Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticide residues in selected marine fish species along the coast of South Africa

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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.

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Summary

Fish consumption is being threatened by the accumulation of hazardous substances within the flesh due to natural occurrences and anthropogenic activities. This tends to undermine the health benefits derived from fish consumption. Human exposure to hazardous compounds has been predominantly through dietary intake and fish in particular. In this study, global priority contaminants, polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) were assessed in some marine fish species of commercial and local consumption importance. The study aims were to assess the occurrences, levels and profiles of selected organic contaminants in marine species covering low trophic (resident) fish species (blacktail and hottentot); middle trophic species (yellowtail and snoek) and the high trophic (predatory) species (tuna and sharks). Also by using diagnostic ratios, the pollution input sources/history was determined and the locations and species with increased burdens, identified. Screened fish were considered safe for consumption based on comparison with regulatory critical values. Extraction of target compounds (analytes) were efficiently carried out following the quick, easy, cheap, efficient, rugged and safe (QuEChERS) method and analysed simultaneously with gas chromatography coupled to mass spectrometry triple quadrupole (GC-MS/MS). Quality control measures were in place and reference material analysed for method validation. Limit of detection, quantitation and percentage recoveries of analytes were within acceptable values. All the analysed species showed evidence of contamination but with variations in profiles and levels. The profile trend was predominantly low molecular weight (LMW) (i.e. non-carcinogenic) PAHs, however, from Port Elizabeth and Dassen Island high molecular weight (HMW) PAHs predominated. PAHs contamination levels in this study ranged from low contamination (<100 µg/kg) in samples from Hout Bay (87.29 ± 1.96 µg/kg wet weight (ww) in hottentot) to a moderate contamination (<1000 µg/kg ww) in tuna dark muscle from False Bay (636.31± 36.03 µg/kg ww). The PAHs acceptable/presence indicator, benzo(*a*)pyrene was found in excess of the European Union (EU) maximum limit (2 µg/kg ww) in fish from 8 locations with highest (29.79 ± 0.81 µg/kg ww) in Blue Shark (False Bay). Intra and inter species variations were observed and considered to be due to fish size, lipid content, feeding habit, trophic level and locational input sources. The hottentot and soupfin shark species were not found with measurable levels of benzo(*a*)pyrene and could be considered acceptable species for human consumption so as to minimize exposure to carcinogenic PAHs. However, the highest DDT level (760.50 ± 484.53 µg/kg ww) was recorded in hottentot (Dassen Island) but was below the Food and Drug Administration (FDA) action level (5000 µg/kg ww). A holistic risk assessment study that will evaluate fish health benefits (nutrients) against the risks (contaminants), plus the effect of heat (cooking methods) on measured contaminants in studied species is recommended for future study.

Opsomming

Die verbruik van vis word bedreig deur die akkumulasie van gevaarlike stowwe in die vlees as gevolg van natuurlike oorsake of antropogeniese aktiwiteite. Dit is geneig om die gesondheidsvoordele wat van vis verbruik verkry word te ondermyn. Menslike blootstelling aan gevaarlike stowwe is hoofsaaklik deur inname vanaf die dieet, in die besonder vis. Die universele prioriteitsbesoedelingsagente, polisikliese aromatiese hidrokoolstowwe (PAHs) en organise-chloried plaagdoders, was in hierdie studie in spesifieke mariene visspesies van kommersiële- en plaaslike verbruikersbelang bestudeer. Die doel van die studie was om die voorkoms, vlakke en profiele van gekose organiese besoedelingsagente in mariene spesies, wat die lae trofiese (residensieël) vis spesies (kolstert en hottentot), middel trofiese spesies (geelstert en snoek) asook die hoë trofiese (roofsugtig) spesies (tuna en haai) verteenwoordig, te beoordeel. Diagnostiese verhoudings was gebruik om besoedelings- invoer bronne/geskiedenis te bepaal en om areas en spesies met 'n verhoogde lading te identifiseer. Gekeurde vis is as veilig vir gebruik beskou gebaseer deur vergelyking met regulatoriese kritiese waardes. Die ekstraksie van teikenkomponente (analiete) was effektief uitgevoer deur die vinnige, maklike, goedkoop, effektiewe, robuste en veilige (QuEChers: quick, easy, cheap, efficient, rugged, safe) metode te volg en was gesamentlik met gas chromatografie verbind met massa spektrometrie driedubbel viervoudig (GC-MS/MS), geanaliseer. Kwaliteitsbeheermaatreëls was opgestel en verwysingsmateriaal geanaliseer vir metode validering. Die limiet van waarneming, kwantifisering en persentasie herwinbaarheid van analiete was binne aanvaarbare vlakke. Al die analiseerde spesies het tekens getoon van kontaminasie, maar met variasie in profiele en vlakke. Die profile tendens was hoofsaaklik lae molekulêre gewig (LMW) (dus nie-karsinogenies) PHAs, alhoewel daar vanaf Port Elizabeth en Dassen Eiland hoë molekulêre gewig (HMW) PAHs voorgekom het. PAHs besoedeling in hierdie studie het gewissel van lae kontaminasie (<100 µg/kg) in monsters vanaf Houtbaai (87.29 ± 1.96 µg/kg nat gewig in hottentot) tot 'n matige kontaminasie (<1000 µg/kg nat gewig) in tuna donker spier vanaf Valsbaai (636.31± 36.03 µg/kg nat gewig). Die PAHs veiligheidsmerker, benso(*a*)pireen het die Europese Unie (EU) maksimum limiet (2 µg/kg nat gewig) oorskrei in vis van 8 lokaliteite met die hoogste vlakke (29.79 ± 0.81 µg/kg ww) in Blouhaai vanaf Valsbaai. Intra- en interspesies variasies was waargeneem en was toegeskryf aan visgrootte, lipied inhoud, voedingswyse, trofiese vlak en plaaslike insetbronne. Die lae trofiese vlak species (kolstert en hottentot) het nie meetbare vlakke van benso(*a*)pireen bevat nie en kan as veiliger species beskou word vir mesnlike gebruik om die blootstelling aan karsinogeniese PHAs te beperk. Die hoogste dichlorodiphenieltrichloroethaan (DDT) vlak (760.50 ± 484.53 µg/kg nat gewig) was egter in hottentot (Dasseneiland) waargeneem, maar was onder die 'Food and Drug Administration'' (FDA) optrede vlak (5000 µg/kg nat gewig). 'n

Holistiese risiko assesseringsstudie rondom gesondheidsvoordele van vis (voedingswaarde) teenoor die risiko's (besoedelingsagente), sowel as die effek van hitte (gaarmaakmetodes) op waargenome besoedelingsagente in die bestudeerde species, word vir toekomstige studie aanbeveel.

Dedication

This work is humbly dedicated to the Triumph of the Immaculate heart of the Blessed Virgin Mary, my Queen and my mother and to the Reign of the Sacred Heart of Jesus Christ, my Lord and King.

Contents

Note

Sixteen Polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticide (OCPs) residues of health importance were screened in selected marine fish species along the coast of South Africa. PAHs have not categorically been grouped as persistent contaminants as they can to some extent be biologically (enzymatically) degraded by some fish. In view of the fact that the same factors (PAHs and OCPs) were assessed in all the species, the work appear to be a repetition and may appear monotonous as similar trends in occurrences and profiles were observed. The extraction and instrumental analytical procedures (materials and methods) was detailed in Chapters 3 and 4 and therefore not repeated in subsequent chapters. All the concentrations (values) of contaminants are reported on fresh weight or weight wet (ww) basis, should it not be indicated along with the value.

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List of abbreviations and acronyms

Ace - Acenaphthene

Acy - Acenaphthylene

Ant - Anthracene

ADI - Acceptable daily intake

BaA - Benzo(*a*)anthracene

BaP - Benzo(*a*)pyrene

BbF - Benzo(*b*)fluoranthene

BkF - Benzo(*k*)fluoranthene

BghiP - Benzo(*g,h,i*)perylene

BHC - Benzenehexachloride

CHLs - Chlordane

DahA - Dibenzo(*a,h*)anthracene

DDE - Dichlorodiphenyldichloroethylene

DDD - Dichlorodiphenyldichloroethane

DDT - Dichlorodiphenyltrichloroethane

Dl – Detection limit

Dw - Dry weight

EDI - Estimated daily intake

EU - European union

FAO - Food and agricultural organization

FDA - Food and drug administration

FLD - Fluorescence detector

Flu - Fluorene

Fluo - Fluoranthene

GC - Gas chromatography

HCB - Hexachlorobenzene

HCH-Hexachlorocyclohexane

HMW- High molecular weight

LC - Liquid chromatography

LMW - Low molecular weight

LOD - Limit of detection

LOQ - Limit of quantification

Lw - Lipid weight

MD - Missing data

MRL - Maximum residue level

MS - Mass spectrometer

NA - Not analysed/assessed

Nap - Naphthalene

ND - Not detected

OCs - Organic contaminants

OCP - Organochlorine pesticides

PAH - Polycyclic aromatic hydrocarbon

PCB - Polychlorinated biphenyl

Phe - Phenanthrene

POP - Persistent organic pollutant

Pyr - Pyrene

TDM - Tuna dark muscle

TLM - Tuna light muscle

Ww - Wet weight

CHAPTER 1

Introduction

Fish is one of the most traded commodities globally and has consequently provided millions of jobs, and has thus offset food security issues in many under developed countries. (FAO, 2012; FAO, 2014). Global fish production (wild and farmed) has progressively increased over time and was estimated at 158 million metric tonnes in 2012, with wild fish capture (marine and inland water) dominating the supply (FAO, 2014). Similarly fish consumption has increased with approximately 19 kg global consumption per capita (FAO, 2014). Fish consumption in developing countries (5.2-17.8 kg consumption per capita) and low-income-highly food insecure countries (4.9-10.9 kg consumption per capita) has also increased over the past 5 decades (FAO, 2014), which highlights the increasing dependence on fish and other aquatic foods for daily dietary requirements (Santerre, 2008).

The benefits of fish consumption are wide and varied and regular consumption is highly recommended (FAO/WHO, 2010). Fish are considered an excellent source of high quality protein that contain essential amino acids (e.g. lysine, methionine and cysteine) (Usydus & Richerts, 2009), vitamins (D and B12), minerals such as Iron, Iodine, Potassium, Calcium, Zinc and Selenium (FAO/WHO, 2010; Mitchel, 2011; Öhrvik, *et al.*, 2012). Most importantly, emphasis on fish consumption has been centred on its low fat (saturated) content and high content of omega 3 and 6 polyunsaturated fatty acids (PUFA) (FAO/WHO, 2010), essential in brain development and reduction of cardio vascular diseases in human (Carlson *et al.*, 2013; Ruxton *et al.,* 2004). Furthermore, fish muscle is comparatively more digestible (FAO, 2012) and relatively cheaper in comparison to other meat sources (Tacon & Metian, 2013). However, these health and economic benefits of fish is greatly threatened by the accumulation of toxic and hazardous (organic and inorganic) substances (Santerre, 2008) that sink into the aquatic environment (Borgå *et al.*, 2004). These hazardous substances emanate from both natural occurrences and human activities.

The global human population is anticipated to be up to 9.6 billion by 2050 (FAO, 2014) and efforts are being made towards the provision of sufficient food to feed and sustain the teeming population. However, as the population keeps increasing, so too has anthropogenic activities (e.g. agriculture, oil exploration/refining, industrialization and waste disposal), use and disposal of chemicals increased; many of which can have varying degrees of toxicity. Heavy industries (oil and gas drilling, ports, harbour automobiles), domestic, municipal and industrial incineration of organic substance, agriculture (use of pesticides and bush burning), anti-fouling paints (used in boats) and disease vector control (malaria) are a few of the main contributors (sources) of harmful contaminants into the environment (UNEP, 2006). These

contaminants are transported through water and air currents into the marine environment (UNEP, 2006) which act as the final repository (Froescheis *et al.*, 2000; Borgå *et al.*, 2004; Tanabe & Ramu, 2012).

In this study, contaminants of focus include the sixteen polycyclic aromatic hydrocarbons (PAHs) listed as priority compounds by US EPA (1998) and organochlorine pesticides (OCPs) listed as POPs by UNEP (2005). The studied OCPs were dichlorodiphenyltrichloroethane (DDT) grouped as (POPs with restricted production and use), benzenehexachlorine (BHC) (also called hexachlorobenzene – HCB) and analogues (alpha, beta, delta and gamma), aldrin, endrin, dieldrin (the latter 3 - POPs not to be manufactured or used), endosulfan (alpha and beta) and more. Following the discovery of the toxic, carcinogenic and mutagenic effects of these compounds on both target and non-target organisms including humans, they were restricted or banned globally (ASTDR, 2002; US EPA, 1998; UNEP, 2005; EFSA, 2006, EFSA, 2008; UNEP, 2009). Thus based on UNEP (2005), priority contaminants were grouped into three categories as those earmarked for total elimination, restricted or permitted usage and those unintentionally produced. The studied contaminants appear under these three catogories with DDT as the only pesticide under restricted usage while the rest of the studied OCPs were grouped as POPs for total elimination. PAHs on the other hand are under the category of unintentionally produced POPs.

DDT is therefore used only in malaria endemic countries such as China, Mexico, India and South Africa (WHO, 2011). In line with EPA's guideline for assessment of contaminants in fish (US EPA, 2000), these target contaminants were chosen from the global list and particularly for their possible prevalence in the study locations (Naidoo & Buckley, 2003; Wells & Leonard, 2006; Degger *et al.*, 2011; Wepener, *et al.*, 2012). Health effects of these contaminants have been reported to include promotion of cancer, neurological and immunological disorders, endocrine disruption, retard development, lower intelligent quotient, cause low birth weight, miscarriage and infertility (ASTDR, 1995, ASTDR, 2002, EFSA, 2006; EFSA, 2008). The target contaminants (PAHs and OCPs) under focus in this study hereby are generally referred to as organic contaminants (OCs) except where stated otherwise.

Coastal and riverine areas are usually heavily populated and South Africa is no exception where more than 30% of the total population reside near the coast (DAFF, 2012). Coastal areas globally have been found to host the citing of heavy industries such as oil and gas exploring and drilling, refineries, ports, harbours, engineering construction companies among others (UNEP, 2006) which are potential sources of marine pollution. Although South Africa has and enforces laws to reduce marine pollution, various matrices have been reported to accumulate these substances such as DDT in sediment (Humphries, 2013), freshwater fish (Barnhoorn *et al.*, 2015) and in breast milk (Channa *et al.*, 2012). These contaminants are

ubiquitous and their spatial transferability (due to no trans boundary limit) have led to the detection of contaminants even in remote places from point source (Channa *et al.*, 2012). At present large knowledge gaps exist in our understanding of contamination levels in edible marine fin fish species within South Africa and the African continent as a whole.

Human exposure to OCs is largely through the diet and particularly via fish and fishery products (Domingo, 2007; FSAI, 2013). Fish samples from a suspected pollution area are therefore assessed and compared with regulatory limits. The assessment of these trace contaminants require efficient extraction of target analytes with minimum interferences of undesirable substances. A quick, easy, cheap, efficient, rugged and safe (QuEChERS) method (Anasstasaides *et al.*, 2003) is a recent method which uses acetonitrile as extracting solvent, salts to partition the polar and non-polar phases (containing target analytes) with further clean up with primary secondary amine (PSA), in addition to the use of centrifugation to provide high throughput in extraction of analytes. The QuEChERS method is time, solvent and labour efficient (Anasstasaides *et al.*, 2003). In addition, different groups of analytes can simultaneously be extracted and analysed using gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) (Kalachova *et al.*, 2013) to reduce cost, time and labour. Validation of the analytical method was necessary to ensure reliability of data and information and this can be done in number of ways such as the use of certified reference materials of known concentration with recoveries within manufacturers' acceptable limit, inter-laboratory analysis and spike recovery method (Cheung *et al.*, 2007, Nyarko *et al.*, 2011; Ramalhosa *et al.*, 2012).

Thus the overarching aim of this dissertation is to provide an overview of occurrences, levels, profiles and diagnostic sources of selected organic contaminants (16 US EPA priority PAH and OCPs) in South African commercial and commonly consumed marine fish species. This was done through a number of objectives:

1. Identify optimal laboratory procedures for the detection of OCs by comparison of Ultra high pressure liquid chromatography with fluorescence detection (UPLC-FLD) and gas chromatography coupled to mass spectrometer triple quadrupole (GC-MS/MS) methods.

2. Assess the variability in occurrences, levels and profiles of contaminants at three species specific trophic levels: Low (blacktail *Diplodus sargus capensis* and hottentot *Pachymetopon blochii*); medium (yellowtail *Seriola lalandi* and snoek *Thyrsites atun*); high (yellowfin tuna *Thunnus albacares*; sharks- blue shark *Prionace glauca*; shortfin mako *Isurus oxyrinchus*; soupfin *Galeorhinus galeus* and smoothhound *Mustelus mustelus*).

3. Examine contamination level with respect to individual species, locations, lipid content and size (tuna for lipid and yellowtail, lipid and size).

4. Identify potential pollution sources and contaminant usage history (current or past).

5. Compare detected OC values with current regulatory data in order to identify if the meat from each species was within acceptable limits for human consumption. Establish acceptable species for human consumption of assessed fish based on comparison of detected OC values with current regulatory data.

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CHAPTER 2

Polycyclic aromatic hydrocarbons (PAHs) and pesticide residues in edible fresh water and marine fish species with emphasis on Africa: A review

Abstract

Regular fish consumption is recommended for its numerous health benefits such as source of animal protein, vitamins, minerals, low (saturated) fat and high polyunsaturated fatty acids (especially omega 3s). Fish is also beneficial in terms of wealth creation, providing livelihood to over 50 million people globally, thus promoting food security. However, these benefits are threatened by fresh water and marine contamination with environmental toxic and hazardous compounds such as polycyclic aromatic hydrocarbons (PAHs) and organochlorinated pesticides (OCPs). Health hazards associated with intake of these toxic compounds include neurologic, reproductive and immune defects. Although numerous reviews on contaminants in marine and freshwater fish species have been published mostly from Europe, America and Asian continents, limited information to date exist within the African continent. Therefore, the current review summarizes available published literature (from 2000 to date) relating to organic (PAHs & OCPs) contamination in fish within the African continent and outlines the consequent health risk of high contamination. Regional variations in profiles and levels of contaminants (PAHs and OCPs) were established, dichlorodiphenyltrichloroethane (DDT) was however, the most prevalent pesticide. Western and Northern Africa reported increased levels of carcinogenic PAHs in marine fish species in levels above EU limit. A number of recommendations have been made which include standardization of analytical methods, increasing research output from understudied coastal areas which have tendencies to higher contamination. In addition, continued monitoring, assessment of contaminants within each country's legislation and the establishment of fish consumption advisories will minimize health risk particularly among vulnerable groups.

Keywords: Fish, Food security, Health-benefits, Contaminants, Health-risks, Africa.

2.1. Introduction

The global fish production industry is enormous, producing an estimated 158 million metric tonnes in 2012 (dominated by wild catches), employing over 58.3 million people and provides food for a growing global population with global fish consumption approximately 19.2kg per capita (FAO, 2014). The African population overall have the least (<10 kg per capita) fish consumption which varies from country to country (FAO, 2014). Nonetheless, fish is widely consumed due to its relatively low cost and its high nutritional value (protein, essential

amino acids, fat soluble vitamins, minerals, and polyunsaturated fatty acids) when compared to traditional livestock (Tacon & Metian, 2013; Usydus *et al.*, 2009; Abouel-Yazeed, 2013). In particular fish are considered excellent sources of nutritionally beneficial omega 3 (predominantly in marine fish) and 6 (predominantly in freshwater fish) fatty acids which are found predominantly in oily/semi-oily fish such as catfish, carp, sardine, cod, tuna, snoek, yellowtail, sea bass and sea bream (Ugoala *et al.*, 2009; Prato & Biandolino, 2012; Abouel-Yazeed, 2013). Essential omega 3 fatty acids such as eicosapentaenoic and docosahexaenoic acids (EPA and DHA) are considered central to the development of brain and nervous systems in infants (Carlson *et al.*, 2013), prevention of arthritis, cardiovascular heart diseases (CVD), Alzheimer's in adults (Sidhu, 2003; Ruxton *et al.,* 2004; EFSA, 2006; Bulliyya, 2002) as well as the promotion of mental health and treatment of inflammatory diseases (Connor, 2000).

Despite the numerous advantages of a high fish based diet, adverse health effects can also exist (Sioen *et al.*, 2008). Fish harvested from polluted aquatic environments can contain chemical contaminants (organic and inorganic) in levels deleterious to human health once consumed (FAO/WHO, 2011). Such contaminants can be naturally present in the environment; however, their concentrations can increase considerably due to domestic, agricultural and industrial activities. One such toxic and persistent group of contaminants, which is of global interest, is the persistent organic pollutants (POPs) (UNEP, 2005; UNEP, 2009). Another group of priority contaminants similar to POPs are the bio-accumulative toxic substances (PBTs) (USEPA, 1998). Some of the global priority compounds (POPs and PBTs) include polychlorinated biphenyls (PCBs), dioxin, furans, polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides (OCPs) such as aldrin, endrin, dieldrin, benzenehexachloride (BHC), dichlorodiphenyltrichloroethane (DDT) and more (UNEP, 2005).

Human exposure to organic contaminants is by direct inhalation and predominantly through the diet (Guo *et al.*, 2010; Van Dyk *et al.*, 2010). Some diets are more contributory than others, for example, diets rich in oily fish harvested from polluted environments can contribute significantly to intake of contaminants. In a food basket survey conducted with 12 food commodities in Catalonia, Spain, fish and sea foods were found to have higher levels of dioxins, furans, polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) (Domingo, 2007). Also, on a global scale following a study conducted among the European Union (EU) member states, fish consumption was reported to contribute 63% of human dietary exposure to POPs, while milk and meat contributed 39% and 32% respectively (FSAI, 2013).

Some of the deleterious effects associated with dietary intake of these contaminants include immune suppression, neurological disorder, reproductive impairment, developmental retardation, cardiovascular disorder, liver disease, infertility and miscarriage (ASTDR, 2002;

USEPA, 2008; de Jager *et al*., 2012). The groups most vulnerable to dietary exposure of contaminants are child bearing women, children below twelve years and subsistence fish farmers (FAO/WHO, 2011; USEPA, 2000). In view of these health problems, there is need to protect the fish and consumers from these contaminants. Regulatory measures/control provided by health and food agencies such as World Health Organization (WHO), Codex, Food and Drug Administration (FDA), and United States of America Environmental Protection Agency contribute to the protection of consumer health and safety.

In many developing countries, poor waste management often occur including indiscriminate dumping of wastes into the rivers and coastal areas (UNEP, 2002). In addition, the use of banned pesticides and related chemicals cannot be ruled out due to the overall poor awareness of banned pesticides amongst the public and peasant rural farmers in particular (UNEP, 2013). Many heavy industries such as oil and gas drilling and extraction, refineries, ports and harbours among others are cited in and around coastal or riverine areas (UNEP, 2006). Thus through human and natural activities, hazardous chemicals from domestic, industrial emissions, effluents and agricultural run-offs enter and pollute the aquatic environment. In a bid to safeguard consumers of possible health risk exposure, assessment of these contaminants in the selected commercial important fish species are conducted against regulatory limits.

The aim of this review therefore was to review PAHs and OCPs (with emphasis on DDTs) profiles and levels in edible fish species within Africa. The review was to identify species and locations with high burden (increased health risk) based on data from published literature (2000 to 2015). Such a comprehensive evaluation will collate relevant information which will be of benefit to governmental and food monitoring agencies, fish industry and consumers to highlight fish safety issues which require attention and updated management strategies. Findings on ecotoxicological studies (i.e. relating to effects of contaminants in fish health) were however excluded from this review. Though such ecotoxicological studies further reveales the prevalence of target contaminants in fish, however, their exclusion from this review was to restrict the scope on edible fish for humans rather than on fish biological development. A review on ecotoxicological studies with respect to fish could be covered where fish is used to monitor environmental or ecological pollution.

2.2. Polycyclic aromatic hydrocarbons (PAHs)

PAHs are lipophilic compounds that consist of two or more benzene rings fused together (EFSA, 2008; Wang *et al*., 2012). PAHs occur naturally as constituents of crude oil, coal, bitumen, and natural gas. PAHs exist as solid and colourless substance in its purest form

for research purposes (USEPA, 2008). The production of PAHs in the environment can be due to anthropogenic activities such as (incomplete) combustion of organic substances, gas flaring and domestic/municipal incinerations (Arias & Spetter, 2009; Bandowe *et al.*, 2014)**.** PAHs with more than four benzene rings are classified as high molecular weights (HMW), which are considered more carcinogenic compared to those of low molecular weight (LMW) (WHO/IARC, 1999). The solubility of PAHs in water decreases with an increase in molecular weight (USEPA, 2008). More than 100 individual PAHs exist (Pule *et al.,* 2012), but 16 priority PAHs (Table 2.1) have been earmarked for global monitoring (US EPA, 2008) and are of focus in this review. Of these priority PAHs, Benzo(*a*)pyrene is considered one of the most potent and was reported as the bio-indicator of PAHs (toxicity) in the reviewed papers, with a maximum European Union (EU) allowable limit of 2 μg/kg in fish (OJEU, 2006). The use of benz(*a*)pyrene as a biomarker in fish is based on the EU regulation adopted by member countries. Although there are other potent PAHs (such as benzo(*a*)anthracene, dibenzo(*a,h*)anthracene, benzo(*b*) fluoranthene, benzo(*k*)fluoranthene, indeno(1,2,3-cd)perylene and chrysene), benzo(*a*)pyrene is mostly detected (50%) in samples in comparison to others (30%) (EFSA, 2008). Furthermore, benzo(*a*) is the only PAH with established oral cancer slope factor (SCF) of 7.3 (mg/kg-d)$^{-1}$ and therefore the potency of other PAHs are determined as benzo(*a*)pyrene equivalent (US EPA, 2000; Skupińska *et al.*, 2004).

PAHs emission can vary considerably over time and space. Globally, annual emission for example, have been reported to drop from 520 Giga gram (Gg) in 2004 (Zhang & Tao, 2009) to 499 Gg in 2008 (Shen *et al.*, 2013), whilst developing countries were reported to emit slightly more carcinogenic PAHs (6.22%) than developed countries (5.73%) (Zhang & Tao, 2009). Largest atmospheric PAHs inputs (63%) were identified to be from domestic and industrial sectors with many African countries contributing significantly due to large use of fossil fuel (Zhang & Tao, 2009).

The fate of PAHs in the environment is reported to be bio-degradable (Doyle *et al.*, 2008) and this implies reduction in accumulation and bio-magnification of PAHs along the food chain (Skupińska *et al.*, 2004). PAHs can be metabolized in higher aquatic vertebrates by enzyme mixed function oxidase (MFO) into forms that are easily excreted out of the system (Hwang *et al.*, 2012; Choudhury *et al.*, 2013). The input sources of PAHs from petroleum (petrogenic) or combustion (pyrogenic) into the environment or biological sample can be determined according to Beg *et al.* (2009) by finding the ratio of LMW: HMW. A diagnostic ratio of LMW: HMW > 1 implies petrogenic (petroleum), while LMW: HMW < 1 implies pyrogenic. Furthermore, ratio of LMW:HMW < 0.5 indicate combustion of liquid fuel (e.g. generating sets, vehicle exhaust fumes and crude oil), while that > 0.5 indicate combustion from grass, wood and coal (Tsymbalyuk *et al.*, 2011). The 16 US EPA priorty PAHs of study

interest are as presented in Table 2.1. Those with 2-3 benzene rings (LMW PAHs) are considered toxic and are of petrogenic (petroleum) sources while those with 4-6 rings (HMW PAHs) are the carcinogenic pyrogenic PAHs.

Table 2.1. Molecular formula, weight and chemical structures and Chemical Abstract Services (CAS) Registry number of 16 priority PAHs by United States Environmental Protection Agency (USEPA) of study focus

Name	Molecular formula	Molecular weight	No of benzene rings	CAS Registry No
Naphthalene (Nap)	$C_{10}H_8$	128	2	91-20-3
Acenaphthylene (Acy)	$C_{12}H_8$	152	3	208-96-8
Acenaphthene (Ace)	$C_{12}H_{10}$	154	3	83-32-9
Fluorene (Flu)	$C_{13}H_{10}$	166	3	86-73-7
Phenanthrene (Phe)	$C_{14}H_{10}$	178	3	85-01-8
Anthracene (Ant)	$C_{14}H_{10}$	178	3	1 20-12-7
Fluoranthene (Fluo)	$C_{16}H_{10}$	202	4	206-44-0
Pyrene (Pyr)	$C_{16}H_{10}$	202	4	129-00-0
Benz(*a*)anthracene (BaA)	$C_{18}H_{12}$	228	4	56-55-3
Chrysene (Chr)	$C_{18}H_{12}$	228	4	218-01-9
Benzo(*b*)fluoranthene (BbF)	$C_{20}H_{12}$	252	5	205-99-2
Benzo(*k*)fluoranthene (BkF)	$C_{20}H_{12}$	252	5	207-08-9
Benzo(*a*)pyrene (BaP)	$C_{20}H_{12}$	252	5	50-32-8
Benzo(*g,h,i*)perylene (BghiP)	$C_{22}H_{12}$	276	6	191-24-2
Dibenz(*a,h*)anthracene (DahA)	$C_{22}H_{14}$	278	5	53-70-3
Indeno(*1,2,3-cd*)pyrene (InP)	$C_{24}H_{14}$	300	6	193-39-05

Source: Boehm (2005); US EPA (2008); EFSA (2008). LMW 2-3 rings; HMW 4-6 rings

2.3. Dichlorodiphenyltrichloroethane (DDT) and other organochlorinated pesticides (OCPs)

DDT ($C_{14}H_9Cl_5$) is an organochlorinated whitish compound, insoluble in water (ASTDR, 2002) with trade names such as genitox, anoflex and pentachlorine (EFSA, 2006). It was first produced in Switzerland in 1874 by Othmar Zeidler but was discovered as a pesticide in 1939 by a Swiss Chemist, Paul Muller (ASTDR, 2002; EFSA, 2006). DDT was used as agricultural pesticide from early 1940's until the early 1970's, when its agricultural use was banned globally (WHO, 2011). However, DDT continues to be used for health purposes in limited countries as

an insecticide in the control of malaria, typhus fever and trypanosomiasis vectors (Wells & Leonard, 2006; WHO, 2011).

DDT can be released into the environment from points of production, application and disposal. In the environment, DDT can be degraded by microbes and/or chemical metabolism into its metabolites dichlorodiphenyldichloroethylene (DDE) and dichlorodiphe-nyltrichloroethane (DDD) (Yu *et al.*, 2011). The sum of these analogues accounts for the total concentration of DDT present in a matrix (EFSA, 2006). The 4,4´-DDE metabolite is more toxic and stable than DDT and is therefore more readily retained in fatty tissue (Hardell *et al.*, 2010; Deribe *et al.*, 2013). In addition, the ratio of DDE metabolite to total DDTs (DDTs i.e. sum of all metabolites) can be used to determine DDT usage as recent (on-going) or has ceased (previous usage) (Deribe *et al.*, 2013). When the ratio of DDE/DDTs is greater than 1, it implies previous or past DDT usage (Naso *et al.*, 2005), whereas DDE/DDTs <1 implies current DDT usage (Coelhan *et al.*, 2006).

The major DDT producing countries include India, China and Democratic Republic of Korea (UNEP, 2008) with India producing by far the greatest quantity (6300 tonnes in 2007). Within Africa, Ethiopia and South Africa formulate DDT using imported ingredients (UNEP, 2008) with the end product distributed to other African countries. During the 2000's, global DDT usage was estimated at 4000 - 5000 tonnes/year with India as the highest consumer (UNEP, 2008). DDT is being used with increasing popularity in numerous African countries (Ethiopia, Mozambique, Namibia, South Africa, Swaziland, Zambia and Zimbabwe) (Wells & Leonard, 2006; UNEP, 2008) due to its efficiency in the control of malaria. Although it was previously banned in South Africa, it was re-introduced in 2000 due to malaria outbreaks and is currently used in Mpumalanga, Limpopo and KwaZulu-Natal provinces of South Africa (Wells & Leonard, 2006). The re-introduction of DDT was however with the approval of World Health Organization (W.H.O), which restricted DDT to be used only in malaria endemic countries of which South Africa is among (WHO, 2011). South Africa as a signatory to the Stockholm convention is allowed to continue the use of DDT as no effective alternative have been found. (Wells & Leonards, 2006).

Other pesticides (herbicides and fungicides among others) used to reduce pre-and post-harvest losses to boost agricultural production within the continent were briefly mentioned in this review. Ansara-ross *et al.* (2012) in a critical review on pesticides in South African freshwaters highlighted the fact that large volumes of pesticides are in use within the continent and through agricultural run-offs get into the aquatic environment. Some of these chemicals have been found to be toxic and hazardous on non-targeted organisms including humans as they persist in the environment. In view of the associated health hazards, pesticides of global concern listed for total eradication include aldrin (used to kill termites, corn rootworm); dieldrin

13

(for textile pests & soil insects), chlordane (for different agricultural produce), endrin (used for mice and rodents) benzohexachloride (BHC) or hexachlorocyclohexane (HCH) and more (UNEP, 2009).

2.4. PAHs, DDTs and OCPs analytical techniques

Assessment of contaminants in fish involves the collection of targeted fish species (usually commonly consumed) from suspected polluted water bodies. According to US EPA (2000) guideline contaminants to be assessed in fish samples should stem from globally priority contaminants prevalent in a given locality. These contaminants are found in trace amounts and so warrant effective and efficient extraction techniques. Numerous extraction methods that can ensure reduced instrumental interferences include: Soxhlet solvent extraction (Wepener *et al.*, 2012), pressurized liquid extraction (PLE), (Helaleh *et al.*, 2012; Ramalhosa *et al.*, 2012), solid-phase extraction (SPE) (Darko *et al.*, 2008), dispersive solid phase extraction (dSPE), microwave assisted extraction (MAE) and the quick, easy, cheap, rugged and the safe (QuEChERS) extraction (Ramalhosa *et al.*, 2009; Gratz *et al.*, 2011). More recent extraction methods such as MAE, PLE, SPE and QuEChERS are less solvent and labour intensive, less time consuming, ensure more throughput and are therefore more efficient and effective in comparison to older methods such as Soxhlet (Han *et al.*, 2014; Gratz *et al.*, 2011).

