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Comparative analysis of organochlorine accumulation in two top predator shark species with contrasting life history traits

By

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Comparative analysis of organochlorine accumulation in two top predator shark species with contrasting life history traits

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ABSTRACT

Sharks vary widely in life history strategies, and possess unique lipid metabolism. Because of this diversity, and organochlorine (OC) lipid association, dynamics of OC accumulation in elasmobranch rich ecosystems may be ineffectively predicted by the typically relied upon indicators; stable isotopes and body size. I compared ontogenetic OC profiles and concentrations between dusky (*Carcharhinus obscurus*) and white (*Carcharodon carcharias*) sharks. OC concentrations were elevated in the larger, higher trophic position (TP) white shark, but despite a tenably faster growth rate, ontogenetic diet shifts to mammal prey, and regional endothermy, slopes of growth dilution corrected concentrations with age were equal between species. Similar ontogenetic TP increases, infrequent white shark seal predation, or inaccurate growth parameters are possible explanations. OCs varied with inshore habitat use and mass in white and dusky sharks respectively. TP and mass predicted interspecific concentrations and may therefore be considered reliable indicators of OC accumulation in these sharks.

DEDICATION

This thesis is dedicated to the memory of Eleanor Mae (“Elly-Mae”) Enns.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/SYMBOLS

2,3,7,8-TCDD	a polychlorinated dibenzo-p-dioxin: 2,3,7,8-tetrachlorodibenzodioxin	2
AhR	aryl hydrocarbon receptor	2
ANCOVA	analysis of covariance	25
ANOSIM	analysis of similarities	23
ANOVA	analysis of variance	23
BDE-71	2,3’,4’,6-tetrabromodiphenyl ether	21
CYP1A1	a member of the cytochrome P450 enzyme superfamily; family 1 member A1	3
DCM	dichloromethane, aka. methylene chloride, technical grade	21
e.g.	*exempli gratia*, ‘for example…’	16
et al.	*et alii*, ‘and others’	1
FL	fork length: snout tip to notch in postventral caudal margin	48
GC-ECD	gas chromatograph electron capture detector	21
GC-MSD	gas chromatograph mass selective detector	21
Hx	hexane, technical grade	21

i.e.
id est, ‘that is to say…’ ……3

K$_{ow}$
octanol-water partition coefficient……1

KZN
KwaZulu-Natal, South Africa……7

KZNSB
KwaZulu-Natal Sharks Board……20

OC
organochlorine……1

OCP
organochlorine pesticide……2

PAH
polycyclic aromatic hydrocarbon……3

PC axes (PC1, PC2, PC3, etc.)
principal component analysis-generated axes……24

PCA
principal component analysis……24

PCB
polychlorinated biphenyl……2

PCL
pre-caudal length: snout tip to upper caudal origin……20

POP
persistent organic pollutant……3

PTFE
polytetrafluoroethylene……21

SCA
stomach contents analysis……8

SE
standard error……43

SIA

stable isotope analysis……………………………………………………………8

SIMPER

percent similarity…………………………………………………...............23

TMF

trophic magnification factor…………………………………………………4

Δ^{15}N

diet-tissue discrimination factor……………………………………………..4

δX

change in ratio of X as compared to a standard, X = either ^{15}N (^{15}N:^{14}N), or ^{13}C (^{13}C:^{12}C)…………………………………………………………………………….23

δ, Δ

delta, 'change in…' ………………………………………………………….....4

δ^{15}N or δ^{13}C

change in ratio of ^{15}N:^{14}N or ^{13}C:^{12}C as compared to a standard ………………..…...4

CHAPTER 1

GENERAL INTRODUCTION

1.1 Introduction

Despite the banning of organochlorine contaminants (OCs) in the majority of countries between 1970 and 1980 (AMAP 1998; Vallack *et al.* 1998), they are still found ubiquitously in the environment. This worldwide distribution is due in large part to their chemical stability, hydrophobicity, and semi-volatility (Smaranda & Gavrilescu 2008). Biological and environmental breakdown processes have slow effects on OCs, as their chlorine component makes them very chemically stable (WHO 1993). Consequently, OCs have long environmental half-lives, and compounds that are more highly chlorinated without adjacent unsubstituted carbon atoms tend to be retained the longest (AMAP 1998). Because of this, and due to their hydrophobicity, these compounds bioaccumulate in the bodies of organisms with passive OC solubilization into lipid compartments, according to the chemical's affinity for lipid matrices (Kainz & Fisk 2009). The tendency of an OC to pass from aqueous solution into lipid (hydrophobicity) is expressed by the octanol-water partition coefficient, K_{ow} (Vallack *et al.* 1998). In aquatic organisms, water-uptake alone (bioconcentration) would produce a steady-state equilibrium concentration of contaminant in the organism's lipid stores, based on the K_{ow} of the chemical and its concentration in the water (Swackhammer 2003). However, since aquatic organisms are exposed to uptake through diet as well, OC dynamics are closely tied with lipid movements along increasing trophic levels of food webs. At high trophic positions, OC concentrations are found to reach much higher levels than would be expected at equilibrium from water concentrations and K_{ow} alone (AMAP 1998;

Swackhammer 2003). This phenomenon is called biomagnification, a specific case of bioaccumulation, and it is defined as the increased accumulation of a contaminant with each trophic level in a food web, beyond the concentration expected from steady-state equilibrium with water concentrations (AMAP 1998; Swackhammer 2003). For very lipophilic compounds (*log* $K_{ow} \geq 5$) with consequent high resistance to transformation/excretion, diet is the main exposure route for upper trophic level consumers (Kainz & Fisk 2009; Thomann 1981).

Biomagnification of PCBs and OCPs can intensify their toxicity. For example, while coplanar PCBs are toxic in small amounts (PCBs 77, 126 and 169: bind to the AhR receptor which induces toxicity similar to 2,3,7,8-TCDD, one of the most toxic compounds ever known), the majority of PCBs obtained by prey ingestion in a food web are non-coplanar and thus do not bind to the AhR receptor (Fischer *et al.* 1998). In this case, physiological dysfunction is not an issue until higher levels are acquired (Giesy & Kannan 1998), which, via biomagnification, can then cause symptoms like immunosuppression, hormonal alterations and tumour production in apex predators (Beland *et al.* 1993). Despite the likelihood of high OC loads in top predators, few studies have attempted to quantify OC accumulation in tropical or subtropical marine apex predator fish, especially sharks (Schlenk *et al.* 2005; Gelsleichter *et al.* 2005). Though no studies have directly demonstrated OC physiological toxicity in sharks, the few observations that have been made demonstrate that high OC levels predominate in sharks; moreso in long-lived, deep-water species (Schlenk *et al.* 2005; Mull *et al.* 2012; Gelsleichter *et al.* 2005; Johnson-Restrepo *et al.* 2005). This is to be expected given the typically high trophic positions and large body sizes of these animals. Larger animals tend to live longer (Speakman 2005), and lifespan is inherent to an organism's OC accumulation potential. In addition to consuming larger-bodied prey (processing more biomass) with increased body size, larger-bodied individuals accumulate OCs to a greater extent than smaller ones do (Paterson *et al.* 2006; Johnston *et al.* 2002). This is because elimination capacities decrease as fish become larger-bodied (Sijm & van der Linde 1995; Fisk *et al.* 1998) owing to an allometric increase in the ratio of an animal's chemical storage capacity to depuration sites with increasing size (LeBlanc 1995). In sharks, other persistent organic pollutants (POPs) have been reported to induce the same physiological mechanisms for toxicity defence (*i.e.* biotransformation or sequesterization) that are present in all vertebrates [CYP1A1 induction by PAH exposure in the redspotted catshark, *Schroederichthys chilensis* (Fuentes-Rios *et al.* 2005)]. Therefore, OCs also have the potential to exert toxic effects on the fitness of individual sharks and adversely affect shark populations. Especially as a compounding stressor to globally declining shark populations due to selective capture via commercial fishing operations and shark fin trade-driven incentives (Worm *et al.* 2013, Ferretti *et al.* 2010), habitat degradation (Ward-Paige *et al.* 2010, Knip *et al.* 2010), and the overarching stressor of climate change (Hollowed *et al.* 2013), potential outcomes of OC exposure on the sustainability of shark populations is an imperative consideration (Gelsleichter *et al.* 2005, Lyons *et al.* 2013).

Since chemical energy is primarily transferred through food webs in the form of lipids (Kainz & Fisk 2009), food web structure may be mapped using POPs (Ramos & González-Solís 2012). OC levels provide an indication of the number of trophic transfers that obtained energy underwent on its way to a consumer, due to the magnification of concentrations with each of these events. OC biomagnification tends to correlate well with $\delta^{15}N$-derived trophic positions (Vander Zanden & Rasmussen 1996; Fisk *et al.* 2001), another widely used tool in ecology for quantifying trophic structure, but one which has many drawbacks and uncertainties at the present time. While each method provides a measure of food web ‘effective’ trophic structure (Williams & Martinez 2004), OC-based tracers are important to be looked at in combination with stable isotopes because of discrepancies such as a wide variety of isotopic ratios at basal trophic levels, and many analytical assumptions (Williams & Martinez 2004). In particular, the enrichment factor of $\delta^{15}N$ between trophic levels ($\Delta^{15}N$) is now thought to be trophic level and ecosystem-specific (Hussey *et al.* 2014; Burkhard *et al.* 2013). OCs may therefore allow ecosystem-specific enrichment factors to be derived based on the ‘Trophic Magnification Factor’ (TMF) of that system; the factor by which biomagnification occurs with successive trophic levels across an entire food web (Burkhard *et al.* 2013). However, the utility of OCs as an accurate predictor of trophic structure within shark-rich food webs remains an important scientific question, given the high diversity of trophic interactions across and between shark species, the wide variation in their life history characteristics, and the resulting high probability of variation in sharks’ ecological roles (Ferretti *et al.* 2010). In addition, few studies have confirmed whether the physiological distinctiveness of sharks from other marine taxa has the potential to diffuse the typically strong relationship between OC concentrations and trophic positions of individuals. Consequently, the level of clarity that OC dynamics may provide to stable isotope–derived trophic structure within shark-rich communities is unknown.

Because of their life history variability, and their physiological distinctiveness from other aquatic vertebrates, predicting OC accumulation in shark species is difficult. The uptake, accumulation and distribution of OCs within a shark-rich system can be affected by migration patterns, resource partitioning, intraguild predation tendency, ontogenetic diet shifts, seasonal lipid changes, and metabolic activity level, while may of these traits may differ depending on shark species, sex, life stage, and reproductive status (Ylitalo *et al.* 2001; Borga *et al.* 2001, 2004; Sims 2005). Physiologically, sharks differ in their metabolic regulation of lipid as compared to other marine taxa, which may alter the dynamics of OCs in systems incorporating sharks. Firstly, this is due to their process of osmoregulation. To retain high enough levels of nitrogenous compounds (*i.e.*, osmolytes) to remain hyperosmotic to seawater, the modification of elasmobranchs' metabolic system affects many processes such as amino acid, methylamine, ketone body and lipid metabolism (Ballantyne & Robinson 2010). For example, elasmobranchs differ from all other vertebrates in their absence of a fatty acid-binding protein in the blood (binding to any such protein is speculated to be disrupted by urea; a chaotropic agent present in high levels in all body fluid compartments) (Ballantyne & Robinson 2010). Secondly, the absence of a swim-bladder in sharks causes buoyancy regulation to result instead from a combination of hydrostatic lift from low density lipid-rich liver stores, and hydrodynamic lift from fin and tail swimming movements (Wetherbee & Nichols 2000). Consequently, storage and mobilization of lipid from shark liver is likely regulated differently due to buoyancy control being a function of priority of that organ (Baldridge 1972). This development may also be responsible for the markedly different biochemical composition of lipids in elasmobranch fishes (Love 1970, Lewis *et al.* 1969), and this has been found to affect the accumulation pattern of hydrophobic contaminants in aquatic organisms (Kainz & Fisk 2009). Third, elasmobranchs differ reproductively in that all fertilization occurs internally and the majority are viviparous (55%), while teleosts exhibit external fertilization and only 2-3% are viviparous (Miller & Kendall 2009; Ballantyne & Robinson 2010). Trophic movements of OCs may be altered due to species-specific reproductive mechanisms of maternal nutrient transfer, even within a category such as viviparity (Oka *et al.* 2006), and due to unique mechanisms in sharks like reproductive oophagy (Lyons & Lowe 2013). Lastly, the presence of regional endothermy in at least lamnid sharks is expected to cause differential OC accumulation due to inducing higher metabolic rates than in ectothermic sharks (Carlson *et al.* 2004). Physiological energy and biomass processing requirements are likely increased in this case as biomass conversion efficiencies are driven down to approximately 0.5-3% in mammalian endotherms, as opposed to up to 87% efficiency in ectotherms (Norstrom 2002).

Enhancing our knowledge of shark species' trophic ecology, and determining the potential extent of the toxic impacts of OCs on shark populations, would further our ability to avoid their extensive decline which could leave supporting trophic structures susceptible to 'trophic cascades'. This is a particular concern in marine systems, where selective over-fishing pressure has been imposed for over six decades, favouring the removal of large-bodied top predator fish (Pauly *et al.* 1998). Predation pressure is consequently released on meso-predators, causing an imbalance in biomass and energy that perpetuates downward through food webs via expansion and suppression of abundances at alternating trophic levels, and potentially disrupting crucial basal resources (Heath *et al.* 2014; Frank *et al.* 2005; Myers *et al.* 2007; Dulvy *et al.* 2004). Alterations in top predator abundance may thus affect the integrity of their entire supporting food webs, and may even alter abiotic ecosystem components such as the biogeochemical balances of carbon and silica in seawater (Estes *et al.* 2011). Structural disruption can also occur simultaneously in multiple geographically discrete food webs due to the high mobility of top predator fish species. Perpetuating consequences of cascades may be lasting, depending on cascade magnitude and species' ability to adapt and/or re-establish in the face of top-down (mesopredatory release) and subsequent bottom-up (resource depletion) effects, collapsing the structure needed to support diverse community assemblages (Heath *et al.* 2014). Implications may also not appear for decades due to initial compensatory mechanisms (*i.e.*, fish with rapid turnover substituting in for predator species who are more susceptible to removal) (Kitchell *et al.* 2002). However, removal of top fish predators as a full functional group is the prospective end result of these continued anthropogenic stresses unless informed management decisions can avoid this.

One study system where cascades have been documented with magnitudes strong enough for conspicuous assemblage changes upon the removal of top shark species, is that of the east coast of South Africa, specifically KwaZulu-Natal (KZN) (Ferretti *et al.* 2010). No question therefore exists about the importance of these shark predators to the underlying structure of this ecosystem. However, the mechanisms of indirect effects and the actual changes in ecosystem structure that influence the patterns of these responses over time are unknown (*i.e.*, directions of response in teleost abundance reversed between two historical periods of large shark decline) (Ferretti *et al.* 2010). A need therefore exists to understand the trophic linkages between these sharks and their prey to gain an understanding of the mechanisms driving cascade outcomes in this system. Substantial characterisation of trophic position, habitat use and dietary niche of numerous key shark species within the KZN food web has been accomplished using both stable isotope and stomach content analyses (SIA/SCA) (Hussey *et al.* 2014). Resolution of potential inconsistencies in these analyses may still be gained with the OC-derived trophic structure of this system, either in confirming species' SCA/SIA-derived trophic ecology, or in resolving inconsistencies surrounding diet discrimination and/or variable signatures due to periods of altered body condition (*i.e.*, fasting/starvation) (Hobson *et al.* 1993). OC-derived trophic structure may prove particularly useful with sharks, because $\delta^{15}N$ is based on a limited time period before tissue turnover (which may reflect periods of atypical feeding behaviour; Hussey *et al.* 2011, 2012). Examination of shark muscle tissue for OCs, which is buffered from body-condition related lipid variation (Watson & Dickson 2001) as opposed to liver stores which are relied upon in these cases (Hussey *et al.* 2010), would provide a robust lifetime-based view of OC accumulation and overall feeding ecology that is resistant to large alterations due to OC bioamplification (Daley *et al.* 2014).

While providing valuable insight into what extent OC biomagnification may be acting as a compounding stressor to the top marine predators of KZN, South Africa, the objective of my MSc research is to quantify the influence of different life history characteristics on OC patterns and concentrations in two top predator shark species. This will further our ability to determine whether OC accumulation provides an accurate reflection of the trophic structure in this system. Because of previous studies on the physiology and life history of lamnid and carcharhinid sharks, and the current SCA/SIA-acquired knowledge into KZN shark trophic ecology, a unique opportunity exists to relate

OC accumulation in these species to differences in these aspects. The dusky (*Carcharhinus obscurus*) and the white *(Carcharodon carcharias*) shark were chosen for this model comparison due to their divergence in particular life history traits that are expected to oppositely affect OC profiles, accumulation, and ontogenetic patterns. These traits include diet shifts in reverse trophic directions with size (Hussey *et al.* 2011, 2012), as well as opposing modes of thermoregulation (Carlson *et al.* 2004). Although recent discrepancy has arisen (Hamady *et al.* 2014), it is still reasonably likely that these species also possess differing growth rates (Semmens *et al.* 2013, Natanson *et al.* 2013, Wintner & Cliff 1999). These latter two differences would translate into different basal metabolic rates (Horodysky *et al.* 2011; Carlson *et al.* 2004), which have also been previously found between carcharhinid and lamnid sharks (Schmid & Murru 1994). Life history similarities shared between these species are those shared by top-predator sharks in general; large-body size, low fecundity, large offspring and late age-at-maturity (Cortés 2000). Most likely due in large part to the limiting nature of these traits on population recruitment in the face of ongoing anthropogenic impacts, each of these species holds a "vulnerable" status (IUCN 2013). Through the efforts of this study, a better understanding may be gained of the trophic ecology of these species, and of the extent that OC biomagnification acts as a compounding stressor to the shark species off the east coast of South Africa. This knowledge may improve shark conservation efforts and management decisions, which may in turn affect the stability of this complete food web system and the resultant health of this entire ecosystem.

