



# **Pollutants in marine biota from three Mascarene islands**

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## Summary

The Indian Ocean is the third-largest body of water on the planet. It is a source of food and livelihood for millions of people—most of them from developing countries. The western Indian Ocean (WIO) extends from the shores of Somalia to South Africa, and as far eastward as the Mauritian Outer Islands. Fewer ecotoxicological studies have been conducted in the WIO compared with other regions, and even less in the Mascarene Basin. This study aims to enhance the ecotoxicological knowledge of three tropical islands in the Mascarene Basin. Four aspects of ecotoxicology are covered in four article manuscripts published or submitted to international journals.

The first ecotoxicological aspect is the transport of pollutants to remote islands through shipwrecks. Shipwrecks cause ecological harm by physically damaging reef systems when grounding and subsequently causing long-term toxicological harm when pollutants leach from the wreck to the surrounding reef system. This has the potential to kill corals, destabilising the base of the coral reef ecosystem.

Secondly, halogenated pollutants were quantified in coral reef biota. This is also the first report of brominated compounds in coral. Brominated and chlorinated compounds were quantified in hard- and soft coral and fish from St. Brandon's Atoll (SBR), Agalega, and Rodrigues for the first time. Soft coral contained higher concentrations than hard coral for all persistent organic pollutants (POPs). Hard coral contained higher concentrations of novel brominated flame retardants than soft coral. Fish consistently had higher concentrations than hard coral but did not differ significantly. The widespread occurrence of pentabromotoluene (PBT) was confirmed in reef biota for the first time, raising the question if PBT should be considered as a candidate POP.

The third article investigated the concentrations of POPs in seabird eggs of fairy terns, sooty terns, and common noddies from SBR. Sooty- and fairy terns forage further offshore and were seemingly exposed to more pollutants. This study also reported POPs in the eggs of a terrestrial species—chicken—from the Mascarene Basin for the first time. This suggests aerial transport of pollutants to the WIO. Concentrations of pollutants in the eggs were lower than other values found in literature.

I quantified perfluoroalkyl substances (PFAS) in the eggs of seabirds and chickens from SBR in the final article – the first for the WIO. Fairy tern eggs contained the highest concentrations of PFAS, followed by sooty terns, then common noddies. Long-chained PFAS were prevalent over short chained PFAS. All chicken eggs contained quantifiable concentrations of PFAS, again suggesting aerial transport.

The concentrations of pollutants in all biota that were quantified were lower than reported from elsewhere. The remote nature and lack of industry in the Mascarene Basin contributed

to low concentrations of pollutants in biota. The Mascarene Basin would be an ideal location to monitor background concentrations of pollutants. The fact that pollutants could be quantified in remote tropical islands in the WIO shows how ubiquitous pollutants are distributed in the environment and may contribute towards identifying candidate POPs.

**Keywords:** Bird eggs; Coral; Fish; Pollution; Western Indian Ocean



## Preface

This is to state that I, Veronica van der Schyff, have chosen to submit my thesis in article format.

All co-authors involved with this study have expressed permission for these articles to be included in this thesis. Authorship statements where each author's involvement is indicated are presented in the Appendix.

The articles have been prepared for submission to the following journals: Marine Environmental Research, Chemosphere, Science of the Total Environment, and Marine Pollution Bulletin. The links to the authors' guidelines of the respective journals are presented here:

Article 1: Marine Environmental Research

<https://www.elsevier.com/journals/marine-environmental-research/0141-1136/guide-for-authors>

Article 2: Chemosphere

<https://www.elsevier.com/journals/chemosphere/0045-6535/guide-for-authors>

Article 3: Science of the Total Environment

<https://www.elsevier.com/journals/science-of-the-total-environment/0048-9697/guide-for-authors>

Article 4: Marine Pollution Bulletin

<https://www.elsevier.com/journals/marine-pollution-bulletin/0025-326x/guide-for-authors>

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## Chapter 1: Introduction

### 1. General Introduction

Ours is truly a “blue planet.” The ocean covers over 70% of the Earth—approximately 360 million km<sup>2</sup> of the surface of the planet is covered by the ocean (Costello *et al.*, 2010; Weast, 1980). Approximately 40% of the world’s human population lives within 100 km of the coast (UN, 2017). All inhabitants of the Earth are dependent on the ocean for ecological services such as oxygen production, food security, and temperature regulation (Ridgewell & Hargreaves, 2007). Unfortunately, these services are compromised by human activity. The ocean absorbs approximately 90 gigatons (Gt) of atmospheric carbon dioxide (CO<sub>2</sub>) every year (Rackley, 2017). As industrialisation increases and the amount of CO<sub>2</sub> emission rises, absorption of CO<sub>2</sub> by the ocean also increases. An increase of oceanic CO<sub>2</sub> leads to a rise in ocean temperature and ocean acidification (Sheppard *et al.*, 2009). The period between 2010 and 2019 was the warmest decade yet on record, with projections indicating a continued steady rise in global temperature (Climate Central, 2020). Marine biota from all regions of the ocean are being pushed to their physiological limits in a warming ocean. A further source of anthropogenic stress on marine biota and ocean systems is the addition of pollution to the ocean (Brown & Howard, 1985; Ramaiah *et al.*, 2002; Kachur *et al.*, 2019). It is thought that the ocean is the final sink for most anthropogenic pollutants (Lohmann *et al.*, 2006; Jamieson *et al.*, 2017; Rochman, 2018). In addition to pollutants emitted directly into the marine environment, the ocean is exposed to both fluvial and aeolian land-based pollutants (Lanceleur *et al.*, 2011; Shevchenko *et al.*, 2016) and affected by the wet deposition of atmospheric pollution through rainfall or snow (Regnery & Püttmann, 2009).

Metallic elements and organic compounds, such as persistent organic pollutants (POPs), perfluoroalkyl substances (PFAS), and novel brominated flame retardants (NBFR), are environmentally hazardous chemicals that are often associated with marine pollution. When these compounds are present at higher concentrations than natural baseline concentrations, it is regarded as contamination (Chapman, 1995). These substances constitute a toxicological threat when present in the environment at elevated concentrations. If the concentration of a contaminant causes adverse effects on a habitat or biota, it is considered as pollution (Chapman, 1995). In this study, the term ‘pollutant’ refers to metals, POPs, PFAS, or NBFR, depending on the context within which the term is used.

The international community attempts to address the emission, distribution, and transmission of environmentally hazardous chemicals through the promulgation of international regulatory instruments (Micklitz, 1991). Some of the most prominent treaties are the Stockholm Convention on Persistent Organic Pollutants, the Basel Convention on the

Control of Transboundary Movements of Hazardous Wastes and Their Disposal, and the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade (Rotterdam Convention, 2010; Basel Convention, 2011; Stockholm Convention, 2019a). Notwithstanding these interventions, the concentrations of pollutants in the ocean are increasing steadily (Visbeck & Schneider, 2018). To further expatiate the situation, a positive correlation exists between ocean acidification and pollution, where one intensifies the other (Zeng *et al.*, 2015). Now more than ever, it is vital to detect and quantify as many pollutants present in the marine environment as possible to understand the extent of the threat presented by marine pollution. What is not understood cannot be managed and mitigated. With more than 80% of the ocean currently unexplored (NOAA, 2018) it stands to reason that a comprehensive ecotoxicological overview, determining the concentrations of metals, POPs, PFAS, and NBFR in the ocean, is long overdue and crucial.

To protect the ocean and all who depend on it, the United Nations developed the United Nations Sustainable Development Goal 14 (SDG 14) as part of Agenda 2030. The goal is to conserve and sustainably use the ocean, seas, and marine resources for sustainable development (UN, 2020) by, amongst others, increasing scientific knowledge to improve ocean health and biodiversity. Target 14A of SDG 14, with its broad focus of increasing scientific knowledge, places emphasis on small island states (UN, 2020). In this context, the western Indian Ocean region (WIO) is identified as a region on which limited scientific literature regarding marine pollution exists (Table 1). The geographic boundaries of the WIO extend from Somalia to the Southern Ocean, stretching as deep as the Mauritian Islands (WIOMSA, 2020). Ten countries are recognised as member states of the western Indian Ocean Marine Science Association (WIOMSA): Somalia, Kenya, Tanzania, Mozambique, South Africa, Madagascar, Comoros, Seychelles, Réunion (France), and Mauritius (WIOMSA, 2020). This study focuses specifically on the Mascarene Basin that forms part of the WIO region.

The entirety of the WIO region is acutely affected by unemployment and poverty (Cinner & David, 2011; Van der Elst *et al.*, 2005). As such, the inhabitants of the WIO countries are dependent on the ocean for income and nourishment. Small-scale fisheries provide between 5% and 99% of the national agricultural export of countries surrounding the WIO (Walmsley *et al.*, 2006). Most of these fisheries are located on coral reefs (McClanahan *et al.*, 2009). Pollution has the potential to adversely impact coral reefs and the fisheries associated therewith (Ko *et al.*, 2014; Khoshnood, 2017; Li *et al.*, 2019) endangering the livelihood of those that depend thereon. Coastal communities in the WIO rely on artisanal fisheries as a primary food source and source of income; therefore, it is vital to understand the extent of pollution in this marine environment, and particularly coral reefs.

Coral reefs cover less than 1% of the ocean's floor but provide a habitat to more than 25% of marine species (Spalding *et al.*, 2001). Coral reefs provide an estimated \$9.6 billion of benefits to coastal communities worldwide. These benefits include revenue through tourism and fisheries, as well as coastal protection and biodiversity (Conservation International, 2008).

Coral reefs are one of the oceanic habitats most vulnerable to damage through anthropogenically-induced stressors (Hugh & Connell, 1999). Some of the best-developed coral reefs are found around islands in the Mascarene Basin of the WIO. It is tempting to think of isolated oceanic islands as being untouched by anthropogenic activities. However, with over 5 trillion pieces of plastic circulating in the ocean (Eriksen *et al.*, 2014), it is inevitable that plastic will end up on isolated beaches (Bouwman *et al.*, 2016). Due to the hydrophobic nature of POPs, they are known to associate with non-water substances, including plastic (Koelmans *et al.*, 2014). The contaminants adsorbed to the plastic will be released on the beach and surrounding ocean. If these contaminants are released onto the coral reefs of isolated oceanic islands, they can biomagnify through the marine food web or bioconcentrate through exposure with toxins in the water column. Another pathway of pollution to isolated oceanic islands is through shipwrecks.

## **1.1 State of the science in the western Indian Ocean**

To date, a limited number of scientific articles have been published on pollution in the Indian Ocean compared to publications focusing on the Atlantic and Pacific oceans — even less on the WIO. Table 1 lists ecotoxicological studies conducted in the WIO that were published in scientific journals. The following criteria were used to ensure that the referenced studies were relevant for determining the extent of the body of scientific publications on pollutants in the WIO:

- 1) Only studies conducted in the western Indian Ocean were included.
- 2) The Southern Ocean (the area south of South Africa) was excluded.
- 3) Countries south of Somalia, including the Island States, were included.
- 4) The Atlantic coast of South Africa was excluded.
- 5) Only peer-reviewed articles published in scientific journals were referenced. Conference proceedings and dissertations/theses were not considered.
- 6) Only studies on natural mediums were considered. Studies involving plastic ingestion by biota were included in Table 1, although microplastic- and plastic debris surveys were excluded.
- 7) Articles on nutrient pollution and eutrophication were not included.
- 8) Only studies written in English were included.



**Table 1.** Previous ecotoxicological studies conducted in the western Indian Ocean

Pollutant	Medium	Country/Region	Collection	Reference
			year	
POPs	Air	Maldives	2004/2005	Würl <i>et al.</i> , 2007
			Collection date	
Metals	Water	Mayotte	unknown	Thomassin <i>et al.</i> 2011
PCBs and petroleum hydrocarbons	Sediment	Tanzania	unknown	Machiwa, 1992a
			Collection date	
Metals	Sediment	Tanzania	unknown	Machiwa, 1992b
Metals and POPs	Sediment	Tanzania	1993	Machiwa, 2000
Metals	Sediment	South Africa	1995/1996	Binning & Baird, 2001
Metals	Sediment	South Africa	1996/1997	Wepener & Vermeulen, 2005
Organochlorine pesticide	Sediment	Kenya	2001	Barasa <i>et al.</i> , 2007
		Kenya/ Tanzania/		
Metals	Sediment	Mozambique	2007	Kamau <i>et al.</i> , 2015
Metals	Sediment	Madagascar	2007	Hervé <i>et al.</i> , 2010
			Collection date	
Metals	Sediment	South Africa	unknown	Newman & Watling, 2007
Metals	Sediment	Tanzania	2010	Rumisha <i>et al.</i> , 2012
Metals	Water and sediment	South Africa	1999/2000	Fatoki & Mathabatha, 2001
	Sediment and biota (periwinkle)	Tanzania	1998	De Wolf <i>et al.</i> , 2001
Metals	Sediment and biota	Tanzania	2000	Mremi & Machiwa, 2003
Metals	Sediment and biota	Tanzania	2005	Mtaga & Machiwa, 2008
PAHs	Sediment and biota (oyster)	Tanzania	2005	Gaspare <i>et al.</i> , 2009
Metals and inorganic pollutants	Sediment and biota	South Africa	2012	Nel <i>et al.</i> , 2015
POPs and PAHs	Sediment and biota (polychaete worms)	Zanzibar (Tanzania)	2015	Mwevura <i>et al.</i> , 2020

Metals	Water, sediment, and biota	Kenya	1997/1998	Mwashote, 2003
POPs	Water, sediment, and biota	Kenya	1998/1999	Wandiga <i>et al.</i> , 2002
Metals	Water, sediment, and biota	Mauritius	1999/2000/2003	Daby, 2006
Metals and POPs	Water, sediment, and biota	Tanzania	2007/2008	Machiwa, 2010
Metals	Water, sediment, and biota	Zanzibar (Tanzania)	2011	Shilla, 2016
PAHs	Water, sediment, and biota	Tanzania	2014	Shilla & Routh, 2018
Plastic	Sharks	South Africa	1978–2000	Cliff <i>et al.</i> , 2002
Metals	Sharks	South Africa	1979/1980	Watling <i>et al.</i> , 1982
Metals	Sharks	South Africa	2011	Naidoo <i>et al.</i> , 2017
			Collection date	
Mercury	Fish	Seychelles	unknown	Matthews, 1983
POPs	Fish	Tanzania	1998/1999	Mwevura <i>et al.</i> , 2002
PBDE	Fish			
PCDDs, PCDFs, and	(Skipjack tuna)	Seychelles	1999	Ueno <i>et al.</i> , 2004
PCBs	Fish			
	(Skipjack tuna)	Seychelles	1999	Ueno <i>et al.</i> , 2005
POPs	Fish	Réunion and South		
	(Albacore tuna)	Africa	2013	Munschy <i>et al.</i> , 2016
			Collection date	
Mercury and Selenium	Fish	Seychelles	unknown	Robinson & Shroff, 2004
	Fish			
POPs and PFAs	(Swordfish)	Seychelles	2013/2014	Munschy <i>et al.</i> , 2020a
	Fish	Réunion and		
Mercury	(Pelagic	Mozambique		
	species)	channel	2004	Kojadinovic <i>et al.</i> , 2006
	Fish	Réunion and		
Metals	(Pelagic	Mozambique		
	species)	channel	2004	Kojadinovic <i>et al.</i> , 2007b
	Fish	Seychelles,		
POPs and PFAs	(Pelagic	Chagos, Somalia,		
	species)	Mozambique	2013-2014	Munschy <i>et al.</i> , 2020b
			Collection date	
Metals	Fish	Tanzania	unknown	Mziray & Kimirei, 2016

			Collection date	
Metals	Fish	Tanzania	unknown	Saria, 2016
	Fish	Réunion and South		
POPs	(Albacore tuna)	Africa	2013/2014	Chouvelon <i>et al.</i> , 2017
Plastic	Fish (Mullet)	South Africa	2014	Naidoo <i>et al.</i> , 2016
POPs	Fish (Milkfish and mullet)	Tanzania	2016	Mwakalapa <i>et al.</i> , 2019
POPs	Fish (Sardine)	South Africa	2017	Wu <i>et al.</i> , 2020
Plastic	Fish	South Africa	2019	Naidoo <i>et al.</i> , 2020
POPs	Fish and invertebrates	South Africa	2019	Erasmus <i>et al.</i> , 2020
Metals	Fish and Squid	South Africa	2017	Uren <i>et al.</i> , 2020
POPs	Squid (Chokka)	South Africa	2017	Wu <i>et al.</i> , 2019 Mshana & Sekadende, 2014
Metals	Octopus	Tanzania	2013	
POPs	Mammal (Cetacean)	South Africa	Collection date unknown	De Kock <i>et al.</i> , 1994
POPs	Mammal (Indo-Pacific bottlenose dolphin)	Zanzibar (Tanzania)	2000–2002	Mwevura <i>et al.</i> , 2010
Metals	Mammal (Indo-Pacific bottlenose dolphin)	Zanzibar (Tanzania)	2000–2004	Mapunda <i>et al.</i> , 2017
POPs and mercury	Mammal (Dolphin)	Réunion	2010/2011	Ditru <i>et al.</i> , 2016
POPs	Mammal (Cetaceans)	South Africa	2012–2015	Aznar-Aleman <i>et al.</i> , 2019
Organochlorines	Birds (Tern eggs and tissue)	Indian Ocean islands (Mauritius/ Seychelles)	1975	Bourne <i>et al.</i> , 1977
Organochlorine pesticides	Birds (Coastal birds)	South Africa	Collection date unknown	De Kock & Randall, 1984
Mercury	Birds	Seychelles	1996-2005	Ramos & Tavares, 2010

Metals	Birds	Réunion	Collection date unknown	Kojadinovic <i>et al.</i> , 2007c
Mercury	Birds	Réunion	Collection date unknown	Kojadinovic <i>et al.</i> , 2007a
POPs	Birds (Tern eggs)	Rodrigues (Mauritius)	2010	Bouwman <i>et al.</i> , 2012
PFAS and POPs	Birds (African penguin)	South Africa	2011/2012	Bouwman <i>et al.</i> , 2015
Metals	Birds (Kelp gull eggs)	South Africa	2011/2012	Van Aswegen <i>et al.</i> , 2019
Plastic	Birds	Réunion/ Juan de Nova	2002–2016	Cartraud <i>et al.</i> , 2019
		South-West Indian Ocean (between Madagascar and Reunion)		
Plastic	Sea turtles	Reunion)	2007-2013	Hoarau <i>et al.</i> , 2014
Metals	Sea turtles	South Africa	2015	Du Preez <i>et al.</i> , 2018
	Benthic Biota			
Metals	(Brown mussel)	South Africa	1974-2009	Greenfield <i>et al.</i> , 2011
Tributyltin (TBT)	Benthic biota (Gastropods)	South Africa	2002	Marshall & Rajkumar, 2003
POPs	Benthic Biota (Brown mussel)	South Africa	Collection date unknown	Degger <i>et al.</i> , 2011
DDT	Benthic biota	South Africa	Collection date unknown	Porter & Schleyer, 2017
Organochlorine pesticides	Benthic biota	South Africa	Collection date unknown	Porter <i>et al.</i> , 2018
Metals	Benthic biota (Coral)	South Africa and Mauritian islands	2014	Van der Schyff <i>et al.</i> , 2020a
	Crustaceans (Giant mud crabs and tiger prawn)			
Metals		Tanzania	2014/2015	Rumisha <i>et al.</i> , 2017

Seventy-two articles were identified on ecotoxicological studies in the WIO that fell within the parameters set. Several studies collected material from multiple countries' in the economic exclusive zones (EEZ). The country in relation to which the most studies were conducted, is South Africa (n=28), followed by Tanzania (n=21), Réunion (France) (n=8), Seychelles (n=8), Mauritius (n=5), Kenya (n=4), and Mozambique (n=4). Only one study each was reported from

the Maldives, Mayotte, Madagascar, Juan de Nova Island (France), Somalia, and the southwestern pelagic Indian Ocean between Réunion and Madagascar. No ecotoxicological studies from Comoros or Tromelin (Joint Mauritian and French administration) Islands were found. It is concerning that only five ecotoxicological studies have been conducted in the Mauritian EEZ.

The articles dealing with pollutants that were quantified in the WIO region were metals (n=29), POPs (n=20), mercury (n=5), plastic (n=5), PAHs (n=2), and TBT (n=1). Ten studies quantified multiple classes of pollutants.

Studies from the wider Indian Ocean have recorded metals in corals from the Red Sea (Ali et al., 2011; El-Sorogy et al., 2012; Mohammed & Dar, 2010), and India (Anu et al., 2007), and PAHs in coral from Taiwan (Ko et al., 2014). No studies on POPs in corals from WIO islands have been published. The specific effects of POPs on corals are largely unknown at this stage. No studies thus far have tested for organochlorines or other POPs in corals from islands in the WIO, even though it is a region that still uses DDT as malaria prevention (Bouwman *et al.*, 2011).

## 1.2 Pollutants

### 1.2.1 Metals

Metals are a group of naturally occurring elements with similar chemical and physical properties. Metals with a specific density of 5 g/cm<sup>3</sup> are generally referred to as “heavy metals” (Newman, 2015). However, the term is also used ambiguously to refer to metals of environmental concern. Modern ecotoxicologists tend to avoid the term “heavy metal” in favour of merely referring to the elements as “metals” (Duffus, 2002).

Certain trace metals are essential elements for life at physiologically regulated concentrations (Bryan, 1971). These naturally occurring metals usually originate from the underlying geology of the region (McCarthy & Rubidge, 2011). Metals are incorporated into the ocean water through hydrothermal vent activity, volcanic ejecta, meteorites, or erosion of terrestrial geology (Kastner, 1999). When metal concentrations are elevated beyond their natural background concentrations due to the influx of anthropogenically-produced metals, it can be considered as contamination. When the concentrations of metals adversely affect biota or their habitat, it is considered pollution (Chapman, 1995). In 2012, the Scientific and Technical Advisory Panel (STAP) of the Global Environment Facility (GEF) classified metal pollution as the number one priority of 22 emerging chemical management issues in developing countries (STAP, 2012).

The elements highlighted in the periodical table (Fig 1) are of concern for this study.

**Periodic Table of the Elements**

1 1A 11A	2 2A 2A											13 3A 3A	14 4A 4A	15 5A 5A	16 6A 6A	17 7A 7A	18 8A 8A	
1 H Hydrogen 1.008																		2 He Helium 4.003
3 Li Lithium 6.941	4 Be Beryllium 9.012											5 B Boron 10.811	6 C Carbon 12.011	7 N Nitrogen 14.007	8 O Oxygen 15.999	9 F Fluorine 18.998	10 Ne Neon 20.180	
11 Na Sodium 22.990	12 Mg Magnesium 24.305	3 III B 3B	4 IV B 4B	5 V B 5B	6 VI B 6B	7 VII B 7B	8 VIII 8	9 VIII 8	10 VIII 8	11 IB 1B	12 IIB 2B	13 Al Aluminum 26.982	14 Si Silicon 28.086	15 P Phosphorus 30.974	16 S Sulfur 32.066	17 Cl Chlorine 35.453	18 Ar Argon 39.948	
19 K Potassium 39.098	20 Ca Calcium 40.078	21 Sc Scandium 44.956	22 Ti Titanium 47.88	23 V Vanadium 50.942	24 Cr Chromium 51.996	25 Mn Manganese 54.938	26 Fe Iron 55.933	27 Co Cobalt 58.933	28 Ni Nickel 58.693	29 Cu Copper 63.546	30 Zn Zinc 65.39	31 Ga Gallium 69.732	32 Ge Germanium 72.61	33 As Arsenic 74.922	34 Se Selenium 78.09	35 Br Bromine 79.904	36 Kr Krypton 84.80	
37 Rb Rubidium 84.468	38 Sr Strontium 87.62	39 Y Yttrium 88.906	40 Zr Zirconium 91.224	41 Nb Niobium 92.906	42 Mo Molybdenum 95.94	43 Tc Technetium 98.907	44 Ru Ruthenium 101.07	45 Rh Rhodium 102.905	46 Pd Palladium 106.42	47 Ag Silver 107.868	48 Cd Cadmium 112.411	49 In Indium 114.818	50 Sn Tin 118.71	51 Sb Antimony 121.760	52 Te Tellurium 127.6	53 I Iodine 126.904	54 Xe Xenon 131.29	
55 Cs Cesium 132.905	56 Ba Barium 137.327	57-71 Lanthanide Series	72 Hf Hafnium 178.49	73 Ta Tantalum 180.948	74 W Tungsten 183.85	75 Re Rhenium 186.207	76 Os Osmium 190.23	77 Ir Iridium 192.22	78 Pt Platinum 195.08	79 Au Gold 196.967	80 Hg Mercury 200.59	81 Tl Thallium 204.383	82 Pb Lead 207.2	83 Bi Bismuth 208.980	84 Po Polonium [209]	85 At Astatine [209]	86 Rn Radon 222.018	
87 Fr Francium 223.020	88 Ra Radium 226.025	89-103 Actinide Series	104 Rf Rutherfordium [261]	105 Db Dubnium [262]	106 Sg Seaborgium [266]	107 Bh Bohrium [264]	108 Hs Hassium [269]	109 Mt Meitnerium [268]	110 Ds Darmstadtium [269]	111 Rg Roentgenium [272]	112 Cn Copernicium [277]	113 Uut Ununtrium unknown	114 Fl Flerovium [289]	115 Uup Ununpentium unknown	116 Lv Livermorium [293]	117 Uus Ununseptium unknown	118 Uuo Ununoctium unknown	
			57 La Lanthanum 138.906	58 Ce Cerium 140.115	59 Pr Praseodymium 140.908	60 Nd Neodymium 144.24	61 Pm Promethium 144.913	62 Sm Samarium 150.36	63 Eu Europium 151.966	64 Gd Gadolinium 157.25	65 Tb Terbium 158.925	66 Dy Dysprosium 162.50	67 Ho Holmium 164.930	68 Er Erbium 167.26	69 Tm Thulium 168.934	70 Yb Ytterbium 173.04	71 Lu Lutetium 174.967	
			89 Ac Actinium 227.028	90 Th Thorium 232.038	91 Pa Protactinium 231.036	92 U Uranium 238.029	93 Np Neptunium 237.048	94 Pu Plutonium 244.064	95 Am Americium 243.061	96 Cm Curium 247.070	97 Bk Berkelium 247.070	98 Cf Californium 251.080	99 Es Einsteinium [254]	100 Fm Fermium 257.095	101 Md Mendelevium 258.1	102 No Nobelium 259.101	103 Lr Lawrencium [262]	

**Fig. 1.** Periodic table of the elements. Elements that are discussed in this thesis are coloured (ThoughtCo, 2020).

### 1.2.2 Persistent Organic Pollutants (POPs)

The term ‘Persistent Organic Pollutants (POPs)’ is defined in the Stockholm Convention: “Persistent Organic Pollutants (POPs) are organic chemical substances; that is, they are carbon-based. They possess a particular combination of physical and chemical properties such that, once released into the environment, they: remain intact for exceptionally long periods (many years); become widely distributed through the environment as a result of natural processes involving soil, water and, most noticeably, air; accumulate in the fatty tissue of living organisms including humans, and are found at higher levels in the food chain, and are toxic to both humans and wildlife.” (Stockholm Convention, 2019b).

POPs are associated with numerous health issues in humans, including endocrine disruption, cancer, obesity, cardiovascular disease, and reproductive issues, to name a few (Alharbi *et al.*, 2018). Similar problems were noted in various organisms with high POPs concentrations in their bodies. Behavioural disturbances were also witnessed in animals with elevated POPs concentrations (Goutte *et al.*, 2018).

In 1962, Rachel Carson brought scientific and societal awareness of POPs to the forefront in her critically acclaimed book *Silent Spring*. Carson documented the adverse environmental effect of pesticides, spurring the United States to ban DDT for agricultural use in 1972 (Grier, 1982). The rest of the world followed suit. The process came to a head in 2001, when the Stockholm Convention on Persistent Organic Pollutants was adopted on 22 May 2001 and

entered into force on 17 May 2004 with 151 signatories from 128 parties. As of 2019, 184 parties are signatories of the Convention (Stockholm Convention, 2019a).

Twelve groups of POPs, known as the “dirty dozen,” were the first chemicals of concern identified in the Stockholm Convention in 2004 (Table 3). Research in the field intensified since 2004, and sixteen new POPs were added (Scheringer *et al.*, 2012) (Table 3). The POPs targeted by the Stockholm convention are listed in the annexes to the convention text and categorised into three categories based on the threat level of the specific POPs listed. Annex A contains a list of chemical substances that must be eliminated. Specific exemptions provided for in the annex apply only to parties that register for them. Parties must take action to eliminate the production of the substance. The use of compounds from Annex B must be restricted, but the chemical may be used under predetermined conditions. Parties are required to minimise the unintentional release of the compounds listed in Annex C, with the goal of continued minimisation and ultimate elimination where possible (Stockholm Convention, 2019c). Certain compounds can be included in both Annexes A and C. All POPs are either pesticides, industrial chemicals, or chemicals that are unintentionally emitted during other processes (Stockholm Convention, 2019c). Table 2 lists all the POPs currently included in the Stockholm Convention, as well as the current candidate POPs. The full compound name, abbreviation, annexe to which it is assigned, and the use and/or emission of the compounds are included.

**Table 2.** The abbreviations, annex, and use of all current and candidate POPs included in the Stockholm Convention.

	<b>Compound</b>	<b>Abbreviation</b>	<b>Annex</b>	<b>Use</b>
Original POPs	Aldrin		A	Pesticide
	Chlordane		A	Pesticide
	Dichlorodiphenyltrichloroethane	DDT	B	Pesticide
	Dieldrin		A	Pesticide
	Endrin		A	Pesticide
	Heptachlor		A	Pesticide
	Hexachlorobenzene	HCB	A & C	Pesticide/ Industrial chemical
	Mirex		A	Pesticide
	Toxaphene	CHB	A	Pesticide
	Polychlorinated biphenyl	PCB	A & C	Industrial chemical/ Unintentional product
	Polychlorinated dibenzo-p-dioxins	PCDD	C	Unintentional product

	Polychlorinated dibenzofuran	PCDF	C	Unintentional product
New POPs	Chlordecone		A	Pesticide
	Dicofol		A	Pesticide
	Hexabromobiphenyl		A	Industrial chemical
	Hexabromocyclododecane	HBCDD or HBCD	A	Industrial chemical
	Hexachlorobutadiene	HCBD	A	Unintentional product
	Hexachlorocyclohexane	HCH	A	Pesticide
	Polybrominated diphenyl ethers	PBDE	A	Industrial chemical
	Pentachlorobenzene	PeCB	A & C	Pesticide/ Industrial chemical/ Unintended product
	Pentachlorophenol and its salts and esters	PCP	A	Pesticide
	Perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride	PFOS and PFOS-F	B	Pesticide/ Industrial chemical
	Perfluorooctanoic acid, its salts, and PFAO-related compounds	PFOA	A	Industrial chemical
	Polychlorinated naphthalenes	PCN	A & C	Industrial chemical/ Unintentional product
	Short-chained chlorinated paraffins	SCCP	A	Industrial chemical
Technical endosulfan and its related isomers		A	Pesticide	
Candidate POPs	Perfluorohexane sulfonic acid	PFHxS		Industrial chemical
	Dechlorane Plus	DP		Industrial chemical
	Methoxychlor			Pesticide

Ten classes of POPs are relevant for this study:

#### 1.2.2.1 Chlordane

*Stockholm Convention classification: Annex A pesticide (Stockholm Convention, 2019d).*

Technical chlordane is a mixture of more than 140 compounds. Trans-chlordane makes up 13% of the mixture, followed by cis-chlordane (11%), trans-nonachlor (5%), heptachlor (5%), and various chlordanes, chlordanes, and nonachlors (Dearth & Hites, 1991; Bindleman



*et al.*, 2002). Chlordane was first produced in 1948, and it was used universally for the next 50 years (Bindleman *et al.*, 2002). Chlordane was a widely used lawn and garden pesticide, but due to toxicity and potential carcinogenicity, the compound was regulated in 1979. For nine years, chlordane was still used as a termiticide in building projects, but production and sale stopped voluntarily in 1988 (Dearth & Hites, 1991). The effects of chronic exposure to chlordane include cancer, congenital disabilities, and mutations. Lethal effects may vary across species, but it is known to kill mallard ducks, bobtail quill, and shrimp (Dearth & Hites, 1991; Stockholm Convention, 2019d).

#### 1.2.2.2 Dichlorodiphenyltrichloroethane (DDT)

*Stockholm Convention classification: Annex B pesticide (Stockholm Convention, 2019d)*

DDT is arguably the most controversial POP (Bouwman *et al.*, 2011). The compound has saved millions of lives through Indoor Residual Spraying (IRS) to prevent malaria. At the same time, severe adverse human health and environmental endpoints have been found (Bouwman *et al.*, 2011). When DDT was temporarily replaced in 1996 by another deltamethrin to combat malaria in South Africa, the death rate, due to malaria, increased dramatically spurring the reintroduction of DDT for IRS in 2000 (Maharaj *et al.*, 2005). The DDT mixture used in IRS is a combination of *para*, *para'*- and *ortho*, *para'*-isomers, of which *p,p'*-DDT is the predominant component (WHO, 1979). DDT can break down aerobically or anaerobically to form the metabolites DDE and DDD, respectively (Guenzi & Beard, 1976). There are legions of research articles available on the effect of DDT on human health (e.g., Jukes, 1971; Bouwman *et al.*, 1990; Beard & Australian Rural Health Research Collaboration, 2006; Eskenazi *et al.*, 2009; Huq *et al.*, 2020). DDT chronically and acutely adversely affects the development, physiology, morphology, and behaviour of humans and animals. The most prominent adverse effects are endocrine disruption. Essentially, DDT creates an abnormal hormonal environment inside contaminated organisms that severely impacts reproductive success (Iwaniuk *et al.*, 2006). It is documented that high DDT concentrations are associated with eggshell thinning in several bird species (Bouwman 2013; 2019).

#### 1.2.2.3 Hexabromocyclododecane (HBCD)

*Stockholm Convention classification: Annex A Industrial Chemical (Stockholm Convention, 2019f)*

Technical HBCD is a mixture of predominantly  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HBCD isomers (Arnot *et al.*, 2009). Collectively, this mixture is a brominated flame retardant (BFR) used extensively in indoor thermal insulation, polystyrene foam, and textile production (Koch *et al.*, 2015).

Typically, HBCD is released into the environment when these products are dumped on waste sites, recycled, or incinerated (Darneurd, 2003). Data on the toxicity of HBCD is lacking in the scientific literature; however, disruption of thyroid- and liver hormones and liver cancer have been associated with elevated HBCD concentrations (Darneurd, 2003; Koch *et al.*, 2015).

#### 1.2.2.4 Hexachlorobenzene (HCB)

*Stockholm Convention classification: Annex A and Annex C pesticide and industrial chemical (Stockholm Convention, 2019d)*

Initially, HCB was developed in 1945 as a fungicide to protect seeds of certain crops. However, it is also a by-product of several commercial chlorination processes (Alvarez *et al.*, 2000; Stockholm Convention, 2019d). The compound is lethal to humans at high concentrations. Between 1954 and 1959, 14% of people who ate grain treated with HCB in Turkey died (Stockholm Convention, 2019d). HCB adversely affects the immune system, liver, reproductive systems, and gene expression of animals and humans (Alvarez *et al.*, 2000).

#### 1.2.2.5 Pentachlorobenzene (PeCB)

*Stockholm Convention classification: Annex A and Annex C pesticide and industrial chemical (Stockholm Convention, 2019f)*

PeCB is used in mixtures to produce pesticides, such as the fungicide quintozone, and in industrial products to increase the viscosity of PCBs. It can also be released during the burning of biomass and solid wastes—particularly old electronic appliances (Bailey *et al.*, 2009). PeCB is closely related to lindane and HCB and is a metabolic by-product of both compounds (Linder *et al.*, 1980). Acute exposure to PeCB is highly toxic to the pancreas. Chronically, it is mildly immunotoxic (Madaj *et al.*, 2018). PeCB concentrations have been quantified in various media worldwide with recent concentrations being overall lower when compared with older studies, indicating a decline in environmental concentrations of the compound (Bailey *et al.*, 2009).

#### 1.2.2.6 Hexachlorocyclohexane (HCH)

*Stockholm Convention classification: Annex A pesticide, with a specific exemption for lindane use as a pharmaceutical for control of scabies and head lice as second-line treatment (Stockholm Convention, 2019f)*

After the Second World War, HCH was one of the most extensively used organochlorine pesticides. Technical HCH is a mixture of eight HCH isomers, with  $\alpha$ -,  $\beta$ -, and  $\gamma$ -HCH being the most prominent isomers (Walker *et al.*, 1999; Vijgen *et al.*, 2011). However,  $\gamma$ -HCH, or

lindane, was isolated in the early 1950s after produce treated by technical HCH was rendered inedible by the mixture's organoleptic properties affecting the palatability of the food items (Vijgen *et al.*, 2011). The agricultural use of lindane is prohibited, but lindane is still used in a few countries for the second-line treatment of human and livestock ectoparasites, such as headlice and scabies (Stockholm Convention, 2019f). However, because lindane is associated with neurotoxicity, reproductive defects, and immunotoxicity, a topical ointment used to treat head lice only contains 1% lindane. This ointment is exclusively recommended as a second-line treatment for head lice and scabies (Stockholm Convention, 2019f; FDA, 2007). The  $\alpha$ - and  $\beta$ - HCH isomers are by-products of lindane production. For every ton of lindane, six to ten tons of  $\alpha$ - and  $\beta$ - HCH are produced (Stockholm Convention, 2019f). During the 60 years that lindane was commercially produced, between four and seven million tons of  $\alpha$ - and  $\beta$ -HCH was produced and discarded into the environment (Vijgen *et al.*, 2011). Of the isomers,  $\alpha$ -HCH is the most carcinogenic and  $\beta$ -HCH may act as an environmental oestrogen causing endocrine disruption (Walker *et al.*, 1999). All HCH isomers pose chronic and acute toxicity to mammals. Immunosuppression, neurological problems, and liver cancer are associated with chronic exposure (Walker *et al.*, 1999).