Further purification of extracted analyte can be achieved using preparative chromatographic techniques using Florisil or gel permeation columns (Polder *et al.*, 2014) or primary secondary amine (PSA) (Han *et al.*, 2014). Liquid and/or gas chromatographic (LC/GC) separation techniques have widely been used in the analysis of various OCs from fish tissue (matrix). The LC or GC coupled to detection devices such as electron capturing (EC), flame ionization (FI), mass spectrometry (MS) detectors can separate and quantify these contaminants (Anyakora *et al.*, 2005; Ssebugere *et al.*, 2014). Separated analytes are identified based on the retention time compared to known external standard (pure) compounds of interest. Whereas, quantification is done by matching known peaks of an external standard with the unknown from a sample or measured from a calibration curve, plotted with the ratio of the response of internal standard to the external standard calibrated over a range of concentrations (Verhaert *et al.*, 2013; Oyo-Ita *et al.*, 2013). Analytical measurements can be validated with analysis of certified reference material (CRM) or spiked samples with known concentration of standards. Acceptability of data is based on recovery of analytes standards within expected satisfactory limits (Manirakiza *et al.*, 2002). Choice of analytical technique is

dependent on many factors such as affordability, trained analytical personnel and type of analysis.

2.5. Methodology

The review sourced for general information on fish, contaminants in fish and health implications from the fishery database, environmental and food regulatory agencies such as Food and Agriculture (FAO), Fish base, Codex United Nations Environmental Programme (UNEP), United States Environmental Protection Agency (US EPA), , World Health Organisation (WHO), Food and Drug Administration (FDA), Official Journal of the European Union (OJEU). Whilst for specific scientific information on the levels, profiles and sauces of target contaminants in fish from African countries data for were generated from the following data base: Science direct, Scopus, Web of knowledge, Google scholar and Food Science Abstracts.

Search terms included persistent organic pollutants or organic contaminants in fish from African countries, DDT, pesticides, PAHs levels in edible fish from African countries, levels and profiles of organic contaminant in African fish species, accumulation of organic contaminants in African fish and health risks. Library search were restricted from 2000 – 2015. Few publications were available within the scope of the study as indicated in Table 2.2. However, papers with focus on target contaminants with respect to fish health and not human health were not reported in the review. In addition published papers with limited access (that could not be readily accessed through the given data base and through Stellenbosch University Library) were also not reviewed.

2.6. Profiles and levels of contaminants in fish from African countries

In developed (e.g. USA, Canada and Europe) and major marine fish producing countries (e.g. China, Japan, USA), information on the levels of contaminants in fish and other seafood is readily available. However, this is not the case in numerous developing countries (e.g. some African countries). Overall, research and monitoring of edible fish species is limited (period of review from 2000 - 2015) in many African countries that have little/no publications, reports and monitoring strategies for contaminants (PAHs, DDTs and OCPs) status (Table 2.2). This may be relatively due to poor economic status of many African countries for such expensive monitoring and assessment studies. Also lack of sophisticated analytical equipment and absence of trained personnel could be contributory to the low output of published data on the subject matter. Furthermore, the assessment of contaminants in fish involves destructive measurement in which complete destruction of fish is required. This can lead to a loss of

industrial revenue. However, the knowledge of contaminant composition and concentration in fish is valuable from human and environmental health points of view.

Africa as a continent is divided into eastern, western, northern, central and southern regions. However, central and southern regions were merged in this review owing to low volume of publication from the central region. The countries that make up each region are given accordingly (Table 2.2) and those contributing to global fish production in figure 2.1. The status of PAHs and OCPs indicating profiles, sources, levels and species across the regions were hereby discussed.

Table 2.2. List of countries within designated African regions, * denote countries where reviewed publications were obtained for current study and in parenthesis number of publications per country

Eastern Africa: Countries	Western Africa: Countries	Central & Southern Africa: Countries	Northern Africa: Countries
Burundi* (1)	Benin* (2)	Democratic Republic of Congo* (1)	Egypt* (6)
Ethiopia* (3)	Ghana* (4)	Central African Republic of Congo	Tunisia* (2)
Tanzania* (2)	Nigeria* (2)	Equitorial Guinea	Libya
Uganda* (2)	Senegal* (1)	Gabon	Algeria
Comoros	Burkina Faso	São Tomé and Principe	Morocco
Djibouti	Cameroon	Botswana* (1)	Western Sahara
Eritrea	Cape Verde	South Africa* (4)	
Kenya	Chad	Angola	
Madagascar	Côte d'Ivoire	Lesotho	
Mauritius	Gambia	Malawi	
Rwanda	Guinea	Mozambique	
Sudan	Guinea Bissau	Namibia	
	Liberia	Swaziland	
	Mali	Zambia	
	Mauritania	Zimbabwe	
	Niger		
	Sierra Leone		
	Togo		

Source (Anon. 2015)
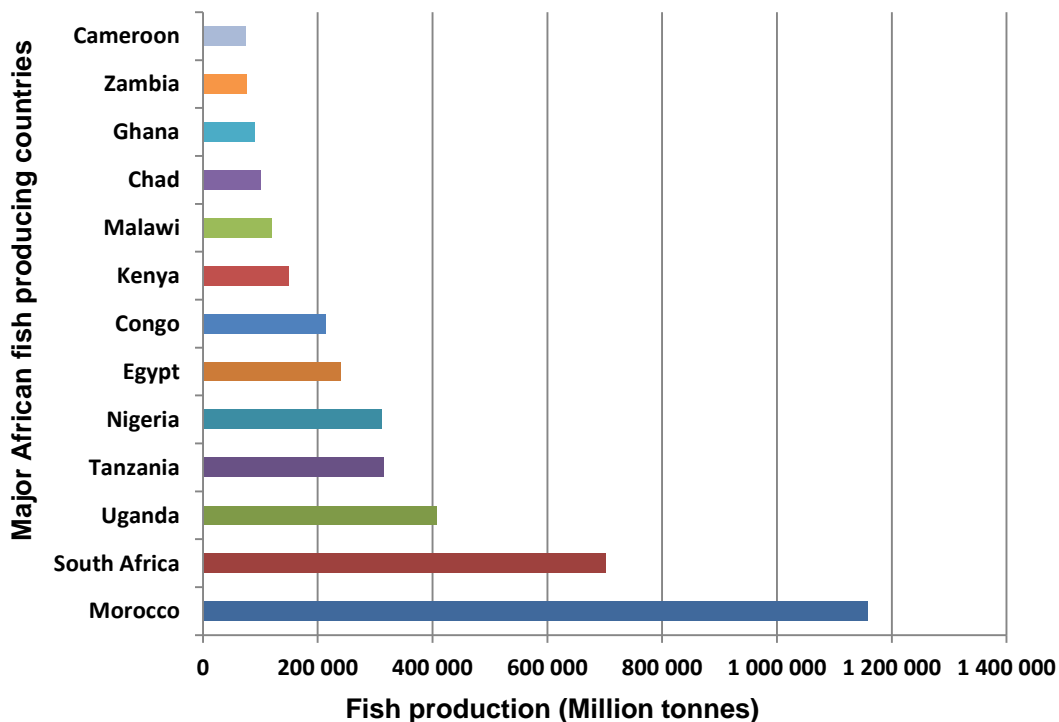
Fig. 2.1 Summary of major fish producing countries from Africa as at 2012. The data includes wild resources only where each country data represents either marine (Morocco & South Africa) or freshwater (remaining countries) catches (FAO, 2014).

2.6.1. Eastern Africa Region

Eastern Africa is made up of 14 countries with seven publications from 4 countries (Table 2.2) available and accessible for review based on set criteria. Fish species analysed from the region (Table 2.3) were predominantly freshwater and included African sharptooth catfish (*Clarias gariepinus)*, Nile tilapia (*Oreochromis niloticus)*, Common carp (*Cyprinus carpio)*, Redbelly tilapia (*Zilli tilapia*), Crucian carp (*Carassius carassius*), Semutundu (*Bagrus docmac*), Mamba (*Protopterus aethiopinus*), Enjunguri (*Haprochromis nigripinnis*) and Giant Cichlid (*Bulengerochromis microlepis*).

A number of contaminants have been assessed and detected in fish from this region such as DDT and metabolites (DDD and DDE), endosulfan (alpha and beta analogues), BHC (alpha, delta, beta and gamma analogues), and chlordane (CHL) (Manirakiza *et al*., 2002; Henry & Kishimba, 2006; Deribe *et al*., 2011; Deribe *et al*., 2013; Yohannes *et al*., 2014; Polder *et al*., 2014). From this region, no publication was found on fish contamination with PAHs within the review criteria and period (2000-2015), which reveals a big gap of knowledge in that respect. Geographical variations were observed in the profiles and levels of these contaminants amongst the various countries in the region. The levels of pesticides detected

17

within the region however, did not exceed the recommended maximum residue level (MRL) of 5000 µg/kg ww or 1000 µg/kg lipid weight (lw) for DDT, (other pesticides, 100-300 µg/kg) (US EPA, 2000). The major sources of these OCPs in the region were from banned and obsolete pesticide stock, current use of pesticide in agriculture (e.g. control of pest in cotton farm), industries and domestic (control of vectors) among others (Manirakiza *et al.*, 2002; Polder *et al.*, 2014).

On a global scale, factors such as fish species, fish size, lipid content, feeding habit and trophic levels were reported to contribute considerably to the accumulation of OCPs in freshwater and marine species. For example variations of OCPs have been observed within same species (Gomes *et al.*, 2013) and within different anatomical positions of same individual fish (Cheung *et al.*, 2007). Because these OCPs accumulate in fatty tissues and biomagnify in the food chain, predatory species tend to have increased burden (Borgå *et al.*, 2004). In line with this global trend, highest concentrations of OCPs (PCBs, DDTs and endosulfan) within the region were found in fatty and high trophic level predatory fish species such as *C. gariepinus* (Deribe *et al.*, 2011; Deribe *et al.*, 2013). Since *C. gariepinus* (catfish) is one of the most commonly consumed species in the continent, the higher frequency of catfish consumption compared to other species, may therefore increase human dietary exposure.

Dichlorodiphenyltrichloroethane (DDT) was the most prevalent OCP in the region based on the little (available) published information. Currently, DDT usage was reported from Ethiopia (Deribe *et al.*, 2011; Deribe *et al.*, 2013) and Tanzania (Polder *et al.*, 2014). In view of DDT's being currently formulated in Ethiopia and widely used in east Africa for control of vectors, may explain its wide detection in the region. Although levels of OCPs and DDT in particular were within acceptable limits, frequent and prolonged consumption of contaminated fish may lead to health risk. An estimation of DDT intake via consumption of *C. gariepinus* based on study in Tanzania (Yohannes *et al.*, 2014), was found to be within the acceptable daily intake (ADI) of 10-30 ng/kg body weight/day (EFSA, 2006; FAO/WHO, 2011). However, cancer risk estimation (over average lifetime of 70 years) based on the same study was considered high when compared to the maximum allowable risk level (RL). The study reported an estimation of 36 deaths in every 100 000 (36×10^{-5}) to occur from the consumption of the contaminated catfish which by far exceeded the normal threshold risk level (RL) of 1×10^{-5} (single death in every 100 000) (US EPA, 2000). This is worrisome and will require strict monitoring to minimize exposure particularly to the vulnerable groups.

Table 2.3. Levels of OCPs in fresh water fish species (showing species with highest burden) from different locations in Eastern Africa. Maximum residue limit (MRL) for DDT 5000 µg/kg wet weight (ww) or 1000 µg/kg lipid weight (lw); other pesticides (100-300 µg/kg) (US EPA, 2000). Acceptable daily intake ADI for DDT 5-30 ng/kg body weight (EFSA, 2006). Note predominant OCP is given as having the highest concentration (applicable to similar tables under this chapter)

Country	Pesticides (method)	Levels (µg/kg)	Location	Fish species	Highlights	Reference
Burundi	ΣDDT ΣHCH ΣEndosulfan (GC-ECD)	68.3-909.1 lw 21.2-288.2 <dl-36.1	Lake Tanganyika	*Chrysichthtys sianenna, Oreochromis niloticus Lates angustifrons, L. stappersii, Bulengerochromis microlepis, Limnothrissa moidon, Stolothrisa moidon*	DDT was predominant *B. microlepis* had the highest level *L. miodon* had the least concentration DDT level was within acceptable limit of 1000 µg/kg (lw) EU MRL DDT status was past usage	Manirakiza *et al.* (2002)
Ethiopia	ΣDDT (GC-MS)	0.05-72.53 ww	Lake Koka	*C. gariepinus O. niloticus, C. carpio & Barbus intermedius*	DDT was the predominant pesticide C. gariepinus had the highest burden of all the pesticides evaluated *C. carpio* had the least concentration of pesticides. No immediate risk with fish consumption. DDT status showed recent usage	Deribe *et al.* (2011)
	ΣDDT (GC-MS)	0.89 – 171.96 ww	Lake Ziway	*C. gariepinus, O. niloticus, Tilapia zilli & Carassius auratus*	*C. gariepinus* had highest level Least level was in *T. zilli* No risk in fish consumption DDT status revealed recent usage	Deribe *et al.* (2013)
	ΣDDT ΣHCH (GC-ECD)	0.77-61.9 ww 0.16-3.54	Lake Ziway	*O. niloticus, T. zilli C. auratus &*	DDT was predominant pesticide Highest burden was in *C. gariepinus*	Yohannes *et al.* (2014)

Country	Pesticides (method)	Levels (µg/kg)	Location	Fish species	Highlights	Reference
				C. gariepinus	Least in *C. auratus* Estimated daily intake (EDI) was lower than acceptable daily intake (ADI) for 30 ng/kg and thus no health risk	
Tanzania	ΣEndosulfan ΣDDT (GC-ECD)	20-80 ww 20-30 ww	Lake Victoria	*O. niloticus* *L. niloticus*	EDI for HCH and DDT were below ADI (no ADI value for endosulfan) No immediate risk with fish consumption Current use of pesticides (DDT & endosulfan)	Henry & Kishimba (2006)
	ΣDDT (GC-ECD)	7.2 -319 lw	Lakes: Victoria, Tanganyika, Nyasa & Babati	*Oreochromis species*	DDT was predominant pesticide Highest DDT was in Tilapia From Tanganyika DDT status as recent usage	Polder *et al.* (2014)
Uganda	ΣDDT (GC-ECD, MSD)	<dl – 104 ww	Lake Edward	*Haprochromis nigripinnis,* *Protopterus aethiopinus, O. niloticus & C. gariepinus*	Highest DDT level was in *P. aethiopinus* Non detection of DDTs in *H. nigripinnis* DDT levels were below FDA/Codex maximum residue limit (MRL) No immediate health risks with species	Ssebugere *et al.* (2009)
	HCH & analogues (GC-HRMS)	14.95– 45.90 lw	Lake Victoria	*L. niloticus & O. niloticus*	Gamma HCH was the predominant analogue *L. niloticus* had the highest HCH burden due to higher lipid content	Ssebugere *et al.* (2014)

Note: Refer to list of acronyms for full meaning

2.6.2. Western Africa Region

Major contributors to global freshwater (capture) fish production (Fig. 2.1) from the region include Nigeria, Ghana, Cameroon and Niger and need monitoring of contaminants to ensure consumption safety. However, in spite of high production rate, published data on contaminants (PAHs & OCPs) in fish based on selecting criteria within the region is scarce (Table 2.2). Ghana had a higher number of publications than other countries in the region probably due to good investment, good institutions, laboratories and trained personnel for assessing contaminants than is obtained in other countries within the same region. Ghana's advantage over other countries in terms of information on contaminants in fish in the region is reflected in proper documentation of available contaminants in their country (Asante *et al*., 2013). Also the relatively high research output in Ghana has resulted in greater awareness in the contaminants presence and its effect on local inhabitants (Asante *et al*., 2013). Fish analysed in Western Africa were comprised of freshwater (*O. niloticus*, *C. gariepinus*, *Tilapia zilli, T. guineensis*) and marine species (*Drapane africana, Cynoglossus senegalensis, Pomadasys peroteti*, *Sardinelle maderensis*, *Pseudotolithus elongates*). The detected contaminants (profiles and highest levels) found in the studied species were shown in Table 2.4.

Contaminants of interest (OCPs and PAHs) in this region were predominantly DDTs, endosulfan, dieldrin, lindane, PCBs, dioxins and furans. Considering the levels and types of contaminants in freshwater species, this review may suggest that fish contamination level were higher in the Western Africa compared to the Eastern Africa. For example, DDT concentration in some freshwater species (e.g. *C. gariepinus*) from Benin (1642 µg/kg lw) (Pazou *et al*., 2006) and Ghana (2205.50 µg/kg lw) (Adu-kumi *et al*., 2010) were apparently higher than species (e.g. *B. microlepis*) from Burundi (909.1 µg/kg lw) in Eastern Africa (Manirakiza *et al*., 2002). However, the comparison being based on different species was not conclusive but only a postulation as such inter-regional comparison requires caution. DDT is currently in use in some countries in the West African region. This was reflected with high levels above the European Union (EU) maximum level (1000 µg/kg lw) in fish from some freshwater bodies such as Ouémé river in Benin (Pazou *et al*., 2014) and Lake Volta in Ghana (Adu-Kumi *et al*., 2010).

On the other hand, studies on fish contamination with PAHs within the region showed no regular trend. For instance, PAHs profile in marine fish species (*S. maderensis* and *G. decadactylus*) from the coast of Ghana (Nyarko *et al*., 2011) showed the predominance of HMW PAHs (e.g. pyrene and benzo(*a*)pyrene) over the LMW PAHs. Also, while benzo(*a*)pyrene was found to exceed the EU maximum limit (2 µg/kg wet weight) which is of significant health risk, no spatial variation of PAHs levels in the studied species were observed.

Conversely, Bandowe *et al.* (2014) in a more recent study, also on PAHs in marine species (*D. africana, C. senegalensis and P. peroteti*), from coastal harbours of Ghana, observed the predominance of the LMW PAHs and benzo(*a*)pyrene was not in excess of the EU limit which implied no immediate cancer risk from the studied species.

Carcinogenic (HMW) PAHs dibenzo(*a,h*)anthracene (564.10 µg/kg dry weight) and benzo(*a*)pyrene (6780 µg/kg) were present at high concentrations in fish from Nigeria (Lagos lagoon and Niger Delta oil producing fishing community) which may be due to oil drilling activities (Anyakora *et al.*, 2005). However, spatial differences in benzo(*a*)pyrene levels were apparent as it was not detected in fish sampled from Lagos lagoon which is also considered a heavily polluted environment (Alani & Drouillar, 2012). This inter-study variation in benzo(*a*)pyrene levels may be due to different pollution sources and species' biological variation. The high levels of benzo(*a*)pyrene in the Nigeria–Niger Delta is indeed worrisome. Thus regular consumption of contaminated fish in the region may have hazardous effect over prolonged period due to accumulation of carcinogenic PAHs (Anyakora *et al.*, 2005; Pazou *et al.*, 2006; Pazou *et al*, 2014).

Table 2.4. Levels of contaminants (OCPs) in fresh water and marine fish species (indicating species with highest burden) from different locations in Western Africa.

Country	PAHs & OCPs (Method)	Levels in fish (µg/Kg)	Location	Fish species	Highlights	Reference
Benin	ΣDDTs (GC-ECD)	130-16420 lipid weight (lw)	Ouémé river (freshwater)	*C. gariepinus, O. niloticus, Z. tilapia, Protopterus annectens*	Highest burden was found in *C. gariepinus* & least in *P. annectens*. No immediate risk in fish consumption as FDA action level (5000 µg/kg) in edible fish was not exceeded. Prolonged consumption of contaminated fish may be of health concern.	Pazou *et al.* (2006)
	ΣDDTs (GC-ECD)	183-580 lw	Lake Nokoue & Cotonou Lagoon (freshwater & marine)	*Hemichromis fasciatus, Ethmalosa fimbriata*	*E. fimbriata* had the highest DDT concentration & *H. faciatus* had the least. Fish consumption is of low health risk. DDT status was past usage.	Pazou *et al.* (2014)
Ghana	ΣDDTs (GC-ECD)	3.4-7.25 lw	Bosomtwi Lake (freshwater)	*Oreochromis sp.* (water & sediment)	Fish accumulated DDTs more than water but less than sediment (values were not inclusive here), risk was not assessed.	Darko *et al.* (2008)
	ΣDDTs (HRGC-HRMS)	28.68-2205.50 lw	Volta, Bosomtwi & Weija Lakes (freshwater)	*O.niloticus & C.gariepinus*	*C. gariepinus* had highest DDT burden (2205.50) above EU maximum (1000 lw) Potential health risk over prolonged consumption.	Adu-kumi *et al.* (2010)

Country	PAHs & OCPs (Method)	Levels in fish (µg/Kg)	Location	Fish species	Highlights	Reference
Ghana cont.	ΣPAHs (GC-FID)	34.04 -54.13 ww	Coastal areas (Chorkor, Tema & Ada-Foah)	*Sardinella maderensis & Galedeoide decadactylus*	Highest PAHs burden was in *G. decadactylus* & lowest in *S. maderensis*. Potential risk in fish indicated as B(*a*)P in al fish analysed exceeded EU standard. HMW PAHs predominated, an indication of pyrogenic PAHs input.	Nyarko *et al.* (2011)
	ΣPAHs (GC-MS)	71-481 dw	Coastal areas (Tema, Takoradi & Elmina)	*Drapane africana, Cynoglossus senegalensis & Pomadasys peroteti*	Highest PAHs burden was in *D. africana* & lowest in *C. senegalensis* (Elmina). No risk of consuming fish muscle as B(*a*)P in fish from all sampled locations were below EU standard. Consumption of fish with guts and gills poses higher health risk, estimated cancer risk with guts & gills were higher than EU guideline. LMW PAHs dominated, an indication of PAHs petrogenic input sources.	Bandowe *et al.* (2014)
Nigeria	ΣPAHs (GC-MS)	100 200.00	Niger Delta Siokolo fishing settlement (marine)	*Pseudomonas elongates*	High potential health risk as B(*a*)P (6780 µg/kg) far exceeded the EU limit of 2 µg/kg ww.	Anyakora *et al.* (2005)
	ΣPAHs (GC-MSD)	44.86 -231.97 dw	Lagos Lagoon (marine)	*Sphyraena barracuda (Barracuda), Caranx hippos (Agaza)* and more	Potential health risk especially with consumption of whole fish as HMW carcinogenic PAHs predominated in whole fish samples.	Alani *et al.* (2012)

Country	PAHs & OCPs (Method)	Levels in fish (µg/Kg)	Location	Fish species	Highlights	Reference
Senegal	ΣDDTs (GC-ECD)	1.9-4.4 (Falia) 2.5-13.1 dw (Fadiouth) 2.8-13.1 dw (wet season) 1.9-6.6 (Dry season)	Mangrove areas (Falia & Fadiouth)	*Bivalves (Molluscs) Arca senilis & Crassostera gasar*	Though higher accumulation in shellfish (1-20 times more than sediment) no immediate health risk in consuming shellfish from the mangroves, most prevalent POC was DDT & status is current usage. Highest DDT level was found in *A. senilis* in wet season and highest DDT in *C. gasar* during the dry season	Bodin *et al.* (2011)

2.6.3. Northern Africa Region

Morocco is the first African country to appear in the top 25 global marine fish producing countries while Egypt also ranked in the top 25 for freshwater species, contributes significantly to global fish production (Fig. 2.1) (FAO, 2014). Appropriate publications and reports on contaminants (OCPs and PAHs) are more in Northern Africa (Table 2.5) largely due to the high research output generated by Egypt and Tunisia which focused on both marine and freshwater species such as *Liza carinata, Solea solea, Alpesdjedaba* spp., *Mugil cephalus* (mullet), *Trachurus capensis* (Horse mackerel), *Oreochromis* sp. (Table 2.5).

Due to the regional shift in species of focus, comparison between the north and the other three regions was made difficult. Nonetheless, it was found that Egypt (Great Bitter Lake) had the highest documented concentration of PAHs (5 800 -218 000 µg/kg ww) within the African continent. HMW (carcinogenic) PAHs (ASTDR, 2002) such as dibenzo(*a,h*)anthracene (533 000 µg/kg ww) and Benzo(*a*)pyrene (1902-32 905.5 µg/kg), were present in the Great Bitter Lake and reached exceptionally high levels exceeding the recommended EU maximum tolerable limit (Said & Agroudy, 2006; Nasr *et al.*, 2012). From Egypt and Tunisia, PAHs input sources of incomplete combustion (pyrogenic) and petroleum (petrogenic), were observed respectively. The high relative concentration of the potent PAHs (benzo(*a*)pyrene and dibenzo(*a,h*)anthracene) suggests that frequent consumption of contaminated fish could be injurious to the health of the local consumers. This therefore calls for greater monitoring and control of pollution from oil spill and industrial emissions to curb further contamination of aquatic foods. The level of contaminants present however, was species specific, for example, while *Mugil* spp*.* and *Alpesdjedaba* spp. contained high levels of PAHs and may pose higher consumption risk, *Rhabdosarguss haffara* and *Liza carinata* had relatively low levels and may be reduce dietary intake (Said, 2007; Mzoughi *et al.*, 2010). Therefore, knowledge of contaminant levels in local fish species can enable consumers to make informed decision on choice of fish to consume and so play a considerable role in consumer health.

Table 2.5. Levels of PAHs & OCPs in freshwater and marine fish species (indicating species with highest burden) from different locations in Northern Africa.

Country	PAHs & OCPs (Method)	Levels in fish	Location	Fish species	Highlights	Reference
Egypt	DDE, , Endosulfan PAHs (GC-ECD)	16. 4 ww (DDE), 124.8 (Endosulfan), 11.1 ww (PAHs)	Lake Temsah (fresh water)	*Mugil cephalus*	Endosulfan was the highest OCP detected in fish muscle (124.8 ng/g) more than the gill & skin. Similar trend was also observed with PAHs but for DDE the gill showed higher burden. DDT was not detected which may imply absence of fresh input from the locality.	Ahmed *et al.* (2001)
	HCB, DDTs (GC-ECD)	20.3 – 33.8 ww (HCB), 18.2 – 20.1(DDT); 15.2 -38.6 (HCB), 28.4-91.3 (DDT)	South Sinai-Suez; Port Said-Dermetta (coastal / marine)	*Bouri Mugil*	Highest conc. of HCB & DDT was found in *Bouri sp*. In all the 4 locations no health risk in fish consumption. Frequent and prolonged consumption over lifetime may pose health concern.	Nemr & Abd-Allah, (2004)
	Total PAHs (GC-FID)	5800-218500 (Great bitter lakes); 68000-623000 (El Temsah) all seasons	Great bitter lakes & El-Temsah (fresh water)	*Rhabdosarguss haffara, Liza carinata, T. zilli, Dahbana sp.*	Highest concentration of 533000 µg/kg found in *Dahbana sp* from Great Bitter lakes (in January). *L. carinata* had the maximum of 68700 µg/kg from El-Temsah (in July). Predominant PAH was dibenzo(*a,h*) anthracene. Major input source based on PHE/ANT ratio >1 implied pyrogenic i.e. from combustion of fossil fuel.	Said & Agroudy, (2006)
	PAHs, DDTs, HCH (GC-FID)	3862-35746 4.89-36.37, 0.3-65.7ww	Western coast of Alexandria	*Euthynnus alleferatus, Scomberomoros commerson, Sphyraena sphyraena, Diplodus vulgaris & Alepes djedaba*	Highest burden of PAHs was in *A. djedaba*. Predominant PAH was B(*a*)P ranged 1902.7-32905.5, exceeded the maximum EU level. PAHs input source(based on LMW/HMW >1) was pyrogenic	Said, (2007)

Country	PAHs & OCPs (Method)	Levels in fish	Location	Fish species	Highlights	Reference
	ΣPAHs (GC-FID)	2440 (Mullet)-3723 (tilapia)	Sharkia Governorate Market	*Oreochromis sp (fresh water) & Marine water Mugil (Mullet)*	Mean ΣPAHs in the two species: Oreochromis (3723) and Mugil (2440) µg/kg. Concentration of B(*a*)P exceeded the EU limit in 55% of marine Mugil and 15% of freshwater Oreochromis	Nasr *et al.* (2012)
	DDT metabolite (pp´DDE) (GC-MS)	1.11-2.5 (catfish) 0.65-1.83 (tilapia)	Elwasta & Mankbad, Assuit (fresh water)	*C. gariepinus & O. niloticus*	Catfish (Elwasta) had higher burden than Tilapia, levels were below EU limit	Yahia & Elsharkawy, (2014)
Tunisia	ΣPAHs and more (GC-FID)	188.85-355.71 ng/g ww	Gulf of Tunis (coastal / marine)	*Mullus surmuletus, Pagellus erythrius Diplodus annularis,Sparusaurata, Pomatomus satatrix, Trachurus capensis*	B(*a*)P was not detected in all fish tissue, highest PAH burden was found in red mullet and least in horse mackerel, predominant PAHs was the LMW and source was found to be petrogenic	Mzoughi *et al.* (2010)
	ΣDDTs, HCB, HCH (GC-ECD, MSD)	54.2-512 ng/g lw (DDTs), 1.70-18.0 (HCB), <dl-58.0 (ΣHCH)	Bizerte lagoon (marine)	*Solea solea*	Bizerte lagoon showed higher burden than Mediterranean Sea (not indicated). Ratio of DDE/DDT found >1 implying previous usage	Ameur *et al.* (2013)

2.6.4. Central and Southern African Region

The Central and Southern Africa were grouped together as information is very limited, even though both regions have countries which play key roles in global fisheries. South Africa is ranked within the top 25 (below Morocco) as key global marine fish (wild) producers while Zambia and Malawi are considered key producers for freshwater fisheries. Major publications were from South Africa which could be an indication of their economic and technological advancement over other countries in the region.

Most research within the region has been restricted to freshwater for fish; however, some studies also focused on marine species (Table 2.6). Contaminants evaluated include PCBs, DDTs, HCH, CHLs & HCB. Within the Congo (Okavango Delta), PCB was the predominant contaminant (<LOQ – 66 µg/kg ww), low DDT level (<LOQ – 11 µg/kg) was found in *Marcusenius* sp (Mormyridae) from Itimbiri River with DDE analogue predominant which indicated past DDT usage (Mbongwe *et al.*, 2014). Within South Africa and Botswana, DDT was the most predominant pesticide analysed in freshwater species (Wepener *et al.*, 2012), however levels were within regulatory limit (5000 µg/kg ww). Within Southern Africa and particularly South Africa, potential health risk (of 5 deaths in every 100 000 consumers) was reported with *C. gariepinus* from polluted Dams (Roodeplaat and Rietvlei) as was observed in a recent study by Barnhoorn *et al.* (2015). The reported cancer risk which exceeded the threshold of 1 in every 100 000 was based on estimation of potential cancer death risk from consumption of fish from Roodeplaat contaminated with endosulfan and lindane (141.4-516.2 and 108.4-514.8 µg/kg ww respectively). A similar health implication was previously observed as total DDT level recorded from Tiger fish (5403.9-6443.9 µg/kg lw) (Wepener *et al.*, 2012) exceeded the EU (inclusive of African countries) maximum level (1000 µg/kg lw) and FDA action level of 5000 µg/kg, which can threaten consumers' health.

In view of the continuous application of DDT and other OCPs within the continent, there is a likelihood of increased dietary exposure particularly of those riverine and coastal dwellers that depend on such fish species. The need therefore to provide advisory services becomes necessary to reduce frequent consumption of identified species with high burden. The consumption of species with reduced burden on the other should be encouraged.

Table 2.6. Levels of contaminants (OCPs) in fresh water and marine fish species (indicating species with highest burden) from different locations in Central and Southern Africa

Country	PAHs & OCPs	Levels in fish	Location	Fish species	Highlights	Reference
Botswana	DDT (GC-ECD)	2.99-7.19 ww	Okavango Delta (fresh water)	C*larias gariepinus, H. vittalus, Tilapia rendali, Cyprinus sp*	*C. gariepinus* showed the highest accumulation. Fish from malaria zone had higher DDT conc. than non-malaria zone	Mbongwe *et al.* (2014)
Congo	DDTs, HCH, CHLs (GC-MS)	< LOQ – 11 ww (DDT), <LOQ- 0.56 (HCH), < LOQ – 0.35 (CHLs)	Congo River Basin (fresh water)	*Marcusenius sp; Shoulderspot Oreochromis, Schilbe grenefelli, Synodontis alberti*	Highest burden of 0.28-15 ng/g ww was found in *Marcusenius sp* a demersal omnivorous fish. The DDT was the dominant contaminant	Verhaert *et al.* (2013)
South Africa	DDT (GC-MS)	77.7-764. ww	Roodeplaat, Rietvlei & Hartbeespoort Dam (fresh water)	*C. gariepinus*	Amongst the predominant contaminants detected in *C.gariepinus* from the three locations were pp´DDE and endosulfan (pesticide).	Barnhoorn *et al.* (2015)
	DDT, HCB (GC-MS)	5403.9-6443.9 lw (DDT); 82.2-355.5 (HCH)	Pongolapoort (fresh water)	*Hydrocynus vittatus*	Total DDTs exceeded EU maximum tolerable level of 1000 ng/g lw. Potential health risk in consuming fish.	Wepener *et al.* (2012)
	PAHs & PCBs (GC-MS GC-ECD)	290-2100 lw (PAHs); 34000-131 000 (PCB)	South African coastline (Cape Town, Port Elizabeth, Saldahna & Tsitsikamma)	*Perna perna*	*P. perna* accumulated PAHs but cannot accumulate Naphthalene	Degger *et al.* (2011)
South Africa Cont.	DDTs (GC-MS, ECD)	0.28-81.49 lw	Nandoni Dam & Xikundu weir (fresh water)	*C. gariepinus, Oreochromis mossambicus*	*C. gariepinus* had the highest accumulation of 81.49 from Xikundu Dam with greater contribution from pp´DDE an indication of past DDT usage or metabolization activities. Fish consumption from the dam is of health concern	Barnhoorn *et al.* (2009)

2.7. Conclusion

In this review, a considerable body of published literature on focus contaminants (PAHs and OCPs) within the African continent was summarized. DDT (including metabolites) was the most widely assessed and dominant pesticide in all the regions. BHC and endosulfan pesticides were found more in fish from Eastern, Central and Southern Africa. Frequent and prolonged consumption of DDT contaminated fish in these regions may predispose consumers to cancer risk. The input source of these pesticides reflected current use for the control of disease vectors and in agriculture. On the other hand, Western and Northern Africa had increased carcinogenic PAHs from coastal fish species which exceeded EU maximum limit. Equally, consumption of such contaminated species might result in increased accumulation within the human system and promote carcinogenic health risk.