1.2 References

AMAP, 1998. Chapter 6: Persistent organic pollutants. IN: AMAP Assessment Report: Arctic Pollution Issues Arctic Monitoring and Assessment Programme (AMAP), Oslo, Norway. pp 183-335.

Baldridge, H.D. Jr. 1972. Accumulation and function of liver oil in Florida sharks. *Copeia* 1972 (2): 306-325.

Ballantyne, J.S., Robinson, J.W. 2010. Freshwater elasmobranchs: a review of their physiology and biochemistry. *J Comp Physiol B* **180**: 475-493.

Beland, P., DeGuise, S., Girard, C., Lagace, A., Martineau, D., Michaud, R., Muir, D.C.G., Norstrom, R.J., Pelletier, E., Ray, S., Shugart, L.R. 1993. Toxic compounds and health and reproductive effects in St. Lawrence beluga whales. *J Great Lakes Res* **19**(4): 766-775.

Borgå, K., Fisk, A.T., Hoekstra, P.F., Muir, D.C.G. 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environ Toxicol Chem* **23**(10): 2367-2385.

Borgå, K., Gabrielsen, G.W., Skaare, J.U. 2001. Biomagnification of organochlorines along a Barents Sea food chain. *Environ Poll* **113**(2): 187-198.

Burkhard, L.P., Borgå, K., Powell, D.E., Leonards, P., Muir D.C.G., Parkerton, T.F., Woodburn, K.B. 2013. *Environ Sci Technol* **47**: 1186-1187.

Carlson, J.K., Goldman, K.J., Lowe, C.G. 2004. Metabolism, energetic demand, and endothermy. IN: Carrier, J.C., Musick, J.A., and Heithaus, M.R (eds). *Biology of sharks and their relatives.* CRC Press, Boca Raton, FL, USA. pp. 203-224.

Cortés, E. 2000. Life history patterns and correlations in sharks. *Rev Fish Sci* **8**(4): 299-344.

Daley, J.M., Paterson, G., Drouillard, K.G. 2014. Bioamplification as a bioaccumulation mechanism for persistent organic pollutants (POPs) in wildlife. IN: Whitacre, D.M. (ed.), *Reviews of Environmental Contamination and Toxicology* 227: 107-155.

Dulvy, N.K., Freckleton, R.P. & Polunin, N.V.C. 2004. Coral reef cascades and the indirect effects of predator removal by exploitation. *Ecol Lett* **7**: 410–416.

Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soulé, M.E., Virtnanen, R., Wardle, D.A. 2011. Trophic downgrading of planet earth. *Science* **333**: 301-306.

Ferretti, F., Worm, B., Britten, G.L., Heithaus, M.R., Lotze, H.K. 2010. Patterns and ecosystem consequences of shark declines in the ocean. *Ecol Lett* **13**: 1055-1071.

Fischer, L. J., Seegal, R. F., Ganey, P. E., Pessah, I. N., & Kodavanti, P. R. 1998. Symposium overview: toxicity of non-coplanar PCBs. *Toxicol Sci* **41**(1): 49–61.

Fisk, A.T., Hobson, K.A., Norstrom, R.J. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. *Environ Sci Technol* **35**: 732-738.

Fisk, A.T., Norstrom, R.J., Cymbalisty, C.D., and Muir D.C.G. 1998. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environ Toxicol Chem* **17**(5): 951-961.

Frank, K.T., Petrie, B., Choi, J.S., Leggett, W.C. 2005. Trophic cascades in a formerly cod-dominated ecosystem. *Science* **308**: 1621-1623.

Fuentes-Rios, D., Orrego, R., Rudolph, A., Mendoza, G., Gavilan, J.F., Barra, R. 2005. EROD activity and biliary fluorescence in *Schroederichthys chilensis* (Guichenot 1848): biomarkers of PAH exposure in coastal environments of the South Pacific Ocean. *Chemosphere* **61**: 192-199.

Gelsleichter, J., Manire, C.A., Szabo, N.J., Cortés, E., Carlson, J., Lombardi-Carlson, L. 2005. Organochlorine concentrations in bonnethead sharks (*Sphyrna tiburo*) from four Florida estuaries. *Arch Environ Contam Toxicol* **48**: 474-483.

Giesy, J.P., Kannan., K. 1998. Dioxin-like and non-dioxin-like toxic effects of polychlorinated biphenyls (PCBs): Implications for risk assessment. *Crit Rev Toxicol* **28**(6): 511-569.

Hamady, L.L., Natanson, L.J., Skomal, G.B., Thorrold, S.R. 2014. Vertebral bomb radiocarbon suggests extreme longevity in White Sharks. *PLoS ONE* **9**(1): e84006. doi:10.1371/journal.pone.0084006

Heath, M.R., Speirs, D.C., Steele, J.H. 2014. Understanding patterns and processes in models of trophic cascades. *Ecol Lett* **17**: 101-114.

Hollowed, A.B., Barange, M., Beamish, R.J., Brander, K., Cochrane, K., Drinkwater, K., Foreman, M.G.G., Hare, J.A., Holt, J., Ito, S., Kim, S., King, J.R., Loeng, H., MacKenzie, B.R., Mueter, F.J., Okey, T.A., Peck, M.A., Radchenko, V.I., Ricel, J.C., Schirripa, M.J., Yatsu, A., Yamanaka, Y. 2013. Projected impacts of climate change on marine fish and fisheries. *ICES J Mar Sci* **70**(5): 1023-1037.

Horodysky, A.Z., Brill, R.W., Bushnell, P.G., Musick, J.A. 2011. Comparative metabolic rates of common western North Atlantic Ocean sciaenid fishes. *J Fish Biol* **79**: 235-255.

Hussey, N.E., Dudley, S.F.J., McCarthy, I.D., Cliff, G., Fisk, A.T. 2011. Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? *Can J Fish Aquat Sci* **68**: 2029-2045.

Hussey, N.E., MacNeil, M.A., McMeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G., Wintner, S.P., Fennessy, S.T., Fisk, A.T. 2014. Rescaling the trophic structure of marine food webs. *Ecology Letters* **17**: 239-250.

Hussey, N.E., McCann, H.M., Cliff, G., Dudley, S.F.J., Wintner, S.P., Fisk, A.T. 2012. Chapter 3: Size-based analysis of diet and trophic position of the white shark, *Carcharodon carcharias*, in South African waters. IN: Domeier, M.L. (ed): *Global perspectives on the biology and life history of the white shark*. CRC Press; Taylor and Francis Group, Boca Raton, FL, USA. pp. 27-49.

Hussey, N.E., Wintner, S.P., Dudley, S.F.J., Cliff, G., Cocks, D.T., MacNeil, M.A. 2010. Maternal investment and size-specific reproductive output in carcharhinid sharks. *J Anim Ecol* **79**: 184-193.

IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. <www.iucnredlist.org>. Downloaded on 31 March 2014.

Johnson-Restrepo, B., Kannan, K., Addink, R., Adams, D.H. 2005. Polybrominated diphenyl ethers and polychlorinated biphenyls in a marine foodweb of coastal Florida. *Environ Sci Technol* **39**: 8243-8250.

Johnston, T.A., Fisk, A.T., Whittle, D.M., D.C.G. Muir. 2002. Variation in organochlorine bioaccumulation by a predatory fish; gender, geography, and data analysis methods. *Environ Sci Technol* **36**: 4238-4244.

Kainz, M.J., Fisk, A.T. 2009. Chapter 5: Integrating lipids and contaminants in aquatic ecology and ecotoxicology. IN: Lipids in Aquatic Ecosystems. Arts, M.T., Kainz M., Brett, M.T. (eds.), Springer, New York, USA. pp. 93-113.

Kitchell J.F., Essington, T.E., Boggs, C.H., Schindler, D.E., Walters, C.J. 2002. The role of sharks and longline fisheries in a pelagic ecosystem of the central pacific. *Ecosystems* **5**: 202-216.

Knip, D.M., Heupel, M.R., Simpfendorfer, C.A. 2010. Sharks in nearshore environments: models, importance, and consequences. *Mar Ecol Prog Ser* **402**: 1-11.

LeBlanc, G.A. 1995. Trophic-level differences in the bioconcentration of chemicals: implications in assessing environmental biomagnification. *Environ Sci Technol* **29**: 154–160.

Lewis, R.W. 1969. The densities of three classes of marine lipids in relation to their possible role as hydrostatic agents. *Lipids* **5**(1): 151-153.

Love, R.M. 1970. The chemical biology of fishes: with a key to the chemical literature. Academic Press, London and New York.

Lyons, K., Carlisle, A., Preti, A., Mull, C.G., Blasius, M., O'Sullivan, J.B., Winkler, C., Lowe, C.G. 2013. Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of young of the year lamniform sharks. *Mar Environ Res* **90**: 27-38.

Lyons, K., Lowe, C.G. 2013. Mechanisms of maternal transfer of organochlorine contaminants and mercury in the common thresher shark (*Alopias vulpinus*). *Can J Fish Aquat Sci* **70**: 1667-1672.

Miller, B.S., Kendall, A.W. 2009. Chapter 1: Fish reproduction. IN: Miller, B.S., Kendall, A.W. *Early life history of marine fishes.* University of California Press. pp 9-37.

Mull, C.G., Blasius, M.E., O'Sullivan, J.B., Lowe, C.G. 2012. Chapter 5: Heavy metals, trace elements, and organochlorine contaminants in muscle and liver tissue of juvenile white sharks, *Carcharodon carcharias*, from the Southern California Bight. IN: Domeier, M.L. (ed). *Global perspectives on the biology and life history of the white shark*. CRC Press; Taylor and Francis Group, Boca Raton, FL, USA. pp. 59-75.

Myers, R.A., Baum, J.K., Shepherd, T.D., Powers, S.P., Peterson, C.H. 2007. Cascading effects of the loss of apex predatory sharks from a coastal ocean. *Science* **315**: 1846–1850.

Natanson, L.J., Gervelis, B.J., Winton, M.V., Hamady, L.L., Gulak, S.J.B., Carlson, J.K. 2013. Validated age and growth estimates for *Carcharhinus obscurus* in the northwestern Atlantic Ocean, with pre- and post management growth comparisons. *Environ Biol Fish*: 10.1007/s10641-013-0189-4.

Norstrom, R.J. 2002. Understanding bioaccumulation of POPs in food webs. Chemical, biological, ecological and environmental considerations. *Environ Sci Poll Res* **9**(5): 300-303.

Oka, M., Arai, T., Shibata, Y., Miyazaki, N. 2006. Reproductive transfer of organochlorines in viviparous surfperch, *Ditrema temmincki*. *Environ Pollut* **142**: 383-387.

Paterson, G., Drouillard, K.G., Haffner, G.D. 2006. An evaluation of stable nitrogen isotopes and polychlorinated biphenyls as bioenergetic tracers in aquatic systems. *Can J Fish Aquat Sci* **63**: 628-641.

Pauly, D., Christensen, V., Dalsgaard, J., Froese, R., Torres, F(Jr.). 1998. Fishing down marine food webs. *Science* **279**: 860-863.

Ramos, R., González-Solís, J. 2012. Trace me if you can: the use of intrinsic biogeochemical markers in marine top predators. *Front Ecol Environ* **10**(5): 258–266.

Schlenk, D., Sapozhnikova, Y., Cliff, G. 2005. Incidence of organochlorine pesticides in muscle and liver tissues of South African great white sharks *Carcharodon carcharias*. *Mar Poll Bull* **50**: 208-236.

Schmid, T.H., Murru, F.L. 1994. Bioenergetics of the bull shark, *Carcharhinus leucas*, maintained in captivity. *Zoo Biol* **13**: 177-185.

Semmens, J.M., Payne, N.L., Huvaneers, C., Sims, D.W., Bruce, B.D. 2013. Feeding requirements of white sharks may be higher than originally thought. *Nature: Sci Reports* **3**(1471): 1-4.

Sijm, D. T., van der Linde, A. 1995. Size-dependent bioconcentration kinetics of hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric relationships. *Environ Sci Technol* **29**(11): 2769-2777.

Sims, D.W. 2005. Differences in habitat selection and reproductive strategies of male and female sharks. IN: Ruckstuhl, K.E., Neuhaus, P. (eds). *Sexual segregation in vertebrates: ecology of the two sexes.* Cambridge University Press, Cambridge, pp 127–140.

Smaranda, C., Gavrilescu, M. 2008. Migration and fate of persistent organic pollutants in the atmosphere- a modelling approach. *EEMJ* **7**(6): 743-761.

Speakman, J. R. 2005. Review: body size, energy metabolism and lifespan. *J Exp Biol* **208**: 1717-1730.

Swackhammer, D.L. 2003. Biomagnification. IN: Bortman, M., Brimblecombe, P., and Cunningham, M.A. (eds.) *Environmental Encyclopedia*. Vol 1. 3rd ed. Gale, Detroit, MI, USA. pp. 144-145.

Thomann, R.V. 1981. Equilibrium model of fate of microcontaminants in diverse aquatic food chains. *Can J Fish Aquat Sci* **38**: 280-296**.**

Vallack, H.W., Bakker, D.J., Brandt, I., Broström-Lundén, E., Brouwer, A., Bull, K.R., Gough, C., Guardans, R., Holoubek, I., Jansson, B., Koch, R., Kuylenstierna, J., Lecloux, A., Mackay, D., McCutcheon, P., Mocarelli, P., Taalman, R.D.F. 1998. Assessment: Controlling persistent organic pollutants- what next? *Environ Toxicol & Pharmacol* **6**: 143-175.

Vander Zanden, M.J., Rasmussen, J.B. 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. *Ecol Monogr* **66**(4): 451-477.

Ward-Paige, C.A., Mora, C., Lotze, H.K., Pattengill-Semmens, C., McClenachan, L., Arias-Castro, E., Myers, R.A. 2010. Large-scale absence of sharks on reefs in the greater-caribbean: a footprint of human pressures. *PLoS ONE* **5**(8): e11968. doi:10.1371/journal.pone.0011968

Watson, R.R., Dickson, K.A. 2001. Enzyme activities support the use of liver lipid-derived ketone bodies as aerobic fuels in muscle tissues of active sharks. *Physiol Biochem Zool* **74**(2): 273-282.

Wetherbee, B.M., Nichols, P.D. 2000. Lipid composition of the liver oil of deep-sea sharks from the Chatham Rise, New Zealand. *Comp Biochem Physiol B* **125**: 511-521.

WHO. 1993. Polychlorinated Biphenyls and Terphenyls, 2nd ed. *Environmental Health Criteria* **140**. Geneva: World Health Organization (http://www.inchem.org/documents/ehc/ehc/ehc140.htm#1.1.2)

Williams, R.J., Martinez, N.D. 2004. Limits to trophic levels and omnivory in complex food webs: theory and data. *Am Nat* **163**(3): 458-468.

Wintner, S.P., Cliff, G. 1999. Age and growth determination of the white shark, *Carcharodon carcharias*, from the east coast of South Africa. *Fish Bull* **97**(1): 153-169.

Worm, B., Davis, B., Kettemer, L., Ward-Paige, C.A., Chapman, D., Heithaus, M.R., Kessel, S.T., and Gruber, S.H. 2013. Global catches, exploitation rates, and rebuilding options for sharks. *Mar Poll* **40**: 194-204.

Ylitalo, G.M., Matkin, C.O., Buzitis, J., Krahn, M.M., Jones, L.L., Rowles, T., Stein, J.E. 2001. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK. *Sci Tot Environ* **281**: 183-203.

CHAPTER 2
COMPARATIVE ORGANOCHLORINE ACCUMULATION IN TWO LARGE, ECOLOGICALLY SIMILAR SHARK SPECIES, WITH SELECT LIFE HISTORY CONTRASTS KEY TO DIVERGENT UPTAKE

2.1 Introduction

The high persistence and global distribution of organochlorines (OCs) has caused them to become some of the most important contaminants to affect environmental health (Reijnders 1994; Turosov *et al.* 2002). Accumulation of OCs in biota is an outcome of their hydrophobicity and resistance to breakdown and excretion, driving diet to be the most important route of exposure to the more hydrophobic OCs to most fish (Thomann & Connelly 1984). Consequently, persistent OC concentrations increase with increasing trophic levels in food webs, and this process is termed biomagnification. Large-bodied, long-lived and high trophic level animals such as birds (Fry 1995, Venier *et al.* 2010, Gómez-Ramírez *et al.* 2010), seals (Vetter *et al.* 1999, Wolkers *et al.* 1998, Muir *et al.* 1988), cetaceans (Krahn *et al.* 2007, Prudente *et al.* 1997, Hobbs *et al.* 2003) and sharks (Fisk *et al*. 2002; Gelsleichter *et al.* 2005, 2007; Mull *et al.* 2012) consequently experience the highest exposure to OCs.

Deleterious toxicity as a result of high OC accumulation in apex predators has been found. This includes tumour production, digestive and glandular lesions, and spinal spondylosis as found in beluga, *Delphinapterus leucas* (Beland *et al.* 1993), immunosuppression as found in *D.leucas*, seal *Phoca vitulina*, and dolphin *Tursiops truncatus* (Beland *et al.* 1993; Ross *et al.* 1996; Lahvis *et al.* 1995), endocrine disruption as in the polar bear *Ursus maritimus* (Oskam *et al.* 2003), and reproductive dysfunction as in the seal species, *Phoca vitulina* and *P. hispida* (Reijnders 1986; Helle 1980).

