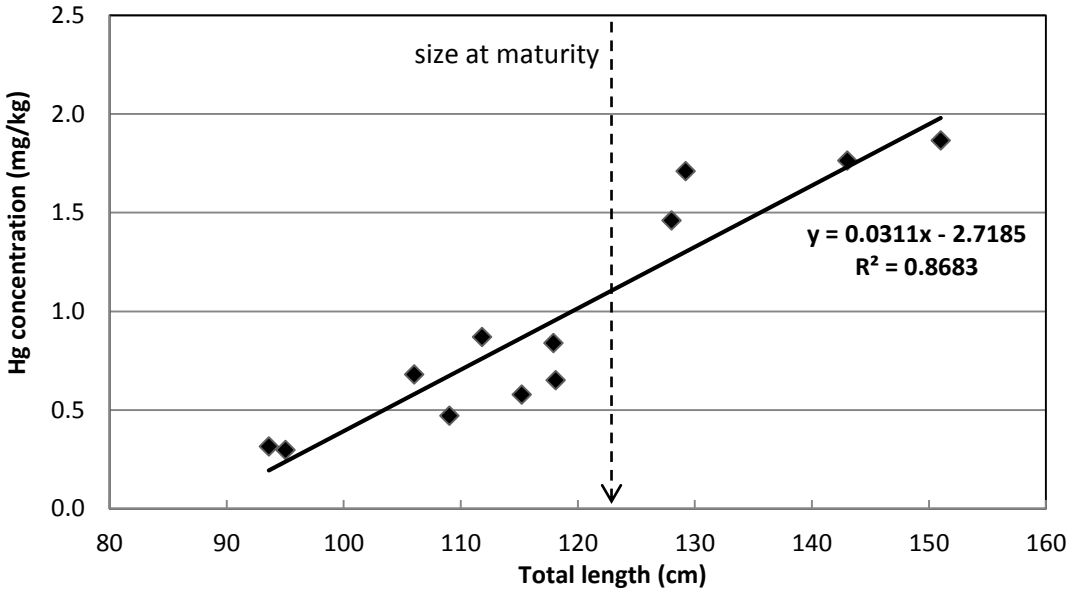


**Figure 9.1** Linear regression between Hg concentration and total length in South African soupfin shark (*Galeorhinus galeus*) (n = 10).

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# Appendix I

**Table A.1** Summaries of metal concentrations measured in shark species in previous studies

| Species | Location | As (MAL = 3.0 mg·kg$^{-1}$) | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 7.538 ± 1.613 | Current study |
| *Isurus oxyrinchus* | South Africa | 1.617 ± 1.257 | Current study |
| *Galeorhinus galeus* | South Africa | 18.409 ± 4.096 | Current study |
| *Mustelus mustelus* | South Africa | 29.497 ± 19.321 | Current study |
| *Mustelus mustelus* | South Africa | 4.595 ± 4.849 | Erasmus *et al.,* 2004 |
| Dogfish | French Atlantic coast | 7.96 – 25.34 | De Gieter *et al.,* 2002 |
| Dogfish | Ostkante | 5.62 – 10.78 | De Gieter *et al.*, 2002 |
| *Scyliorhinus canicula* | North sea and channel | 21.3 – 64.0 | De Gieter *et al.*, 2002 |
| *Squalus megalops* | South Africa | 45.468 ± 15.418 | Erasmus *et al.*, 2004 |