#### 1.2.2.7 Mirex

*Stockholm Convention classification: Annex A pesticide (Stockholm Convention, 2019c)*

Mirex was developed in 1959 as an insecticidal bait to control imported fire ants in the United States (Waters *et al.*, 1977; Kaiser, 1978) The compound was also used as flame retardant under the name Dechlorane (Kaiser, 1974). However, the United States Environmental Protection Agency (USEPA) was worried by the widespread use of the relatively unknown pesticide when mirex was applied to 120 million acres of land (Kaiser, 1978). Studies were conducted soon after that indicated the tumorigenic effects of mirex on mammals (Innes *et al.*, 1969). Like all POPs, mirex is a known endocrine disruptive chemical (Heinzow, 2009). It is unusually resistant to metabolic activity and can be readily excreted from an organism (Pope, 2014). Mirex is one of the most persistent POPs, with a half-life of up to 10 years (Stockholm Convention, 2019d).

#### 1.2.2.8. Polybrominated diphenyl ethers (PBDEs)

*Stockholm Convention classification: Annex A industrial Chemical (Stockholm Convention, 2019f)*

PBDEs are the most used BFR (Groga *et al.*, 2013). These compounds reduce fire hazards by interfering with the combustion of polymeric materials and are used in various applications,

including electronic appliances, building materials, plastics, and textiles (Rahman *et al.*, 2001; Gorga *et al.*, 2013). Commercially produced PBDE is typically a mixture of different brominated diphenyl ethers (BDEs), their homologues, and isomers. Penta-BDE (PeBDE), octa-BDE (OBDE), and deca-BDE (DeBDE) (Rahman *et al.*, 2001) are the main components of such mixtures. The isomer, BDE-209, is the main component of DeBDE (Ross *et al.*, 2009). The European Union banned the use of DeBDE in electrical and electronic applications since 1 July 2008 (European Court of Justice, 2008; Covaci *et al.* 2011). The harmful effects of PBDE include adverse endocrine, metabolic, reproduction, and neurological effects (Pradhan *et al.*, 2013).

#### 1.2.2.9 Polychlorinated biphenyl (PCB)

*Stockholm Convention classification: Industrial Chemical under Annex A, but with specific exemptions under Annex C (Stockholm Convention, 2019d)*

PCBs are chemical mixtures created by fractional distillation of the products of the catalytic chlorination of biphenyl (Addison, 1983). They can either be directly produced or are incidental by-products of other chemical reactions (Stockholm Convention, 2019d). PCBs were most often used as chemical components in dielectric fluids, insulators, flame-resistant plasticisers, and hydraulic fluids (Addison, 1983; Jafarabadi *et al.*, 2018). These compounds were used since the early 1930s, but production dramatically decreased in the late 1970s when the persistent and hazardous nature of the compounds were realised (Addison, 1983). The production of PCBs was officially banned in the US by the American Environmental Protection Agency in 1979 (EPA, 2016) and internationally by the Stockholm Convention in 2001 (Porta & Zumeta, 2002).

PCBs are usually released into the environment through improper commercial use and disposal after intended production, or by accidental spills or municipal solid waste incinerators (Jafarabadi *et al.*, 2018). Ninety-three PCB congeners, including PCB-5, -11, and -52, are “unintentionally produced PCBs,” which appear in the environment, although they are not commercially produced (Basu *et al.*, 2009; Bartlett *et al.*, 2019). Elevated concentrations of PCBs are associated with hepatotoxicity, immunotoxicity, and reproductive toxicity in various organisms (Eisler, 2007). Tumour growths, deleterious neurodevelopmental, and endocrine disruptive effects also stem from PCB exposure (Brouwer *et al.*, 1999).

#### 1.2.2.10 Toxaphene (CHB)

*Stockholm Convention classification: Annex A pesticide (Stockholm Convention, 2019d)*

Toxaphene is a complex mixture of polychlorinated camphenes. It was produced between 1945 and the 1980s, primarily as an insecticide for the cotton industry. However, it proved to be an effective piscicide to help rid the aquaculture industry of fish that are undesirable to aquaculture or sport fishers (Saleh, 1991). In 1970, polydopen— a mixture of toxaphene and DDT— was used as a substitute for DDT in several countries, including countries bordering on the WIO, such as Tanzania. When toxaphene production was banned in America by the USEPA, the rest of the world followed suit (de Geus *et al.*, 1999). Toxaphene is highly toxic to aquatic organisms—even more so to marine organisms than to their freshwater counterparts (de Geus *et al.*, 1999). Hepatotoxicity, carcinogenicity, and mutagenicity are some of the dangerous impacts of toxaphenes on organisms (de Geus *et al.*, 1999; Stockholm Convention, 2019d).

### 1.2.3 Per- and polyfluoroalkyl substances (PFAS)

PFAS are anthropogenically produced fluorinated compounds used in a wide range of products such as firefighting foam, paper, textiles, food containers, and anti-stick surfaces of appliances (Buck *et al.*, 2011; Konwick *et al.*, 2008; McCarthy *et al.*, 2017). PFAS are closely related to POPs. Two PFAS compounds have recently been added to the Stockholm Convention (Stockholm Convention, 2019c) and are now classified as POPs. Perfluorooctanoic acid (PFOA) is classified as an Annex A POP by the Stockholm Convention, while Perfluorooctane sulfonic acid (PFOS) is categorised as an Annex B POP (Stockholm Convention, 2019c). Perfluorohexane sulfonic acid (PFHxS) is listed as a chemical under consideration to be added to the Convention (Stockholm Convention, 2019e). All these compounds are immunotoxic, hepatotoxic, and potentially carcinogenic (Stockholm Convention 2019c, 2019e).

While most POPs accumulate in lipids, PFAS bind strongly to proteins (Newman, 2015). Like POPs, PFAS are persistent and bio-accumulative in the environment with adverse effects on human and environmental health (Giesy & Kannan, 2002). Documentaries such as “The Devil we know” and “No Defense” have thrust the danger of PFAS contamination in drinking water into the spotlight (Anon, 2020; GreatLakesNow, 2020). PFAS compounds are often associated with surface water contamination due to their surfactant properties (Ju *et al.*, 2008). The surfactant properties are due to the molecular composition of PFAS. The hydrophilic head of the molecule, consisting of the chemical functional group, is submerged in water, while the tail section (which is simultaneously hydro- and lipophobic) is exposed to the air. The tail section is made of a carbon chain with fluoride molecules (McCarthy *et al.*, 2017; ITRC, 2018; EPA, 2019). PFAS can either be deposited directly into the environment from industrial activities or be the result of the breakdown of neutral precursors, such as fluorotelomer alcohols (FTOH) (Schenker *et al.*, 2008). After photooxidation of FTOH, PFAS are deposited

through atmospheric deposition such as rain or snow (Ellis *et al.*, 2004), often in very remote areas, such as the Arctic, Antarctic, and island systems (Jahnke, 2007; Huber *et al.*, 2015; Munoz *et al.*, 2017a).

#### 1.2.4 Novel Brominated Flame Retardants (NBFRs)

Novel Brominated Flame Retardants (also known as “alternative,” “emerging,” “new,” “current-use,” or “non-PBDE” brominated flame retardants) is a blanket term for relatively new compounds used *in lieu* of traditional PBDEs after the latter was banned or restricted from use (Covaci *et al.*, 2011). Even though these compounds are used to mitigate the adverse effects of PBDE and traditional BFRs, but still function as a flame retardant, NBFRs share much of the same characteristics as traditional BFRs. NBFRs and BFRs have similar dangers of toxicity, bioaccumulation, and long-range transport (Covachi *et al.*, 2011; Ezechiáš *et al.*, 2014). Certain NBFR, such as Dechlorane Plus (DP), have been cleared for high production volume (>1000 t/y) (Zhou *et al.*, 2014). Because of the toxicity hazard and production volume, DP is listed as a compound proposed for listing under the Stockholm Convention (Stockholm Convention, 2019e). Other NBFR such as tetrabromophthalate (TBPH) and tetrabromobenzoate (TBB) have been confirmed to be endocrine disruptive (Saunders *et al.*, 2015).

Most NBFR are not yet candidate compounds considered by the Stockholm Convention or similar treaties. However, the European Commission has urged the European Union to keep a wary eye on toxicity studies and concentrations of NBFR in food to make pre-emptive decisions regarding the regulation of these compounds (Borg, 2014).

## 2 Problem statement

### 2.1. Research questions

Insufficient knowledge is available on pollution in the Mauritian Outer Islands in the Mascarene Basin region of the western Indian Ocean. Although marine ecotoxicology is the focus of several studies worldwide, very few studies have focussed on pollutants in biota from the Mascarene region. At the outset of this study, the only studies on pollutants in biota in the Mascarene Basin region were a study on metals in corals from the Mauritian Outer Islands (Van der Schyff *et al.*, 2020a), a study on POPs in common noddies and sooty terns from Rodrigues Island (Bouwman *et al.*, 2012), a study that quantified DDE and PCBs in terns from St. Brandon's Atoll in 1975 (Bourne *et al.*, 1977), and eight studies on biota from Reunion. (Table 1). When this is compared with the volume of toxicological research conducted on coral reef biota in other areas, such as the Great Barrier Reef or Florida, the extent of the void becomes apparent. As a result, conservation strategies of this region do not include monitoring or mitigation of toxicological threats because the extent of toxicological threats to the biota of the Mascarene Basin is unknown.

The main research questions underpinning this study are:

- At what concentrations and compositional patterns are anthropogenic pollutants present in coral, fish, and seabirds from the Mauritian Outer Islands in the Mascarene Basin?
- Which factors are associated with the concentrations and patterns of pollutants observed?
- Based on the results, what are the opportunities that coral islands offer for pollution monitoring?

To answer effectively the research questions, the different research objectives are stated below. A hypothesis—that will either be accepted or rejected—is linked with each objective.

### 2.2. Objectives and hypotheses

*General objective:*

To provide a comprehensive overview of the ecotoxicological state of knowledge of the the western Indian Ocean with a specific focus on quantifying pollutants in marine biota from coral reefs of the Mauritian Outer Islands.

*Hypothesis 1: Pollutants will be present in all biota from the Mauritian Outer Islands.*

*Specific objectives:*

1. To investigate shipwrecks as a vector of pollutants to remote islands by determining associated ecological and toxicological effects.

*Hypothesis 2: Shallow shipwrecks cause quantifiable ecological damage to reefs of isolated tropical islands and serve as a vector for pollutants to these islands.*

2. To determine POPs and NBFR concentrations in coral and coral reef fish from the Mascarene region.

*Hypothesis 3: Fish will contain higher POPs concentrations than corals due to biomagnification at a higher trophic level.*

3. To determine pollutant concentrations in biota from higher trophic positions in the tropical island food web by quantifying POPs and PFAS in the eggs of seabirds from the Mascarene region.

*Hypothesis 4: Seabird eggs will contain quantifiable, but low concentrations of POPs and PFAs.*

4. To determine the factors that may explain the concentrations and patterns of pollutants.

*Hypothesis 5: Pollutants are transported to remote islands in the WIO through multiple pathways.*

*Hypothesis 6: Different islands will have different concentrations and patterns of pollutants.*

*Hypothesis 7: Different coral reef biota will have different compositional patterns of pollutants.*

5. Based on the results, what are the opportunities that coral islands offer for pollution monitoring?



*Hypothesis 8: Remote oceanic islands can be used as reference sites to determine regional background concentrations of pollutants against which concentrations of marine pollution elsewhere can be compared.*

### 2.3. Structure of the thesis

Chapter One is aimed at contextualising the state of the science of marine ecotoxicology, with the emphasis on the WIO region. A summary of all significant publications on ecotoxicology from the WIO is presented. The pollutants of concern for this study are discussed to provide the reader with a lens with which to view this thesis.

Chapters Two to Five are presented in article format:

(i) Chapter Two focuses on the way shipwrecks acts as a source of pollution to remote oceanic islands and the environmental consequences thereof. This chapter is the first case study in which the effects of a shallow shipwreck are examined in the WIO region. Metal contamination was quantified on coral and macroalgae from the area surrounding the wreck site. The article was published in the journal *Marine Environmental Research* (Van der Schyff *et al.*, 2020b);

(ii) Chapter Three presents the first initial assessment on the concentrations of persistent organic pollutants (POPs) in five coral and three fish genera from three Mauritian Outer Islands. This chapter represents a manuscript submitted to *Chemosphere* (submitted on 27<sup>th</sup> July 2020; manuscript number: CHEM75582);

(iii) Chapter Four focusses on POPs in the eggs of three seabird species from St. Brandon's Atoll. The article was submitted to the journal *Science of the Total Environment* (submitted on 15<sup>th</sup> June 2020; manuscript number: STOTEN-D-20-14100);

(iv) Chapter Five quantified perfluoroalkyl substances (PFAS) in the eggs of three seabird species from St. Brandon's Atoll. The article was published in the journal *Marine Pollution Bulletin* (Van der Schyff *et al.*, 2020c).

Chapter Six summarises the study and contains recommendations.

### 3. Site description and sample collection

#### 3.1. Indian Ocean

The Indian Ocean is the third largest water body on Earth, after the Pacific and Atlantic oceans. The Indian Ocean contains approximately 20% of the world's ocean water and is bordered by the Australian, African, and Asian landmasses (Gritzner, 2009). To the south, the Indian Ocean is bordered by the Southern Ocean.

Before the 1960s, the Indian Ocean was considered as an area of little importance. This perspective was changed by the large-scale oil exploitation in the Persian Gulf, an extension of the Indian Ocean. Since the late 1960s, the Indian Ocean and associated seas and gulfs have been at the centre of international geopolitical tension, attributed to oil production, associated shipping lanes, and conflicts of EEZs. In 2009, 46.5% of the world's major conflicts took place around the Indian Ocean (Boucard & Crumplin, 2010).

Despite the political turmoil above the waves, the Indian Ocean, particularly the WIO, is regarded as one of the less ecologically disturbed areas in the world. Endemism in marine organisms is high at 22% when compared with 6% endemism in the eastern Indian Ocean and 13% in the Red Sea (WWF, 2018).

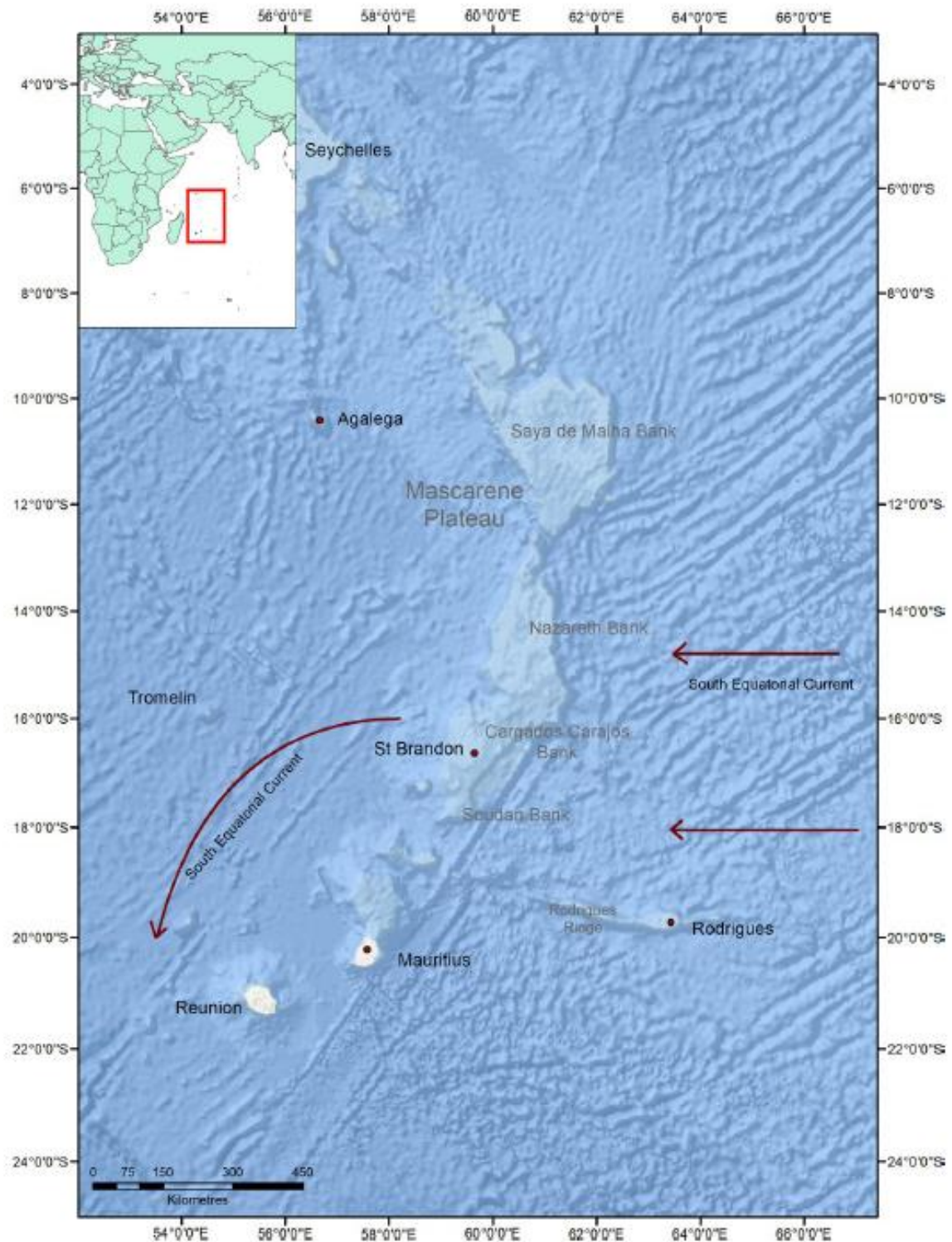
#### 3.2. Mascarene region

The Mascarene Islands comprise of Réunion, Mauritius, and Rodrigues (Fig. 2) (Thébaud *et al.*, 2009). In this thesis, all the Mauritian Outer Islands are also included in the definition of the Mascarene Islands.

The EEZ of Mauritius encompasses 1.9 million km<sup>2</sup> of the western Indian Ocean, with a further 396 000 km<sup>2</sup> under a joint management agreement with the government of Seychelles (Bhagooli & Kaullysing, 2018). The geopolitical presence of Mauritius extends beyond the island of Mauritius to the Mauritian Outer Islands. The geographic positions of these islands, relative to Mauritius, are as follows: Rodrigues (617 km east), St. Brandon's Atoll (470, km north), and Agalega (1 100 km north; Fig. 2). There is a long-running dispute between Mauritius and Britain regarding the sovereignty of the Chagos Archipelago (2 200 km north-east of Mauritius). Only Rodrigues, St. Brandon's Atoll, and Agalega were study sites for this study wherefrom samples were collected.

The major oceanographic features of the Mauritian EEZ include: the Saya de Malha Bank, Nazareth Bank, Rodrigues Ridge, Cargados Carajos Bank, and the Soudan Bank (Bhagooli

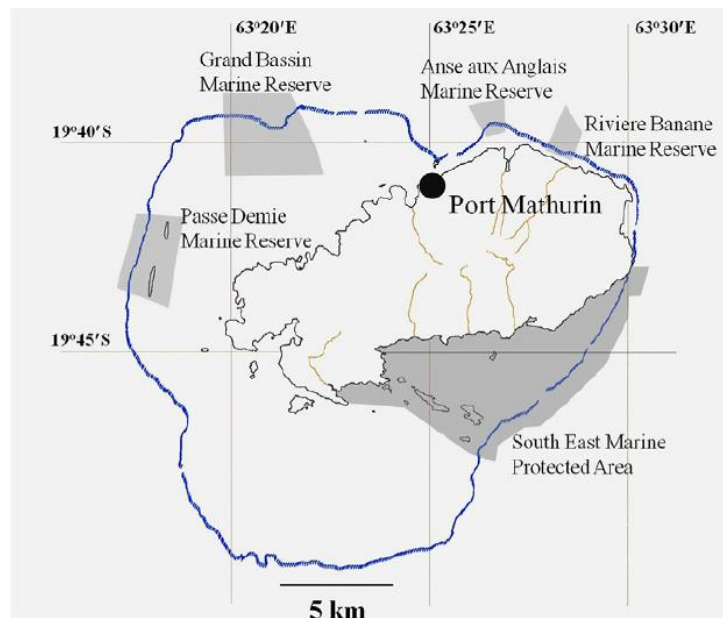
& Kaullysing, 2018; Fig. 2). All these features are located on the Mascarene Plateau. The Mauritian Republic is a signatory of the Stockholm Convention on Persistent Organic Pollutants (UNTC, 2020).



**Fig. 2.** The location of the Mascarene Basin in the western Indian Ocean with the three Mauritian Outer Islands (Rodrigues, St. Brandon's, Agalega) and oceanographic features indicated (Bhagooli & Kaullysing, 2018).

### 3.2.1. Rodrigues

Rodrigues is the largest of the Mauritian Outer Islands, with a permanent population of approximately 42 000 (Statistics Mauritius, 2019). It boasts the oldest and best-developed coral reef in the WIO, with a lagoon covering 200 km<sup>2</sup> (Fig. 3). The island is of volcanic origin, but not on the same hotspot that created Mauritius and Réunion. A lava flow from the Pliocene epoch created the base on which the fringing coral reef developed (Rees *et al.*, 2005; Turner & Klaus, 2005). Maritime traffic primarily consists of several small boats, mainly used by fishermen, as well as a supply ferry from Mauritius that visits the island regularly. The local airline (Air Mauritius) services the island with two flights daily (Statistics Mauritius, 2019; van der Schyff *et al.*, 2020a).



**Fig. 3.** Map of the island of Rodrigues (Bhagooli & Kaullysing, 2018). The reef is indicated in blue.

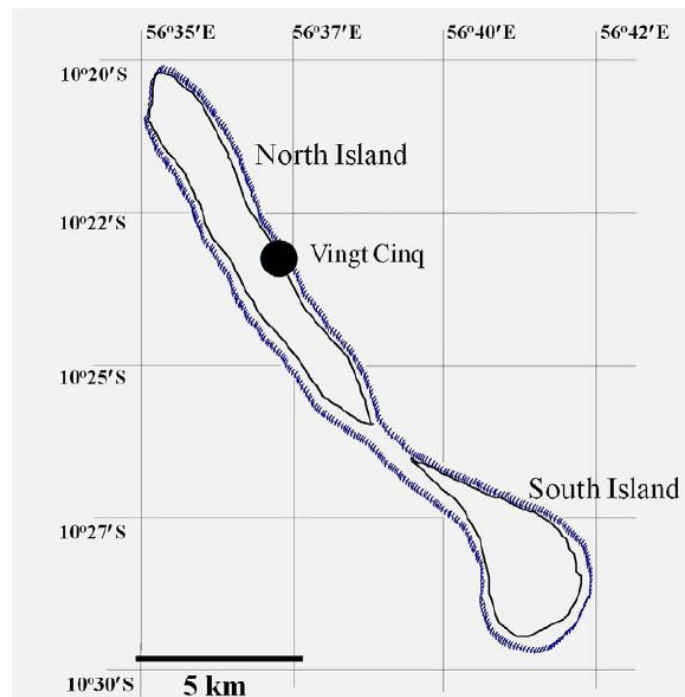


**Fig. 4.** Views of Rodrigues Island. a) The southern lagoon with *Ille au Chat*, b) the lagoon at *Baie de L'Este* with a large channel, and c) *True d'Agent* an isolated beach. (Mascarene Coral

Island Expedition, 2014). All three sites are located in the South East Marine Protected Area (Fig. 3)

### 3.2.2. Agalega

Agalega consists of two small islands connected by a shallow lagoon. The island system is located approximately 1100 km north of Mauritius and supports a population of approximately 300 people. At low tide, a tractor ferries people between islands. A ferry from Mauritius provides essential supplies to the island every three months. The only economic activity on Agalega is coconut harvesting and processing (Statistics Mauritius, 2019). No tourist activities are present on Agalega.



**Fig. 5.** Map of the island of Agalega (Bhagooli & Kaullysing, 2018). The surrounding reef is indicated in blue.

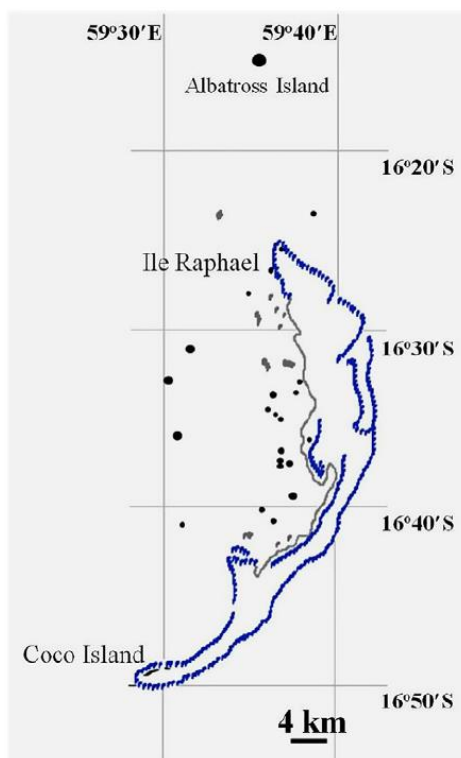


**Fig. 6.** Views of Agalega Island. a) The beach near the sampling site at the southern tip of North Island, b) view of the lagoon between the North and South islands, and c) Agalega's international port (Mascarene Coral Island Expedition, 2014).

### 3.3.3. St. Brandon's Atoll

St. Brandon's Atoll is a remote archipelago approximately 450 km north of Mauritius. The atoll forms part of the Cargados Carajos Shoal located on the Cargados Carajos Bank in the Mascarene Plateau. The atoll consists of approximately 19 sandbars and 24 vegetated islands, connected by a 200 km<sup>2</sup> shallow lagoon (Quod, 1999). Patches of coral are present in the lagoon, but the reef crest is a relatively well-developed reef. Several shipwrecks are present on the reef crest and the outer edges of the lagoon (Bouwman *et al.*, 2016; Evans *et al.*, 2016). The atoll system is located in the centre of the South Equatorial Current (Bhagooli & Kaullysing, 2018; Fig. 2).

No people permanently reside on St. Brandon's Atoll. Approximately 40 fishermen, weather personnel, and members of the National Coast Guard live on a rotational basis the atoll—mostly on *Ile Raphael* and *Ile du Sud* (Bouwman *et al.*, 2016; Evans *et al.*, 2016). A few exclusive yacht charters enable eco-tourists and recreational anglers to visit the atoll. Most residents of Mauritius refer to the atoll as St. Brandon, but it is also known as St. Brandon's Atoll, St. Brandon's Island, and St. Brandon's Rock (van der Schyff *et al.*, 2020a).



**Fig. 7.** Map of the St. Brandon's Atoll (Bhagooli & Kaullysing, 2018). Islets are indicated in black, and the reef in blue.





**Fig. 8.** Views of St. Brandon's Atoll. a) The research base, surrounded by a patchy coral reef, b) a fairy tern over the lagoon, and c) a fossil coral reef exposed due to lower sea level (Mascarene Coral Island Expedition, 2014).

## **Chapter 2. Impacts of a shallow shipwreck on a coral reef: A case study from St. Brandon's Atoll, Mauritius, Indian Ocean.**

### **Foreword to Article 1: "Impacts of a shallow shipwreck on a coral reef: A case study from St. Brandon's Atoll, Mauritius, Indian Ocean."**

Pollution has been quantified in all corners of the globe—even in extremely remote locations such as Antarctica (Munoz *et al.*, 2017b), the high Arctic (Sørmo *et al.*, 2008), and the deep ocean (Jamieson *et al.*, 2017). Certain pollutants adsorb to plastics (Ogata *et al.*, 2009) and are transported to remote locations where they can leach out into the environment (Mato *et al.*, 2001). However, marine debris may not be the only vector of pollutants to isolated islands, shipwrecks can also contribute. St. Brandon's Atoll, the least inhabited of the Mauritian Outer Islands, accumulates high volumes of long-range transported plastics (Bouwman *et al.*, 2016). Nevertheless, only a small temporary population of fishermen, weather station personnel, and National Coast Guard reside on the atoll—mostly on the northern island of *Ile Raphael*. In addition, these people typically do not reside on the atoll for longer than two months at a time. Their direct impact on pollution in the atoll system is thus negligible. Furthermore, very few tourists visit the atoll due to its remote location. Most of the tourists visiting the islands do so with exclusive catamarans (Pers. Com. Merven, 2014). Therefore, the effect of tourism on pollution in the atoll is also negligible. The question then stands: How are pollutants transported to isolated oceanic islands? While on St. Brandon's Atoll during the Mascarene Coral Island Expedition (MCIE), we were notified of a shallow shipwreck that was causing visible ecological damage to the surrounding reef. We conducted an opportunistic study on the wreck and surrounding areas to investigate its effect as potential vector of pollutants.

The relevance of this this manuscript is highlighted by the ecological disaster faced by the Republic of Mauritius in August 2020. The Japanese bulk carrier *MV Wakashio* ran aground on the reef offshore of Pointe d'Esny, in the south of Mauritius, on the 25<sup>th</sup> July 2020. On the 6<sup>th</sup> of August 2020, the ship began to leak oil. As of the time of writing (11<sup>th</sup> of August 2020), more than 1000 metric tons of oil have leaked out of the wreck. Pointe d'Esny is located close to two of Mauritius's most prominent conservation areas: *Ile au Aigrettes* nature reserve—the only area populated mainly by endemic Mauritian wildlife (Feare, 2018), and Blue Bay Marine Park, an area designated as a wetland of international importance (RAMSAR, 2008).



In this article, potential consequences of a shallow shipwreck (such as the *MV Wakashio*) to a coral reef is examined, which may aid in the mitigation of the wreck and recovery of the surrounding coral reef of Pointe d'Esny.

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[https://www.sciencedirect.com/science/article/pii/S0141113619304866?casa\\_token=tpNpSkFd3qkAAAAA:9SOcB\\_WxHijUPYqlstM1R8F2-TP01WIJb-YjTpRpIld089YI2MExnfxWwYjJPQdCVI-aRv96hA8](https://www.sciencedirect.com/science/article/pii/S0141113619304866?casa_token=tpNpSkFd3qkAAAAA:9SOcB_WxHijUPYqlstM1R8F2-TP01WIJb-YjTpRpIld089YI2MExnfxWwYjJPQdCVI-aRv96hA8)

## **Impacts of a shallow shipwreck on a coral reef: A case study from St. Brandon's Atoll, Mauritius, Indian Ocean.**

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### **Abstract**

Shallow shipwrecks can have severe ecological and toxicological impacts on coral atolls. In 2012, a tuna longliner ran aground on the reef crest of St Brandon's Atoll, Mauritius, broke up into three pieces which was moved by currents and storms into the lagoon. In the months following the grounding, the coral around the wreck became dead and black. Down-current from the wreck, a dense bloom of filamentous algae (*Ulva sp.*) attached to coral occurred. To determine the ecological effects of the wreck on the system, the marine biota around the wreck, in the algal bloom, and fish reference zones were counted in 2014. Metal concentrations in reference and affected coral was determined using inductively coupled plasma mass spectrometry (ICP/MS). A pronounced difference was seen in the metal concentration pattern between coral from the wreck- and algal zones, and the coral reference zone. While the wreck zone contained the highest abundance of fish, the fish reference zone had the highest species diversity but with fewer fish. We also counted eleven Critically Endangered hawksbill sea turtles *Eretmochelys imbricata* and significantly more sea cucumbers in the algal zone than the reference zones. The effects of shipwrecks on coral reefs must be considered a threat over periods of years and should be studied further.

**Keywords:** Algal bloom; black reefs; coral; ecotoxicology; fish; sea cucumbers

## 1. Introduction

Worldwide, the health of coral reefs is declining (Gardner *et al.*, 2003; Porter & Schleyer, 2017; Hughes *et al.*, 2018; Baumann *et al.*, 2019). Some of the causes are global such as rising sea temperatures (Carpenter *et al.*, 2008) and ocean acidification (Millero *et al.*, 2009). Others are localised threats, including damage caused by storms and recreational divers (Sheppard *et al.*, 2009), or chemical threats such as oil spills and plastic debris (Allen *et al.*, 2017).

Shipwrecks are often found on the shallow reef crests or back reefs of oceanic atolls, posing an intricate ecological conundrum as their presence impacts both positively and negatively on reefs. Shipwrecks provide structure for the formation of new coral reefs and shelter for fish and other sea-life, increasing the species richness and diversity of an area (Perkol-Finkel *et al.*, 2006; Consoli *et al.*, 2015). However, shipwrecks on coral reefs present a threat to coral reefs as they often cause physical damage to the reef and pollute the broader environment (Evans *et al.*, 2016; Kelly *et al.*, 2012; Yusuf, 2014). Physical damage is caused when strong wave action, storms, and strong currents break up the structure of ships, often pushing them across the reef crest into deeper waters, leaving scars on the lagoon floor. Outbreaks of coral diseases are also associated with shipwrecks (Raymundo *et al.*, 2018). Chemical pollution occurs from the ship's fuel, cargo, and structure, influencing the ecology and toxicology of the surrounding reefs (Barrett, 2011). Shipping activities are also vectors for several invasive marine organisms. Macroalgae spores, including spores of several *Ulva* species have been recorded in ships ballast water (Flagella *et al.*, 2007; Aguirre-Macedo *et al.*, 2008). Species of the genus *Ulva* are opportunistic filamentous algae with a traditionally coastal distribution. It is now found in several sites outside the traditional known distribution (Zetuche-González *et al.*, 2009; Yuping *et al.*, 2015). Algal bloom growth resulting from macroalgae spores released from shipping activities can cause coral mortality by overgrowing coral colonies and smothering the organisms as they grow (Jompa & McCook, 2003), outcompeting corals for resources, and reducing coral larvae settlement (McCook *et al.*, 2001).

Numerous ships have been wrecked on St Brandon's Atoll (SBR) (Hancock, 2018). Although some of the wrecks have been recovered, such as the Team Vestas Wind racing yacht that stranded during the 2014 Volvo Ocean Challenge (Bouwman *et al.*, 2016), many wrecks are not salvaged and remain on the atoll for years.

In October 2014, during an expedition to SBR to collect coral, fish, and plastic for pollutant analyses, we were made aware of the wreck of a 79.5 ton tuna longliner that ran aground on the south-eastern edge of SBR of the atoll reef crest in 2012 (Merven, J. pers. obs. 2014) (Fig. 1). The vessel subsequently broke into three segments (the bow section that contained the cargo hold, the stern section with the engine room and fuel tanks, and the centre

wheelhouse section) that were shifted by currents and storms across the lagoon to where they are now, approximately 6 km down current from the initial point of impact (Fig. 1). The bow and stern are approximately 850 m, with the wheelhouse approximately 670 m perpendicular from the stern (Fig. 1). Our initial observations showed that this wreck and its subsequent breakup caused physical damage to the lagoon, and apparently contributed to a dense concentrated (approximately 40 hectare) algal bloom. An *Ulva spp.* bloom developed on coral heads down-current from the wreck, approximately 100 m from the wheelhouse (Fig. 1). Such a bloom has not been observed before on SBR. (Merven, J. pers. obs. 2014).

The coral in the direct vicinity of the wreck fragments were noticeably blackened. Black reefs associated with shipwrecks have been recorded in the Pacific Ocean, but never before in the Indian Ocean (Schroeder *et al.*, 2008; Kelly *et al.*, 2012; Mangubhai & Obura, 2019). This posed a unique opportunity to study the ecological and toxicological effects of a shipwreck on a near-pristine island system in the western Indian Ocean.

There is no framework or comprehensive method of risk assessment for shipwrecks (Landquist *et al.*, 2013). The aim of this research was to investigate the impact of the wreck using a two-fold approach with two objectives: examining the data from fish and sea cucumber transects, and measuring the metals in coral and algae collected from the wreck and reference zones.

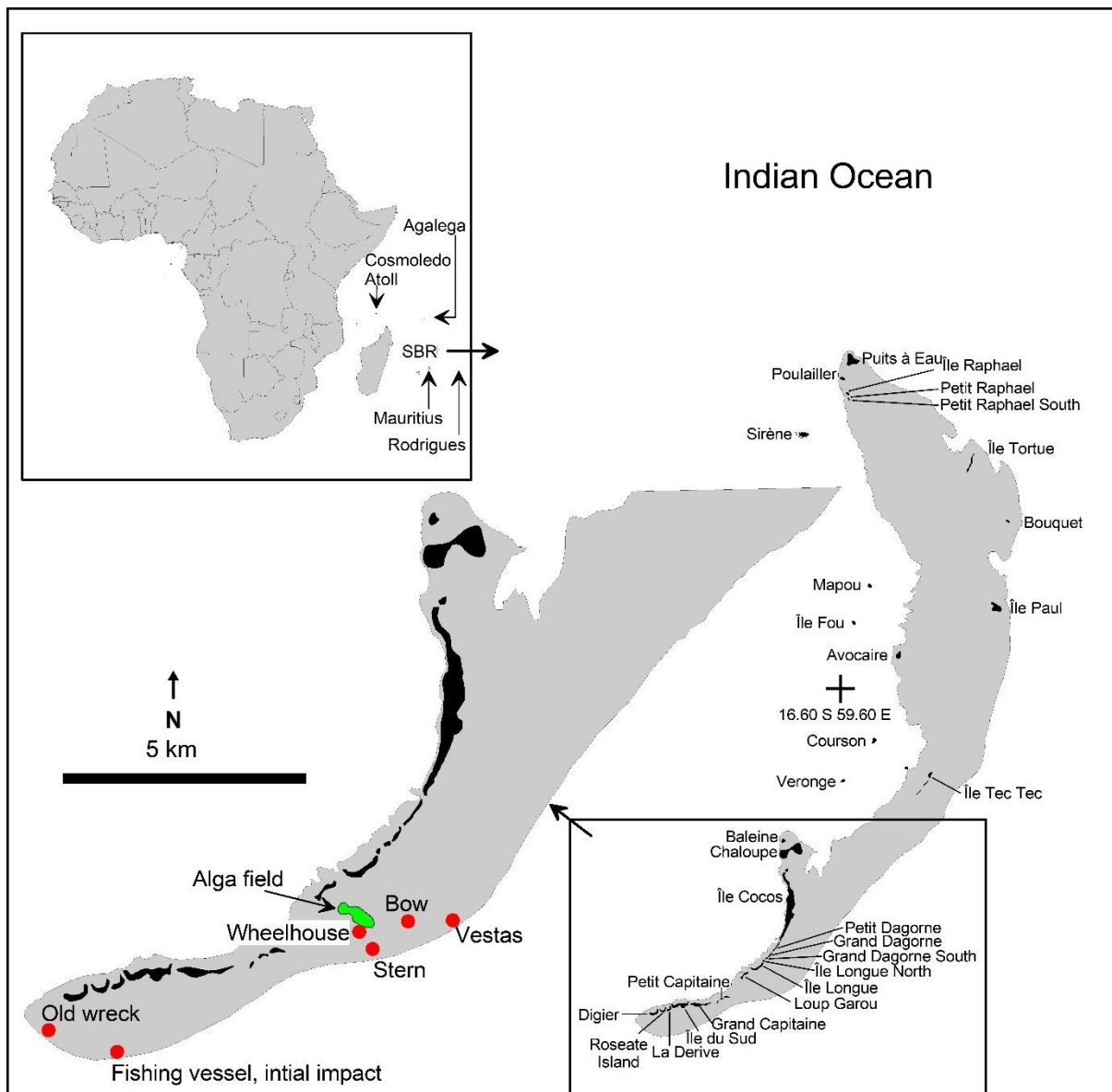
We hypothesized that corals from the wreck- and algal zones would have a higher concentration of metals than corals from the coral reference zone. Furthermore, we predicted that the undisturbed reef reference (fish reference zone) would have a higher fish species richness, and that the wreck zone would have a higher fish abundance due to the structure afforded by the wreck pieces.