Variation in methodologies as well as fish composition, age, species, sex, season and location made comparisons between studies difficult. Greater standardization of methods and procedures would limit such comparison biases and is recommended for future work. In general, published literature on focus contaminants particularly PAHs in edible fish species from the continent is limited. Future research should also examine the effect of processing (cooking methods) on these contaminants and their bioavailability in human system (as studies in this regard are very scarce). This becomes essential as assessment based on fresh (raw) fish meat may not be actual level ingested after cooking. Therefore, research promotion, investment and specialized education is required in order to increase the research output and our understanding of contaminants presence, concentration and effects from edible marine and freshwater species.

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CHAPTER 3

Assessment of organic contaminants (PAHs and pesticides) in yellowfin tuna (*Thunnus albacares*) muscles: QuEChERS and GC-MS/MS analysis

Abstract

Thunnus albacares (yellowfin tuna) is one of the most commonly consumed marine fish species globally and in South Africa. Organic contaminants (OCs) of health concern and global interest, namely dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), benzenehexachloride (BHC) and some other organochlorine pesticides (OCPs) were evaluated in the dark (TDM) and light (TLM) muscle portions of yellowfin tuna and their concentrations compared to recommended limits. Extraction of contaminants was done with QuEChERS method and analysed with GC-MS/MS. The mean concentration of total DDT was significantly higher ($p < 0.05$) in TLM (229.09 ± 2.04 µg/kg ww, than in TDM (4.47 ± 1.64 µg/kg); while mean concentration of total PAHs, BHC and OCPs were higher ($p < 0.05$) in TDM (636.61 ± 36.03 µg/kg, 4.23 ± 0.54 µg/kg and 12.12 ± 2.90 µg/kg respectively) than TLM (369.41 ± 64.72 µg/kg, 1.39 ± 0.13 µg/kg and 5.36 ± 0.9 µg/kg). Concentrations of DDT, BHC and other OCPs in both muscles were below European Union (EU) maximum residue levels. However, benzo(*a*)pyrene, the acceptable indicator for PAHs, exceeded the EU recommended maximum level in both TDM and TLM. This study indicates that yellowfin tuna had high levels of PAHs whilst simultaneously having low levels of DDT, BHC and OCPs. Due to the associated human health hazards associated with these chemicals and their persistence in the environment, the levels recorded in the yellowfin tuna are of concern and warrants further investigation and monitoring.

Keywords: Tuna muscles, PAHs, Pesticides, QuEChERS, GC-MS/MS

3.1. Introduction

The United States of Environmental Protection Agency (US EPA) and United Nations Environmental Program (UNEP) earmarked over 20 persistent organic pollutants (POPs) for restricted usage or total ban due to their deleterious effects on humans (US EPA, 1998; UNEP, 2005; UNEP, 2009). These POPs include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), organochlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT), aldrin (used to kill termites, corn rootworm), dieldrin (for controlling textile pests and soil insects), chlordane (for different agricultural produce), endrin (against mice and rodents), and benzohexachloride (BHC) or hexachlorocyclohexane (HCH) (UNEP, 2009). The POPs considered in this study were DDT and metabolites (4,4´-

DDD, 4,4´-DDE and 4,4´-DDT), 16 US EPA priority PAHs and organochlorine pesticide (OCPs). These compounds are in this context referred to as organic contaminants (OCs).

The accumulation of OCs in marine biota is largely due to anthropogenic pollution. The South African coastline (area under study) is one of the busiest in the world (Donoghue & Marshall, 2003; Moldan & Jackson, 2005) with numerous oil spill incidents which contribute to marine pollution (Shahidul Islam & Tanaka, 2004). In addition, the use of pesticides (registered) is widely employed in agriculture but focus is on the banned pesticides (UNEP, 2005). Furthermore, DDT, an insecticide useful in the prevention of malaria is currently used in malaria hot spots within the country (Well & Leonard, 2006; WHO, 2011). Each of the aforementioned pollutants can be present in the marine environment resulting in accumulation in the flora and fauna which inhabit it.

Fish is a dietary source of micro and macro nutrients essential for good human health. Marine fish in particular are considered an excellent source of omega 3 polyunsaturated fatty acids (Özogul *et al.*, 2007, Ugoala *et al.*, 2008; Abouel-Yazeed, 2013) which are necessary for brain development in infants and the reduction of coronary heart diseases in adult (FAO, 2008; Usydus & Szlinder-Richert, 2012; Carlson *et al.*, 2013; Longley, *et al.*, 2014). However, fish particularly oily ones, can also contain high levels of toxic contaminants released into the aquatic environment from natural and human activities (FSAI, 2013). Fish have been found to contribute significantly to dietary intake of OCs when compared to other foods. For instance in a food basket survey conducted in Catalonia, Spain with 12 commodities, fish and other sea foods were found to contain higher levels of dioxin, furans, PCBs and PAHs (Domingo, 2007). In a more global study among European Union member countries fish was found to contribute 63% of human dietary intake of OCs when compared to milk (39%) and meat (32%) (FSAI, 2013). The dietary intake of these OCs can have deleterious effects on humans over a period of time. Some of the reported effects include developmental retardation, promotion of cancer, cardiovascular disorder, miscarriage, infertility and more (ASTDR, 2002; WHO/IARC, 1999; Jager *et al.*, 2012).

Thunnus albacares (yellowfin tuna) is one of the most widely traded and commonly consumed marine fish species (FAO, 2014). Global production (capture) of yellowfin tuna increased from 4.6 million metric tons (MMT) in 2011 to over 7 million metric tons in 2012 (FAO, 2014) largely reflecting the economic demand and increase in consumption. Tuna muscle is composed of dark and light meat (Nishioka *et al.*, 2007; Chaijan & Panpipat, 2009; Liu *et al.*, 2014)**,** hereafter referred to as tuna dark muscle (TDM) and tuna light muscle (TLM). TLM is the commonly consumed portion and is usually prepared into tuna steaks, loins or processed as canned tuna (Herpandi *et al.*, 2011) while TDM has traditionally been viewed as of little or no commercial value (Sánchez-Zapata *et al.*, 2011). Recent studies, however, have

found that TDM has superior concentration of vitamin B12 (Nishioka *et al.*, 2007), lipids (Liu *et al.*, 2014) and iron (Sánchez-Zapata *et al.*, 2011) compared to TLM, all of which are beneficial when incorporated in the human diet. In addition, the high water and oil holding capacities of TDM (8.37 g of water/g fish and 8.11 g of oil/g of fish, respectively) can be exploited in making of food emulsions and gels in the food industry (Sánchez-Zapata *et al.*, 2011; Saidi *et al.*, 2013; Saidi *et al.*, 2014). Therefore, there is a potential for the commercial development and sale of TDM-based products for human consumption. However, the high lipid content can be problematic where contaminants such as OCs are more prominent in fatty tissues resulting in a potential trade off.

Due to the prevalenece of OCs in marine environments and their negative health effects in humans, these OCs were examined in locally (South Africa) caught yellowfin tuna. The variation in concentrations of DDT, pesticides and PAHs between light and dark muscle was determined. Such information can be used to inform regulatory bodies, fish processors, fishers and the consumers with regards to contamination levles in portion specific (TDM and TLM) sections of yellowfin fish.

3.2. Materials and methods

3.2.1. Chemicals and materials

Analytical grade acetonitrile (purity > 98 %) purchased from Stargate Science, South Africa was used as extraction solvent. High purity analytical standards ($\geq$99.9 %) used for instrumental calibration were purchased from Sigma Aldrich (USA) (with catalogue number M-610). The Accustandard polynuclear aromatic hydrocarbon (PAH) mix comprised of 16 US EPA priority PAHs (acenaphthene, acenaphthylene, anthracene, benz(*a*)anthracene, benzo(*a)*pyrene, benzo(*b*)fluoranthene, benzo(*g,h,i*)perylene, benzo(*k*)fluoranthene, chrysene, dibenzo(*a,h*) anthracene, fluoranthene, fluorene, indeno(1*,2,3-cd*)pyrene, naphthalene, phenanthrene and pyrene), and EPA Pesticide mix (4,4´-DDT, 4,4´-DDE, 4,4´-DDD, aldrin, chlordane, endrin, endosulfan 1 and 2, heptachlor, benzenehexachloride (BHC-alpha, beta, delta & gamma analogues), eldrin aldehyde, endosulfan sulfate). Internal standards used were deuterated 4,4´-DDT-d8, naphthalene d8, acenaphthene d10, chrysene d12 and perylene d12. Certified reference material (CRM) of fish origin could not be purchased due to logistical restrictions; therefore, a soil certified reference material (CRM 141.50G) PAH-Loamy clay was used. QuEChERS pre-packed extraction kits (50 mL Teflon centrifuge/extraction tube and 15 mL Teflon centrifuge/clean-up tube containing extraction salts and clean-up agents, respectively) were used.

All standard stock solutions were prepared in acetonitrile then diluted to the desired concentrations. A 7-point GC calibration of mix standard solutions was made at concentration levels: 10, 20, 40, 60, 100, 200 and 300 µg/L. To each calibration solution, the internal standards mixture (50µg/L) was added. The extraction solvent acetonitrile containing the internal standards mixture (50µg/L) was freshly prepared before extraction.

3.2.2. Sample collection and preparation

Twelve yellowfin tuna (*Thunnus albacares*) were harvested in the vicinity of False Bay (34.2198° S, 18.6400° E), South Africa (Bosch *et al.*, 2016) by local line fishermen and transported in a crate covered with ice to the Stellenbosch University laboratory (were they caught on the same day?). On arrival, the fish were rinsed under fresh running tap water, de-headed and skinned. The dark and light muscles from the 12 individual tuna fish with an average weight of 47.79 ± 15.05 kg were homogenized, vacuum packed separately in polyethylene bags and stored at -20ºC till further analysis.

3.2.3. Total lipid determination

Total lipid (crude fat) was determined (Lee *et al.*, 1996) using 5 g of homogenized TDM and TLM extracted with 50 mL of chloroform methanol in the ratio of 1:2 v/v. A hand held mixer (bamix) was used to mix the fish and solvent thoroughly for 1 min, which was then filtered into a separating funnel and with the addition of 20 ml of 5% salt solution, the polar and non-polar phases were separated after standing for 60 min. The polar phase was decanted into an Erlenmeyer flask and 5 ml extract transferred into a pre-weighed fat beaker which was dried in a sand bath to a constant weight (45 min) and the percentage lipid gravimetrically calculated.

3.2.4 Extraction of OCs

The target OCs (16 US EPA PAHs, DDT and other OCPs) were extracted from TDM and TLM (from 12 individual fish) according to the AOAC (2007.01) quick, easy, cheap, efficient, rugged and safe (QuEChERS) method (with modification). This was done by weighing 5 gram of the fish homogenate into a 50 mL QuEChERS Teflon centrifuge tube. Milli-Q-water (5 mL) was added, shaken (1 min) to mix thoroughly, where after 10 ml acetonitrile containing 50 µg/L mixed internal standards (deuterated DDT-d8, PAHs - naphthalene-d8, acenaphthene d10, chrysene d12 and perylene d12 & pesticides) was added. Each tube was allowed to stand for 15 minutes, thereafter QuEChERS pre-mix salt (6 g $MgSO_4$ and 1.5 g NaCl) was added, shaken vigorously for 1 min (for quick dispersion of the salts into the fish homogenate), vortexed for 3 min and centrifuged at 4 000 revolution per minute (rpm) for 4

min. The vortex and centrifuge increased the separation and clarification of the lipid and aqueous phases. An aliquot of 6 mL from the supernatant (acetonitrile or lipid phase) was transferred into the (QuEChERS) 15 mL dispersive solid phase (dSP) clean-up Teflon centrifuge tube containing 900 mg $MgSO_4$, 300 mg PSA (primary secondary amine) and 150 mg C18 (sorbent). Then the individual tubes and content were again shaken and vortexed for 1 min and centrifuged at 4000 rpm for 4 min. Finally, 1 ml of the purified extract was transferred into a GC labelled vial, corked and analysed with GC-MS/MS.

3.2.5. Instrumental analysis

Simultaneous analysis of OCs from purified fish extract was carried out according to a modified method described by Kalachova *et al*. (2012) with a GC Thermo Scientific TRACE^{TM} 1310 (USA) automated injection, coupled to TSQ 8000 Mass Spectrometer detector (MSD) (USA). The GC column length was 15 m, with an internal diameter (ID) of 0.25 mm and thickness of 0.25 µm (P/N 13620-127). The initial oven temperature was 75°C, which was held for 3 min, where after it was increased by 10°C/min to 200°C, held for 10 min at this temperature before being heated to 320°C, and held for 2 min with a total run time of 34 min. The injector temperature was 275°C and the injector line temperature was 250°C. The injection was set to splitless mode with flow rate of 50ml/min while the carrier gas was helium at a flow rate of 1.15 ml/min. The GC/MSD was operated with a programmable temperature vaporizer (PTV) and the mass spectrometer was set in electron ionization (EI) mode. The ionization source temperature was set at 250°C and the emission current was 75 µA Argon as collision gas. Identification of target analytes were based on the matching of sample peak area and the retention time (RT) with that of reference standard, allowing a RT tolerance intervals of 2.5-4% of the reference standard within same analytical batch. While quantification (µg/L wet weight) was based on the response ratio of the sample peak area against concentration of the analyte to that of the internal standard. The concentration of the contaminant in fish was expressed as µg/kg wet weight (ww) which was calculated from the detected concentration (in the injected volume) divided by the sample mass and multiplied by the dilution factor.

3.2.6. Quality control

Procedural blank samples were analysed in each batch of 20 samples to monitor for correspondence of peaks with target analytes. Validation of method was achieved using certified reference material (CRM) PAH – loamy clay (Cheung *et al*., 2007; Nyarko *et al*., 2011) obtained from Sigma-Aldrich (USA) which was extracted in the same manner as the fish samples and percentage recoveries were calculated for each analyte. The use of multiple internal standards was necessary to ensure that internal standards with retention time (RT) close to the target analytes were used for identification. Limits of detection (LOD) and

quantitation (LOQ) for the analytes were calculated based on calibration standards (n = 7) signal to noise ratio of 3:1 and 10:1 respectively (Saadati *et al.*, 2013). Blank sample values (particularly for naphthalene) were subtracted from sample values and carry-over of analytes was checked by running solvent blanks after each batch of 10 samples.

3.2.7. Statistical data analysis

All data generated were tested for normality and were found to be normally distributed except for DDT that was log transformed. Differences in the concentration of contaminants between TDM and TLM (n=12) were statistically analysed using one-way analysis of variance (ANOVA) (Statistica version 12.0, Statsoft Inc., USA). Significant differences at 95% confidence level (P ≤ 0.05) between contaminants concentrations in TDM and TLM were tested using Mann-Whitney *U* test.

3.3. Results and discussion

3.3.1. Lipid contents of dark and light muscle

The TDM had significantly higher (p<0.05) lipid content (6.83 ± 0.6 %) compared to the TLM (4.13 ± 0.40 %) which is in agreement with results found in skipjack tuna (*Katsuwonus pelamis*) (Liu *et al.*, 2014) and bluefin tuna (*Thunnus orientalis*) (Hisamichi *et al.*, 2010) harvested from southern, central and northern regions, off the coast of Japan. The TDM is the primary energy store in tuna species, facilitating long distance swimming with minimum fatigue (Liu *et al.*, 2013). Therefore, the higher lipid content observed in the TDM in the current and previous studies was expected. A previous study (Sánchez-Zapata, 2011) on yellowfin tuna reported considerably lower lipid content (4.87%) in the TDM portion of the muscle compared to the current study. These differences are likely due to a number of physiological and environmental factors such as age, sex, diet and season, all which can affect the lipid content of dark muscle in yellowfin tuna fish (Sriket *et al.*, 2007) and can considerably affect the level of energy used and stored in fish.

3.3.2. Detection and quantification of OCs

The calibration curves of all the target analytes were linear with correlation coefficient (r^2) from 0.996 to 1.00. The target OCs (16 PAHs, DDTs and other OCPs) analysed were successfully separated with identification parameters highlighted (Table 3.1). The overall average percentage (analytes) recovery based on CRM-PAH was 73.35 % (ranged from 47.40 - 117.70 %) (Table 3.2) which was within mean recoveries level for acceptable results (Bolaños *et al.*, 2007; Ssebugere *et al.*, 2009). The limit of detection (LOD) for pesticides, PAHs and DDTs were 0.99 µg/L; 1.70 µg/L and 3.12 µg/L respectively while the limit of quantification (LOQ) in similar order were 3.28 µg/L; 5.67 µg/L and 10.38 µg/L.

46

Table 3.1. Target OCs in tuna muscles analyzed simultaneously with GC-MS/MS and settings

Name	RT (min)	Q1 Mass (Da)	Product Mass (Da)	Collision Energy (Volts)
Naphthalene_d8	5.16	136.00	108.00	12.00
Naphthalene	5.21	102.00	76.00	14.00
Acenaphthylene	9.90	151.90	151.00	16.00
Acenaphthene_d10	10.31	164.00	162.20	16.00
Acenaphthene	10.38	153.60	152.00	28.00
Fluorene	11.64	166.20	165.00	16.00
BHC alpha	13.02	216.80	181.00	18.00
Dieldrin	13.45	276.80	241.00	12.00
BHC Beta	13.67	216.90	181.00	25.00
BHC Delta	13.72	216.90	181.00	14.00
Phenanthrene	13.94	178.00	151.50	26.00
Anthracene	14.03	178.00	151.90	20.00
Endosulfan I	14.56	240.90	206.00	10.00
Heptachlor	15.14	271.90	236.90	12.00
Aldrin	15.81	292.90	220.00	22.00
Heptachlor Epoxide	16.61	352.90	316.90	14.00
Fluoranthene	16.72	202.00	200.00	32.00
Pyrene	17.18	201.90	200.10	34.00
4,4-DDE	17.78	317.90	246.00	18.00
Endrin	18.16	280.80	243.20	12.00
Endosulfan II	18.35	240.90	206.00	22.00
4,4-DDD	18.56	235.00	165.10	18.00
Endrin Aldehyde	18.71	344.90	244.90	14.00
Endosulfan Sulfate	19.14	271.80	236.90	12.00
4,4-DDT_d8	19.18	243.10	173.20	20.00
4,4-DDT	19.22	235.00	164.90	20.00
Chrysene	20.09	228.00	225.90	28.00
Chrysene_d12	20.16	240.10	236.20	30.00
Benzo(*a*)anthracene	20.17	228.00	226.10	28.00
Benzo(*b*)fluoranthene	22.42	252.00	250.10	25.00
Benzo(*k*)fluoranthene	22.50	252.00	250.10	30.00
Benzo(*a*)pyrene	23.10	252.00	250.10	32.00
Perylene_d12	23.31	264.10	260.10	32.00
Indeno(*1,2,3-cd*)pyrene	25.18	276.00	274.10	36.00
Dibenzo(*a,h*)anthracene	25.29	278.00	276.10	30.00
Benzo(*g,h,i*)perylene	25.58	276.00	274.10	38.00

Table 3.2. Percentage recovery of 16 United States Environmental Protection Agency (USEPA) priority PAHs from certified reference material (CRM) of soil origin, PAHs (only) for validation of analytical method

Analyte	Con. ng/g	Error level ±	ng in 5g sample	ng/ml in 15ml MeCN	Error level in 15ml MeCN	Acceptable low level in MeCN	Measured concentration in MeCN after extraction, ng/mL	Acceptable high level in MeCN	Measured Conc. in solid, ng/g	Certified value, µg/Kg	Accepted low level in solid, µg/Kg	% Recovery
Nap	524	69.4	2620	174.7	23.1	151.5	154.9	197.8	465	524	455	88.7
Acy	592	55.0	2960	197.3	18.3	179.0	129.1	215.7	387	592	537	65.4
Ace	413	40.0	2065	137.7	13.3	124.3	107.5	151.0	322	413	373	78.1
Flu	388	30.0	1940	129.3	10.0	119.3	125.6	139.3	377	388	358	97.1
Phe	537	43.0	2685	179.0	14.3	164.7	173.0	193.3	519	537	494	96.6
Ant	460	59.0	2300	153.3	19.7	133.7	149.7	173.0	449	460	401	97.6
Fluo	531	45.0	2655	177.0	15.0	162.0	191.3	192.0	574	531	486	108.1
Pyr	420	42.0	2100	140.0	14.0	126.0	151.3	154.0	454	420	378	108.1
Benz(a)A	94.4	5.6	472	31.5	1.9	29.6	33.6	33.3	101	94	89	106.8
Chry	102	5.0	510	34.0	1.7	32.3	34.1	35.7	102	102	97	100.3
B(b)F	240	13.0	1200	80.0	4.3	75.7	68.7	84.3	206	240	227	85.9
B(k)F	341	35.6	1705	113.7	11.9	101.8	106.5	125.5	319	341	305	93.7
B(a)P	128	8.7	640	42.7	2.9	39.8	20.2	45.6	61	128	119	47.4
IndP	240	23.0	1200	80.0	7.7	72.3	94.2	87.7	282	240	217	117.7
B(a,h)A	62	3.6	310	20.7	1.2.0	19.5	21.7	21.9	65	62	58	105.0
B(ghi)P	229	21	1145	76.3	7.0	69.3	75.3	83.3	226	229	208	98.6

MeCN- acetonitrile

Table 3.3 Concentrations (mean ± SE, p-value and range) of detected OCs (µg/kg ww) in tuna dark (TDM) and light (TLM) muscles compared to critical value (maximum recommended standards)

Compounds	TDM (mean)	TLM	p-value	TDM (Range)	TLM (Range)	Critical value	Ref
4-4´DDT	4..47 ± 1.64	229.09 ± 2.04	0.03	0.15-94.39	12.06-4855.30	5000	USEPA, 2000
BHC A	ND	ND	ND	ND	ND	100-300	
BHC B	1.17 ± 0.16	0.74 ± 0.16	0.07	1.06-2.71	ND-1.37	50-100	
BHC D	0.89 ± 0.24	0.89 ± 0.24	0.04	ND-3.10	0.06-0.41		
BHC G	2.17 ± 0.23	0.50 ± 0.20	0.01	BD-4.61	0.40-1.20		
OCPs							
Aldrin	ND	ND	ND	ND	ND		
Endos 1	3.95 ± 1.2	ND		ND-6.27	ND		
Endos 2	3.66 ± 1.50	ND		ND-18.7	ND		
Dieldrin	ND	0.24 ± 0.08		ND	ND-1.17		
Endrin	4.50 ± 2.12	ND		ND-34.52	ND		
Eldrin A	ND	1.09 ± 0.4		ND-18.7	ND-6.54		
Endos S	ND	4.03 ± 1.3		ND	ND-17.25		
PAHs							
Nap	132.68 ± 16.2	153.07 ± 18.2	0.44	88.73-175.60	60.37-336.89	Benz(a)pyrene	OJEU, 2006; USEPA, 1998
Acy	18.22 ± 0.65	3.97 ± 0.61	0.01	16.67-22.14	2.81-9.06	2 µg/kg ww	
Ace	1.51 ± 0.60	2.22 ± 0.64	0.44	ND- 4.68	ND-7.55		
Flu	33.06 ± 3.66	14.83 ± 3.16	0.02	24.55 - 53.05	3.84-39.84		
Phe	54.82 ± 3.16	26.25 ± 3.86	0.00	49.57-78.27	10.39-54.90		
Ant	34. 32 ± 0.49	7.98 ± 0.59	0.00	31.73 - 38.16	6.54 -9.33		
Fluo	8.33 ± 0.81	23.34 ± 0.28	0.04	5.63 - 36.41	10.53 -73.21		

Compounds	TDM (mean)	TLM	p-value	TDM (Range)	TLM (Range)	Critical value	Ref
Pyr	29.93 ± 1.03	26.74 ± 0.34	0.75	25.61- 59.85	5.02 -102.98		
Chry	77.48 ± 2.37	ND		73.75-111.01	ND		
B(*a*)A	29.90 ± 0.97	ND		ND-80.18	ND		
B(*b*)F	73.47 ± 0.47	ND		48.36-96.96	ND		
B(*k*)F	ND	26.72 ± 0.53		ND	7.84-97.76		
B(*a*)P	22.11 ± 0.42	17.53 ± 1.60	0.90	19.80-43.03	8.47-57.38		
InP	69.74 ± 0.79	ND		72.19-116.71	ND		
D(*ah*)A	29.53 ± 1.42	22.60 ± 0.24	0.52	27.14-74.15	3.52-93.50		
B(*ghi*)P	21.54 ± 1.40	44.18 ± 0.45	0.28	ND-74.45	ND-188.69		

Significant differences in OCs concentrations between TDM and TLM at $p \leq 0.05$.

3.3.3 PAHs concentration in TDM and TLM

The sum of 16 USEPA PAHs analysed in this study was higher (p<0.05) in TDM (636.61± 36.03 µg/kg ww) than TLM (369.41 ± 64.72 µg/kg ww) (Table 3.3). There were no significant differences between TDM and TLM in 6 out of the 16 PAHs (naphthalene, acenaphthene, pyrene, benz(*a*)pyrene, dibenzo(*a,h*)anthracene and benz(*g,h,i*)perylene) (Table 3.3). This could be due to the fact that in addition to lipid content, the chemical properties of individual PAH can also affect their accumulation (Baumard *et al.*, 1998). Thus PAHs that were more lipophilic accumulated in the fatty matrix as the fat content of TDM was higher (p<0.05) than TLM. In the current study, 15 of the 16 PAHs were detected in TDM with only benzo(*k*)fluoranthene being undetected, while four were undetected in the leaner TLM namely: chrysene, benz(*a*)anthracene, benz(*b*)Fluoranthene and indeno(*1,2,3-cd*) pyrene. The non-detected PAHs (Table 3.3) in the current study were mostly the high molecular weight (HMW) PAHs usually produced from incomplete combustion. The non-detection of some of these PAHs was in line with other published observations (Nasr *et al.*, 2012; Cheung *et al.*, 2007). For example, Cheung *et al.* (2007), in China did not detect dibenzo(*a*)anthracene, benzo(*a*)pyrene, benzo(*b*) fluoranthene and benzo(*k*) fluoranthene in all assessed muscles of both marine and freshwater species analyzed. The non-detection of certain compounds could be an indication of absence of compounds in the sample or concentration below the detection limit.

Recent information on PAHs contamination in marine fin fish within South Africa is scarce. This was confirmed in a recent critical review on the state of marine pollution within South Africa by Wepener & Degger (2012) which reported that the monitoring of metal contaminants dominated the OCs studies within South Africa. Degger *et al.* (2011) using *Perna perna* (brown mussel) and a non biological semi-permeable membrane device (SPMD) assessed the accumulation of OCs (PAHs and PCBs) from five harbours (Saldanha Bay, Cape Town, Port Elizabeth and Richards Bay, with Tsitsikama as a reference point) along the South African coastline. However, the discrepancies found between the accumulation of contaminants in the biological and non-biological matrices (with a significant correlation between mussel and SPMDs existing only in florurene out of the 15 PAHs analyzed) and the non-bioaccumulation of naphthalene by mussel limits a direct comparison of their study with this study (on fin fish).

The concentration of PAHs found in the current study was compared with some studies on marine fish species across the globe. For example, the levels of PAHs from the current study were lower than that reported in marine species from Nigeria and Egypt (Table 3.4). Benzo(*a*)pyrene (BaP), the acceptable indicator of PAHs in fish under current study, exceeded the EU recommended maximum value of 2 µg/kg ww in fish (US EPA, 2000) in both the TDM

and TLM (22.11 ± 0.42 µg/kg ww and 17.53 ± 1.60 µg/kg ww, respectively). Equally excessive levels were reported in marine species by Anyakora et al. (2005); Said, (2007); Nyarko *et al.* (2009) from Nigeria, Egypt and Ghana (Table 3.4).

The ratio of low/ high molecular weight (LMW/HMW) PAHs or Phenanthrene/ Anthracene was > 1, which was indication of pollution source from petroleum (Beg *et al.*, 2009). Furthermore, the predominance of naphthalene from the study was another determining factor confirming the input source of PAHs as petrogenic (Irwin *et al.*, 1997). This could be attributed to several oil spills and seeps from oil tankers along the South African coast (Attwood, 2000; UNEP, 2006). In addition, there are refinery activities near the coastline of South Africa that may contribute to the source of PAHs (Donoghue & Marshall, 2003) into the marine environment. Furthermore, PAHs can be spatially transferred from distant regions (UNEP, 2009) and therefore not conclusive to localise the input as completely generated from the country alone.

3.3.4 Concentration of DDTs in TDM and TLM

The mean concentration of total DDTs (sum of 4,4´-DDD, 4,4´-DDE and 4,4´-DDT) in TLM (229.09 ± 2.04 µg/kg) was significantly ($p = 0.03$) higher than in TDM (4.47 ± 1.64 µg/kg) (Table 3.3). The predominant analogue was 4,4´-DDT in both TDM and TLM which could be seen as a current input of DDT in the environment (Naso *et al.*, 2005; Deribe *et al.*, 2013). DDT level from the current study was found to be within acceptable level as it was lower than the US Food and Drug Administration (FDA) Action level (5000 µg/kg ww) (US EPA, 2000). Due to the lack of published literature on DDT levels in TDM and TLM, no direct comparisons could be made between studies. Nonetheless, DDT levels have been measured in numerous marine species and a selected few are summarized in Table 3.4. Despite the lack of literature on DDT in TDM, it was assumed that dark muscle would contain higher levels due to its higher lipid content but it was not observed to be so in the current study. The association of DDT with lipid content has been found to vary where Ameur *et al.* (2013) reported no correlation in *Solea solea*, whilst Zhou *et al.* (2007) reported a positive correlation in *Cynolossus abbreviaius*.

It is not unusual to have such a variation in OCs accumulation within different muscle sections in the same fish. For instance, Hisamichi *et al.* (2010) in Japan, reported variation in concentrations of OCs (mercury, PCBs, DDTs and chlordanes) in axial (akami) and ventral (toro) muscles of tuna species (Pacific bluefin, yellowfin and albacores) harvested from southern, central and northern regions of Japan. The ventral muscle in all their analysed species had significantly higher OCs; for example, the DDT concentration in Pacific bluefin was significantly higher (1384 ± 1169 µg/kg ww) compared to the axial (6.5 ± 8.4 µg/kg ww). Similarly, Cheung *et al.* (2007) found a similar trend with PAHs and DDTs in freshwater and

marine fish species (Table 3.4) purchased from local markets in China, and this current study was in agreement with their findings. Although the TDM (usually found beneath the fish skin and backbone) is part of the axial muscle and had a higher lipid content (in this study) than the TLM, it could be said that the muscle position (axial or ventral) also contribute significantly to the accumulation of OCs.

Information on current DDT levels in South African marine fin fish is scarce for comparison with the current study. However, a baseline data from a previous study on DDT in marine species reported a range of 2 µg/kg (muscle) -141000 µg/kg (digestive tract) in *Thyrsites atun* (snoek) (Aucamp *et al.*, 1971). As both fish species are migratory and predatory, a comparison of the snoek baseline data with the current study is justifiable and showed an increased level in muscle in the range (12.06-4855.30 µg/kg) of DDT levels. This could be explained by the current DDT usage, which limits the use only for health purposes as an indoor residual spray (IRS) in the control of the malaria vector. In addition, other sources of environmental DDT other than IRS have been reported which can lead to increased DDT level observed in current study. DDT have been reported to be used in antifouling paints used to maintain undecked boats (Li *et al.*, 2006; UNEP, 2006) and also as constituent of Dicofol which is an approved pesticide in the country (De la Cal *et al.*, 2008; Quinn *et al.*, 2011).

3.3.5 Concentrations of BHCs and other OCPs pesticides in TDM and TLM

The mean concentration of total BHCs (mean sum of beta, delta and gamma analogues) detected in TDM was significantly ($p<0.05$) higher than TLM (4.23 ± 0.42 µg/kg ww and 1.40 ± 0.42 µg/kg ww, respectively). Alpha BHC analogue was not detected in all samples while the gamma BHC analogue (also known as Lindane) was the dominant with mean value of 2.18 ± 0.24 (TDM) and 0.50 ± 0.23 (TLM). These results were in agreement with the finding of Barnhoorn *et al.* (2015) which showed that lindane (BHC gamma) was one of the predominant pesticides in *Clarias gariepinus* from freshwater dams in South Africa with levels > 200 µg/kg ww.

The ratio of gamma BHC analogue to the total BHCs (from the analysed fish samples) was 0.5, which could be interpreted as fresh input of the pesticides to the sampled location. Barnhoorn *et al.* (2015) also reported fresh input of OCPs including BHCs which could be attributed to it still being used in agriculture for treatment of corn and canola seeds (Batterman *et al.*, 2008). However, this is not conclusive as BHC has been banned in South Africa since 1983 (Batterman *et al.*, 2008) and the long spatial transferability of the pesticide could be contributory to its environmental prevalence and reflectance in detected levels.