| Species | Location | Zn | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 3.519 ± 0.795 | Current study |
| *Isurus oxyrinchus* | South Africa | 2.888 ± 0.500 | Current study |
| *Isurus oxyrinchus* | Pacific ocean | 4.00 | Vlieg *et al.,* 1993 |
| *Galeorhinus galeus* | South Africa | 2.824 ± 0.155 | Current study |
| *Galeorhinus galeus* | Atlantic ocean | 2.12 | Vas, 1991 |
| *Mustelus mustelus* | South Africa | 4.346 ± 0.411 | Current study |
| *Mustelus mustelus* | South Africa | 5.210 ± 2.589 | Erasmus *et al*., 2004 |
| *Mustelus mustelus* | Mediterranean | 3.38 | Storelli *et al.*, 2011 |
| *Galeocerdo cuvier* | Japan | 4.72 ± 3.28 | Endo *et al.,* 2008 |
| *Carcharhinus albimarginatus* | Japan | 3.40 ± 0.81 | Endo *et al.*, 2008 |
| *Squalus megalops* | South Africa | 112.432 ± 57.536 | Erasmus *et al.*, 2004 |
| *Sphyrna zygaena* | Mediterranean | 6.97 | Storelli *et al.*, 2003 |

| Species | Location | Fe | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 2.393 ± 0.9039 | Current study |
| *Isurus oxyrinchus* | South Africa | 4.242 ± 1.1377 | Current study |
| *Galeorhinus galeus* | South Africa | 2.355 ± 0.4469 | Current study |
| *Mustelus mustelus* | South Africa | 3.139 ± 0.6770 | Current study |
| *Mustelus mustelus* | South Africa | 36.626 ± 26.515 | Erasmus *et al.*, 2004 |
| *Galeocerdo cuvier* | Japan | 3.10 ± 1.47 | Endo *et al.*, 2008 |
| *Carcharhinus albimarginatus* | Japan | 3.26 ± 1.93 | Endo *et al.*, 2008 |
| *Squalus megalops* | South Africa | 328.512 ± 129.900 | Erasmus *et al*., 2004 |

| Species | Location | Al | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 2.270 ± 2.118 | Current study |
| *Isurus oxyrinchus* | South Africa | 1.283 ± 1.336 | Current study |
| *Galeorhinus galeus* | South Africa | 0.723 ± 0.311 | Current study |
| *Mustelus mustelus* | South Africa | 1.246 ± 0.416 | Current study |
| *Mustelus mustelus* | South Africa | 41.142 ± 20.120 | Erasmus *et al.*, 2004 |
| *Squalus megalops* | South Africa | 31.840 ± 24.578 | Erasmus *et al.*, 2004 |

| Species | Location | Hg (MAL = 1.0 mg·kg$^{-1}$) | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 0.521 ± 0.198 | Current study |
| *Prionace glauca* | South Eastern Pacific | 0.014 ± 0.09 | Lopez *et al.*, 2013 |
| *Prionace glauca* | North East Pacific | 1.03 ± 0.08 | Barrera-García *et al*., 2012 |
| *Prionace glauca* | North East Pacific | 1.39 ± 1.58 | Escobar-Sanchez *et al.,* 2011 |
| *Prionace glauca* | North Pacific | 0.82 ± 0.34 | Maz-Courrau & López-Vera, 2006 |
| *Prionace glauca* | South East Atlantic | 0.76 | Dias *et al.*, 2008 |
| *Prionace glauca* | Atlantic ocean (Azores) | 0.22 ± 1.3 | Branco *et al.*, 2007 |
| *Prionace glauca* | Atlantic ocean (Equator) | 0.68 ± 2.5 | Branco *et al.*, 2007 |
| *Prionace glauca* | Adriatic sea | 0.38 | Storelli *et al.*, 2001 |
| *Isurus oxyrinchus* | South Africa | 1.743 ± 0.516 | Current study |
| *Isurus oxyrinchus* | New Jersey | 1.83 ± 0.17 | Burger & Gochfeld, 2011 |
| *Isurus oxyrinchus* | South Eastern Pacific | 0.006 ± 0.001 | Lopez *et al.*, 2013 |
| *Isurus oxyrinchus* | North Pacific | 0.4 | Velez 2009 |
| *Isurus oxyrinchus* | South West Pacific | 1.58 | Vlieg *et al.*, 1993 |
| *Isurus oxyrinchus* | North Pacific | 1.05 ± 0.82 | Maz-Courrau *et al.*, 2012 |
| *Galeorhinus galeus* | South Africa | 0.958 ± 0.5815 | Current study |
| *Mustelus mustelus* | South Africa | 0.999 ± 0.7005 | Current study |
| *Mustelus mustelus* | Adriatic sea (Italy) | 0.31 ± 0.06 | Storelli *et al.*, 2002 |
| *Mustelus mustelus* | Mediterranean | 1.77 | Storelli *et al.*, 2011 |
| *Galeocerdo cuvier* | Japan | 0.78 ± 0.29 | Endo *et al.*, 2008 |
| *Carcharhinus albimarginatus* | Japan | 1.80 ± 0.45 | Endo *et al.*, 2008 |
| *Carcharodon leucas* | Florida | 0.77 ± 0.32 | Adams & McMichael, 1999 |
| *Carcharhinus limbatus* | Florida | 0.77 ± 0.71 | Adams & McMichael, 1999 |
| *Rhizoprionodon terraenovae* | Florida | 1.06 ± 0.63 | Adams & McMichael, 1999 |
| *Sphyrna tiburo* | Florida | 0.50 ± 0.36 | Adams & McMichael, 1999 |