## **2. Materials and methods**

### *2.1. Study area and sampling zones*

St. Brandon's Atoll, forming part of the Cargados Carajos Shoals in the Mascarene Basin, is a 200 km<sup>2</sup> coral reef island approximately 450 km north-east of Mauritius in the Indian Ocean (Evans *et al.*, 2016; Quod, 1999; Fig. 1). SBR is located upstream of where the South Equatorial Current splits at the coast of Madagascar. The current flows from the north of Australia and the Indonesian island chain to the western Indian Ocean (Schott & McCreary Jr., 2001). There are no permanent residents on SBR, but there is a small permanent but rotating human presence of approximately 40 fishermen, meteorological station personnel, and National Coast Guards of Mauritius (Bouwman *et al.*, 2016). The effect of the people residing on the atoll on the pollution of the area is considered minimal, except in the areas of settlements at Ile du Sud and Ile Raphael. Most of the pollution on the atoll is from external

sources, such as marine debris and pollutants transported by ocean currents (Bouwman *et al.*, 2016). The wreck is located beyond where the Atoll's inhabitants normally venture.



**Fig. 1.** Location of the shipwreck sections and the algal bloom relative to St. Brandon's Atoll.

Four zones were selected for surveys and sample collection. The water depth at all zones varied between one and three metres and strong currents from the south-west are dominant throughout the year. The respective zones are described as follow:

- The wreck zone is the area where the three wreck segments were located within eyeshot of each other, with the bow and stern separated by approximately one kilometre, and the wheelhouse approximately 500 m west from the stern (Fig. 1). The coral in the immediate vicinity (ca. 20-50 m radius) of each section of wreck was dead and blackened (Fig. 2) with

many large fish present. Each wreck section was individually surveyed for fish, sea cucumber, and coral, and sampled for blackened coral.



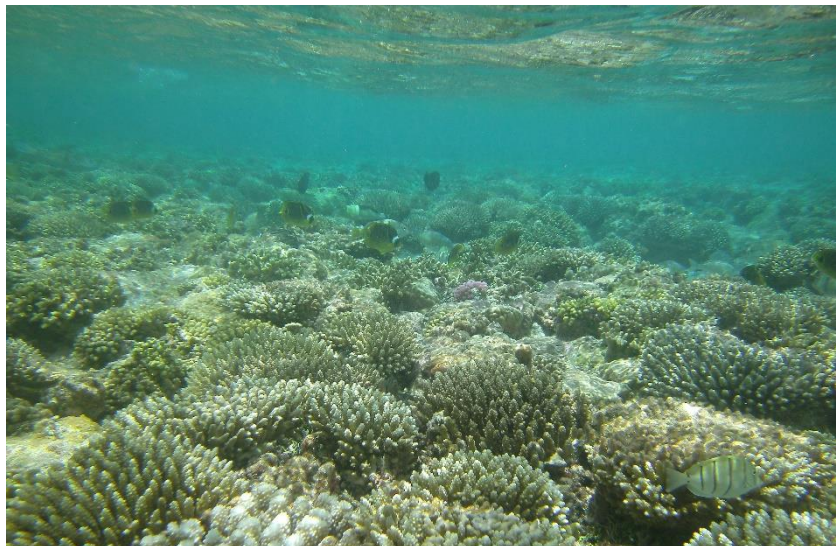
**Fig. 2.** Blackened coral in the wreck zone.

- The algal zone comprised 40 hectare of the lagoon where filamentous algae (*Ulva* spp.) grew down-current from the wreck (Fig. 1). This algal bloom developed following the stranding of the fishing vessel in 2012 (Merven, J. pers. obs. related in 2014). We circumnavigated the algal zone with a small boat while recording the track with a GPS (Fig. 1). The algae occurred in long strands, up to 10 m, attached to coral heads. Much of the coral underneath the attached algae appeared dead or heavily stressed. This is evidenced by bleached sections of coral observed under the algal strands (see videos in Supplemental Materials). Many coral heads were broken, presumably due to the hydrodynamic drag of the strong currents imparted on the heads by the algae (Fig. 3) as the broken coral heads were down-current from their attachments. Strands of algae and coral were collected and analysed for metals. Fish and sea cucumber abundance surveys were also conducted in this zone.



**Fig. 3.** Macroalgae strands over a coral colony in the algal zone.

- The fish reference zone is an area that was not affected by the current flowing from the wreck (Fig. 1). No algal growth occurred here. The corals from this zone appeared healthy (Fig. 4). Fish and sea cucumbers were surveyed here as a reference state.



**Fig. 4.** The fish reference zone reef, without macroalgae growth with seemingly healthy coral.

- The coral reference zone is located on the other side of the atoll, where the effect of the wreck and algae was deemed negligible. Coral genera were found in this area were similar to that in the algal zone and wreck zone. Samples of live colonies of *Pocillopora* spp. and *Stylophora* spp. were collected here. The reference corals were not collected from the fish



reference site due to the proximity of the wreck— metals from the wreck may have contaminated corals down current.

## 2.2. Fish and sea cucumber surveys

Three, 50 m transects were surveyed in the algal zone, and four, 50 m transects in the fish reference zone. Fish and sea cucumber species and abundance were recorded while snorkelling, recording fish and sea cucumbers within 5 m either side of the transect line (50 m rope). The parallel transects were about 100 m apart, measured by GPS. An area of 5000 m<sup>2</sup> was surveyed in the fish reference zone. Fish and sea cucumber abundance and species within a 50 m radius circle (an area of 7850 m<sup>2</sup>) around each wreck piece was recorded.

## 2.3. Coral and algae sampling

*Pocillopora* and *Stylophora* hard corals occurred in abundance in the wreck zone, algal zone, and coral reference zone. Fragments of live coral were collected from the algal zone and coral reference zone. In the wreck zone, where all coral close to the wreck were dead, fragments of dead colonies of both species were collected. The fragments of the two coral species per area were pooled, placed in plastic bags, and frozen at -20°C. Algal strands were collected from the centre of the algal zone and stored in high-density polyethylene bottles. Small paint chip fragments from the wreck were collected. The biological samples were shipped frozen to South Africa (Import permit No. P0075626) for chemical analysis in a frozen state.

## 2.4. Laboratory analysis

Coral fragments, algal strands, and paint chip samples were analysed for metal concentrations at EcoAnalytica Laboratory in Potchefstroom, South Africa, according to the United States Environmental Protection Agency (EPA 3051A) (EPA 1996). Two grams of finely ground, freeze-dried samples were heat- and acid digested using reagent grade 33% hydrochloric acid, 50% hydrogen peroxide, and 65% concentrated nitric acid (Associated Chemical Enterprises). The solution was heated and diluted to 50 ml with deionised water (18MΩ·cm) water, before analyses of 31 elements using inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7500c ICP-MS with standard quartz chamber and micromist-type nebulizer). A 1 µg/l solution of cerium, lithium, and thallium for low-oxide/low interference levels (≤ 1.5%) was used to maintain sensitivity across the mass range. External calibration was employed using certified ULTRASPEC mixed multi-element stock standard solutions (De Bruyn Spectroscopic Solutions) that contained all the elements of interest at different concentration levels. Full calibration sets and quality control check standards were run frequently. The detection limits for each element were calculated from the calibration curves



with each calibration run. A standard reference material (SRM 1944 - New York/New Jersey Waterway Sediment) was used for quality control. The concentrations of the certified elements were within 20% of certified values. Concentrations are expressed as milligram per kilogram on a dry mass basis (mg/kg dm).

## 2.5. Statistical analysis

Graphpad Prism 8.1.0 ([www.graphpad.com](http://www.graphpad.com)) was used for summary statistics and comparisons. Because the datasets were not normally distributed, we used the non-parametric, Kruskal-Wallis tests to test for variance between the metal concentrations of coral from the wreck zone, algal zone, and coral reference zone. The Dunn's multiple comparisons test was used to test the differences between pairs of samples. Significance was selected at  $p < 0.05$ .

Nonmetric multidimensional scaling (NMS), using MjM Software PC-ORD 7.07 ([www.pcord.com](http://www.pcord.com)) was conducted to ordinate relative metal proportions in corals and algae. The data were relativised per element in order to obtain a proportional profile ('fingerprint') of metals in each sample. "Gower-ignore-0" was used as the distance measure. Five hundred runs of real data were used, from random starting conditions. A maximum of six axes were allowed. A second NMS was run with the same parameters to ordinate the relative fish diversity in the different zones. "Gower-ignore-0" was, again, used as the relative measure of distance.

## 3. Results

### 3.1. Visual observations of the 'dead' zones of the wreck segments, and the algal field

There were no live corals within a 10 m radius around each of the wreck segments, nor on the wreck segments themselves. The dead coral was blackened and broken. Black band diseases was disregarded as the cause of the coloration because the entirety of the affected colonies were black; the distinctive black band separating live tissue and dead white tissue (Barneah *et al.*, 2007) was not seen in the affected area. Between the wreck segments and the algal field, live coral was present, but these appeared stressed with few fish present in the area. However, no quantitative surveys were conducted here.

The algal field was located about 100 m away, down-current, from the closest wreck segment (Fig. 1). This algal field was a striking phenomenon, as shown in Fig. 3. Underwater, the damage to coral heads was quite apparent, probably caused by the hydrodynamic drag of the algae strands. The coral also appeared stressed, especially where the rhizoids of the algae were attached to the coral, and where the algae strands covered the coral (see Supplemental Materials).

### 3.2. Metals in corals

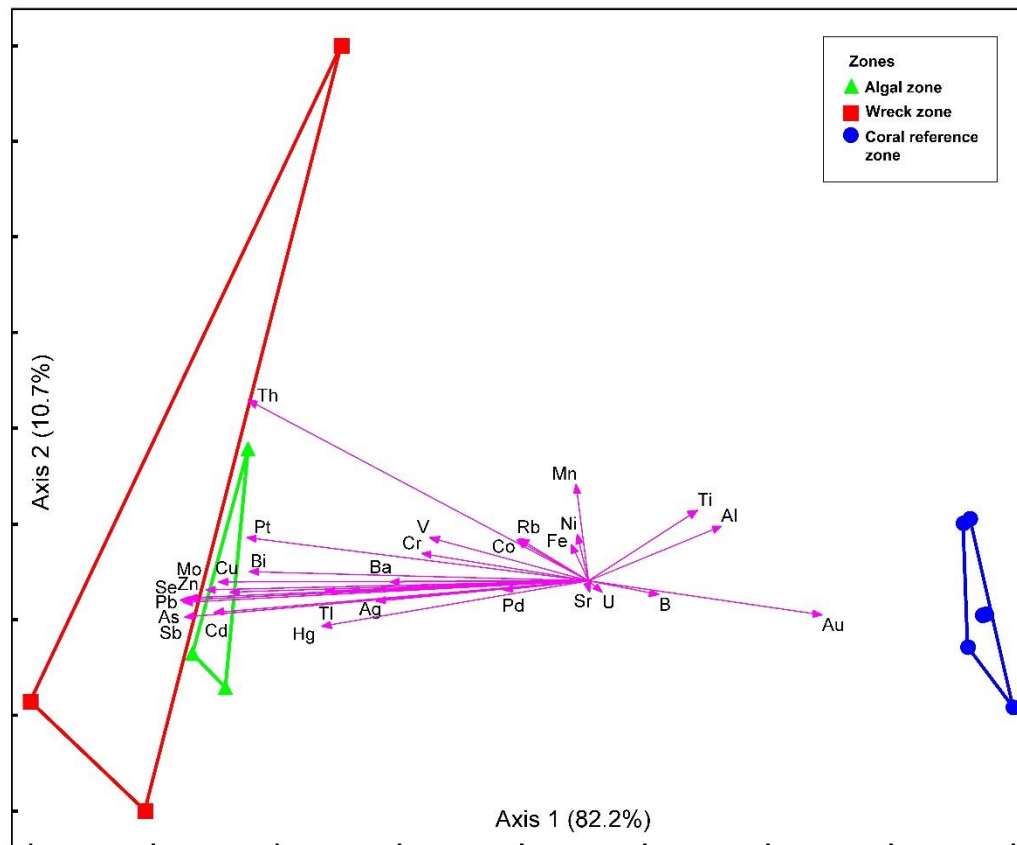
Mean concentrations are summarised in Table 1. There were no significant differences between the coral samples from the three zones for Al, Ti, V, Mn, Fe, Co, Ni, and Hg. The wreck zone corals had significantly higher concentrations of Cr, Zn, and As than the coral reference zone. The algal zone corals had significantly higher concentrations of Cu, Cd, Pb, and U compared with the coral reference zone.

**Table 1.** Mean metal concentration (mg/kg dm) and standard deviations (in bracket) of paint chips (n=3) from the wrecks, *Ulva spp.* algae strands (n=3) from the centre of the algal zone, pooled coral fragments from the algal zone (n=3), wreck zone (n=3), and coral reference zone (n=6).

	Al	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	As	Cd	Hg	Pb	U
<b>Paint chips</b>	22000	6200	160	1500	2400	240000	100	160	102000	13000	14	0.095	0.082	1000	7.1
	(2600)	(8000)	(140)	(1300)	(3300)	(330000)	(63)	(180)	(140000)	(17000)	(19)	(0.071)	(0.079)	(760)	(4.7)
<b>Algae strands</b>	9.5	2.2	1.5	41	3	140	0.37	9.7	7.6	4.7	5.7	0.44	0.075	1.6	0.25
<b>Ulva spp.</b>	(1)	(0.45)	(0.36)	(7)	(0.1)	(24)	(0.034)	(2.7)	(5.3)	(0.85)	(3)	(0.17)	(0.033)	(0.68)	(0.053)
<b>Coral fragments</b>															
<b>WZ</b>	120	9.1	1.4	*43	12	1300	2.5	15	2.6	*3	*2.4	0.055	0.042	0.69	2.3
	(105)	(7.6)	(0.36)	(9.2)	(9.4)	(603)	(0.98)	(8.02)	(0.43)	(0.17)	(0.19)	(0.017)	(0.031)	(0.091)	(0.15)
<b>AZ</b>	95	4.2	0.71	26	8.9	1360	2.1	17	*3.3	2.8	1.1	*0.4	0.029	*0.84	*0.47
	(20)	(1.03)	(0.16)	(2.9)	(3.1)	(180)	(0.29)	(4)	(0.37)	(0.32)	(0.47)	(0.1)	(0.0057)	(0.3)	(0.023)
<b>CR</b>	325	18	0.79	*24	15	1960	2.6	24	*0.22	*0.15	*0.034	*0.0025	0.017	*0.0083	*2.7
	(122)	(6.7)	(0.48)	(8.2)	(11)	(717)	(1.1)	(11)	(0.015)	(0.072)	(0.072)	(0.00035)	(0.0021)	(0.0039)	(0.039)
<b>KW</b>	0.0156	0.0466	0.2299	0.0253	0.6676	0.2971	0.7685	0.6529	0.0005	0.0027	0.0003	0.0003	0.0819	0.0027	0.0102

The three convex hulls in the NMS graph (Fig. 5) represent the relativised metal compositional pattern of corals sampled from the algal zone, wreck zone, and coral reference zone. A final stress of 3.59 was achieved after 40 iterations, needing only two axes to ordinate the sample points – axis one represents more than 80% of the variance. Clarke's rule of thumb interprets ordinations with a stress of less than 5 as excellent with no prospect of misinterpretation (McCune & Grace, 2002). None of the convex hulls overlapped, indicating that the metallic composition of the corals in each of the zones was different. However, the metallic 'fingerprints' of the corals from the algal zone and wreck zone were far more similar, and both clearly distinct from the corals from the coral reference zone. The convex hulls from the affected zones were associated with higher relative proportions of metals such as Cu, As, Cd, and Pb, while the coral from the coral reference zone had higher relative proportions of

Au, B, Al, and Ti. The concentrations of all metals shown in Fig. 5 can be found in Supplementary Table 1 (Table S1).



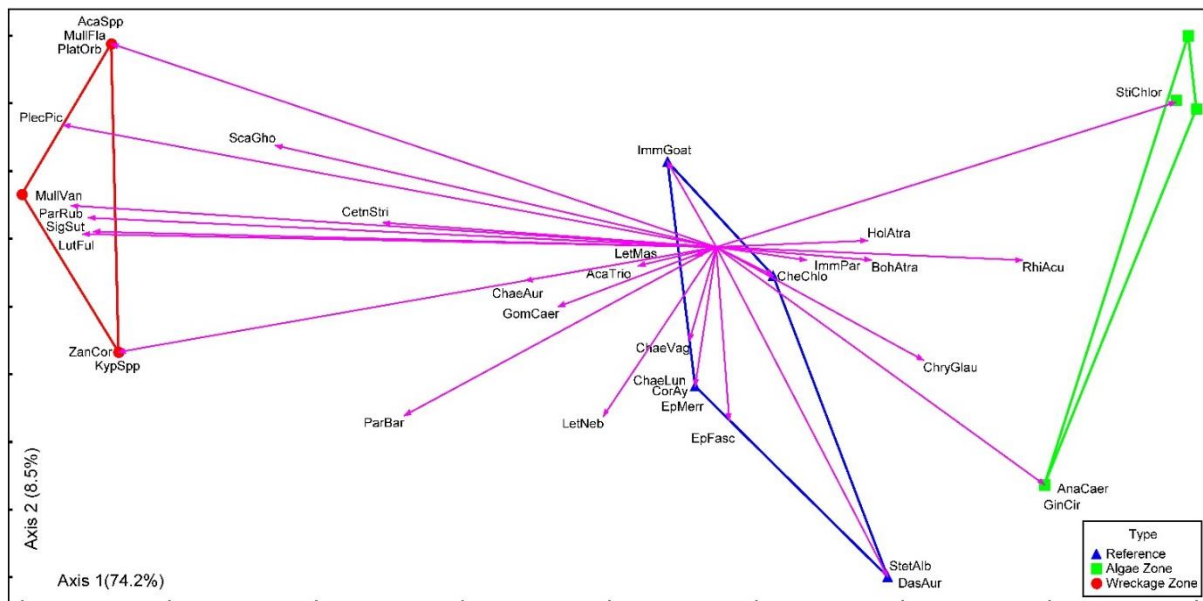
**Fig. 5.** Non-metric, multidimensionally scaled ordination of the relativized metal compositional patterns ('fingerprints') of corals collected from the wreck zone (squares), algal zone (triangles), and the healthy coral reference zone (circles).

### 3.3. Fish and sea cucumber community compositions

The results of the fish and sea cucumber surveys are listed in Table 2, showing the high density of fish associated with the wreck zone, highest species richness associated with the fish reference zone, and the highest density of sea cucumbers with the algal zone. (Table 2). The algal zone had the lowest species richness and species diversity (Shannon and Simpson indices), and the fish reference zone the highest.

**Table 2.** Fish and sea cucumber species richness and density. The Shannon and Simpson indices were calculated from the fish reference zone, algal zone, and the wreck zones.

Zone	Species richness	Fish / m <sup>2</sup>	Sea cucumbers / m <sup>2</sup>	Shannon	Simpson
AZ	5	0.82	1.5	0.72	1.6
WZ	19	4.9	0.025	1.91	3.8
FR	25	1.9	0.095	2.19	4.8



**Fig. 6.** Non-metric, multidimensionally scaled, ordination of relativized fish and sea cucumber composition of the wreck zone, algal zone, and fish reference zone. The vectors represent fish and sea cucumber species. The key to scientific and common names of the organisms are provided in Table 3.

The fish and sea cucumber compositions of the three zones were very different from one another (Fig. 6) indicated by the non-overlap of the convex hulls. Two dimensions were needed, reached after 55 iterations, a stress < 0.00001, with a final stress of 2.87; again a very good representation. Most of the fish species were associated with the wreck zone, but were also present in the fish reference zone. Whilst only a few fish species ordinated towards the algal zone, both sea cucumber species (*Holothuria atra* and *Stichopus chloronotus*) ordinated towards the algal zone convex hull, representing the high occurrence of sea cucumbers there. Although they were not found on the other transects and at the wreck sites, 11 hawksbill sea turtles (*Eretmochelys imbricata*) were seen feeding on the algae in the algal zone. Two nurse sharks (*Ginglymostoma cirratum*) were also seen in the algal zone. The fish that was most often seen in the algal zone was the herbivorous *Chrysiptera glauca* (Branch *et al.*, 2016). White-banded triggerfish (*Rhinecanthus aculeatus*) were the second most abundant fish in the algal zone.

**Table 3.** The species name and common names of the fish and sea cucumbers in Fig. 6.

<b>NMS abbreviation</b>	<b>Species name</b>	<b>Common name</b>
AcaSpp		Surgeon fish
AcaTrio	<i>Acanthurus triostegus</i>	Convict surgeonfish
AnaCaer	<i>Anampses caeruleopunctatus</i>	Bluespotted wrasse
ChaeAur	<i>Chaetodon Auriga</i>	Threadfin butterflyfish
ChaeLun	<i>Chaetodon lunula</i>	Raccoon butterflyfish
ChaeVag	<i>Chaetodon vagabundus</i>	Vagabond butterflyfish
CheChlo	<i>Cheilinus chlorourus</i>	Floral wrasse
ChryGlau	<i>Chrysiptera glauca</i>	Grey demoiselle
CorAy	<i>Coris aygula</i>	Clown coris
CtenStri	<i>Ctenochaetus striatus</i>	Striated surgeonfish
DasAur	<i>Dascyllus auruanus</i>	Whitetail dascyllus
EpFasc	<i>Epinephelus fasciatus</i>	Blacktip grouper
EpMerr	<i>Epinephelus merra</i>	Honeycomb grouper
GinCir	<i>Ginglymostoma cirratum</i>	Nurse shark
GomCaer	<i>Gomphosus caeruleus</i>	Green birdmouth wrasse
ImmGoat		Small immature goatfish
ImmPar		Small immature parrotfish
KypSpp	<i>Kyphosus spp</i>	Grey chub
LetMas	<i>Lethrinus mahsena</i>	Sky emperor
LetNeb	<i>Lethrinus nebulosus</i>	Spangled emperor
LutFul	<i>Lutjanus fulviflamma</i>	Dory snapper
MullFla	<i>Mulloidichthys flavolineatus</i>	Yellowstripe goatfish
MullVan	<i>Mulloidichthys vanicolensis</i>	Yellowfin goatfish
ParBar	<i>Parupeneus barberinus</i>	Dash-and-dot-goatfish
ParRub	<i>Parupeneus rubescens</i>	Rosy goatfish
PlatOrb	<i>Platax orbicularis</i>	Orbicular batfish
PlecPic	<i>plectorhinchus picus</i>	Painted sweetlips
RhiAcu	<i>Rhinecanthus aculeatus</i>	White-banded triggerfish
ScaGho	<i>Scarus ghobban</i>	Blue-barred parrot
SigSut	<i>Siganus sutor</i>	Shoemaker spinefoot
StetAlb	<i>Stethojulis albovittata</i>	Bluelined wrasse
ZanCor	<i>Zanclus cornutus</i>	Moorish idol
BohAtra	<i>Bohadschia atra</i>	Sea cucumber
HolAtra	<i>Holothuria atra</i>	Lollyfish sea cucumber
StiChlor	<i>Stichopus chloronotus</i>	Greenfish sea cucumber

## 4. Discussion

### 4.1. Metals in corals

Pollution originating from fossil fuel spillage (Vouk & Priver, 1983; Prego & Cobelo-García, 2004; Landquist *et al.*, 2013), corrosion of electronic waste (Hahladakis *et al.*, 2013), and antifouling paint (IMO, 2002) are usually associated with shipwrecks. Lead, Hg, Se, As, Cu, Zn, Cd are associated with fossil fuels (Vouk & Priver, 1983; Prego & Cobelo-García, 2004). All these elements were present in higher concentrations in coral from the affected area (wreck zone and algal zone combined). Cadmium, Se, Hg, Pb, and Zn are metals that have been implicated in endocrine disruption in invertebrates such as sea cucumbers that were present in large numbers in the algal zone (Depledge & Billingham, 1999). Chronic exposure to oil can cause reproductive failure, cellular degradation, and atrophy in *Stylophora* corals (Peters *et al.*, 1981). Elevated concentrations of Zn and Cd are metals known to be associated with so-called green tide algae blooms (Ge *et al.*, 2017). Corals from the affected areas contained higher concentrations of these metals than corals from the coral reference zone. It is evident that the presence of the wreck amplified these metal concentrations beyond the background concentrations of the natural reef.

In addition, it was found that the paint from the wreck contained high concentrations of Cu (103 000 mg/kg), Fe (702 000 mg/kg), Al (59 000 mg/kg) Ti (18 000 mg/kg), and Pb (1 800 mg/kg). Since restrictions were placed on maritime companies by the International Maritime Organisation (IMO) to eliminate the use of tributyltin (TBT) as an antifoulant, copper-based replacements have become increasingly popular (IMO, 2002). High concentrations of Cu and Zn originating from antifoulant paint had been found in sediment around other shipwrecks in previous studies (Jones, 2007). The wreck in SBR had been painted in a shipyard in Mauritius before its last voyage (Merven, J. Pers Obs. 2014). This fresh coat of paint may be a reason for the high Cu concentration in the paint of the wrecked ship.

Lead was also found in paint samples from the wreck, at 1 800 mg/kg dm. This Pb most likely caused lead concentrations two orders of magnitude higher in algal zone and the wreck zone coral than in coral from the coral reference zone (Table 2). Despite the strong movement of banning lead from paints, it is still found in hull paints. Although lead in paint is rather insoluble and therefore will leach slower than Cu or Zn (Turner, 2014), abrasion of the hulls, as was seen through the scouring of the coral reef lagoon, will release paint chips.

Although most metals were found in significantly higher concentrations in coral from the wreck-affected areas, some metals did not follow this same pattern. Iron, Al, Ti, Co, Ni, and U concentrations were lower in the affected area than in the coral reference zone. This was

unexpected, because the coral reference zone was situated on the other side of the atoll, and these corals were isolated from the effects of the wreck in question.

#### 4.2. Algal bloom

Coral thrives in nutrient poor waters (Sheppard *et al.*, 2009). Shipwrecks have the potential to create a nutrient influx that promotes algal growth that out competes coral growth (Sheppard *et al.*, 2009). The nutrients promoting algal growth might have been released when the cargo hold and the ballast water tank of the fishing vessel broke after being dragged over the reef crest by currents and waves (Littler *et al.*, 2006; Humanes *et al.*, 2016). The ballast water that ships require for stabilization are known vectors of *Ulva spp.* spores (Flagella *et al.*, 2007; Aguirre-Macedo *et al.*, 2008). We speculate that the initial *Ulva spp.* spores that caused the bloom were transported by ballast water.

The wreck in question was a tuna longliner. It stands to reason that the cargo hold would contain bait and the tuna that were caught prior to the wreckage event. These fish would have thawed and decayed. When the cargo hold containing the fish tissue broke open, the contents would have been released to the reef in a nutrient influx from the decomposed fish. We do not know whether this release occurred all of a sudden when the hull was breached, or gradually over time through slow leakage. Both scenarios would favour bottom-up eutrophication (Littler & Littler, 1984) which could play a large role in an algae-coral phase shift, favouring algal growth over coral growth.

Normally, algal growth on live and healthy coral would be visibly absent due to intense grazing by herbivores and active cleaning of the surface by the coral itself (Nugues *et al.*, 2004). However, since the corals in the algal zone presumably were compromised by *inter alia* elevated metal concentrations (Table 1) and possibly nutrient and fuel oil release, the natural cleaning mechanisms of the coral. Herbivory by small fish was insufficient to prevent the algal growth.

#### 4.3. Fish and sea cucumber community structure

Fig. 6 shows that while the wreck zone had the highest fish abundance, the fish reference zone contained the highest species diversity. The fish that occurred in the wreck zone were predominantly large and at a higher trophic level (Branch *et al.*, 2016).

Even though some tourism does occur on the atoll, small-scale commercial fishing is the main economic activity practiced in the area. Frozen and salted fish are transferred from the outer atoll system to the Mauritian main island. Fishers mainly target spangled emperors (*Lethrinus nebulosus*) and sky emperors (*Lethrinus mahsena*) — both predatory fish that were found in abundance around the wreck.

Coralivorous fish, such as species of the genus *Chaetodon* (butterfly fish), were mostly found in the fish reference zone. This makes sense as the coral of the fish reference zone was, on face value, the healthiest. The coral in the fish reference zone did not exhibit the blackened coloration of the coral from the wreck zone, and were unaffected by the algal bloom of the algal zone.

Macroalgae blooms can change biochemical cycles and the community structures, and decrease biodiversity of an area (Choi *et al.*, 2010). This was seen in SBR, where the algal zone had the lowest species richness and species diversity (Table 2). The majority of fish found between the algae strands were either herbivorous or predators of sea cucumbers. Most of the fish found in the algal zone were also large individuals. Several hawksbill sea turtles were found in the algal zone, but not in the other areas, suggesting that they feed on the algae and associated metals (Table 1).

The organisms that were most often encountered in the algal zone were sea cucumbers. We surmise that the high numbers of sea cucumbers were attracted to the affected area due to the detritus biomass resulting from the broken reef and algal bloom upon which they could feed. Elsewhere in the SBR lagoon, the observation of high numbers of sea cucumbers in the track of the wreck being pushed by currents through the coral of the lagoon supports this deduction.

Owing to their effective defence mechanism, very few fish actively prey on sea cucumbers. However, predation by trigger fish and emperor fish have been recorded (Dance *et al.*, 2003). Even though no predation was observed during our study, it is likely that the trigger fish and emperor fish of the algal zone hunt sea cucumbers there (Branch *et al.*, 2016).

#### 4.4. Potential for trophic transfer

The most abundant organism group we counted in the algal zone was the sea cucumbers (Table 2). Although collection permits obtained for this research did not allow collection of sea cucumbers, the effects of metals on the organism can be inferred through the literature. Li *et al.* (2016) found that elevated Cu, Cd, and Zn concentrations contributed to negative effects on the sea cucumbers' respiration, muscle tissue, and intestinal tract. Sea cucumbers are seldom the target prey of fish, but several fish will prey on them if other prey is scarce. Emperor fish and trigger fish are known to prey opportunistically on sea cucumbers (Branch *et al.*, 2016; Dance *et al.*, 2003). Approximately 80% of SBR's fish export consists of emperor fish, *Lethrinus mahsena* (Boistol *et al.*, 2011). If the sea cucumbers contain these and other metals in their tissue, trophic transfer will be expected to occur through biomagnification if the fish prey on the echinoderms (Newman, 2010). People can be exposed to these metals when they eat the fish containing metals (Pheiffer *et al.*, 2014). Fish such as butterfly fish and parrot fish



that prey on coral and coral associated algae are also at risk of being affected by elevated levels of metals through biomagnification (Newman, 2010).

Eleven hawksbill sea turtles were found feeding on the algal bloom. Hawksbill sea turtles are considered Critically Endangered by the International Union for Conservation of Nature (IUCN) Red List (Mortimer & Donnelly, 2008). They are exposed to metals in algae through ingestion. Metals such as Cu, As, and Pb are elevated in algae from the bloom area and are known to affect sea turtle physiology adversely (Du Preez *et al.*, 2018).

## **5. Assessment and recommendations**

The shallow shipwreck we surveyed caused an ecological shift in species distribution and benthic cover. Around the wreck zone and algal zone, the corals were visibly negatively affected or dead. The coral from the affected area and coral reference zone all contained quantifiable concentrations of several metals detrimental to the environment.

Due to the remoteness of SBR, no follow-up study was possible to determine whether the wreck- and algal zones had completely recovered. The algae had largely dissipated after a large storm had swept through the atoll in 2015 (Merven, J. pers. com. 2015). However, the toxicological effects may remain in the environment for a long time as the metals would persist due to the presence and ongoing breakdown of the wreck and remain longer in coral and coral rubble. Because of the potential bioaccumulation of pollutants by emperor fish and sea turtles, and the species' close association with shipwreck structures, consideration should be given to removing shipwrecks from the atoll as soon as possible without causing more damage. Furthermore, fish and other foods obtained near shipwrecks may contain elevated metal concentrations, detrimental to human health, that need to be monitored.

Studying the effects of major disturbances such as shipwrecks on reefs are difficult as such disturbances itself are not predictable, desirable, replicable, or easily restorable. Before-and-after-control studies are therefore only possible in previously surveyed areas. However, our findings show that the occurrences of shipwrecks on coral reefs needs to be included as a potential impact factor on coral reef ecosystems. The effects of a shipwreck seem not only to be limited to the impact point itself, or even the subsequent location of any wreck; down-current effects need also be considered.

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## Supplementary material

### **Impacts of a shallow shipwreck on a coral reef: A case study from St. Brandon's Rock, Mauritius, Indian Ocean.**

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**Table S1.** Averages and standard deviations (mg/kg dm) of metals in coral shown on the non-metric scaling graph (Fig. 5)

	<b>B</b>	<b>Al</b>	<b>Ti</b>	<b>V</b>	<b>Cr</b>	<b>Mn</b>	<b>Fe</b>	<b>Co</b>	<b>Ni</b>	<b>Cu</b>	<b>Zn</b>	<b>As</b>	<b>Se</b>	<b>Bi</b>	<b>Rb</b>
<b>Algal zone coral</b>	31.02	95.3	4.24	0.71	25.6	8.86	1362	2.12	16.96	3.33	2.84	1.11	0.81	0.0072	0.27
Average and SD	(2.13)	(20.04)	(1.03)	(0.16)	(2.87)	(3.08)	(180)	(0.29)	(3.94)	(0.37)	(0.32)	(0.47)	(0.15)	(0.003)	(0.014)
<b>Wreck zone coral</b>	23.2	120	9.12	1.39	42.7	12	1300	2.54	14.65	2.61	2.99	2.37	0.92	0.0065	0.36
Average and SD	(2.16)	(105)	(7.56)	(0.36)	(9.15)	(9.4)	(603)	(0.98)	(8.02)	(0.43)	(0.17)	(0.19)	(0.14)	(0.0013)	(0.14)
<b>Coral reference zone</b>	64.5	324	17.5	0.79	23.8	15.2	1960	2.64	23.7	0.22	0.15	0.034	0.012	0.00082	0.36
Average and SD	(6.4)	(122)	(6.71)	(0.48)	(8.2)	(11.4)	(717)	(1.12)	(11)	(0.015)	(0.078)	(0.072)	(0.0016)	(0.000012)	(0.17)
	<b>Sr</b>	<b>Mo</b>	<b>Pd</b>	<b>Ag</b>	<b>Cd</b>	<b>Sb</b>	<b>Ba</b>	<b>Pt</b>	<b>Au</b>	<b>Hg</b>	<b>Tl</b>	<b>Pb</b>	<b>Th</b>	<b>U</b>	
<b>Algal zone coral</b>	4944	0.34	10.4	0.35	0.4	0.042	13.6	0.01	0.0072	0.029	0.0031	0.84	0.012	0.47	
Average and SD	(156)	(0.018)	(0.28)	(0.019)	(0.1)	(0.02)	(0.53)	(0.0044)	(0.0025)	(0.0057)	(0.0007)	(0.3)	(0.0016)	(0.023)	
<b>Wreck zone coral</b>	4790	0.3	10.6	0.47	0.055	0.046	17.1	0.0069	0.000029	0.042	0.0033	0.68	0.023	2.25	
Average and SD	(420)	(0.0049)	(0.92)	(0.05)	(0.017)	(0.0063)	(3.4)	(0.0015)	(0.000041)	(0.031)	(0.0013)	(0.091)	(0.019)	(0.15)	
<b>Coral reference zone</b>	8130	0.022	11.2	0.22	0.0025	0.0012	9.54	0.0008	0.022	0.017	0.0012	0.0083	0.00031	2.67	
Average and SD	(547)	(0.00038)	(0.6)	(0.47)	(0.00035)	(0.00022)	(2.43)	(0.00008)	(0.00058)	(0.0021)	(0.00002)	(0.0039)	(0.000053)	(0.039)	

### Supplementary videos

<https://www.youtube.com/channel/UC0rea-ZPAFw1ixE4v2J1PiQ>



### **Chapter 3. Differences in concentrations and compositions of chlorinated and brominated POPs and novel brominated flame retardants in hard coral, soft coral, and reef fish**

#### **Foreword to Article 2: “Differences in concentrations and compositions of chlorinated and brominated POPs and novel brominated flame retardants in hard coral, soft coral, and reef fish”**

The Mascarene Basin of the Western Indian Ocean is one of the most remote localities on Earth. This basin includes Mauritius, Rodrigues, and Réunion (Thébault *et al.*, 2009) but for the purpose of this study, Agalega, and St. Brandon’s Atoll are included as well. All three study islands are surrounded by prominent coral reefs (Van der Schyff *et al.*, 2020a). Despite their remoteness, the islands are affected by long-range transport of pollutants through air, ocean currents, plastic debris, and shipwrecks (Würl *et al.*, 2006; Bouwman *et al.*, 2016; Van der Schyff *et al.*, 2020b). As stated in section 1.1 (State of the science in the western Indian Ocean), limited research has been conducted on pollutants in the coral reef ecosystems of the WIO. POPs have previously been quantified in coral from the Maputoland reefs of Sodwana, South Africa (Porter & Schleyer, 2017; Porter *et al.*, 2018). Only metals have previously been quantified in coral from islands in the Mascarene Basin (Van der Schyff *et al.*, 2020a, 2020b). In the following article, persistent organic pollutants (POPs) and novel brominated flame retardants (NBFRs) were quantified in hard coral, soft coral, and coral reef fish for the first time in the Mascarene Basin. Three species of coral reef fish—*Chaetodon zanzibarensis* (Zanzibar butterflyfish; a corallivore), *Acanthurus blochii* (Ringtail surgeonfish; a herbivore), and *Scarus rubroviolaceus* (Ember parrotfish; a herbivore scraper) were collected to determine differences in pollutant composition between species. Three genera of hard corals (*Acropora*, *Pocillopora*, and *Fungia*), and two soft coral genera (*Sarcophyton* and *Sinularia*) were collected. The morphology and ecology of hard- and soft coral differ significantly between each other (Sheppard *et al.*, 2009), and it is known that the different coral types interact differently with pollutants (Jafarabadi *et al.*, 2018; Van der Schyff *et al.*, 2020a). This article offers insight on the differences in pollutant concentration and composition between different functional groups of coral reef organisms, as well as differences between islands in the WIO.