All other pesticides detected were summed up as total organochlorine pesticides (∑OCPs) (Fig 3.1) and these include Endosulfan 1 and 2, dieldrin; endrin, eldrin aldehyde and

endosulfan sulfate. Aldrin, heptachlor and chlordane were not detected in any of the muscles analysed. Similar to the BHCs, the average concentration of OCPs was significantly ($p < 0.05$) higher in TDM than TLM which could be attributed to the lipophilic nature of OCPs, causing higher accumulation in the TDM which also had higher lipid content. Levels of these pesticides found in both TDM and TLM were far below the EU maximum recommended levels (Table 3.3) and therefore do not suggest immediate risk to tuna fish consumers. Due to the persistence and long spatial transfer of these pesticides (Braune *et al.*, 2005), their detection in samples could not be linked to or confirm their current use within the country. However, adequate monitoring of illegal use of banned pesticides and enforcement of already existing regulations should be maintained.
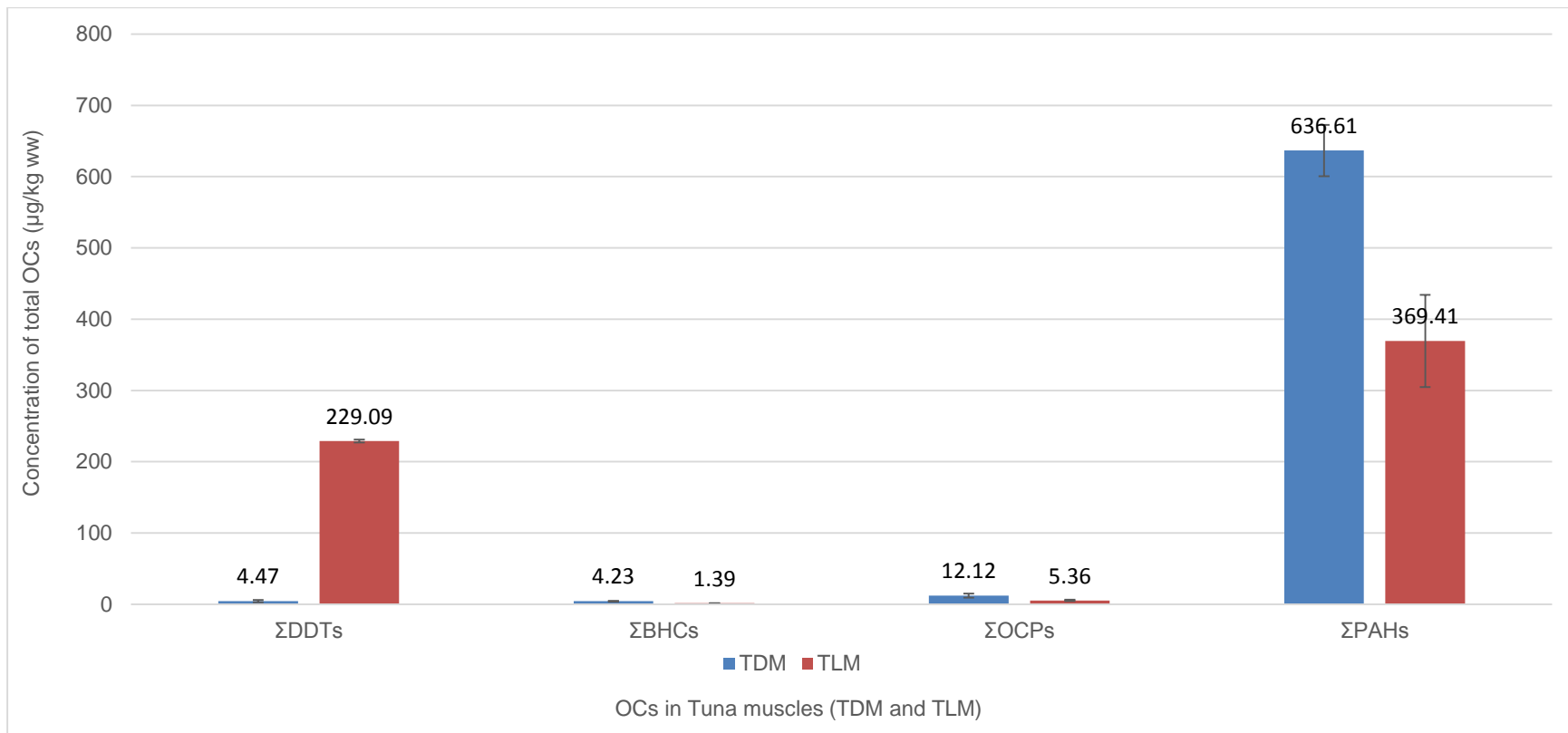
Figure 3.1. Total concentrations (mean ± standard error) of OCs (DDTs, BHCs, OCPs and PAHs) showing variation between tuna dark and tuna light muscles (TDM and TLM).

Table 3.4. Concentration (µg/kg wet weight) of OCs from current study compared with some OCs data in marine fin fish species across the globe (2007- date)

Fish species	Portion	DDTs	PAHs	B(a)P	BHC	OCPs	Country	Reference
Thunnus albacares (Yellowfin tuna)	Dark muscle	4.47 ± 1.64	636.61 ± 36.03	22.01 ± 3.41	4.23 ± 0.54	12.12 ± 2.89	South Africa	Current study
	Light muscle	229.09 ± 2.04	369.41 ± 64.72	17.53 ± 1.60	1.40 ± 0.25	5.37 ± 2.89		
Exocoetus volitans (flying fish)	Muscle	9.23	NA	NA	NA	NA	Brazil	Dias *et al.* (2013)
Scomber japonicus (Chub mackerel)	Muscle	7.33 ± 3.86	NA	NA	NA	NA	Portugal	Ramalhosa *et al.* (2012)
Belone belone (Garfish)	Muscle	1.50	NA	NA	NA	NA	Bulgaria	Georgieva *et al.* (2012)
Oncorhynchus nerka (Sockeye salmon)	Muscle	6.90	NA	NA	NA	NA	USA	Hardell *et al.* (2010)
Sardinella longiceps (oil sardine)	Muscle	24.00 ± 13.00	NA	NA	562.00 ± 129.00	740.00 ± 24.00	India	Muralidharan *et al.* (2009)
Alepedes djedaba	Muscle	16.40	35746.00	9464.50	16.35	NA	Egypt	Said, (2007)
Scomber Scombrus (Atlantic mackerel)	Muscle	NA	63.33	NA	NA	NA	Italy	Perugini *et al.* (2007)
Trachinotus blochii (snunose pompano)	Ventral	10 18 .00	67.20	NA	NA	NA	China	Cheung *et al.* (2007)
	Axial	409.00	48.70	NA	NA	NA		
Cynoglossus robustus (Tongue sole)	Ventral	36.20	145.00	NA	NA	NA		
	Axial	15.50	37.50	NA	NA	NA		
Pseudmonas elongatus (marine fish)	Muscle	NA	100,200.00	67890.00	NA	NA	Nigeria	Anyakora *et al.* (2005)

NA - not analysed

3.4. Conclusion

This study has provided baseline data on the levels of OCs (DDTs, PAHs, BHCs and OCPs) in tuna dark (TDM) and tuna light (TLM) muscle. The total concentration of DDTs was found to be significantly higher in TLM (the more edible portion) than in the TDM and this may be of concern especially among frequent tuna fish consumers. However, urgent need to further investigate the effect of cooking on DDT in fish will help to address the concern on consumption safety.TDM on the other hand had significantly higher concentrations of PAHs, BHCs and OCPs than TLM. This suggests that the incorporation of TDM in food products in view of its previously highlighted nutritional benefits may require such processing that will eliminate intake of the detected contaminants to avoid some health hazards particularly from benzo(*a*)pyrene. Further investigation into these postulations is recommended. Although the concentrations of the DDTs and pesticides (BHCs and OCPs) were relatively lower than recommended maximum levels in edible fish as indicated by the US FDA, which may suggest the acceptability of fish as fit for human consumption. However, prolonged consumption may result in body build-up of these compounds over time assuming the levels in the raw fish is retained after cooking. In view of the fact that the assessed tuna fish muscles (TDM and TLM) had benzo(*a)*pyrene in levels above the EU recommended maximum level (2 µg/kg ww), there is need to further investigate the possibility of human dietary exposure to PAHs via the consumption of tuna fish species. However, such study requires caution not to focus on raw fish only but include ready to eat fish to establish factual risk.

Input sources of DDTs could be attributed to current usage within the country; however, the presence of BHCs (lindane) could be linked to persistence and long spatial transfer, while the detection of PAHs in fish products may be attributed to petroleum pollution. With the migratory nature of tuna fish, it becomes difficult to conclude input of OCs to study location. However, the control of coastal oil spills and seeps may need to be re-enforced to reduce marine oil pollution.

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61

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CHAPTER 4

Detection and quantification of PAHs in South African snoek (*Thyrsites atun)* and soupfin shark (*Galeorhinus galeus*): A comparison of UPLC-FLD and GC-MS/MS methods

Abstract

Polycyclic aromatic hydrocarbons (PAHs) are of health concern due to their toxic, carcinogenic and mutagenic effects to humans. PAHs can enter the aquatic environment; accumulate in organisms such as fish and biomagnify in the food web. This study evaluated 16 priority PAHs in marine fish species (*T. atun* and *G. galeus)* using gas chromatography-tandem mass spectrometry (GC-MS/MS) and ultra performance liquid chromatography with fluorescence detection (UPLC-FLD). In snoek GC-MS/MS detected 4 PAHs, whilst UPLC-FLD detected 9 PAHs. In Soupfin shark, GC-MS/MS detected 4 PAHs and UPLC-FLD detected 5. GC-MS/MS had detection limit range of 0.56 (acenaphthene)-3.31 (naphthalene) µg/kg whereas LC-FLD had 0.004 (benzo(*g,h,i*)perylene) - 0.013 (chrysene) µg/kg. Most of the measured PAHs in the two fish species using both GC-MS/MS and UPLC-FLD of PAHs showed no significant variation ($p > 0.05$) in concentrations except with fluoranthene in snoek and phenanthrene in soupfin. Intra-class correlation of the two methods revealed an agreement range from 0.03 (fluoranthene) to 0.98 (fluorene) and consistency from 0.23 - 0.98 in snoek; whilst in soupfin an agreement range of 0.02 (phenanthrene) to 0.63 (naphthalene), consistency: 0.04 - 69. Both methods showed that snoek had higher ($p = 0.000$) PAHs concentration than Soupfin shark. Input source of PAHs identified by both methods was petrogenic with dominance of naphthalene. The non detection (by the two methods) of benzo(*a*)pyrene used for PAHs acceptability (2 µg/kg) by the EU member states and the dominance of non-carcinogenic PAHs, in the fish analysed may suggest studied snoek and soupfin to be of acceptable quality.

Keywords: PAHs, marine-fish, GC-MS, UPLC/FLD, detection, quantitation and detection-limits

4.1. Introduction

Polycyclic aromatic hydrocarbons are also referred to as polynuclear aromatic hydrocarbons (PNAHs) are a group of compounds of global interest due to their toxicity, carcinogenicity, environmental prevalence and persistence (Boehm, 2005, US EPA, 2008; FSAI, 2009). PAHs are formed by the fusion of two or more benzene rings with naphthalene as the simplest having 2 benzene rings (EFSA, 2008; Lee, 2010). Based on the number of

benzene rings PAHs are grouped into low molecular weight (LMW) and high molecular weight PAHs (HMW). The LMW PAHs have less than 4 benzene rings and are acutely toxic while the HMW have 4 or more benzene rings and are carcinogenic and mutagenic (WHO/IARC, 1999; Tsymbalyuk *et al.*, 2011). PAHs are lipophilic and are thus weakly soluble in water which decreases with increase in the number of benzene rings (Skupinkska *et al.*, 2004).

There are over 100 PAHs that have been identified (Boehm, 2005), however the United States Environmental Protection Agency (USEPA, 2008) listed 16 priority PAHs of global interest. These are: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo(*a*)anthracene; benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenz(*a,h*)anthracene, indeno(*1,2,3-cd*)pyrene and benzo(*g,h,i*)perylene. PAHs can be released into the environment from natural sources such as: coal, petroleum and other crude oil deposits and anthropogenic activities such as wild fire, domestic, municipal and industrial incineration (USEPA, 2008). Through these various sources PAHs can enter the aquatic environment, due to their lipophilic nature accumulate in lipid matrices and biomagnify in the food web. PAHs have widely been detected in various environmental and biological matrices confirming their prevalence. Some of these matrices include dust (Garrison *et al.*, 2014), ambient air (Sram *et al.*, 2007; Zhang &Tao, 2009), sediment (Sibiya *et al.*, 2013); freshwater fish (Wang *et al.*, 2012; Musa *et al.*, 2014) and marine fish (Nyarko *et al.*, 2009; Hwang *et al.*, 2012). Fish and in particular marine fish species have been reported to contribute significantly to human dietary exposure to PAHs (Domingo, 2007; Cheung *et al.*, 2007). The accumulation of PAHs in fish undermines the nutritional health benefits associated with fish consumption such as good sources of amino acids and polyunsatuarated fatty acids (Usydus *et al.*, 2009). These essential nutrients, for example omega 3 fatty acids predominantly found in marine fish species (Ugoala *et al.*, 2009) play a vital role in infant brain development and prevention of heart diseases (Sidhu, 2003; Carlson *et al.*, 2013).

To ensure consumption safety of aquatic foods such as fish, there is need to assess and monitor these contaminants (USEPA, 2000) against the safety limits provided by local and international regulatory agencies such as the World Health Organisation, (WHO). In view of the hazardous effects of PAHs, even in trace amounts, it is necessary to choose analytical instruments that can detect with high selectivity and sensitivity the target analytes (Wise *et al.*, 2015). Chromatographic analysis have been widely used for such assessments as it allows for the separation of compounds into its basic constituents and which are consequently detected with an attached detector (CDER, 1994; Lee, 2010). There are various factors which determine the type of chromatographic instrument to be used for analysis such as the stability of the target compounds at high temperature and the volatility of the compounds (CDER, 1994;

Boehm, 2005). In this study, focus is on gas and liquid chromatography (GC and LC) coupled to a mass spectrometer (MS) and fluorescence detectors (FLD) respectively. The principle of separation in LC is based on the affinity of the target compound to either the mobile (usually polar solvent) or stationary phase (CDER, 1994; Wise *et al.*, 2015). If the target compound is more soluble in the mobile phase the elution time is short whereas if attracted to the stationary phase the compound will take a longer time to elute. In the case of GC, the target compounds must be volatilizable and the principle is similar to the LC but with gas such as Helium as the carrier gas (Filigenzi *et al.*, 2011).   UPLC is a form of LC where a much smaller particle size column packing material is employed sub 2 micron which results in higher efficiency and better separation. The smaller particle size columns are run at a much higher backpressure (typically 7000-12000 psi) than conventional HPLC (1000-3000 psi range) which necessitates an ultra-high pressure LC system that can cope with these pressures.

Information on PAHs profile and levels in marine fish species within the African continent and South Africa in particular is scarce. However, a recent monitoring of PAHs and polychlorinated biphenyl (PCBs) from five harbours along the South African coastline was conducted by Degger *et al.* (2011). The study which used brown mussel (*Perna perna*) and a semi-permeable membrane device – (SPMD) as biological and non-biological matrices respectively, confirmed the prevalence of PAHs within the studied environment. Their study reported discrepancies with the PAHs profile and concentrations as measured by both methods. For instance, the biological matrix showed Saldanha Bay as the most contaminated whilst with the SPMD, it was Port Elizabeth. Furthermore on the levels of PAHs, brown mussel was in the range of 290-2100 µg/kg lipid weight (lw) whereas the SPMD ranged from 260-720 µg/kg lw. In addition brown mussel could not accummulate naphthalene which is an important determining factor in the input sources of PAHs (Boehm *et al.*, 2005; Degger *et al.*, 2011). This supports the evaluation of PAHs (profile and concentrations) using marine fish species like snoek (*Thyrsites atun*) and Soupfin shark (*Galeorhinus galeus*); both are large sized, predatory and migratory fish species commonly consumed within South Africa. The study was therefore aimed to assess and compare PAHs profiles and levels in two South African marine fish species (*T.atun* and *G. galeus*) in a single (same) extraction but diverse measurement techniques (GC-MS/MS and UPLC-FLD).

4.2. Materials and methods

4.2.1 Chemicals and standards

Polynuclear aromatic hydrocarbons (PAHs) standards mix purchased from Sigma Aldrich (USA) with catalogue number M-610 PAHs – consisting of 16 United States

Environmental Protection Agency's (USEPA) priority list of PAHs (purity $\geq$99.9 %) were used for instrumental (GC and LC) calibrations. These PAHs were: acenaphthene, acenaphthylene, anthracene, benz(*a*)anthracene, benzo(a)pyrene, benzo(*b*)fluoranthene, benzo(*g,h,i*)perylene, benzo(*k*)fluoranthene, chrysene, dibenzo(*a,h*) anthracene, fluoranthene, fluorene, indeno(*1,2,3-cd*)pyrene, naphthalene, phenanthrene and pyrene. Deuterated naphthalene d8; acenaphthene d10; chrysene d12 and perylene d12 were used as internal standards. Pre-packed extraction kits (QuEChERS 50 mL Teflon centrifuge/extraction tube and 15 mL Teflon centrifuge/clean-up tube containing extraction salts and clean-up agents respectively) and acetonitrile (analytical grade) used were purchased from Stargate Scientific, South Africa.

The standards stock solution were prepared in acetonitrile and further diluted to the desired concentrations for the calibration of instruments (GC and LC). Six concentration levels for GC (2.5, 5.0, 10.0, 25, 50 and 75 µg/L) and seven for LC (0.01, 0.1, 0.5, 1.0, 5.0, 10.0, and 50 µg/L) were used for the calibrations. To the calibration solution, the internal standard (50 µg/L) mix was added at each level. Extraction solvent acetonitrile containing the internal standards mix (50 µg/L) was freshly prepared before each extraction.

4.2.2 Sample preparation

Snoek and soupfin shark were used for this study (Table 4.1) with average weights of 4.041± 0.42 kg and 7.440 ± 0.31 kg, while average length (Total) were 103.05 ± 4.56 cm and 115.50 ± 15.44 cm for the respectively. Fish samples (10 per species) were harvested by local line fishermen from Port Elizabeth (33.9581° S, 25.6000° E) (South Africa) and were conveyed in crates (covered with ice) to the Stellenbosch University laboratory. The studied fish were harvested on the same date per species per location with the collaboration of the department of Agriculture, Forestry and Fisheries (DAFF). Each fish was thoroughly washed (after measurements) under running tap water, de-headed, eviscerated, skinned and filleted. The fillets were homogenized on an individual basis, vacuum packed and stored at -20°C pending further chemical analysis. A total of ten individual fish per species was used for the analysis.

4.2.3 Extraction procedure

The extraction procedure for this and for all other chapters was the same. The detailed method of extraction has been given in chapter three and therefore was not repeated.

Table 4.1. Biometric measurements for snoek (*Thyrsites atun*) and soupfin shark (*Galeorhinus galeus*) used for detection of PAHs

Common name	Scientific name	Weight (kg)	Total length (cm)	Sex
Snoek	*Thyrsites atun*	3.54	101.60	NA
Snoek	*Thyrsites atun*	3.62	100.30	NA
Snoek	*Thyrsites atun*	3.65	97.70	NA
Snoek	*Thyrsites atun*	4.10	106.00	NA
Snoek	*Thyrsites atun*	4.85	112.50	NA
Snoek	*Thyrsites atun*	3.90	104.50	NA
Snoek	*Thyrsites atun*	4.05	103.50	NA
Snoek	*Thyrsites atun*	4.55	106.40	NA
Snoek	*Thyrsites atun*	4.25	100.50	NA
Snoek	*Thyrsites atun*	3.90	97.50	NA
Soupfin shark	*Galeorhinus galeus*	6.30	106.00	female
Soupfin shark	*Galeorhinus galeus*	9.50	128.00	male
Soupfin shark	*Galeorhinus galeus*	9.05	129.20	female
Soupfin shark	*Galeorhinus galeus*	6.10	109.00	female
Soupfin shark	*Galeorhinus galeus*	7.150	118.10	male
Soupfin shark	*Galeorhinus galeus*	14.30	143.00	male
Soupfin shark	*Galeorhinus galeus*	4.00	95.00	female
Soupfin shark	*Galeorhinus galeus*	7.45	117.90	male
Soupfin shark	*Galeorhinus galeus*	3.50	93.60	female
Soupfin shark	*Galeorhinus galeus*	7.05	115.20	male

N A– not assessed

4.3. Instrumental (GCMS/MS and LC/FLD) analysis of fish extract for PAHs

4.3.1 PAHs analysis with GC-MS/MS

The final clear fish extract was analyzed for the 16 priority PAHs following a modified method described by Kalachova *et al*. (2012) using a GC Thermo Scientific TRACE™ 1310 (USA) (automated injection) which was coupled to TSQ 8000 Mass Spectrometer (MS)(USA). Separation was performed on a non-polar (95% dimethylpolysiloxane) capillary column Restek –Rxi ®-5Sil MS w/Intrega-Guard ® (15 m, 0.25 mm ID, 0.25 µm film thickness) part number 13620-127. The initial oven temperature was 75°C, held for 3 min and finally increased to 320°C at 10°C/min, and held at this temperature for further 3 min with a total run time of 31 min. The injector temperature was maintained at 275°C, injection was splitless with the split flow set at 50 ml/min for 2 min. Gas saver was activated for 5 min at 20 ml/min. Helium was used as carrier gas at a flow rate of 1 ml/min. Both the injector and transfer line

temperatures were held at 250°C. The ionization source temperature was set at 250°C with emission current of 75 µA and Argon was used as the collision gas. Analytes were identified based on matching sample peak area and retention time (RT) with that of a known reference standard (RT) with a tolerance intervals of 2.5-4% of the known reference RT (within same analytical batch). Quantification (µg/L wet weight) was based on the response ratio of the peak area against concentration of the analyte to that of the internal standard.

4.3.2. PAHs analysis with UPLC-FLD

The UPLC-FLD was also used to analyse the 16 priority PAHs following the method adapted from Pule *et al.* (2012). This was carried out using a Waters Acquity ultra-performance liquid chromatograph (UPLC) (Waters, Massachusetts, USA) fitted to a Waters Acquity fluorescence detector (FLD) and Waters photo diode array (PDA) detector. The separation of target analytes was achieved within a Waters Acquity UPLC HSS T3 column (2.1 x 150 mm and particle size of 1.8 µm). The mobile phase consisted of water (solvent A) and acetonitrile (solvent B), at a column temperature of 35°C. The gradient started at 35% acetonitrile in water for 0.05 minutes followed by a concave gradient (curve 5) over 15 min to 75% acetonitrile and a linear gradient over 2 min to 77% acetonitrile and re-equilibration at initial conditions for 2 min to yield a total run time of 25 min. The injection volume was 5 µL and a flow rate of 0.35 mL/min. The detection was based on the optimum response of the analytes at the excitation and emission wavelengths of 260 and 220-800 nm respectively. The acquisition of data was done with Waters Masslynx version 4.1. The final concentration of the contaminant in the fish was expressed as µg/kg wet weight (ww) and was calculated from the detected concentration divided by the sample weight and multiplied by the dilution factor.

4.4. Statistical analysis

The data generated were tested for normality and outliers were removed. One way analysis of variance (ANOVA) was used to identify if significant variation existed between PAHs level measured by the two diverse methods (GC-MS/MS and UPLC-FLD) for both species. Posthoc separation of means was done and significant differences established at 95% confidence level ($p < 0.05$). Statistical package Statistica version 12.0 (Statsoft Inc., USA, 2012) was used. Only PAHs commonly detected by both methods were analyzed while PAHs below limits of detection (LOD) were treated as not detected (ND). A reliability test of data was done with intra-class correlation (ICC) by comparing the ratings of individual PAHs measured by GC versus LC against the total ratings.

4.5 Results and Discussion

4.5.1. Comparison of PAHs profiles in fish samples as detected with GC-MS/MS and LC-FLD

The GC-MS/MS and UPLC-FLD successfully separated (resolved) the 16 PAHs (reference standards) within a run time of 31 and 20 min respectively (Figures 4.1 & 4.2). Hwang *et al.* (2012) with GC-MS/MS reported a run time of 42 min whilst with LC-FLD, Ramalhosa *et al.* (2009) and Pule *et al.* (2012) reported run times of 30 and 14 min, respectively. The run times for both the GC-MS/MS and UPLC-FLD observed in this study were within acceptable limits. The PAHs were identified by comparing the peak area and retention time of the known in the reference standard, with the unknown in the fish extract. The limits of detection (LODs) and quantification (LOQs) were calculated from a calibration standard on the basis of 3 and 10 times the signal to noise ratio respectively (Saadati *et al.*, 2013). The identification parameters: retention time (RT), LODs, LOQs and correlation coefficient (R^2) of PAHs as detected by the two methods are given in Table 4.2. These parameters were found to be in agreement with published work under similar conditions. For instance Pule *et al.* (2012) reported a limit of detection range from 0.03 µg/kg ww in benz(*a*)anthracene to 0.62 for naphthalene. In our current study, UPLC-FLD gave a lower limit range of 0.004 µg/kg (benzo(*g,h,i*)perylene to 0.013 µg/kg ww (chrysene). Whilst with the GC-MS/MS, LOD ranged from 0.56 (acenaphthene) to 3.31 µg/kg ww (naphthalene). The current LOD with GC-MS/MS was higher than what Hwang *et al.* (2012) reported in a similar analysis: a range of 0.01 (fluorene) to 0.05 µg/kg (naphthalene). The higher LOD could be attributed to factors such as column type, mobile and stationary phase constituents which are all known to affect the instrument's sensitivity and selectivity (CDER, 1994).

The GC-MS/MS did not have co-elution of any of the analysed 16 PAHs (standards), whereas with the UPLC-FLD, acenaphthene co-eluted with the isotopically labelled internal standard (deuterated acenaphthene) and dibenzo(*a,h*)anthracene co-eluted with indeno(*1,2,3-cd*)perylene. In addition, UPLC-FLD did not detect acenaphthylene which was attributed to its inability to emit under FLD (Perugini, *et al.*, 2007; Gratz *et al.*, 2011). A comparison of the PAHs profile in the two fish species as detected by the two methods showed that for snoek the GC-MS/MS detection was dominated by the LMW PAHs (naphthalene, fluorene and phenanthrene) with naphthalene as the most abundant. Whilst the UPLC-FLD, detected more number of HMW PAHs (fluoranthene, pyrene, chrysene, benzo(*k*)fluoranthene and indeno(*1,2,3-cd*)perylene) than the LMW PAHs (naphthalene, fluorene, phenanthrene and anthracene) (Table 4.3). However, in soupfin shark GC-MS/MS detected three LMW PAHs (naphthalene, fluorene and anthracene) and none of the HMW PAHs. Conversely, the

UPLC-FLD detected four LMW PAHs (naphthalene, fluorene, phenanthrene and anthracene) and one HMW PAHs (pyrene) (Table 4.3).

The observed variations in PAHs profile as detected by the two analytical methods highlight the sensitivity and selectivity of each technique. Moreover, the GC-MS/MS detection limit observed from this study was high which thus affected its detection ability (Cajka & Hajslova, 2011). The majority of the HMW PAHs present were below the detection limit for the GC-MS/MS and were considered as not detected (Table 4.3). However, this trend was peculiar to this analysis, as in a previous analysis with tuna fish extracts (chapter 3), GC-MS/MS detected HMW PAHs such as fluoranthene, chrysene, benz(*a*)pyrene and indeno(*1,2,3-cd*)perylene. The source of variation in the detected PAHs profile from the two methods could be explained as due to relatively rapid breakdown and possible loss of some PAHs under different analytical conditions (Cajka & Hajslova, 2011).

PAHs profiles in fish may have species effect which may be so in view of the different metabolization pathways of PAHs by different fish species (USEPA, 2000). As pertaining to the two fish species, it is known that HMW PAHs some large fish can transform faster than the LMW as some experimental and physical factors can promote PAHs biotransformation (Gert-Jan deMaagd & Vethaak, 1998). The high detection of LMW PAHs (especially naphthalene, fluorene, and phenanthrene) by both methods in the studied fish samples could be explained by some of these factors. For example the fact that LMW PAHs are more hydrophobic, readily and directly taken up by fish via the polluted water unlike the HMW that attach to particles and sediment (Brown & Peake, 2006), will contribute to higher accumulation and invariable in measurable or detectable concentrations. Also the LMW PAHs dominate in aquatic environment where pollution source is from petroleum oil (Boehm, 2005). Furthermore the LMW PAHs due to low transformation are retained in the fish longer than the HMW which can be excreted after breakdown (Gert-Jan de Maagd & Vethaak, 1998).

PAHs input sources in the two species could be said to be petrogenic as the overall dominant PAH from both methods was naphthalene. Furthermore, using the diagnostic ratio of LMW/HMW with a value greater than 1 to imply petrogenic origins (Brown & Peake, 2006; Karlsson & Viklander, 2008) was observed in this study and thus confirm input sources to be from petroleum. This was in agreement with the finding of our previous study on the assessment of organic contaminants in tuna dark and light muscle (Chapter 3). Degger *et al*. (2011) also reported that the South African marine environment (the study areas were harbours) was contaminated with PAHs from petroleum sources. This emphasizes the impact of oil tanker spillages and other releases that could emanate from refinery and drilling activities. The regulatory, monitoring and control of petroleum and allied products into the

marine environment by the department of Environment and Tourism (DEAT) should be intensified.
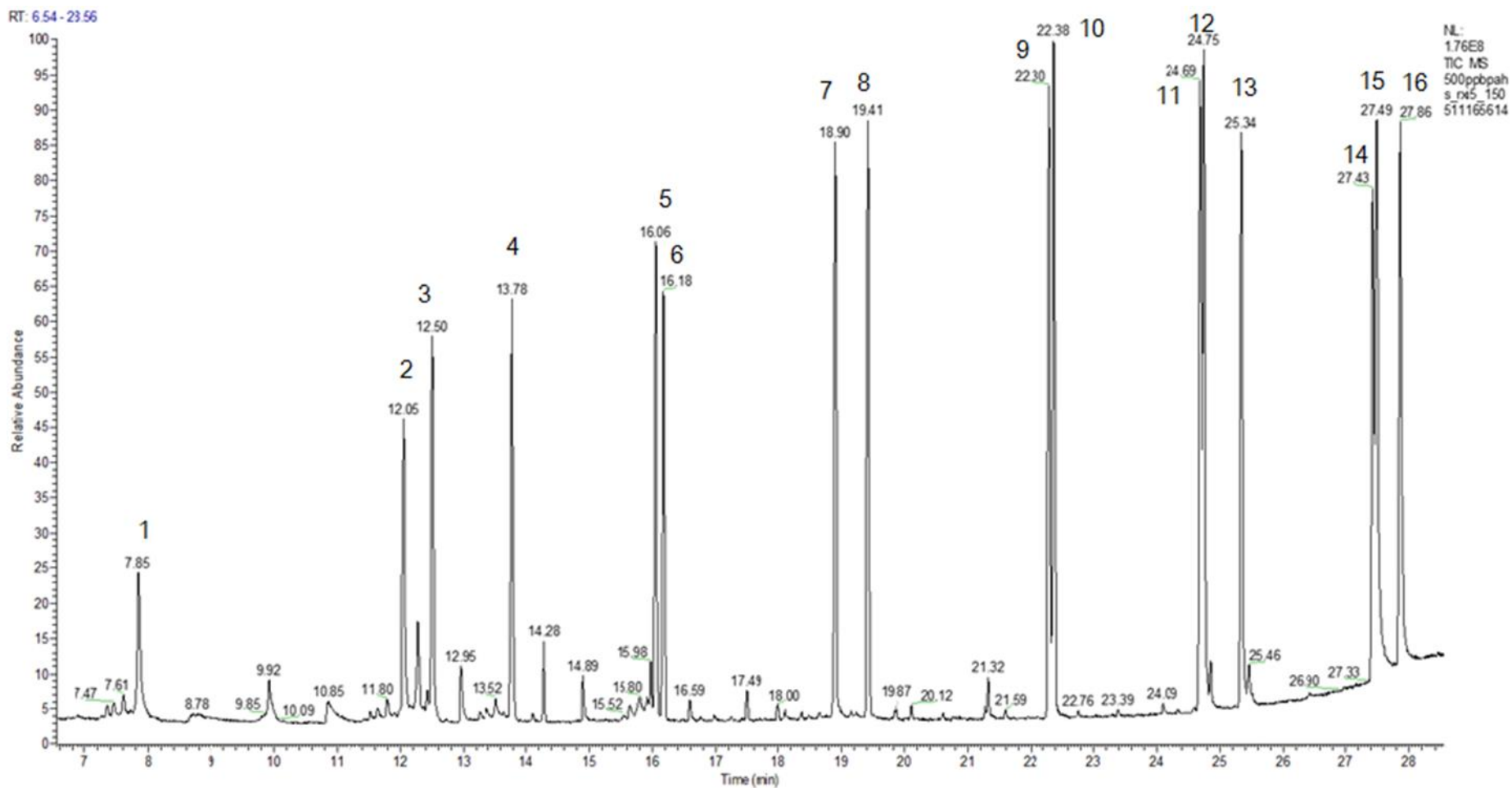
Figure 4.1. GC total ion chromatogram (TIC) showing the 16 US EPA priority PAHs in standard solution (100 µg/L) as detected on GC-MS/MS. List of the PAHs with the corresponding retention time were given in Table 4.2. [1 Napthalene. 2 Acenaphthylene. 3 Acenapthene. 4 Fluorene. 5 Phenanthrene. 6 Anthracene. 7 Fluoranthene. 8 Pyrene. 9 Chrysene. 10 Benzo(*a*)anthracene. 11 Benzo(b)fluoranthene. 12 Benzo(*k*)fluoranthene. 13 Benzo(*a*)pyrene. 14 Indeno(*1,2,3-cd*)pyrene. 15 Dibenzo(*a,h*)anthracene. 16 Benzo*(g,h,i)*perylene]
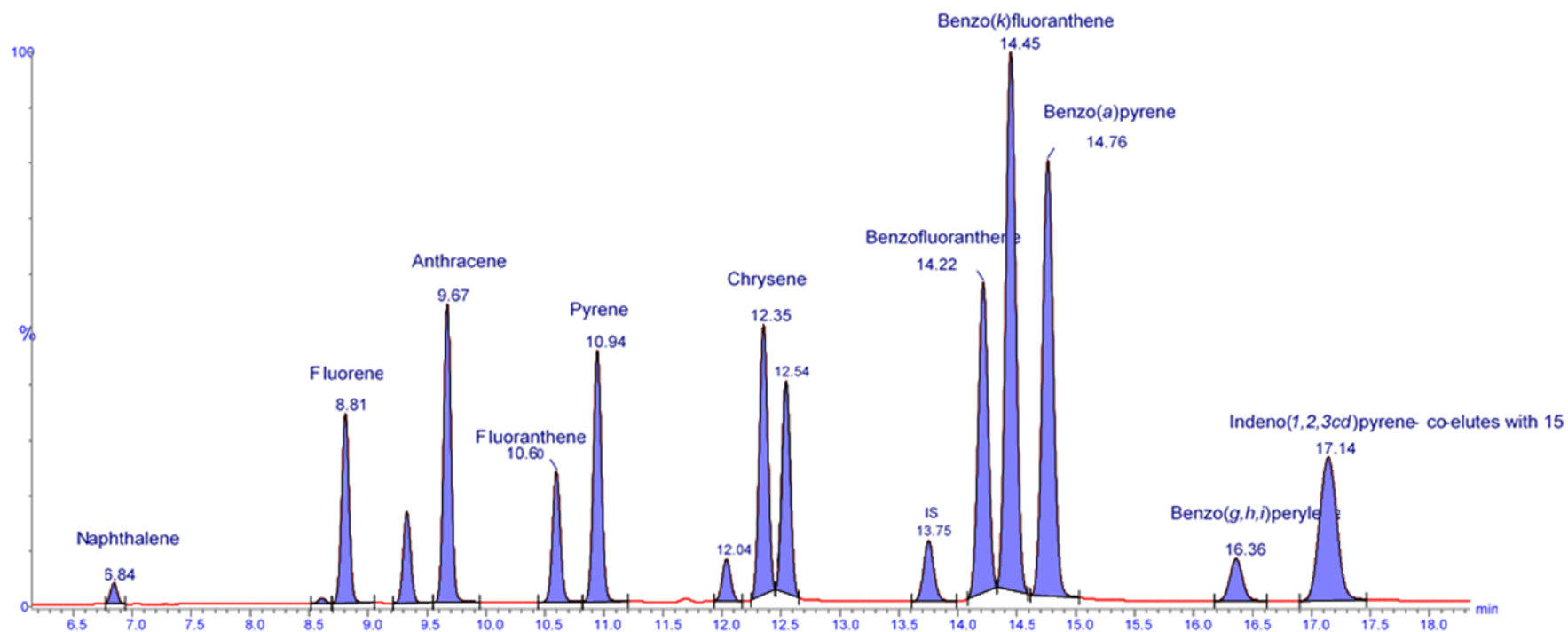
Figure 4.2. Chromatogram of the 16 priority PAHs standard solution (100 µg/L) as analyzed with liquid chromatography, fluorescence detector (LC-FLD). Acenaphthylene was not detected as it does not emit under the FLD. Acenaphthene co-eluted with its isotopically labelled internal standard and also dibenzo(*a,h*)anthracene co-eluted with indeno(*1,2,3-cd*)pyrene.