| Species | Location | Se | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 0.258 ± 0.063 | Current study |
| *Prionace glauca* | Atlantic ocean (Azores) | 0.084 ± 0.30 | Branco *et al.*, 2007 |
| *Prionace glauca* | Atlantic ocean (Equator) | 0.23 ± 0.46 | Branco *et al.*, 2007 |
| *Isurus oxyrinchus* | South Africa | 0.293 ± 0.073 | Current study |
| *Isurus oxyrinchus* | New Jersey | 0.26 ± 0.014 | Burger & Gochfeld, 2011 |
| *Galeorhinus galeus* | South Africa | 0.374 ± 0.129 | Current study |
| *Mustelus mustelus* | South Africa | 0.718 ± 0.464 | Current study |
| *Xiphias gladius* | Atlantic ocean (Azores) | 0.18 ± 1.2 | Branco *et al.*, 2007 |
| *Xiphias gladius* | Atlantic ocean (Equator) | 0.36 ± 0.73 | Branco *et al.*, 2007 |
| *Thunnus albacares* | New Jersey | 0.47 ± 0.027 | Burger & Gochfeld, 2011 |

| Species | Location | Cu | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 0.235 ± 0.0558 | Current study |
| *Prionace glauca* | Atlantic ocean | 0.24 | Vas, 1991 |
| *Isurus oxyrinchus* | South Africa | 0.252 ± 0.0240 | Current study |
| *Isurus oxyrinchus* | Pacific ocean | 0.35 | Vlieg *et al.*, 1993 |
| *Galeorhinus galeus* | South Africa | 0.239 ± 0.0955 | Current study |
| *Galeorhinus galeus* | Atlantic ocean | 0.44 | Vas, 1991 |
| *Mustelus mustelus* | South Africa | 0.297 ± 0.0702 | Current study |

| | | | |
|---|---|---|---|
| *Mustelus mustelus* | South Africa | 0.668±0.410 | Erasmus *et al*., 2004 |
| *Mustelus mustelus* | Mediterranean | 0.71 | Storelli *et al.*, 2011 |
| *Squalus megalops* | South Africa | 14.824 ± 23.576 | Erasmus *et al.*, 2004 |
| *Sphyrna zygaena* | Mediterranean | 1.45 | Storelli *et al.*, 2003 |
| *Centrophorus granulosus* | Mediterranean | 0.36 | Hornung *et al.*, 1993 |

| Species | Location | Mn | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 0.076 ± 0.010 | Current study |
| *Isurus oxyrinchus* | South Africa | 0.093 ± 0.027 | Current study |
| *Galeorhinus galeus* | South Africa | 0.107 ± 0.014 | Current study |
| *Mustelus mustelus* | South Africa | 0.083 ± 0.015 | Current study |
| *Mustelus mustelus* | South Africa | 0.429 ± 0.199 | Erasmus *et al.*, 2004 |
| *Squalus megalops* | South Africa | 10.575 ± 9.828 | Erasmus *et al.*, 2004 |