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## Differences in concentrations and compositions of chlorinated and brominated POPs and novel brominated flame retardants in hard coral, soft coral, and reef fish

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### Abstract

Persistent organic pollutants (POPs) are anthropogenic halogenated pollutants of concern for human and environmental health. Since POPs are regulated by international treaties, alternative compounds such as novel brominated flame retardants (NBFRs) are increasingly used. There are no data on POPs or NBFRs in coral reef biota from tropical islands in the western Indian Ocean (WIO). For this assessment, three hard coral genera, two soft coral genera, and three species of coral reef fish were collected from Rodrigues, Agalega, and St. Brandon's Atoll (Republic of Mauritius) in the Mascarene Basin of the WIO. Seven compounds—PBT,  $\gamma$ -HCH, *p,p'*-DDE, HCB, BDE-47, BDE-209, and PCB-11—were quantifiable in all samples. Hard coral consistently contained the lowest concentrations of POPs. Concentrations of all POPs classes in soft coral and fish did not differ significantly, but hard corals contained higher concentrations of NBFRs than soft corals. PBDEs occurred at the highest concentrations in coral, while PCBs had the highest concentrations in fish. The presence of BDE-209 and BDE-47 suggests long-range transport to, uptake by, and breakdown of BDE-209 in coral reef ecosystems. We quantified dechlorane plus (DP), a candidate POP, in coral reef biota. Pentabromotoluene measured in all samples raise concern regarding the long-range transport of this compound. Agalega and St. Brandon's Atoll can be considered as locations to monitor changes in background concentrations of pollutants in the WIO due to their remoteness. Because the different taxa we sampled had different concentrations and patterns of POPs and NBFRs, future surveys should stratify sampling accordingly.

**Keywords:** Coral reefs; ecotoxicology; emerging contaminants; Mascarene Basin; Western Indian Ocean

## 1. Introduction

Coral reefs are highly biodiverse and dynamic marine ecosystems (Spalding *et al.*, 2001). Reef-building corals provide essential ecological services by providing habitat for more than a quarter of all marine species (Spalding *et al.*, 2001) and livelihood for millions of people from coastal communities (Cinner *et al.*, 2014). Due to their sessile nature, coral colonies interact with their environment primarily through chemical cues (Richmond, 1997). Chemoreceptors in the polyps are used to identify prey (Thorington & Hessinger, 1988), participate in spawning events (Zhang *et al.*, 2019), defend themselves from predators, and to communicate with other coral colonies (Quévrain *et al.*, 2014). Various coral reef biota, including corals, produce chemical compounds such as halogenated natural products (Gribble, 1999) and other compounds; some of which are used in modern medicine, cosmetics, and nutritional supplements (Bruckner, 2002). Coral has long been listed as a medicinal substance in Korea (Heo, 1615). Certain naturally occurring halogens produced by marine benthic organisms are modestly effective against certain human breast cancer cell lines, and polio and herpes viruses (Gribble, 2004). The fish and other macro-vertebrates of the reef also perform essential ecological services to the reef system. Herbivores, such as parrotfish (Family Scaridae) and surgeonfish (Family Acanthuridae), cause a bottom-up cascade effect on the reef. By clearing an area of algae, they provide space for coral larvae recruitment (Burkepile & Hay, 2008). Butterflyfish (Family Chaetodontidae) have been shown as an indicator of coral reef health because they were recorded leaving a chemically contaminated reef before the corals themselves showed signs of damage (Reese, 1995).

Coral reefs and their associated biota are threatened by several anthropogenically induced effects, including ocean warming, acidification, and pollution (Sheppard *et al.*, 2009; Rackley, 2017; Kachur *et al.*, 2019; Van der Schyff *et al.*, 2020c). Persistent organic pollutants (POPs) are halogenated organic compounds of anthropogenic origin. POPs are priority compounds worldwide because of their established environmental persistence, toxicity, and long-range transfer potential (Stockholm Convention, 2019). Due to their hydrophobic properties, POPs can adsorb to suspended matter and marine debris such as plastic and be transported to remote island systems (Ogata *et al.*, 2009; Bouwman *et al.*, 2016). Tropical and sub-tropical islands are often surrounded by coral reefs, and the contaminants associated with plastic can be leached into the reef system (Bouwman *et al.*, 2016). Novel brominated flame retardants (NBFRs) are a class of brominated compounds used as alternatives to polybrominated diphenyl ethers (PBDEs) after the production of the latter class was discontinued. The effects of NBFRs on marine biota are not as extensively studied as the effects of POPs, but the compounds are known to exhibit persistent, bioaccumulative, and toxic properties (Covaci *et*

*al.*, 2011). Both POPs and NBFRs are endocrine disruptive compounds (Gregoraszczyk & Ptak, 2013; Saunders *et al.*, 2013) which may impede the natural functioning of an organism's physiological activity, possibly affecting the functioning of reef systems.

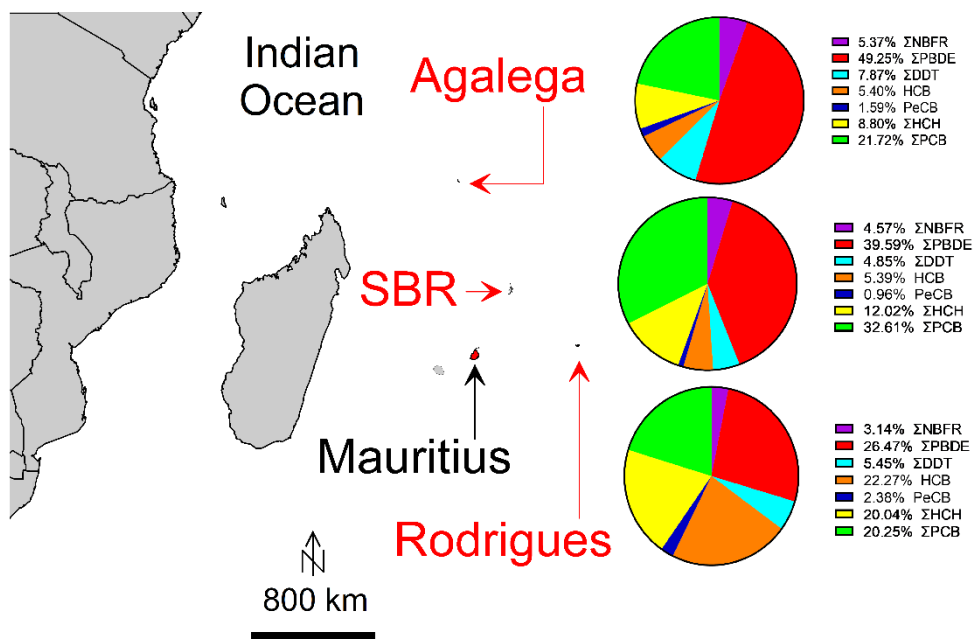
The Mascarene Islands in the western Indian Ocean (WIO) consist of Réunion, Mauritius, and the associated Mauritian Outer Islands (Garnier & Desarthe, 2013). Approximately 705 km<sup>2</sup> of coral reefs are found in the Mascarene Basin associated with the various islands (Turner & Klaus, 2005). In the Mascarene region, POPs have been quantified in birds (Bourne *et al.*, 1977; Bouwman *et al.*, 2012; Van der Schyff *et al.*, 2020b), tuna (Ueno *et al.*, 2004; Ueno *et al.*, 2005; Chouvelon *et al.*, 2017), swordfish (Munschy *et al.*, 2020), and dolphins (Dirtu *et al.*, 2016). The fact that POPs are quantified in animals in high trophic positions highlights the need to examine the POPs concentration in organisms lower in the marine food web, due to the known bioaccumulative potential of POPs (Gobas *et al.*, 2009). Only one study quantified pollutants (metals) in coral from the Mauritian Outer islands (Van der Schyff *et al.*, 2020a), but no studies have quantified pollutants in coral reef fish from there. To our knowledge, no study has quantified PBDEs or NBFRs in coral anywhere. This study aims to provide an initial assessment of POPs and NBFRs in hard and soft coral, and coral reef fish, from three Mauritian Outer Islands. Since coral colonies are sessile, and small coral reef fish from isolated coral islands are not prone to travel long distances (O'Donnell *et al.*, 2017), POPs found in these organisms would have to be transported there by other means. This study provides new information regarding the presence, concentrations, and relative compositions of brominated and chlorinated compounds in corals and fish from remote tropical islands, including information that may be relevant when considering the listing of additional POPs. A criterion when considering candidate POPs is the potential to be transported over long distances, by air, biota, or by water movement combined with proven harmful effects on biota (Stockholm Convention, 2019).

## **2. Materials and methods**

### *2.1. Site description*

The Mauritian Outer Islands (Fig. 1) are semi-autonomous under the governance of the Republic of Mauritius (Bhagooli & Kaullysing, 2018). Rodrigues is the largest of the Mauritian Outer Islands, 620km east of Mauritius, with a population of approximately 43 000 residents (Statistics Mauritius, 2019). The island hosts the best-developed coral reef system of the western Indian Ocean (Rees *et al.*, 2005) with 200 km<sup>2</sup> reef flat surrounding the island (Turner & Klaus, 2005). Agalega is the most remote Outer Island. It is located 1 100 km north of Mauritius and has a population of approximately 270 residents (Statistics Mauritius, 2019). The only economic activity is the harvesting and processing of coconuts (Van der Schyff *et*

*al.*, 2020a). A small, well-developed fringing reef that ends in a deep drop-off is present around the island. St. Brandon's Atoll (also known as St. Brandon, St. Brandon's Rock, or St. Brandon's Island) forms part of the Cargados Carajos Shoals. The atoll is located 450 km northeast of Mauritius, is approximately 200 km<sup>2</sup> in size, consisting of approximately 24 vegetated islands and 19 sandbars (Quod, 1999; Evans *et al.*, 2016; Van der Schyff *et al.*, 2020a). Personnel of a small weather station, two fishing stations, and members of the National Coast Guard make up a rotating population with no permanent human residents. Several shipwrecks (varying ages and stages of breakdown) are present on the reef crest (van der Schyff, 2020b).



**Fig. 1.** Map of Southern Africa and the western Indian Ocean, indicating the sampling localities for this study. SBR indicates St. Brandon's Atoll. The pie graphs depict the percentage contribution of the pollutant load per island.

## 2.2. Sample collection and ethical approval

Fragments of approximately 10 cm (but no larger than 1/8<sup>th</sup> of the total colony size) were collected from five colonies of each coral genus from each island. The coral colony fragments comprised of both tissue and skeleton. Because genetic studies were not conducted to identify corals to species level, the coral samples were pooled and considered per genus. Three genera of hard corals (*Acropora*, *Pocillopora*, and *Fungia*), and two soft coral genera (*Sarcophyton* and *Sinularia*) were collected from Agalega and Rodrigues (Figs. S1a-e). *Sarcophyton* colonies were not found in the lagoon of St. Brandon's Atoll—all other genera were collected from there. Fragments of hard coral were carefully removed with a pair of side

cutters and soft coral fragments with a diving knife. Whole colonies of *Fungia* were collected because they occur as single free-living polyps (Chadwick-Furman *et al.*, 2010). The collected fragments were placed in secure collection bags lined with a high-density plastic bag. Each genus was placed in a separate bag to avoid contamination. In all, 70 coral samples were collected.

Five individuals of three species of coral reef fish were collected with the help of local fishermen. Zanzibar butterflyfish (*Chaetodon zanzibarensis*), and ember parrotfish (*Scarus rubroviolaceus*) were collected from Rodrigues, Agalega and St. Brandon's Atoll (Figs. S2a-b). Ringtail surgeonfish (*Acanthurus blochii*) was collected from Rodrigues and St Brandon's Atoll but not collected from Agalega due to time and weather constraints (Fig. S2c). *Chaetodon zanzibarensis* are corallivorous, feeding primarily on the living tissue of hard- and soft corals (Pratchett *et al.*, 2004). Ember parrotfish primarily feed on epilithic algae, brown algae, and crustose coralline algae that they scrape from hard reef substrates with a specialised beak. They also eat coral polyps, although this constitutes less than 1% of their diet (Gromova & Maktotin, 2019). Ringtail surgeonfish primarily feed on algae films on sandy substrates (Fishbase, 2020). All fish species are common in the Mascarene Basin and considered as "Least Concern" by the IUCN (Meyer & Pratchett, 2010; Choat *et al.*, 2012a, Choat *et al.*, 2012b). Samples from Agalega and St. Brandon's Atoll were collected in October 2014, while samples from Rodrigues were collected in March 2015. In all, 35 fish samples were collected.

Biological samples were collected from the Mauritian Outer Islands with permission from the Outer Island Development Corporation, the National Parks and the Conservation Service of the Ministry of Agro-Industry, Food Production, and Security. Ethical approval for the study was obtained from the North-West University (NWU-01275-19-S9).

### 2.3. Extraction and clean-up

Table 1 lists all the compounds that were analysed for during this study.

The chemical analyses were performed by the accredited Research Centre for Toxic Compounds in the Environment (EN ISO/IEC 17025:2005), at the Masaryk University, Czech Republic. Prior to analyses, the samples were pooled per genus per island (21 pools), homogenised and freeze-dried at -96°C for 48 hours. Ten grams of pooled freeze-dried coral, and 0.9 g – 5 g pooled freeze-dried fish tissue was used for extraction. The target compounds were extracted using the ultrasonic extraction method (Gómez *et al.*, 2020). The extraction process for coral and fish samples differed slightly. The target compounds from corals were extracted in 50 mL analytical dichloromethane for three 15-minute cycles. The fish tissues were extracted in a 20 mL 3:1 *n*-hexane: dichloromethane mixture for three 15-minute cycles.

Clean-up was achieved on a sulphuric acid (44% w/w) modified silica (8 g) column with 2 g pre-baked sodium sulphate. Elution was obtained with a 40 mL 1:1 *n*-hexane:

dichloromethane mixture. The extracts were evaporated using a nitrogen Turbovap evaporator (Biotage, Sweden). 50  $\mu$ L of nonane solvent was added to the evaporated sample. Extracts were spiked with  $^{13}\text{C}$ -PCB95 (Absolute Standards, USA),  $^{13}\text{C}$ -BDE77, and  $^{13}\text{C}$ -BDE138 (Wellington, Canada). All extraction and elution solvents were of analytical grade.

**Table 1.** The compound group, name, abbreviations, and individual congeners of contaminants analysed for this study

Compound class	Compound name	Abbreviation	Congeners/isomers
Organochlorine pesticides (OCP)	Hexachlorobenzene	HCB	
	Pentachlorobenzene	PeCB	
	Hexachlorocyclohexane	HCH	$\alpha$ -, $\beta$ -, $\gamma$ -, $\delta$ -, $\epsilon$ -HCH
	Dichlorodiphenyltrichloroethane	DDT	$p,p'$ -DDT $p,p'$ -DDD $p,p'$ -DDE $o,p'$ -DDT $o,p'$ -DDD $o,p'$ -DDE
Industrial chemicals	Polychlorinated biphenyls	PCB	PCB -9, -11, -28, -52, -101, -118, -138, -180
Brominated flame retardants	Polybrominated diphenyl ethers	PBDE	BDE-28, -47, -66, -85, -99, -100, -153, -154, -183, -209
		NBFR	Pentabromobenzene (PBBZ) 1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE) Hexabromobenzene (HBB) Pentabromomethylbenzene (PBEB) 2,3,5,6-Tetrabromo- <i>p</i> -xylene (pTBX) Pentabromotoluene (PBT) $\alpha$ -1,2,5,6-Tetrabromocyclooctane (alphaTBCO) $\alpha$ -Tetrabromocyclohexane (alphaTBECH) Hexachlorocyclopentenyl-dibromocyclooctane (HCDBCO) 2,3-Dibromopropyl 2,4,6-tribromophenyl ether (DPTE) 2,3,6-Tribromophenylether (BATE) anti-Dechlorane Plus (antiDP) syn-Dechlorane Plus (synDP) Tris(2,3-dibromopropyl)isocyanurate (T23BPIC) Tetrabromo- <i>o</i> -chlorotoluene (TBCT) $\beta$ -1,2,5,6-Tetrabromocyclooctane (betaTBCO) $\beta$ -Tetrabromocyclohexane (betaTBECH) Decabromodiphenylethane (DBDPE)

#### 2.4. OCPs and PCBs instrumental analyses

The separation and detection of OCPs and PCBs were performed on a gas chromatography triple quadrupole-tandem mass spectrometer system (GC-MS/MS)(Agilent QQQ 7000B; Agilent Technologies, USA) combined with an Agilent 7890 gas-chromatograph equipped with a 60 m x 0.25 mm x 0.25  $\mu$ m Rxi-5Sil-MS column (Restek, France). The MS was operated in positive electron ionisation (EI+) mode using multiple reaction monitoring (MRM). The injection was splitless 3  $\mu$ L at 280°C with helium as the carrier gas at 1.5 mL/min.

The GC's temperature was programmed first at 80°C (1.5 min hold), then at 40°C/min to 200°C/min (18 min hold), and finally ramped at 5°C/min to 305°C/min (2 min hold). The temperature of the GC-MS transfer line was 310°C and the ion source was 250°C. Quantification was done by using MassHunter Quantitative Analysis (version B.06.00) software.

### 2.5. PBDEs and NBFRs instrumental analyses

The separation and detection of PBDEs and NBFRs were performed using gas chromatography- high-resolution mass spectrometry (GC/HRMS)— A 7890A GC (Agilent Technologies, USA) equipped with a 15 m x 0.25 mm x 0.1 µm Rtx-1614 capillary column (Restek, USA) coupled to an AutoSpec Premier MS (Waters, Micromass, USA). The MS was operated in positive ion mode with selective ion monitoring (EI+SIM) at a resolution of >10 000. The resolution was set at >5000 when BDE-209 was quantified. The injection was 2 µL splitless at 280°C for PBDEs and 250°C for NBFRs. Helium was the carrier gas at a constant flow of 1 mL/min for PBDEs and 1.2 mL/min for NBFRs; after 15 min, the flow was increased to 1.4 mL/min. The temperature and hold settings differed for the PBDEs and NBFRs analyses. The GC temperature programme for the analysis of PBDEs was 80°C (1 min hold), increased at 20°C/min to 250°C, increased at 1.5°C/min to 260°C (2 min hold), and then at 25°C/min to 320°C (4.5 min hold). The GC temperature programme for the analysis of NBFRs started at 80°C (1 min hold), ramped at 30°C/min to 140°C, increased by 4°C/min to 175°C, then at 8°C/min to 270°C, and finally 15°C/min to 325°C with a 5 min hold.

### 2.6. Quality Control

Before extraction, the samples were spiked with surrogate and internal standards. For PCBs and OCPs, 50 µl of 20 ng/mL of the following standards were injected: <sup>13</sup>C<sub>6</sub>: PeCB, HCB, β-HCH, γ-HCH; <sup>13</sup>C<sub>12</sub>: *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, PCB-19, PCB-104, and PCB-178 (Absolute Standards, USA). 50 µl of 20 ng/mL of the following standards were used for PBDEs: <sup>13</sup>C labelled BDE-28, -47, -99, -100, -153, -154, and -183; and 50 µl of 100 ng/mL <sup>13</sup>C-209 as a standard for BDE-209 (Wellington, Canada). The internal standards used for NBFRs were: 50 µl of 20 ng/mL <sup>13</sup>C-BTBPE, <sup>13</sup>C-HBB, <sup>13</sup>C-aDP, <sup>13</sup>C-sDP, <sup>13</sup>C-PBBZ, and 30 ng/mL <sup>13</sup>C-DBDPE (Wellington, Canada).

Most of the quantification obtained by the chemical analyses was corrected for recovery using the isotope dilution analytical method (Chropeňová *et al.*, 2016). Some NBFRs—DPTE, α-, and β-TBECH—were quantified using the syringe standard (<sup>13</sup>C-BDE77) and not recovery corrected. Limits of quantification (LOQ) are sample and compound-specific. LOQs of BDEs and NBFRs were calculated as concentrations corresponding to 9:1 signal-to-noise-ratio in



the chromatograms of individual compounds in the respective samples. The signal-to-noise-ratio to determine the LOQs of OCPs and PCBs was set at 10:1 of the individual compounds in the calibration standards with the lowest concentrations of PCBs and OCPs (1 ng/mL). Since sample size were small, no lipid content determinations could be done, so that concentrations are reported on a wet mass (wm) basis.

### 2.7. Statistical analyses

The compounds that were quantified in all sample pools were compared between hard coral, soft coral, and fish tissue using Graphpad Prism 8.0.2 ([www.graphpad.com](http://www.graphpad.com)). All hard coral collected were pooled into the functional group "hard coral". Similarly, all soft coral collected were pooled as "soft coral", and all fish tissue were pooled as "fish". The concentrations of POPs and NBFRs in biota from the Mauritian Outer Islands were not normally distributed and, therefore, the Kruskal-Wallis test was used to compare medians. To distinguish between which functional group statistically significant differences occurred, Dunn's post-hoc test was applied. Significance was set at  $p < 0.05$ . A "part-of-a-whole" analysis was also conducted on GraphPad Prism 8.0.2. The proportions of chemical compounds are expressed as percentages.

Non-metric multidimensional scaling (NMS) was conducted to ordinate POPs proportions in coral and fish from St. Brandon's Atoll, Rodrigues, and Agalega islands using MjM Software PC-ORD 7.03 ([www.pcord.com](http://www.pcord.com)). Compounds that were only quantified in one sample pool were excluded from the ordination. The data was relativised per sample pool to obtain a 'chemical fingerprint' of the POPs profile. Two hundred and fifty runs of real and randomised data with random starting conditions were used. The Gower-ignore-zero coefficient was used as a relative measurement of distance. Convex hulls were used to denote the 'chemical fingerprint' of each functional group. The less these hulls overlap, or the farther they are apart, indicates less commonality in relative chemical composition.

## 3. Results

### 3.1. Analytical results

The wet mass concentrations of POPs and NBFRs in corals are presented in Table 2, and fish data in Table 3. Butterflyfish tissue from Rodrigues could not be analysed due to small tissue size. The concentrations of the following compounds did not exceed the LOQ in any sample: PCB-9, -118, -101, -138, and -180; PBDE-85, -153, -154, and -183; *o,p'*-DDT, *o,p'*-DDE, and  $\epsilon$ -HCH; pTBX,  $\alpha$ -TBCO,  $\beta$ -TBCO, HCDBCO, BATE, T23BPIC, TBCT, and DBDPE.

**Table 2.** Concentrations (ng/g wm) of POPs in pooled coral samples (n=5) from different coral genera from three islands. The sum of compound classes are indicated in bold. SBR indicates St. Brandon's Atoll.

Genus	Island	PBBZ	BTBPE	HBB	PBEB	PBT	$\alpha$ TBECH	$\beta$ TBECH	antiDP	synDP	$\Sigma$ NBFR	$\alpha$ HCH	$\beta$ HCH	$\gamma$ HCH	$\delta$ HCH	$\Sigma$ HCH
<i>Acropora</i>	Agalega	<LOQ	<LOQ	<LOQ	0.00031	0.046	0.0014	0.001	0.002	<LOQ	<b>0.051</b>	<LOQ	<LOQ	0.013	<LOQ	<b>0.013</b>
<i>Acropora</i>	Rodrigues	<LOQ	<LOQ	<LOQ	<LOQ	0.0092	0.00067	0.0006	0.0017	<LOQ	<b>0.012</b>	<LOQ	<LOQ	0.0094	<LOQ	<b>0.0094</b>
<i>Acropora</i>	SBR	<LOQ	<LOQ	<LOQ	<LOQ	0.026	0.0012	0.0009	<LOQ	0.0017	<b>0.03</b>	<LOQ	<LOQ	0.035	<LOQ	<b>0.035</b>
<i>Pocillopora</i>	Agalega	<LOQ	<LOQ	<LOQ	<LOQ	0.02	0.0013	0.001	0.0017	0.0015	<b>0.026</b>	<LOQ	<LOQ	0.0066	<LOQ	<b>0.0066</b>
<i>Pocillopora</i>	Rodrigues	<LOQ	<LOQ	<LOQ	<LOQ	0.014	0.0011	0.00087	0.0023	0.0019	<b>0.02</b>	<LOQ	<LOQ	0.0039	<LOQ	<b>0.0039</b>
<i>Pocillopora</i>	SBR	<LOQ	<LOQ	<LOQ	<LOQ	0.027	0.00087	0.00073	0.0014	<LOQ	<b>0.03</b>	<LOQ	<LOQ	0.011	<LOQ	<b>0.011</b>
<i>Fungia</i>	Agalega	<LOQ	<LOQ	<LOQ	<LOQ	0.0071	<LOQ	0.00059	<LOQ	0.00099	<b>0.0087</b>	<LOQ	<LOQ	0.0048	<LOQ	<b>0.0048</b>
<i>Fungia</i>	SBR	<LOQ	<LOQ	<LOQ	<LOQ	0.0067	0.0015	0.00094	0.0015	0.0012	<b>0.012</b>	<LOQ	<LOQ	0.017	<LOQ	<b>0.017</b>
<i>Fungia</i>	Rodrigues	<LOQ	<LOQ	<LOQ	<LOQ	0.0088	0.00092	0.0007	0.0011	0.0011	<b>0.013</b>	<LOQ	<LOQ	0.0063	<LOQ	<b>0.0063</b>
<i>Sarcophyton</i>	Agalega	0.0036	<LOQ	<LOQ	<LOQ	0.0098	<LOQ	<LOQ	0.0028	<LOQ	<b>0.016</b>	<LOQ	0.12	0.052	<LOQ	<b>0.172</b>
<i>Sarcophyton</i>	Rodrigues	<LOQ	<LOQ	<LOQ	<LOQ	0.015	<LOQ	<LOQ	<LOQ	<LOQ	<b>0.015</b>	<LOQ	0.26	0.073	<LOQ	<b>0.333</b>
<i>Sinularia</i>	Agalega	<LOQ	0.0053	<LOQ	<LOQ	0.011	<LOQ	<LOQ	0.0029	0.0018	<b>0.021</b>	<LOQ	0.038	0.034	<LOQ	<b>0.072</b>
<i>Sinularia</i>	Rodrigues	0.0027	<LOQ	0.0041	<LOQ	0.0069	<LOQ	<LOQ	0.0016	<LOQ	<b>0.015</b>	0.0042	<LOQ	0.053	<LOQ	<b>0.0572</b>
<i>Sinularia</i>	SBR	<LOQ	<LOQ	<LOQ	<LOQ	0.0096	<LOQ	0.0014	0.0022	<LOQ	<b>0.013</b>	<LOQ	<LOQ	0.18	0.1	<b>0.28</b>

Genus	Island	<i>p,p'</i> -DDD	<i>p,p'</i> -DDE	<i>p,p'</i> -DDT	$\Sigma$ DDT	PeCB	HCB	PBDE28	PBDE47	PBDE66	PBDE99	PBDE209	$\Sigma$ BDE	PCB 28	PCB 52	PCB 153	PCB 11	$\Sigma$ PCB
<i>Acropora</i>	Agalega	<LOQ	0.027	0.0058	<b>0.033</b>	0.004	0.0072	0.00024	0.0014	<LOQ	0.00084	0.036	<b>0.039</b>	0.0035	<LOQ	0.0066	0.048	<b>0.058</b>
<i>Acropora</i>	Rodrigues	<LOQ	0.011	<LOQ	<b>0.011</b>	0.0044	0.0053	0.00014	0.0017	<LOQ	0.00015	0.19	<b>0.19</b>	0.0035	<LOQ	<LOQ	0.033	<b>0.037</b>
<i>Acropora</i>	SBR	<LOQ	0.013	<LOQ	<b>0.013</b>	0.004	0.0072	0.0004	0.0026	<LOQ	0.00031	0.18	<b>0.18</b>	0.0043	0.003	<LOQ	0.076	<b>0.083</b>
<i>Pocillopora</i>	Agalega	<LOQ	0.009	<LOQ	<b>0.009</b>	0.0039	0.005	<LOQ	0.0006	<LOQ	0.00034	0.15	<b>0.15</b>	0.0028	0.0027	<LOQ	0.042	<b>0.048</b>
<i>Pocillopora</i>	Rodrigues	<LOQ	0.0077	<LOQ	<b>0.0077</b>	0.0061	0.0051	<LOQ	0.0005	<LOQ	<LOQ	0.038	<b>0.039</b>	<LOQ	<LOQ	<LOQ	0.032	<b>0.032</b>
<i>Pocillopora</i>	SBR	<LOQ	0.019	<LOQ	<b>0.019</b>	0.0045	0.0046	<LOQ	0.0015	<LOQ	0.00035	0.53	<b>0.53</b>	0.0028	<LOQ	<LOQ	0.034	<b>0.037</b>
<i>Fungia</i>	Agalega	<LOQ	0.0039	<LOQ	<b>0.0039</b>	0.0039	0.0045	<LOQ	0.00031	<LOQ	<LOQ	0.016	<b>0.016</b>	0.0023	<LOQ	<LOQ	0.046	<b>0.048</b>
<i>Fungia</i>	SBR	<LOQ	0.008	0.011	<b>0.019</b>	0.0042	0.0061	0.00049	0.004	<LOQ	<LOQ	0.065	<b>0.069</b>	0.0026	<LOQ	<LOQ	0.071	<b>0.074</b>
<i>Fungia</i>	Rodrigues	<LOQ	0.0046	<LOQ	<b>0.0046</b>	0.0047	0.004	<LOQ	0.00021	<LOQ	<LOQ	0.043	<b>0.043</b>	<LOQ	<LOQ	<LOQ	0.032	<b>0.032</b>
<i>Sarcophyton</i>	Agalega	0.024	0.014	<LOQ	<b>0.038</b>	0.012	0.073	0.0031	0.013	<LOQ	<LOQ	0.089	<b>0.11</b>	<LOQ	0.0068	<LOQ	0.12	<b>0.13</b>
<i>Sarcophyton</i>	Rodrigues	0.036	0.049	<LOQ	<b>0.085</b>	0.03	0.75	0.044	0.22	0.011	<LOQ	0.062	<b>0.34</b>	<LOQ	<LOQ	<LOQ	0.18	<b>0.18</b>
<i>Sinularia</i>	Agalega	0.024	0.02	<LOQ	<b>0.044</b>	<LOQ	0.035	0.0029	0.017	<LOQ	0.0015	1.6	<b>1.6</b>	<LOQ	0.0058	<LOQ	0.14	<b>0.15</b>
<i>Sinularia</i>	Rodrigues	0.012	0.016	<LOQ	<b>0.028</b>	0.0082	0.026	0.0086	0.052	0.0043	<LOQ	0.048	<b>0.11</b>	<LOQ	0.0037	<LOQ	0.11	<b>0.11</b>
<i>Sinularia</i>	SBR	0.015	0.012	<LOQ	<b>0.027</b>	0.0074	0.05	0.0051	0.026	<LOQ	0.0011	0.13	<b>0.16</b>	0.01	0.0063	<LOQ	0.13	<b>0.15</b>

**Table 3.** Concentrations (ng/g wm) of POPs in pooled coral reef fish muscle samples (n=5) of three fish species from three islands. The sum of compound classes are indicated in bold.

Genus	Island	BTBPE	PBT	$\alpha$ TBECH	$\beta$ TBECH	antiDP	synDP	DPTE	$\Sigma$ NBFR	$\beta$ HCH	$\gamma$ HCH	$\Sigma$ HCH	<i>p,p'</i> -DDD	<i>p,p'</i> -DDE	$\Sigma$ DDT
<i>Acanthurus</i>	SBR	0.0055	0.021	0.0015	0.001	0.0054	0.0034	<LOQ	<b>0.038</b>	<LOQ	0.074	<b>0.074</b>	<LOQ	0.031	<b>0.031</b>
<i>Acanthurus</i>	Rodrigues	<LOQ	0.0085	<LOQ	<LOQ	0.0041	0.0031	<LOQ	<b>0.016</b>	<LOQ	0.32	<b>0.32</b>	0.015	0.037	<b>0.052</b>
<i>Chaetodon</i>	SBR	<LOQ	0.052	<LOQ	0.0026	0.017	0.012	<LOQ	<b>0.084</b>	<LOQ	0.12	<b>0.12</b>	<LOQ	0.11	<b>0.11</b>
<i>Chaetodon</i>	Agalega	0.007	0.087	0.0036	0.0031	0.0042	0.0036	<LOQ	<b>0.11</b>	0.018	0.05	<b>0.068</b>	<LOQ	0.21	<b>0.21</b>
<i>Scarus</i>	SBR	0.0018	0.0069	0.0008	<LOQ	0.0016	0.0011	<LOQ	<b>0.012</b>	<LOQ	0.038	<b>0.038</b>	<LOQ	0.013	<b>0.013</b>
<i>Scarus</i>	Rodrigues	0.0034	0.012	0.0025	0.0017	0.0031	0.0021	0.0078	<b>0.033</b>	<LOQ	0.058	<b>0.058</b>	<LOQ	0.026	<b>0.026</b>
<i>Scarus</i>	Agalega	<LOQ	0.0084	0.002	0.0015	0.002	0.001	<LOQ	<b>0.015</b>	<LOQ	0.066	<b>0.066</b>	<LOQ	0.022	<b>0.022</b>

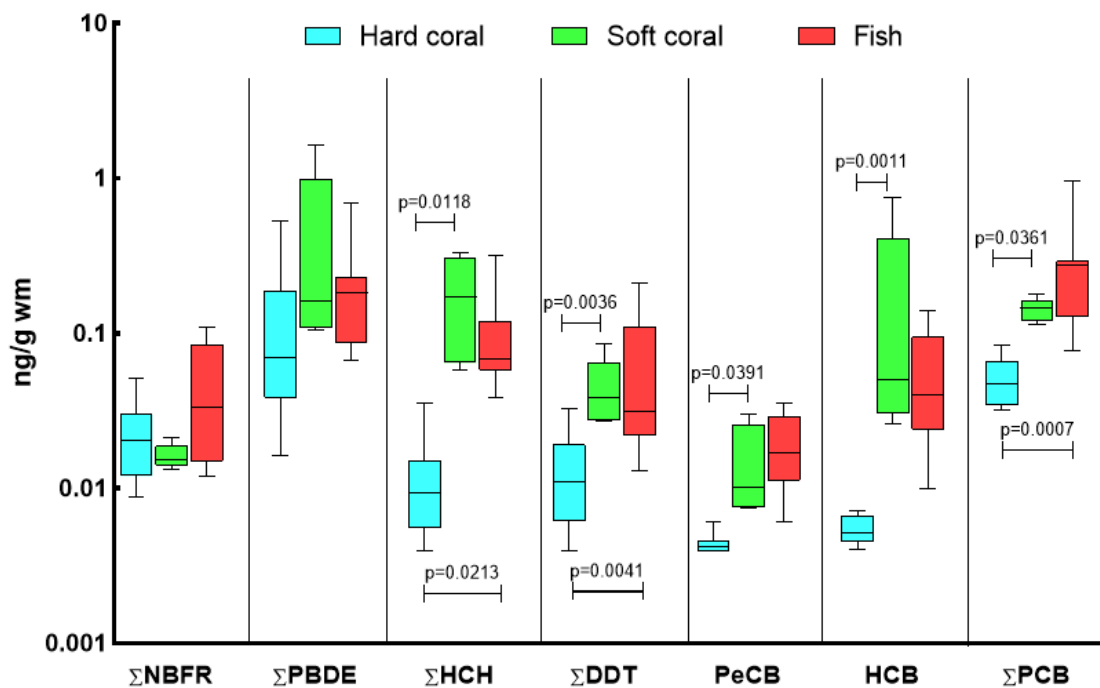
Genus	Island	PeCB	HCb	PBDE28	PBDE47	PBDE209	$\Sigma$ BDE	PCB 28	PCB 52	PCB 153	PCB 11	$\Sigma$ PCB
<i>Acanthurus</i>	SBR	0.02	0.04	<LOQ	0.012	0.17	<b>0.18</b>	0.015	0.017	<LOQ	0.14	<b>0.17</b>
<i>Acanthurus</i>	Rodrigues	0.027	0.061	<LOQ	0.0043	0.18	<b>0.18</b>	0.014	0.0086	<LOQ	0.25	<b>0.27</b>
<i>Chaetodon</i>	SBR	<LOQ	0.14	<LOQ	0.018	0.68	<b>0.7</b>	0.071	0.079	<LOQ	0.82	<b>0.97</b>
<i>Chaetodon</i>	Agalega	0.035	0.095	0.0012	0.031	0.2	<b>0.23</b>	0.021	0.017	0.053	0.2	<b>0.291</b>
<i>Scarus</i>	SBR	0.006	0.01	<LOQ	0.00034	0.066	<b>0.066</b>	0.0051	0.0056	<LOQ	0.067	<b>0.077</b>
<i>Scarus</i>	Rodrigues	0.013	0.024	0.00049	0.0019	0.13	<b>0.13</b>	0.011	0.008	<LOQ	0.11	<b>0.13</b>
<i>Scarus</i>	Agalega	0.014	0.027	<LOQ	0.0013	0.086	<b>0.087</b>	0.0083	0.0073	<LOQ	0.26	<b>0.28</b>

Twenty-two compounds were present at quantifiable concentrations in at least one sample pool. Seven compounds—PBT,  $\gamma$ -HCH, *p,p'*-DDE, HCB, BDE-47, BDE-209, and PCB-11—were quantifiable in all sample pools. The highest concentration of a compound found in this study was from a soft coral—a sample pool of *Sinularia* from Agalega—containing 1.59 ng/g wm of PBDE-209. PBDEs were the compound class with the highest proportion in all biota from the Mauritian Outer Islands (Fig. 1) and the highest concentrations in most corals (Table 2). PCBs were the compound class with the highest concentrations in fish tissue (Table 2; Fig. 2).