Table 4.2. Limits of detection (LOD) and quantification (LOQ), retention time (RT) and R^2 values of each analyte (16 priority PAHs) as analyzed with gas chromatography/mass spectrometer (GC-MS/MS) and Liquid chromatography/Fluorescence detector (UPLC-FLD).

PAHs	GC-MS/MS				UPLC-FLD			
	LOD (µg/kg)	LOQ (µg/kg)	R^2	RT in min	LOD (µg/kg)	LOQ (µg/kg)	R^2	RT in min
Naphthalene	3.315	11.049	0.997	7.85	0.010	0.034	0.999	6.84
Acenaphthylene	1.102	3.674	0.998	12.05	ND			
Acenaphthene	0.556	1.852	0.996	12.50	Co-elution	Co-elution		8.77
Fluorene	1.617	5.389	0.997	13.77	0.010	0.034	0.998	8.81
Phenanthrene	2.257	7.525	0.99	16.06	0.009	0.031	0.999	9.33
Anthracene	1.349	4.495	0.991	16.18	0.009	0.029	0.998	9.66
Fluoranthene	2.404	8.013	0.994	18.90	0.009	0.030	0.999	10.6
Pyrene	1.737	5.788	0.992	19.41	0.007	0.024	0.999	10.94
Chrysene	2.835	9.449	0.991	22.30	0.013	0.043	0.999	12.35
Benzo(a)anthracene	2.274	7.581	0.992	22.38	0.010	0.032	0.999	12.55
Benzo(b)fluoranthene	1.666	5.552	0.997	24.69	0.006	0.020	0.999	14.22
Benzo(k)fluoranthene	1.532	5.107	0.996	24.75	0.009	0.029	0.999	14.47
Benzo(a)pyrene	2.369	7.896	0.996	25.34	0.007	0.022	0.999	14.77
Indeno(*1,2,3-cd*)pyrene	2.089	7.896	0.997	27.43	0.010	0.033	0.999	17.12
Dibenz(*a,h*)anthracene*	1.774	5.912	0.997	27.49	Co-elution			
Benzo(*g,h,i*)perylene	1.537	5.123	0.998	27.86	0.004	0.012	0.999	16.37

RT=retention time
Indeno (*1,2,3-cd*) pyrene eluted last in LC/FLD (co-elution with dibenz(*a,h*)anthracene
Acenaphthene co-eluted with isotopically labelled internal standard
ND-not detected (acenaphthylene do no emit under FLD)

Table 4.3. Comparison of 16 United States Environmental Protection Agency (US EPA) priority PAHs (profile) from marine fish samples (snoek and soupfin shark) as analyzed with GC-MS/MS and LC-FLD

Fish species	GC-MS/MS			UPLC-FLD		
	PAHs detected	PAHs Not detected	PAHs Co-eluted/<LOD	PAHs detected	PAHs Not detected	PAHs Co-eluted
Snoek	Naphthalene Fluorene Phenanthrene Pyrene	Anthracene Chrysene Benzo(*a*)anthracene Benzo(*k*)fluoranthene Indeno(*1,2,3-cd*)pyrene Benzo(*g,h,i*)perylene	Acenaphthylene Acenaphthene Fluoranthene Benz(a)pyrene	Naphthalene Fluorene Phenanthrene Anthracene Fluoranthene Pyrene Chrysene Benz(*k*)fluoranthene Indeno(*1,2,3-cd*)pyrene	Acenaphthylene Benzo(*a*)anthracene Benzo(*b*)fluoranthene Benzo(*a*)pyrene Benzo(*g,h,i*)perylene	Acenaphthene Dibenz(*a,h*)anthracene
Soupfin shark	Naphthalene Fluorene Anthracene Phenanthene	Acenaphthene Chrysene Benz(*a*)anthracene Benzo(*b*)fluoranthene Benzo(*k*)fluoranthene Indeno(*1,2,3-cd*)pyrene Dibenzo(*a,h*)anthracene Benzo(*g,h,i*)perylene	Acenaphthylene Fluorene Fluoranthene Pyrene Benz(*a*)pyrene	Naphthalene Fluorene Anthracene Phenanthrene Pyrene	Acenaphthylene Fluoranthene Benz(a)pyrene Indeno(1,2,3-cd)pyrene Dibenzo(a)anthracene Benzo(g,h,i)perylene	Acenaphthene

PAH - polycyclic aromatic hydrocarbon

4.5.2 Comparison of PAHs concentrations in the fish extracts

The concentrations of the commonly detected PAHs in both methods were compared (Table 4.4). Thus all the HMW PAHs detected and quantified only with the LC-FLD could not be compared with GC-MS/MS values (as these PAHs were found below detection limits). In snoek, no significant differences ($p \geq 0.05$) were observed in the four PAHs (naphthalene, fluorene, phenanthrene and pyrene) out of the five as measured by both methods (Tables 4.3 & 4.4). Fluoranthene showed significant variation ($p < 0.05$) between the two methods with GC-MS/MS measuring 0.28 ± 0.05 µg/kg ww whilst UPLC-FLD measured a higher concentration of 1.08 ± 0.08 µg/kg ww. The total PAHs as measured with the GC-MS/MS in the snoek samples (122.94 ± 10.65 µg/kg ww) was not different ($p = 0.11$) from that measured with the LC/FLD (144.18 ± 10.65 µg/kg ww).

In soupfin shark, the phenanthrene concentration varied significantly ($p < 0.05$) with GC-MS/MS measuring 8.81 ± 2.73 µg/kg ww and UPLC-FLD at 0.73 ± 0.09 µg/kg ww (Table 4.4). Furthermore the total PAHs concentration with GC-MS/MS (113.49 ± 4.26 µg/kg ww) was higher ($p<0.05$) than that measured with UPLC-FLD (100.67 ± 3.72 µg/kg ww). Fluoranthene and phenanthrene were also observed to vary with the methods measurement in snoek and soupfin respectively (Table 4.4). It could be assumed that possible loss of analyte through evaporation may have occurred under analytical conditions (Brown & Peak, 2006). GC-MS/MS gave a lower concentration of total PAHs in snoek (122.94 ± 9.96 µg/kg ww) than UPLC-FLD (144.18 ± 11.29 µg/kg ww), whereas in soupfin the opposite was noted (Table 4.4). In general, PAHs total concentration could be affected by factors associated with experimental methodology (such as properties of the analytes), or with biological factors such as fish size and species (USEPA, 2000). Different fish species have different abilities to metabolize PAHs which may affect the residual levels (Gert-Jan de Maagd & Vethaak, 1998).

Further comparison of the two methods to determine the validity of the data was done with intra-class correlation (ICC) based on a 2D scatter plot. The individual PAH measured on the GC-MS/MS was compared with the same PAH measured on the UPLC-FLD to determine their ICC agreement and consistency (e.g. GC-naphthalene versus LC-naphthalene). The ICC values obtained (Table 4.4) apart from fluoranthene, ranged from 0.43 (naphthalene) to 0.98 (fluorene), such a range from weak positive to a very strong positive correlation could indicate that the observed variation may be analyte specific than instrumental. Comparison of the current study with published data is limited as data under similar analytical conditions are scarce. Berset *et al.* (1999) reported HRGC-MS/MS as superior to UPLC-FLD in measuring PAHs in a soil matrix, whereas Wise *et al.* (2015) reported LC to be more sensitive and selective in PAHs analysis (in a biological matrix) if an appropriate column is used. In this

study, the UPLC-FLD detected a higher number of PAHs and had lower limits of detection than the GC-MS/MS.

The toxicity of the PAHs analogues were not determined in this study as the fish was deemed safe in view of the non-detection of potent benzo(*a*)pyrene by both GC-MS/MS and UPLC-FLD, which is the International (EU and FAO/WHO) PAHs acceptability indicator. Further investigation with smaller, non-migratory and non-predatory fish may be necessary to further understand the accumulation and health risk implications of PAHs within South African marine fish species.

Table 4.4**.** Concentrations (µg/kg ww) of PAHs in snoek (*Thyrsites atun*) and soupfin shark (*Galeorhinus galeus*) commonly detected and measured on both GC-MS/MS and UPLC-FLD, indicating p-value, intra-class agreement and consistency between the two methods and also r- values based on intra-class correlation (ICC).

Species	PAHs	GC-MS/MS concentration µg/kg ww	UPLC-FLD concentration µg/kg ww	p-value	ICC agreement	ICC consistency	r-value
Snoek	Naphthalene	108.63 ± 10.63	129.29 ± 12.34	0.11	0.43	0.48	0.37
	Fluorene	5.03 ± 1.84	5.29 ± 1.59	0.50	0.98	0.98	0.77
	Phenanthrene	7.79 ± 0.97	7.20 ± 1.14	0.37	0.82	0.82	0.61
	Fluoranthene	0.28 ± 0.5	1.08 ± 0.08	0.03	0.03	0.23	0.43
	Pyrene	1.20 ± 0.13	1.32 ± 0.15	0.46	0.43	0.42	0.37
	Σ PAHs	122.94 ± 9.96	144.18 ± 11.29	0.11	0.32	0.34	0.30
Soupfin	Naphthalene	102.29 ± 2.68	97.71 ± 3.58	0.10	0.63	0.69	0.52
	Fluorene	2.39 ± 0.22	2.23 ± 0.18	0.55	0.21	0.20	0.07
	Phenanthrene	8.81 ± 2.73	0.73 ± 0.09	0.02	0.02	0.04	0.58
	Σ PAHs	113.49 ± 4.26	100.67 ± 3.72	0.001	0.50	0.78	0.85

BGC-MS/MS – gas chromatography/mass spectrometer
UPLC-FLD – liquid chromatography/ fluorescence detector
PAHs – polycyclic aromatic hydrocarbons

4.6. Conclusion

The ability of the UPLC-FLD to detect both LMW and HMW PAHs (based on this study) would suggest this methodology to be superior to GC-MS/MS in PAHs profiling. The UPLC-FLD with lower detection limit could be an explanation to its detection of more of the LMW and HMW PAHs in comparison to the GC-MS/MS. In other words the GC-MS/MS could not detect PAHs with very low concentration thereby having greater portion of the HMW PAHs as below detection limit or not detected. However, it may become necessary to combine both methods

for validation if more sensitive assessments are required as the PAHs concentration as quantified by GC-MS/MS in soupfin was relatively higher than UPLC-FLD. MS/MS is a more selective detection method than FLD and can therefore be used as a confirmation for the presence of compounds detected on FLD..

The dominance of LMW (non-carcinogenic) PAHs, particularly naphthalene, in the investigated marine species (snoek and soupfin shark) confirms a petrogenic input sources. However, some large fish species (of the types studied) can enzymatically metabolize and excrete the HMW PAHs, which may be have contributed to the observed low detection and concentrations of HMW in studied species by both methods. Overall, both methods (GC-MS/MS and UPLC-FLD agreed that snoek had higher level of PAHs contamination than the soupfin shark. This could be attributed to the possibility of Soupfin shark transforming and excreting PAHs more efficiently than snoek and species diverse food source. However, both postulations require further investigation to have a clear understanding of species specificity in accumulation of contaminants.

4.7. References

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CHAPTER 5

Polycyclic aromatic hydrocarbons (PAHs) and organochlorinated pesticides (OCPs) in yellowtail (*Seriola lalandi*) from three spatially distinct locations along the coast of South Africa: Levels, sources and fish size effect.

Abstract

Polycyclic aromatic hydrocarbons (PAHs) and organochlorinated pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT), endosulfan and benzenhexachloride (BHC) were evaluated in yellowtail (*Seriola lalandi*) fish species.These hardous compounds were studied in view of their prevalence in the country and fish were sampled from three locations: Port Elizabeth, Yzerfontein and Struis Bay. The aim of the study was to investigate the profiles, levels and sources of PAHs and pesticides in yellowtail from the selected locations in relation to fish size and lipid content. Significant variations ($p < 0.05$) were observed in the levels of PAHs measured in fish sampled from the three locations. Fish from Port Elizabeth had the highest PAHs concentrations (533.95 ± 34.36), followed by Yzerfontein (221.40 ± 33.03) and Struis Bay (88.97 ± 2.83) µg/kg wet weight. Benzo(*a*)pyrene (PAHs biomarker) exceeded the recommended EU limit (2 µg/kg) in samples from Port Elizabeth and Yzerfontein whereas samples from Struis Bay did not exceed. DDT was detected only in samples from Port Elizabeth and Yzerfontein with mean total concentrations (7.48 ± 5.18 and 11.14 ± 1.44 respectively) not significantly different. Fish size (weight) correlated positively with lipid content (0.65; $p < 0.01$) and a stronger positive correlation with ΣPAHs (0.83; $p < 0.01$). PAHs input source in fish from Port Elizabeth reflected a mixture of petrogenic and pyrogenic whereas, Yzerfontein and Struis Bay showed input source as petrogenic. In conclusion, consumption of large sized fish in locations with high PAHs burden can predispose consumers to health risk. Further investigation into human dietary exposure with the species is recommended.

Keywords: *Seriola lalandi*, organic contaminants, fish size, fish fat, health implication

5.1. Introduction

Sequel to the Stockholm convention on persistent organic pollutants (POPs) in 2002, some hazardous compounds have been identified for global eradication or control based on their persistence, ubiquity, toxicity and carcinogenicity (US EPA, 1998; UNEP, 2005; UNEP, 2009). These hazardous compounds remain in the environment due to their low degradation, spatial transferability and lipophilicity which promotes their accumulation in fatty tissues and thus biomagnify in the food web (US EPA, 2000; Kaushik & Kaushik, 2007; Haritash &

Kaushik, 2009). Such hazardous compounds considered in this study include polycyclic aromatic hydrocarbons (PAHs) and organochlorinated pesticides (OCPs). PAHs are a group of compounds formed by the fusion of two or more benzene rings with napthalene (2 benzene rings) as the simplest (Boehm, 2005). Though more than 100 PAHs have been isolated from various matrices, the 16 PAHs US EPA priority PAHs already mentioned in chapter 3 were considered. In addition to the PAHs, OCPs such as dichlorodiphenyltrichloroethane (DDT), benzenehexachloride (BHC), endosulfan, aldrin, eldrin and dieldrin among others were also evaluated in *S. lalandi.* In line with the US EPA recommendations for choice of contaminants to be studied (US EPA, 2000); the target contaminants (PAHs and OCPs) were selected based on their prevalence and use within the country (Degger *et al*., 2011; Ansara-ross *et al*., 2012; Barnhoon *et al*., 2015). These compounds hither to be referred to as organic contaminants (OCs) except where otherwise stated.

Organochlorine contaminants accumulates in fish mainly through contaminated diet and water in polluted aquatic environment with oily fish accumulating higher levels (FSAI, 2013). A number of biological factors such as fish size, sex, age, feeding habit and lipid content (among others) can affect the accumulation of contaminants in fish (Kleinow *et al*., 1987). Once the contaminants are ingested into the fish redistribution into different tissues portions based on binding affinity for example, the lipophilic ones can be trapped in more fatty tissues such as liver (Zhou *et al*., 2013). Furthermore, enzymatic biotransformation of these contaminants can lead to their breakdown into by-products that can be stored in the fish or eliminated (Ramesh *et al*., 2004; Bandowe *et al*., 2014). However, no conclusive trend had been reported with these factors with respect to accumulation of contaminants for they are interwoven but fish species as well as contaminant type were reported as major determining factors.

Frequent consumption of fish contaminated with OCs may hamper the associated health benefits from fish particularly the dietary contribution of omega 3 and 6 fatty acids (FAO, 2011). In view of the earlier stated fact (chapter 2) that human dietary exposure to the OCs were reported to be significantly higher via fish and sea foods (ASTDR, 1995; Skupińska *et al*.**,** 2004; Domingo, 2007), hence the relevance to assess the OCs levels and sources in one of the South African commonly consumed marine species (*S.lalandi*).

Yellowtail (*Seriola lalandi*) is an important commercial and recreational marine linefish species and is among the most commonly consumed fish species in South Africa (NSW, 2010; WWF, 2011). It is pelagic as well as demersal species (benthopelagic) and widely distributed in places such as the Atlantic, Pacific and Indian temperate waters (NSW, 2010), whilst distribution within South Africa include KwaZulu-Natal, Western and Eastern Cape Provinces.

Its current status is being over fished and annual catch was reported to have declined from 700 tonnes (1995) to 400 tonnes in 2011 (Kerwath, 2013).

Currently information on concentration of OCs in yellowtail species is scarce globally and South Africa in particular. The study was therefore aimed at evaluating the concentrations and profiles of OCs in yellowtail (*Seriola lalandi*) marine fish harvested from Yzerfontein, Port Elizabeth and Struis bay, on the coast of South Africa. In addition the size, lipid content and locational distribution of OCs in yellowtail species from the three study locations were compared.

5.2. Materials and methods

5.2.1.Chemicals and standards

The chemicals and standards used for the study were as previously detailed in chapter 3.

5.2.2 Sample collection and preparation

Ten yellowtail were harvested from each of (if looked at Table 5.1) three different locations namely Port Elizabeth (33.9581° S, 25.6000° E) in the Eastern Cape Province and Yzerfontein (33.3330° S, 18.1620° E) and Struis Bay (34.8044° S, 20.0575° E) both in the Western Cape Province. Fish samples were harvested by local line fishermen in collaboration with the Department of Agriculture, Fisheries and Forestry (DAFF), which were sampled from these locations on different dates. Harvested fish were transported in crates covered with ice to the Stellenbosch University laboratory and fish biometric data; total length (cm) and weight (g), were recorded. Fish samples were rinsed under clean tap water and thereafter eviscerated, beheaded, skinned and filleted. Finally samples were homogenized on an individual basis, vacuum packed and stored at -20ºC until further chemical analysis.

Table 5.1. Average (± Std. Error) weight, length and lipid content of yellowtail (Seriola lalandi) sampled from Port Elizabeth, Yzerfontein and Struis Bay assessed for persistent organic contaminants OCs (PAHs and OCPs)

Location	Sample size (n)	Weight (g)	Length (cm)	Lipid (%)
Port Elizabeth	10	13925.00 ± 321.48	123.75 ± 1.81	3.605 ± 0.22
Yzerfontein	10	3236.30 ± 131.61	72.48 ± 1.01	2.771 ± 0.06
Struis Bay	10	6955.00 ± 415.56	98.18 ± 2.59	NA

NA: Not analysed

5.2.3 Experimental analysis

Lipid content determination of the studied yellowtail was as earlier described in chapter 3. Also extraction and instrumental analysis of target analytes were same as in chapter 3.

5.2.4. Statistical Analysis.

All data were checked for normality and homogeneity with Kolmogorov-Smirnov and Levene's F tests respectively. Differences in contaminants concentrations in fish sampled from the three locations were compared using analysis of variance (one way-ANOVA). But where the equality test failed, non-parametric tests (Mann Whitney *U* test or Kruskal Wallis) were used. A post hoc test was further carried out to establish significant variation of means. Correlations of fish weight with lipid content and OCs were established using both Pearson and Spearman's correlation which showed similar result. However, Pearson's correlation with stronger positive values was presented. As some PAHs were below detection limit they were not statistically assessed. In addition, ANOVA evaluation was limited, where only PAHs common (detected) in at least two locations were considered for statistical analysis. Significant differences and correlations were established at 95% confidence limit ($p < 0.05$) and all statistics was done using Statistica version 12.3 (Statsoft Inc., USA, 2012).

5.3. Results and Discussion

5.3.1 Lipid content in yellowtail species *S. lalandi*

A summary of the weight (kg), total length (cm) and % lipid of yellowtail sampled from Port Elizabeth, Struis Bay and Yzerfontein is given in Table 5.1. Fish sampled from Port Elizabeth were significantly higher in size (weight and length) ($p < 0.05$) than fish from both Yzerfontein and Struis Bay and also had significantly higher ($p < 0.05$) lipid content compared to Yzerfontein (Table 5.1). Lipid content of samples from Struis Bay was unfortunately not determined due to some unavoidable logistic issues (samples from this location were lately received, as such no sufficient time and slot for booking/use of the laboratory in iew of deadline for submission of dissertation). The variation in the lipid content of fish (inter and intra species) have been attributed to a number of factors such as age, size, sex and feeding habits (Baumard, 1998; Covaci *et al.*, 2006; Sriket *et al.*, 2007). The observed variation in the lipid content of studied species sampled from different study locations could be accommodated by these factors. In a recent study on yellowtail species from South Africa involving farmed and wild species, lipid content of 4.29 ± 1.63 % for wild with no significant difference from the farmed (3.72 ± 1.29 %) were observed by O'Neill *et al.* (2015). Burke (2011) in another study

with farmed yellowtail off coast of Port Elizabeth, South Africa recorded lipid content of 4.3 ± 0.23 %. Thus variation within species could be attributed to the mentioned factors.

5.3.2 PAHs in *S. lalandi*

Of the 16 PAHs analysed in yellowtail fish from Port Elizabeth, Yzerfontein and Struis Bay, two (acenaphthene and benzo(*k*)fluoranthene), five (benzo(k)fluoranthene, chrysene, benzo(*a*)anthracene, benzo(*b*)fluoranthene and indeno(*1,2,3-cd*)pyrene) and seven (acenaphthene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, indeno(*1,2,3-cd*)perylene, dibenz(*a,h*)anthracene and benzo(*g,h,i*) perylene) PAHs were not detected respectively. Most of the non-detected PAHs (in fish from Yzerfontein and Struis Bay) were predominantly HMW PAHs which usually emanate from incomplete combustion of organic substances. This trend in low detection of HMW and high detection of LMW PAHs in fish has been observed in previous published studies (Moraleda-Cibirán *et al.*, 2015; Xu *et al.*, 2011; Wang *et al.*, 2010; Perugini *et al.,* 2007) and may be due to uptake pathways and longevity in the body (Gert-Jan de Maagd & Vethaak, 1998). HMW PAHs are taken up predominantly through diet whilst LMW PAHs are mostly accumulated through water (Zhao *et al.*, 2014). Furthermore, HMW PAHs can be metabolized by some large fish and be excreted (Ramesh *et al.*, 2004) thereby reducing accumulation and concentration (retention) in the fish body. A summary of yellowtail PAHs concentrations (mean, S. E and range) from the three study location were given in Table 5.2.

The most abundant PAHs were naphthalene, phenanthrene and anthracene (in descending order), where the highest concentration of naphthalene (137.33 ± 15.17 µg/kg) was found in fish sampled from Port Elizabeth. The dominance of naphthalene in fish tissue was previously observed in *Thunnus albacares* (Chapter three) whilst a study on global PAHs emission observed that naphthalene's was the overall dominant PAH, often present at up to 50% more than other PAHs present (Zhang & Tao., 2009). Naphthalene is predominantly derived from anthropogenic sources such as oil and gas drilling, crude oil, petroleum refining and bitumen among others (*Skupiŋska et al*., 2004; Lee *et a*l., 2010). Therefore, the high abundance of naphthalene relative to other PAHs may be due to its high input into the environment through industrial and domestic emissions (Zhang & Tao, 2009), in particular petroleum sources (Karlsson & Viklander, 2008). In addition, the higher concentrations of LMW PAHs such naphthalene, acenaphthylene, phenanthrene, fluorene and anthracene in fish may also be attributed to their higher solubility in water compared to HMW PAHs (Brown & Peake**,** 2006). Therefore, LMW PAHs are readily bioavailable through fish gill and skin diffusion whereas the HMW PAHs attach to particles and sediment and must be consumed through diet (Gert-Jan deMaagd & Vethaak, 1998; Brown & Peake**,** 2006; Karlsson & Viklander, 2008).

Table 5.2. Concentration (mean and range) in µg/kg wet weight of persistent organic contaminants (OCs): polycyclic aromatic hydrocarbons (PAHs), dichlorodiphenyltrichloroethane (DDT) and organochlorine pesticides (OCPs) detected in yellowtail species (*Seriola lalandi*) sampled from Yzerfontein, Port Elizabeth and Struis Bay, coast of South Africa. Different alphabet letter(s) on the same row denote significant difference (p<0.05).

Location	Yzerfontein		Port Elizabeth		Struis Bay	
Compound	Mean ± S.E	Range	Mean ± S.E	Range	Mean ± S.E	Range
Naphthalene	137.33**a** ± 15.17	55.75-244.09	132.47**a** ± 8.41	93.98-163.87	84.59**b** ± 3.41	68.21-100.63
Acenaphthylene	3.05**a** ± 0.13	2.30-3.77	17.84**b** ± 0.53	16.44-21.21	0.74**c** ± 0.07	0.18-1.03
Fluorene	10.40**b** ± 2.36	5.01-30.97	29.33**a** ± 2.18	24.31-42.31	0.05**c** ± 0.03	<dl-0.31
Phenanthrene	16.02**b** ± 3.44	9.70-45.08	52.54**a** ± 2.27	48.56-72.15	0.24**c** ± 0.15	<dl-0.45
Anthracene	10.09**b** ±0.24	8.99-11.56	32.68**a** ± 0.7	31.01-37.90	2.95**c** ± 2.02	<dl-17.90
Fluoranthene	10.66**a** ± 0.06	10.44-10.89	7.21**b** ± 2.02	<dl-24.59	0.09**c** ± 0.05	<dl-0.41
Pyrene	23.60**b** ± 16.77	5.70-174.51	28.42**a** ± 2.07	26.09-47.04	0.05**c** ± 0.03	<dl-0.25
Benz(*a*)pyrene	6.16 ± 0.92	4.10 -10.10	29.35 ± 2.38	17.33-38.04	0.25**c**± 0.07	<dl-0.62
Dibenz(*a,h*)anthracene	1.69 ± 0.70	<dl-4.97	24.13± 4.58	<dl-49.59	ND	ND
Benz (*g,h,i*)perylene	2.39 ± 0.98	<dl-6.05	5.13 ± 5.13	<dl-51.32	ND	ND
DDT	11.14 ± 1.44	2.03-16.55	7.48 ± 5.18	0.45- 53.93	ND	
BHC beta	0.26 ± 0.14	<dl-1.22	1.08 ± 0.31	<dl-2.54	ND	
BHC gamma	0.28 ± 0.19	<dl-1.68	1.94 ± 0.23	<dl-2.86	ND	
Endrin	0.40 ± 0.11	<dl-1.01	1.29 ± 0.52	<dl-5.30	ND	