| Species | Location | Cr | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 0.010 ± 0.011 | Current study |
| *Isurus oxyrinchus* | South Africa | 0.009 ± 0.007 | Current study |
| *Galeorhinus galeus* | South Africa | 0.014 ± 0.014 | Current study |
| *Mustelus mustelus* | South Africa | 0.045 ± 0.030 | Current study |
| *Mustelus mustelus* | South Africa | 0.066 ± 0.050 | Erasmus *et al.*, 2004 |
| *Mustelus mustelus* | Mediterranean | 0.13 | Storelli *et al.*, 2011 |
| *Squalus megalops* | South Africa | 2.438 ± 2.625 | Erasmus *et al*., 2004 |
| *Sphyrna zygaena* | Mediterranean sea | 0.18 | Storelli *et al.*, 2003 |
| *Carcharhinus limbatus* | Atlantic ocean | 0.44 | Núnez-Nogueira, 2005 |
| *Heterodontus portusjacksoni* | Pacific ocean | 0.14 | Gibbs & Miskiewicz, 1995 |

| Species | Location | Cd (MAL = 1.0 mg·kg$^{-1}$) | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 0.009 ± 0.0046 | Current study |
| *Prionace glauca* | Atlantic ocean | 0.45 | Vas, 1991 |
| *Isurus oxyrinchus* | South Africa | 0.005 ± 0.0030 | Current study |
| *Galeorhinus galeus* | South Africa | 0.004 ± 0.0024 | Current study |
| *Galeorhinus galeus* | Atlantic ocean | <0.02 | Vas, 1991 |
| *Mustelus mustelus* | South Africa | 0.037 ± 0.0210 | Current study |
| *Mustelus mustelus* | South Africa | 0.170 ± 1.145 | Erasmus *et al*., 2004 |
| *Mustelus mustelus* | Mediterranean | 0.01 | Storelli *et al.*, 2011 |
| *Squalus megalops* | South Africa | 3.959 ± 1.857 | Erasmus *et al.*, 2004 |
| *Sphyrna zygaena* | Mediterranean | 0.03 | Storelli *et al.*, 2003 |
| *Centrophorus granulosus* | Mediterranean | 0.06 | Hornung *et al.*, 1993 |
| *Etmopterus spinax* | Mediterranean | 0.08 | Hornung *et al.*, 1993 |

| Species | Location | Pb (MAL = 0.5 mg·kg$^{-1}$) | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 0.004 ± 0.0036 | Current study |
| *Prionace glauca* | South Eastern Pacific | 2.244 ± 0.81 | Lopez *et al.*, 2013 |
| *Prionace glauca* | Atlantic ocean | <0.02 | Vas, 1991 |
| *Isurus oxyrinchus* | South Africa | 0.014 ± 0.0064 | Current study |
| *Isurus oxyrinchus* | South Eastern Pacific | 0.848 ± 0.47 | Lopez *et al.*, 2013 |
| *Isurus oxyrinchus* | North Pacific | 0.29 | Velez 2009 |
| *Galeorhinus galeus* | South Africa | 0.001 ± 0.0000 | Current study |
| *Galeorhinus galeus* | Atlantic ocean | 0.16 | Vas, 1991 |
| *Mustelus mustelus* | South Africa | 0.031 ± 0.0189 | Current study |
| *Mustelus mustelus* | South Africa | 0.128 ± 0.106 | Erasmus *et al.*, 2004 |
| *Mustelus mustelus* | Mediterranean | 0.06 | Storelli *et al.*, 2011 |
| *Squalus megalops* | South Africa | 11.862 ± 13.748 | Erasmus *et al*., 2004 |
| *Sphyrna zygaena* | Mediterranean sea | 0.02 | Storelli *et al.*, 2003 |
| *Scyliorhinus canicula* | Atlantic ocean | 0.35 | Vas, 1991 |

| Species | Location | Co | Reference |
|---|---|---|---|
| *Prionace glauca* | South Africa | 0.003 ± 0.002 | Current study |
| *Isurus oxyrinchus* | South Africa | 0.001 ± 0.0007 | Current study |
| *Galeorhinus galeus* | South Africa | 0.002 ± 0.002 | Current study |
| *Mustelus mustelus* | South Africa | 0.002 ± 0.0009 | Current study |
| *Mustelus mustelus* | South Africa | 0.016 ± 0.015 | Erasmus *et al.*, 2004 |
| *Squalus megalops* | South Africa | 2.585 ± 2.583 | Erasmus *et al.*, 2004 |