### 3.2. Differences between taxa

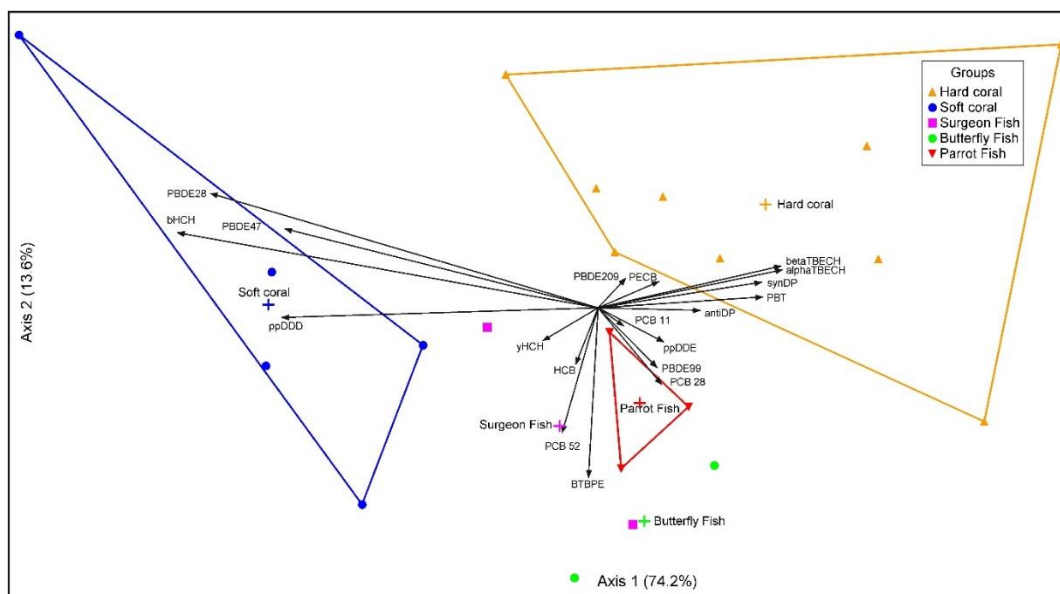
Because the concentrations of contaminants in coral reef biota from the Mauritian Outer Islands were very low, the sum of the contaminant classes; NBFRs, BDEs, DDTs, HCHs, and PCBs, as well as single compounds HCB and PeCB, are reported. The organisms were further pooled according to their functional groups: hard coral, soft coral, and fish. The concentrations of the sum of compound classes found in all pooled samples are presented in Fig. 2.

There were no significant differences between the coral reef functional groups for either of the brominated compound classes ( $\Sigma$ BDE and  $\Sigma$ NBFRs). Hard coral contained significantly ( $p < 0.05$ ) lower concentrations compared with soft coral and fish tissue for  $\Sigma$ DDT,  $\Sigma$ HCH, PeCB, and  $\Sigma$ PCB. HCB was also significantly lower than hard coral than in soft coral.



**Fig. 2.** Box-and-whisker plot (horizontal lines are medians, 95% confidence intervals, minima, and maxima) of the concentrations of the sums of compound class concentrations in all sample pools of hard coral, soft coral, and fish. Kruskal-Wallis test with Dunn's multiple comparisons was conducted. The p-values indicate statistically significant differences between functional groups. Y-axis is on a log scale.

The non-metric multidimensionally-scaled (NMS) ordination shows a clear distinction between the different taxa (Fig. 3). The pattern of ordinations has a final stress value of 12.44 that is considered to have little risk of misinterpretation. Ordinations for most ecological studies have a final stress value of between 10 and 20 (McCune & Grace, 2002). A final instability of <0.0001 was obtained after 33 iterations. Real and randomised data were used for 250 runs, with random starting conditions. A Monte Carlo test of significance of each dimensionality tested the probability of stress having been derived by chance.

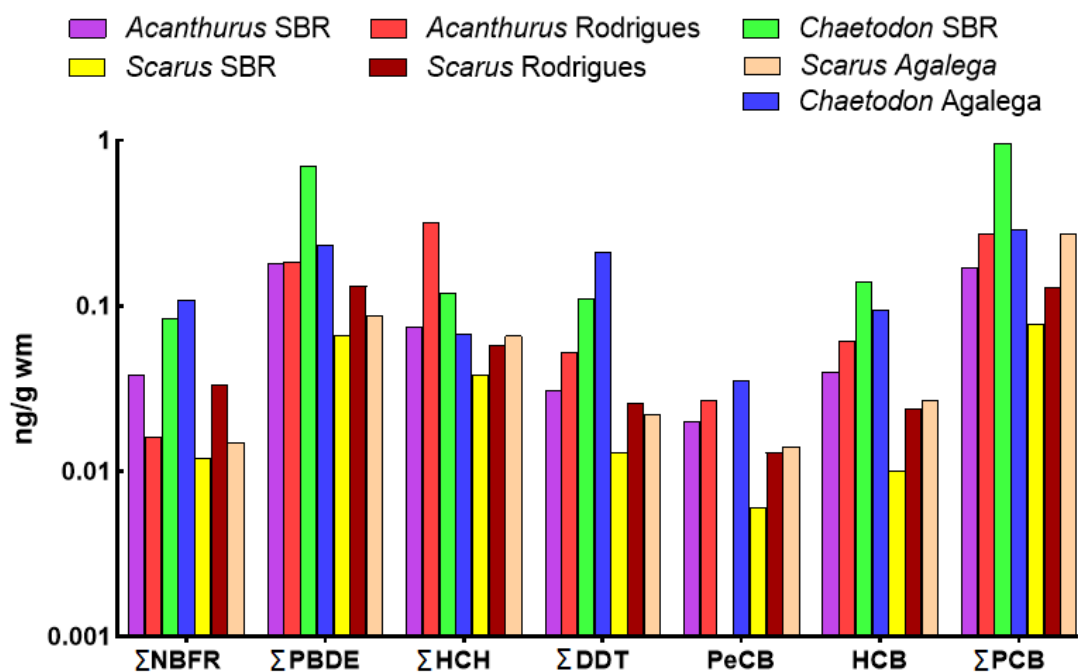


**Fig. 3.** Non-metric multidimensionally-scaled (NMS) ordination of the relative contributions of POPs and NBRs in coral and coral reef fish from the Mauritian Outer Islands. Axis 1 explains 74.2% of the ordination, and Axis 2, 13.6%. The ordination had a final stress value of 12.44, and a final instability of <0.0001. The coloured crosses (+) indicate group centroids of the convex hulls of hard coral, soft coral, and three coral reef fish species.

The convex hulls representing hard coral, soft coral, and fish did not overlap, indicating dissimilarities in contaminant composition between the different taxa. All NBRs, except

BTBPE, ordinated towards the convex hull representing hard coral. All PCBs ordinated towards the convex hulls representing fish.

Butterflyfish (*Chaetodon*) contained the highest concentrations of most pollutants, except for  $\Sigma$ HCH (Fig. 4). Of the twenty-two quantified compounds, butterflyfish from St. Brandon's Atoll contained the highest concentrations of seven compounds, and butterflyfish from Agalega contained the highest concentration of five.

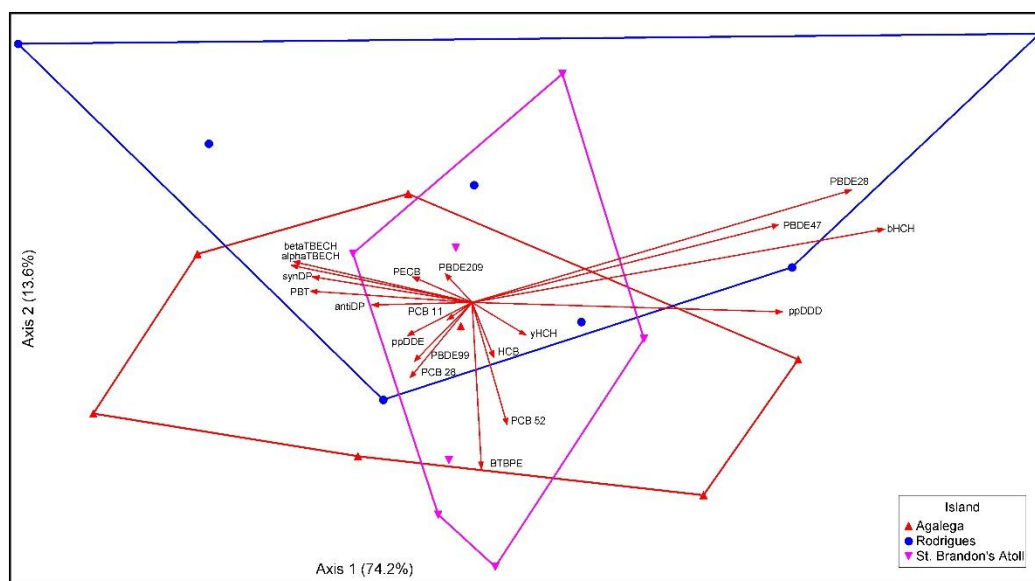


**Fig. 4.** Bar graph of the total concentrations of compound groups in different fish species from three islands. Y-axis is on a log scale.

### 3.3. Differences between islands

Fig. 1 indicates the proportion each compound class contributed per island for all three functional groups combined. In all island systems,  $\Sigma$ BDEs contributed the greatest proportion. The second-largest contaminant class was  $\Sigma$ PCBs for Agalega and St. Brandon's Atoll, but HCB was the compound that contributed the second-highest proportion in Rodrigues.

There were no statistically significant differences for any compound class between the three islands. The NMS ordination (Fig. 5) shows significant overlaps of the convex hulls representing the Mauritian Outer Islands, indicating similarities in the chemical composition of POPs and NBFRs in the combined biota from the islands. The convex hull representing Rodrigues is larger than the hulls representing the other islands, indicating larger variation within the chemical composition of biota samples from Rodrigues.



**Fig. 5.** Non-metric multidimensionally-scaled (NMS) ordination of the relative contributions of POPs and NBRs in biota from the Mauritian Outer Islands. The coloured crosses (+) indicate group centroids of the three islands. Axis 1 explains 74.2% of the ordination, and Axis 2, 13.6%. The ordination had a final stress value of 12.44, and a final instability of <0.0001.

To gain a better understanding of the difference in pollutant composition in coral and reef fish populations between islands, a taxon comparison factor (TCF) was calculated to compare the concentration of contaminants in biota relative to the different islands. The TCF we defined as the ratio of compounds in two sympatrically occurring taxa, whether trophically related or not. A TCF value close to one indicates near-similarity in the concentrations between two different taxa. A TCF of two denotes double the concentration in one taxon compared with the other, and a TCF of 10 would indicate an order of magnitude difference. The TCF is a helpful tool to detect proportional differences in the concentrations of contaminants in biota between islands, but it does not indicate statistically significant differences. Table 4 lists the TCFs for the different functional groups for the contaminants that were quantified in all sample pools, per island. To ensure consistency, only biota that were collected from all islands were used to calculate TCFs. *Sarcophyton* soft coral, butterflyfish, and surgeonfish are excluded from this analysis.

**Table 4.** The taxon comparison factors (TCF) of the compound classes for the different functional groups between three islands. A dash (–) indicates no data. SBR refers to St. Brandon’s Atoll.

		Agalega/SBR	Rodrigues/SBR	Rodrigues/Agalega
ΣNBRF	Hard coral	1.2	0.6	0.5
	Soft coral ( <i>Sinularia</i> )	1.6	1.2	0.7
	Fish ( <i>Scarus</i> )	1.2	2.7	2.2
ΣHCH	Hard coral	1.2	0.3	0.8
	Soft coral ( <i>Sinularia</i> )	0.3	0.2	0.8
	Fish ( <i>Scarus</i> )	1.8	1.5	0.9
ΣDDT	Hard coral	0.9	0.5	0.5
	Soft coral ( <i>Sinularia</i> )	1.6	1	0.6
	Fish ( <i>Scarus</i> )	1.7	2	1.2
PeCB	Hard coral	0.7	1.2	1.3
	Soft coral ( <i>Sinularia</i> )	-	1.1	–
	Fish ( <i>Scarus</i> )	2.3	2.1	0.9
HCB	Hard coral	0.9	0.8	0.9
	Soft coral ( <i>Sinularia</i> )	0.7	0.5	0.7
	Fish ( <i>Scarus</i> )	2.7	2.4	0.9
ΣBDE	Hard coral	0.3	0.3	1.3
	Soft coral ( <i>Sinularia</i> )	10	0.7	0.07
	Fish ( <i>Scarus</i> )	1.4	2	1.5
ΣPCB	Hard coral	1.6	0.6	0.7
	Soft coral ( <i>Sinularia</i> )	1	0.8	0.8
	Fish ( <i>Scarus</i> )	3.5	1.7	0.5

The TCFs of the concentrations of contaminants in corals typically did not vary much between the different islands. The exception was ΣBDE, where the concentrations in *Sinularia* from Agalega was an order of magnitude higher than either Rodrigues or SBR, indicated by a TCF value of 0.07 and 10. PeCB was not present above quantifiable concentrations in *Sinularia* from Agalega. The concentrations of contaminants in fish from St. Brandon’s Atoll was typically lower than the other islands. A TCF value two or higher was seen when fish from Rodrigues and St. Brandon’s Atoll were compared with each other for all compounds, except ΣHCH and ΣPCB. The TCF value of fish from Agalega compared with St. Brandon’s Atoll was higher than two for PeCB, HCB, and ΣPCB.

## 4. Discussion

### 4.1. Comparisons of pollutant composition per taxon

Hard coral consistently contained lower concentrations of all POPs than soft coral (Fig. 2). The inverse was observed for NBRFs where hard coral consistently contained a higher concentration than soft coral, but still lower concentrations than in fish. For this reason, a



distinction will be made when discussing POPs and NBFRs. This important difference may indicate different interactions between POPs and NBFRs with coral type.

Soft corals have shown higher accumulation of several contaminants such as PCBs, metals, and pesticides than hard coral in other studies (Jafarabadi *et al.*, 2018; Porter *et al.*, 2018; Van der Schyff *et al.*, 2020a). The concentration of all pollutants in fish were comparable with soft coral (Fig. 2), with no statistically significant differences between the functional groups.

Hard corals have proportionally less organic tissue than soft coral (Sheppard *et al.*, 2009). The major component of a hard coral colony is the aragonite skeleton covered by a thin tissue layer (Sheppard *et al.*, 2009). Zooxanthellae—symbiotic dinoflagellate algae—occur within the tissue layer of hard corals and supply the coral with oxygen, carbon, and photosynthates (Baker *et al.*, 2008, Imbs *et al.*, 2010). Soft corals have a more substantial organic layer with proportionally fewer zooxanthellae covering a calcite skeleton. Soft corals are more reliant on suspension-feeding than their stony counterparts (Sheppard *et al.*, 2009). The fact that soft corals are more reliant on heterotrophic feeding might make these corals more susceptible to biomagnification of pollutants through prey items than hard coral that relies more on photosynthesis. Planktonic organisms are known to accumulate POPs (Hording *et al.*, 1997), enabling bioconcentration in corals. Hard corals have also been reported to ingest microplastic particles when feeding, as because plastic particles release phagostimulants that cause the corals to identify incorrectly the particles as food items (Hall *et al.*, 2015; Allen *et al.*, 2017; Hankins *et al.*, 2018). POPs have the potential to either be adsorbed to or absorbed by plastic particles due to the hydrophobic properties of POPs (Mato *et al.*, 2001). The extent of plastic uptake in soft corals are not as well understood. The spawning event of both hard and soft corals may affect the POPs concentrations in the colony. Most lipids in the coral tissue are located in their reproductive tissues (Ward *et al.*, 1995). It is unknown whether pollutants are transferred from the coral colony to gametes or planulae as is the case with other invertebrate organisms (Bakker *et al.*, 2013; Saxton *et al.*, 2013; Fajana *et al.*, 2020). All coral colonies possess a mucus layer consisting of exudates containing polysaccharides, polymeric glycoproteins, and lipids that overlays the tissue of the colony (Klaus *et al.*, 2007; Bythell & Wild, 2011). The mucus layer is more pronounced in soft coral than in hard coral. The mucus of certain soft coral, including *Sarcophyton*, contain higher concentrations of lipids than hard coral mucus (Meikle *et al.*, 1988). It is yet unknown whether an extensive mucus layer may buffer a coral colony from POPs or increase the uptake by the polyps of the contaminants, but this data suggests that the mucus layer may facilitate greater accumulation of POPs in soft coral.

Hard coral contained higher concentrations of NBFRs than soft coral (Figs. 2 and 3). Corals use chemoreceptors to catch prey items from the water columns. It is also known that hard coral will also actively take up microplastic particles (Allen *et al.*, 2017). NBFRs may induce a similar response in hard corals. Certain anthropogenic halogenated compounds such as NBFRs are structurally similar to certain natural brominated compounds (Covaci *et al.*, 2011; Wu *et al.*, 2019). There are more than 1600 naturally occurring brominated compounds. Most of these compounds occur in the marine environment, *inter alia* in marine plants, algae, sponges, bryozoans, and coral (Gribble, 1999). It stands to reason that corals may misidentify NBFRs as naturally occurring brominated compounds and either actively ingest the compounds or be reluctant to expel the ingested compounds. To our knowledge, no study has thus far compared the chemical properties and behaviour of NBFRs and naturally occurring brominated compounds. This is a gap in the scientific literature. Fish may also ingest NBFRs, but the metabolism and excretion thereof need further investigation.

Fish may be exposed to POPs through biomagnification via food items, as well as bioconcentration of pollutants from the water when present through their gills and dermal cells (Jafarabadi *et al.*, 2019). High lipophilicity and low solubility of PCBs favour high bioconcentration, bioaccumulation, and low excretion from fish (Miao *et al.*, 2000). The lipid content of fish bodies is higher than the lipid content of coral colonies, and pollutants may be retained more extensively, providing opportunity and lipid mass to accumulate pollutants (Miao *et al.*, 2000). The lipid concentration, and by association, the POPs concentration, may vary between fish depending on the age, sex, and reproductive status of the individual (Sprague *et al.*, 2012).

Corallivorous butterflyfish (*Chaetodon*) from St. Brandon's Atoll and Agalega contained the highest concentrations of most individual compounds (Table 3) and compound groups (Fig. 4). Direct biomagnification from pollutants in coral tissue is expected to impact accumulation in butterflyfish. PCBs are known to sorb to algae (Miao *et al.*, 2000), which may be a pathway of PCB contamination to herbivorous fish, such as parrotfish and surgeonfish. Parrotfish typically contained the lowest concentrations of pollutants between the fish species (Fig. 4), with ringtail surgeonfish showing intermediate concentrations. Fish from the Mauritian Outer Islands may also directly ingest plastic fragments and the potential pollutants associated with it. Microplastic particles—predominantly fibres—were found in the gastrointestinal tract of coral reef fish from the Red Sea (Baalkhuyur *et al.*, 2018).

The different functional groups sampled in this study had different patterns and concentrations of pollution. Biota from different trophic positions and ecological niches do not interact with pollutants in the same manner. This indicates the importance of stratifying sampling when collecting biota in the field. A skewed perspective on the ecotoxicological state

of a reef may result from studying only one taxon or functional group—A factor that future studies should bear in mind.

#### 4.2. Possible sources of pollutants

Li *et al.* (2011) quantified PBDE in air samples from the open Indian Ocean. BDE-47 contributed more than 60% of the total PBDEs that were quantified. This was ascribed to e-waste recovery activities from the Indian subcontinent (Li *et al.*, 2011). Low concentrations of PBDEs were found in tern eggs from Rodrigues and St. Brandon's Atoll (Bouwman *et al.*, 2012; unpublished data). Wet deposition of PBDE over the ocean is found to be highest in the Intertropical Convergence Zone (ITCZ) (Jurado *et al.*, 2005). However, the Mauritian Outer Islands fall outside of the southern limits of the ITCZ. The southern ITCZ extends as far as 8° south of the equator (Schneider *et al.*, 2014). Agalega, the most northern island in this study, is located 10°S (Bhagooli & Kaullysing, 2018). The island system may still experience a larger wet deposition of PBDEs than the other islands, as PBDEs made up 49% of the total pollutant concentration in biota (Fig. 1). The other prominent PBDE congener that was quantified in all biological samples from the Mauritian Outer Islands is BDE-209—the primary constituent of deca-BDE (Ross *et al.*, 2009). Models have shown BDE-209 to be the least concern of the BDEs in PBDE mixtures due to its low volatility and high hydrophobicity (Tittlemier *et al.*, 2002). The compound is thought to have a low potential for long-range transfer and bioaccumulation, and as such, deca-BDE mixture is produced and used the most volume wise out of all PBDE mixtures (Earnshaw *et al.*, 2013). Despite the claim of being less prone to long-range transport, BDE-209 had been quantified in biota from remote regions such as the Arctic, the Antarctic, and pelagic environments (Hale *et al.*, 2008; Sørmo *et al.*, 2008; Aznar-Alemay *et al.*, 2019). BDE-209 was also quantified in all coral and coral reef fish tissues from the remote Mascarene Basin (Table 2), proving that it does undergo long-range transport. Although BDE-209 is relatively non-toxic, it breaks down to more toxic but less brominated compounds such as BDE-47 (Gandhi *et al.*, 2011). The fact that all coral and fish tissue pools from the Mauritian Outer Islands contained both BDE-209 and BDE-47 might indicate to such a breakdown process.

Lesser chlorinated PCBs, such as PCB-11 and -28 had a higher detection frequency than higher chlorinated PCBs in corals. Lesser chlorinated PCBs in remote locations are often a result of atmospheric deposition (Wania & Daly, 2002). The same pattern of PCB contamination was also found in Iran, where coral tissue and zooxanthellae algae contained higher concentrations of di- and tri-CBs than other PCB congeners (Jafarabadi *et al.*, 2018). Fish contained high detection frequencies of PCB-52, a tetra-CB. PCB compounds in this

study were found at a higher detection frequency in hard coral and fish than in soft coral (Table 2 & 3).

Compounds such as PBDEs are incorporated in plastic production to reduce the flammability of the product (Gallen *et al.*, 2014). POPs associated with plastic particles can be transported through ocean current to areas far from their point of origin (Ogata *et al.*, 2009). The concentration of certain other POPs compounds, such as PCB or DDT has been found to be an order of magnitude higher in plastic particles than the surrounding waters (Mato *et al.*, 2001). Up to 80% of marine plastic debris originates from land-based sources and are redistributed through ocean currents (Verster & Bouwman, 2020). It is thought that coral islands may re-concentrate pollutants associated with debris (Bouwman *et al.*, 2016). The accumulation rate of plastics on the beaches of Indian Ocean islands vary between geographic regions (Barnes, 2004). Plastic debris are visible on the beaches of all three sampling islands (personal observation, Van der Schyff, 2014).

Another source of pollutants to coral reefs of subtropical islands is the redistribution of POPs through seabird excrement. Seabirds are exposed to pollutants in their foraging ranges but can excrete pollutants at the roosts and breeding colonies—thus releasing pollutants to the surrounding coral reefs (Cipro *et al.*, 2019). A study in Antarctica indicated that large penguin colonies are an important redistributor of HCB and PCBs to the surrounding environment (Cipro *et al.*, 2019). More than one million birds breed on St. Brandon's Atoll (Evans *et al.*, 2016). The breeding colonies of species such as sooty terns (*Onychoprion fuscatus*), fairy terns (*Gygis alba*), and common noddies (*Anous stolidus*) may be a secondary source of pollutants to the islands where they breed.

#### 4.3. Contamination composition per island

Rodrigues is the largest and most industrialised of the Mauritian Outer Islands (Bhagooli & Kaullysing, 2018). The island is primarily supplied with power by a 11 MWh fuel-driven generator installed in Port Mathurin. However, in accordance with the National Implementation Plan of Mauritius for the Stockholm Convention, no PCB contaminated oils are used in the transformers (Bouwman *et al.*, 2012). Plastic accumulation on Rodrigues is relatively low. In 2004, the density of plastic accumulation for the island was calculated at 4.41 items/km/year (Barnes, 2004). The relatively higher concentrations of pollutants in biota from Rodrigues can be ascribed to areas of high fuel combustion, particularly from the harbour and airport, accidental oil spills, and waste incineration (Pozo *et al.*, 2017). St. Brandon's Atoll does not have a permanent human population. It is postulated that most of the pollution in biota from the atoll is transported by plastic debris. The plastic density of St. Brandon's Atoll was calculated at 0.76 items/m shoreline (Bouwman *et al.*, 2016). The presence of shipwrecks on

the reef crest may also contribute to pollutants being transported to the atoll (Van der Schyff, 2020c). Agalega does have a permanent human population, but little industrial activity. Less plastic debris is found in the beaches of Agalega than of St. Brandon's Atoll (personal observation, Van der Schyff, 2014). Transport of pollution through plastic debris and waste incineration are the most likely sources of pollutants to coral reef biota of Agalega.

Other studies have found low concentrations of pollutants in biota from higher trophic level from the Mascarene Basin (Ueno *et al.*, 2004, 2005; Bouwman *et al.*, 2012; Munsch *et al.*, 2020). Corals and coral reef fish from relatively low tropic positions contained even lower concentrations of POPs and NBFs.

The distribution of pollutants in the Mascarene Basin seems to be rather homogenous between different islands (Fig. 5; Table 4).

#### 4.4. Comparison with other studies

In order to determine the global pattern of chlorinated and brominated contaminants' accumulation in coral reefs, the concentrations of POPs in coral and fish found in this study were compared with results from others (Supplementary tables S1 and S2). Only studies that quantified multiple contaminant groups were included in order to determine compositional patterns per region.

When the concentrations of contaminants in coral reef biota from the Mascarene Basin are compared with contaminants in coral reef biota collected elsewhere, it is clear that concentrations are lower in the Mascarene Basin than most other locations (Supplementary Tables S1 and S2). Concentrations of HCH and DDT were up to five orders of magnitude higher in soft coral from South Africa than the Mascarene Basin (Porter *et al.*, 2018). HCB and HCH concentrations in coral (*Porites evermanni*) from Bikini Atoll in the Pacific Ocean were slightly higher, but comparable to concentrations found in the corals from the Mascarene Basin (Wang *et al.*, 2008). Concentrations of HCB in hard corals in this study are comparable to that in coral from Florida in 1991 (Glynn *et al.*, 1995). Hard corals (*Montastraea annularis*) from the Virgin Island in the Caribbean, collected in 2010, were screened for 68 compounds, but did not contain quantifiable concentrations of DDT, PeCB, HCB, or PBDE (Bargar *et al.*, 2013).

When the different studies from different locations are examined, different classes of POPs are dominant in coral from the different regions. In Egypt, PCBs was the most prominent compound class (El Nemr *et al.*, 2013), while DDT was found at the highest proportions in the French Frigate Shoals (Wang *et al.*, 2008). In hard corals from both Florida and South Africa, HCH was found at higher concentrations than other compounds (Glynn *et al.*, 1995; Porter *et al.*, 2018). In the Mascarene Basin, PBDE was the most prominent compound in all coral (Supplementary Table S1).

When the concentrations of contaminants in coral reef fish from the Mascarene Basin are compared with the results from other studies, a similar trend occurs. All concentrations of pollutants found in other studies were higher than what was quantified in the Mascarene Basin. DDT was found at higher concentrations than HCH in fish tissue from French Polynesia (Roche *et al.*, 2011) and from Yongxing Island and Xuande Atoll in the South China Sea (Sun *et al.*, 2014; 2017). PCBs was the compound class quantified at the highest concentrations in fish tissue from Natuna Island in the South China Sea (Hao *et al.*, 2010) as well as the Mascarene Basin (Supplementary Table S2).

The concentrations of pollutants in coral reef biota in this study are some of the lowest in reported literature. The small human populations and remote localities on the island systems are thought to be the main reason for the low concentrations in biota from the Mascarene Basin, making the islands suitable reference sites to monitor background values of POPs in the western Indian Ocean (Van der Schyff *et al.*, 2020b).

#### 4.5. Implication to policy

No NBFRs are currently included in the Stockholm Convention. Dechlorane Plus is listed as a candidate POP with the potential to be included in the Convention (Stockholm Convention, 2019). One of the criteria of a candidate POP, as stipulated by the Stockholm Convention, is the potential to be transported over long distances, by air, biota, or by water movement (Stockholm Convention, 2019). Because *syn*DP was quantified in 66% of all sample pools, and *anti*DP in 86% of sample pools, it would appear that the compounds are subjected to long-range transport. Pentabromotoluene (PBT) was quantified in 100% of sample pools, indicating high potential for long-range transport to tropical islands. In previous studies, the compound was confirmed to be toxic (Feng *et al.*, 2013) and bioaccumulative (Zang *et al.*, 2011). PBT may therefore be considered as a candidate POP by the Stockholm Convention. Other NBFRs found at high detection frequencies were  $\alpha$ - and  $\beta$ -TBECH with detection frequencies of 61% and 71%, respectively. The concentrations of NBFRs in coral reef biota should be monitored closely.

## 5. Conclusions

Corals and coral reef fish from the Mauritian Outer Islands contained very low concentrations of POPs and NBFRs. Concentrations of pollutants in soft coral and fish did not differ significantly between any compound classes, but hard coral fragments had significantly lower concentrations of  $\Sigma$ DDT, HCB, PeCB, and  $\Sigma$ PCB than either soft coral or fish. Hard coral and fish contained higher concentrations of NBFRs than soft coral. The compounds PBT,

lindane, *p,p'*-DDE, HCH, BDE-47, BDE-209, and PCB-11 were quantified in all sample pools from all three islands. PBDE was the compound class proportionally the highest in coral from all islands, while PCB was the compound class with the highest concentrations in fish tissue. The presence of both BDE-209 and BDE-47 in all sample pools reflect the danger of long-range transport and breakdown of BDE-209 in coral reef ecosystems. Long-range transport of pollutants via plastic debris and aerial deposition are likely the main vectors of pollutant transport to the islands, with a secondary concentration effect from nesting marine birds. The presence of PBT in all sample pools raise concern regarding the long-range transport of the compound and it may well be considered as a possible POP. The small human population and remote location of the Mauritian Outer Islands may serve as a buffer to pollutant accumulation. Because of the low concentrations of pollutants in coral reef biota, the Mauritian Outer Islands—particularly Agalega and St. Brandon's Atoll could be useful for identifying potential new POPs, and as monitoring sites to study the long-term background concentrations of pollutants in the WIO.

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## Supplementary materials

### **Differences in concentrations and compositions of chlorinated and brominated POPs and novel brominated flame retardants in hard coral, soft coral, and reef fish**

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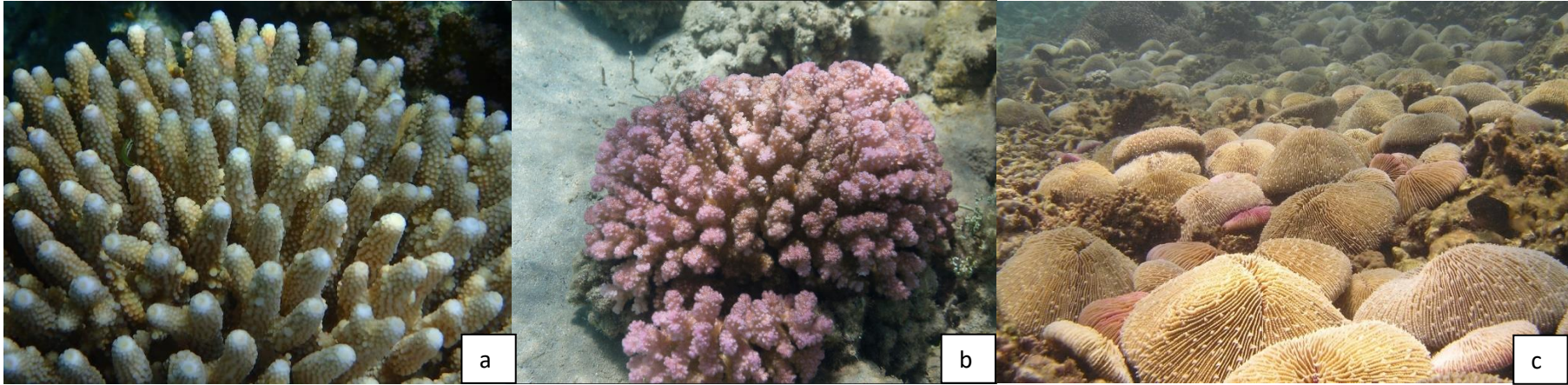
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**Table S1.** Comparative POPs concentrations in hard and soft coral from other studies compared with concentrations measured in this study. If samples were collected from multiple sampling points within the same geographic region, the range of means of the concentrations of contaminants (ng/g) are presented.

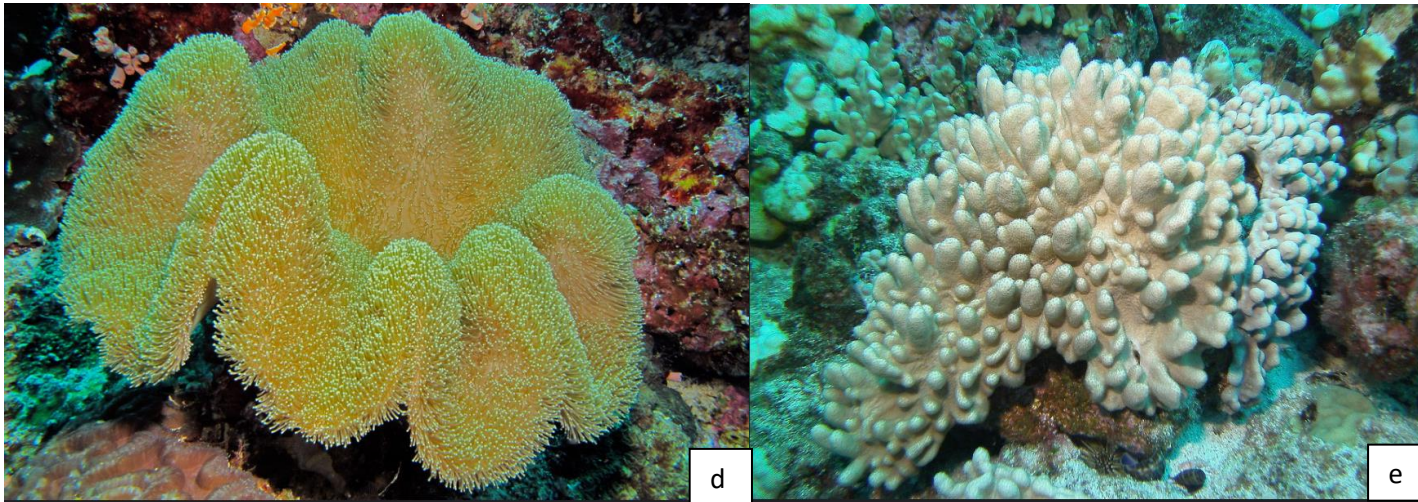
Species	Type	Location	Region	Unit	ΣHCH	p,p'-DDD	p,p'-DDE	p,p'-DDT	ΣDDT	PECB	HCB	ΣBDE	ΣPCB	
<i>Acropora sp.</i>	Hard	Mascarene Basin	Mauritius	ng/g wm	<b>0.0094-0.35</b>		<b>0.011-0.027</b>	<b>&lt;LOQ-0.0058</b>	<b>0.011-0.033</b>	<b>0.004-0.0044</b>	<b>0.0053-0.0072</b>	<b>0.038-0.19</b>	<b>0.036-0.083</b>	<b>This study</b>
<i>Acropora sp.</i>	Hard	Red Sea	Egypt	ng/g	0.8-14.6	0.3-6.1	0.1-37	0.6-11.3	11-11.7				6.2-48.3	El Nemr et al., 2004
<i>Pocillopora sp.</i>	Hard	Mascarene Basin	Mauritius	ng/g wm	<b>0.0039-0.011</b>		<b>0.0077-0.019</b>		<b>0.0077-0.019</b>	<b>0.0039-0.0061</b>	<b>0.0046-0.0051</b>	<b>0.039-0.53</b>	<b>0.032-0.048</b>	<b>This study</b>
<i>Fungia sp.</i>	Hard	Mascarene Basin	Mauritius	ng/g wm	<b>0.0048-0.017</b>		<b>0.0039-0.008</b>	<b>&lt;LOQ-0.0011</b>	<b>0.0039-0.019</b>	<b>0.0039-0.0047</b>	<b>0.004-0.0061</b>	<b>0.016-0.069</b>	<b>0.032-0.074</b>	<b>This study</b>
<i>Porites evermanni</i>	Hard	Bikini Atoll	French Frigate Shoals	ng/g dw	0.008-0.082				0.88-0.21		0.007.0-0.26			Wang et al., 2008
<i>Porites evermanni</i>	Hard	Tern Island	French Frigate Shoals	ng/g dw	0.086-0.63				0.59-3.2		0.003-0.045			Wang et al., 2008
<i>Porites astereoides</i>	Hard	Florida	USA	ng/g ww	nd-0.11				0.01-0.06		nd-0.01			Glynn et al., 1995
<i>Millepora alcicornis</i>	Hard	Florida	USA	ng/g ww	0.06-0.82				0.22-0.45		0.01-0.06			Glynn et al., 1995
<i>Montastraea annularis</i>	Hard	Virgin Island	Caribbean	ng/g wm		<0.01	<0.02	<0.01		<0.25	<0.25	<0.0005		Bargar et al., 2013
<i>Sarcophyton sp.</i>	Soft	Mascarene Basin	Mauritius	ng/g wm	<b>0.17-0.33</b>	<b>0.024-0.036</b>	<b>0.014-0.049</b>		<b>0.038-0.085</b>	<b>0.012-0.03</b>	<b>0.073-0.75</b>	<b>0.11-0.34</b>	<b>0.13-0.18</b>	<b>This study</b>
<i>Sarcophyton glaucum</i>	Soft	Maputuland	South Africa	ng/g wm	78-290	52-91	31-76	3.9-16	88-170					Porter et al., 2018
<i>Sinularia sp.</i>	Soft	Mascarene Basin	Mauritius	ng/g wm	<b>0.057-0.28</b>	<b>0.012-0.024</b>	<b>0.012-0.2</b>		<b>0.027-0.044</b>	<b>&lt;LOQ-0.0082</b>	<b>0.026-0.05</b>	<b>0.11-1.62</b>	<b>0.011-0.15</b>	<b>This study</b>
<i>Sinularia gravis</i>	Soft	Maputuland	South Africa	ng/g wm	120-420	61-160	29-120	5.6-140	170-370					Porter et al., 2018

**Table S2.** Comparative POPs concentrations in coral reef fish from other studies compared with concentrations measured in this study. If samples were collected from multiple sampling points within the same geographic region, the range of means of the concentrations of contaminants (ng/g) are presented.