Although there is no direct evidence of toxic physiological effects in sharks to date, the potential for OCs to adversely impact the fitness of shark populations exists, because long-term, chronic exposure effects are unknown and may manifest in fish through inconspicuous behavioural toxicity (e.g., neurotransmitter or sensory system disruption; Scott & Sloman 2004). These unseen effects alter social and reproductive behaviours essential for population recruitment and this disruption may not be recognizable until populations have declined. Moreover, documented OC levels in some shark species are higher than threshold levels that have resulted in detrimental effects in other aquatic vertebrate taxa (Gelsleichter & Walker 2010). Especially as a compounding stressor to globally declining shark populations (Dulvy *et al*. 2014), resulting from selective capture via commercial fishing operations and shark fin trade-driven incentives (Worm *et al.* 2013, Ferretti *et al.* 2010), habitat degradation (Ward-Paige *et al.* 2010, Knip *et al.* 2010), and the overarching stressor of climate change (Hollowed *et al.* 2013), potential outcomes of OC exposure on the sustainability of shark populations is an imperative consideration (Gelsleichter *et al.* 2005, Lyons *et al.* 2013).

Predicting OC levels in sharks is a challenge for several reasons. Sharks are unique compared to other vertebrates in their metabolic regulation of lipid stores, as a result of their specific osmoregulatory strategy and use of their lipid-rich livers for buoyancy control (Ballantyne & Robinson 2010; Baldridge 1972). Many shark species also vary greatly in life history characteristics that would be expected to affect contaminant accumulation, such as growth rates, feeding rates, ontogenetic diet shifts, and metabolic capacity (Cortés 2000; Pastor *et al.* 1996; McIntyre & Beauchamp 2007; Norstrom 2002). Because of the wide variation in physiological and ecological processes governing OC accumulation in sharks, a thorough breakdown of these effects on OC loading is required for a clear understanding of OC exposure to shark populations. OC dynamics in sharks are nonetheless under-studied.

The Southern Hemisphere has generally been regarded as less intensely contaminated by OCs (Loganathan & Kannan 1994) as the majority of their production took place in the Northern Hemisphere, and global distillation has subsequently driven distributions toward the Arctic pole. However, due to global fractionation, compounds that are most highly chlorinated (and most problematic for bioaccumulation and biomagnification in food webs) are said to remain more closely tied to their latitudes of use (Vallack *et al.* 1998). Southern Hemispheric countries such as South Africa contain many highly industrialized areas that used OCs, in practices such as smelting, mining, manufacture and transport industries, as well as large and intensive agricultural practices, prior to their global ban (Bouwman *et al.* 2008). Additionally in South Africa, the historic dumping of PCB-containing products at Richard’s Bay, Durban, East London and Port Elizabeth provided sources of OCs into the South African marine environment that augmented loadings from land run-off and global aerial transport (Cockcroft 1999).

Dusky (*Carcharhinus obscurus*) and white (*Carcharodon carcharias*) sharks are two species that inhabit South Africa’s marine ecosystem, and possess many similar life history traits such as large body size, large offspring and late age-at-maturity (Cortés 2000). However, these species differ in at least two key life history characteristics that would be expected to directly influence OC exposure and accumulation; variable diet shifts with increasing animal size (Hussey *et al.* 2009, 2011, 2012), and different thermoregulatory modes (endothermic vs. ectothermic) which translates into different basal metabolic rates (Carlson *et al.* 2004). In addition, these species may possess different rates of somatic growth (Figure 2.1: Natanson *et al.* 2013; Wintner *et al.* 1999). Because of these distinct differences, but retention of other similar life history parameters common among most large sharks, these two species provide an excellent model to illustrate the variable influence of these particular life history characteristics on sharks' OC dynamics through the comparison of their ontogenetic accumulation patterns.

It would be expected that the life history attributes of the white shark would drive higher OC accumulation than the dusky shark over ontogeny. First, at a body size of ≥2 m, white sharks shift much of their predation effort from teleost towards elasmobranch and mammal prey (particularly the Cape Fur Seal, *Arctocephalus pusillus pusillus*) (Hussey *et al.* 2012; Fallows *et al.* 2012). Conversely, while examination of stomach contents of large dusky sharks has revealed the consumption of relatively high trophic level prey species such as other elasmobranchs, an overall depletion of $\delta^{15}N$ values with increasing shark size has been observed (Hussey *et al.* 2011). This size-related decline in trophic position is thought to relate to an ontogenetic change from coastal occupation to foraging at the edge of the continental shelf on abundant schools of lower trophic level fish prey (Hussey *et al.* 2011). Larger and older white sharks would therefore be exposed to more contaminated prey than dusky sharks, due to higher OC biomagnification of the prey consumed. Second, regional endothermy in lamnid sharks is said to drive greater energy requirements, as they possess higher basal metabolic rates than ectothermic sharks despite the maintenence of elevated temperatures in only select areas of the body (Carlson *et al.* 2004). This would result in white sharks consuming a larger amount of prey biomass than the ectothermic dusky shark (Semmens *et al.* 2013). In general, endotherms including mammals and birds require over an order of magnitude more biomass consumption to meet their energetic demands, with this being the most parsimonious explanation for much greater contaminant biomagnification (Fisk *et al.* 2001; Norstrom 2002). Last, while growth rate is often only referred to as a factor in contaminant elimination via growth dilution, higher growth rates in fish may also cause differential accumulation of organochlorines between species because elimination capacities decrease with increasing fish body size (Fisk et al. 1998; Sijm & van der Linde 1995). This results both from a lower rate of tissue turnover due to decreasing mass-specific metabolic rates with body size (Schimdt-Nielsen 1986), and from an allometric increase in the ratio of an animal’s chemical storage to depuration capacity (LeBlanc 1995). Simultaneously, depuration rates have a parabolic relationship with log Kow, with maximum elimination time at log Kow 7 (Fisk et al. 1998).

Evidence of differential OC accumulation in shark species that possess similar life history traits but with different ontogenetic diet shifts, endothermy, and growth rate may help to determine the weight of each effect on overall OC exposure. The specific objectives of this study were; (a) to compare observed concentrations and patterns of OCs between the two species, (b) to determine if OC concentrations in the sharks can be explained by relationships with age, body mass, or stable isotope indicators of trophic position and habitat use, and (c) to quantify and contrast the relationships between growth-dilution corrected OC concentration and age in these two species.

2.2 Methods

Sample collection

Dusky and white shark tissue samples were taken from animals caught in bather protection nets set approximately 400 m offshore, and at depths of 10 - 14 m at designated beaches along the KwaZulu-Natal (KZN) coast off Southern Africa (see Dudley 1997 for specific details). Between 2005 and 2012, white muscle tissue was removed alongside the vertebral column anterior to the first dorsal fin from *C. carcharias* (n = 57; 31 female, 26 male, 1600-3630 mm precaudal length [PCL]), and *C. obscurus* (n = 54; 27 female, 27 male, 1060 -2700 mm PCL) during routine dissections by KZN Sharks Board (KZNSB) staff. PCL (mm), mass (kg), and sex were recorded for all individuals. Muscle tissue samples were immediately stored frozen (-20°C). Samples were lyphosized (48 hours), and shipped to the Chemical Tracer Lab at the Great Lakes Institute for Environmental Science (GLIER, University of Windsor, Ontario, Canada) in 2009 and 2012, for both OC contaminant and stable isotopes analysis.

OC Extraction and Analysis

Using the method of Lazar *et al.* (1992) with modification, muscle tissue was analyzed for 40 PCB congeners (Table 1), p,p'-DDT and metabolites p,p'-DDE and p,p'-DDD, chlorobenzenes (pentachlorobenzene, 1,2,3,4-tetrachlorobenzene, 1,2,4,5-tetrachlorobenzene and hexachlorobenzene), hexachlorocyclohexanes (α-HCH, β-HCH, γ-HCH), chlordanes (heptachlor epoxide, oxychlordane, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor), octachlorostyrene, mirex, and dieldrin. All materials contacting the sample (glass, aluminum, PTFE) were rinsed three times with acetone and hexane before use.

Briefly, solid/liquid column extraction using 15 mL 1:1 hexane (Hx):dichloromethane (DCM) (% v/v) was carried out on 1 g freeze-dried muscle tissue previously weighed and desiccated in 10 g dried anhydrous sodium sulfate (Na_2SO_4). Internal standards of 50µl of 694ng/mL PCB 34, and 100µl of 500ng/mL BDE 71 were added. Column eluate was rotaevaporated (Heidolph Hei-Vap Advantage®, Model 561-01110-00, Heidolph Instruments, GmbH & Co. KG, Schwabach, Germany) and brought to 10 mL in Hx. One mL of this solution was dried at 110°C for 1 hour for gravimetric lipid determination. Lipid was removed only from samples containing over 0.15 g of lipid via gel permeation chromatography. Subsequent florisil separation involved elution through 6 g activated florisil, and elution volumes per fraction included 50 mL of hexane (fraction 1), 50 mL 15:85 DCM:Hx (% v/v) and 130 mL 60:40 DCM: Hx (% v/v). Eluate was rotaevaporated, brought to 1 mL in isooctane, and capped in 32 mm x 12 mm (2 mL) amber glass wide crimp-top autosampler vials (Agilent Technologies Canada Ltd., Mississauga Ontario, Canada) before analysis on a GC-ECD (GC System Model 6890A, Autosampler Tray Model 7683, Agilent Technologies, Santa Clara, CA, USA).

Blanks and identical fish (carp) reference homogenate were run with each sample set to account for variation in results caused by the method itself, and were within the criteria for the GLIER Organics Analysis Laboratory, certified by CALA (Canadian Association for Laboratory Accreditation Inc.). Verification of compounds was performed on a subset of samples with GC-MSD. Recovery percentages ranged from

41.96 – 101.54%, and blank and recovery correction was performed for all samples. All chemicals were purchased from Fisher Scientific Ltd. (Nepean Ontario, Canada).

Carbon and Nitrogen Stable Isotope Determination

Stable isotope analysis was conducted as per Hussey *et al.* (2012). Briefly, freeze-dried samples were homogenized, lipid extracted according to standard protocols, and analyzed on a continuous flow isotope ratio mass spectrometer (Finnigan MAT Delta^{plus}; Thermo Finnigan, San Jose, CA). Atmospheric nitrogen was used as the reference standard for $\delta^{15}N$, and Pee Dee Belemnite Carbonate for $\delta^{13}C$. Stable isotope values were expressed as the deviation from standards in parts per thousand (‰). Analytical precision expressed as the standard deviation of internal lab standard fish muscle and NIST 8414 bovine standard (n= 7 for each) was respectively; 0.11‰ and 0.10‰ for $\delta^{15}N$, and 0.15‰ and 0.12‰ for $\delta^{13}C$. Analytical accuracy based on difference from NIST standards were within 0.14‰ of certified values for $\delta^{13}C$ of both sucrose (NIST 8542; n=58) and L-glutamic acid (NIST 8573; n=57), and within 0.24‰ of certified values for $\delta^{15}N$ of L-glutamic acid (NIST 8573; n=57), ammonium sulfate (NIST 8548; n=57), and nitrate (NIST 8549; n=18).

Data Analysis

All data analyses were performed using the statistical software, “R” (reference index version 3.0.1; R Core Team, 2013). Appropriate non-parametric tests were selected where log-transformation did not normalize distributions or homogenize variance between species. Log K_{ow} values were obtained from a number of sources (Sangster 2013, Hansch *et al.* 1995, Shen & Wania 2005, Veith *et al.* 1979, Simpson *et al.* 1995, Mortimer & Connell 1995). Ages of sharks were calculated from precaudal lengths (PCL) using published parameters of von Bertalanffy equations for the two species (white; Wintner & Cliff 1999, dusky; Natanson *et al.* 2013). The equation was modified to solve for age as per Frazier & Loefer (2013). Because of the likelihood for maternal OC transfer to bias results (Olin *et al.* 2014; Mull *et al.* 2013), no neonates were included in comparisons between species.

Differences in δ^{15}N values and log-transformed mass (kg) between species were determined using a one-way ANOVA, whereas a two-tailed Exact Wilcoxon Rank-Sum Test (Package "exactRankTests"; Hothorn & Hornik 2013) was used to compare differences in δ^{13}C between species.

To determine whether differences in ontogenetic contaminant profiles occurred between species, non-transformed compound concentrations were converted to relative proportions via division by the most abundant and highly recalcitrant compound; p,p'-DDE. Proportions were then assessed for difference using ANOSIM on a Bray-Curtis dissimilarity matrix, followed by SIMPER (Package "vegan"; Oksanen *et al.* 2013) to determine compounds contributing most to species' dissimilarity. The effect of life stage on intraspecific OC profiles was also examined by converting non-transformed concentrations into 'p_i': the proportion of a compound's concentration in an individual to the summed concentrations for that contaminant in the species overall. A Shannon's Diversity Index value was then computed for individual sharks using $H = -(\Sigma p_i \mathit{ln} p_i)$, to assess variance in the diversity of compound profiles as a function of life stage (i.e., differential mobility and consequent habitat range) according to compound presence/absence and abundance. H values were then log-transformed and pair-wise differences were tested between life stage groups using One-Way ANOVA. Life stages were defined in PCL, for dusky sharks as <100 cm neonate, 101 – 155 cm juvenile, 155 cm – 210 cm subadult, and >210 cm adult (Romine *et al.* 2009) and for white sharks as <150 cm neonate, 150 – 200 cm small juvenile, 200 cm – 256 cm large juvenile, >256 cm subadult (Bruce & Bradford 2012).

To determine whether overall OC profiles within each species correlated with shark $\delta^{15}N$, $\delta^{13}C$, mass and sex, two separate principal component analyses (PCA, one for each species) were performed using vegan on correlation matrices of white and dusky shark contaminant proportions (*i.e.*, p,p'-DDE standardized) following removal of the three most extreme outliers (one juvenile, and two adult dusky sharks; median absolute deviation test statistic ≥ 15). Proportions were used to account for contamination variability across the suite of contaminants as a whole (a more highly contaminated individual would tend to have higher levels of all compounds, and vice versa). Shark $\delta^{15}N$, $\delta^{13}C$, mass and sex (predictor variables) were then regressed (using additive multiple linear regression) against PC scores from the first three axes (PC1, PC2, PC3: dependent variables) to explore how variation in contaminant profiles in each species was related to these shark characteristics. Because $\delta^{15}N$ and $\delta^{13}C$, as well as $\delta^{15}N$ and mass, were highly correlated, $\delta^{15}N$ and sex were included in one set of regressions and $\delta^{13}C$, mass and sex included in a separate set of regressions.

To test for species differences in actual compound concentrations, only the four compounds that were found in every individual of each species were considered (p,p'-DDE, *t*-nonachlor, and PCBs 180 and 187), and concentrations were log-transformed prior to analysis. Accumulation differences between species, and relationships between concentrations and predictor variables are expected to be well exemplified by these congeners because they are highly biomagnifying (Alava & Gobas 2012; Fisk *et al.* 2001; Walters *et al.* 2011), as expected for compounds at a log K_{ow} near 7 (range 6.35-6.96) (Walters *et al.* 2011; Fisk *et al.* 1998). Concentration differences in these compounds between species were tested using either two-tailed Exact Wilcoxon Rank-Sum Test, Student's t-Test, or Welch's t-Test, dependant on data normality and variance homogeneity. To examine ontogenetic trends, correlations between concentrations of these compounds and shark mass, $\delta^{15}N$, and $\delta^{13}C$ were investigated (excluding neonates) using either Linear Regression or Spearman's ρ.

Finally, due to the potential for differences in growth-dilution to confound a proper comparison of uptake rates between species, concentrations of these four compounds were growth-dilution corrected by standardizing to shark mass. Because observed concentrations could only have been growth-diluted to an extent equal to shark size, concentrations were multiplied by the masses of the particular individuals they were found in. Species' differences in slopes of the relationship between concentrations and age were then tested via ANCOVA. This analysis was undertaken both with white shark ages calculated according to Wintner & Cliff (1999), and with these white shark ages doubled given potential age underestimation (Hamady *et al.* 2014). Dusky shark individuals with ages calculated as per Natanson *et al.* 2013, were included in each species' comparison only as corresponded to the white shark age range; specifically, 1-13 and 1-26 years of age (8 dusky shark ages fell between 13- 26 years, therefore they were added to the latter analysis).

2.3 Results

Body Size, Age, and Stable Isotopes

White and dusky sharks were found to differ significantly in δ^{15}N (F = 109.6), mass (F = 11.3), and δ^{13}C (W = 232) ($p < .01$ for all, Figure 2.2). Polynomial regression was a better fit than linear regression between *log* mass and both δ^{13}C and δ^{15}N using Akaike's Information Criteria, and significant relationships were found for both δ^{13}C and δ^{15}N in the dusky shark, and δ^{13}C in the white shark (r^2 = 0.26, 0.58 and 0.12, respectively, and $p < .05$ for all; Figure 2.2).

OC Compound Ontogenetic Profiles

Comparison of overall OC accumulation pattern via ANOSIM revealed a significant difference in OC profiles between species (R = 0.5, $p < .01$; Figure 2.3). Nine compounds were responsible for 52.5% of the 54.6% total dissimilarity between species; PCBs 138 (11.1%), 153 (7.9%), 180 (7.3%), *t*-nonachlor (6.2%), and PCBs 33 (4.8%), 52 (4.6%), 44 (3.8%), 187 (3.4%), and 31/28 (3.4%); SIMPER, Figure 2.3). For white sharks, 13 compounds were found in all individuals (*cis*-chlordane, *t*-nonachlor, *cis*-nonachlor, p,p'-DDE, PCBs 31/28, 153, 156/171, 170, 180, 183, 187, 194, 199) contrasting only 4 compounds found in every dusky shark (p,p'-DDE, *t*-nonachlor, and PCBs 180 and 187).