# CHAPTER 10:

## General discussion and conclusion

Fish meat is recommended internationally as part of a healthy diet as it is a food source high in protein providing essential minerals, amino acids and omega-3 fatty acids (Kris-Etherton *et al*., 2002; Limin *et al.*, 2006). It also contributes substantially to food security, especially in countries with extended coastlines, such as South Africa (Bell *et al*., 2009; Isaacs, 2013). However, despite the benefits, fish meat consumption may also carry potential hazards in the form of metal contaminants, which are absorbed into fish tissue from the environment due to both natural and anthropogenic sources and when ingested above certain quantities, can have harmful effects on human health. Metal contaminants can occur in a wide variety of food products, but metals commonly accumulated to toxic levels in fish tissue include arsenic (As), mercury (Hg), cadmium (Cd) and lead (Pb). These metals therefore need to be continually monitored in fish and fish products available for consumption. The toxicity of metals, however, varies with regards to the chemical forms of the metals present, their distribution across fish carcasses and concentrations in different sized fish and species. Monitoring of specific toxic metal components in fish could therefore require extensive sampling and analyses adding to processing time and costs. Research on the toxicity, distribution and final concentrations of metals in various fish could therefore help to optimise more accurate and efficient sampling and analytical techniques. Such research on South African marine fish is extremely limited (Wepener & Degger, 2012); therefore this study is one of the first to comprehensively investigate metal concentrations in important South African marine fish species.

It was confirmed that when inductively coupled plasma mass spectrometry (ICP-MS), which is currently commonly used for total metal analyses and monitoring, is coupled to high pressure liquid chromatography (HPLC), this online system (HPLC-ICP-MS) can be used as an accurate and effective method for Hg speciation in fish muscle samples. This separation and quantification of total Hg into its individual Hg species (inorganic Hg, methylmercury and ethylmercury) showed that methylmercury (MeHg), which is one of the toxic Hg species, is the predominant Hg form present in fish muscle with concentrations of ethylmercury (EthHg) as the other toxic Hg species being negligible (Bosch *et al.*, 2016a). Methylmercury is therefore the actual Hg component which should be monitored to determine the true Hg toxicity in fish muscle.

Investigating the distribution of both total metal and Hg species concentrations across the carcass of larger fish species with distinctly different muscle types showed that sampling from the dark muscle for total Hg monitoring could result in falsely high indications of toxic Hg concentrations as higher total Hg concentrations were measured in the dark muscle due to an increased non-toxic inorganic Hg component (Bosch *et al.*, 2016b). Therefore, consistent sampling from the dorsal anterior (light muscle) portions of fish should provide monitoring results that are representative of both the total metal concentrations across the

edible muscle portions (light muscle) of the fish as well as Hg toxicity across the entire carcass muscle (light and dark muscle). Samples can therefore be taken without harming the industrial quality of fish fillets as offcuts of whole fillet or muscle pieces could be used. As several metals as well as the toxic Hg component were found to vary with varying fish size, subsamples taken for monitoring fish safety should include fish sizes representing all size categories present in the total catch in order to obtain results that represent the food safety of the total catch.