Species	Common name	Diet	Location	Region	Unit	ΣHCH	ΣDDT	ΣBDE	ΣPCB	ΣDP	
<i>Chaetodon zanzibarensis</i>	Butterflyfish	Corallivore	Mascarene Basin	Mauritius	ng/g wm	0.068-0.12	0.031-0.052	0.2-0.7	0.29-0.97	0.008-0.029	This study
<i>Scarus rubroviolaceus</i>	Parrotfish	Herbivore	Mascarene Basin	Mauritius	ng/g lm	0.038-0.066	0.013-0.026	0.066-0.13	0.078-0.28	0.003-0.005	This study
<i>Acanthurus blochii</i>	Surgeonfish	Herbivore	Mascarene Basin	Mauritius	ng/g wm	0.074-0.32	0.031-0.052	0.18-0.18	0.17-0.27	0.007-0.008	This study
<i>Chlorurus sordidus</i>	Parrotfish	Herbivore	French Polynesia	Polynesia	ng/g wm	8.6-149	172-1088				Roche <i>et al.</i> , 2011
<i>Epinephelus merra</i>	Grouper	Piscivorous	French Polynesia	Polynesia	ng/g wm	nd-114	64.2-813				Roche <i>et al.</i> , 2011
<i>Chlorurus bowersi</i>	Parrotfish	Herbivore	Xuande Atoll	South China sea	ng/g lm		56.2	17.5	16.9	1.32	Sun <i>et al.</i> , 2017
<i>Selar crumenophthalmus</i>	Scad	Filter feeder	Xuande Atoll	South China sea	ng/g lm		83.2	24.4	43.6	0.93	Sun <i>et al.</i> , 2017
<i>Muraenesox talabonoides</i>	Pike conger	Piscivorous	Xuande Atoll	South China sea	ng/g lm		272.7	51.2	1041	6.45	Sun <i>et al.</i> , 2017
<i>Saurida undosquamis</i>	Lizardfish	Carnivorous	Natuna Island	South China sea	ng/g lm		16.7	5.55	26.4		Hao <i>et al.</i> , 2014
<i>Decapterus russelli</i>	Scad	Filter feeder	Natuna Island	South China sea	ng/g lm		24.4	7.64	31.9		Hao <i>et al.</i> , 2014
<i>Upeneus bensasi</i>	Goatfish	Carnivorous	Natuna Island	South China sea	ng/g lm		10.8	2.85	14.3		Hao <i>et al.</i> , 2014
<i>Trachinocephalus myops</i>	Lizardfish	Carnivorous	Natuna Island	South China sea	ng/g lm		40.3	7.82	48.8		Hao <i>et al.</i> , 2014
<i>Priacanthus macracanthus</i>	Bigeye	Carnivorous	Natuna Island	South China sea	ng/g lm		7.99	3.13	24.9		Hao <i>et al.</i> , 2014
<i>Plectorhinchus diagrammus</i>	Sweetlip	Carnivorous	Yongxing Island	South China sea	ng/g lm		40	11	11		Sun <i>et al.</i> , 2014
<i>Parupeneus chrysopleuron</i>	Goatfish	Carnivorous	Yongxing Island	South China sea	ng/g lm		265	46	89		Sun <i>et al.</i> , 2014
<i>Lethrinus atkinsoni</i>	Emperor	Carnivorous	Yongxing Island	South China sea	ng/g lm		37	10	27		Sun <i>et al.</i> , 2014
<i>Cephalopholis argus</i>	Grouper	Carnivorous	Yongxing Island	South China sea	ng/g lm		64	5.8	17		Sun <i>et al.</i> , 2014
<i>Epinephelus fasciatus</i>	Reef cod	Carnivorous	Yongxing Island	South China sea	ng/g lm		93	6.9	18		Sun <i>et al.</i> , 2014

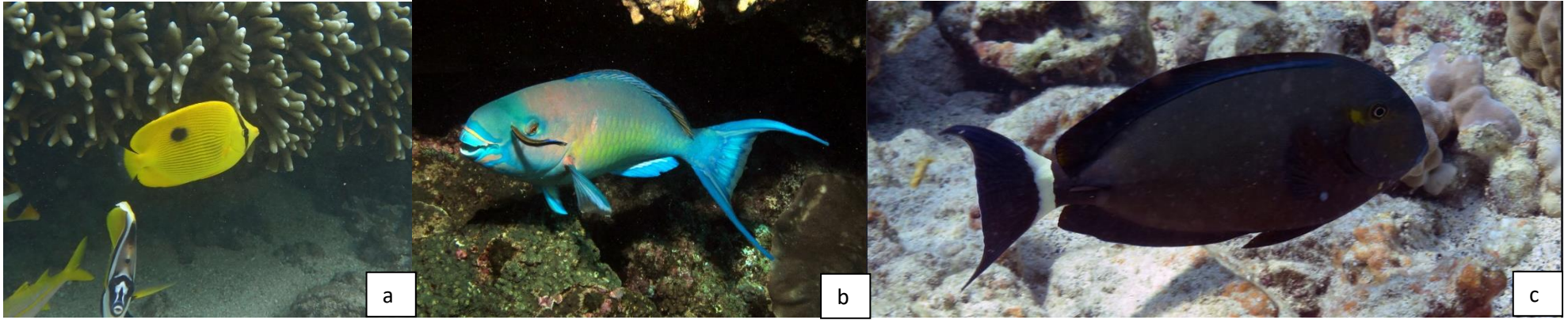


**Fig. S1a-c.** Representatives of the hard corals sampled. 1a – *Acropora* spp. (Hans Hillewaert). 1b – *Pocillopora* spp. (Nikolai Vladimirov). 1c – *Fungia* spp. (Nicolas Ory). For creative commons licence information, see below.



**Fig. S1d-e.** Representatives of the soft corals sampled. 1d- *Sarcophyton* spp. (Bernard Dupont). 1e – *Sinularia* spp. (Barry Fackler). For creative commons licence information, see below.





**Fig. S2a-c:** The different coral reef fish we sampled. 2a - Zanzibar butterflyfish (*Chaetodon zanzibarensis*) (Philippe Bourjon and Elisabeth Morcel); background *Porites spp.* coral. 2b - Ember parrotfish (*Scarus rubroviolaceus*) (Derek Keats), together with cleaner wrasse. 2c - Ringtail surgeonfish (*Acanthurus blochii*) (Kris Bruland). For creative commons licence information, see below.

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Fig. S1a

Author: Hans Hillewaert

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Fig. S1b

Author: Nikolai Vladimirov

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Fig. S1c.

Author: Nicolas Ory

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Fig. S1d.

Author: Bernard Dupont

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Fig. S1e.

Author: Barry Fackler

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Fig. S2a.

Author: Philippe Bourjon and Elisabeth Morcel

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Fig. S2b.

Author: Derek Keats

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Fig. S2c.

Author: Kris Bruland

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## Chapter 4. Persistent Organic Pollutants in sea bird eggs from the Indian Ocean's Mascarene Basin

### Foreword to Article 3: “Persistent Organic Pollutants in sea bird eggs from the Indian Ocean's Mascarene Basin”

After pollutants were quantified in biota from low trophic positions in the coral reef food web, the next step was to quantify pollutants in species from higher trophic positions to obtain a holistic perspective on the ecotoxicological state of islands in the Mascarene Basin. To do this, eggs were collected from three species of seabirds abundantly breeding on St. Brandon's Atoll—fairy terns (*Gygis alba*), sooty terns (*Onychoprion fuscatus*), and common noddies (*Anous stolidus*). Bird eggs are good indicators of environmental pollution (Braune, 2007; Burger *et al.*, 2004; Van der Schyff *et al.*, 2016). The concentrations of pollutants in the egg provide an accurate representation of the pollutant load of the mother's body (Ackerman *et al.*, 2016). One study, conducted in 1975, quantified DDT and PCB in fairy tern eggs from St. Brandon's Atoll (Bourne *et al.*, 1977), but those concentrations are no longer representative of the pollutant load in the Mascarene Basin in modern times. The most recent study on pollutants in seabird eggs from the Mascarene Basin was conducted on Rodrigues (Bouwman *et al.*, 2012). However, as Rodrigues and St. Brandon's Atoll are over 400 km apart, it is important to understand the threat that POPs pose to the seabird population of St. Brandon's Atoll by quantifying the pollutants in the eggs of the breeding colonies.

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## Persistent Organic Pollutants in sea bird eggs from the Indian Ocean's Mascarene Basin

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### Abstract

We report the concentrations of persistent organic pollutants (POPs) in seabird eggs from St. Brandon's Atoll, a tropical island system in the western Indian Ocean, for the first time in 45 years. Ten eggs each of sooty terns (*Onychoprion fuscatus*), fairy terns (*Gygis alba*), and common noddies (*Anous stolidus*) were collected from the atoll. For a terrestrial reference, we analysed three feral chicken (*Gallus gallus domesticus*) eggs from the same location. Sooty tern eggs contained the highest mean concentrations of  $\Sigma$ Chlordane (0.21 ng/g wm),  $\Sigma$ PCB (1.5 ng/g wm), and  $\Sigma$ PBDE (1.1 ng/g wm). Fairy tern eggs contained the highest mean concentrations of HCB (0.68 ng/g wm) and  $\Sigma$ Toxaphene (0.83 ng/g wm). The chicken eggs contained the highest mean concentrations of  $\Sigma$ DDT (2.6 ng/g wm), while common noddy eggs contained the highest mean concentrations of  $\Sigma$ HCH (0.5 ng/g wm). We surmise that the differences in chemical composition between species reflect different pollutant compositions in prey from different foraging ranges. The birds foraging offshore contained higher POPs concentrations than the nearshore foragers. The latter reported earlier inter-island differences in contaminant concentrations are seen between eggs of the same species from St. Brandon's Atoll and Rodrigues Island, 520 km south-east. Concentrations of contaminants found in this study are lower than values quantified by other studies, making St. Brandon's Atoll an ideal reference site to determine background concentrations of POPs in the sub-tropical Indian Ocean.

**Keywords:** Biomonitoring; DDT; Mauritius; PBDE; PCB; Seabird eggs

## 1. Introduction

Persistent organic pollutants (POPs) are toxic and resistant to environmental degradation, consisting of pesticides, industrial chemicals, and by-products of industrial processes (Alharbi *et al.*, 2018; Stockholm Convention, 2019). POPs can contaminate remote, uninhabited regions by long-range aerial transport (Scheringer *et al.*, 2004), wet precipitation (Hamers *et al.*, 2001), water movement such as currents (Ilyina *et al.*, 2006), or adsorbing to plastic debris (Ogata *et al.*, 2009). They also tend to biomagnify through the food web (Newman, 2015). Adverse environmental and human health effects are associated with elevated POPs concentrations, *inter alia* endocrine disruption, cancers, reproductive impairment, and behavioural changes (Alharbi *et al.*, 2018, Goutte *et al.*, 2018).

The major shipping route between South-East Asia and South Africa crosses the environmentally important Mascarene Basin and the Saya de Malha Bank in the western Indian Ocean (Au & Pitman, 1986; Le Corre *et al.*, 2012). This section of the western Indian Ocean is also a prolific fishing ground (IOTC, 2008) supporting the economy and livelihoods of thousands in many different countries. Maritime traffic poses the risk of chemical pollution in the region, through either accidental oil spills or ongoing fuel combustion (Le Corre *et al.*, 2012).

Quantification of POPs in biota from the western Indian Ocean region is sparse. POPs have been quantified in terns from Rodrigues Island (Bouwman *et al.*, 2012) and St. Brandon's Atoll (Bourne *et al.*, 1977), penguins from South Africa (Bouwman *et al.*, 2015), dolphins from Zanzibar (Mwevura *et al.*, 2010), mussels from Mayotte (Thomassin *et al.*, 2011); tuna from Reunion and Seychelles (Ueno *et al.*, 2004; Ueno *et al.*, 2005; Chouvelon *et al.*, 2017), and coral from South Africa (Porter *et al.*, 2018). A recent study quantified polyfluoroalkyl substances (PFAS) in the eggs of terns from St. Brandon's Atoll (van der Schyff *et al.*, 2020c).

Terns of the family Laridae (Subfamily Sterninae) are small, predominantly marine birds with a wide distribution (Schreiber & Burger, 2001). Three abundant tern species in the western Indian Ocean are fairy tern (also known as the white tern; *Gygis alba*), sooty tern (*Onychoprion fuscatus*), and common noddy (also known as brown noddy; *Anous stolidus*) (Fig. 1). The predominant feeding strategy of these terns is to catch fish and other small prey chased to the surface by predators, such as tuna and dolphins (Ashmole, 1968; Jaquemet *et al.*, 2004).



**Fig. 1.** Photographs of a) fairy tern, b) sooty tern, and c) common noddy from St. Brandon's Atoll.

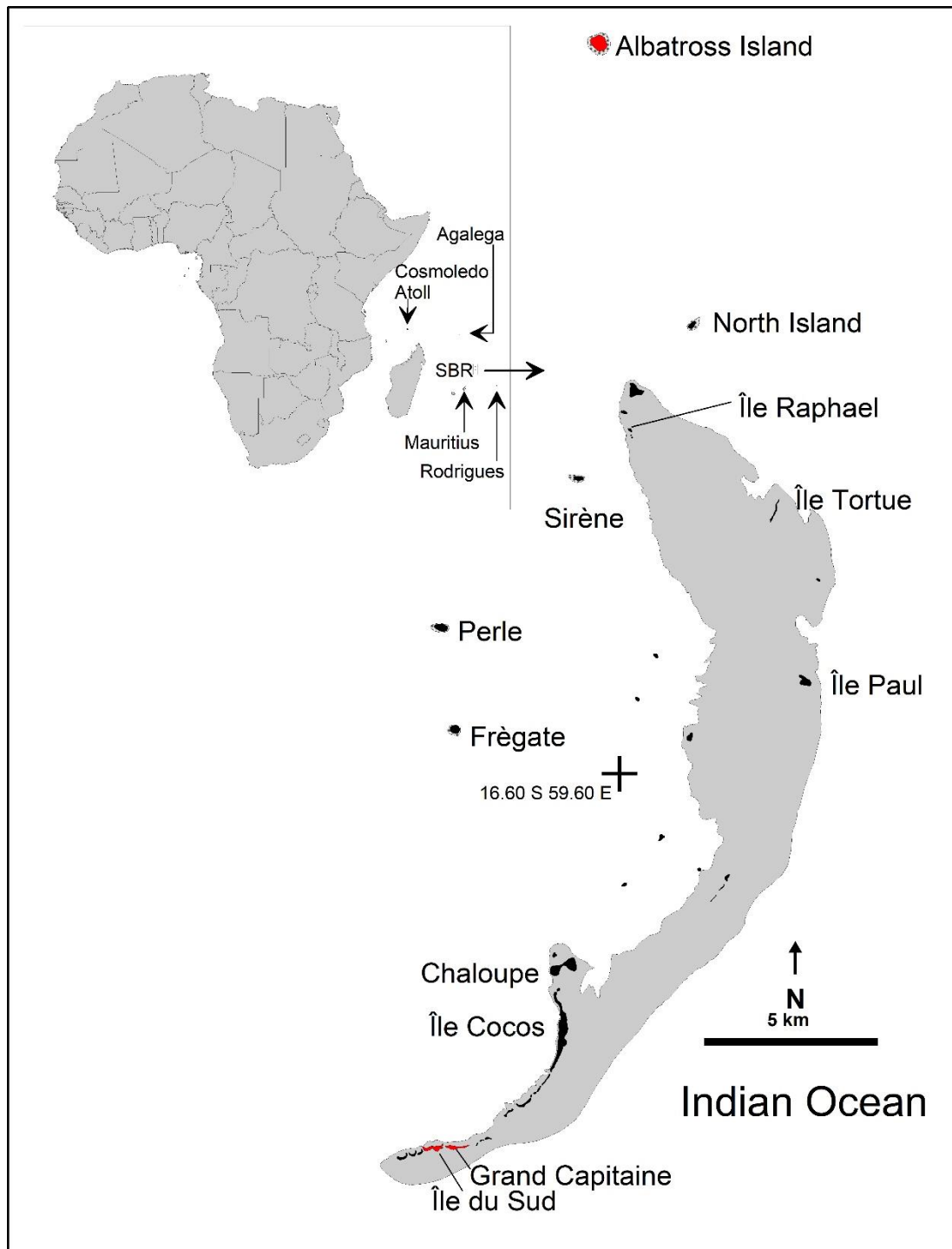
Given that the same prey types are exploited by terns, tuna, and dolphins in the western Indian Ocean (Le Corre *et al.*, 2012), the pollutant concentrations found in the seabirds may be an indication of the pollutant load in other economic and environmental keystone marine predators. A healthy seabird population is vital for the functioning of coral reefs where they nest, as their excrement provides nutrients for the reef inhabitants and increases reef productivity (Graham *et al.*, 2018; Savage, 2019). However, pollutants in seabirds pose a risk to the entire island system, as seabirds are secondary pollution sources, excreting pollutants at roosts and breeding colonies, thus releasing them to the surrounding ecosystem that is often coral reefs (Cipro *et al.*, 2019). The aim of this study was to quantify POPs in the eggs of three tern species nesting on St. Brandon's Atoll. The concentrations of contaminants in the eggs are interpreted according to the species' different forage ranges and compared with concentrations found by Bouwman *et al.* (2012), where the same compounds were quantified in common noddies and sooty tern eggs collected from Rodrigues Island— which is also within the Mascarene Basin, but 520 km to the south-east of St. Brandon's Atoll (Fig. 2). The relative compositional pattern of the eggs from the different islands will also be compared to determine if the location (islands) plays a larger role than the species themselves. This will provide a greater understanding of POPs contamination in breeding seabirds from the Mascarene Basin.

## **2. Materials and Methods**

### *2.1. Site description*

St. Brandon's Atoll (alternatively known as St. Brandon, St. Brandon's Island, or St. Brandon's Rock), forming part of the Cargados Carajos Shoals, is a remote archipelago, belonging to the Republic of Mauritius. The island system is located approximately 450 km north-east of Mauritius (Fig. 2). St. Brandon's Atoll consists of approximately 19 sandbars and 24 sparsely vegetated islands, connected by a shallow lagoon covering approximately 200

km<sup>2</sup> (Quod, 1999; Evans *et al.*, 2016) (Fig. 2). Several shipwrecks are present on the reef crest (van der Schyff *et al.*, 2020b). The closest prominent landmasses are Seychelles (1380 km north), Madagascar (990 km west), Reunion (670 km south), and Mauritius (450 km south). These areas are, however, not directly linked with St. Brandon's Atoll through oceanic currents. Only the South Equatorial Current from western Australia directly affects St. Brandon's Atoll (New *et al.*, 2007). There are no permanent residents on the atoll, but approximately 40 fishermen and a few Meteorological Service and National Coast Guard personnel reside there on a rotational basis (Bouwman *et al.*, 2016). A few exclusive yacht charters facilitate small-scale ecotourism and recreational fishing.



**Fig. 2.** Map of St. Brandon's Atoll in the Indian Ocean. Islands and sandbars are black, and the lagoon in grey. Islands that were sampled are indicated in red.

## 2.2. Egg collection and preparation

The eggs were collected in October 2010, with permission from the National Parks and Conservation Service of the Ministry of Agro-Industry, Food Production and Security, Mauritius, as well as the Outer Islands Development Corporation. Ethical approval was

obtained from the North-West University (NWU-00055-07-S3). Ten eggs per species of fairy terns, sooty terns, and common noddies were collected from nests on the ground and in the trees. The eggs were wrapped in aluminium foil (acetone- and hexane rinsed) and frozen. Common noddy and fairy tern eggs were sampled from Grande Capitaine in the southern cluster area of the atoll (Fig. 2). Sooty tern eggs were collected from Albatross Island, the most northern island of the atoll (Fig. 2). For a "terrestrial reference", three eggs from a small flock of free-roaming feral chickens (*Gallus gallus domesticus*) were collected from *Ile du Sud*, in the South of the atoll.

The frozen eggs were transported to the University of Mauritius, where they were thawed and homogenised with an ultrasound homogeniser. The homogeniser probe was thoroughly cleaned between each sample to prevent the cross-contamination of the samples. The probe was wiped with tissue paper, rinsed with soapy water, double distilled water, and finally rinsed with analytical grade acetone and hexane, respectively. The homogenised content was individually frozen in 50 ml high-density polyethylene containers and transported to Norway in a frozen state.

### 2.3. Chemical analyses

The chemical analyses were performed at the Laboratory of Environmental Toxicology, Norwegian University of Life Sciences (NMBU), Oslo, Norway. The laboratory is accredited by the Norwegian Accreditation for testing the analysed chemicals in biological material according to the requirements of the NS-EN ISO/IEC 17025 (TEST 137).

#### 2.3.1. Extraction

The compounds analysed are indicated in Table 1. In brief, 2–3 g of each homogenised sample was weighed in centrifuge tubes. The following internal standards were used: PCB-29, PCB-112, and PCB-207 (Ultra Scientific, RI, USA); BDE-77, BDE-119, BDE-181 and <sup>13</sup>C12-BDE-209 (Cambridge Isotope Laboratories, Inc., MA, USA); and 2-endo,3-exo,6-exo,8,9,10,10-heptachlorobornane (DE-TOX 409; LGC Standards GmbH, Germany). After adding solvents (HPLC grade), distilled water, and 6% NaCl, the samples were homogenised again with an Ultra-Turrax®. Lipids were extracted twice with cyclohexane and acetone (3:2) using an ultrasonic homogeniser followed by centrifugation and separation. The lipid removal was performed using ≥97.5% H<sub>2</sub>SO<sub>4</sub> (Fluka Analytical®). The lipid determination was done gravimetrically using 1 mL aliquot of the fat extract.

**Table 1.** Classification, names, abbreviations, and congeners of compounds analysed for in this study.

Compound group	Compound name	Compound abbreviation	Individual congeners/isomers
Organochlorine pesticides	Hexachlorobenzene	HCB	
	Hexachlorocyclohexane	HCH	$\alpha$ -, $\beta$ -, $\gamma$ -HCH
	Chlordane	CHL	Oxychlordane, cis-chlordane, trans-chlordane
	1,1-Bis(4-chlorophenyl)-2,2,2-trichloroethane	DDT	<i>p,p'</i> -DDT, <i>p,p'</i> -DDE, <i>p,p'</i> -DDD
	Mirex		
	Toxaphene	CHB	CHB-26, -40, -41, -44, -50, -62
Industrial chemicals	Polychlorinated biphenyl	PCB	PCB-28, -52, -66, -74, -99, -101, -105, -118, -138, -149, -153, -180, -183, -187, 194
Brominated flame retardants	Polybrominated diphenyl ethers	PBDE	BDE- 28, -47, -99, -100, -153, -154, -183, -206, -207, -208
	Hexabromocyclododecane	HBCD	

The extraction and clean-up procedures were based on Brevik (1987) and described and referenced in Bouwman *et al.* (2015) and Polder *et al.* (2014).

### 2.3.2. Separation and detection

Separation and detection of the organochlorine pesticides and PCBs were performed on a high-resolution gas chromatograph (HRGC) (Agilent 6890 Series gas chromatography system; Agilent Technologies) equipped with an autosampler (Agilent 7683 Series; Agilent Technologies). These were connected to a 1 m long pre-column and a dual column system (SPB-5 and SPB-1701) (60 m, 0.25 mm ID, 0.25  $\mu$ m film thickness; Supelco) and coupled to two  $^{63}\text{Ni}$  micro electron capture detectors (Agilent 6890  $\mu$ -ECD). The temperature program and other GC-ECD conditions were described elsewhere (Polder *et al.*, 2008). Separation and detection of CHBs were performed on an HRGC coupled to a low-resolution mass spectrometric (LRMS) detector operating in electron capture negative ion (ECNI) mode with selected ion monitoring (SIM). Separation and detection of PBDEs (except from BDE 209) and HBCD were performed on an HRGC–LRMS. For BDE 209, 10  $\mu$ L were injected into an HRGC–LRMS configured with a programmable temperature vaporisation (PTV) injector (Agilent Technologies). GC-details, temperature programs, and target ions for CHBs, PBDEs, HBCD, and BDE 209 were described elsewhere (Polder *et al.*, 2014).

### 2.3.3. Analytical quality

Every analytical series included three procedural blanks (solvents), one blind (non-spiked commercial chicken egg), two spiked samples of commercial chicken eggs for recoveries, and the laboratory's reference materials (LRMs). Harp seal (*Pagophilus groenlandicus*) blubber was used as the LRM for testing performance of all compounds except CHBs, and fat of minke whale (*Balaenoptera acutorostrata*) was the LRM for testing of CHBs. The lowest levels of detection (LODs) for individual compounds were defined as three times the noise level. A full list of LODs, relative recoveries, and detection frequencies for the individual compounds is presented in the supplementary information (Table S1). In addition to the LRMs, analytical quality during the period of analyses was successfully achieved by routinely analysing Certified Reference Materials (CRM; CRM 2525), and by participation in intercalibration tests such as Quasimeme, Arctic Monitoring and Assessment Programme (AMAP) and the Northern Contaminant Programme (NCP).

### 2.4. Statistical analyses

Summary statistical analyses were conducted using Graphpad Prism 8.0.2 for Windows ([www.graphpad.com](http://www.graphpad.com)). The concentrations of POPs in seabird eggs from St. Brandon's Atoll were not equally distributed and were therefore compared using non-parametric Kruskal-Wallis tests. Dunn's post-hoc tests were conducted to compare the differences between species. Significance was set at  $p < 0.05$ . Chicken eggs were excluded from Kruskal-Wallis tests because of the small sample size.

Non-metric multidimensional scaling (NMS) was conducted to ordinate POPs compound proportions in eggs of the four species collected from St. Brandon's Atoll and Rodrigues Island using MjM Software PC-ORD 7.03 ([www.pcord.com](http://www.pcord.com)). To obtain a 'chemical fingerprint' of the POPs profile in each sample, the data was relativised per compound. The Gower-ignore-zero coefficient was used as a relative measurement of distance. Two hundred and fifty runs of real randomised data with random starting conditions were used. Ordinations for most ecological studies have a final stress value of between 10 and 20 (McCune & Grace, 2002). The patterns of ordinations with a final stress value under 10 is considered to have little risk of misinterpretation. Real randomised data were used for 250 runs, with random starting conditions. A Monte Carlo test of significance of each dimensionality tested the probability of stress having been derived by chance.



### 3. Results

The wet mass (wm) concentrations of POPs are presented in Table 2. All discussions and statistical analyses are based on wet mass. However, lipid-based data will be presented in Table 3 for comparisons with other reports. The toxaphene congener, CHB-44, could not be analysed because it coeluted with another compound. Technical difficulties arose with the analyses of  $\alpha$ -HCH and PCB-66, and these compounds will be excluded from the discussion. BDE-206, -207, -208, and -209 were analysed but not quantified. BDE- 28 (<0.01 ng/g) , -74 (<0.036 ng/g), -99 (<0.044 ng/g), -101 (<0.033 ng/g), -128 (<0.015 ng/g), -149 (<0.028 ng/g), -156 (<0.018 ng/g), -157 (<0.025 ng/g), and -170 (<0.016 ng/g) concentrations were not quantifiable above LOD. Many compound concentrations were very low—close to the LOD. For this reason, levels below quantification (LO Q) will not be discussed.

#### 3.1. Egg mass and lipid percentages.

The mean egg content mass of fairy terns (10.43 g) was significantly lighter than both sooty tern (22.48 g) and common noddy egg content (23.06 g) ( $p=0.0011$  and  $p=0.0002$  respectively). The mass of the egg contents of sooty terns and common noddies did not differ significantly ( $p>0.9999$ ).

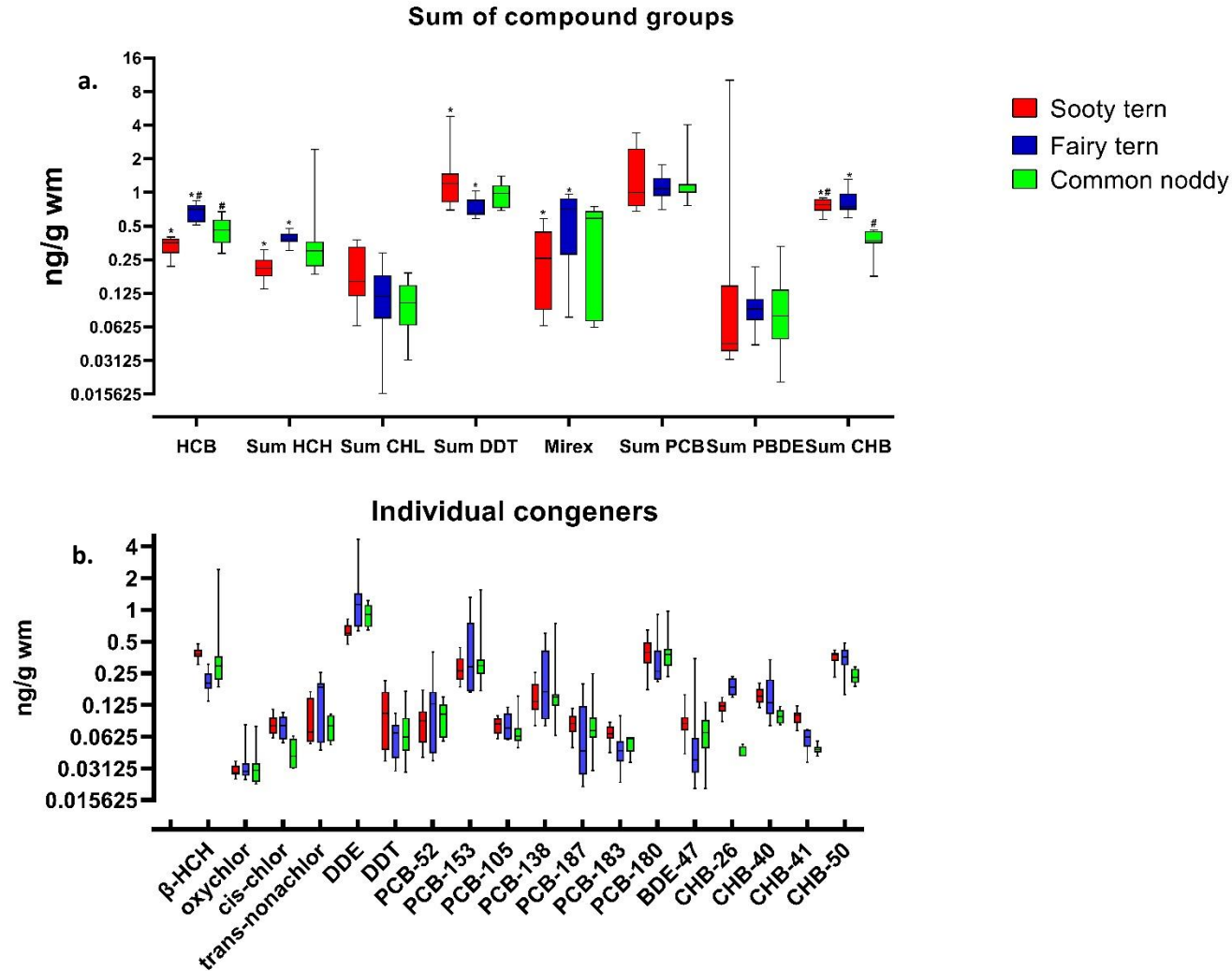
Lipid percentages did not vary significantly between the eggs of the different species. However, fairy tern eggs contained fewer lipids (9.65%) than either sooty terns (11.11%) or common noddies (11.97%). Chicken eggs contained 11.21% lipids.

#### 3.2. Differences between seabird species

There were no statistically significant ( $p< 0.05$ ) differences between the seabird species for  $\Sigma$ PBDE concentrations. Only BDE-47 differed significantly, where post-hoc comparisons showed that fairy terns had a significantly higher concentration than sooty terns ( $p=0.0175$ ). There were no differences between species for  $\Sigma$ PCB concentrations; the only congener with a significant difference was PCB-183, where fairy terns had significantly higher concentrations than sooty terns ( $p=0.0296$ ). There were no significant differences for  $\Sigma$ CHL concentrations between the species (Fig. 3), but common noddies had statistically lower concentrations of cis-chlordane than either sooty terns or fairy terns ( $p=0.0064$  and  $p=0.0024$  respectively). Fairy tern eggs contained significantly more HCB than sooty terns ( $p<0.0001$ ; Fig. 3). This was also the case for  $\beta$ -HCH ( $p=0.0001$ ) and  $\Sigma$ HCH ( $p=0.0002$ ; Fig. 3). However, fairy tern eggs had significantly lower concentrations of DDE and  $\Sigma$ DDT when compared with sooty tern eggs ( $p=0.0024$  and  $p=0.0034$ , respectively; Fig. 3). Fairy terns contained higher concentrations of

DDE in their eggs compared with common noddy eggs ( $p=0.0023$ ). There were no significant differences between the three species for  $p,p'$ -DDD or  $p,p'$ -DDT.

Toxaphene was the chemical group with the most variance between species. Common noddies had significantly lower CHB-26 concentrations than sooty terns ( $p=0.0002$ ). Fairy terns also had significantly lower concentrations of CHB-26 than sooty terns ( $p=0.0058$ ). Common noddies had statistically lower concentrations of CHB-40 than either fairy terns or sooty terns ( $p=0.002$  compared with fairy terns, and  $p=0.033$  compared with sooty terns). Similarly, common noddy eggs contained significantly less CHB-50 compared with fairy terns and sooty terns ( $p=0.0023$  and  $p=0.0112$  for fairy and sooty terns, respectively). Fairy terns had significantly higher concentrations of CHB-41 in their eggs than sooty terns ( $p=0.0169$ ) and common noddies ( $p<0.0001$ ). Common noddy eggs contained significantly less  $\Sigma$ CHB than either fairy ( $p=0.0004$ ) or sooty terns ( $p=0.0005$ ) (Fig. 3).



**Fig. 3.** Box and whisker plots (horizontal lines are medians, 95% confidence intervals, minima, and maxima) of **(a)** total POPs concentrations and **(b)** individual POPs congener concentrations in fairy terns, sooty terns, and common noddies. An asterisk (\*) or pound sign (#) indicate a value with a significant difference between species (Kruskal-Wallis test with Dunn's multiple comparisons).

**Table 2.** Concentrations of POPs on a wet mass basis (ng/g wm; wet mass) in fairy tern, sooty tern, common noddy, and feral chicken eggs from St. Brandon's Atoll. The number of samples with quantifiable amounts (Pos) are presented in the left-hand columns.