No significant differences in H values were found among life stages of dusky sharks ($p > .05$ for all; Figure 2.4a), indicating compound profiles were consistent over ontogeny. For white sharks, neonates showed a trend of more diverse compound profiles than all other life stages, but neonates were only significantly different to juveniles ($p < .01$; Figure 2.4b).

Relationships between intraspecific OC compound profiles and predictor variables

The first three PC axes extracted from the PCAs performed on white and dusky shark contaminant proportions explained 69.7% and 53.6% of the total contaminant variation, respectively. For white sharks, only $\delta^{13}C$ was significantly related to PC2 (Figure 2.5a; no other significant relationships were found for any predictor with PC1, PC2 or PC3) via the following univariate relationship: $PC2 = 0.100 \times \delta^{13}C + 1.477$, $r^2=0.170$, ($p < .01$). Proportions of PCBs 153, 138, 128, 180, 170, 194 and 206 in white sharks all loaded positively and heavily on PC2 (loadings > 0.714; Table 2.3) and PCBs 128, 180 and 206 were significantly positively correlated with $\delta^{13}C$ (Pearson's $r > .29$, $p < .05$). For dusky sharks, PC1 was positively related to mass (Figure 2.5b; $PC1 = 0.236 \times log_{10}(\text{mass}) + (-0.474)$, $r^2 = 0.227$, $p < .01$). Only mirex and PCB 194 loaded positively, although weakly (loading = 0.16 and 0.12, respectively) on PC1 of the dusky shark PCA, but only mirex was significantly positively correlated to dusky shark mass ($r = 0.44$, $p < .05$). Proportions of several compounds (PCBs 31/28, 44, 95, 99, *c*-chlordane and *t*-nonachlor) loaded negatively and heavily (loadings < -0.70; Table 2.3) on PC1 but only PCBs 95, 99 and *c*-chlordane were significantly, negatively correlated with dusky shark mass ($r = -0.40, -0.35, -0.57$, respectively, all $p < .05$). No other relationships were found between PC axes and mass, $\delta^{15}N$, $\delta^{13}C$, or sex in dusky sharks.

OC Concentrations

OC concentrations were different between species for 2 of the 4 examined compounds, *t*-nonachlor and p,p'-DDE, with elevated levels in the white shark (Table 2.2; $p < .001$; log K_{ow} 6.35 and 6.96, respectively).

Relationships between OC concentrations and predictor variables

In dusky sharks, significant negative relationships were found between $\delta^{15}N$ and p,p'-DDE ($r^2 = 0.11$), and between $\delta^{13}C$ and p,p'-DDE ($r_s^2 = 0.10$), PCB 180 ($r^2 = 0.15$) and PCB 187 ($r^2 = 0.12$) concentrations ($p < .05$ for all), while a positive relationship was found between mass and p,p'-DDE ($r^2 = 0.22$, $p < .01$, Figure 2.6a). For white sharks, a positive relationship was found between PCB 180 and $\delta^{13}C$ ($r^2 = 0.17$, $p < .01$, Figure 2.6c). Among all sharks, *t*-nonachlor and p,p'-DDE had positive relationships with $\delta^{15}N$ ($r^2 = 0.38$ [Figure 2.6b], $r_s^2 = 0.06$, respectively), $\delta^{13}C$ ($r^2 = 0.34$, $r_s^2 = 0.08$, respectively), and mass ($r_s^2 = 0.06$, $r^2 = 0.12$, respectively) ($p < .05$ for all).

Differences in OC accumulation with age

Slopes of the relationship between growth-dilution corrected OC concentrations and age as calculated from Wintner & Cliff (1999) and Natanson *et al.* (2013) did not differ significantly between species for any of the four compounds, whether or not white shark ages were doubled as may increase accuracy according to Hamady *et al.* (2014) (Figure 2.7; ANCOVA interaction, $p > .05$ for all).

2.4 Discussion

This study compared OC ontogenetic profiles, concentrations and accumulation patterns in two shark species that contrast each other in some important life history parameters. Among individuals of both species, OC concentrations had significant relationships with variables generally associated with OC dynamics in fish, but fewer were found intraspecifically. Despite the distinct life history characteristics of these species, slopes of the relationship between OC concentrations and age did not differ between them. However, concentrations of the highly biomagnifying compounds, *t*-nonachlor and p,p’-DDE, were significantly elevated in the higher trophic level and larger-bodied white shark. This work provides insight on the comparative physiology and trophic ecology of these species, indicating that life-history complexity among shark species may hinder the accuracy of simple prediction models at the single-species level, but that trophic structure may still accurately predict OC dynamics in elasmobranch-rich food webs.

Muscle p,p’-DDE concentrations of dusky and white sharks in this study were higher than blubber levels reported from other marine apex predators caught at KZN [Minke whale (*Balaenoptera acutorostrata*), fin whale (*B. physalus*), and sperm whale (*Physeter macrocephalus*)], but were similar to levels reported previously in KZN white sharks (Schlenk *et al.* 2005) (Table 2.2, lipid-basis). Notably, lipid-based levels of p,p’-DDT in sharks from this current study were lower than previous reports of KZN cetaceans and white sharks, with a marked decrease from the cetaceans sampled in 1974 (by an order of magnitude on average), and a lesser decrease from the white sharks sampled in 2003 (by approximately half) (Table 2.2). This suggests that KZN white sharks have not been as intensely exposed to DDT in recent years, and have now metabolized the majority of their p,p’-DDT burden to p,p’-DDE. Lipid-based concentrations of PCBs 138, 149, 153 and 180 quantified here are less than half the level observed in *O.orca* blubber from the Crozet Archipelago, the most southerly comparable location (Noël *et al.* 2009), but are over an order of magnitude higher than previously found in portuguese dogfish liver (*Centroscymnus coelolepis*), from the northwestern African Atlantic Ocean (Serrano *et al.* 1997). While this would suggest higher contamination in the Southern Hemisphere, comparable levels of PCBs were found in the shortnose velvet dogfish (*Centroscymnus cryptacanthus*), and nearly double the amount of PCB 153, as well as over an order of magnitude higher PCB 118 were found in the leafscale gulper shark (*Centrophorus squamosus*), both also from the northwestern African Atlantic. It is therefore likely that such low levels in the portugese dogfish are instead a result of their being an extremely deep-living benthic species (Sion *et al.* 2004), rarely encountering the highly contaminated food sources from shallower coastal habitats (Bor *et al.* 2007).

To compare OC levels in muscle tissue between shark species from different geographic locations, approximate wet weight concentrations were calculated by applying a moisture content of 75.4% to dry weight concentrations. This value was determined from the regression line of the relationship between measured wet and freeze-dried weights of muscle from KZNSB sharks, which was the same for all (8) species ($y = 3.9255x + 0.1863$, $r^2 = 0.97$, $p < 0.001$). KZN white shark p,p'-DDE concentrations were nearly equal to concentrations reported from bonnethead sharks (*Sphyrna tiburo*) sampled from Florida, a short-lived, low trophic-level species primarily eating crustacean and molluscan prey (Gelsleichter *et al.* 2005; Cortés *et al.* 1996). KZN dusky and previously sampled whites from Schlenk *et al.* (2005) contained approximately half this amount. This may indicate that for Southern Hemispheric animals, only those compounds with the highest propensity for biomagnification are likely to reach levels that are readily available to low trophic level organisms in the Northern Hemisphere, and that the above-mentioned conversion to the p,p'-DDE metabolite now provides white sharks with similar levels of this particular compound. Higher trophic level sharks (blacktip, *Carcharhinus limbatus* and sandbar, *Carcharhinus plumbeus*) from the Florida and Georgia regions of the U.S. were found to have much higher wet weight concentrations in liver as would be expected, however these values were not expressed in lipid weight, so a portion of this increase is a result of higher lipid concentration in liver than muscle tissue.

White and dusky sharks had significantly different OC profiles, indicating differential habitat use and/or feeding ecology of these species over ontogeny. For the neonate life-stage, white sharks showed a high amount of variation in the diversity (H) of compounds, a result attributed to maternal offloading which likely impacts first born young to a greater extent due to receiving adult burdens at their highest point (Mull *et al.* 2013, Lyons *et al.* 2013). Differences in $\delta^{13}C$ profiles between dusky and white sharks also indicate potential variation in habitat use across all life stages, with the higher $\delta^{13}C$ values of the white shark suggesting comparatively more inshore feeding over ontogeny, a behaviour supported by previous satellite tracking data (Bonfil *et al.* 2005). The overall contaminant profile of white sharks is in agreement with this, given a comparatively more even contamination across the full suite of analysed compounds (i.e., as proportions of p,p'-DDE), and the increased extent of contamination of inshore habitats (Bor *et al.* 2007). Conversely, dusky shark contamination was predominated by the three compounds contributing most to dissimilarity between the species' profiles; PCB 153, 138 and 180. These congeners, along with p,p'-DDE, are highly persistent, consistently biomagnifying efficiently through food webs (Kelly 2006; Serrano *et al.* 2003) and may be expected to predominate in typically less-contaminated pelagic food webs (as hexa and hepta-chlorinated PCB congeners are shown to predominate in pelagic species; Goutte *et al.* 2013). This is supported by the fact that subadult and adult dusky sharks predominantly occur and feed in the pelagic habitat at the edge of the continental shelf (Hussey *et al.* 2012; Rogers *et al.* 2013). Differential habitat use between these species is further indicated by the fact that δ^{13}C values correlated positively with the composite (PCA) profile response, indicating a proportionate increase in select contaminants (PCBs 128, 180 and 206) with increased inshore predominance of the white shark and therefore increased contaminant exposure (Bor *et al.* 2007). The overall more depleted δ^{13}C values of dusky sharks may suggest their predominant occurrence at the edge of the continental shelf, precluding the observation of this same relationship. Decreasing proportions of PCBs 95, 99 and *c*-chlordane with dusky size are likely the combined result of these compounds not being very highly bioaccumulative, and decreasing in contamination intensity with ontogenetic movement of the dusky offshore.

While a positive relationship between body size and OC concentrations is well-documented for fish species (Ernst *et al.* 1976; Nabawi *et al.* 1987; Olsson *et al.* 2000; McIntyre & Beauchamp 2007), which also persist when increased lipid with size is accounted for (Johnston *et al.* 2002), the absence of positive relationships between OC concentration and body size observed in the white shark is not an unusual finding when compared to other top trophic level marine predators. High trophic level mammal and shark species commonly have a negative or null relationship between OC concentrations and body size, such as the Killer Whale, *Orcinus orca* (southern Alaska; Ylitalo *et al.* 2001, southern Indian Ocean; Noël *et al.* 2009), salmon shark, *Lamna ditropis*, thresher shark, *Alopias vulpinus*, (southern California; Lyons *et al.* 2013), and white sharks sampled from southern California (Mull *et al.* 2012, Lyons *et al.* 2013). Lyons *et al.* (2013) suggested that positive relationships between OC concentrations and size in lamnids may occur only after an age when growth rate has decreased enough for dilution to have a reduced effect on bioaccumulation. White sharks in this study, however, only included individuals of an age that would correspond to positive growth (Table 2.1). Given the scenario suggested by Lyons *et al.* (2013), it is possible that dusky sharks may indeed grow much slower than white sharks as was previously thought (Wintner & Cliff 1999), which results in the positive relationships of the overall compound profile PCA response, and p,p'-DDE concentrations to be observed with mass. Alternatively, the greater ability for mass to predict OC levels in dusky sharks may be contingent upon the fact that dusky shark individuals sampled in this study are included up to at least 40 years old.

Our expectation that key differences in life-history traits between species would result in higher white shark OC accumulation with age (obscured by growth dilution) was not supported by slope differences. Neither an ontogenetic diet shift to higher trophic level and more contaminated mammal prey, nor regional endothermy driving increased biomass intake per unit time, or a possible higher growth rate enhancing OC uptake and assimilation in white sharks, was sufficient to drive an increased slope of OC accumulation with age when compared to the dusky. The high similarity in slopes overall may be partly explained by the fact that declining $\delta^{15}N$ values with mass in the dusky shark may not be a result of switching to feeding on only lower trophic level prey, i.e., small schooling fish. Instead dusky sharks may feed on large pelagic prey with comparable levels of OC contamination to that in white shark prey. Slopes may also be similar due to a lower frequency of seal-predation by white sharks than expected, high individual variability in predation success on mammal prey (Laroche *et al.* 2008; De Vos

& O'Riain 2010; Hussey *et al.* 2012), or because adult, large dusky sharks also incorporate elasmobranch, dolphin and other mammal prey (Dudley *et al.* 2005; Hussey *et al.* 2011) in similar proportions as subadult white sharks. Consequently, the observed decline in $\delta^{15}N$ values of larger dusky sharks is likely a result of a lower ecosystem baseline value in the pelagic foodweb (Hussey *et al.* 2011). This is further supported by the positive linear relationship between concentrations of p,p'-DDE and mass, and none with $\delta^{15}N$, providing evidence that body size is a better predictor of OC concentrations in the dusky shark. Although these scenarios may account for this unexpected comparative OC accumulation, the possibility that currently available von Bertalanffy growth parameters are inaccurate for these species may also be questioned as a confounding factor.

Our hypothesis that white sharks contain higher concentrations of biomagnifying OCs than the dusky shark was verified by elevated concentrations of p,p'-DDE, and *t*-nonachlor. This is a result of their higher trophic position, as indicated by significantly higher $\delta^{15}N$ values and body masses of white sharks. Because of a positive relationship between these compounds and both $\delta^{15}N$ and mass among all shark individuals, it is apparent that body size also plays a role in determining OC burdens between shark species of different sizes. This is expected due to decreasing elimination capacity with fish body size (Fisk *et al.* 1998; Sijm & van der Linde 1995). As detailed above, although positive relationships between OC concentrations and white shark size were not observed, this was likely due to not sampling larger individuals and resultant masking by growth-dilution (Lyons *et al.* 2013). Because fish (including sharks) have indeterminate growth and generally consume prey as gape size allows (Heupel *et al.* 2014), verification of the importance of body size through comparisons of species with similar trophic positions but different body sizes is difficult (Borgå *et al.* 2004). Here it has been well demonstrated given the high similarity in accumulation patterns with age, but overall higher concentrations in the larger-bodied white shark. However, this does not preclude differing trophic positions (Hussey *et al.* 2014) as an important determinant of OC levels as well, because although positive relationships between OC concentrations and $\delta^{15}N$ were absent within each species, a positive overall trend among all shark individuals was observed with $\delta^{15}N$. Given the fundamental mechanism of OC biomagnification and the structure of trophic systems, trophic level is a consistently reliable indicator of OC concentrations in food webs (LeBlanc 1995; Borgå *et al.* 2004).

Summary

With growth-dilution correction, slopes of white and dusky shark OC accumulation with age were similar. Examples of significantly higher levels of some readily biomagnifying compounds were found in the white shark, attributable to higher trophic level feeding and larger body size. No correlations were found in white sharks between mass and OC concentrations, but this relationship was positive in dusky sharks and may be attributed to either more rapid growth-dilution in white sharks, or the inclusion of much older dusky shark individuals in the analyses. Inshore habitat use was found to be an important predictor of OC variation in white sharks and was absent in dusky sharks due to the predominant occurrence of subadult and mature individuals in pelagic food webs at the edge of the continental shelf. In conclusion, while $\delta^{15}N$ and mass did not predict OC concentrations in both species intraspecifically, they adequately predicted levels of some of the most highly biomagnifying OC compounds between these species.

2.5 References

Alava, J.J., Gobas, F.A.P.C. 2012. Chapter 4. Assessing biomagnification and trophic transport of persistent organic pollutants in the food chain of the Galapagos Sea Lion (*Zalophus wollebaeki*): conservation and management implications. IN: Romero, A., Keith, E.O. (eds). 2012. *New Approaches to the Study of Marine Mammals*. InTech — Open Access Company. pp. 77-108. (http://www.intechopen.com/books/new-approaches-to-the-study-of-marine-mammals).

Baldridge, H.D. Jr. 1972. Accumulation and function of liver oil in Florida sharks. *Copeia* **1972**(2): 306-325.

Ballantyne, J.S., Robinson, J.W. 2010. Freshwater elasmobranchs: a review of their physiology and biochemistry. *J Comp Physiol B* **180**: 475-493.

Beland, P., DeGuise, S., Girard, C., Lagace, A., Martineau, D., Michaud, R., Muir, D.C.G., Norstrom, R.J., Pelletier, E., Ray, S., Shugart, L.R. 1993. Toxic compounds and health and reproductive effects in St. Lawrence beluga whales. *J Great Lakes Res* **19**(4): 766-775.

Bonfil, R., Meÿer, M., Scholl, M.C., Johnson, R., O'Brien, S., Oosthuizen, H., Swanson, S., Kotze, D., Paterson, M. 2005. Transoceanic migration, spatial dynamics, and population linkages of white sharks. *Science* **310**: 100-103.

Borgå, K., Fisk, A.T., Hoekstra, P.F., Muir, D.C.G. 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environ Toxicol Chem* **23**(10): 2367-2385.

Bor, S.K., Simbauni, J.A., Oyugi, M.P., Njue, W.M. Chhabra, S.C. 2007. Short communication: organochlorine residues in tissues of marine fauna along the coast of Kenya-Mombasa Island. *Bull. Chem Soc Ethiop* **21**(1): 145-149.

Bouwman, H., Polder, A., Venter, B., Skaare, J.U. 2008. Organochlorine contaminants in cormorant, darter, egret, and ibis eggs from South Africa. *Chemosphere* **71**: 227-241.