Strong relationships found between total Hg and MeHg in all fish species studied indicate that toxic Hg concentrations could be accurately determined from total Hg measurements. In yellowfin tuna (*Thunnus albacares*) a prediction equation with weight as covariate ($cMeHg = 0.073 + 1.365 \cdot ctHg - 0.008 \cdot w$) can be used to calculate the MeHg concentrations from total Hg measurements. In all other fish species investigated [blacktail (*Diplodus sargus capensis*), hottentot (*Pachymetopon blochii*), yellowtail (*Seriola lalandi*), snoek (*Thyrsites atun*), blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), soupfin (*Galeorhinus galeus*) and smoothhound (*Mustelus mustelus*)] total Hg measurements can be used as a direct indication of MeHg concentrations ($cMeHg = ctHg$) as the contribution of other Hg components to total Hg concentrations are negligible.

These accurate and effective methods, improved sampling protocol and MeHg prediction equations could benefit the fishing and processing industry by saving costs of additional methods and equipment and preventing discard of catches falsely classified as toxic or unsuitable for consumption. It could also help protect the consumer from being exposed to toxic metal levels due to biased sampling and monitoring results.

Metal analyses can, however, only be done once fish have reached the processing plant and analytical results are usually only obtained once processing has been completed. Therefore if recordable or visible fish parameters could be used to estimate which fish would be likely to contain toxic metal concentrations, the processing industry could save significant amounts of time and costs spent on analyses and lost due to discard of product found to be unsuitable for human consumption by rejecting such fish before it reaches the processing line. However, few consistent trends in the effects of recordable or visible parameters on metal concentrations were found in the current study. Too much intermetal and interspecific variation was found to extrapolate results from one species to similar trophic position species and fish metal concentrations were not significantly correlated to environmental pollution. In some fish species (yellowfin tuna, yellowtail and soupfin shark), fish size could be used as an indicator of toxicity levels of certain metals. Such information could be used to motivate the fisher persons to target fish considered safer for consumption (yellowfin tuna < 70 kg for example), which could help reduce unnecessary losses for the fish processing industry and in turn protect the consumer from being exposed to toxic metal concentrations through fish consumption.

Metal concentrations in fish should be individually investigated as metal accumulation is both metal and species specific. Table 10.1 presents a summary of the mean metal concentrations in hottentot (*P.*

*blochii*), blacktail (*D. sargus capensis*), snoek (*T. atun*), yellowtail (*S. lalandi*), blue shark (*P. glauca*), shortfin mako (*I. oxyrinchus*), soupfin shark (*G. galeus*), smoothhound shark (*Mustelus mustelus*) and yellowfin tuna (*T. albacares*) with regards to regulatory limits for safe consumption where applicable. Most metals did not display considerable differences in concentrations among species or trophic levels with a select few exceptions. Concentrations of Zn, Fe, Se and Cu were higher in yellowfin tuna than all other fish species, but still well within maximum allowable limits (MALs) and upper limits (ULs) and Fe possibly adding to recommended daily intake of essential Fe (8 $mg \cdot day^{-1}$). Fish species can be grouped into 3 categories according to As toxicity: hottentot, snoek and yellowtail were low in As (< 1.5 $mg \cdot kg^{-1}$); blacktail, shortfin mako and yellowfin tuna had intermediate concentrations (1.5 – 3.0 $mg \cdot kg^{-1}$); and blue shark, soupfin and smoothhound had high concentrations of As (> 3.0 $mg \cdot kg^{-1}$). Mercury was the only metal displaying inter-specific differences between fish in diverse trophic levels with higher concentrations observed in high trophic level fish. Even though several of the fish species studied (hottentot, blacktail, snoek, yellowtail, blue shark and yellowfin tuna) contained mean Hg concentrations considered safe for human consumption according to MALs, excessive consumption of such fish could lead to Hg intake which exceeds the provisional tolerable weekly intake (PTWI) and could lead to negative health effects. Daily consumption (150 g portions) of hottentot by the average adult (70 kg) is considered safe according to the PTWI (1.6 $\mu g \cdot kg^{-1}$ body weight) for Hg, whereas blacktail should be limited to three portions a week and snoek to two portions a week. On average (including yellowtail of all sizes), four portions of yellowtail can be safely consumed per week; however, as Hg concentrations increase with yellowtail size, the consumption of larger yellowtail (12 - 14 kg) should be limited to just two portions a week. One portion of blue shark per week can be safely consumed without exceeding the PTWI, whereas one portion of yellowfin tuna could already be in excess of the maximum PTWI for Hg. Weekly consumption of yellowfin tuna should therefore be limited to smaller sized fish as Hg concentrations are increased with increasing fish size. It is also recommended that catches of yellowfin tuna above 70 kg be avoided as such individuals are likely to contain toxic Hg concentrations. Single portions of shortfin mako, smoothhound and mature soupfin (above 120 cm total length) sharks all provide Hg quantities exceeding the PTWI, with shortfin mako providing more than double the PTWI; therefore normal human consumption should be limited or avoided in order to ensure safe Hg intakes.