ND- not detected; Values: mean ± standard error of mean

In general, significant variations were observed in the mean concentration of total PAHs in fish from the three locations where yellowtail fish from Port Elizabeth had higher ($p<0.05$) PAHs concentration (533.95 ± 34.36 µg/kg) compared to Yzerfontein (221.40 ± 33.03 µg/kg) and Struis Bay (52.54 ± 2.27 µg/kg). In view of the associated toxic and carcinogenic effects of PAHs caused by binding of PAHs (breakdown products) to human genetic cells, the elevated PAHs burden in fish particularly from Port Elizabeth is of health interest and need further investigation. The fish sampled from Port Elizabeth, which is considered an industrial area (Moore & Breetzke, 2013), had slightly higher percentage of HMW PAHs (50.39%) in comparison to LMW PAHs (49.61%) (Fig. 5.1) but not significantly different (Fig 5.2) in terms of concentration. The PAHs input sources were both from petrogenic as well as pyrogenic which may be a reflection of activities at the harbours, ports and the accumulation of fumes into the marine environment from automobile industries. In addition, where the ratio of LMW/HMW is <1, pyrogenic sources are indicated as the main contributors (Brown & Peake, 2006). The significant differences observed between the total LMW PAHs and total HMW PAHs (Yzerfontein and Struis Bay) shown in Fig. 5.3, reflects the dominance of LMW over the HMW in these locations. However, fish sampled from Port Elizabeth had an average ratio of LMW/HMW as 0.984 whilst some individual fish had a ratio >1 which suggests that mixed contamination from both petroleum and combustion inputs with a slight dominance of pyrogenic sources. According to Zhang & Tao (2009), the spatial variations in PAHs concentrations is a reflection of emission source and quantity.
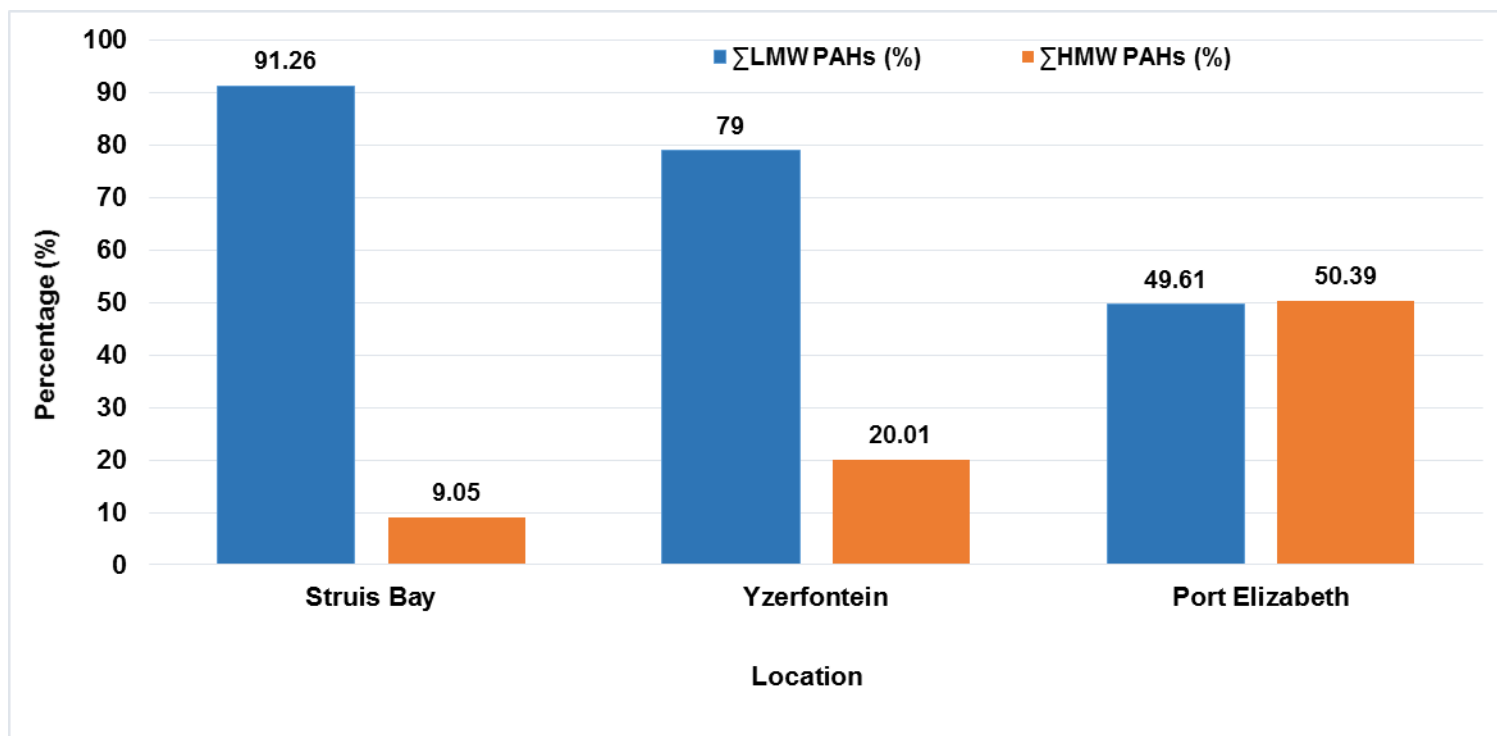
Figure 5.1. Percentage distribution of low molecular weight (LMW) and high molecular weight (HMW) PAHs in yellowtail (*Seriola lalandi*) fish species from Struis Bay, Yzerfontein and Port Elizabeth along the coast of South Africa.
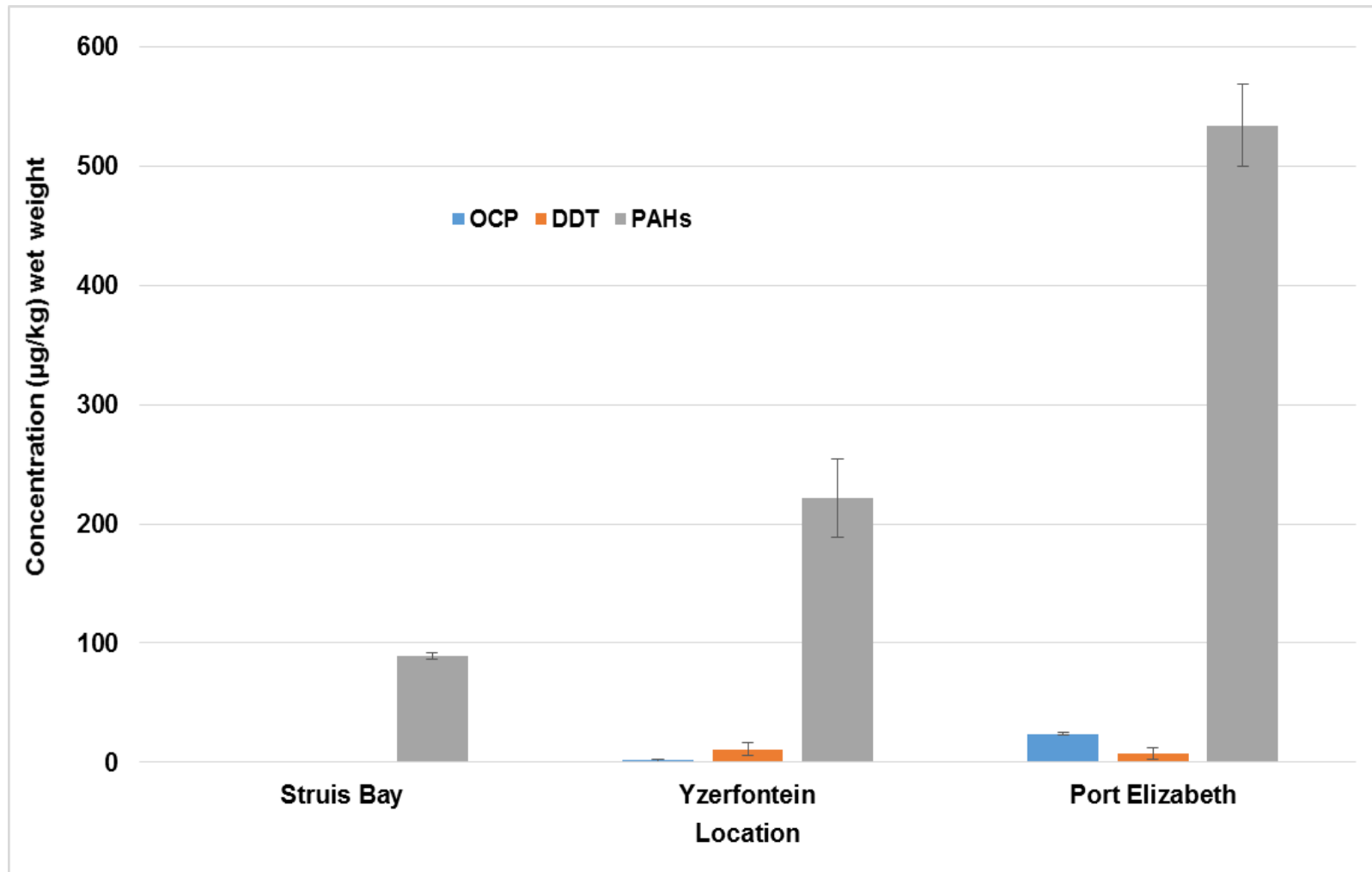
Fig.5.2. Total concentration (mean ± SE) of persistent organic contaminants (ΣOCPs, ΣDDT and ΣPAHs) in yellowtail (*Seriola lalandi*) from Struis Bay, Yzerfontein and Port Elizabeth along the coast of South Africa.
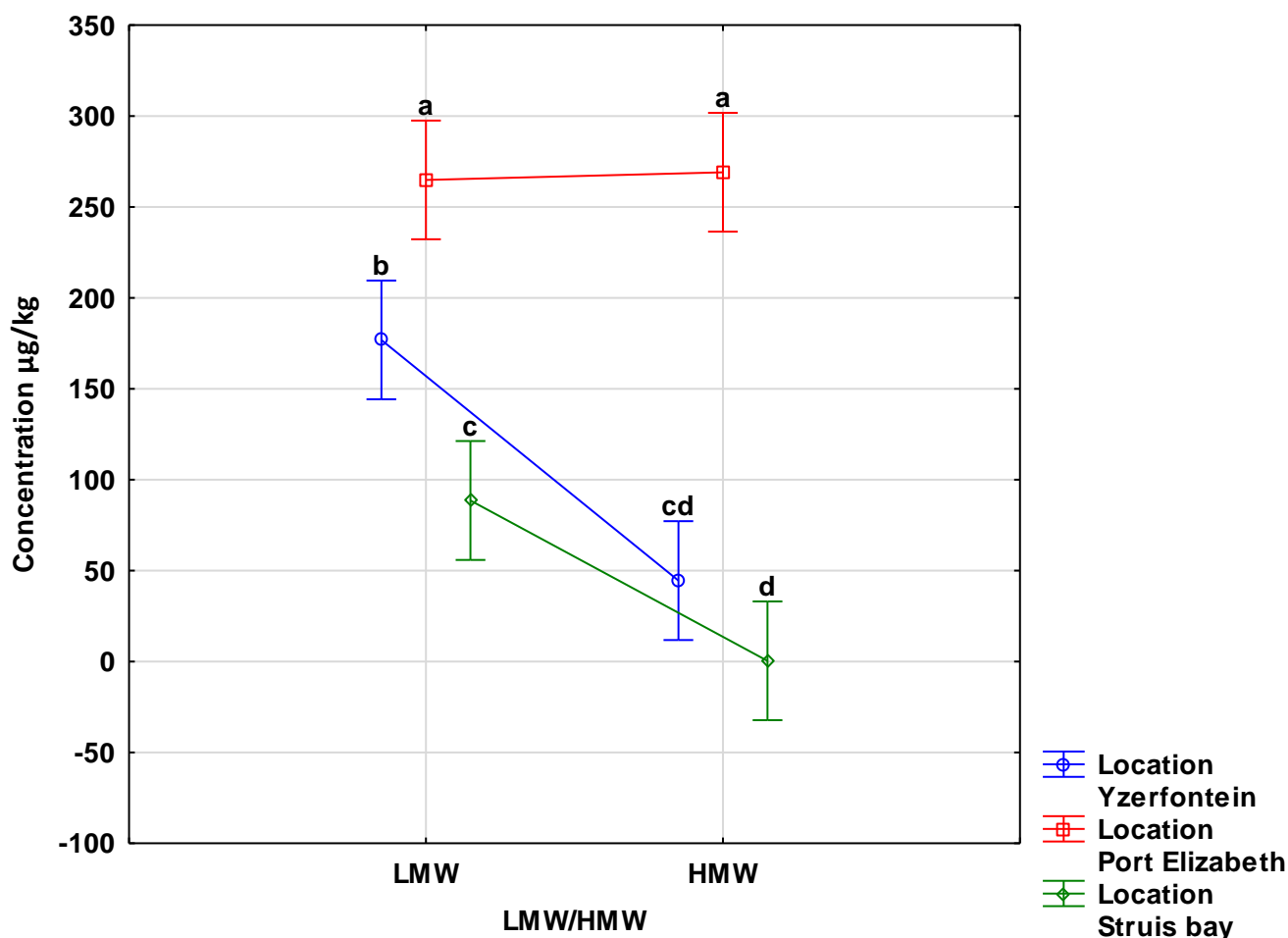
Figure 5.3. Distribution of Low molecular weight and high molecular weight PAHs in yellowtail across three study locations highlighting significant differences (p<0.05) with different alphabets letters

The non-biological SPMD in contrast to the biological transplanted object (mussel) accumulated more HMW PAHs such as chrysene, benzo(*b*) fluoranthene and benzo(*k*) fluoranthene which except for benzo(*k*) fluoranthene were also amongst the PAHs detected in fish sampled from Port Elizabeth (in this study). Benz(*a*)pyrene the PAHs acceptability indicator was found to exceed the EU maximum limit of 2 µg/kg ww in all the samples from Port Elizabeth and Yzerfontein (28.42 ± 2.07 and 6.16 ± 0.92 respectively). Fish sampled from Struis Bay, (rarely contaminated with the assessed OCs) had benz(*a*)pyrene concentration of 0.25 ± 0.07 µg/kg ww which was less than the EU limit. The exceeding of the benz(*a*)pyrene EU limit in fish is considered a health concern and as the study is baseline, further investigation would be needed to generate enough evidence for advisory purposes. Similar reports of exceeding the EU limit in benzo(*a*)pyrene were also observed in marine fish species sampled

from Ghana (Bandowe *et al.*, 2014), Nigeria (Anyakora *et al.*, 2005), Egypt (Said, 2007). The metabolites of carcinogenic PAHs such as benzo(*a*)pyrene can form complexes by binding with cellular DNA and can alter genetic sequence and consequently can promote cancer (Peltonen & Dipple, 1995 as cited by Al-Saleh & Al-Doush, 2002). Owing to the associated carcinogenic effects of benzo(*a*)pyrene considered as one of the most potent PAHs (*Skupińska et al.*, 2004), there is need to protect fish consumers from dietary exposure of fish with increased benzo(*a*)pyrene burden by avoiding fish species identified as benzo(*a*)pyrene accumulators.

Contamination of fish with PAHs according to Baumard *et al.* (1998) can be classified based on measured concentrations into: low contamination (0-100); moderately contaminated (100-1000); highly contaminated (1000-5000) and very highly contaminated if greater than 5000 ng/g. Thus based on this classification the fish sampled from Port Elizabeth and Yzerfontein could be said to be moderately contaminated (100-1000 µg/kg) with PAHs while fish from Struis Bay had low contamination with PAHs. The uptake of PAHs and distribution of PAHs in fish can be affected by a number of factors such as fish anatomical section (Cheung *et al.*, 2007), species (Du *et al.,* 2012; Gomes *et al.*, 2013; Zhou *et al.,* 2013), size (Domingo, 2007), gender (Bodiguel *et al.*, 2009) and biotransformation ability (Borgå *et al.,* 2004). For instance variation in PAHs profiles and concentrations were observed in different muscle portions (dark and light) of *T. albacares* fish (chapter 3). Also anatomical variations in ventral and axial muscles of marine and freshwater species were reported by Cheung *et al.* (2007). Therefore choice of fish portion, size and species among other factors can increase or reduce human dietary exposure to PAHs. Thus consumers can make better informed decision on selection of fish.

5.3.3 DDTs and OCPs in *S. lalandi*

DDT was detected in Yzerfontein and Port Elizabeth and not detected in all samples from Struis Bay. Struis Bay an old fishing harbour in Western Cape, South Africa (DAFF, 2010) is a settlement for peasant dwellers (Anon, 2015) and not with dense population and industrial activities as Yzerfontein and Port Elizabeth probably do not have application of DDT in the area. The use of DDT as anti-fouling agent in paint used for undecked boat maintenance (Li *et al.* 2006 as in Zhang *et al.*, 2013; Qui *et al.*, 2009) and agricultural practices that may involve the use of Dicofol pesticides, synthesised from DDT (De la Cal *et al.*, 2008) which can increase environmental DDT (other than from control of malaria) may not be common practices in Struis Bay. These may explain the low level of OCs and in particular the non-detection of DDT in the fish sampled from Struis Bay.

The more stable DDT analogues (DDE and DDD) were below detection limit in all the sampled fish from all the three locations which indicated their low concentrations and also the non-degradation of the fresh DDT input. Overall, the total concentration of 4,4'-DDT (the only analogue detected) was significantly higher ($p<0.05$) in Yzerfontein compared to Port Elizabeth (Fig 5.3). The predominance of DDT (4,4'-DDT analogue) was an indication of fresh input or current DDT usage in the environment (Eqani *et al.*, 2013), where a greater usage of DDT may have occurred in Yzerfontein than the other locations. The higher concentration of DDT in fish from Yzerfontein (an indication of higher DDT pollution) may be a reflection of increased application of antifouling DDT containing paints in the care of undecked fishing boats. Yzerfontein has more fishing activities than other locations and is reported to provide about 60 % of line fishes in the western coast (Anon, 2015b)

The spatial transferability of OCs such as DDT can result to detection even in places/matrices remote from points of production, use or emission. For instance in a study by Channa *et al.* (2012) on prenatal exposure to DDT in malaria and non-malaria regions of South Africa, DDT was equally detected among women from the non-malaria zone though at a lower concentrations than the malaria endemic regions. Again, the detection of DDT in fish especially non-resident species may not provide actual environmental pollution history. Despite the presence of DDT in yellowtail from Yzerfontein and Port Elizabeth, the concentrations were considerably lower than the Food and Drug Administration (FDA) action level of 5000 µg/kg (legal level of confiscation) (US EPA, 2000). This suggests that yellowtail caught in the three study locations are considered acceptable for human consumption with respect to DDT.

The other pesticides detected in fish from Port Elizabeth were BHCs (beta and gamma analogues), endrin and endosulfan (5.1 & 5.2). BHCs (beta, delta and gamma analogues), endrin and eldrin aldehyde were detected in fish sampled from Yzerfontein (Table 5.2) and none from Struis Bay. The concentrations of all detected pesticides excluding DDT were summed up as total OCPs (∑OCPs) where the concentration of ∑OCPs in fish from Port Elizabeth was ($p<0.5$) higher than fish from Yzerfontein. The detection of these pesticides, except for DDT which is currently being used in the country, may reflect their persistence, illegal application and possibly as metabolites from industrial incineration (Ameur *et al.*, 2013). A list of banned pesticides in South Africa include aldrin, endrin, mirex, chlordane and heptachlor (Naidoo & Buckley, 2003), therefore the detection of endosulfan and BHCs though on global list of banned pesticides but not included in banned list in South Africa and could still be in use. This calls for further investigation in order to conform to the Stockholm treaty of which South Africa is a signatory. Nevertheless the levels of the studied pesticides in all the

sampled fish from the locations under investigation were below the EU maximum residue levels (MRLs) for pesticides in the range of 50-300 µg/kg ww (US EPA, 2000).

5.3.4 Relationship between fish size (weight), lipid content and OCs concentrations

Yellowtail (n=20) size (weight) had a strong positive correlation with lipid content (r=0.65); p< 0.01 (Table 5.3). The increased lipid content with body weight could be a reflection of growth variation where older fish accumulate more lipid as energy reserve for migration and reproduction (Couderc *et al.*, 2015). Thus larger fish with increased surface area (intracellular and extracellular muscles which contribute to overall weight) would be expected to have more oil than smaller sized fish. Thus lipid content in fish could be associated with growth rate (a function of age, weight and length) (Covaci *et al.*, 2006). A strong negative correlation (r=0.67; p<0.001) between lipid content of fish and size (length) was observed in fish species (e.g. *Carasius auratus gibelio* and *Abramis brama*) from Romania, though the study did not determine relationship with weight (Covaci *et al.*, 2006). However, information regarding the relationship between fish size (weight) and lipid content is limited for extensive comparison.

In this study, a strong positive correlation was observed with fish weight and total PAHs (r=0.83; p<0.01) as well as some individual carcinogenic PAHs benzo(*a*)pyrene, (r=0.81; p<0.01), anthracene (r=0.78; p<0.01) dibenz(*a,h*)anthracene (r=0.77; p<0.01) (Table 5.3). Similar to increased lipid content in older fish, the lipophilic nature of PAHs leads to their binding and accumulating in fatty matrices. Thus higher lipid content of investigated species will imply increase in the accumulation of PAHs particularly the carcinogenic PAHs. The positive correlation existing between these PAHs and fish weight may be as a function of prolonged exposure spanning over period of juvenile to adult stage (maturity) and in addition to larger surface area in adult fish. Similar result of positive correlation (r=0.99; P<0.05) with total PAHs and fish weight was observed in *Siganus rivulatus* from Lebanon (Barbour *et al.*, 2008), with chub and horse mackerel (Ramalhosa *et al.*, 2012). In any case fish species effect cannot be ruled out as playing significant role to these interactions of fish parameters and contaminant. As different species vary in their degree of enzymatic breakdown which may give rise to products that be excreted and consequently affect the residual level of contaminant. In view of this, the observed relationship was not definitive and may require further investigation and comparison with different species may be a misleading interpretation. For instance, Perugini *et al.* (2007) observed no significant correlation with total PAHs and weight, length and trophic levels of evaluated marine species (e.g. *Merluccius merluccuius* and *Scomber scombrus*).

Table 5.3. Significant correlations (positive) of fish weight to fish lipid content, organic contaminants (PAHs and OCPs) detected in yellowtail fish (*Seriola lalandi*)

Variable 1	Variable 2	Correlation value (Pearson)	p-value
Fish weight	Lipid content	0.65	<0.01
Fish weight	Acenaphthylene	0.87	<0.01
Fish weight	Fluorene	0.69	<0.01
Fish weight	Phenanthrene	0.74	<0.01
Fish weight	Anthracene	0.78	<0.01
Fish weight	Benz(*a*)pyrene	0.81	<0.01
Fish weight	Dibenz(*a*)A	0.77	<0.01
Fish weight	Total PAHs	0.83	<0.01
Fish weight	BHC beta	0.51	<0.01
Fish weight	BHC gamma	0.80	<0.01
Fish weight	∑BHC	0.71	<0.01
Fish weight	∑OCPs	0.64	<0.01

Refer to list of acronyms

5.4. Conclusion.

The variations in concentrations of evaluated contaminants in the fish sampled from the three locations (Yzerfontein, Port Elizabeth and Struis Bay) were attributed to fish size, lipid content and locational effects. The study revealed that fish from Port Elizabeth (despite the size and lipid content) had the highest contamination of PAHs and pesticides. This was followed by Yzerfontein whilst fish from Struis Bay had the least contamination. The pollution input sources of PAHs from Port Elizabeth showed a mixture of petrogenic and pyrogenic possibly due to industrial and harbour activities. Whilst from Yzerfontein and Struis Bay both locations revealed input as predominantly petrogenic sources which may be due to refineries and shipping activities within the province. The bioconcentration of OCs particularly the carcinogenic (HMW) PAHs and OCPs (DDT and BHCs) revealed increased concentration with fish size. This implication may need further investigation for development of a possible fish weight-OCs predictable model which will be beneficial in determining the critical size of yellowtail above which consumption might be harmful. Overall, all detected OCPs (DDT and BHCs) were below the EU limits and therefore designated acceptable for human consumption in all sites sampled; however, Benz(*a*)pyrene the PAH acceptable marker was found to exceed the EU maximum limit of 2 µg/kg ww for yellowtail sampled in Port Elizabeth and Yzerfontein.

Therefore, fish consumers in particular, food (fish) regulatory agencies and those in fish business (fishing & industries) need an increased awareness of current prevalence and composition of these chemical contaminants in these locally consumed and commercially important fish species. Owing to the prevalence of some of the banned pesticides, a greater monitoring and control of product distribution and use is needed to prevent further environmental contamination.

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CHAPTER 6

Comparative evaluation of PAHs and pesticides levels in three different shark species from the coast of South Africa: Blue sharks (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*) and smoothhound (*Mustelus mustelus*).

Abstract

Sharks are becoming an important marine species for human consumption in many parts of the world. As a consequence, the export/import trade of shark meat is increasing, from South Africa to places like Australia. However, sharks by their predatory nature can bioaccumulate toxic and carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAHs), dichlorodiphenyltrichloroethane (DDT) and other organochlorine pesticides (OCPs). Therefore, these contaminants were assessed in three commercially important shark species: Blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*) and smoothhound (*Mustelus mustelus*) from the coast of South Africa. Simultaneous extraction of target compounds was done based on QuEChERS method and analysed with GC-MS/MS. The study revealed similar uptake of all the contaminants in the studied sharks as no significant variation ($p \geq 0.05$) was observed in all the compounds analysed (though blue shark had relatively higher concentrations of all the contaminants). The only detected DDT analogue was 4-4´DDT with average concentration in the sharks below the FDA recommended action level of 5000 µg /kg. The predominance of 4-4´DDT analogue is an indication of fresh DDT input. Ratio of low molecular weight PAHs to high molecular weight PAHs was > 1 in all the sharks, which indicated input source as petrogenic. The benzo(*a*)pyrene, PAHs acceptability indicator exceeded the European Union (EU) recommended acceptable limit of 2 µg/kg wet weight in all the analysed sharks and this could be of health concern and calls for risk investigation.

Keywords: Sharks, South Africa, marine, organic contaminants, PAHs, DDT, health-concern

6.1. Introduction

Global monitoring and awareness of hazardous compounds have increased after the United Nations (UN) 2001 convention on persistent organic pollutants (POPs) in Stockholm, Sweden (UNEP, 2005). These hazardous compounds were earmarked for global eradication, restricted usage or control based on their prevalence, toxicity, carcinogenic effects, ability to accumulate and biomagnify in food web (ASTDR, 2002; UNEP, 2005). Similarly, persistent bioaccumulative toxic substances (PBTs) have been listed by United States Environmental Protection Agency (US-EPA, 1998) for global monitoring and eradication.

Some of these priority compounds include polycyclic aromatic hydrocarbons (PAHs), organochlorinated pesticides (OCPs) such as dichlorodiphenyltrichloroethane (DDT), benzenehexachloride (BHC), endosulfan, aldrin, endrin and chlordane among others. While PAHs can occur naturally as constituents of crude oil, bitumen, coal or produced from incomplete combustion of organic materials, OCPs are basically man-made chemicals. These organic compounds can therefore be released at various points, from production, utilization and disposal can become prevalent in the environment (DAFF, 2010). DDT was previously used as agro pesticide until its global ban as agro pesticide but restricted to health sector for control of disease vectors (WHO, 2011). Presently, DDT is used as an indoor residual spray (IRS) for control of malaria vector in malaria endemic zones Limpopo, Mpumalanga and KwaZulu-Natal Provinces of South Africa (Wells & Leonard, 2006) and so can prevail in these locations and beyond via spatial transfer.

A number of published studies within South Africa have confirmed the prevalence of DDT and metabolites in various matrices such as air (Batterman *et al.,* 2008; Naude & Rohwer, 2012); human breast milk (Bouwman *et al.*, 2006; Bouwman *et al.*, 2012), surface water and sediment (Sibali *et al.*, 2009) and freshwater fish (McHugh *et al.*, 2011). DDT is considered the most widely studied and detected pesticide in South African freshwater systems (Ansara-ross *et al.*, 2012); however little research exists for DDT prevalence in marine species. PAHs on the other hand, due to its ubiquitous nature have been considered a major threat to the entire ecosystem. PAHs entry into the marine environment can be through atmospheric emissions especially for pyrogenic (combustion) PAHs or through water current as seeps, spills or run offs (petrogenic) PAHs.

The South African coastline is a very busy shipping route and thus has witnessed numerous oil spills and seeps (Donoghue & Marshall, 2003; Moldan & Jackson, 2005). Also numerous industries are located around the coast such as petroleum refineries, automobiles, ports and harbours. In addition emissions from domestic, municipal and industrial incineration (Al-Saleh & Al-Doush, 2002; UNEP, 2006; Dong *et al.*, 2012), are all possible sources of PAHs contamination. PAHs have been detected in numerous coastal harbours of various commercial and recreational activities (Cape Town, Port Elizabeth, Richards Bay, Saldanha Bay and Tsitsikamma) (Degger *et al.*, 2011) which highlights the large scale prevalence of PAH contaminants.

Human exposure to these environmental contaminants can be through direct inhalation but the major exposure route is via the diet (Guo *et al.*, 2010; Van Dyk *et al.*, 2010). Fish and in particular marine species contribute significantly to human dietary exposure to these contaminats (Domingo, 2007; FSAI, 2013). Although fish are excellent sources of

essential micro and macro nutrients, the frequent and prolonged consumption of contaminated fish may hamper the health benefits obtained (Ugoala *et al.*, 2009; Usydus *et al.*, 2009; FAO, 2011). Some of the ingested contaminants can cause health defects such as reproductive and developmental retardation (de Jager *et al.*, 2009); infertility, low birth weight, miscarriage, (Dalvie, *et al.*, 2004; Borman *et al.*, 2009); immune suppression, neurological impairment and more (WHO/IARC, 1999; ASTDR 2002).

The assessment and monitoring of contaminants in target species such as commonly consumed or commercially important species, bottom feeders or predatory species has been suggested by the US EPA, (2000). In line with the EPA guideline, shark species which are predatory and of commercial importance were screened for selected contaminants in the current study. The species assessed were: blue shark (*Prionace glauca)*, shortfin mako shark (*Isurus oxyrinchus*) and smoothhound (*Mustelus mustelus*) (Da Silva & Bürgener, 2007). In South Africa there are over 180 sharks species (inclusive of other elasmobranchs) caught from different commercial and recreational fishery ports with an annual average landing estimated at 4 000 tonnes (DAFF, 2010). Sharks are generally not considered a commonly consumed species in South Africa but are commercially important due to the growing export trade to Australia (Da Silva & Bürgener**,** 2007), Italy and Uruguay while sharks fins are also exported to countries such as Japan (DAFF, 2010).

Information on contaminants (DDTs, PAHs and OCPs) in marine fish species and sharks is limited from South Africa (Wepener & Degger, 2012; Degger *et al.*, 2012). However, recently, Bosch *et al.* (2016) reported heavy metal contamination levels in smoothhound shark species within South Africa and found that approximately 37% of the studied sharks contained total mercury level above the maximum limit. Thus the aim of the study was to evaluate and compare the levels of contaminants (PAHs, DDTs and OCPs) in three different commercially important sharks from South African coastline determine its acceptability for consumption based on the regulatory requirements.

6.2. Materials and methods

6.2.1. Sampling and Sample preparation.

Three different shark species (*P. glauca*, *I. oxyrinchus* and *M. mustelus*) were harvested from different locations but on different dates per species by local line fishermen in collaboration with the South African Department of Agriculture, Forestry and Fisheries (DAFF). *P. glauca* (n=5) was sampled from Port Elizabeth (33.9581° S, 25.6000° E) in the Eastern Cape, *I. oxyrinchus* (n=10) from Hout Bay (34.0333° S, 18.3500° E) and *M. mustelus* (n=10) from Langebaan (33.0917° S, 18.0333° E) both in the Western Cape of South Africa. The

biometric measurements (weights and lengths) for the studied species were taken with the exception of *I. oxyrinchus'* weight (were received from DAFF already filleted). Figure 6.1 summarises the various measurements taken for each species. Each fish (*P. glauca* and *M. mustelus*) was de-headed, de-gutted, skinned and dissected (fillets) then rinsed under clear running tap water. Homogenized samples were then vacuum packed and stored at - 20°C prior to the chemical analyses.

Table 6.1. Biometric measurements of Mako (*Isurus oxyrinchus*), blue (*Prionace glauca)* and smoothhound (*Mustelus mustelus*) sampled from different locations along the coast of South Africa evaluated for levels and profiles of organic contaminants (PAHs and pesticides).

Sample ID	Location	Common name	Weight (kg)	Total length (cm)	Gender
MCF1	Cape St Francis	Mako shark	MD	182.0	female
MCF2	Cape St Francis	Mako shark	MD	196.0	male
MCF3	Cape St Francis	Mako shark	MD	210.0	female
MCF4	Cape St Francis	Mako shark	MD	196.0	female
MCF5	Cape St Francis	Mako shark	MD	207.0	male
MCF6	Cape St Francis	Mako shark	MD	186.0	male
MCF7	Cape St Francis	Mako shark	MD	186.0	male
MCF8	Cape St Francis	Mako shark	MD	205.0	female
MCF9	Cape St Francis	Mako shark	MD	202.0	female
MCF10	Cape St Francis	Mako shark	MD	166.0	MD
BHB1	Hout Bay	Blue shark	6.45	124.0	male
BHB2	Hout Bay	Blue shark	6.20	126.0	female
BHB3	Hout Bay	Blue shark	4.35	108.0	female
BHB4	Hout Bay	Blue shark	11.10	142.5	female
BHB5	Hout Bay	Blue shark	16.85	125.0	female
SHS 10	Langebaan	Smoothound	20.00	149.4	female
SHS 20	Langebaan	Smoothound	16.68	160.3	female
SHS 23	Langebaan	Smoothound	1.86	80.1	female
SHS 38	Langebaan	Smoothound	6.80	122.2	female
SHS 45	Langebaan	Smoothound	16.60	149.9	female
SHS 46	Langebaan	Smoothound	24.60	165.2	female
SHS 49	Langebaan	Smoothound	4.50	113.4	male
SHS 53	Langebaan	Smoothound	7.40	112.0	female
SHS 63	Langebaan	Smoothound	10.90	139.0	female
SHS 64	Langebaan	Smoothound	15.40	149.6	female

MD- missing data

6.2.2. Extraction and analytical procedures

The method of extraction and instrumental analysis were as previously described in chapter 3. The target analytes extracted and analysed were 16 United States Environmental Protection Agency (US EPA) priority PAHs: Naphthalene (Nap), acenaphthylene (Acy), acenaphthelene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fluo), chrysene (Chr), pyrene (Pyr), benzo(*a*)anthracene (BaA), benzo(*b*)fluoranthene (BbF), benzo(*k*)fluoranthene (B*k*F), benzo(*a*)pyrene (BaP), dibenzo(*a,h*)anthracene (BahA), indeno(*1,2,3-cd*)pyrene (IP), benzo(*g,h,i*)perylene.

Pesticides: Dichlorodiphenyltrichloroethane (DDT), Dichlorodiphenyldichloroethyne (DDE) and Dichlorodiphenyldichloroethane (DDD), aldrin, endrin, dieldrin, endosulfan (alpha & beta), benzenehexachloride (BHC) alpha, beta, delta and gamma analogues, heptachlor, endosulfan sulfate and eldrin aldehyde.

6.2.3. Statistical analysis

All data generated were tested for normality and homogeneity with Kolmogorov-Smirnov and Levene's F tests respectively and were found to be normally distributed and in conformity to homogeneity test. Differences in the concentration of contaminants (16 US EPA PAHs, DDTs, BHCs and OCPs) in the three sharks were statistically analysed using one-way analysis of variance (ANOVA) (Statistica version 12.0, Statsoft Inc., USA, 2012) and Post-hoc separation of means with significant differences at 95% confidence level ($p < 0.05$) was established. Analytes below detection limit were treated as not detected and excluded from statistical analysis.

6.3. Results and Discussion

6.3.1. Biometric measurements

The Blue and Smoothhound sharks' average weights were 8.99 ± 2.26 kg and 1.41 ± 0.21 kg respectively. The mean length for Blue, shortfin Mako and Smoothhound sharks were 125.10 ± 5.46 cm; 193.60 ± 4.31 cm and 134.11 ± 8.42 cm respectively; no significant variation (p≥0.05) between Blue and Smoothhound sharks was found but both Blue and Smoothhound sharks were significantly (p<0.05) shorter than the Mako shark sampled. Similar variation was reported in comparable species in Korea where the average length and weight of Blue, Mako and Smoothhound sharks was 112 ± 20 cm and 22 ± 8.2 kg, 118 ± 9.8 cm and 44 ± 26 kg, 60 cm (no indication of Standard deviation) and 1.6 ± 0.8 kg respectively (Lee *et al.*, 2015). Based on size, the Blue, Mako and Smoothhound sharks assessed in the current study are therefore considered equally mature adult sharks to those examined by Lee *et al.* (2015).Their study

assessed persistent organochlorines in 13 different shark species (including Blue, Mako and Smoothhound) and considering the comparable sizes between studies, inter comparison of OCPs (PAHs not inclusive) is possible. The individual length of all the studied sharks is outlined in Table 6.1.

6.3.2. Organochlorine pesticides (OCPs) concentration and inter-species comparison in blue, shortfin mako and smoothhound sharks.

DDT concentration in all the analysed sharks (muscles) ranged from 19.07-23.06 µg/kg ww. DDT and metabolites (DDD and DDE) were evaluated but only the 4,4´-DDT analogue was detected in measurable levels (above detection limits); therefore only 4,4´-DDT was DDT concentration. The concentration of total DDT was not significantly (p≥0.05) different in the three sharks muscles evaluated. The Blue and Mako sharks have similar habitat (pelagic sharks) and but similar feeding strategies (aggressive carnivores) with smoothhound (Lee et al., 2015; Shark Trust, 2010), leading to similar uptake mechanism, which may expalin the non-significant in DDT levels between species.

Baseline data on DDT level in marine fish species from South Africa was conducted more than four decades ago by Aucamp *et al.* (1971) which reported a DDT range of 2 µg/kg (muscle) -141000 µg/kg (organ) of snoek species (*Thyrsites atun*). Upon comparison, the current study showed evidence of increased DDT level in shark muscle in comparison to the baseline (snoek). A number of biological factors (the different species feeding habits, season, sex, size and fat content) may have contributed to the elevated levels in current study. Furthermore, the re-introduction of DDT in the country from 2002 after the initial global ban in early 70's (Wells & Leonard, 2006) and other sources of DDT into the environment other than for malaria control are all possible sources to the increased concentration recorded in this study. For instance Dicofol synthesised from DDT is an approved agro pesticide containing impurities of DDT as ingredient (De la Cal *et al.*, 2008) and is in widespread use in South Africa (Quinn *et al.*, 2011) and via run off can enter the marine environment. Again the use of antifouling paint is still widely used for maintaining undecked fishing boats in the coastal areas (Li *et al.*, 2006; UNEP, 2006) can continually increase the DDT in the aquatic environment. The levels of DDT in the studied shark species do not imply locational effect (places they were harvested from) due to their migratory nature and so do not reflect input as precisely from areas caught.

The detection of 4,4-´DDT in the shark samples (as the only detected analogue) was considered an indication of fresh input of DDT in the environment (Naso *et al.*, 2005; Coelhan *et al.*, 2006). The level of DDT from this study was in agreement with the range reported by

Zhou *et al.* (2013). In their study on the sources and distribution of organochlorine pesticides DDTs, hexachlorocyclohexane (HCH) in dog shark (*Musteleus griseus*), from China (Zhoushan fishing ground), DDTs ranged from 7.27-26.62 ng/g ww, while HCH ranged 2.67-3.35 and chlordanes was 0.54-0.61. In this study, endrin was the only pesticide within detectable limit in the three sharks at no significant difference ($p > 0.05$) but with relatively the Blue shark had the highest concentration (7.32 ± 1.31). The low concentration of endrin found in the sharks (which is below maximum residue level) could reflect its low input into the marine system.