Bruce, B.D., Bradford, R. 2012. Chapter 17: Habitat use and spatial dynamics of juvenile white sharks, *Carcharodon carcharias,* in Eastern Australia. IN: Domeier, M.L. (ed): *Global perspectives on the biology and life history of the white shark*. CRC Press; Taylor and Francis Group, Boca Raton, FL, USA. pp. 225-253.

Carlson, J.K., Goldman, K.J., Lowe, C.G. 2004. Metabolism, energetic demand, and endothermy. IN: Carrier, J.C., Musick, J.A., and Heithaus, M.R (eds). *Biology of sharks and their relatives.* CRC Press, Boca Raton, FL, USA. pp. 203-224.

Cliff, G., Dudley, S.F.J., Davis, B. 1989. Sharks caught in the protective gill nets off Natal, South Africa. 2. The great white shark *Carcharodon carcharias* (Linnaeus). *S Afr J mar Sci* **8**: 131-144.

Cliff, G., Wilson, R.B. 1986. Natal Sharks Board's field guide to sharks and other marine animals. Durban; Natal Sharks Board: 57 pp.

Cockcroft, V.G. 1999. Organochlorine levels in cetaceans from South Africa: a review. *J Cetacean Res Manage* (Special Issue 1): 169-176.

Cortés, E. 2000. Life history patterns and correlations in sharks. *Rev Fish Sci* **8**(4): 299-344.

Cortés, E., Gruber, S.H. 1990. Diet, feeding habits and estimates of daily ration of young lemon sharks, *Negaprion brevirostris* (Poey). *Copeia* **1990**(1): 204-218.

Cortés, E., Manire, C.A., Hueter, R.E. 1996. Diet, feeding habits, and diel feeding chronology of the bonnethead shark, *Sphyrna tiburo*, in Southwest Florida. *Bull Mar Sci* **58**(2): 353-367.

De Vos, A., O'Riain, M.J. 2010. Sharks shape the geometry of a selfish herd: experimental evidence from seal decoys. *Biol Lett* **6**: 48–50.

Dudley, S.F.J., Cliff, G., Zungu, M.P., Smale, M.J. 2005. Sharks caught in the protective gill nets off KwaZulu-Natal, South Africa. 10. The dusky shark *Carcharhinus obscurus* (Lesueur 1818). *African J Mar Sci* **27**(1): 107–127.

Dudley, S.F.J. 1997. A comparison of the shark control programs of New South Wales and Queensland (Australia) and KwaZulu-Natal (South Africa). *Ocean Coast Mgmt* **34**: 1–27.

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., Carlson, J.K., Davidson, L.N.K., Fordham, S.V., Francis, M.P., Pollock, C.M., Simpfendorfer, C.A., Burgess, G.H., Carpenter, K.E., Compagno, L.J.V., Ebert, D.A., Gibson, C., Huepel, M.R., Livingstone, S.R., Sanciangco, J.C., Stevens, J.D., Valenti, S., White, W.T. 2014. Extinction risk and conservation of the world's sharks and rays. eLife 2014;3:e00590.

Ernst, W., Goerke, H., Eder, G., Schaefer, R.G. 1976. Residues of chlorinated hydrocarbons in marine organisms in relation to size and ecological parameters. I. PCB, DDT, DDE, and DDD in fishes and molluscs from the English Channel. *Bull Environ Contam Toxicol* **15**(1): 55-65.

Fallows, C., Martin, R.A., Hammerschlag, N. 2012. Chapter 9: Comparisons between white shark-pinniped interactions at Seal Island (South Africa) with other sites in California. IN: Domeier, M.L. (ed): *Global perspectives on the biology and life history of the white shark*. CRC Press; Taylor and Francis Group, Boca Raton, FL, USA. pp. 105-118.

Ferretti, F., Worm, B., Britten, G.L., Heithaus, M.R., Lotze, H.K. 2010. Patterns and ecosystem consequences of shark declines in the ocean. *Ecol Lett* **13**: 1055-1071.

Fisk, A.T., Hobson, K.A., Norstrom, R.J. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. *Environ Sci Technol* **35**: 732-738.

Fisk, A.T., Norstrom, R.J., Cymbalisty, C.D., Muir, D.C.G. 1998. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water partition coefficient. *Environ Toxicol Chem* **17**(5): 951-961.

Fisk, A.T., Tittlemier, S. A., Pranschke, J. L., Norstrom, R. J. 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. *Ecology* **83**(8): 2162-2172.

Frazier, B.S. and Loefer, J.K. 2013. Update to maximum observed age of Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) in the western North Atlantic Ocean based on a direct age estimate of a long term recapture. SEDAR34-WP-06. SEDAR, North Charleston, SC. 5 pp.

Gelsleichter, J., Manire, C.A.., Szabo, N.J., Cortés, E., Carlson, J., Lombardi-Carlson, L. 2005. Organochlorine concentrations in bonnethead sharks (*Sphyrna tiburo*) from four Florida estuaries. *Arch Environ Contam Toxicol* **48**: 474-483.

Gelsleichter, J., Szabo, N.J., Morris, J.J. 2007. Organochlorine contaminants in juvenile sandbar and blacktip sharks from major nursery areas on the East coast of the United States. *Amer Fish Soc Symp* **50**: 153-164.

Gelsleichter, J., Walker, C.J. 2010. Pollutant exposure and effects in sharks and their relatives, IN: Carrier, J.C. *et al.* (eds). 2010. *Sharks and their relatives II: Biodiversity, adaptive physiology, and conservation.* CRC Marine Biology Series: pp. 491-537.

Goutte, A., Chevreuil, M., Alliot, F., Chastel, O., Cherel, Y., Eléaume, M., Massé, G. 2013. Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica. *Mar Poll Bull* **77**: 82-89.

Hamady, L.L., Natanson, L.J., Skomal, G.B., Thorrold, S.R. 2014. Vertebral bomb radiocarbon suggests extreme longevity in white sharks. *PLoS ONE* **9**(1): e84006. doi:10.1371/journal.pone.0084006

Hansch, C., Leo, A., Hoekman, D. 1995. Exploring QSAR. Hydrophobic, electronic and steric constants. Amer Chemical Society, Washington, DC.

Helle, E., 1980. Lowered reproductive capacity in female ringed seals (*Phoca hispida*) in the Bothnian Bay, northern Baltic Sea, with special reference to uterine occlusions. *Ann Zool Fenn* **17**: 147-158.

Henry, J., Best, P.B. 1983. Organochlorine residues in whales landed at Durban, South Africa. *Mar Poll Bull* **14**(6): 223-227.

Heupel, M.R., Knip, D.M., Simpfendorfer, C.A., Dulvy, N.K. 2014. Sizing up the ecological role of sharks as predators. *Mar Ecol Prog Ser* **495**: 291-298.

Hollowed, A.B., Barange, M., Beamish, R.J., Brander, K., Cochrane, K., Drinkwater, K., Foreman, M.G.G., Hare, J.A., Holt, J., Ito, S., Kim, S., King, J.R., Loeng, H., MacKenzie, B.R., Mueter, F.J., Okey, T.A., Peck, M.A., Radchenko, V.I., Ricel, J.C., Schirripa, M.J., Yatsu, A., Yamanaka, Y. 2013. Projected impacts of climate change on marine fish and fisheries. *ICES J Mar Sci* **70**(5): 1023-1037.

Hothorn, T., Hornik, K. 2013. exactRankTests: Exact Distributions for Rank and Permutation Tests. R package version 0.8-27. (http://CRAN.R-project.org/package=exactRankTests)

Hussey, N.E., Dudley, S.F.J., McCarthy, I.D., Cliff, G., Fisk, A.T. 2011. Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? *Can J Fish Aquat Sci* **68**: 2029-2045.

Hussey, N.E., MacNeil, M.A., McMeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G., Wintner, S.P., Fennessy, S.T., Fisk, A.T. 2014. Rescaling the trophic structure of marine food webs. *Ecology Letters* **17**: 239-250.

Hussey, N.E., McCann, H.M., Cliff, G., Dudley, S.F.J., Wintner, S.P., Fisk, A.T. 2012. Chapter 3: Size-based analysis of diet and trophic position of the white shark, *Carcharodon carcharias*, in South African waters. IN: Domeier, M.L. (ed): *Global perspectives on the biology and life history of the white shark*. CRC Press; Taylor and Francis Group, Boca Raton, FL, USA. pp. 27-49.

Hussey, N.E., McCarthy, I.D., Dudley, S.F.J., Mann, B.Q. 2009. Nursery grounds, movement patterns and growth rates of dusky sharks, *Carcharhinus obscurus*: a long-term tag and release study in South African waters. *Mar Freshw Res* **60**: 571-583.

Johnston, T.A., Fisk, A.T., Whittle, D.M., D.C.G. Muir. 2002. Variation in organochlorine bioaccumulation by a predatory fish; gender, geography, and data analysis methods. *Environ Sci Technol* **36**: 4238-4244.

Kelly, B.C. 2006. Bioaccumulation potential of organic contaminants in an arctic marine food web. School of Resource and Environmental Management, Ph.D. Simon Fraser Univ., Vancouver BC (2005).

Knip, D.M., Heupel, M.R., Simpfendorfer, C.A. 2010. Sharks in nearshore environments: models, importance, and consequences. *Mar Ecol Prog Ser* **402**: 1-11.

Lahvis, G.P., Wells, R.S., Kuehl, D.W., Stewart, J.L., Rhinehart, H.L., Via, C.S. 1995. Decreased lymphocyte responses in free ranging bottlenose dolphins (*Tursiops truncatus*) are associated with increased concentrations of PCBs and DDT in peripheral blood. *Environ Health Perspect* **103**: 67-72.

Laroche, R.K., Kock, A.A., Dill, L.M., Oosthuizen, W.H. 2008. Running the gauntlet: a predator-prey game between sharks and two age classes of seals. *Anim Behav* **76**: 1901-1917.

Lazar, R., Edwards, R.C., Metcalfe, C.D., Metcalfe, T., Gobas, F.A.P.C., and Haffner, G.D. 1992. A simple, novel method for the quantitative analysis of coplanar (non-ortho substituted) polychlorinated biphenyls in environmental samples. *Chemosphere* **25**(4): 493-504.

LeBlanc, G.A. 1995. Trophic-level differences in the bioconcentration of chemicals: implications in assessing environmental biomagnification. *Environ Sci Technol* **29**: 154–160.

Loganathan, B.G., Kannan, K. 1994. Global organochlorine contamination trends: an overview. *Ambio* **23**(3): 187-191.

Lyons, K., Carlisle, A., Preti, A., Mull, C.G., Blasius, M., O'Sullivan, J.B., Winkler, C., Lowe, C.G. 2013. Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of young of the year lamniform sharks. *Mar Environ Res* **90**: 27-38.

McCosker, J.E. 1987. The white shark, *Carcharodon carcharias*, has a warm stomach. *Copeia* **1987**(1): 195-197.

McIntyre, J.K., Beauchamp, D.A. 2007. Age and trophic position dominate bioaccumulation of mercury and organochlorines in the food web of Lake Washington. *Sci Tot Environ* **372**: 571-584.

Mortimer, M.R., and Connell, D.W. 1995. A model of the environmental fate of chlorohydrocarbon contaminants associated with Sydney sewage discharges. *Chemosphere* **30**: 2021-2038.

Mull, C.G., Blasius, M.E., O'Sullivan, J.B., Lowe, C.G. 2012. Chapter 5: Heavy metals, trace elements, and organochlorine contaminants in muscle and liver tissue of juvenile white sharks, *Carcharodon carcharias*, from the Southern California Bight. IN: *Global perspectives on the biology and life history of the white shark.* Domeier, M.L. (ed.) CRC Press; Taylor and Francis Group, Boca Raton, FL, USA. pp. 59-75.

Mull, C.G., Lyons, K., Blasius, M.E., Winkler, C., O'Sullivan, J.B., Lowe, C.G. 2013. Evidence of maternal offloading of organic contaminants in white sharks

(*Carcharodon carcharias*). *PLoS ONE* **8**(4): e62886. doi:10.1371/journal.pone.0062886

Nabawi, A.E., Heinzow, B., Kruse, H. 1987. Residue levels of organochlorine chemicals and polychlorinated biphenyls in fish from the Alexandria Region, Egypt. *Arch Environ Contam Toxicol* **16**: 689-696.

Natanson, L.J., Gervelis, B.J., Winton, M.V., Hamady, L.L., Gulak, S.J.B., Carlson, J.K. 2013. Validated age and growth estimates for *Carcharhinus obscurus* in the northwestern Atlantic Ocean, with pre- and post management growth comparisons. *Environ Biol Fish*: 10.1007/s10641-013-0189-4.

Natanson, L.J., Kohler, N.E. 1996. A preliminary estimate of age and growth of the dusky shark *Carcharhinus obscurus* from the south-west Indian Ocean, with comparisons to the western North Atlantic population. *S Afr J Mar Sci* **17**: 217-224.

Noël, M., Barrett-Lennard, L., Guinet, C., Dangerfield, N., Ross, P.S. 2009. Persistent organic pollutants (POPs) in killer whales (*Orcinus orca*) from the Crozet Archipelago, southern Indian Ocean. *Mar Environ Res* **68**: 196-202.

Norstrom, R.J. 2002. Understanding bioaccumulation of POPs in food webs. Chemical, biological, ecological and environmental considerations. *Environ Sci Poll Res* **9**(5): 300-303.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H. 2013. vegan: Community Ecology Package. R package version 2.0-9. (http://CRAN.R-project.org/package=vegan)

Olin, J., Beaudry, M., Fisk, A.T., Paterson, G. 2014. Age-related polychlorinated biphenyl dynamics in immature bull sharks (*Carcharhinus leucas*). *Environ Toxicol Chem* **33**(1): 35-43.

Olsson, A., Valters, K., Burreau, S. 2000. Concentrations of organochlorine substances in relation to fish size and trophic position: A study on perch (*Perca fluviatilis* L.). *Environ Sci Technol* **34**: 4878-4886.

Oskam, I.C., Ropstad, E., Dahl, E., Lie, E., Derocher, A.E., Wiig, O., Larsen, S., Wiger, R., Skaare, J.U. 2003. Organochlorines affect the major androgenic hormone, testosterone, in male polar bears (*Ursus maritimus*) at Svalbard. *J Toxicol Environ Health A* **66**(22): 2119-2139.

Pastor, D., Boix, J., Fernandez, V., Albaigés, J. 1996. Bioaccumulation of organochlorinated contaminants in three estuarine fish species (*Mullus barbatus, Mugil cephalus and Dicentrarcus labrax*). *Mar Poll Bull* **32**(3): 257-262.

Reijnders, P.J.H., 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. *Nature* **324**: 456-457.

Reijnders, P.J.H. 1994. Toxicokinetics of chlorobiphenyls and associated physiological responses in marine mammals with particular reference to their potential for ecotoxicological risk assessment. *Sci Tot Environ* **154**: 229-236.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (http://www.R-project.org/)

Rogers, P.J., Huvaneers, C., Goldsworthy, S.D., Mitchell, J.G., Seuront, L. 2013. Broad-scale movements and pelagic habitat of the dusky shark *Carcharhinus obscurus* off Southern Australia determined using pop-up satellite archival tags. *Fish Oceanogr* **22**(2): 102–112.

Romine, J., Musick, J., Burgess, G. 2009. Demographic analyses of the dusky shark, *Carcharhinus obscurus,* in the Northwest Atlantic incorporating hooking mortality estimates and revised reproductive parameters. *Environ Biol Fish* **84**: 277-289.

Ross, P.S., de Swart, R.L., Timmerman, H.H., Reijnders, P.J.H., Vos, J.G., van Loveren, H. and Osterhaus, A.D.M.E. 1996. Suppression of natural killer cell activity in harbour seals (*Phoca vitulina*) fed Baltic Sea herring. *Aquat Toxicol* **34**: 71-84.

Sangster, J. 2013. LOGKOW© – a databank of evaluated octanol-water partition coefficients (Log P). Sangster Research Laboratories, Montreal. *Accessed October 28, 2013* (http://logkow.cisti.nrc.ca/logkow/search.html).

Scott, G.R., Sloman, K.A. 2004. Review - The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. *Aquat Toxicol* **68**: 369-392.

Semmens, J.M., Payne, N.L., Huvaneers, C., Sims, D.W., Bruce, B.D. 2013. Feeding requirements of white sharks may be higher than originally thought. *Nature: Sci Reports* **3**(1471): 1-4.

Serrano, R., Fernandez, M.A., Hernandez, L.M., Hernandez, M., Pascual, P., Rabanal, R.M., Gonzalez, J. 1997. Coplanar polychlorinated biphenyl congeners in shark livers from the north-western African Atlantic ocean. *Bull Environ Contam Toxicol* **58**: 150-157.

Serrano, R., Simal-Julián, A., Pitarch, E., Hernández, F., Varó, I., Navarro, J.C. 2003. Biomagnification study on organochlorine compounds in marine aquaculture: the sea bass (Dicentrarchus labrax) as a model. *Environ Sci Technol* **37**: 3375–3381.

Shen, L., Wania, F. 2005. Compilation, evaluation, and selection of physical-chemical property data for organochlorine pesticides. *J Chem Eng Data* **50**: 740-768.

Sijm, D.T.H.M., Selnen, W., Opperhuizen, A. 1992. Life-cycle biomagnification study in fish. *Environ Sci Technol* **26**(11): 2162-2174.

Sijm, D. T., van der Linde, A. 1995. Size-dependent bioconcentration kinetics of hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric relationships. *Environ Sci Technol* **29**(11): 2769-2777.

Simpson, C.D., Wilcock, R.J., Smith, T.J., Wilkins, A.L., Langdon, A.G. 1995. Determination of octanol-water partition coefficients for the major components of technical chlordane. *Environ Contam Toxicol* **55**: 149-153.