	Fairy tern (n=10)						Sooty tern (n=10)						Common noddy (n=10)						Chicken (n=3)					
	Pos	Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max
Lipid %	10	9.65	9.84	2.04	6.4	13.03	10	11.11	10.43	2.08	8.17	14.82	10	11.97	11.87	9.34	9.7	14.82	3	11.21	11.69	1.09	9.7	12.25
HCB	10	0.68	0.71	0.12	0.51	0.85	10	0.33	0.35	0.06	0.22	0.4	10	0.47	0.46	0.12	0.28	0.67	3	0.24	0.25	0.045	0.19	0.27
B-HCH	10	0.39	0.38	0.047 0.00001	0.3	0.48	10	0.21	0.2	0.051	0.14	0.31	10	0.49	0.29	0.68	0.19	2.4	3	0.44	0.13	0.59	0.077	1.1
y-HCH	2	0.019	0.019	5	<LOD	0.019	1	0.014	0.014	0	<LOD	0.014	1	0.015	0.015	0	<LOD	0.015	1	0.016	0.016	0	<LOD	0.016
<b>ΣHCH</b>	<b>10</b>	<b>0.39</b>	<b>0.38</b>	<b>0.049</b>	<b>0.3</b>	<b>0.48</b>	<b>10</b>	<b>0.21</b>	<b>0.21</b>	<b>0.051</b>	<b>0.14</b>	<b>0.31</b>	<b>10</b>	<b>0.5</b>	<b>0.3</b>	<b>0.68</b>	<b>0.19</b>	<b>2.4</b>	<b>3</b>	<b>0.45</b>	<b>0.15</b>	<b>0.58</b>	<b>0.077</b>	<b>1.1</b>
oxychlordane	7	0.03	0.028	0.004	<LOD	0.037	8	0.036	0.029	0.019	<LOD	0.081	8	0.034	0.03	0.018	<LOD	0.078	0					
cis-chlordane	9	0.082	0.079	0.017	<LOD	0.11	10	0.079	0.08	0.02	0.055	0.11	10	0.045	0.041	0.014	0.031	0.063	1	0.034	0.034	0	<LOD	0.034
trans-nonachlor	4	0.09	0.069	0.052	<LOD	0.17	7	0.14	0.19	0.085	<LOD	0.26	4	0.078	0.079	0.021	<LOD	0.1	1	0.055	0.055	0	<LOD	0.055
<b>ΣCHLs</b>	<b>10</b>	<b>0.13</b>	<b>0.12</b>	<b>0.08</b>	<b>0</b>	<b>0.29</b>	<b>10</b>	<b>0.21</b>	<b>0.16</b>	<b>0.12</b>	<b>0.064</b>	<b>0.38</b>	<b>10</b>	<b>0.1</b>	<b>0.1</b>	<b>0.052</b>	<b>0.032</b>	<b>0.19</b>	<b>3</b>	<b>0.03</b>	<b>0.034</b>	<b>0.028</b>	<b>0</b>	<b>0.055</b>
p,p'-DDE	10	0.63	0.6	0.098	0.47	0.82	10	1.4	1.1	1.2	0.63	4.6	10	0.89	0.9	0.21	0.64	1.2	3	1.5	1.5	0.3	1.3	1.9
p,p'-DDD	0						2	0.068	0.068	0.0023	<LOD	0.07	1	0.039	0.039	0	<LOD	0.039	2	0.55	0.55	0.4	<LOD	0.83
p,p'-DDT	10	0.11	0.1	0.064	0.037	0.21	10	0.064	0.068	0.024	0.03	0.1	10	0.072	0.062	0.04	0.029	0.17	2	1.1	1.1	0.17	<LOD	1.2
<b>ΣDDT</b>	<b>10</b>	<b>0.74</b>	<b>0.65</b>	<b>0.15</b>	<b>0.58</b>	<b>1</b>	<b>10</b>	<b>1.5</b>	<b>1.2</b>	<b>1.2</b>	<b>0.7</b>	<b>4.8</b>	<b>10</b>	<b>0.97</b>	<b>0.98</b>	<b>0.23</b>	<b>0.69</b>	<b>1.4</b>	<b>3</b>	<b>2.6</b>	<b>2.4</b>	<b>0.64</b>	<b>2.1</b>	<b>3.3</b>
Mirex	10	0.61	0.71	0.34	0.076	0.96	10	0.28	0.26	0.18	0.064	0.58	10	0.46	0.58	0.28	0.062	0.75	3	0.11	0.084	0.063	0.062	0.18
CHB 26	10	0.12	0.12	0.018	0.087	0.15	10	0.19	0.19	0.032	0.15	0.23	0						0					
CHB 40	10	0.15	0.15	0.026	0.12	0.2	10	0.16	0.13	0.079	0.079	0.34	10	0.1	0.096	0.014	0.081	0.12	0					
CHB 41	10	0.097	0.10	0.015	0.071	0.12	10	0.06	0.062	0.012	0.036	0.073	10	0.047	0.047	0.0046	0.041	0.057	0					
CHB 50	10	0.36	0.37	0.055	0.23	0.42	10	0.35	0.36	0.089	0.16	0.49	9	0.24	0.23	0.035	<LOD	0.29	0					
CHB 62	3	0.35	0.29	0.15	<LOD	0.52	1	0.18	0.18	0	<LOD	0.18	0						0					
<b>ΣCHB</b>	<b>10</b>	<b>0.83</b>	<b>0.75</b>	<b>0.22</b>	<b>0.59</b>	<b>1.3</b>	<b>10</b>	<b>0.77</b>	<b>0.77</b>	<b>0.1</b>	<b>0.57</b>	<b>0.89</b>	<b>10</b>	<b>0.38</b>	<b>0.37</b>	<b>0.083</b>	<b>0.18</b>	<b>0.46</b>	<b>0</b>					
PCB-28	0					0.025	3	0.11	0.041	0.13	<LOD	0.25	6	0.047	0.047	0.015	<LOD	0.064	2	0.04	0.04	0.012	<LOD	0.049
PCB-52	9	0.088	0.088	0.041	<LOD	0.17	8	0.14	0.13	0.12	<LOD	0.4	8	0.097	0.1	0.034	<LOD	0.15	2	0.095	0.095	0.022	<LOD	0.11
PCB-118	2	0.059	0.059	0.0099	<LOD	0.066	5	0.14	0.17	0.058	<LOD	0.2	3	0.12	0.072	0.092	<LOD	0.23	2	0.073	0.073	0.048	<LOD	0.11
PCB-153	10	0.29	0.26	0.09	0.19	0.44	10	0.49	0.29	0.43	0.17	1.3	10	0.41	0.3	0.41	0.17	1.6	3	0.28	0.073	0.35	0.073	0.69
PCB-105	10	0.081	0.083	0.013	0.06	0.099	10	0.081	0.076	0.024	0.059	0.12	10	0.072	0.063	0.029	0.049	0.15	1	0.056	0.056	0	<LOD	0.056
PCB-138	10	0.15	0.13	0.053	0.08	0.25	10	0.25	0.17	0.19	0.079	0.6	10	0.2	0.15	0.2	0.064	0.75	3	0.16	0.07	0.18	0.045	0.37
PCB-187	10	0.083	0.084	0.02	0.049	0.12	10	0.074	0.046	0.064	0.021	0.2	10	0.087	0.071	0.06	0.03	0.25	1	0.1	0.1	0	<LOD	0.1
PCB-183	8	0.068	0.067	0.013	<LOD	0.086	8	0.05	0.046	0.023	<LOD	0.099	7	0.055	0.06	0.01	<LOD	0.062	1	0.039	0.039	0	<LOD	0.039
PCB-180	10	0.4	0.39	0.14	0.18	0.64	10	0.36	0.26	0.23	0.21	0.91	10	0.41	0.38	0.21	0.23	0.97	3	0.14	0.037	0.19	0.024	0.37
PCB-194	1	0.036	0.036	0	<LOD	0.036	4	0.044	0.047	0.024	<LOD	0.069	2	0.055	0.055	0.046	<LOD	0.087	2	0.023	0.023	0.016	<LOD	0.034
<b>ΣPCB</b>	<b>10</b>	<b>1.2</b>	<b>1.1</b>	<b>0.32</b>	<b>0.7</b>	<b>1.8</b>	<b>10</b>	<b>1.5</b>	<b>1</b>	<b>1</b>	<b>0.68</b>	<b>3.4</b>	<b>10</b>	<b>1.4</b>	<b>1.2</b>	<b>0.96</b>	<b>0.76</b>	<b>4</b>	<b>3</b>	<b>0.8</b>	<b>0.3</b>	<b>0.9</b>	<b>0.26</b>	<b>1.8</b>
BDE-47	10	0.087	0.084	0.03	0.043	0.16	10	0.07	0.038	0.098	0.02	0.34	10	0.071	0.069	0.033	0.02	0.13	2	0.026	0.026	0.0052	0.023	0.03
BDE-99	0					0.031	2	0.12	0.12	0.11	<LOD	0.19	2	0.08	0.08	0.086	<LOD	0.14	3	0.15	0.091	0.12	0.072	0.28
BDE-100	0						3	0.057	0.054	0.018	<LOD	0.077	1	0.054	0.054	0	<LOD	0.054	1	0.054	0.054	0	0.054	0.054
BDE-153	0						2	0.69	0.69	0.96	<LOD	1.4	0						0					
BDE-154	2	0.023	0.023	0.0085	<LOD	0.029	4	0.091	0.035	0.13	<LOD	0.28	1	0.011	0.011	0	<LOD	0.011	0					
BDE-183	0						1		7.9		<LOD	7.9	1		0.13		<LOD	0.13	0					
<b>ΣBDE</b>	<b>10</b>	<b>0.099</b>	<b>0.091</b>	<b>0.047</b>	<b>0.043</b>	<b>0.22</b>	<b>10</b>	<b>1.1</b>	<b>0.044</b>	<b>3.2</b>	<b>0.032</b>	<b>10</b>	<b>10</b>	<b>0.11</b>	<b>0.079</b>	<b>0.091</b>	<b>&lt;LOD</b>	<b>0.33</b>	<b>3</b>	<b>0.18</b>	<b>0.13</b>	<b>0.11</b>	<b>0.11</b>	<b>0.31</b>
HBCD	1	0.61	0.61	0	<LOD	0.61	1	0.61	0.61	0	<LOD	0.61	1	31	31	0			1	31	31	0	31	31

**Table 3.** Concentrations of POPs on a lipid mass basis (ng/g lm; lipid mass) in fairy tern, sooty tern, common noddy, and feral chicken eggs from St. Brandon's Atoll. The number of samples with quantifiable amounts (Pos) is presented in the left-hand columns.

	Fairy tern (n=10)						Sooty tern (n=10)						Common noddy (n=10)						Chicken (n=3)					
	Pos	Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max	Pos	Mean	Median	SD	Min	Max
<b>HCB</b>	<b>10</b>	<b>2.8</b>	<b>2.7</b>	<b>1.1</b>	<b>1.5</b>	<b>5.2</b>	<b>10</b>	<b>1.2</b>	<b>1.1</b>	<b>0.43</b>	<b>0.53</b>	<b>1.7</b>	<b>10</b>	<b>1.5</b>	<b>1.4</b>	<b>0.48</b>	<b>0.78</b>	<b>2.3</b>	<b>3</b>	<b>0.72</b>	<b>0.66</b>	<b>0.16</b>	<b>0.6</b>	<b>0.91</b>
bHCH	10	1.5	1.4	0.52	0.98	2.6	10	0.72	0.73	0.25	0.35	1	10	1.5	0.87	1.9	0.51	6.7	3	1.2	0.43	1.5	0.26	3
yHCH	10	0.014	0	0.029	< LO D	0.07	6	0.0058	0	0.014	< LO D	0.035	6	0.0074	0	0.018	< LO D	0.045	1	0.052	0.052	0	0.052	0.052
<b>ΣHCH</b>	<b>10</b>	<b>1.5</b>	<b>1.5</b>	<b>0.52</b>	<b>0.98</b>	<b>2.6</b>	<b>10</b>	<b>0.72</b>	<b>0.73</b>	<b>0.25</b>	<b>0.35</b>	<b>1</b>	<b>10</b>	<b>1.5</b>	<b>0.9</b>	<b>1.9</b>	<b>0.51</b>	<b>6.7</b>	<b>3</b>	<b>1.2</b>	<b>0.49</b>	<b>1.5</b>	<b>0.26</b>	<b>3</b>
oxychlorodane	10	0.092	0.12	0.066	<LOD	0.17	8	0.12	0.12	0.044	0.066	0.22	8	0.11	0.093	0.048	0.067	0.22	0					
Cis-chlorodane	10	0.31	0.29	0.16	<LOD	0.57	10	0.28	0.22	0.12	0.15	0.43	10	0.14	0.13	0.051	0.087	0.22	1	0.11	0.11	0	0.11	0.11
Trans-nonachlor	10	0.17	0	0.32	<LOD	1	10	0.4	0.21	0.42	<LOD	1	7	0.13	0.18	0.13	<LOD	0.32	1	0.15	0.15	0	0.15	0.15
<b>ΣCHLs</b>	<b>10</b>	<b>0.57</b>	<b>0.56</b>	<b>0.48</b>	<b>&lt;LOD</b>	<b>1.8</b>	<b>10</b>	<b>0.78</b>	<b>0.51</b>	<b>0.56</b>	<b>0.17</b>	<b>1.5</b>	<b>10</b>	<b>0.32</b>	<b>0.33</b>	<b>0.15</b>	<b>0.088</b>	<b>0.6</b>	<b>3</b>	<b>0.087</b>	<b>0.11</b>	<b>0.077</b>	<b>&lt;LOD</b>	<b>0.15</b>
p,p'-DDE	10	2.5	2.5	0.81	1.5	3.9	10	4.2	3.5	2.6	2.5	11	10	2.7	2.9	0.6	1.8	3.5	3	4.8	4.9	1.3	3.4	6
p,p'-DDD	0						2	0.21	0.21	0.066	0.16	0.26	1	0.13	0.13	0	0.13	0.13	2	1.5	1.5	0.94	0.87	2.2
p,p'-DDT	10	0.45	0.44	0.3	0.097	1	10	0.22	0.26	0.096	0.076	0.3	10	0.22	0.2	0.1	0.078	0.42	2	3.5	3.5	0.49	3.2	3.9
<b>ΣDDT</b>	<b>10</b>	<b>2.9</b>	<b>3</b>	<b>1.1</b>	<b>1.6</b>	<b>4.9</b>	<b>10</b>	<b>4.5</b>	<b>3.6</b>	<b>2.6</b>	<b>3</b>	<b>12</b>	<b>10</b>	<b>3</b>	<b>3.1</b>	<b>0.65</b>	<b>1.9</b>	<b>3.7</b>	<b>3</b>	<b>8.1</b>	<b>8.1</b>	<b>2.6</b>	<b>5.6</b>	<b>11</b>
<b>Mirex</b>	<b>10</b>	<b>2.6</b>	<b>3.1</b>	<b>1.6</b>	<b>0.23</b>	<b>4.6</b>	<b>10</b>	<b>1.1</b>	<b>0.92</b>	<b>0.8</b>	<b>0.17</b>	<b>2.2</b>	<b>10</b>	<b>1.5</b>	<b>1.7</b>	<b>0.95</b>	<b>0.17</b>	<b>2.7</b>	<b>3</b>	<b>0.34</b>	<b>0.22</b>	<b>0.23</b>	<b>0.2</b>	<b>0.6</b>
CHB-26	10	0.47	0.43	0.14	0.34	0.77	10	0.65	0.58	0.22	0.39	0.91	4	0.13	0.13	0.017	0.11	0.15	0					
CHB-40	10	0.6	0.6	0.17	0.4	0.88	10	0.55	0.5	0.28	0.23	0.91	10	0.3	0.29	0.07	0.21	0.4	0					
CHB-41	10	0.38	0.35	0.12	0.26	0.66	10	0.21	0.2	0.081	0.12	0.33	10	0.15	0.15	0.028	0.12	0.2	0					
CHB-50	10	1.4	1.4	0.49	0.77	2.3	10	1.2	1.2	0.49	0.42	2.2	9	0.74	0.69	0.17	0.52	0.99	0					
CHB-62	3	1	0.98	0.39	0.62	1.4	1	0.47	0.47	0	0.47	0.47	0						0					
<b>ΣCHB</b>	<b>10</b>	<b>3.2</b>	<b>2.9</b>	<b>0.73</b>	<b>2.3</b>	<b>4.6</b>	<b>10</b>	<b>2.7</b>	<b>2.3</b>	<b>0.84</b>	<b>1.6</b>	<b>4.1</b>	<b>10</b>	<b>1.2</b>	<b>1.2</b>	<b>0.33</b>	<b>0.48</b>	<b>1.6</b>	<b>3</b>	<b>0.049</b>	<b>0</b>	<b>0.085</b>	<b>0</b>	<b>0.15</b>
PCB-28	2	0.076	0.076	0.018	0.064	0.089	3	0.36	0.1	0.46	0.097	0.89	6	0.15	0.14	0.054	0.089	0.22	2	0.12	0.12	0.056	0.084	0.16
PCB-52	9	0.39	0.32	0.3	0.13	1.1	8	0.52	0.55	0.44	0.093	1.4	8	0.3	0.33	0.12	0.16	0.46	2	0.31	0.31	0.079	0.26	0.37
PCB-118	2	0.22	0.22	0.034	0.19	0.24	5	0.39	0.43	0.11	0.22	0.51	3	0.36	0.22	0.24	0.22	0.63	2	0.23	0.23	0.17	0.1	0.35
PCB-153	10	1.1	1.3	0.44	0.55	1.6	10	1.5	0.89	1.1	0.63	3.5	10	1.2	0.86	1.1	0.58	4.3	3	0.88	0.24	1.2	0.19	2.2
PCB-105	10	0.32	0.31	0.086	0.18	0.47	10	0.26	0.27	0.037	0.2	0.31	10	0.22	0.19	0.081	0.15	0.42	1	0.18	0.18	0	0.18	0.18
PCB-138	10	0.6	0.58	0.26	0.29	0.94	10	0.75	0.53	0.49	0.29	1.6	10	0.58	0.43	0.53	0.22	2.1	3	0.51	0.19	0.59	0.15	1.2
PCB-187	10	0.32	0.32	0.091	0.19	0.44	10	0.23	0.15	0.16	0.076	0.53	10	0.26	0.23	0.16	0.1	0.68	1	0.33	0.33	0	0.33	0.33
PCB-183	8	0.29	0.29	0.066	0.17	0.37	8	0.18	0.17	0.084	0.057	0.35	7	0.17	0.18	0.033	0.12	0.22	1	0.12	0.12	0	0.12	0.12
PCB-180	10	1.6	1.4	0.63	0.68	2.5	10	1.1	0.96	0.52	0.77	2.4	10	1.2	1.1	0.54	0.79	2.7	3	0.46	0.099	0.63	0.081	1.2
PCB-194	1	0.13	0.13	0	0.13	0.13	4	0.12	0.12	0.059	0.048	0.18	2	0.15	0.15	0.12	0.067	0.24	2	0.07	0.07	0.056	0.03	0.11
<b>ΣPCB</b>	<b>10</b>	<b>4.6</b>	<b>4.8</b>	<b>1.7</b>	<b>2.4</b>	<b>6.8</b>	<b>10</b>	<b>4.7</b>	<b>3.6</b>	<b>2.5</b>	<b>2.5</b>	<b>9.1</b>	<b>10</b>	<b>4.1</b>	<b>3.4</b>	<b>2.6</b>	<b>2.6</b>	<b>11</b>	<b>3</b>	<b>2.5</b>	<b>1</b>	<b>2.9</b>	<b>0.7</b>	<b>5.9</b>
FBDE-47	10	0.35	0.31	0.17	0.14	0.57	10	0.24	0.14	0.35	0.048	1.2	10	0.22	0.2	0.12	0.054	0.41	2	0.08	0.08	0.028	0.06	0.1
BDE-99	3	0.095	0.089	0.018	0.081	0.11	2	0.39	0.39	0.41	0.1	0.68	2	0.25	0.25	0.27	0.056	0.44	3	0.47	0.24	0.4	0.23	0.93
BDE-100	0						5	0.12	0.1	0.092	0.039	0.27	1	0.17	0.17	0	0.17	0.17	1	0.17	0.17	0	0.17	0.17
BDE-153	0						2	2.4	2.4	3.4	0.039	4.8	0						0					
BDE-154	2	0.085	0.085	0.033	0.062	0.11	4	0.3	0.086	0.47	0.041	1	1	0.033	0.033	0	0.033	0.033	0					
BDE-183	0						1	28	28	0	28	28	1	0.33	0.33	0	0.33	0.33	0					
<b>ΣBDE</b>	<b>10</b>	<b>0.4</b>	<b>0.42</b>	<b>0.21</b>	<b>0.14</b>	<b>0.8</b>	<b>10</b>	<b>3.8</b>	<b>0.17</b>	<b>11</b>	<b>0.1</b>	<b>36</b>	<b>10</b>	<b>0.33</b>	<b>0.25</b>	<b>0.28</b>	<b>0.054</b>	<b>1</b>	<b>3</b>	<b>0.58</b>	<b>0.4</b>	<b>0.4</b>	<b>0.3</b>	<b>1</b>
<b>HBCD</b>	<b>0</b>						<b>1</b>	<b>2.2</b>	<b>2.2</b>	<b>0</b>	<b>2.2</b>	<b>2.2</b>	<b>0</b>						<b>1</b>	<b>103</b>	<b>103</b>	<b>0</b>	<b>103</b>	<b>103</b>

## 4. Discussion

### 4.1. Differences in pollutant composition per species

Sooty tern eggs contained the highest mean concentrations of most compounds (15 compounds), followed by fairy tern eggs (eleven compounds), feral chicken eggs (six compounds), and common noddy eggs (four compounds) (Table 2). This is in contrast with the PFAS species composition of St. Brandon's Atoll, where fairy tern eggs contained the highest concentrations of most PFAS compounds, followed by sooty terns and common noddies (van der Schyff *et al.*, 2020c).

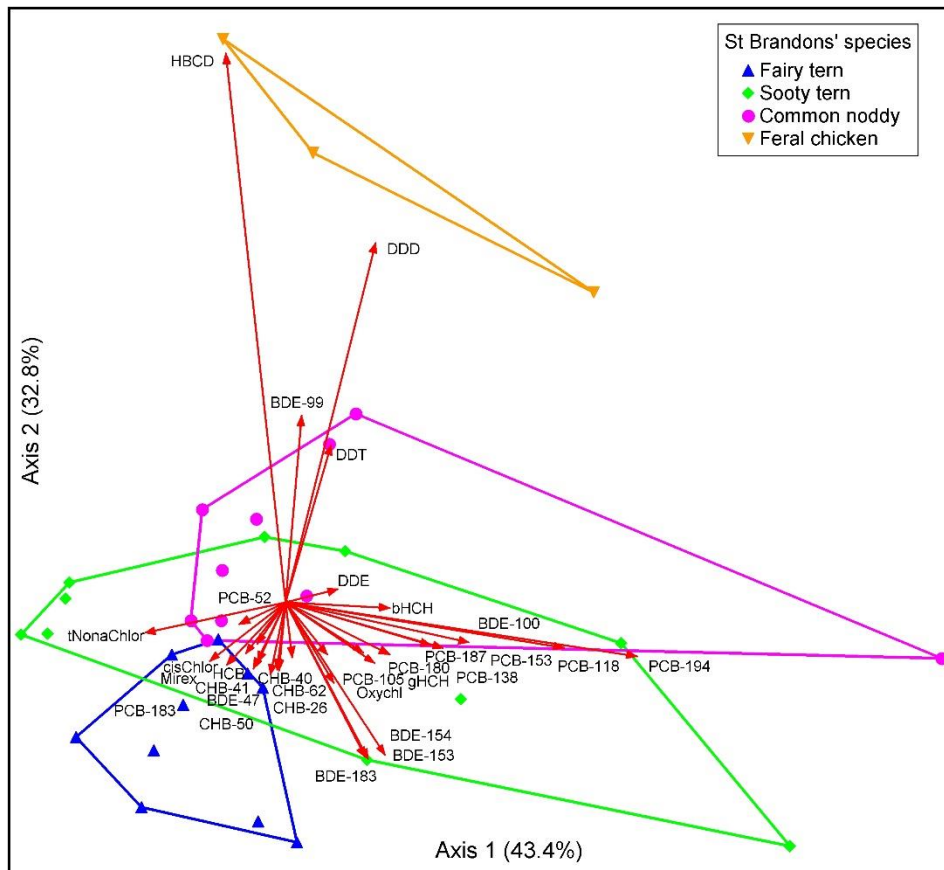
All three seabird species are of similar size and mass, with a mostly overlapping diet (Carthy *et al.* 2009). Flying fish, fish larvae, and squid make up the bulk of the diets, but the ratios differ between species and breeding seasons (Carthy *et al.* 2009; Shealer, 2001). The three species also have comparable lifespans, with the maximum recorded age of common noddies being 27, sooty terns being 36, and fairy terns living up to 37 years (Schreiber & Burger, 2001).

POPs concentrations increase as lipid concentrations decrease (Alava *et al.*, 2006). Sooty tern eggs from Rodrigues had 17% more lipids than common noddies from the same island (Bouwman *et al.*, 2012). This was ascribed to the fact that sooty terns have a broader foraging range, and the higher lipid percentage was necessary to accommodate more extended periods away from the nest (Bouwman *et al.*, 2012). The same pattern was not reflected in the eggs collected from St. Brandon's Atoll. Common noddies and sooty tern eggs had 12% and 11% lipid content, respectively, while fairy tern eggs contained the lowest lipid percentage at 9.7%. Embryo development affects the lipid composition of the egg (Romanoff, 1932). One explanation of the different lipid concentrations in the eggs could be that the species were at different phases of their respective breeding seasons when sampling occurred. For this reason, we mainly base our statistics and discussion of concentrations on a wet mass basis.

Fig. 4 illustrates the non-metric multi-dimensionally scaled (NMS) ordination of the POPs in the eggs from the four bird species from St. Brandon's Atoll. The final stress of the graph was 9.065, indicating high confidence in the patterns derived by the ordination (McCune & Grace, 2002). The convex hull (fingerprint) representing sooty terns overlapped substantially with the hulls of both fairy terns and common noddies. However, the hulls of the common noddy and fairy tern did not overlap due to different relative compositions of POPs, suggesting different compositions in their prey. Feral chickens ordinated separate from any of the seabird species. The convex hulls representing sooty tern and common noddy eggs were relatively large, compared with that of the fairy tern eggs. This indicates greater chemical variability in the eggs from these species, while fairy terns had a relatively smaller variability. Most vectors

representing chemical compounds ordinated in the direction of the convex hulls representing fairy terns and sooty terns. The vectors representing HBCD, p,p'-DDD, and BDE-99 distinctly ordinated towards the convex hull representing feral chicken eggs. The differences in the vectors further support differences of POPs in compositions of their respective prey.

One biological factor that may explain the ordination seen in the NMS graph (Fig. 4) is the different foraging ranges of the seabird species that likely represent different POPs compositions in their respective prey. Sooty terns can forage over 2,000 km from their breeding colony (Jaeger *et al.*, 2017). Approximately one month before breeding, sooty terns from the Seychelles embark on a foraging exodus to the Saya de Malha Bank in the Mascarene Basin (Jaeger *et al.*, 2017). If sooty terns from the Seychelles preferred the Saya de Malha Bank to closer foraging sites, it is possible that the sooty terns from St. Brandon's Atoll could embark on a similar journey to the same area. Fairy terns forage approximately 300 km from their breeding locality (Shealer, 2001). It is possible that fairy terns from St. Brandon's Atoll forage on the western edge of the Saya de Malha Bank, as indicated by the congruent POPs composition seen in the fairy terns and sooty terns from St. Brandon's Atoll (Figs. 3 and 4). This may be proven through isotopic analyses as the topic for future studies. Common noddies, on the other hand, rarely venture further than 50 km offshore (Shealer, 2001; Carthy *et al.*, 2009). From Fig. 4, one may deduce that most of the pollutant congeners that common noddies are exposed to are located at offshore foraging ranges.



**Fig. 4.** Non-metric multidimensional scaled graph (NMS) ordination of the relative contributions of POPs in seabird eggs from St. Brandon's Atoll. The convex hulls represent eggs of different species sampled from the atoll system. Axis 1 explained 43.4% ordination, and Axis 2, 32.8%. The final stress was 9.065, and the final instability was < 0.0001.

Three feral chicken eggs collected from *Ile du Sud* on St. Brandon's Atoll (Fig. 2) served as a terrestrial reference. These eggs contained very low PBDE concentrations. No toxaphene congeners were quantifiable. However, most PCB and organochlorine compounds were present above their LO Qs. It was particularly interesting to find that mirex was present in all these samples. *p,p'*-DDD, the anoxic breakdown product of *p,p'*-DDT was also present in two of the eggs. One egg contained 30.86 ng/g wm of HBCD. It is supposed that the chickens are exposed to POPs either directly eating beached plastics to which the POPs are adsorbed (Lwanga *et al.*, 2017) or through rainwater containing volatile POPs compounds, such as PCB-28 and -52 (Hamers *et al.*, 2001; Wurl & Obbard, 2005). This may explain the different relative POPs compositions from the terns that get their food directly from the ocean.



#### 4.2. Potential sources of pollutants

Several micro- and mesoplastic particles were seen in several fairy tern and common noddy nests during an expedition to St. Brandon's Atoll in 2014 (Bouwman *et al.*, 2016; van der Schyff *et al.*, 2020c). Most of the plastic debris located on the islands of St. Brandon's Atoll originate from other countries (based on product brands) and are transported to the islands by means of long-range transport via ocean currents (Bouwman *et al.*, 2016). Due to the hydrophobic properties of POPs, the chemicals tend to be absorbed by or adsorbed to plastic particles and transported to areas far from their area of origin (Ogata *et al.*, 2009). The concentrations of compounds such as DDT and PCBs can be an order of magnitude higher in a plastic particle than in the surrounding water (Mato *et al.*, 2001).

Seabirds are particularly vulnerable to POPs bioaccumulation due to their high trophic position in the marine food web (Dias *et al.* 2018). A study in Hawaii revealed that fairy terns, sooty terns, and common noddies had plastic particles in their guts, albeit in lower concentrations than other marine bird species (Sileo *et al.*, 1989). Similarly, 15% of sooty terns from a study conducted on Réunion Island had plastic in their gut. Interestingly, only fibres were found in the gut contents of sooty terns from the western Indian Ocean (Cartaud *et al.*, 2019). This was not entirely unexpected as microplastic ingestion by marine fish is considered a common occurrence (Lusher *et al.*, 2013), and microplastic and the pollutants associated therewith can be transported via trophic transfer from a prey item to predators, such as terns (Carbery *et al.*, 2018).

In addition to catching prey in flight (flying fish), the seabirds in this study hunt by "dipping"—snatching prey from the sea surface while in flight (Shealer, 2001). The sea surface microlayer is an area of POPs enrichment compared with the underlying water column (Wurl & Obbard, 2005). The birds may, therefore, be exposed to POPs not only through predation but also through direct uptake from the surface layer of the ocean.

Elevated concentrations of PCBs and HCB are found in ship-breaking areas in Bangladesh (Nøst *et al.*, 2015). Several shipwrecks are in various states of breakdown on the reef crest of St. Brandon's Atoll (van der Schyff *et al.*, 2020b) and are possibly a more localised source of contamination to the atoll system.

#### 4.3. Comparison with other studies

The concentrations of contaminants in seabird eggs from St. Brandon's Atoll were lower than all other studies in reported literature (Table 4; Bouwman *et al.*, 2012; 2015). The concentrations in these eggs were closely followed by eggs from Rodrigues Island, also in the Mascarene Basin of the western Indian Ocean (Bouwman *et al.*, 2012). This contrasts with high concentrations of various POPs found in brown boobies from Cagarras (Cunha *et al.*, 2012), an island system close to the industrialised city of Rio de Janeiro in the Atlantic Ocean.

The isolated nature of the islands in the Mascarene Basin seems to protect them from exposure to toxic compounds due to distance and concomitant dilution of pollutants (van der Schyff *et al.*, 2020c).

The isolated geographic location of St Brandon's Atoll, therefore, seems to buffer the system from pollutants. Although the African continent may seem like a significant pollution source to the Mauritian Outer Islands, the prevailing oceanic and wind currents in this region are predominantly from the west across the vastness of the central Indian Ocean (Woodberry *et al.*, 1989; New *et al.*, 2007). The warm tropical water of the western Indian Ocean may also provide an additional buffer against POPs accumulation. It is known that biota from the Arctic and Antarctic regions are more susceptible to POPs accumulation than biota from the temperate and tropic regions (Borgå *et al.*, 2004). Increased lipids in cold-water zooplankton and other cold-water biota facilitate greater biomagnification of POPs through trophic levels (Borgå *et al.*, 2004; Sobek *et al.*, 2010). Lower seawater temperature is also thought to favour thermodynamically passive partitioning of POPs to organic matrices (Sobek *et al.*, 2010). Biota from sub-tropical regions, such as the Mascarene Basin, are exempted from these phenomena as relatively higher water temperatures do not necessitate a higher lipid content required for warmth. Thus, the abiotic factors of the tropical ecosystem result in relatively less POPs partitioning in biota. This may be why birds from regions almost as geographically isolated as St. Brandon's Atoll, such as Antarctica, contain higher POPs concentrations than those found in this study (Corsolini *et al.*, 2011).

The oldest study to report POPs concentrations in terns from St. Brandon's Atoll was conducted in 1975 (Bourne *et al.*, 1977). They reported 5 n/g ww *p,p'*-DDE in the eggs of fairy terns that were collected from St. Brandon's Atoll in 1975. DDT was widely used since the early 1960s (Maharaj *et al.*, 2005), which could explain the high concentration of the *p,p'*-DDE metabolite in terns from St. Brandon's Atoll at that time. The current concentrations, measured in eggs that were collected in 2010, are at least fivefold lower when compared with eggs collected 35 years ago, most likely reflecting a drop in the background concentrations due to the almost complete banning of DDT. The remoteness of this atoll suggests that this and other similar remote sampling sites would be suitable sites from which to monitor global background concentrations in the tropics, akin to remote sites in the polar regions.

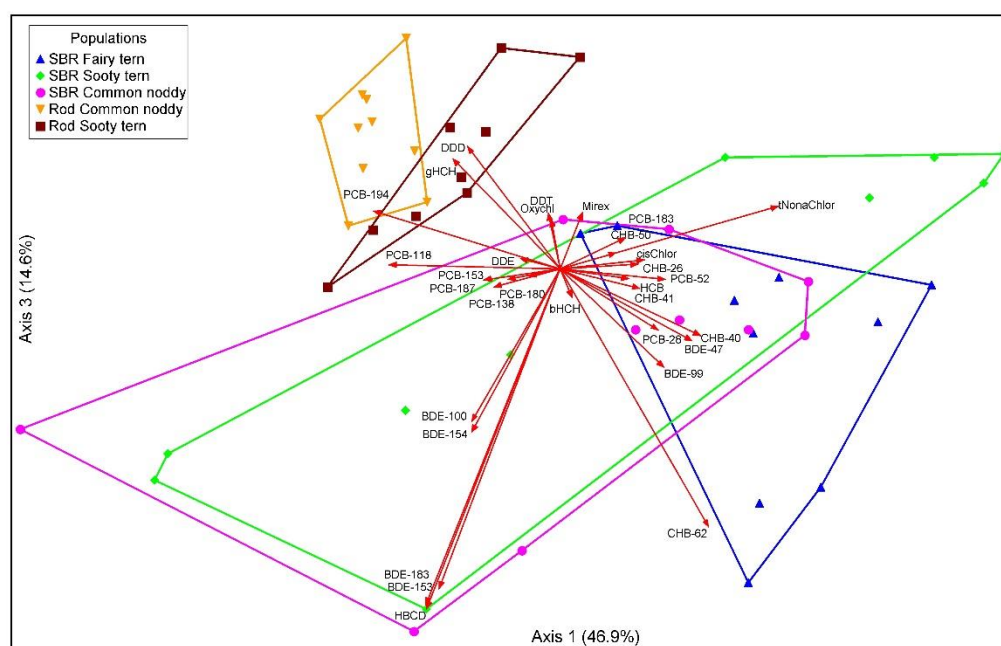
The terrestrial species in this study, feral chickens, exceeded the concentrations of several POPs compared with common noddies (Table 2). Rats, mice, and various reptile species are present on most of the islands in the Mascarene Basin (Cole *et al.*, 2005; Russell *et al.*, 2016). However, no studies have thus far focussed on the concentration of POPs in terrestrial wildlife in the Mascarene Basin. This is a vital gap in the scientific literature.

**Table 4.** POPs concentrations (ng/g wm) in piscivorous marine bird eggs in studies from the past ten years. Older publications on POPs in birds are referenced in tables in Bouwman *et al.* (2012; 2015). Concentrations found in this study are indicated in bold.

Species	N	Locality	Collected	HCB	ΣHCH	ΣCHL	p,p'-DDE	p,p'-DDT	ΣDDT	Mirex	ΣPCB	ΣBDE	Reference
Common noddy	10	Rodrigues	2008	0.39	0.26	0.09	1.7	0.21	1.9	0.96	2.2	0.08	Bouwman <i>et al.</i> (2012)
<b>Common noddy</b>	<b>10</b>	<b>St. Brandon's Atoll</b>	<b>2010</b>	<b>0.47</b>	<b>0.5</b>	<b>0.1</b>	<b>0.89</b>	<b>0.072</b>	<b>0.97</b>	<b>0.46</b>	<b>1.4</b>	<b>0.11</b>	<b>This study</b>
Sooty tern	10	Rodrigues	2008	0.41	0.22	0.15	2.8	0.18	3.1	0.69	2.6	0.07	Bouwman <i>et al.</i> (2012)
<b>Sooty tern</b>	<b>10</b>	<b>St. Brandon's Atoll</b>	<b>2010</b>	<b>0.33</b>	<b>0.21</b>	<b>0.21</b>	<b>1.4</b>	<b>0.64</b>	<b>1.5</b>	<b>0.28</b>	<b>1.5</b>	<b>1.1</b>	<b>This study</b>
<b>Fairy tern</b>	<b>5</b>	<b>St. Brandon's Atoll</b>	<b>2010</b>	<b>0.68</b>	<b>0.39</b>	<b>0.31</b>	<b>0.63</b>	<b>0.11</b>	<b>0.74</b>	<b>0.61</b>	<b>1.2</b>	<b>0.099</b>	<b>This study</b>
Arctic tern	6	Iceland	2002/2004	5.7	0.65	0.82	28.2				56		Jörundsdóttir <i>et al.</i> (2010)
Adélie penguin	37	Antarctica (East)	2003/2005	4.7			4.3				22		Corsolini <i>et al.</i> (2011)
Adélie penguin	9	Antarctica (West)	Unclear	7.6	1.8		19	0.49			12		Corsolini <i>et al.</i> (2011)
African penguin	10	Robben island	2011	2	2	0.62	25	0.27	25	0.3	42	0.34	Bouwman <i>et al.</i> (2015)
African penguin	10	Bird island	2012	2.2	2.2	0.78	50	0.9	51	1.1	64	0.6	Bouwman <i>et al.</i> (2015)
Brown booby	8	St Peter and St Paul (Brazil)	2007				10		10		50		Cunha <i>et al.</i> (2012)
Brown booby	8	Abrolhos (Brazil)	2007				20		30		20		Cunha <i>et al.</i> (2012)
Brown booby	8	Cagarras (Brazil)	2007				1020		1800		8400		Cunha <i>et al.</i> (2012)
Herring gull	10	Sweden	2008					19.5			205	8.3	Carlsson <i>et al.</i> (2011)
Herring gull	10	Norway (Strömstad)	2008						19.5		205.4	8.3	Carlsson <i>et al.</i> (2011)
Audouin's gull	18	Chafarina Islands	2007									9.72	Roscales <i>et al.</i> (2016)
Yellow-legged gull	19	Chafarina Islands	2007									6.52	Roscales <i>et al.</i> (2016)
Yellow-legged gull	10	Chafarina Islands	2016	0.82			61.7	0.19					Zapata <i>et al.</i> (2018)
Yellow-legged gull	36	Atlantic Islands of Galicia	2016	3.61			31.4	0.13					Zapata <i>et al.</i> (2018)
Common eider	10	Norway (Strömstad)	2008						3.8		11.4	1.4	Carlsson <i>et al.</i> (2011)
Common eider	10	Sweden	2008					20.1			108	0.6	Carlsson <i>et al.</i> (2011)
Magnificent frigate bird	5	West Indies (Barbuda)	2010		0.42	1.95				26.14	2.83	110.62	Trefry <i>et al.</i> (2013)

#### 4.4. Focus on the Mascarene Basin

Because of the comparability of this study with Bouwman *et al.* (2012), it was possible to directly compare datasets on an NMS graph (Fig. 5). The eggs in Bouwman *et al.* (2012) were collected from the same species and analysed for the same compounds at the Norwegian School of Life Sciences. The same analytical protocol and quality assurance were conducted in both studies (Bouwman *et al.*, 2012). The ordination had a final stress of 8.99 and a final instability of <0.0001, making the ordination reliable for accurate interpretation (McCune & Grace, 2002).