Despite the fact that South Africa formulates DDT (WHO, 2011) for distribution to other African countries and its current use as indoor residual spray (IRS) in malaria endemic areas such as the KwaZulu Natal and Limpopo provinces (Jaga & Dharmani, 2003; Channa *et al.*, 2012), levels found in studied sharks were within FDA action level (threshold value) of 5000 µg/kg ww to warrant any health implication. In a previous study on DDT in yellowfin tuna dark and light muscle (chapter 3) harvested from False Bay, the average concentration of total DDT was significantly higher than the observed concentration from sharks (current study). These variations could be attributed to a number of factors such as species specific (Gomes *et al.*, 2013), habitat and/or trophic level (Kong *et al.,* 2005; Verhaert *et al.,* 2013). In order to compare the current DDT levels detected with those reported in other studies (globally) a summary table has been collated and is presented in Table 6.3

6.3.3 Comparison of PAHs concentration and profiles in the studied sharks (Blue, Mako and Smoothhound)

The average concentration of total PAHs was 508.09 ± 27.10 µg/kg ww, 458.28 ±13.39 µg/kg ww 489.40 ± 16.13. µg/kg ww in Blue Mako and Smoothhound sharks (Fig 6.2). No significant differences ($p \geq 0.05$) were observed in the PAHs concentration between the three studied sharks, though; Blue shark had relatively higher level. The non-significant variation in PAHs levels among the three sharks (as in DDT) could indicate comparable feeding habits (predators) as the majority of PAH are accumulated through food and the external water medium (Zhou *et al.*, 2013; Cascaes *et al.*, 2014). The level of PAHs in fish is greatly affected by the ability of the fish species to enzymatically transform PAHs into by-products that can be excreted or accumulated (Zhao *et al.*, 2014).

The Blue and Mako sharks are pelagic species and feed on preys such as marine birds, squid, and other smaller bony fish (Lee *et al.*, 2015) whilst the Smoothhound can be found mostly inshore around estuaries and shallow bays but has similar feeding habit as blue and mako shark (Shark Trust, 2010). In view of the aggressive predatory nature of these shark

species, they are highly susceptible to high contaminants burdens (Gelsleichter, *et al.*, 2005; Weijs *et al.*, 2015). Therefore, the observed comparable total PAHs in the three sharks concentration may be a reflection of their similar predatory nature and exposure to marine pollution. Though their study evaluated OCPs and not PAHs, however, the uptake of contaminants in the aquatic environment is usually through water and diet (Zhou *et al.*, 2013; Cascaes *et al.*, 2014). Limited (published) information on PAHs level in sharks for comparison with current study. Nonetheless, total PAHs concentration was possible for other predatory species examined within the current dissertation. Soupfin shark (*Galeorhinus galeus*) sampled from Port Elizabeth evaluated for PAHs (chapter 4) had lower mean total PAHs concentration (113.49 ± 4.26 µg/kg ww) while tuna dark muscle (*Thunnus albacares*) from False Bay had higher mean total PAHs concentration (636.61± 36.93 µg/kg ww) than present study.

Benzo(*a*)pyrene is of HMW which are more lipophilic and usually get adsorbed unto particles and are absorbed through dietary intake, bioaccumulate and biomagnify in organisms. Benzo(*a*)pyrene is regarded as the most potent PAH and was found to exceed the European Union (EU) recommended limit of 2 µg/kg ww in all the studied sharks with Blue shark having the highest level of 29.71 ± 0.89 µg/kg ww. Al-Hassan *et al.* (2000) assessed PAHs in various shark species within the Arabian Gulf and found that on average all species had concentration of benz(*a*)pyrene from 4.66-18.5 µg/g ww, which similar to the current study, exceeded the EU recommended limit.

Table 6.2 summarised the PAHs profile detected from the studied shark species which consists of both LMW (toxic) as well as the HMW (carcinogenic) PAHs. The most abundant PAH detected was naphthalene with mean concentrations (in the sharks) in descending order as Blue (153.20 ± 20.90) > Mako (122.13 ± 10.40) >Smoothhound (135.30 ± 10.53) µg/kg ww. The dominance of naphthalene is an indication of petroleum contamination (Boehm, 2005). The profile trend was similar to previous the results of previous chapters in this dissertation (chapters 3 and 4) which emphasises the source of PAHs pollution into the South African marine as to petrogenic (from petroleum products). In addition, the diagnostic ratio of phenanthrene to anthracene (PHE/ANT) and/or that of sum of the LMW/HMW PAHs (which was greater than 1) also signals PAHs input in the marine from petroleum sources (Naso *et al.*, 2005; Coelhan *et al.*, 2006). The abundance of LMW PAHs in fish muscles have been attributed to the low breakdown of LMW and thus longer retention in fish muscles compared to HMW PAHs that are enzymatically metabolized (Gert-Jan de Maagd & Vethaak, 1998). Again the LMW PAHs are readily bioavailable to fish through water ingested through fish gill or skin in comparison to the HMW PAHs available through diet (Brown & Peake, 2006).

On a global comparison of concentration of PAHs in shark species, limited information exists. However, Al-Hassan *et al.* (2000) assessed PAHs and aromatic hydrocarbons (AHs) in different sharks (Table 6.3) from the Arabian Gulf using GC-MS/MS and the result of their study showed a high concentration of PAHs with saw-toothed reef shark having the highest burden of 72.96 µg/g (72 960 µg/kg). However, the liver and gills contributed to this high burden as the level found in the shark muscle was only 1020 µg/kg. Al-Hassan *et al.* (2000) equally reported the dominance of LMW particularly naphthalene in the studied sharks and similar uptake of studied contaminants (PAHs and AHs). This suggests that there was high petroleum (LMW PAHs) input into the areas inhabited by the sharks in the current study and those assessed in the Arabian Gulf (Al-Hassan *et al.*, 2000) irrespective of mobility depth and feeding habit. High PAHs concentrations of 100.2 g/kg (100,200.00 µg/kg) ww and 3862-35,746 µg/kg ww have been reported in marine species from the Niger Delta oil producing area in Nigeria (Anyakora *et al.*, 2005) and the coast of Alexandra, in Egypt (Said, 2007) respectively. These buttress the health hazards which marine fish consumers may be predisposed to and should therefore require further investigation to protect premature death that can arise from the accumulation of toxic and carcinogenic PAHs in human organs and can promote cancer related issues.

In general, no significant variation was observed in the three sharks analysed although they were sampled from different locations. Similar findings were reported by Moraleda-Cibrián *et al.* (2015) who assessed PAHs, PCBs and DDTs in European hake (*Merluccius merluccius*) from seven locations and found no significant variations in all the contaminants' concentration in fish sampled from all the study locations. The non-significant locational variation shows that the assessed species were exposed to similar source of contaminants (Moraleda-Cibrián *et al.*, 2015). On the other hand, Lee *et al.* (2015) in the assessment of contaminants (DDTs, OCPs and polychlorinated biphenyls (PCBs) in 13 sharks from Korea observed variations in contaminants concentration among studied species and locations. Again different factors (age, gender, lipid content and breakdown ability) may interplay to result in the significant variations or otherwise in intra and inter fish species and locations. The more persistent contaminants such as PCBs, with higher degree of chlorination can resist breakdown in tissue and therefore can accumulate more over long exposure (Lee *et al.* 2015). Whereas, PAHs can be readily metabolized in some larger fish by the mixed function oxidase (MFO) enzyme and will be excreted (Ramesh *et al.,* 2004).

Table 6.2. Summary of detected priority PAHs (mean ± S.E; (µg/kg wet weight) in three different sharks (blue shark, mako and Smoothhound) from coastal areas of South Africa analyzed with gas chromatography coupled to mass spectrometry triple quadrupole (GCMS/MS)

Detected PAHs	Number of benzene ring (*PAHs)	Blue Shark (n=5)	Mako shark (n=10)	Smoothhound (n=10)
Naphthalene	2	153.20 ± 20.90	122.13 ± 10.40	135.30 ± 10.53
Acenaphthylene	3	9.99 ± 0.12	10.08 ± 0.08	10.29 ± 0.08
Fluorene	3	19.18 ± 0.66	20.09 ± 0.33	20.10 ± 0.40
Phenanthrene	3	46.37 ± 0.22	46.42 ± 0.16	46.84 ± 0.19
Anthracene	3	33.52 ± 0.21	34.03 ± 0.22	33.75 ± 0.18
Pyrene	4	20.53 ± 5.13	20.56 ± 3.43	23.25 ± 2.59
Chrysene	4	64.97 ± 0.14	64.72 ± 0.06	58.40 ± 6.49
Ben(*b*)fluoranthene	4	45.97 ± 0.86	40.88 ± 4.57	47.72 ± 0.86
Benzo(*a*)pyrene	4	29.71 ± 0.89	26.14 ± 2.93	29. 58 ± 1.06
ΣPAHs (mean)		508.09 ± 27.10	458.29 ± 13.39	489.40 ± 16.13
ΣDDTs(4-4´DDT)		20.94 ± 0.56	20.86 ± 0.38	20.09 ± 0.39
ΣOCPs (endrin)		7.32 ± 1.31	5.20 ± 0.73	4.71 ± 0.87

Note: Values are mean ± standard error (S.E)

*Benzene ring: 2-3 (Low molecular weight -LMW PAHs); 4-6 (high molecular weight -HMW PAHs)

Table 6.3. Concentration (µg/kg) of organic contaminants (OCs) in Sharks from current study compared with some sharks species across the globe

Country	Sampling site	Species sharks	OCs studied	Levels detected	Remarks	Refs
South Africa	Coast of South Africa	Blue shark (*Prionace glauca*), Shortfin mako (Isurus paucus) & Smooth hound (Mustelus mustelus)	DDT, OCPs & PAHs	Highest DDT (20.94 ± 0.56 µg/kg ww) and PAHs (508.09 ± 27.10 µg/kg) were found in *P. glauca*	No significant variation in OC levels among the three sharks	Current study
India	Indian lagoon (near shore of Atlantic ocean)	Atlantic stingrays (*Dasyatis sabina*), bonnethead (*Sphyrna tiburo*), blacktip sharks (*Carcharhinus limbatus*)	PCB, OCPs e.g. DDT	*C. limbatus* DDT was highest mother shark (1056 ±49), embryo (256 ± 11) lw	Maternal transfer observed and age could be a factor as mother's level was higher than the embryo	Weijs *et al.* (2015)
Korea	Coastal water area of Korea	Spiny dogfish(*Squalus acanthias*),blue shark (*Prionace glauca*), Pelagic tresher shark (*Alopias pelgicus*), shortfin mako*(Isurus axyrinchus)*, Cloudy dogfish (*Scyliorhiru torazame*), Oceanic whitetip shark (*Carcharhinus longimanus*), Shortnose spurdog (*Squalus megalops*), Smooth hammerhead (*Sphyrna zygaena*), Crocodileshark (*Pseudocarcharias Kamoharai*) & more	PCB, OCPs e.g. DDT	DDT total mean (88.5 ± 290) for all sharks, mako 107 ± 273 lw; Blue shark (88.5 ± 290)	Inter and intra species variation in OCs concentration. DDT was predominant OCP and Mako shark from Indian ocean had highest concentration.	Lee *et al.* (2015).
Brazil	Praia Grande	Megamouth shark (*Megachasma pelagios*)	OCPs	0.19-0.47 ng/g (LOD)	Low levels of OCs	De Moura *et al.* (2015)
Greenland	Kong Oscars Fjord	Greenland shark (*Somniosus microcephalus*)	PCB, DDT and more	0.594 ng/g ww (DDT)	low level	Corsolini *et al.* (2014)
Brazil	Brazilian coast	Sword fish and blue shark, Blacktip reef shark (*Carcharhinus melanopterus*)	PCBs & DDT	0.52 ng/g ww DDT (blue shark)	EDI < ADI 20 µg/kg bw	De Azevedo e Silva *et al.* (2007)

Country	Sampling site	Species sharks	OCs studied	Levels detected	Remarks	Refs
USA (Florida)	4 Bays in Florida	Bonnethead (*Sphyrna tiburo)*	PCB, DDTs	8.00 ± 3.28 - 20.11± 3.14 lw (DDT)	No maternal transfer was observed. Irrespective of depth studied sharks had detectable levels of PAHs and AHs	Gelsleichter *et al.* (2005)
Kuwait	Arabian Gulf	Milk sharks (*Rhizoprinodon acutus*), Saw-toothed reef sharks (*Carcharhius sorrah*), Arabian carpet nurse shark (*Chilosyllim arabicum*), Black tipreef shark (*Caecharhius melanopterus*) and more.	PAHs and AHs	72.97 µg/g ww, highest total PAHs was in *C. sorrah*	No correlation was observed with fish size and PAHs, species and locational variations were observed	Al-Hassan *et al.* (2000)

6.4. Conclusion

The mako, blue and smoothhound sharks sampled from diverse spatial locations showed similar concentrations of organic contaminants OCs (OCPs and PAHs). Although fresh DDT input was detected, the levels of OCP (DDT and endrin) were found relatively low (below FDA action limit) which may be due to improved chemical management and control (use and disposal). The level of PAHs (toxic and carcinogenic) concentration in the studied sharks are worrisome and in particular the high level of benzo(*a*)pyrene which exceeded the EU maximum limit. The study which focused on screening of sharks in comparison to the regulatory standards found the assessed sharks to be contaminated with carcinogenic benzo(*a*)pyrene but not with organochlorine pesticides especially DDT. Effort should be intensified by regulatory government authorities such as the department of environmental affairs (DEA) through the existing national marine or coastal management programme, towards minimizing oil pollution in the coastal areas to minimize health risk. However, the study did not investigate the possible risk of consuming shark fish with studied contaminants (PAHs and OCPs) and would recommend in view of the commercial relevance of the species, that further investigation should be carried out considering both raw and ready to eat (processed) shark meats.

6.5. References

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CHAPTER 7

Occurrences, levels and profiles of PAHs and OCP in marine fish species from different locations along the South African coastline: Blacktail (*Diplodus sargus capensis*) *and* Hottentot (*Pachymetopon blochii*) species

Abstract

Organic contaminants (OCs) including polycyclic aromatic hydrocarbons (PAHs) and dichlorodiphenyltrichloroethane (DDT) were assessed in Blacktail (*Diplodus sargus capensis*) *and* Hottentot (*Pachymetopon blochii*) from the coast of South Africa. Hottentots (*Pachymetopon blochii*) were sampled from Dassen Island, Hout Bay, Hondeklip, Lamberts Bay, Kalk Bay and Saldahna Bay, whilst blacktail (*Diplodus sargus capensis*) were sampled from Mossel Bay, Saldahna Bay, Muzeinberg, Witsand, Port Elizabeth and Durban. The aim of the study was to assess the variation in occurrences, levels and profiles of target OCs in selected species across the study locations. The highest (405.45 ± 140.31 µg/kg) and lowest (87.28 ± 1.96 µg/kg) PAHs concentrations in hottentot were from Dassen Island and Saldahna Bay respectively; whereas, PAHs concentration was highest in blacktail sampled from Mossel Bay (437.38 ± 16.33 µg/kg) and the lowest in samples from Durban (147.22 ± 14.63 µg/kg). The predominant PAHs profiles in both species were the low molecular weight (LMW), except in samples from Dassen Island and Port Elizabeth where HMW predominated. Naphthalene was the most abundant LMW PAHs while benzo(*a*)pyrene was not detected in hottentot but exceeded EU level (2 µg/kg) in blacktail from two locations (Mossel and Saldhna Bays) only. Average DDT concentrations of 760.50 ± 484.53 and 3.88 ± 0.32 µg/kg were recorded in hottentot fish from Dassen Island and Hout Bay respectively. DDT was neither detected in hottentot from other locations nor in any blacktail from all the six sampling locations. The non-detection of benzo(*a*)pyrene and few other carcinogenic PAHs in hottentot could be species associated and may be considered relatively as a more acceptable species.

Keywords: South Africa, marine, organic contaminants, PAHs, DDT, health-concern

7.1. Introduction

South Africa, at the tip of the continent is embedded by two oceans, Indian and Atlantic and has a coastline (Fig. 7.1) which is estimated to be over 3000 km stretching from Ponta do Ouro in the East to Orange River in the West. The coastline has rich marine resources of diverse species (DEA, 2014; Griffiths *et al.*, 2010; Turpie & Wilson, 2011; WWF, 2011). The coastline is divided into three major bio-regions (East, South and West coasts) based on temperature differences due to the influence of warm Agulhas and cold Benguela currents from Indian and Atlantic oceans respectively. The East (sub-tropical) coast which receives

warm Agulhas current from the Indian Ocean produces high diversity of marine species but with low fish resources, while the West coast with the influence of cold Benguela current from the Atlantic Ocean, yields less marine diversity but high fish resources (DEA, 2014; Turpie & Wilson, 2011). The South coast is between both ocean currents and consequently supports mixture of species.

South African fish production has grown substantially with total annual fish production of marine fisheries as at 2012 estimated at over 700,000 tonnes (DAFF 2012, FAO, 2012). The fishing activities in South Africa comprise of the offshore, nearshore (commercial) and inshore (subsistence) for both local and international markets (WWF, 2011; DAFF, 2011; DAFF, 2012). The country has over 147 fishing communities with an estimation of 29,000 as subsistence fish farmers (Turpie & Wilson, 2011; DAFF, 2012; WWF, 2011). The major marine fish species of importance in South Africa include: Hake, snoek, swordfish, tuna, yellowtail, shark, blacktail and hottentot (WWF, 2011; Baust *et al*., 2015).

Blacktail and Hottentots are resident marine fish species among the commonly consumed fish in South Africa (Baust *et al*., 2015). Blacktail is usually distinguished with dark spot mark on the tail (Mann, 1992) with distribution mostly around the Eastern and Western Cape, whilst the hottentot are distributed along the entire South African coast. Additionally, both species have similar feeding habits; both are omnivores adults while deviation has been identified during the juvenile phase (juvenile blacktail are omnivores) (Froese & Paule, 2015). Resident species were reported to accumulate higher levels contaminants (Hunt *et al*., 1999) which can lead to further biomagnification of contaminants along the food chain. In addition, the marine environment have been reported as the repository of contaminants (Froescheis *et al*., 2000) and it is anticipated that resident aquatic organisms such as blacktail and hottentots used for the assessment of contaminants will reflect the actual environmental contaminants for effective monitoring. Given that approximately one third of South Africa's population reside within close proximity to the coast (DEA, 2013), and depend on the marine environment for their dietary requirements, it is essential that the risk of consuming fish is assessed. Therefore, the aim of this study was to investigate PAHs and OCPs profiles and levels in blacktail and hottentot species sampled from six different locations for each species along the coast of South Africa. The study also identified locations with increased contaminant levels and possible input sources of organic contaminants were postulated.

7.2. Materials and methods

7.2.1. Sampling and sample preparations

Blacktail and hottentot were harvested by local line fishermen in collaboration with the Department of Agriculture, Forestry and Fisheries (DAFF) Fish were harvested on different dates per species per location. Blacktail were sampled from: Durban (29.8833° S, 31.0500° E), Mossel Bay (34.1833° S, 22.1333° E), Muizenberg (34.1050° S, 18.4717° E), Port Elizabeth (33.9581° S, 25.6000° E), Saldanha Bay (33.0347° S, 18.0097° E) and Witsand (34.3950° S, 20.8410° E), whilst the Hottentots were sampled from: Dassen Island (33.4167° S, 18.0833° E), Hout Bay (34.0333° S, 18.3500° E), Hondeklip Bay (30.3167° S, 17.2667° E), Lamberts Bay (32.0833° S, 18.3000° E), Kalk Bay (34.1278° S, 18.4483° E) and Saldahna Bay (33.0347° S, 18.0097° E) (Fig. 7.1). Biometric data (length and weight) of the fish were taken (Table 7.1). Fish were rinsed under clean running (tap) water, beheaded and eviscerated, skinned and filleted. Finally, the fish were homogenized (a total of 10 individual fish per species was used), vacuum packed and stored at -20°C till further analysis.

Table 7.1. Biometric data (Mean (SE) of hottentot (*Pachymetopon blochii*) and blacktail (*Diplodus sargus capensis*) sampled from different locations along the coast of South Africa

Location	Species	Sample size (n)	Weight (g)	Total length (cm)	Fork length (cm)
Dassen Island	Hottentot	10	748.89 (15.920)	32.37 (0.29)	30.01 (0.34)
Hout bay	Hottentot	10	590.59 (30.69)	32.71 (0.64)	30.18 (0.54)
Hondeklip	Hottentot	10	587.71 (14.90)	31.38 (0.17)	28.80 (0.17)
Kalk bay	Hottentot	10	496.65 (22.17)	29.78 (0.48)	27.11 (0.45)
Lamberts Bay	Hottentot	10	450.85 (16.98)	28.67 (0.39)	26.23 (0.35)
Saldahna	Hottentot	7 10	469.80 (65.51)	29.07 (1.31)	26.44 (1.15)
Durban	Blacktail	10	487.33 (36.18)	29.84 (0.65)	26.22 (0.57)
Mossel Bay	Blacktail	10	550.43 (18.83)	32.08 (0.39)	28.19 (0.36)
Muizenberg	Blacktail	10	637.56 (27.44)	32.88 (0.49)	28.47 (0.43)
Port Elizabeth	Blacktail	8	464.70 (46.96)	32.59 (2.17)	28.68 (2.14)
Saldahna	Blacktail	10	825.00 (47.24)	35.42 (0.60)	31.22 (0.53)
Witsand	Blacktail	10	197.53 (13.82)	23.10 (0.59)	20.14 (0.52)

Figure 7.1. Sampling locations (★) blacktail (*Diplodus sargus capensis*) and hottentot (*Pachymetopon blochii*) distributed along the South African coastlineGoogle map as edited by Adesuyi, S. A. (2015). Geology Department, Stellenbosch University.

7.2.2 Extraction and Instrumental analysis.

The extraction and analytical procedures were as already detailed in chapter 3.

7.2.3. Statistical analysis.

Data were tested for normality and homogeneity using Kolmogorov-Smirnov and Levene's F tests respectively. There was no inter-species (i.e. between blacktail and hottentot) comparison rather all comparisons were based on spatial intra-species comparisons (between locations) of the detected organic contaminants. ANOVAs tested for potential differences of normally distributed and homoscedastic data. Where data failed the ANOVA test assumptions, differences in concentration levels were determined using the non-parametric Mann Whitney *U* test. Post-hoc analysis was conducted where significant differences were observed. T-test was used to compare the differences between the sum of LMW PAHs and the HMW in each species per location. All analytes found below limit of detection were considered as not detected and excluded from statistical analysis. All statistical analysis was done using Statistica version 12 (Statsoft Inc., USA, 2012) where the confidence level was set at 95 % (p<05).

7.3. Results and Discussion

7.3.1 PAHs levels in *P. blochii* from six different locations along the coast of South Africa: Dassen Island, Hout Bay, Hondeklip, Kalk Bay, Lamberts Bay and Saldahna Bay

Total PAHs concentration in hottentot species sampled from six different locations showed significant variations (p<0.05) among these locations as given in Table 7.2. Fish from Dassen Island had significantly higher (p<0.05) concentration (405.45 ± 140.31 µg/kg ww) than samples from other locations. No significant variation was found between fish sampled from Hondeklip and Kalk Bay (Table 7.2) which could mean similar pollution. Whilst fish from Saldahna Bay was the least contaminated with mean total concentration of 87.29 ± 1.96 µg/kg ww reflecting possibly a low pollution input. The concentration of total PAHs in fish from the studied areas in descending order were as follows: Dassen Island>Lamberts Bay>Kalk Bay>Hondeklip>Hout Bay>Saldahna Bay. The intra species variation in concentration of PAHs observed may therefore indicate variation due to locational input sources as spatial variation is a reflection of environmental emission and distribution of PAHs (Zhang & Tao, 2009).

The most abundant PAHs in hottentot fish was naphthalene with highest concentration from Kalk Bay (262.60 ± 46.44 µg/kg ww), significantly higher (p<0.05) than naphthalene

levels in fish from other locations while naphthalene levels from Dassen Island, Hout Bay and Saldahna Bay did not vary significantly (p>0.05) (Table 7.2).

The dominance of naphthalene over other PAHs similarly observed by Zhao *et al.* (2014) and Bandowe *et al.* (2014) from China and Ghana respectively have been considered an indication of pollution from crude oil and petroleum sources (Karlsson & Viklander, 2008). In general the low molecular weight (2-3 benzene rings) PAHs dominated the PAH profile in the hottentot species which was a similar trend observed in other related studies (chapters 3,4, 5 & 6) with predatory and migratory species. This suggests a trend in pollution input trend of petroleum in which national drilling, refineries, ports and harbours activities near the coast likely contributed significantly to overall pollution input (UNEP, 2006). Given the hazardous effects of PAHs (both toxic and carcinogenic ones), urgent effort to investigate the petroleum pollution and enhance monitoring and control of human exposure through consumption of contaminated fish is required.

However, benzo(*a*)pyrene which is one of the most toxic PAHs and used by the FAO/WHO and EU (at a maximum acceptable limit of 2 µg/kg) as the PAHs indicator was not detected in hottentot analyzed from any of the six locations. This could be as a result of species inability to bioaccumulate or ability to metabolize and excrete benzo(*a*)pyrene (Boehm, 2005). Ramalhosa *et al.* (2009) also did not detect benzo(*a*)pyrene in 89 individual fish (e.g. Horse mackerel, chub mackerel and sardine) sampled in Portuguese waters proposing that non-contamination of fish with benzo(*a*)pyrene as a common positive occurrence. Thus the present results in this study suggest that hottentot species may be one of the safer fish species for consumption in South Africa as it has a reduced dietary exposure to benzo(*a*)pyrene. Nonetheless, the non-detection of benzo(*a*)pyrene in hottentot species should further be investigated incorporating seasonal and gender factors to confirm the bioaccumulation of the most potent PAH. The various PAHs (profile) detected in hottentot from the six sampling locations in descending order of concentration were:

Dassen Island: Nap>Phe>Flu>Ant>Acy>Ace

Hout Bay: Nap>Flu>Ant>Phe>Acy>Ace

Hondeklip: Nap>Phe>Flu>Ace

Kalk Bay: Nap>Phe>Flu>Ace

Lamberts Bay: Nap>Phe>Flu>Ant>Acy>Ace

Saldahna Bay: Nap>Flu>Phe>Acy

Table7.2. Concentration (mean ± Standard error; and range) of polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in hottentot (*Pachymetopon blochii*) fish muscle sampled from six locations along the coast of South Africa. ND=Not detected; values (μg/kg wet weight) with different alphabet letter(s) on the same row denote significantly different (p<0.05).

Compounds	Dassen Island	Hout Bay	Hondeklip	Kalk Bay	Lamberts Bay	Saldahna Bay
Nap	117.70 ± 6.3**c**	105.35 ± 11.70**c**	224.33 ± 45.88**ab**	262.60 ± 40.44**a**	157.88 ± 18.38**cb**	81.32 ± 5.82**c**
	(68.50-138.54)	(11.61-148.45)	(60.49-407.26)	(39.58-412.35)	(143.72-205.88)	(48.35-91.18)
Acy	2.22 ± 0.65**bc**	2.31 ± 0.18**b**	ND	ND	3.72 ± 0.13**a**	1.03 ± 0.42**c**
	(<dl-6.23)	(1.62-3.66)	ND	ND	(2.94-4.54)	(0.06-3.47)
Ace	1.82 ± 0.24**ab**	1.52 ± 0.12**b**	1.47 ± 0.16**b**	1.38 ± 0.36**b**	2.06 ± 0.19**a**	ND
	(0.93-3.06)	(0.98-1.98)	(0.70-1.94)	(0.84-1.86)	(1.41-3.32)	ND
Flu	9.09 ± 1.20**a**	6.86 ± 0.23**ac**	5.14 ± 0.35**cb**	6.34 ± 0.31**ac**	8.37 ± 1.87**ab**	3.54 ± 3.34**c**
	(5.43-14.66)	(6.10-8.54)	(3.37-6.50)	(5.40-8.63)	(4.27-23..11)	(<dl-23.57)
Phe	11.61 ± 3.87**b**	4.26 ± 0.11**b**	7.13 ± 0.16**b**	7.40 ± 0.28**b**	22.19 ± 8.28**a**	1.16 ± 0.80**b**
	(<dl-31.41)	(3.57-4.76)	(6.09-7.86)	(6.53-8.91)	(8.73-91.67)	(<dl-5.91)
Ant	5.10 ± 2.27**a**	6.00 ± 2.28**a**	ND	ND	6.68 ± 2.46**a**	ND
	(<dl-18.87)	(<dl-21.59)	ND	ND	(3.29-28.28)	ND
Fluo	39.83 ± 22.32**a**	2.48 ± 0.05**a**	ND	ND	ND	0.10 ± 0.02**a**
	(<dl-195.40)	(2.26-2.62)	ND	ND	ND	(0.06-0.18)
Pyr	51.69 ± 28.26**a**	5.06 ± 0.07**b**	3.73 ± 0.25**b**	3.56 ± 0.19**b**	25.19 ± 13.24**ab**	0.14 ± 0.02**b**
	(43.54-261.86)	(4.67-5.38)	(2.94-5.65)	(2.92-4.72)	(8.07-141.98)	(0.06-0.22)
Chr	34.02 ± 13.13**a**	3.06 ± 0.01**b**	3.31 ± 0.01**b**	3.29 ± 0.02**b**	39.99 ± 18.75**a**	ND
	(3.01-107.35)	(3.02-3.11)	(3.27-3.36)	(3.25-3.39)	(11.25-190.10)	ND
BbF	78 50 ± 42. 58**a**	1.79 ± 0.29**b**	ND	ND	34.72 ± 16.08**ab**	ND
	(0.90-381.01)	(0.34-2.74)	ND	ND	(1.59-282.81)	ND
BkF	53.88 ± 28.70**a**	2.53 ± 0.10**a**	ND	ND	ND	ND
	(2.15-145.17)	(2.12-2.97)	ND	ND	ND	ND
BaA	ND	ND	ND	ND	ND	ND
BaP	ND	ND	ND	ND	ND	ND
DahA	ND	ND	ND	ND	ND	ND
IndP	ND	ND	ND	ND	ND	ND
BghiP	ND	ND	ND	ND	ND	ND
Σ PAHs	405.45 ± 140.31**a**	141.22 ± 12.86**cb**	246.97 ± 46.46**ac**	286.44 ± 40.33**ac**	301.34 ± 44.58**ab**	87.29 ± 1.96**c**
	(131.79-1408.32)	(45.00-199.83)	(74.90-444.89)	(61.46-432.98)	(201.47-660.53)	(79.94-92.45)
DDT (4-4´DDT)	760.50 ± 484.53	3.88 ± 0.32	ND	ND	ND	ND
	(<dl-4604.31)	(2.50-5.87)	ND	ND	ND	ND
BHC beta	0.87 ± 0.39**b**	8.69 ± 5.56**c**	0.33 ± 0.07**b**	0.22 ± 0.07**b**	1.26 ± 0.61**b**	ND
	(0.28-3.89)	(<dl-48.50)	(<dl-0.53)	(<dl-0.48)	(0.44-6.52)	ND

The above profile distribution could be considered to be the species-specific uptake of PAHs. This was marked with the most and least abundant LMW PAHs as naphthalene and acenaphthene respectively irrespective of location (except for Saldahna which had no acenaphthene). Also each PAH has specific properties such as solubility and metabolic pathway which can affect its uptake and accumulation in fish (Ramesh *et al.*, 2004). Furthermore fish biological factors (age, sex and lipid content, feeding habit and habitat) and environmental factors (season and pollution input sources) may also affect the relative abundance and eventual uptake of each PAH by fish (Cascaes *et al.***,** 2014; Lee *et al.*, 2015).

Low molecular weight (LMW) PAHs such as those detected in hottentot (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene) from Dassen Island, Hout bay and Lamberts Bay are considered toxic. On the other, hand high molecular weight (HMW) PAHs such as fluoranthene, pyrene, chrysene, benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a,h*)anthracene, indeno(*1,2,3-cd*)pyrene and benzo(*g,h,i*)perylene are considered carcinogenic and were found in all the fish from all locations. A summary of all PAH concentrations for each location and statistical output are presented on Table 7.2.

The diagnostic input of PAH into the environment can be determined based on the relationship between LMW and HMW measured from the fish samples. The predominance of LMW implies input from petroleum while input from combustion is characterised by the dominance of the HMW PAHs. A comparison (t-test) between total LMW PAHs (Σ LMW PAHs) and HMW PAHs (Σ HMW PAHs) in fish from the same location and among locations showed that significant variations ($p < 0.05$) existed both inter and intra spatially (Fig 7.2). Fish from Dassen Island had Σ HMW PAHs (257.91 µg/kg) significantly higher ($p < 0.05$) than Σ LMW PAHs (147.54 µg/kg) whereas all other locations showed reversed situation. PAHs pollution source in Dassen Island could be considered pyrogenic whilst the rest locations showed petrogenic. In addition the ratio of Σ LMW PAHs/ Σ HMW PAHs for Dassen Island was 0.57 was an indication of combustion possibly from coal or wood (Tsymbalyuk *et al.,* 2011). The use of wood and coal as fuel within the Island cannot be ruled out in view of the poor management of the area as there is currently no electricity in operational offices (Cape Nature, 2012).