Sion, L., Bozzano, A., D'Onghia, G., Capezzuto, F., Franza, M. 2004. Chondrichthyes species in deep waters of the Mediterranean Sea. *Sci Mar* **68**(Suppl 3): 153-162.

Thomann, R.V., Connolly, J.P. 1984. Model of PCB in the Lake Michigan lake trout food chain. *Environ Sci Technol* **18**(2): 65-71.

Turosov, V., Rakitsy, V., Tomatis, L. 2002. Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistance, and risks. *Environ Health Perspect* **110**(2): 125-128.

Vallack, H.W., Bakker, D.J., Brandt, I., Broström-Lundén, E., Brouwer, A., Bull, K.R., Gough, C., Guardans, R., Holoubek, I., Jansson, B., Koch, R., Kuylenstierna, J., Lecloux, A., Mackay, D., McCutcheon, P., Mocarelli, P., Taalman, R.D.F. 1998. Assessment: Controlling persistent organic pollutants- what next? *Environ Toxicol & Pharmacol* **6**: 143-175.

Veith, G.D., Defoe, D.L., Bergstedt, B.V. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. *J Fish Res Board Can* **26**: 1040-1048.

Walters, D.M., Mills, M.A., Cade, B.S., Burkard, L.P. 2011. Trophic magnification of PCBs and its relationship to the octanol-water partition coefficient. *Environ Sci Technol* **45**: 3917–3924.

Ward-Paige, C.A., Mora, C., Lotze, H.K., Pattengill-Semmens, C., McClenachan, L., Arias-Castro, E., Myers, R.A. 2010. Large-scale absence of sharks on reefs in the greater-caribbean: a footprint of human pressures. PLoS ONE 5(8): e11968. doi:10.1371/journal.pone.0011968

Wintner, S.P., Cliff, G. 1999. Age and growth determination of the white shark, *Carcharodon carcharias*, from the east coast of South Africa. *Fish Bull* **97**(1): 153-169.

West, J.E., O'Neil, S.M. 1995. Accumulation of mercury and polychlorinated biphenyls in quillback rockfish (*Sebastes maliger*) from Puget Sound, Washington. *Puget Sound Research '95 Proceedings Volume 2*. Puget Sound Water Quality Authority, Olympia, WA, USA. pp. 666-677.

Worm, B., Davis, B., Kettemer, L., Ward-Paige, C.A., Chapman, D., Heithaus, M.R., Kessel, S.T., and Gruber, S.H. 2013. Global catches, exploitation rates, and rebuilding options for sharks. *Mar Poll* **40**: 194-204.

Ylitalo, G.M., Matkin, C.O., Buzitis, J., Krahn, M.M., Jones, L.L., Rowles, T., Stein, J.E. 2001. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK. *Sci Tot Environ* **281**: 183-203.

Table 2.1. Mean ± 1SE of species' parameters, and observed OC concentrations (ng/g dry wt) for *C. carcharias* and *C. obscurus*, sampled from KZN, South Africa, 2005-2012 (excluding neonates).

	White Shark *Carcharodon carcharias*	**Dusky Shark** *Carcharhinus obscurus*
n (sex)	53 (28 F, 25 M)	42 (22 F, 20 M)
PCL (mm)	2192.6 ± 56.7	1965.3 ± 75.3
Mass (kg)	196.5 ± 20.3	136.6 ± 14.0
Lipid %	1.5 ± 0.1	1.3 ± 0.2
$\delta^{15}N$	15.6 ± 0.1	13.8 ± 0.2
$\delta^{13}C$	-14.7 ± 0.1	-15.8 ± 0.1
HCB	0.2 ± 0.0	0.1 ± 0.0
Dieldrin	1.0 ± 0.1	0.3 ± 0.1
Mirex	0.8 ± 0.1	0.4 ± 0.1
β–HCH	0.1 ± 0.0	0.1 ± 0.0
γ–HCH	0.1 ± 0.0	0.1 ± 0.0
ΣHCH	**0.2 ± 0.0**	**0.2 ± 0.0**
trans-Chlordane	0.7 ± 0.1	0.3 ± 0.0
cis-Chlordane	0.3 ± 0.0	0.2 ± 0.0
trans-Nonachlor	4.6 ± 0.3	1.0 ± 0.1
cis-Nonachlor	0.2 ± 0.0	0.1 ± 0.0
Oxychlordane	0.1 ± 0.0	0.2 ± 0.0
Heptachlor epoxide	0.2 ± 0.0	0.2 ± 0.0
ΣCHL	**6.2 ± 0.4**	**1.8 ± 0.2**
p,p'-DDE	20.2 ± 2.7	9.3 ± 2.2
p,p'-DDT	0.2 ± 0.0	0.2 ± 0.1
ΣDDT	**20.4 ± 2.7**	**9.5 ± 2.2**
PCB 18/17	0.7 ± 0.1	0.2 ± 0.1
PCB 31/28	1.3 ± 0.2	0.7 ± 0.1
PCB 33	1.0 ± 0.2	0.9 ± 0.2
PCB 52	1.9 ± 0.3	0.9 ± 0.1
PCB 49	0.7 ± 0.1	0.6 ± 0.1
PCB 44	1.1 ± 0.1	0.7 ± 0.1
PCB 70	1.4 ± 0.3	0.3 ± 0.1
PCB 95	1.6 ± 0.3	0.4 ± 0.1
PCB 101	1.8 ± 0.5	0.4 ± 0.1
PCB 99	0.8 ± 0.2	0.4 ± 0.0
PCB 110	1.0 ± 0.3	0.5 ± 0.1
PCB 151/82	0.6 ± 0.1	0.2 ± 0.0
PCB 149	1.1 ± 0.1	0.2 ± 0.0
PCB 118	1.2 ± 0.2	0.7 ± 0.1
PCB 153	5.5 ± 0.6	5.0 ± 0.9
PCB 105/132	0.6 ± 0.1	0.5 ± 0.1

Table 2.1 (*continued*). Mean ± 1SE of species' parameters, and observed OC concentrations (ng/g dry wt) for *C. carcharias* and *C. obscurus*, sampled from KZN, South Africa, 2005-2012 (excluding neonates).

	White Shark *Carcharodon carcharias*	**Dusky Shark** *Carcharhinus obscurus*
PCB 138	5.2 ± 0.4	4.4 ± 0.7
PCB 158	0.3 ± 0.0	0.3 ± 0.1
PCB 187	1.7 ± 0.2	2.1 ± 0.5
PCB 183	0.7 ± 0.1	0.8 ± 0.2
PCB 128	1.7 ± 0.2	1.5 ± 0.7
PCB 177	0.5 ± 0.0	0.4 ± 0.1
PCB 156/171	0.6 ± 0.1	0.5 ± 0.1
PCB 180	3.4 ± 0.3	4.2 ± 1.0
PCB 191	0.1 ± 0.0	0.1 ± 0.0
PCB 170	1.0 ± 0.1	1.5 ± 0.4
PCB 199	0.6 ± 0.1	0.9 ± 0.2
PCB 195/208	0.3 ± 0.0	0.3 ± 0.1
PCB 194	0.4 ± 0.0	0.7 ± 0.2
PCB 206	0.1 ± 0.0	0.3 ± 0.1
PCB 209	0.1 ± 0.0	0.1 ± 0.0
ΣPCB	**38.5 ± 3.4**	**30.5 ± 4.7**

Table 2.2. Comparison of OC concentrations in KZN white and dusky sharks sampled 2005-2012 with published levels in other marine predators.

Class-Tissue Citation	Location	Species	*n*	Compound (ng/g, mean ± SE)														
				H. Epox	γ-HCH	*t*-non	*cis*-non	*t*-chl	*cis*-chl	Diel	p,p’-DDE	p,p’-DDT	149	118	153	138	180	170
Mammalia - Blubber																		
Henry & Best 1983	Durban Whaling Station (29°53'S 31 °03'E)	Minke Whale *Balaenoptera acutorostrata*	28[a]	-	-	-	-	-	-	24.7 ± 4.8	64.5 ± 18.0	215.6 ± 69.6	-	-	-	-	-	-
Henry & Best 1983	Durban Whaling Station (29°53'S 31 °03'E)	Fin Whale *B. physalus*	6[a]	-	-	-	-	-	-	ND	91.9 ± 40.9	115.0 ± 72.6	-	-	-	-	-	-
Henry & Best 1983	Durban Whaling Station (29°53'S 31 °03'E)	Sperm Whale *Physeter macrocephalus*	12[a]	-	-	-	-	-	-	ND	351.3 ± 38.8	364.9 ± 106.7	-	-	-	-	-	-
Noël *et al.* 2009	Crozet Archipelago, southern Indian Ocean	Killer Whale (*Orcinus orca*)	9[a]	-	-	-	-	-	-	-	-	-	860 ± 180	-	2670 ± 380	1580 ± 260	860 ± 150	-
Chondrichthyes - Liver																		
Serrano *et al.* 1997	Canary Islands, North African Atlantic Ocean	Portuguese Dogfish *Centroscymnus coelolepis*	9[a]	-	-	-	-	-	-	-	-	-	2.3 ± 0.7	20.6 ± 6.0	45.7 ± 18.3	45.0 ± 27.1	29.3 ± 11.3	2.5 ± 0.9
Serrano *et al.* 1997	Canary Islands, North African Atlantic Ocean	Shortnose Velvet Dogfish *C. cryptacanthus*	1[a]	-	-	-	-	-	-	-	-	-	NA	NA	297.8	253.3	124.4	23.4
Serrano *et al.* 1997	Canary Islands, North African Atlantic Ocean	Leafscale Gulper Shark *Centrophorus squamosus*	1[a]	-	-	-	-	-	-	-	-	-	138.9	1251	1056.5	67.3	12.2	56.8
Schlenk *et al.* 2005	Kwazulu-Natal, South Africa	White Shark *Carcharodon carcharias*	3[a]	26.1 ± 12.4	3.8 ± 0.2	-	-	-	-	-	1114.2 ± 379.0	55.5 ± 14.0	-	-	-	-	-	-
Gelsleichter *et al.* 2007	U.S. East Coast	Sandbar Shark *Carcharhinus plumbeus*	23[b]	2.5 ± 0.3	-	65.4 ± 10.1	36.6 ± 5.8	2.0 ± 0.9	7.7 ± 1.4	2.7 ± 1.4	445.6 ± 71.8	16.5 ± 4.3	-	-	-	-	-	-

Table 2.2 (cont'd). Comparison of OC concentrations in KZN white and dusky sharks sampled 2005-2012 with published levels in other marine predators.

Class-Tissue Citation	Location	Species	*n*	Compound (ng/g, mean ± SE)														
				H. Epox	γ-HCH	*t*-non	*cis*-non	*t*-chl	*cis*-chl	Diel	p,p'-DDE	p,p'-DDT	149	118	153	138	180	170
Gelsleichter *et al.* 2007	U.S. East Coast	Blacktip Shark *C. limbatus*	25[b]	0.68 ± 0.1	-	41.7 ± 6.8	19.7 ± 3.2	1.7 ± 0.2	10.2 ± 1.8	1.7 ± 0.6	127.0 ± 29.2	4.5 ± 2.1	-	-	-	-	-	-
Chondrichthyes - Muscle																		
Gelsleichter *et al.* 2005	Florida Bay, Tampa Bay, Apalachicola Bay, U.S.	Bonnethead Shark *Sphyrna tiburo*	50[b]	0.4 ± 0.3	-	3.1 ± 0.6	2.2 ± 0.8	6.5 ± 0.7	4.0 ± 0.5	0.2 ± 0.9	4.5 ± 0.5	13.9 ± 2.0	-	-	-	-	-	-
Schlenk *et al.* 2005	Kwazulu-Natal, South Africa	White Shark *C. carcharias*	3[b]	1.5 ± 0.6	0.9 ± 0.4	-	-	-	-	-	1.6 ± 0.2	1.8 ± 0.6	-	-	-	-	-	-
This Study	South African West Coast	Dusky Shark *C. obscurus*	42[c]	0.04 ± 0.01	0.01 ± 0.002	0.3 ± 0.03	0.01 ± 0.002	0.1 ± 0.01	0.04 ± 0.004	0.1 ± 0.01	2.3 ± 0.6	0.1 ± 0.01	0.1 ± 0.01	0.2 ± 0.02	1.3 ± 0.2	1.1 ± 0.2	1.1 ± 0.3	0.4 ± 0.1
This Study	South African West Coast	White Shark *C. carcharias*	53[c]	0.1 ± 0.01	0.02 ± 0.004	1.1 ± 0.1	0.1 ± 0.01	0.2 ± 0.02	0.1 ± 0.01	0.2 ± 0.03	5.0 ± 0.7	0.1 ± 0.01	0.3 ± 0.03	0.3 ± 0.04	1.4 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	0.3 ± 0.02
This Study	South African West Coast	Dusky Shark *C. obscurus*	42[a]	35.0 ± 19.4	5.8 ± 1.4	133.1 ± 26.4	6.4 ± 1.7	47.1 ± 9.5	25.8 ± 6.4	45.4 ± 14.8	1114.3 ± 302.7	35.8 ± 17.1	34.3 ± 14.5	107.0 ± 36.7	614.0 ± 153.4	542.9 ± 125.7	520.6 ± 153.5	174.8 ± 50.2
This Study	South African West Coast	White Shark *C. carcharias*	53[a]	20.1 ± 6.9	5.8 ± 1.0	373.3 ± 34.8	17.9 ± 3.1	52.3 ± 7.0	26.5 ± 3.0	95.0 ± 18.8	1802.9 ± 339.2	18.2 ± 3.2	89.3 ± 13.7	102.8 ± 19.4	462.4 ± 67.1	422.7 ± 53.4	278.4 ± 32.1	87.3 ± 11.4

[a] ng/g lipid wt, [b] ng/g wet weight, [c] ng/g wet wt with weight-based moisture content 75.40%, taken from previously determined regression line of relationship between wet and freeze-dried weights of muscle tissue of NSB sharks. The same relationship occurs for all species; $y = 3.9255x + 0.1863$, $r^2 = 0.97$, $p < 0.001$.

Table 3.3. Compound loadings on individuals species PCA axes with significant regressions against mass and $\delta^{13}C$ for dusky and white sharks respectively.

Compound	**PC1 – Dusky Shark** $\lambda = 9.97$	**PC2 – White Shark** $\lambda = 10.03$
HCB	-0.60	-0.18
β-HCH	-0.67	-0.13
γ-HCH	-0.56	-0.01
PCB 18/17	-0.48	-0.45
PCB 31/28	-0.77	-0.26
PCB 33	-0.28	-0.12
PCB 52	-0.61	-0.44
PCB 49	-0.24	-0.34
PCB 44	-0.76	-0.05
Heptachlor Epoxide	-0.30	0.22
Oxychlordane	-0.43	0.44
PCB 70	-0.28	-0.56
PCB 95	-0.70	-0.60
t-chlordane	-0.67	-0.34
PCB 101	-0.63	-0.53
PCB 99	-0.71	-0.37
cis-chlordane	-0.75	-0.39
t-nonachlor	-0.76	0.43
Dieldrin	-0.57	-0.39
PCB 110	-0.58	-0.43
PCB 151/82	-0.43	-0.58
PCB 149	-0.59	-0.06
PCB 118	-0.49	0.13
cis-nonachlor	-0.61	0.05
PCB 153	-0.13	0.76
PCB 105/132	-0.40	0.49
p,p'-DDT	-0.30	-0.10
PCB 138	-0.38	0.71
PCB 158	-0.49	0.40
PCB 187	-0.15	0.67
PCB 183	-0.20	0.52
PCB 128	0.08	0.88
PCB 177	-0.50	0.62
PCB 156/171	-0.71	0.45
PCB 180	-0.27	0.76
PCB 191	-0.24	-0.43
Mirex	0.16	0.57
PCB 170	-0.04	0.81
PCB 199	-0.10	0.65
PCB 195/208	-0.18	0.21
PCB 194	0.12	0.71
PCB 206	-0.16	0.74
PCB 209	-0.24	0.22