The one major limitation of this study was the sourcing of certain species from specific locations which proved more difficult than expected as fishing was dependent on environmental conditions and size ranges were limited for many of the species as they tend to appear in schools of similar sizes. This might have led to unavoidable sample bias in several cases. In addition, a sample population such as smoothhound from Langebaan lagoon, which is a closed environmental system, could differ from other South African smoothhound populations. Therefore these results provide baseline information, but require further research in order to be extrapolated to the whole of South Africa. Also, total metal and metal species accumulations in fish muscle are clearly species specific, therefore even though certain trends could be noticed from the species currently studied; this study merely represents a baseline and motivation for similar

studies on the many other important fish species such as kabeljou, red roman, haarders, white stumpnose, geelbek and many more consumed in South Africa. Such extended research could help health authorities to set up more specific fish consumption guidelines for South African consumer as the examples given in the current study. These guidelines will, however, need frequent monitoring and adjustment as metal pollution increases.

The current study also provides a strong baseline for further research on the speciation of other metals such as As. Even though As has been found in levels exceeding the maximum guideline levels for human consumption in several species, no conclusion on true As toxicity can be drawn from the current research as it is assumed that As is mostly present in its non-toxic form in fish tissue (Goyer & Clarkson, 2001; Castro-González & Méndez-Armenta, 2008). Further, more detailed studies on speciation of this metal are required to more accurately estimate or determine the safety of commercial fish meat in South Africa in terms of As toxicity.

Overall, this study provided sampling protocol recommendations for the accurate and effective measurement of total and toxic metal concentrations in fish muscle samples and subsamples and provides a baseline for the concentrations and distribution of 16 metals in muscle of South African marine fish in terms of species, muscle type, fish size and location. Such information (as summarised in Table 10.1) can be valuable when considered by the fishing and processing industry as well as health authorities for setting up consumer recommendations.

**Table 10.1** Mean metal concentration in mg·kg$^{-1}$ (standard deviation) (**number of individual samples exceeding the maximum allowable limits**) per fish species sampled off the South African coast compared to maximum allowable limits (MAL) and upper limits (UL) of South African, American and European legislation.