The level of PAHs in fish from all the studied locations based on Baumard *et al.* (1998) can be summarised (range value) as moderately contaminated (100-1000 µg/kg) with the exception of fish from Saldahna Bay with low contamination range (0-100 µg/kg). Although benzo(*a*)pyrene was not detected, the high concentration of HMW PAHs which are the

carcinogenic PAHs (and more deleterious to health) (ASTDR, 2002) could be issue of health concern particularly to the Island dwellers that may depend on seafood for their animal protein.
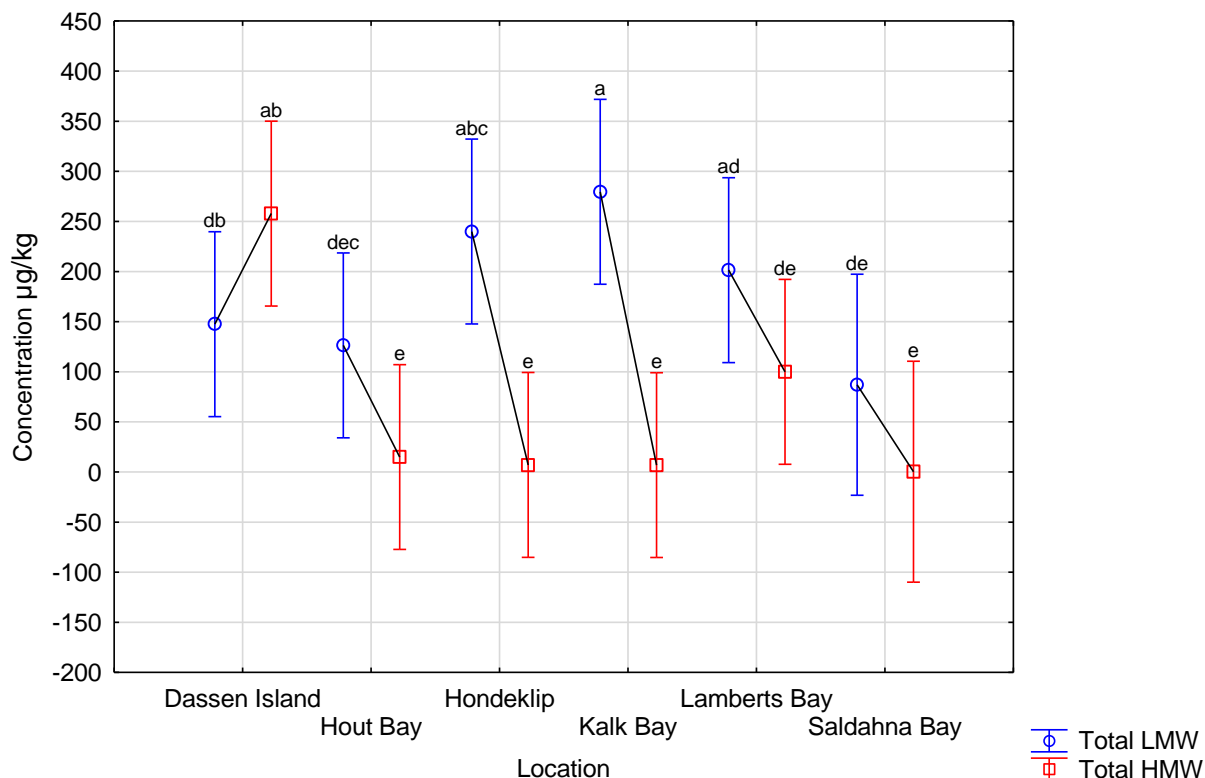

Figure 7.2. Concentration of low molecular weight and high molecular weight (LMW & HMW) PAHs in hottentot (*Pachymetopon blochii*) from six different locations (n=10 per location) on the coast of South Africa.   Values are mean ± Standard error μg/kg ww; different alphabets letter(s) denote significant variations ($p<0.05$) between LMW and HMW PAHs.

7.3.2 Organochlorine pesticides in *P. blochii*

DDT and its analogues DDD and DDE were not detected in fish from four of the sampling locations: Hondeklip, Kalk, Lamberts and Saldahna Bays. However, average concentration of DDT (predominantly 4,4´-DDT analogue) in hottentot from Dassen Island (760.50 ± 484.53 μg/kg ww) was significantly higher ($p<0.05$) than that in fish from Hout Bay (3.88 ± 0.32 μg/kg) and also comparatively higher than levels observed in previous studies with middle and high trophic species in previous chapters (3, 5 and 6). The high level of DDT found in fish from Dassen Island in comparison to other locations could not be readily explained in view of its distance from the malaria endemic zones where DDT is used as IRS. This therefore may be a reflection of other sources of DDT into the environment other than from IRS.

Apart from the use of DDT as an IRS for malaria control (WHO, 2011), DDT is also incorporated in the production of Dicofol and may be retained as impurities, an approved agro pesticide (De la Cal *et al.*, 2008). DDT impurities may be introduced into the areas other than the DDT spray zone. Dicofol is an organochlorine pesticide and is used for protection of crops and fruits such as beans, cabbage, apples, pears, peas and more in South Africa (Quinn *et al.*, 2011). Thus the increased DDT concentration (though < FDA action level of 5000 µg/kg) found in fish from Dassen Island may suggest possible use of Dicofol pesticide in agriculture in that area. Agriculture is one of the major economic activities in Dassen Island (Cape Nature, 2012) and use of pesticides can be used to boost agriculture. Further investigation in the use of Dicofol containing DDT impurities as agro pesticide in the country may be necessary to mitigate increased bioaccumulation in fish and other food commodities. Again undecked boats are means of transportation from/to the Island and possible maintenance with antifouling paint containing DDT (Li *et al.*, 2006; UNEP, 2006), may be contributory to the input of DDT in the area. Although DDT level in fish from Dassen Island in this current study did not exceed the FDA level, however, frequent and prolonged consumption of fish contaminated with DDT might predispose consumers to DDT associated carcinogenic health risks (ASTDR, 1995; ASTDR, 2002).

BHC beta analogue was detected in all the fish samples from the study locations except in Durban but in very low levels. Ryan *et al.* (2012) reported a decrease of BHC in a study on persistent organic in South African waters. The decreased and current low levels of BHCs detected in South African waters (current study; Ryan *et al.*, 2012) reflects a decline in usage likely due to the global banning (including South Africa) more than two decades. Although low, it was noted that Hout Bay hottentot had the highest BHC average concentration of 8.69 ± 5.56 µg/kg was observed in fish from Hout Bay (Table 7.2) which may reflect comparatively greater application in the locality.

Of the 12 OCPs (excluding DDT) assessed only BHC beta analogue was detected in fish from all the study locations. The non-detection or low detection OCPs could be due to low concentrations in fish below instrumental detection, non-application of such pesticides in study locations (banned globally), non-ability to accumulate and/or metabolize and excrete these OCPs (Covaci *et al.*, 2003; Weber *et al.*, 2003; Adu-Kumi *et al.*, 2010)

7.3.3 Comparison of PAHs concentration in *D. sargus* from six different locations:

Total PAHs concentration in blacktail varied considerably between locations (Table 7.3) with concentrations in descending order as follows: Mossel Bay>Saldahna Bay>Port Elizabeth> Muizenberg>Witsand>Durban. The total PAHs in blacktail varied on average between 147.21± 14.63 µg/kg ww sampled from Durban and 437.38 ± 16.33 µg/kg ww from

Mossel Bay. Mossel Bay, Saldahna Bay and Port Elizabeth did not vary significantly (p≥0.05) (Table 7.3), while fish from Witsand and Muizenberg have comparable PAHs concentrations (p≥0.05) but varied significantly higher than Durban but lower than the rest locations. Blacktail from Durban had significantly lower level of total PAH (p<0.05; 147.21± 14.63 µg/kg) than all other sites. The current overall PAH trend is similar to that reported in earlier chapters (3, 5 & 6) with benzo(*a*)pyrene found above EU levels in fish sampled from Mossel Bay and Saldahna Bay. Whilst blacktail from the rest locations were not found to have benzo(*a*)pyrene in detectable levels. In all the study locations, the average ∑LMW PAHs in blacktail were significantly higher than ∑HMW PAHs. The predominance of LMW over the HMW reflected the source of contamination as petroleum (Tsymbalyuk, *et al.*, 2011).
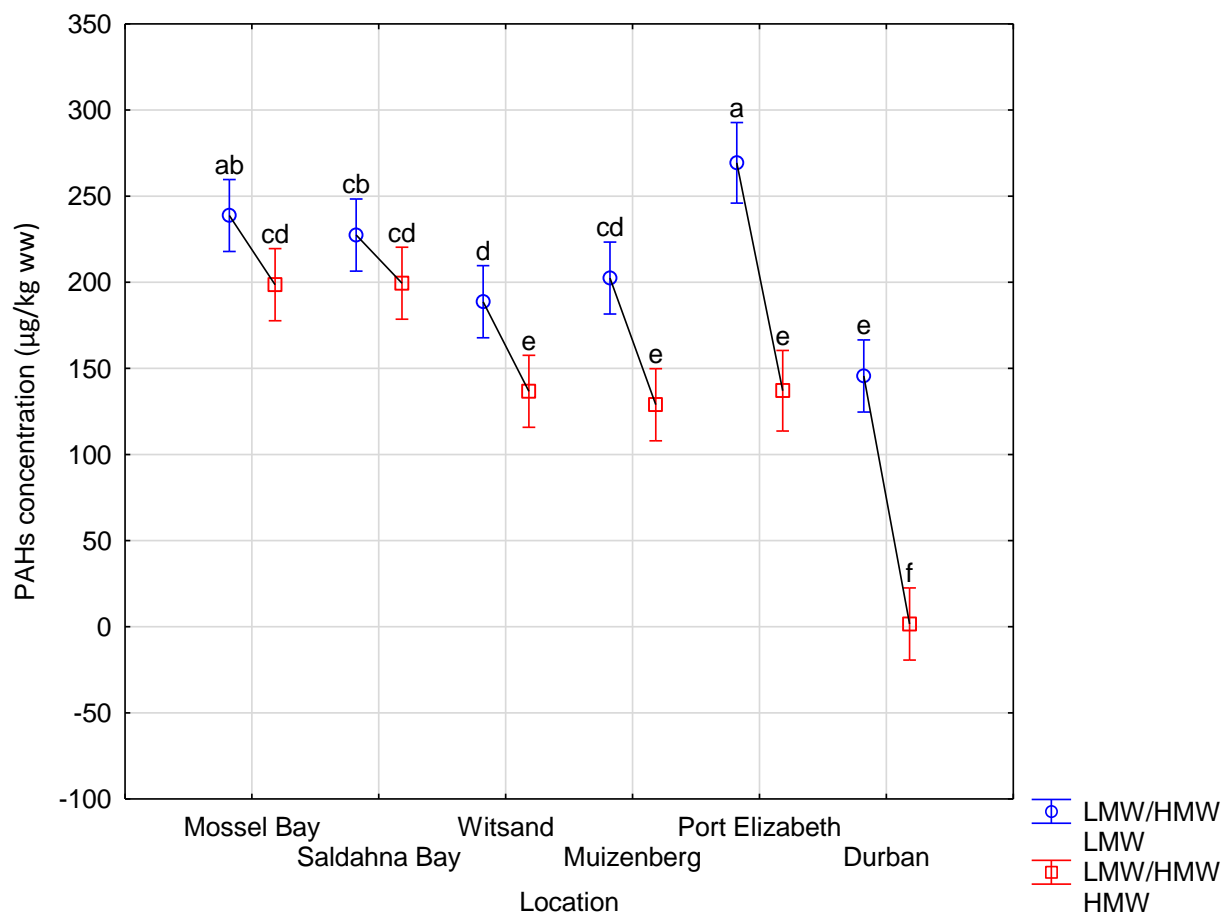

Figure 7.3. Concentration of low molecular weight and high molecular weight (LMW and total HMW) PAHs in Blacktail (*Diplodus sargus capensis*) sampled from six different locations along the coast of South Africa. Values are mean ± Standard error µg/kg ww; different alphabets letter denote significant variations (p<0.05) between LMW and HMW PAHs.

Table 7.3. Concentration (mean ± Standard error; and range) of polycyclic aromatic hydrocarbons (PAHs) and organochlorine pesticides (OCPs) in blacktail (*Diplodus sargus capensis*) fish muscle sampled from six different locations along the coast of South Africa. ND= Not detected; values (µg/kg wet weight) with different alphabet letter(s) on the same row) denote significant differences ($p < 0.05$).

Analyte	Mossel Bay	Saldahna Bay	Witsand	Muizenberg	Port Elizabeth	Durban
Nap	136.40 ± 17.32**a** (72.41-249.42)	127.17 ± 7.46**a** (93.74-172.02)	76.47 ± 15.56**b** (ND-138.21)	95.83 ± 18.70**ab** (ND-165.60)	138.17 ± 16.52**a** (67.16-200.98)	113.68 ± 12.43**ab** (67.41-182.82)
Acy	9.17 ± 0.13**b** (8.72-9.97)	9.08 ± 0.13**b** (8.64-9.80)	11.94 ± 0.74**a** (10.04-15.84)	10.15 ± 0.09**a** (9.82-10.67)	11.12 ± 0.71**ab** (9.72-16.03)	0.70 ± 0.09**c** (0.40-1.18)
Ace	1.15 ± 0.15**b** (ND-1.84)	1.06 ± 0.20**b** (0.80-1.40)	ND ND	ND ND	ND ND	2.09 ± 1.58**a** (ND-4.37)
Flu	13.41 ± 0.10**b** (12.98-14.05)	13.09 ± 0.28**b** (11.21-14.04)	17.03 ± 4.36**ab** (4.91-35.08)	15.65 ± 2.34**b** (5.70-22.32)	24.39 ± 2.78**a** (19.54-43.53)	16.77 ± 3.17**ab** (2.71-30.46)
Phe	52.32 ± 0.25**a** (52-121-53.24)	51.31 ± 0.45**a** (49.57-54.35)	48.67 ± 0.93**b** (45.99-53.92)	47.00 ± 0.29**b** (45.53-48.39)	48.42 ± 1.49**b** (45.73-58.44)	10.98 ± 1.36**c** (2.35-17.17)
Ant	26.29 ± 0.16**d** (25.65-27.19)	25.73 ± 0.11**d** (25.42-26.56)	34.57 ± 0.36**b** (33.52-37.15)	33.84 ± 0.14**c** (33.33-34.46)	47.26 ± 0.48**a** (45.73-49.73)	1.41 ± 0.19**e** (0.95-2.21)
Pyr	36.91 ± 0.19**a** (36.02-37.85)	36.47 ± 0.17**b** (35.75-37.01)	26.30 ± 0.12**c** (25.53-26.83)	25.82 ± 0.09**d** (25.50-26.36)	25.98 ± 0.12**cd** (25.67-26.55)	1.21 ± 0.07**e** (0.88-1.47)
Chr	38.58 ± 4.29**b** (ND-44.21)	42.77 ± 0.10**b** (42.46-43.54)	64. 82 ± 0.06*a* (64.59-65.14)	58.33 ± 6.48**a** (ND-65.28)	64.66 ± 0.08**a** (ND-65.21)	ND ND
BbF	ND ND	ND ND	45.59 ± 0.82**a** (42.06-51.56)	44.77 ± 0.98**a** (41.54-51.14)	46.38 ± 0.53**a** (44.86-48.71)	ND ND
BaP	28.99 ± 0.93**a** (25.77-33.00)	27.85 ± 0.83**b** (25.21-33.21)	ND ND	ND ND	ND ND	ND ND
InP	42.10 ±0.02**a** (42.02-42.23)	42.09 ± 0.04**a** (42.06-42.19)	ND ND	ND ND	ND ND	ND ND
DbA	15.13 ± 1.68**a** (ND-17.05)	13.44 ± 2.24**a** (ND-17.05)	ND ND	ND ND	ND ND	ND ND
BghiP	36.91 ± 0.02**a** 36.86-37.09)	36.87 ± 0.01**a** (36.85-36.92)	ND ND	ND ND	ND ND	ND ND
Fluo	ND	ND	ND	ND	ND	ND
BaA	ND	ND	ND	ND	ND	ND
BkF	ND	ND	ND	ND	ND	ND
Total PAHs	437.38 ± 16.33**a** (380.28-532.54)	426.93 ± 8.96**a** (384.41-477.22)	325.40 ± 13.71**b** (233.00-379.74)	331.39 ± 16.85**b** (251.25-396.37)	406.38 ± 13.79**a** (359.50-461.84)	147.22 ± 14.63**c** (92.39-229.80)
Endosulfan	34.86 ± 6.23 (3.52-69.33)	30.92 ± 4.19 (16.40-60.60)	ND	ND	ND	ND

137

7.3.4 Organochlorine pesticides concentrations in *D. sargus*

Endosulfan (beta) was only detected in blacktail from Mossel (3.52-69.33 µg/kg) and Saldahna Bays (16.37-60.60 µg/kg) and no significant variation was observed between the two locations. The detection of endosulfan in blacktail from the two locations would be interpreted as possible usage in these locations. The use of endosulfan could be confirmed as it is currently not included in the in South Africa's list of banned pesticides (chlordane, heptachlor, aldrin, dieldrin, endrin and mirex) (Naidoo & Buckley, 2003), despite its inclusion in global target contaminants in edible fish (US EPA, 2000).

DDT though still in use in the malaria endemic zones of the country with W.H.O's permission, was however, not detected in blacktail fish from all the study locations which may reflect effective regulation or species-specific effect. Fish ability to accumulate chemicals from their aquatic environment can vary due to different metabolic activities, feeding habit, age, gender and size. For example Lee *et al.* (2015) in a study with 13 different sharks in Korea, observed species effect among 3 shark species (blacktip reef shark, blue shark and shortfin mako shark) which had significantly higher concentration of DDT (among other contaminants) than other 10 sharks species. In addition, blacktail is carnivorous as juvenile and omnivorous as adult (Mann, 1992) which implies that feeding at different stage of life (age effect) can affect the accumulation of contaminant given that most contaminants are taken up via food. In general the detected levels of pesticide residue (endosulfan) in fish from Mossel and Saldahna Bays were below the critical EU maximum residue level (MRL) of 100 µg/kg.

7.4. Conclusion

The predominant PAH in blacktail from the six sampled locations was naphthalene which implied petroleum pollution along the coastal areas. Similar trend was observed with the hottentot species except for Dassen Island that the LMW PAHs predominated an indication of pyrogenic source. Benzo(*a*)pyrene and DDT were not detected in hottentot and blacktail respectively from all the study locations whilst Benz(*a*)pyrene was detected in levels above EU in blacktail from Mossel and Saldahna Bays. The studied low trophic and resident species could better reflect the locational inputs of contaminants. Thus the hottentot species could be considered a more acceptable species considering the non accumulation of benzo(*a*)pyrene and some other carcinogenic PAHs. In general the data generated from the study with resident marine fish species (blacktail and hottentot) reflected differences in locational pollution level along the South African coastline. This may require enforecement of best management practices in places with increased burden such as Dassen Island (hottentot), Mossel Bay and Saldahna Bay (blacktail). The enforcement of regulatory laws such as

water and sediment quality guideline, agricultural pesticide use and coastal management policies will reduce the input of contaminants into the aquatic environment.

However, when considering human exposure of these contaminants via fish consumption, it is not only a function of the contaminants concentrations but in addition the volume of fish consumed, the frequency and duration of exposure should be reckoned with. Furthermore the method of cooking can increase or reduce some of these contaminants particularly the PAHs. It is therefore necessary to research further into the human dietary exposure to these contaminants particularly in those locations (e.g. Dassen Island and Port Elizabeth) due to their increased burden. It is suggested that factors such as season, gender, fish size and anatomical portions be considered in future studies.

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139

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CHAPTER 8

General discussion and conclusion

Natural and human activities in the contemporary world have significantly contributed to perennial environmental pollution (Skupińska *et al.*, 2004; UNEP, 2005; EFSA, 2008; Tanabe & Ramu, 2012). These have impacted on the entire ecosystem such that man at the apex of food chain remains the most vulnerable to the associated hazards of polluted environment (ASTDR, 2002). Through dietary intake of contaminated foods of which fish, particularly marine oily species have been indicted as most probable source, humans are continuously exposed to these toxic organic and inorganic substances (Dougherty *et al.*, 2000; Domingo, 2007; FSAI, 2013). Thus the expected benefits of fish consumption, is being threatened by the accumulation of these hazardous toxic and carcinogenic substances.

The study did not emphasize the health risk estimation via the consumption of contaminated fish but rather based acceptability of fish screened based on the provided FDA and EU regulatory maximum limits (US EPA, 2000; OJEU, 2006). However, for a more protective measure (to protect the vulnerable fish consumer groups particularly the subsistence fishers) US EPA (2000), guideline stipulated that DDT level of 14.2 µg/kg be taken as the threshold value above which there is a potential health risk. But in view of the fact that the study was not limited to this group of consumers, it is suggested a study to further investigate intake of contaminated fish by this group be conducted. Due to a lack of local guidelines, the FDA action level for the general public was used to establish fish safety.

Numerous published work have estimated health risks due to fish consumption however, these were based mostly on raw fish (Jiang *et al.*, 2005; Dai *et al.*, 2011; Dhananjayan & Muralidharan, 2012; Man *et al.*, 2013). A more authentic method for estimating health risk of contaminants via fish consumption, according to Domingo *et al.* (2007) and Du *et al.* (2012) is based on a net balance of fish nutrients (especially omega 3) against the studied contaminants. Furthermore, in a critical review by Domingo (2011), it was revealed that some of these contaminants level may be increased or reduced depending on the food item and cooking method. Thus effect of heat (different cooking methods) have been reported to affect the final concentration of contaminants particularly PAHs in some investigated fish (Perelló *et al* 2009).

In addition, studies have further shown that not all the measured concentration of contaminants in a given food material are digested and assimilated into the body. These further raises the question on the current practice of risk assessment of contaminants merely based only on detected levels in the raw flesh. Therefore, it is suggested that for a valid and holistic risk estimation of intake of contaminants (especially for PAHs) via the studied fish, should take into account the net

balance of the contaminant concentration as in ready to eat fish (and if possible to estimate the concentration assimilated after digestion). This study was initially designed and proposed to cover and establish a health-benefit–risk study which was to access the benefits (nutrients) against the risk (contaminants) according to Domingo *et al.* (2007). However, due to the huge financial cost implication of the study and time constraint, these objectives were excluded from this study. In view of these limitations, the acceptability of the fish for consumption was evaluated based on provisional regulatory standards of FDA and EU. Thus fish species from this study with highest (increased) burden and their corresponding sampling locations were as given in Table 8.1, while Table 8.2 summarises al the studiedfish species, locations, trophic levels and ontaminant levels.

Table 8.1. Summary of species showing locations with highest and lowest concentrations of target contaminants (PAHs and DDTs only), other OCPs were of low concentration and were not considered a health threat.

Species	Location	PAH	B(*a*)P	DDT	Remarks
Blue shark	False Bay	508.09 ± 27.10	29.71 ± 0.89	20.86 ± 0.38	Critical value for PAHs based on Benzo(*a*)pyrene, 2 µg/kg ww (OJEU, 2006)
Yellowtail	Port Elizabeth	533.95 ± 34.36	29.35 ± 2.38	7.48 ± 5.18	
Blacktail	Mossel bay	437.38 ± 16.33	28.99 ± 0.93	ND	The DDT critical level of DDT as 5000 µg/kg (US EPA, 2000)
Hottentot	Dassen Island	405.45 ± 140.31	ND	760.50 ± 484. 53	
Tuna (dark) muscle	False Bay	636.61 ± 36.03	22.01 ± 3.41		
Tuna light	False Bay	369.41 ± 64.72	17.53 ± 1.60	229.09 ± 2.04	
Yellowtail	Struis Bay	88.97 ± 2.83	0.25 ± 0.07	ND	
Hottentot	Saldahna Bay	87.29 ± 1.96	ND	ND	
Soupfin	Port Elizabeth	102.29 ± 2.68	ND	ND	
Blacktail	Durban	147.22 ± 14.63	ND	ND	

Values are mean ± Standard error (µg/kg wet weight)

Table 8.2. General overview of levels mean ± Std. Error (critical value) µg/kg ww of PAHs, BaP, DDT & OCPs of studied species indicating respective locations

Species (muscle)	Scientific name	Trophic level	Location	Σ PAHs	BaP (2 µg/kg)	DDT (5000)	ΣOCPs (300)
Tuna (dark)	*Thunnus albacares*	High trophic level	False Bay	636.61 ± 36.03	22.01 ± 3.41	4.47 ± 1.64	12.12 ± 2.9
Tuna light	*T. albacares*	High trophic level	False Bay	369.41 ± 64.72	17.53 ± 1.60	229.09 ± 2.04	5.36 ± 0.9
Mako shark	*Isurus oxyrinchus*	High trophic level	Cape Francis	458.29 ± 13.37	26.14 2.93	20.94 ± 0.56	ND
Blue shark	*Prionace glauca*	High trophic level	False Bay	508.09 ± 27.10	29.71 0.89	20.86 ± 0.38	ND
Smoothhound	*Mustelus mustelus*	High trophic level	Langebaan	489.40 ± 16.13	29.58 1.06	20.09 ± 0.39	ND
Soupfin	*Galeorhinus galeus*	High trophic level	Port Elizabeth	102.29 ± 2.68	ND	NA	NA
Yellowtail	*Seriola lalandi*	High trophic level	Port Elizabeth	533.95 ± 34.36	29.35 2.38	7.48 ± 5.18	23.96 ± 5.98
Yellowtail	*S.lalandi*	Middle trophic level	Yzerfontein	221.40 ± 33.03	6.16 0.92	11.14 ± 1.44	1.69 ± 0.88
Yellowtail	*S.lalandi*	Middle trophic level	Struis Bay	88.97± 2.83	0.25 0.07	ND	ND
Snoek	*Thyrsites atun*	Middle trophic level	Dassen Island	122.94 ± 9.96	ND	NA	NA
Blacktail	*Diplodus sargus capensis*	Low trophic level	Mossel bay	437.38 ± 16.33	28.99 ± 0.93	ND	ND
Blacktail	*D.sargus capensis*	Low trophic level	Muizenberg	331.39 ± 16.85	27.85 ± 0.83	ND	ND
Blacktail	*D.sargus capensis*	Low trophic level	Saldahna	426.93 ± 8.96	ND	ND	ND
Blacktail	*D.sargus capensis*	Low trophic level	Witsand	325.40 ± 13.71	ND	ND	ND
Blacktail	*D.sargus capensis*	Low trophic level	Durban	147.22 ± 14.63	ND	ND	ND
Blacktail	*D.sargus capensis*	Low trophic level	Port Elizabeth	406.38 ± 13.79	ND	ND	ND
Hottentot	*Pachymetopon blochii*	Low trophic level	Dassen Island	405.45 ± 140.31	ND	760.50 ± 484 53	0.87 ± 0.39
Hottentot	*P. blochii*	Low trophic level	Hout Bay	141.22 ± 12.86	ND	3.88 ± 0.32	8.69 ± 5.56
Hottentot	*P. blochii*	Low trophic level	Hondeklip	246.97 ± 46.46	ND	ND	0.33 ± 0.07
Hottentot	*P. blochii*	Low trophic level	Kalk Bay	286.44 ± 40.33	ND	ND	0.22 ± 0.07
Hottentot	*P. blochii*	Low trophic level	Lamberts bay	301.24 ± 44 58	ND	ND	1.26 ± 0.61
Hottentot	*P. blochii*	Low trophic level	Saldahna Bay	87.29 ± 1.96	ND	ND	ND

145

The sixteen priority PAHs (US EPA, 1998; US EPA, 2000) and organochlorine pesticides (UNEP, 2005) were screened in selected marine species (hottentot, blacktail, yellowfin tuna, yellowtail, snoek, blue shark, mako shark, soupfin and smoothhound sharks) from different locations on the coast of South Africa. The aim of which was to establish the trend, profiles, sources and levels of studied contaminants. The study revealed the occurrences of PAH contaminants in the selected species from all the 21 study locations with a profile trend of predominantly low molecular weight (LMW) PAHs (2-3 benzene rings) in all species from 19 locations (except at Port Elizabeth and Dassen Island).The implication of this findings suggest that the input of PAHs into the South African marine environment was of a common source to these study locations.

Based on a ratio of LMW to HMW PAHs > 1 (as petrogenic) diagnostic measurement (Naso *et al.*, 2005; Coelhan *et al.*, 2006; Brown & Peake, 2006; Karlsson & Viklander, 2008), the common input sources of PAHs was determined to be from petroleum. In addition the dominance of naphthalene (over other PAHs) as an indicator of pollution from petroleum (petrogenic) (Irwin *et al.*, 1997; Boehm, 2005) further confirms the source of the marine pollution. Other abundant LMW PAHs were fluorene, phenanthrene and anthracene. The input of PAHs into the South African marine environment have been reported to be due to the entry of oil from oil tanker spills, drilling and refineries usually carried out near the coastal areas (Attwood, 2000; UNEP, 2006). On the other hand the dominance of the LMW PAHs in investigated fish can be due to a number of factors such as the input volume of petroleum into the marina as they enter in the form of colloids. Thus LMW are readily bioavailable to fish (being more soluble in water) through the fish skin and gills than HMW conveyed via air currents attached to particles that then sink as sediment. In general therefore the retention (concentration) of PAHs is a function of the biotransformation and excretion ability of species with HMW being readily metabolized and excreted (Gert-Jan deMaagd & Vethaak, 1998; Kaushik & Kaushik, 2007).

The PAHs profile trend observed from the study was in agreement with some published observations from Northern China (Xu *et al.*, 2011) and Ghana (Bandowe *et al.*, 2014). However, for yellowtail fish from Port Elizabeth the input was considered to be a mixture of both petrogenic and pyrogenic as the percentage distribution of LMW (49.61 %) and HMW (50. 39 %) were not statistically significant different. In soupfin and blacktail from Port Elizabeth though not sampled from the same point and period, the PAHs input reflected petrogenic origin. The longevity of species affecting the duration of exposure and volume of uptake from the environment based on species biotransformation ability is indicated in the observed variations.

146

The concentration of PAHs from all the study locations fell within two range levels (Baumard *et al.*, 1998) of low contamination (<100 µg/kg) (measured in hottentot from Saldahna Bay (87.29 ± 1.96) and yellowtail from Struis Bay (88.97 ± 2.83 µg/kg) and moderate contamination (<1000 µg/kg) in fish from the remaining locations. In general the highest mean concentration of total PAHs considering all studied species was found in yellowfin tuna dark muscle (636.61 ± 36.03 µg/kg ww) from False Bay, this was followed by yellowtail from Port Elizabeth (533.95 ± 34.36 µg/kg ww) and thirdly by Blue Shark from False Bay (508.09 ± 27.10 µg/kg ww). Predatory species have been known to bioaccumulate higher levels of OCs from (contaminated) diets (Gelsleichter, *et al.*, 2005; Lee *et al.*, 2015; Weijs *et al.*, 2015).

PAHs acceptability indicator benzo(*a*)pyrene, was found in concentrations below the maximum limit (2 µg/kg ww) only in yellowtail from Struis Bay (Chapter 5), whereas, in the other 5 locations (False Bay, Cape Francis, Langebaan and Port Elizabeth), it exceeded the EU maximum limit. However, benzo(*a*)pyrene was not detected in soupfin (high trophic level) and hottentot (resident and low trophic level). This could possibly mean that these species, though with high PAH levels, could not bioaccumulate benzo(*a*)pyrene which belong to HMW PAH. The studied predatory species (tuna, yellowtail and the sharks), observed with higher levels above the maximum limit may need further investigation so as to establish consumption health risks with concerned population groups such as immunologically challenged (HIV positive) consumers. This observed variation between the predatory and non-predatory species, in the bioconcentration of benzo(*a*)pyrene may be due to variation in feeding habit (Kleinow *et al.*, 1987). Also, the observed intra species variation in the level of benzo(*a*)pyrene in yellowtail from Struis Bay (0.25 ± 0.07 µg/kg ww) and from Port Elizabeth (29.35 ± 2.38 µg/kg ww) could be due to locational, size and /or lipid effect (Kleinow *et al.*, 1987; Said, 2007; Gomes *et al.*, 2013).

The OCPs screened in the studied fish included DDT, BHC (alpha, beta, delta, and gamma) endosulfan (alpha and beta), aldrin, eldrin, dieldrin, aldrin aldehyde, endosulfan sulfate. Only a few OCPs (DDT, BHC and endosulfan) were detected in few species and in concentrations below maximum limits. The most common pesticide detected was DDT and the 4,4´-DDT analogue was most abundant; while the other DDT analogues (DDE and DDD) were found below detection limit. The highest concentration (760.50 ± 484.53 µg/kg ww) found in hottentot from Dassen Island while the least overall mean DDT level was 3.88 ± 0.32 µg/kg ww (in hottentot) from Hout Bay. The predominance of 4,4´-DDT analogue was an indication of current DDT application or usage. However, DDT prevalence in the environment does not necessarily imply its usage for IRS but could also be introduced through Dicofol pesticide or from paint containing DDT as antifouling agent. The need to urgently look into the use of DDT containing paint and pesticides should be revisited by appropriate government

147

ministries/departments (agriculture, health and environment) through their pesticide management agencies to forestall additional environmental input in view of its global restriction to health sector only. In summary therefore the following were covered in this study:

- Optimal laboratory procedures for a simultaneous detection of PAHs and organochlorine pesticides in marine fish species using gas chromatography coupled to mass spectrometer triple quadrupole (GC-MS/MS) was achieved (chapters 3,5,6 & 7). Also by a comparison of GC-MS/MS and Ultra high pressure liquid chromatography with fluorescence detection (UPLC-FLD), optimal analytical procedures for PAHs (only) in marine fish was achieved (Chapter 4).

- The level of PAHs contamination in the studied marine fish species were found to be within low contamination (<100 $\mu g/kg$) in 2 fish species hottentot (Saldhna Bay) and yellowtail (Struis Bay). While the rest species from the remaining locations had contaminantion level within moderate contamination (<1000 $\mu g/kg$).

- Tuna dark and light muscles were assessed for muscle type variation (Chapter 3), yellowtail was studied with respect to locations fish size and lipid content effect (Chapter 5).

- Diagnostic ratio of detected contaminants (for PAHs in particular LMW/HMW) were used to estimate contaminant source and for DDT as only 4,4´-DDT metabolite was predominantly detected, it denotes fresh DDT usage.

- Detected OC levels were compared with international regulatory limits (South Africa as a member of EU, in absence of national limits of studied contaminants in fish) in order to identify if the meat from each species was within acceptable limits for human consumption. The maximum acceptable limit of 2 µg/kg for benz(*a*)pyrene as indicator of PAHs by EU and FAO/WHO in raw fish was exceeded in all the studied high trophic level species except in soupfin (Tuna and Sharks) (Chapters 3-6).

- The occurrence of these priority contaminants (especially PAHs) in the selected commercially important and consumable species, in levels above the

148

EU and FAO/WHO's benzo(*a*)pyrene acceptable limit, calls for further investigation.

Thus the need for joint government initiated collaborative and participatory research with stakeholders such as government departments (water, agriculture, fisheries, environment and health), regulatory agencies (e.g food, pesticide, drugs and cosmetics), research institutes (e.g. universities and research organizations), industries (e.g. fishing, chemical producing/using), organized private sectors, fish farmers and consumers representatives/associations. This will promote better understanding of pollution input, sources and best waste management in the country. Holistic assessment of nutrients, contaminants and bioavailabilty studies if conducted will give more factual information to the general public. In view of the huge financial requirements for study of this nature, only such a joint collaborative study will be confirmatory to the present screening study.

It is also recommended that future study be extended to fresh water and other marine species such as molluscs, (such as clams, mussels, oysters and scallops), crustaceans (such as shrimp, crab, lobster and Barnacles) and echinoderms (such as sea urchins and sea cucumbers) as these are important consumable sea foods to South African people. Furthermore, future research should be geared towards mitigation of these contaminants so as not to undermine the associated fish health benefits, by investigating/ researching into the best bio-processing or cooking method(s) suited to remove or significantly reduced these contaminants.

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150

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153