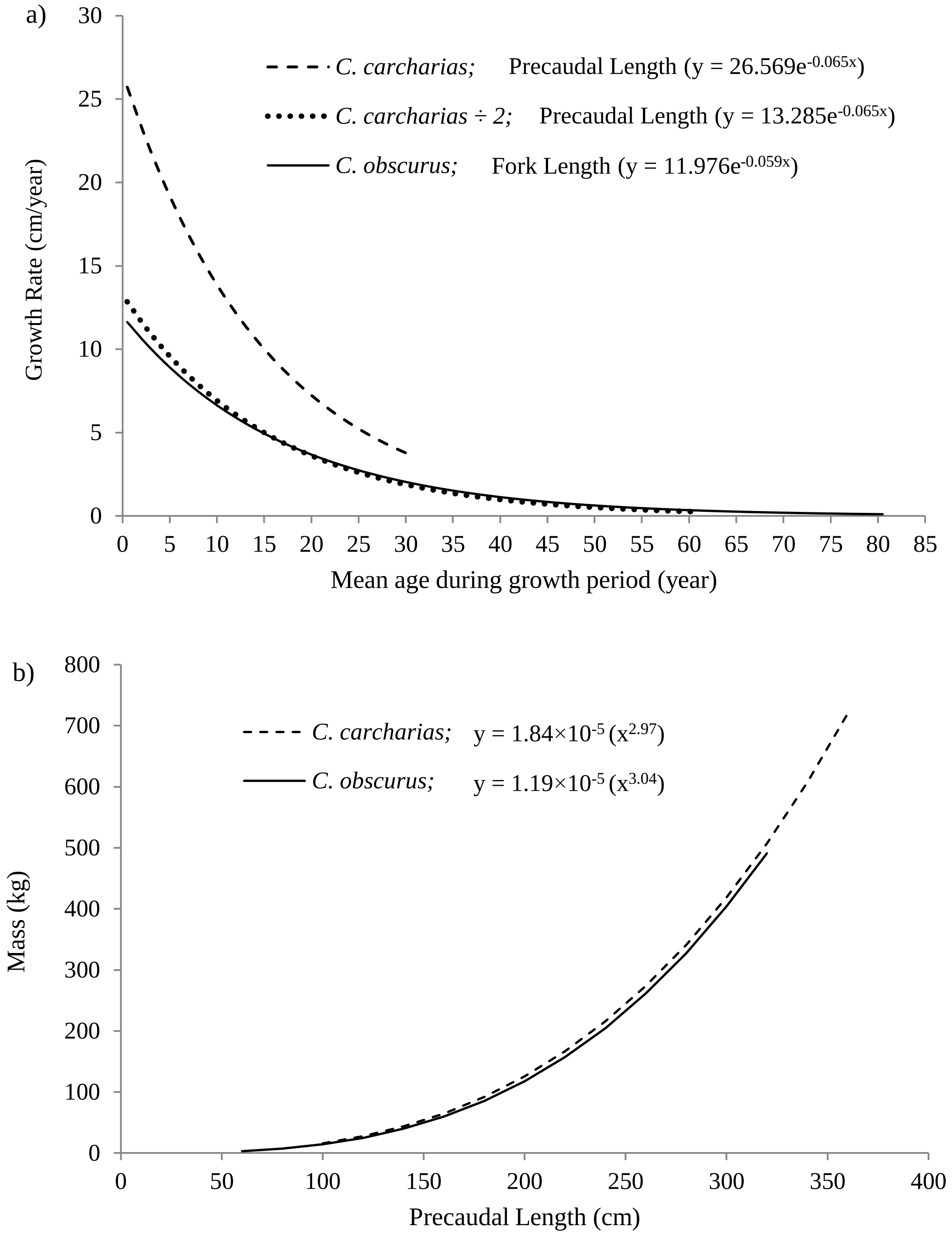


Figure 2.1. Comparative growth between South African dusky and white sharks; a) growth rate at age as per von Bertalanffy growth curves from Natanson *et al.* (2013), and Wintner & Cliff (1999) respectively, with white shark growth rate also halved due to potentially underestimated longevity (Hamady *et al.* 2014), and b) length-mass curves obtained from Cliff *et al.* (1989), and Dudley *et al.* (2005). Maximum ages were inferred from von Bertalanffy parameters, using maximum reported lengths caught in South African waters; a 328 cm (FL) *C. obscurus* caught at the KZNSB (Cliff & Wilson 1986; Natanson & Kohler 1996), and a 485 cm (PCL) *C. carcharias* caught off the Southern Cape (Cliff *et al.* 1989).

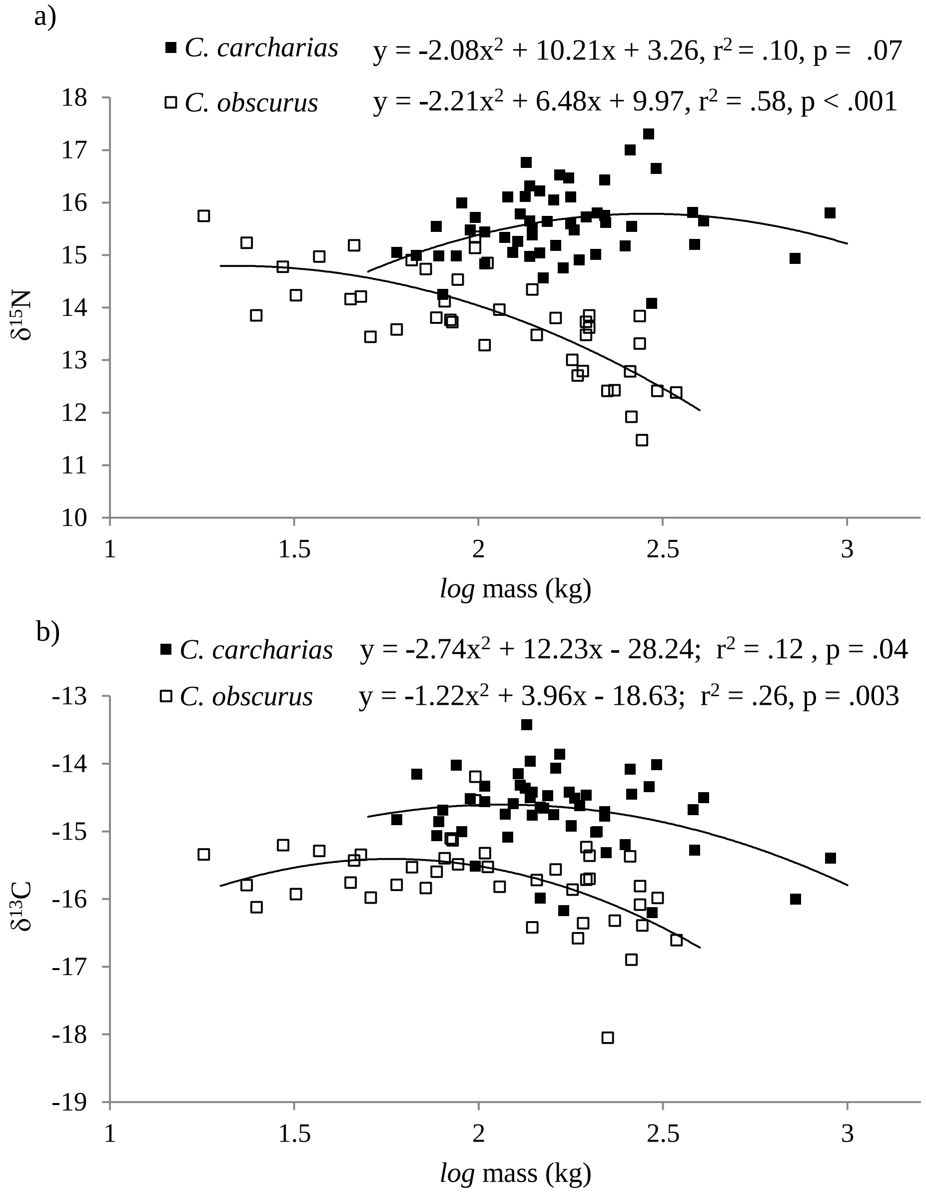


Figure 2.2. Quadratic regression of log-transformed mass vs. stable isotopes; (a) $\delta^{15}N$, and (b) $\delta^{13}C$ in *C. carcharias* and *C. obscurus* muscle tissue, sampled from KZN, South Africa, 2005-2012 (excluding neonates).

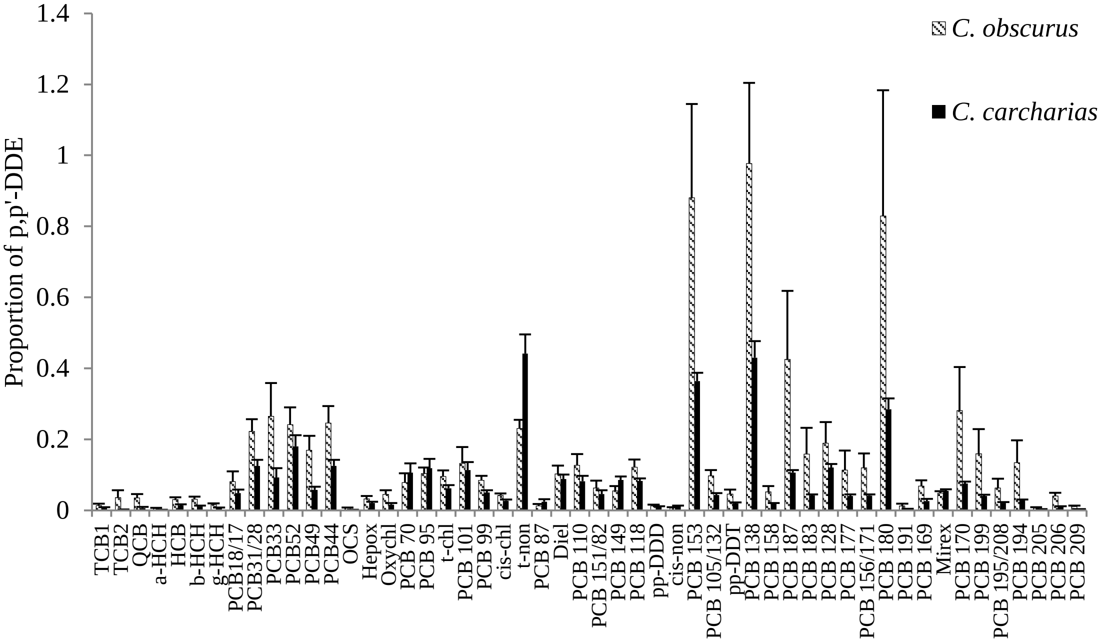
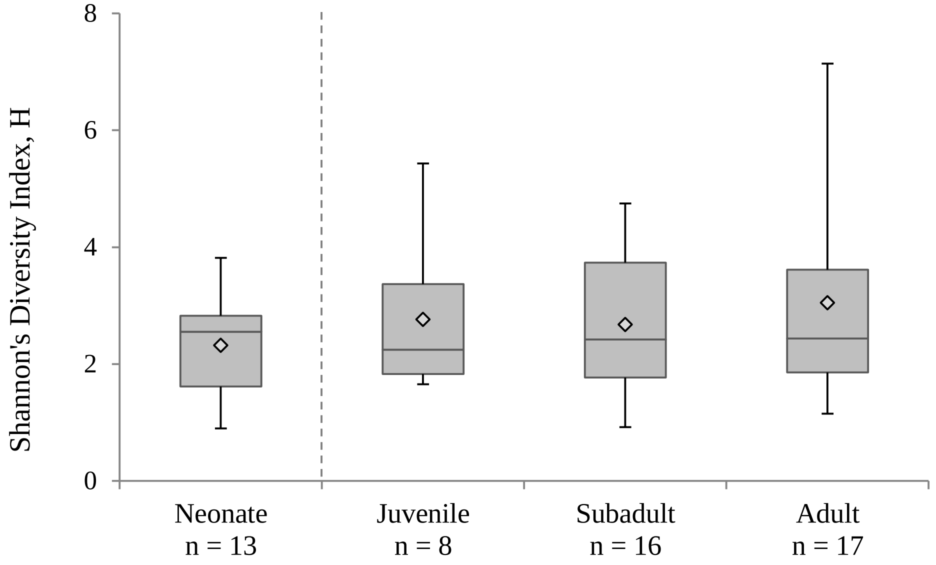


Figure 2.3. Differences in dusky and white shark contaminant profiles sampled from KZN, South Africa, 2005-2012, compared across all analyzed compounds as proportion of p,p'-DDE (excluding neonates). Mean and standard error are represented by bar and error bar values respectively.

a) *C. obscurus*



b) *C. carcharias*

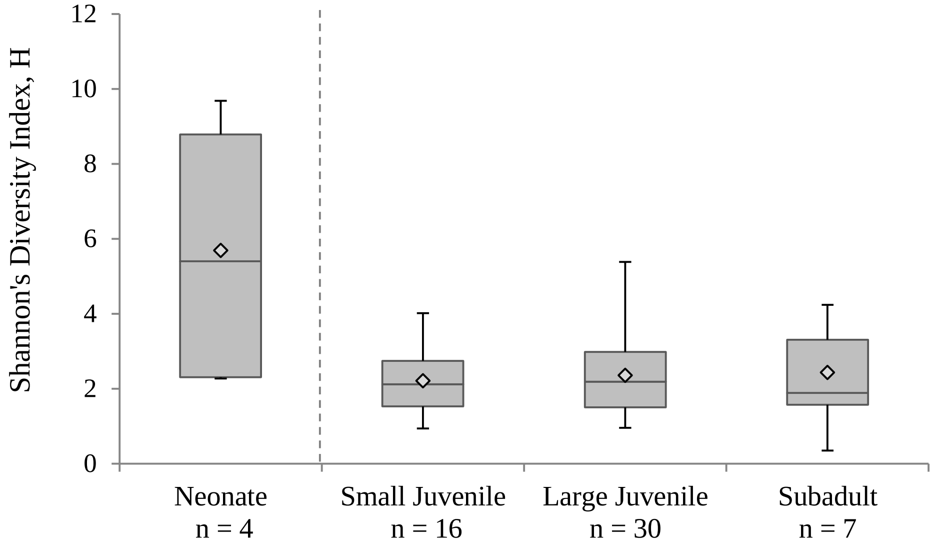


Figure 2.4. Box-plots displaying differences in Shannon's Diversity (H) values between sharks of different life stages for (a) dusky and (b) white sharks, sampled from KZN, South Africa, 2005-2012. Box limits represent 1st and 3rd quartiles, error bars represent minimum and maximum values, centre line displays median values and diamonds represent the mean.

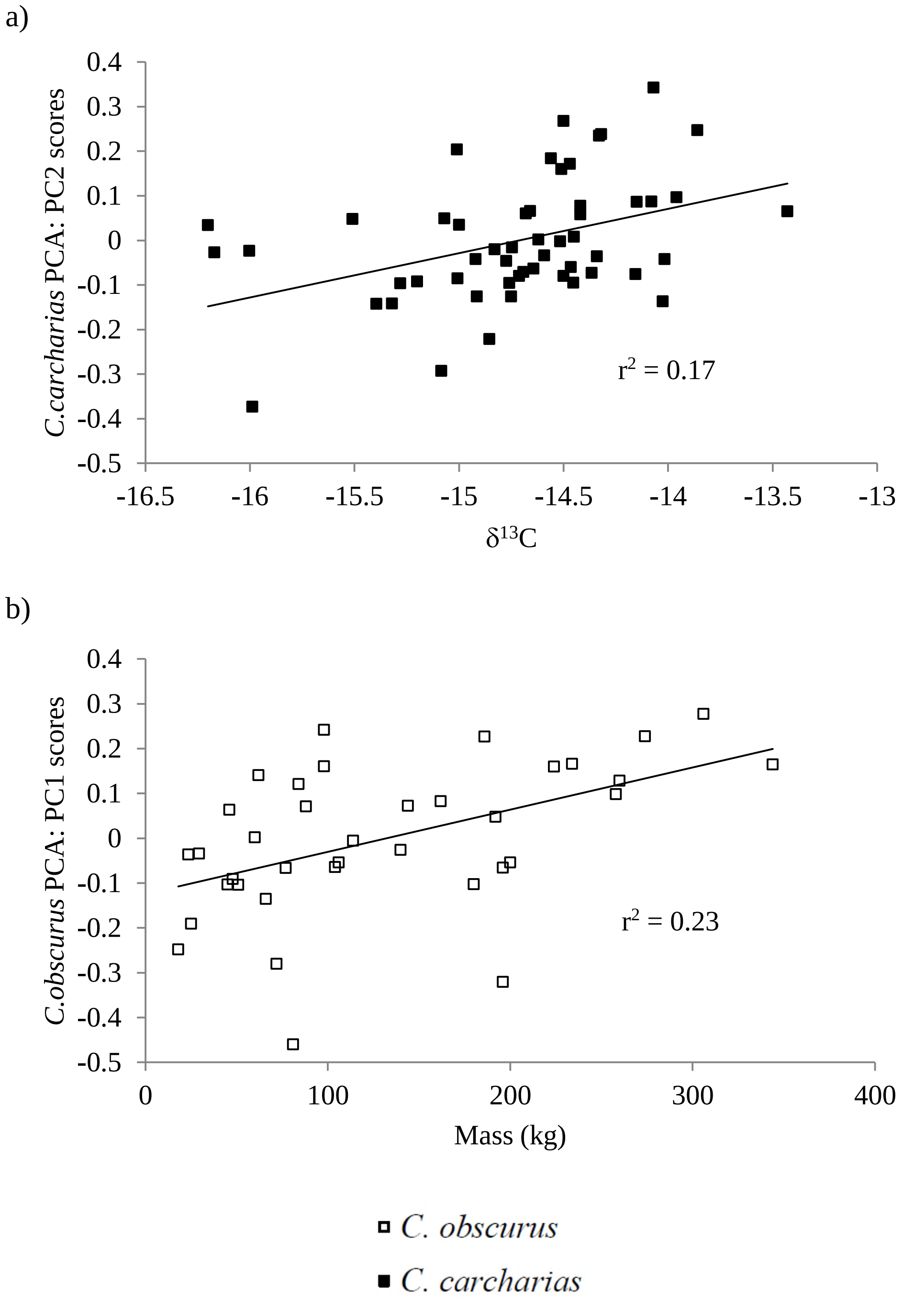


Figure 2.5. Significant regressions found between intraspecific PCA ordination axes (constructed from data as proportions of p,p'-DDE) and shark mass, δ^{13}C, and δ^{15}N values in white and dusky sharks sampled from KZN, 2005-2012. a) White shark PC2 (accounting for 23.3% of data variation) vs. δ^{13}C. Proportions of PCBs 153, 138, 128, 180, 170, 194 and 206 loaded positively (> 0.714) on PC2. b) Dusky shark PC1 (accounting for 23.2% of data variation) vs. mass. Proportions of only mirex and PCB 194 loaded positively but weakly (< 0.2) on PC1.