| | *P. blochii* (n = 58) | *D. sargus capensis* (n = 76) | *T. atun* (n = 20) | *S. lalandi* (n = 36) | *P. glauca* (n = 10) | *I. oxyrinchus* (n = 10) | *G. galeus* (n = 12) | *M. mustelus* (n = 30) | *T. albacares* (n = 14) | SA[1] (MAL) | USA[2] (UL) | EU[3] (MAL) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| As | 0.77 (0.218) | 1.82 (1.223) (**13**) | 0.61 (0.210) | 0.98 (0.431) | 7.54 (1.613) (**10**) | 1.62 (1.257) (**1**) | 18.41 (4.096) (**12**) | 29.50 (19.321) (**30**) | 1.53 (0.488) | 3.0 mg·kg$^{-1}$ | UL not established | - |
| Zn | 3.35 (0.529) | 3.70 (0.702) | 4.62 (0.699) | 4.02 (0.462) | 3.52 (0.795) | 2.89 (0.500) | 2.82 (0.155) | 4.35 (0.411) | 8.41 (2.304) | - | 40 mg·day$^{-1}$ | - |
| Fe | 2.85 (1.024) | 3.66 (1.407) | 2.80 (0.873) | 5.41 (1.521) | 2.39 (0.904) | 4.24 (1.138) | 2.36 (0.447) | 3.14 (0.677) | 29.82 (9.369) | - | 45 mg·day$^{-1}$ | - |
| Al | 1.14 (1.342) | 1.89 (1.996) | 3.61 (9.369) | 1.58 (2.084) | 2.27 (2.118) | 1.28 (1.336) | 0.72 (0.311) | 1.25 (0.416) | 0.81 (0.647) | - | - | - |
| Hg | 0.10 (0.070)* | 0.19 (0.116) (**1**)* | 0.27 (0.121) (**2**)* | 0.16 (0.093)* | 0.52 (0.198)** | 1.74 (0.516) (**10**)** | 0.96 (0.581) (**4**)** | 1.00 (0.701) (**12**)** | 0.77 (0.238) (**4**)** | 0.5 mg·kg$^{-1}$* 1.0 mg·kg$^{-1}$** | - | 0.5 mg·kg$^{-1}$* 1.0 mg·kg$^{-1}$** |
| Se | 0.21 (0.081) | 0.28 (0.131) | 0.41 (0.048) | 0.39 (0.042) | 0.26 (0.064) | 0.29 (0.073) | 0.37 (0.129) | 0.72 (0.464) | 1.79 (0.613) | - | 0.4 mg·day$^{-1}$ | - |
| Cu | 0.26 (0.162) | 0.27 (0.120) | 0.29 (0.047) | 0.47 (0.130) | 0.24 (0.056) | 0.25 (0.024) | 0.24 (0.096) | 0.30 (0.070) | 1.20 (0.323) | - | 10 mg·day$^{-1}$ | - |
| Mn | 0.14 (0.053) | 0.14 (0.214) | 0.29 (0.134) | 0.09 (0.042) | 0.08 (0.010) | 0.09 (0.027) | 0.11 (0.013) | 0.08 (0.015) | 0.11 (0.030) | - | 11 mg·day$^{-1}$ | - |
| Cr | 0.02 (0.037) | 0.04 (0.068) | 0.03 (0.039) | 0.006 (0.0046) | 0.01 (0.010) | 0.009 (0.0073) | 0.01 (0.014) | 0.05 (0.030) | 0.06 (0.063) | - | UL not established | - |
| Ni | 0.06 (0.103) | 0.04 (0.088) | 0.02 (0.027) | 0.01 (0.016) | 0.01 (0.007) | 0.02 (0.006) | 0.01 (0.008) | 0.19 (0.224) | 0.03 (0.014) | - | 1 mg·day$^{-1}$ | - |
| Cd | 0.005 (0.0048) | 0.005 (0.0115) | 0.008 (0.0038) | 0.004 (0.0026) | 0.009 (0.0046) | 0.005 (0.0030) | 0.004 (0.0024) | 0.04 (0.021) | 0.02 (0.014) | 1.0 mg·kg$^{-1}$ | - | 0.1 mg·kg$^{-1}$ |
| Pb | 0.005 (0.0097) | 0.03 (0.031) | 0.009 (0.0052) | 0.005 (0.0067) | 0.004 (0.0036) | 0.01 (0.006) | 0.001 (0.0000) | 0.03 (0.019) | 0.009 (0.0071) | 0.5 mg·kg$^{-1}$ | - | 0.3 mg·kg$^{-1}$ |
| Co | 0.002 (0.0014) | 0.009 (0.0089) | 0.008 (0.0062) | 0.003 (0.0013) | 0.003 (0.0018) | 0.001 (0.0007) | 0.002 (0.0023) | 0.002 (0.0009) | 0.008 (0.0029) | - | - | - |

[1]Department of Health, 2004; [2]Food and Nutrition Board, 2000; 2001; [3]Commission Regulation (EC), 2001; 2006; 2008. *For fish species in general; **For predatory fish species

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