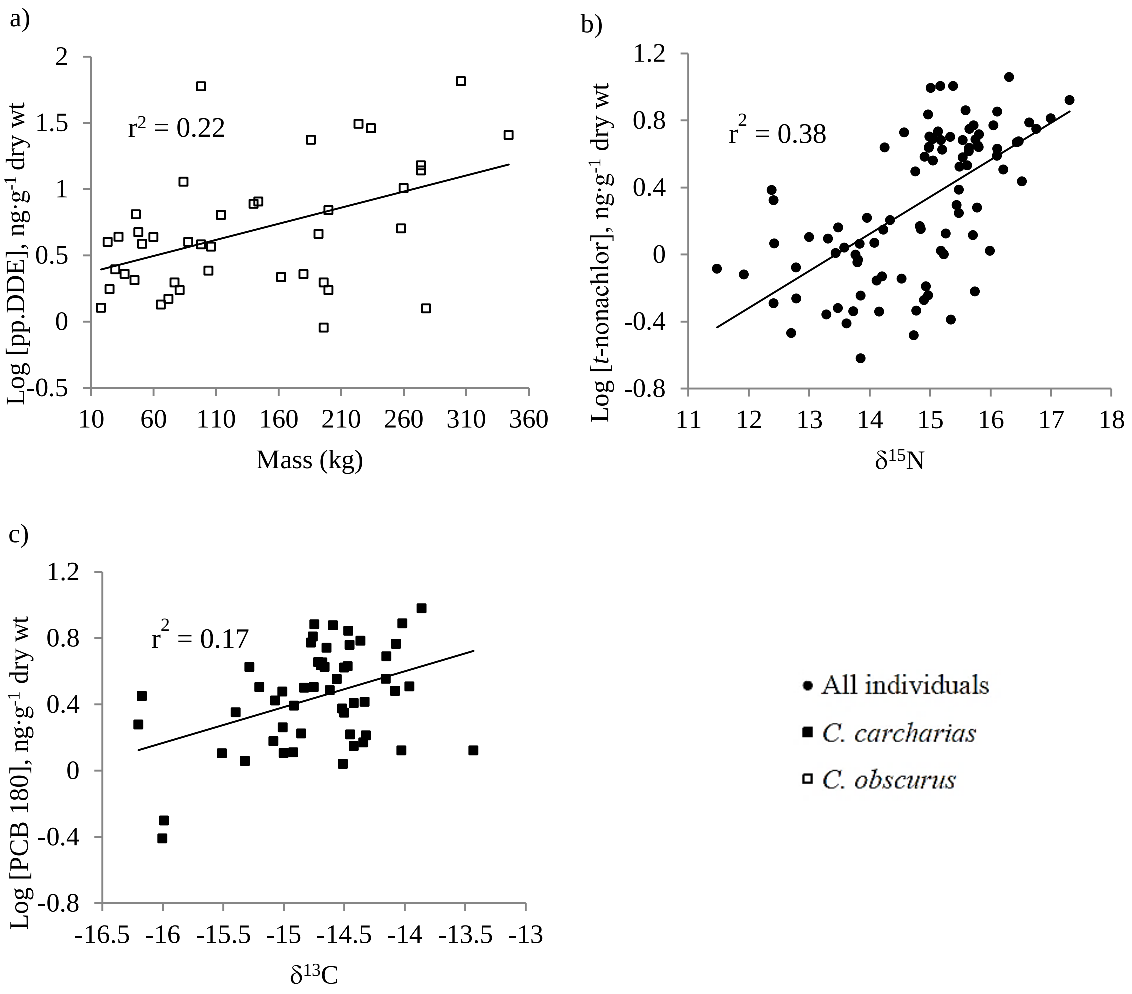


Figure 2.6. Examples of significant relationships between muscle OC concentrations and shark predictor variables when excluding neonates; a) p,p'-DDE vs. mass in the dusky shark, b) *t*-nonachlor vs. $\delta^{15}N$ in all individuals, and c) PCB 180 vs. $\delta^{13}C$ in the white shark. Species were sampled from the coast of KZN, South Africa, 2005-2012.

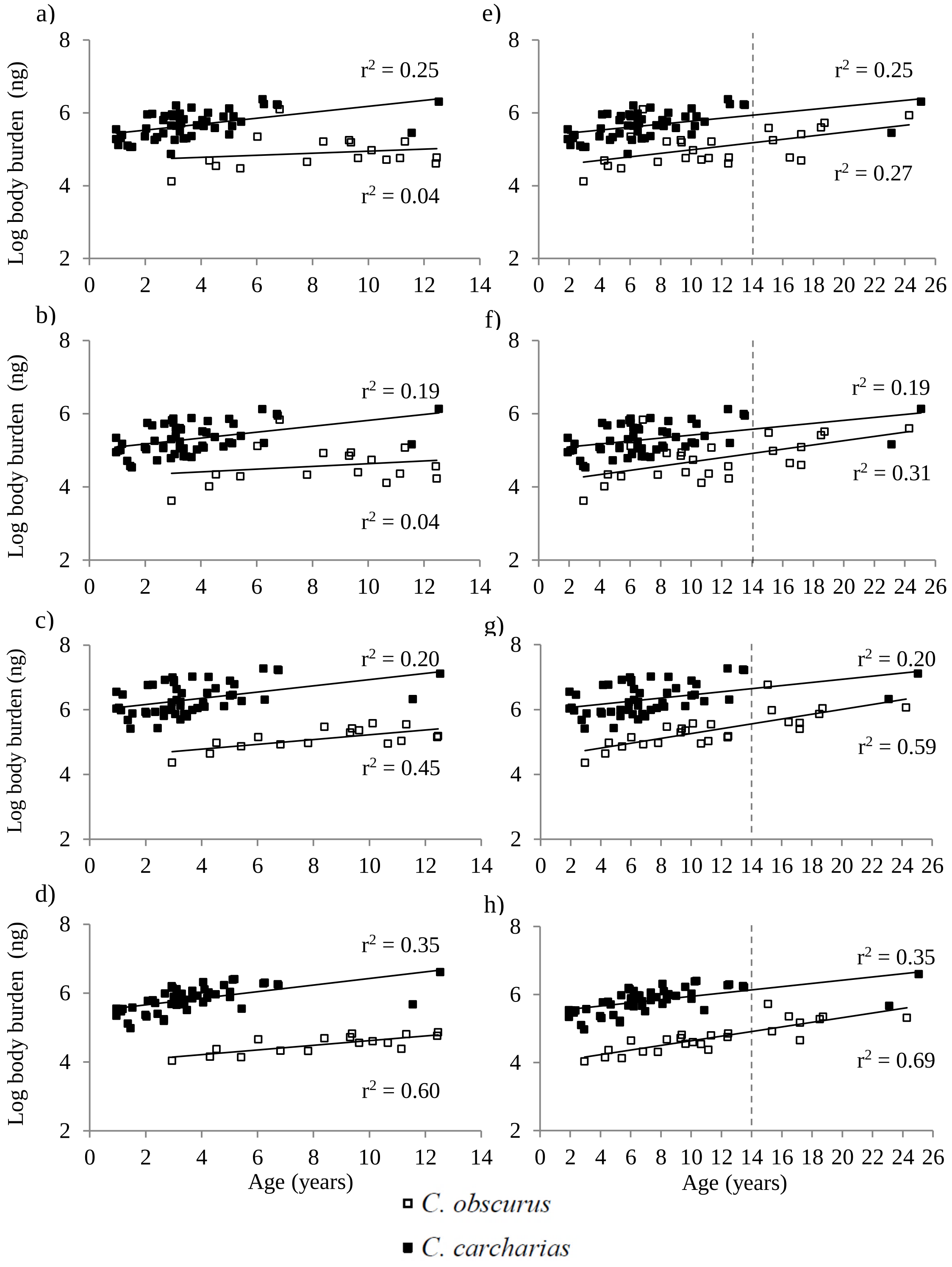


Figure 2.7. Growth-dilution corrected concentrations of PCBs 180 (a, e), 187 (b, f), p,p'-DDE (c, g), and *t*-nonachlor (d, h) vs. age for white and dusky sharks from KZN, South Africa (excluding neonates). a-d; white shark ages as per Wintner & Cliff (1999), e-h; white shark ages doubled and 8 additional dusky sharks added (after dotted-line). ANCOVA interaction term; p > .05 for all.

CHAPTER 3

GENERAL DISCUSSION

3.1 Summary

It is important to understand the mechanisms behind the bioaccumulative processes of hydrophobic contaminants in marine systems, not only to provide general information on the most highly exposed species and the potential for toxic impacts, but also to gain insight into the movements of energy, in the form of lipids, throughout food web structures. In this thesis I examined organochlorine contaminant (OC) accumulation in two ecologically comparable top predator shark species (*Carcharodon carcharias* and *Carcharhinus obscurus*) from the east coast of South Africa, with the life history contrasts of ontogenetic divergence in prey trophic position, thermoregulatory mode and tenably, different growth rates. Specifically, this thesis highlighted comparative OC profiles, concentrations, and accumulation patterns with ontogeny.

Profiles of OCs were observed with respect to species and life stages within species, and the composite proportional response of all analysed chemicals were examined for relationships with the individual shark attributes of $\delta^{15}N$, $\delta^{13}C$, sex and mass. Statistical assessment revealed significantly different OC profiles between species. This difference was driven by higher proportions of the PCBs 153, 138 and 180 in the dusky shark, which are some expectedly predominant congeners in pelagic regions (Goutte *et al.* 2013). This result is supported by these species' $\delta^{13}C$ values which suggest comparatively more offshore residency in the dusky shark, and by the fact that subadult and adult dusky sharks are known to reside at the edge of the continental shelf (Hussey *et* *al.* 2011; Rogers *et al.* 2013). Intraspecific profiles did not differ in diversity across life stages except in neonate white sharks, representing efficient maternal transfer in this species (Mull *et al.* 2013; Lyons & Lowe 2013). More prevalent inshore habitat use in the white shark was found to provide a more even abundance of the full suite of (analysed) compounds available in the South African ecosystem, increasing the proportions of 5 PCB congeners in particular (153, 128, 180, 170, 206). Overall, I discovered that compared to the dusky shark, the white shark possesses a more even profile of contamination, which is intensified with a greater prevalence of inshore feeding as indicated by $\delta^{13}C$ values.

Body size was a predictor of OC concentrations within the dusky shark only. More rapid growth-dilution is implicated in obscuring any relationships with body size in the white shark (Lyons *et al.* 2013). This observation supports the likelihood that white sharks do have higher growth rates than dusky sharks, despite recent indications of enhanced longevity (Hamady *et al.* 2014). To avoid the effect of differential growth-dilution on the comparison of gross ontogenetic uptake, accumulation slopes with age were compared between species using mass-based concentrations to correct for decreases resulting from growth-dilution. Despite ontogenetic diet shifting to mammal prey, regional endothermy, and likely faster growth rate, slopes of growth-dilution corrected concentrations with age in the white shark were equal to those of the dusky shark. This is an important observation because it allows for clarification of the ontogenetically declining $\delta^{15}N$ values within dusky sharks, by providing evidence of similar OC accumulation to the white shark which increases in $\delta^{15}N$ ontogenetically. This would provide support against the hypothesis that this pattern is a result of diet shifting to lower trophic level prey with ontogeny, and instead the likelihood that progressive movement toward the continental shelf provides lower $\delta^{15}N$ due to lower baseline values in that system (Hussey *et al.* 2011). This demonstrates that movement to or between systems with variable baseline stable isotope signatures must be a pivotal consideration in ecological studies (Post 2002; Jardine *et al.* 2006).

Due to the white shark's higher trophic position (Hussey *et al.* 2014), and accompanying greater body size, they were expected to possess elevated levels of OCs. This was true for p,p'-DDE and *t*-nonachlor, two of the four notoriously highly biomagnifying compounds (also PCBs 180 and 187), chosen to exemplify differences in net (growth-diluted) concentrations between these species. These two OCPs have been previously found to biomagnify to a greater extent (one order of magnitude more) than PCBs 180 and 187, from prey fish to piscivorous Galapagos sea lions, *Zalophus wollebaeki* (Alava & Gobas 2012). Similar concentrations of these two PCB congeners further exemplifies comparable ontogenetic accumulation between these species, and the fact that significantly higher levels of only powerfully biomagnifying compounds were found supports that trophic position (along with the accompanying larger body size of white sharks) is responsible for the heightened accumulation. While comparatively more inshore habitat use (*i.e.,* significantly elevated $\delta^{13}C$ values) may further contribute to high contamination in white sharks, this is not the most parsimonious explanation for their elevated levels of p,p'-DDE and *t*-nonachlor. Based on a significant negative relationship between PCB 180 concentrations and $\delta^{13}C$ in both species, white sharks would be expected to contain higher concentrations of PCB 180 than the dusky shark, but this is not the case. This study has therefore highlighted the role of body size/trophic structure, and its precedence as a predictor of the accumulation of OCs in shark species despite variations in life history.

3.2 Implications and Future Directions

By beginning with an examination of the expected divergence in OC accumulation between two shark species, this study can be extended to apply the concepts of body size and trophic position as predictors of OC accumulation in food webs that incorporate many shark species, such as the KZN marine ecosystem. OCs are likely useful as tracers of trophic structure and trophic ecology of species in this system, regardless of these complexities introduced by variable life-history characteristics. However, this study was limited in the size range of included white sharks, due to rare capture of adults in KZNSB nets. Further insight into the effects of diet-shifts to mammals on white shark OC burdens may be provided by the examination of larger, older white sharks, in case the younger individuals included here showed low accumulation with age due to a low frequency of seal predation. This may be obtainable through a muscle biopsy of large white sharks that are captured and tagged, and could also provide valuable information about which individuals specialize at the seal-hunting strategy. In addition, more shark species should be examined, particularly those with more extensively diverse physiology and trophic ecology, such as the enormous filter-feeding whale shark, *Rhincodon typus*. The question remains whether trophic position and body size still drive accumulation potential in the face of such exceedingly large differences in physiology and feeding behaviour.

Other aspects of chemical bioaccumulation may also still be examined, for example, in light of specific physico-chemical properties of certain biomagnifying contaminants, and whether they render trophic level and body size more or less important as drivers of levels found in sharks and/or shark-rich ecosystems. Because so few studies have examined the physiology of OCs in sharks in terms of both their toxicity and metabolism (Gelsleichter & Walker 2010), and many cases exist where species or class-specific metabolic biotransformation ability determines the presence and abundance of certain OCs (Borgå *et al.* 2004), more investigation is required to understand whether a specific benchmark chemical may be utilized to best discriminate trophic structure within the KZN food web. This is particularly important also because of the current discrepancies in stable isotope analyses, and the avenue provided by biomagnifying contaminants for clarification of SIA-derived trophic structure (Soto *et al.* 2013).

3.3 References

Alava, J.J., Gobas, F.A.P.C. 2012. Chapter 4. Assessing biomagnification and trophic transport of persistent organic pollutants in the food chain of the Galapagos Sea Lion (*Zalophus wollebaeki*): conservation and management implications. IN: Romero, A., Keith, E.O. (eds). 2012. *New Approaches to the Study of Marine Mammals*. InTech — Open Access Company. pp. 77-108. (http://www.intechopen.com/books/new-approaches-to-the-study-of-marine-mammals).

Borgå, K., Fisk, A.T., Hoekstra, P.F., Muir, D.C.G. 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environ Toxicol Chem* **23**(10): 2367-2385.

Gelsleichter, J., Walker, C.J. 2010. Pollutant exposure and effects in sharks and their relatives, IN: Carrier, J.C. *et al.* (eds). 2010. *Sharks and their relatives II: Biodiversity, adaptive physiology, and conservation.* CRC Marine Biology Series: pp. 491-537.

Goutte, A., Chevreuil, M., Alliot, F., Chastel, O., Cherel, Y., Eléaume, M., Massé, G. 2013. Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica. *Mar Poll Bull* **77**: 82-89.

Hamady, L.L., Natanson, L.J., Skomal, G.B., Thorrold, S.R. 2014. Vertebral bomb radiocarbon suggests extreme longevity in white sharks. *PLoS ONE* **9**(1): e84006. doi:10.1371/journal.pone.0084006

Hussey, N.E., Dudley, S.F.J., McCarthy, I.D., Cliff, G., Fisk, A.T. 2011. Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? *Can J Fish Aquat Sci* **68**: 2029-2045.

Hussey, N.E., MacNeil, M.A., McMeans, B.C., Olin, J.A., Dudley, S.F.J., Cliff, G., Wintner, S.P., Fennessy, S.T., Fisk, A.T. 2014. Rescaling the trophic structure of marine food webs. *Ecology Letters* **17**: 239-250.

Jardine, T.D., Kidd, K.A., Fisk, A.T. 2006. Critical review: applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. *Environ Sci Technol* **40**(24): 7501–7511.

Lyons, K., Carlisle, A., Preti, A., Mull, C.G., Blasius, M., O'Sullivan, J.B., Winkler, C., Lowe, C.G. 2013. Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of young of the year lamniform sharks. *Mar Environ Res* **90**: 27-38.

Lyons, K., Lowe, C.G. 2013. Mechanisms of maternal transfer of organochlorine contaminants and mercury in the common thresher shark (*Alopias vulpinus*). *Can J Fish Aquat Sci* **70**: 1667-1672.

Mull, C.G., Lyons, K., Blasius, M.E., Winkler, C., O'Sullivan, J.B., Lowe, C.G. 2013. Evidence of maternal offloading of organic contaminants in white sharks (*Carcharodon carcharias*). *PLoS ONE* **8**(4): e62886. doi:10.1371/journal.pone.0062886

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**(3): 703-718.

Rogers, P.J., Huvaneers, C., Goldsworthy, S.D., Mitchell, J.G., Seuront, L. 2013. Broad-scale movements and pelagic habitat of the dusky shark *Carcharhinus obscurus* off Southern Australia determined using pop-up satellite archival tags. *Fish Oceanogr* **22**(2): 102–112.

Soto, D.X., Gacia, E., Catalan, J. 2013. Freshwater food web studies: a plea for multiple tracer approach. *Limnetica* **32**(1): 97-106.

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