**Fig. 5.** Non-metric multidimensional scaled graph (NMS) ordination of the relative compositions of POPs in seabird eggs from St. Brandon's Atoll (SBR) and Rodrigues' (Rod) islands. The convex hulls represent eggs of different species sampled from the two island systems. Axis 1 explained 46.9% ordination, and Axis 3, 14.6%.

Comparing Figs. 4 and 5, species differences in POPs compositions seen in Fig. 4 for St. Brandon's Atoll (non-overlapping hulls for fairy tern and common noddy in Fig. 4) disappears when combined with the data from Rodrigues. In Fig. 5, the two islands separate, but the species from the respective islands are now comparable. The convex hulls representing species from Rodrigues are smaller than from St. Brandon's Atoll, indicating less variability in pollutant concentrations. The NMS ordination of the pollutant composition in sooty terns and common noddies from Rodrigues is included in supplementary Fig. 1 (S2).

There is a clear difference between the convex hulls representing populations from the different islands (Fig. 5). There is enough distance between the two islands to result in compositional differences in POPs composition (non-congruence of convex hulls of populations from different island systems). Species play less of a role than location when considering the POPs compositions, indicating that the two systems, although only 520 km apart, are influenced by oceanographic and biological factors that result in the differences, irrespective of species.

The two islands have vastly different reef systems and sub-oceanic features, such as seamounts and ocean ridges which may cause differentiation in pollutant concentrations and distributions. Rodrigues boasts a well-developed fringe reef, while St. Brandon's Atoll's reef comprises of patchy coral in the lagoon and reef crest (van der Schyff *et al.*, 2020a). Rodrigues has a stable human population of approximately 40 000 people, which may cause a more substantial pollutant influx than in St. Brandon's Atoll, which has a temporal occupation of about 40 people (Bouwman *et al.*, 2012; van der Schyff *et al.*, 2020a). More PCB congeners were quantifiable in the eggs from Rodrigues (n=19; Bouwman *et al.*, 2012) than in the eggs from St. Brandon's Atoll (n=10). However, it is interesting that the majority of the pollutant vectors ordinated towards St. Brandon's Atoll. The vectors represent relativised concentrations, therefore not absolute concentrations. This means that compounds such as *p,p'*-DDD and  $\gamma$ -HCH occurred in higher proportions in eggs from Rodrigues than eggs from St. Brandon's Atoll. Plastic-associated compounds occurred in relatively higher proportions in St. Brandon eggs. One reason might be that St. Brandon's Atoll has more plastic debris on the beaches and reef system than Rodrigues (Bouwman *et al.*, 2016). Another explanation may be attributed to the prevalence of the South Equatorial Current that affects St. Brandon's Atoll, but not Rodrigues. This current passes Australia and the Indo-Pacific islands, where it may accumulate pollutants in the industrialised countries and transport it to oceanic islands (Schott & McCreary Jr., 2001). Another explanation may be that the terns from St. Brandon's Atoll forage in the major shipping lanes between South Africa and India, where shipping activity may be a pollution source in the open ocean (Jaeger *et al.*, 2017), while Rodrigues has less shipping traffic in the surrounding waters. However, eggs from both islands have some of the lowest POPs concentrations in reported literature (Table 4).

All terns in this study have a commensal relationship with subsurface predators, such as tuna. Tuna from the western Indian Ocean region have been analysed for the presence of several POPs congeners (Ueno *et al.*, 2004; 2005). Tuna collected from the western Indian Ocean (Seychelles and Réunion) contained lower concentrations of PCBs, BDEs, and organochlorines than tuna collected anywhere else globally. Because terns and tuna largely share prey populations, it can be surmised that POPs are present in relatively low concentrations in the avian food web of the western Indian Ocean.

## **5. Conclusion**

In St. Brandon's Atoll, sooty tern eggs contained the highest mean concentrations of most POPs, followed by fairy terns, feral chickens, and common noddies. POPs were present in the terrestrial environment of St. Brandon's Atoll, as indicated by POPs concentrations of feral chicken eggs. This implies long-range transport of POPs to remote islands through rainfall and plastic as vectors. It is proposed that the differences in species composition between seabird species eggs can primarily be ascribed to the differences in foraging ranges, and therefore different exposures from different foraging zones. Fairy terns and sooty terns forage further offshore and are seemingly exposed to a higher concentration of most congeners, while common noddies (nearshore foragers) contain lower concentrations of most POPs. The concentrations of POPs found in this study are lower than any other reported in literature. The next lowest concentrations were found in sooty terns and common noddies from Rodrigues, also located in the Mascarene Basin. The isolated nature of the island systems, some abiotic characteristics of tropical islands, and oceanographic features of the western Indian Ocean may explain the lower concentrations of POPs when compared with other regions. The remoteness of the islands also suggests that these are suitable localities from which to monitor global background trends of POPs in the tropics.

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## Supplementary materials

### Persistent Organic Pollutants in sea bird eggs from the Indian Ocean's Mascarene Basin

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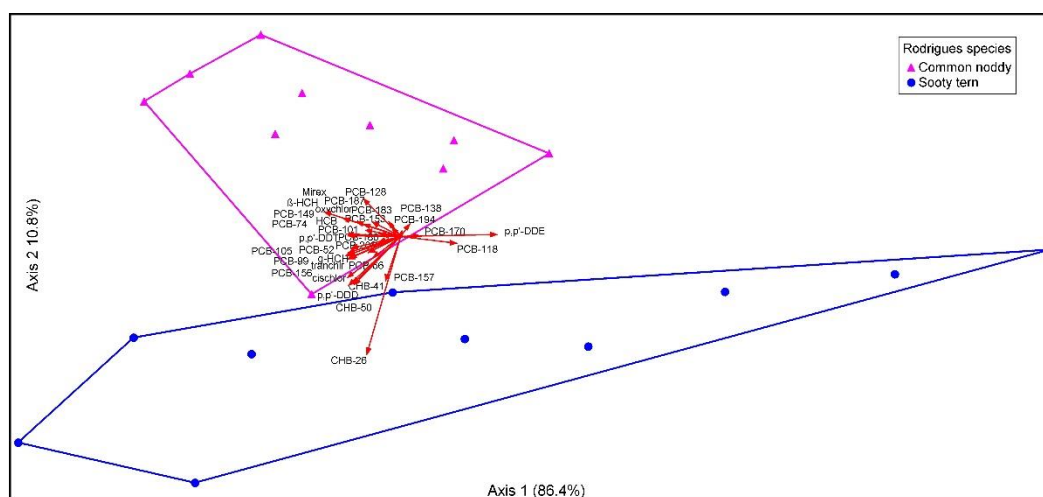
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### Supplemental Materials



**Fig. S1.** NMS biplot of related compounds and individual eggs of sooty terns and common noddies collected from Rodrigues Island in 2010. The final stress was 6.46 for the two-dimensional solution. Axis 1 explains 86% of the ordination, and Axis 2 10.8%, for a cumulative of 97.2% (Bouwman *et al.*, 2012).

**Table S1.** The lowest level of detection (LOD), relative recovery percentage (RR (%)), and detection frequency (DF (%)) of compounds analysed during this study

	LOD	RR (%)	DF (%)
HCB	0.015	109	100
$\beta$ -HCH	0.015	109	100
$\gamma$ -HCH	0.011	86	15.2
oxychlordane	0.025	98	69.7
<i>cis</i> -chlordane	0.025	104	91
<i>trans</i> -nonaklor	0.028	80	48.5
<i>p,p'</i> -DDE	0.016	100	100
<i>p,p'</i> -DDD	0.025	104	15.2
<i>p,p'</i> -DDT	0.019	113	97
Mirex	0.03	107	100
PCB-28	0.029	100	39.4
PCB-52	0.034	95	81.8
PCB-74	0.036	175	0
PCB-101	0.033	110	0
PCB-99	0.044	113	0
PCB-149	0.028	114	0
PCB-118	0.03	118	36.4
PCB-153	0.034	122	100
PCB-105	0.02	106	94
PCB-138	0.025	109	100
PCB-187	0.018	127	94
PCB-183	0.021	120	72.7
PCB-180	0.021	108	100
PCB-194	0.019	98	27.3
BDE-28	0.015	91	72.7
BDE-47	0.2	89	97
BDE-99	0.035	106	30.3
BDE-100	0.03	106	21
BDE-153	0.3	102	6.1
BDE-154	0.02	105	21.2
BDE-183	0.03	108	6.1
HBCD	0.06	88	6.1
CHB-26	0.06	96	0
CHB-40	0.04	110	90.9
CHB-41	0.03	101	90.9
CHB-50	0.055	87	90.9
CHB-62	0.24	108	12.1

## Chapter 5. Perfluoroalkyl substances (PFAS) in tern eggs from St. Brandon's Atoll, Indian Ocean

### Foreword to Article 4: "Perfluoroalkyl substances (PFAS) in tern eggs from St. Brandon's Atoll, Indian Ocean."

Brominated and chlorinated pollutants (as quantified in Chapter 4) are but two classes of pollutants that are present in the environment. Other halogenic compounds, such as fluorinated compounds can also adversely affect biota. Certain perfluoroalkyl substances, such as PFOA and PFOS, are recognised as POPs by the Stockholm Convention (Stockholm Convention, 2019c). A unique feature of PFAS is the fact that it is water-soluble due to its molecular structure (McCarthy *et al.*, 2017). The fact that the compounds are water-soluble can lead to compounds accumulating in the open ocean (Yamashita *et al.*, 2008). Little is known regarding the environmental fate of the PFAS compounds—for example, if the compounds are reconcentrated around oceanic islands? In order to determine whether PFAS are present in the island ecosystems of the WIO, fairy tern, sooty tern, and common noddies eggs were analysed for PFAS compounds. This article showcases the first data of PFAS in biota from the Mascarene Basin.

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## Perfluoroalkyl substances (PFAS) in tern eggs from St. Brandon's Atoll, Indian Ocean

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### Abstract

Per- and polyfluoroalkyl substances (PFAS) are anthropogenic fluorinated compounds of concern for human and environmental health. There is no data on PFAS concentrations in marine bird eggs from the Western Indian Ocean. We analysed eight PFAS in eggs of fairy terns (*Gygis alba*), sooty terns (*Sterna fuscata*), and common noddies (*Anous stolidus*) from St. Brandon's Atoll. Fairy tern eggs contained the highest concentrations, followed by sooty terns and common noddies. Perfluoroundecanoic acid (PFUdA) had the highest mean concentration (2.3 ng/g wm), followed by perfluorooctane sulfonic acid (PFOS) (2.0 ng/g wm), and perfluorononanoic acid (PFNA) (0.93 ng/g wm) in fairy tern eggs. Concentrations of all PFAS were lower than values found in literature. PFOS and PFOA concentrations were three orders of magnitude lower than toxicity reference values and levels of lowest-observed-adverse-effect-level concentrations. Eggs from St. Brandon's would be useful to monitor background changes on a regional and perhaps global scale.

**Keywords:** Biomonitoring; Perfluoroalkyl acids; Perfluorooctane sulfonate (PFOS); Seabird eggs; western Indian Ocean



## 1. Introduction

Per- and polyfluoroalkyl substances (PFAS) have gained international awareness after being detected and quantified in major cities' drinking water, food, and in an assortment of wildlife all over the globe (Bossi *et al.*, 2005; McCarthy *et al.*, 2017; Sedlak *et al.*, 2017; Kaboré *et al.*, 2018; Boone *et al.*, 2019). PFAS are anthropogenically produced fluorinated compounds (Buck *et al.*, 2011). PFAS are used in a variety of commercial products, such as anti-stick coating on appliances, carpets, clothes, food containers, firefighting foams, and paper (Konwick *et al.*, 2008; McCarthy *et al.*, 2017) because of its oil- and water repellent characteristics. PFAS are linear or branched carbon chains with fluoride molecules attached thereto with a variety of reactive components at one end such as sulfonic or carboxylic acid (Newman, 2015). The strength of the carbon-fluorine bonds and highly oxidised state make the PFAS molecules difficult to break down chemically, biologically, and thermally (Fair & Houde, 2018; Newman, 2015). The combination of properties makes many PFAS bind strongly to proteins, rather than accumulate in lipids such as many persistent organic pollutants do (POPs; Newman, 2015).

PFAS are long-lived and bioaccumulative in the environment with adverse effects on human and environmental health (Giesy & Kannan, 2002, 2010). Long chained PFAS compounds (compounds with eight or more carbon atoms) are more bioaccumulative than short-chained compounds (Taniyasu *et al.*, 2008). Some PFAS, such as perfluorooctane sulfonic acid (PFOS) and perfluorooctane sulfonic acid (PFOA) are classified as POPs by the Stockholm Convention on Persistent Organic Pollutants, whilst others, such as perfluorohexane sulphonic acid (PFHxS), are under consideration to be listed as a new POP (Stockholm Convention, 2019).

PFAS can either be deposited directly into the environment from industrial activities, or result as a breakdown product of neutral precursors such as fluorotelomer alcohols (FTOH). Compounds within the perfluoroalkyl carboxylic acids (PFCA) group tend to be the main breakdown products of FTOH (Schenker *et al.*, 2008).

The open ocean is often thought to be the final sink for PFOS and PFOA, due to their high water solubility (Yamashita *et al.*, 2008). Despite PFAS being studied in a wide range of biota (e.g. Giesy & Kannan, 2002; Braune & Letcher, 2013; Grønnestad *et al.*, 2016; Lesch *et al.*, 2017), no literature exists pertaining to PFAS in seabirds from the Western Indian Ocean (WIO), other than in African penguin (*Spheniscus demersus*) eggs from the east coast of South Africa (Bouwman *et al.*, 2015).

The seabird colonies on St. Brandon's Atoll north of Mauritius have attracted scientific interest since the 1970s (e.g. Pocklington *et al.*, 1972; Bourne *et al.*, 1977; Williams &

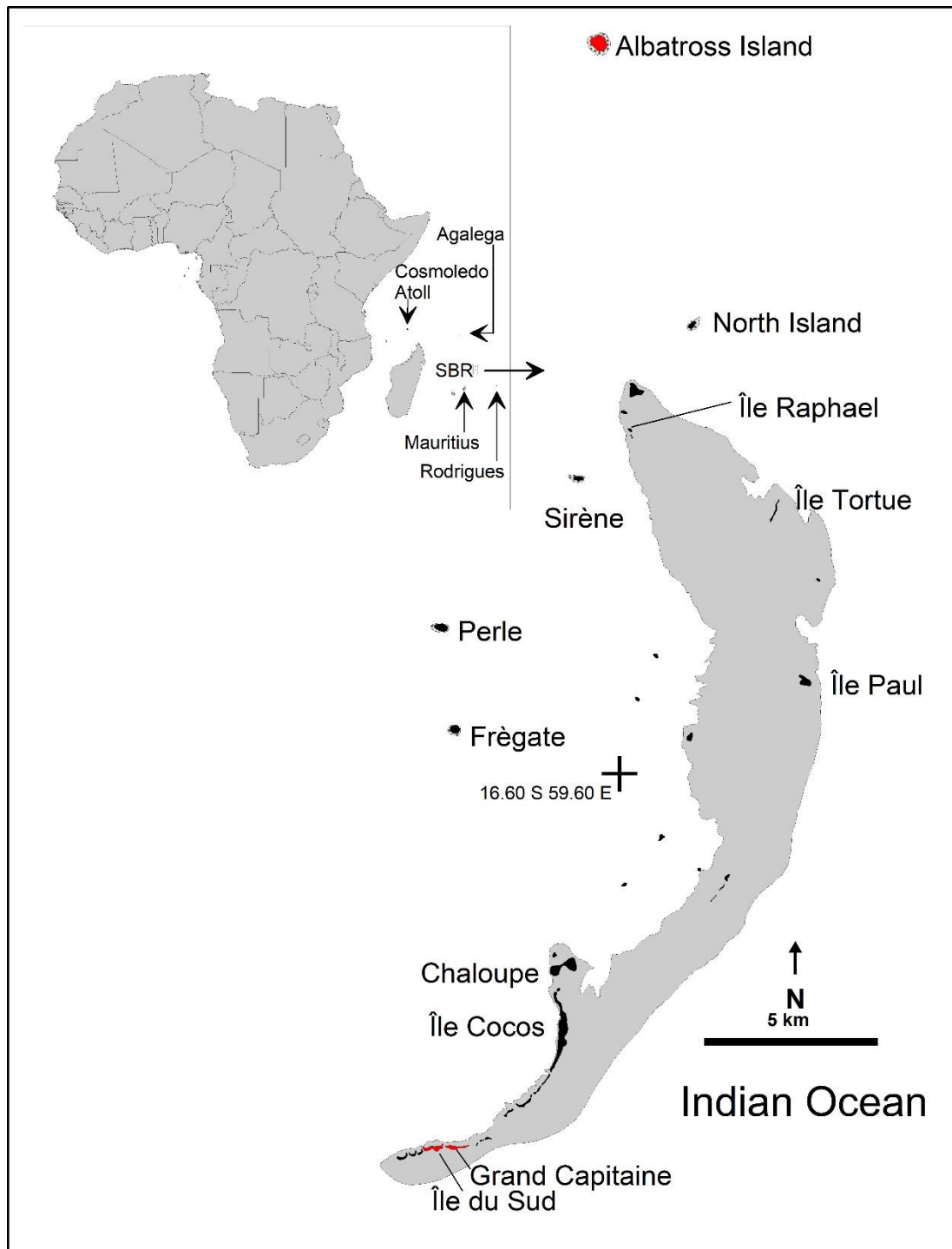
Rowlands, 1980). In 2010, a bird survey found more than one million individuals of seven breeding species (Evans *et al.*, 2016). Seabird guano is known to enhance coral reef functioning and productivity (Graham *et al.*, 2018; Savage, 2019). It is therefore vital to study potential threats to seabirds on coral islands to ensure the wellbeing of the atoll's coral reef system.

The primary aim of this study is to quantify PFAS in the eggs of seabirds breeding on St. Brandon's Atoll, an isolated oceanic system in the WIO (Fig. 1). The secondary aim is to determine whether the PFAS concentrations within the seabird eggs from St. Brandon's might pose a threat to the species studied by comparing concentrations found in this study with toxicity reference values (TRVs). Because St. Brandon's is isolated, we hypothesise that PFAS will occur in low (compared with other studies) but quantifiable concentrations in the eggs of all species sampled. For the same reason, we expect that concentrations will be lower than reported TRV values.

## **2. Materials and Methods**

### *2.1. Site description*

St. Brandon's Atoll is a remote archipelago in the WIO, approximately 450 km northeast of Mauritius, and belongs to the Republic of Mauritius (Fig. 1). St. Brandon's Atoll (also known as St. Brandon, St. Brandon's Island, or St. Brandon's Rock) forms part of the Cargados Carajos Shoal. The archipelago consists of about 24 small, vegetated islands and 19 sandbars, connected by a shallow lagoon covering approximately 200 km<sup>2</sup> (Quod, 1999; Evans *et al.*, 2016) (Fig. 1). There is a flock of feral chickens and a colony of rabbits introduced to *Ile du Sud* (pers. obs. van der Schyff, 2014), and rats and mice on several islands (Feare *et al.*, 2007). There are no permanent residents, but approximately 40 fishermen, National Coast Guard, and Meteorological Services personnel rotate every few months (Bouwman *et al.*, 2016). A few exclusive charters enable recreational fishermen and eco-tourists to visit the atoll. Otherwise, there are no industries on this atoll. Several shipwrecks, in various stages of break-down, are located on the outer reef crest of the atoll (van der Schyff *et al.*, 2020a). St. Brandon's is affected by the South Equatorial Current (Schott & McCreary Jr., 2001), but not by currents from Mauritius, Seychelles, or Madagascar.



**Fig. 1.** Map of St. Brandon's Atoll in the Indian Ocean. Islands and sandbars are black, and the lagoon in grey. Islands that were sampled from are indicated in red.

## 2.2. Species description and collection process

There have been several studies on environmental pollution using bird eggs (e.g. Verreault *et al.*, 2007; Holmström *et al.*, 2010; van der Schyff *et al.*, 2016; Bouwman *et al.*, 2019). Eggs are often easy to collect and handle and can be stored frozen for long periods (Zhang & Ma,

2011). Concentrations of contaminants in bird eggs give a relatively accurate representation of the concentrations present in the mother's body (Ackerman *et al.*, 2016). For these reasons, we chose to collect eggs from three wide-ranging species that breed on the remote St. Brandon's Atoll to use as bio indicators of PFAS pollution in the WIO.

Three tern species (Family Laridae, Subfamily Sterninae) were selected due to their relatively high trophic position in the marine food web and their abundance on St. Brandon's. The population status of all three species are classified as "least concern" (IUCN, 2020a, 2020b, 2020c). Ten eggs from separate nests of fairy terns (also known as white terns) common noddies (also known as brown noddies), and sooty terns were collected in October 2010. More information on these species can be found in Bouwman *et al.* (2012). Eggs were wrapped in pre-cleaned foil (rinsed with hexane and acetone), and frozen individually on the same day. Fairy tern and common noddy eggs were collected from *Grande Capitaine* in the southern cluster area of the atoll (Fig. 1). Sooty tern eggs were collected from Albatross island— the most northern island of the atoll system (Fig. 1). Three eggs of a small, semi-feral flock of chickens (*Gallus gallus*) on *Ile du Sud* were also collected as a 'terrestrial' reference.

The eggs were collected with the appropriate ethics approval from the North-West University (NWU-00055-07-S3). Permission to collect eggs was obtained from two authorities: the National Parks and Conservation Service of the Ministry of Agro Industry, Food Production and Security, Mauritius, and the Outer Islands Development Corporation. Permission for importation of the eggs to Norway was given by the Norwegian Food Safety Authority.

### 2.3. Laboratory analysis

The eggs collected from St. Brandon's were analysed at the Laboratory of Environmental Toxicology at the Norwegian University of Life Sciences. The concentration of eight PFAS were analysed — two PFSA (perfluoroalkyl sulfonic acid) compounds, and six PFCA compounds (Table 1). The samples were analysed following the methodology of Grønnestad *et al.* (2016).

**Table 1.** Abbreviations, full names, carbon chain length, and limit of detection (LOD) concentrations of PFAS compounds analysed during this study.

	Carbon chain length	LOD (ng/g wm)
<b>PFASs</b>	Perfluoroalkyl sulfonic acids	
PFHxS	C6	0.043
PFOS	C8	0.076
<b>PFCAs</b>	Perfluoroalkyl carboxylic acids	
PFOA	C8	0.017
PFNA	C9	0.029
PFDA	C10	0.004
PFUdA	C11	0.004
PFDoA	C12	0.009
PFTriA	C13	0.053

*Internal standards:* 1 mL homogenized egg was weighed in high density plastic Falcon® centrifuge tubes (VWR International, LLC Randor, USA). <sup>13</sup>C-labeled PFAS mix (containing MPFOA, MPFNA, MPFUdA, MPFDoA, MPFOS, and O<sup>18</sup> MPFHxS) (Wellington laboratories) was used as an internal standard (IS). A concentration of 1 µg/mL for PFASs and 500 ng/mL for PFCAs IS were added to the samples to a concentration of 20 ng/mL in the final extracts. No individual IS were available for PFTriA. The relative recovery rate for PFAS in the eggs ranged from 86% to 110%.

*Extraction:* 5 mL methanol was added to each sample (Rathburn chemicals. Walkerburn, Scotland) and shaken for 10 seconds on a Whirlymixer (MS2 Minishaker, IKA®, MA, USA) and mixed for 30 min in a Vibrax machine (Vibrax VXR, IKA®, MA, USA). The samples were then centrifuged for 10 min at 3000 rpm. The supernatant was extracted and added to new Falcon® tubes. The remaining deposits were extracted once again with 5 mL methanol added. This was mixed with a spatula first, and then 10 seconds on the Whirlymixer, and finally mixed for 30 min on the Vibrax machine. The samples were centrifuged again for 10 min at 3000 rpm. The supernatant was extracted and pooled with the supernatant from the first extraction. The total supernatant mixture was evaporated to 2 mL using heat blocks (37°C) under a gentle nitrogen gas flow (Purity: 99.6%, Aga AS, Oslo, Norway).

*Clean-up:* Between 0.2 g and 0.3 g activated coal (ENVI-Carb™, Sigma-Aldrich, Oslo, Norway) was added to each sample. The samples were mixed on the Whirlymixer for 10 seconds and centrifuged at 3500 rpm for 15 min. The supernatant was transferred to new Falcon® tubes. 1 mL methanol was added to the deposit and the clean-up step with activated coal was repeated. The total supernatant was evaporated to dryness on heat blocks (37°C) under a nitrogen gas flow. A 1 mL methanol and water solution (1:1) was added to each

extract, mixed on the vortex mixer, and centrifuged at 3000 rpm for 10 min. The supernatant was transferred to vials with plastic inlets (200  $\mu$ L).

#### 2.4. PFAS quantification

The samples were analysed by high-performance liquid chromatography coupled to tandem spectrometry (HPLC-MS/MS) (API 3000, LC/MS/MS System) using a negative electrospray ionization source. Analyte separation was performed using a Discovery C18 column (150 mm x 2.1 mm, 5  $\mu$ m particle size); a Supelguard Discovery C18 column (20 mm x 2.1 mm, 5  $\mu$ m particle size) was used as a guard pre-column. Both columns were from Supelco (Sigma-Aldrich, Oslo, Norway). Analyte detection was performed using an AB Sciex API 3000 triple quadrupole mass spectrometer. 5  $\mu$ L of the prepared extract was injected.

A calibration curve was constructed with matrix-matched calibrations, created with native analyte concentrations ranging between 0-100 ng/ mL while the isotope labelled IS were kept at a constant concentration of 20 ng/mL. The accepted criterion of the range of coefficient of determination of the calibration curve for different compounds was  $R^2 > 0.995$ . Concentrations were calculated from the chromatographic data using the instrument control and data processing program Analyst<sup>®</sup> Software Version 1.6. The limits of detection (LOD) of the PFAS compounds were derived from three times the signal to noise ratio, unless a higher signal was recorded in the blank sample. The LODs ranged between 0.004 to 0.076 ng/g wm (wet mass). Blank samples were procedural blanks which contained solvents and IS following the entire sample preparation. Blank contributions were subtracted from final concentrations. Table 1 shows the LOD for all PFAS.

#### 2.5. Statistical analysis

We used Graphpad Prism 8.0.2 ([www.graphpad.com](http://www.graphpad.com)) for summary statistics. Because the data sets were not normally distributed, we used Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison tests. Significance was set at  $p < 0.05$ . Outliers were identified using ROUT ( $Q = 1\%$ ). Only one outlier (a common noddy egg) was identified. The data from this egg were excluded from the Kruskal-Wallis test and Dunn's multiple comparisons.

Nonmetric multidimensional scaling (NMS) was conducted to ordinate PFAS compound proportions in different eggs from different bird species using MjM Software PC-ORD 7.03 ([www.pcord.com](http://www.pcord.com)). The data were unrelativised per compound in order to obtain a profile 'fingerprint' of PFAS compounds in each sample. The Sørensen coefficient was used as a relative measurement of distance (McCune & Grace, 2002). Two-hundred and fifty runs of real randomised data were used, with random starting conditions. Monte Carlo tests of significance of each dimensionality to test the probability of stress having been derived by chance were done.

### 3. Results

#### 3.1. PFAS occurrences in bird eggs.

The mean, median, standard deviation, minimum, and maximum concentrations of eight PFAS in eggs from all species are presented in Table 2. Because PFAS have oleophobic and hydrophobic properties as opposed to most POPs that are lipophilic (Newman, 2015), it was opinioned by McCarthy *et al.* (2017) to not calculate PFAS concentrations against lipid mass. We therefore only present the concentrations in ng/g wm (Table 2).

PFOS, PFOA, PFNA, PFDA, and PFUdA were present at quantifiable concentrations in 100% of the samples. The detection frequency of PFHxS was 56%, PFTriA was 94%, and PFDoA was 97%. PFUdA was the compound found at the highest mean concentrations (2.3 ng/g wm), followed by PFOS (2.03 ng/g wm), and PFNA (0.93 ng/g wm). The common noddy egg that was excluded from the statistical analysis as an outlier contained 40.8 ng/g wm PFOA, 32.8 ng/g wm PFNA, and 14.4 ng/g wm PFDA.

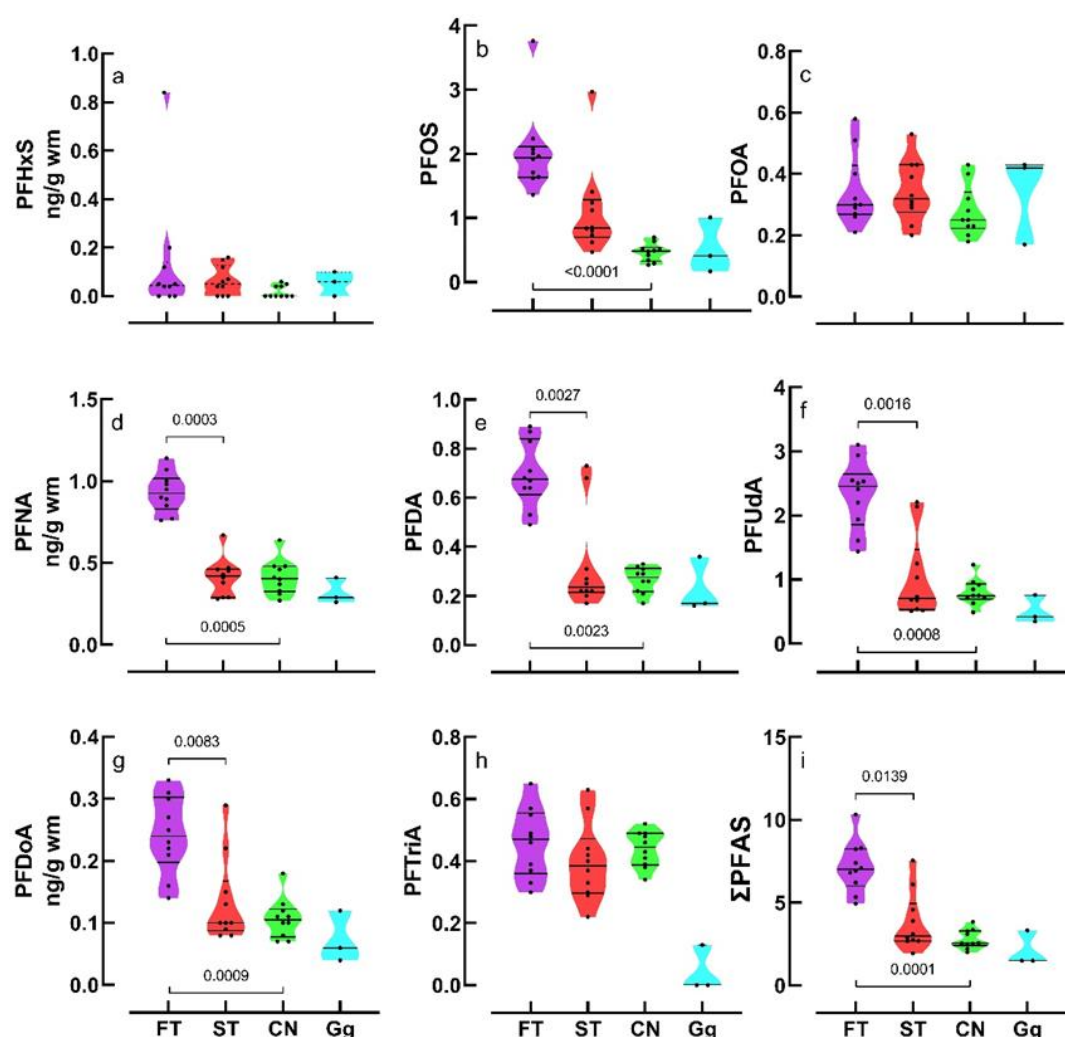
**Table 2.** PFAS concentrations (ng/g wm) in eggs of fairy terns, sooty terns, common noddies, and feral chickens from St. Brandon’s Atoll. Lipid percentages of eggs are indicated.

	Fairy tern (n=10)					Sooty tern (n=10)					Common noddy (n=10)						Feral chicken (n=3)				
	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Mean	Median	SD	Min	Max	Outlier (n=1)	Mean	Median	SD	Min	Max
Lipid %	9.65	9.84	2.04	6.4	13.03	11.11	10.43	2.08	8.17	14.82	11.97	11.87	1.34	9.7	14.82	12.31	11.21	11.69	1.09	9.7	12.25
PFHxS	0.13	0.045	0.24	<LOD	0.84	0.064	0.05	0.058	<LOD	0.16	0.048	0.045	0.0083	<LOD	0.06	<LOD	0.053	0.06	0.05	<LOD	0.1
PFOS	2.03	1.9	0.63	1.4	3.8	1.1	0.85	0.68	0.47	3	0.46	0.49	0.13	0.27	0.7	0.33	0.53	0.41	0.43	0.17	1.01
PFOA	0.34	0.3	0.11	0.21	0.58	0.34	0.32	0.1	0.2	0.53	0.28	0.25	0.078	0.18	0.43	40.81	0.34	0.42	0.15	0.17	0.43
PFNA	0.93	0.93	0.12	0.76	1.1	0.41	0.42	0.11	0.28	0.67	0.42	0.41	0.1	0.27	0.64	32.8	0.32	0.29	0.079	0.26	0.41
PFDA	0.7	0.68	0.13	0.49	0.89	0.33	0.24	0.19	0.17	0.73	0.27	0.28	0.05	0.17	0.33	14.42	0.23	0.17	0.11	0.16	0.36
PFUdA	2.3	2.5	0.51	1.4	3.1	1.03	0.71	0.61	0.51	2.2	0.8	0.75	0.19	0.49	1.23	6.6	0.51	0.42	0.22	0.35	0.76
PFDoA	0.24	0.24	0.059	0.14	0.33	0.13	0.1	0.066	0.08	0.29	0.11	0.11	0.031	0.07	0.18	<LOD	0.073	0.06	0.042	0.04	0.12
PFTriA	0.46	0.47	0.11	0.3	0.65	0.4	0.39	0.12	0.22	0.63	0.44	0.45	0.055	0.34	0.52	3.18	0.13	0.13	0	<LOD	0.13
<b>ΣPFAS</b>	<b>7.2</b>	<b>7</b>	<b>1.5</b>	<b>4.9</b>	<b>10.3</b>	<b>3.8</b>	<b>2.99</b>	<b>1.7</b>	<b>1.9</b>	<b>7.6</b>	<b>2.8</b>	<b>2.6</b>	<b>0.55</b>	<b>2</b>	<b>3.84</b>	<b>98.14</b>	<b>2.756</b>	<b>1.83</b>	<b>1.156</b>	<b>1.15</b>	<b>3.32</b>



### 3.2. Differences between bird species

There were no significant differences ( $p < 0.05$ ) for any of the PFAS between sooty terns and common noddies. There was a significant difference between fairy terns and common noddies for PFOS ( $p < 0.0001$ ), PFNA ( $p = 0.0005$ ), PFDA ( $p = 0.0023$ ), PFUdA ( $p = 0.0008$ ), and PFDoA ( $p = 0.0009$ ). Concentrations in sooty- and fairy tern eggs differed significantly for PFNA ( $p = 0.0003$ ), PFDA ( $p = 0.0027$ ), PFUdA ( $p = 0.0016$ ), and PFDoA ( $p = 0.0083$ ). There were no significant differences between any of the species for PFHxS, PFOA, and PFTrIA (Fig. 2). With the exception of PFOA, the feral chickens from *Ile du Sud* had the lowest concentrations of most PFAS compounds. Fairy terns had the highest mean concentrations of most compounds. PFAS concentrations in chicken eggs are indicated on the violin graph (Fig. 2) for comparison, but are not included in statistical analyses.



**Fig. 2.** Violin graphs with mean PFAS concentrations (ng/g ww), standard deviations, and p-values of Kruskal-Wallis one way ANOVA with Dunn's multiple PFAS concentrations in fairy tern (FT), sooty tern (ST), common noddy (CN), and chicken (Gg) eggs from St. Brandon's Atoll. Only significant ( $p < 0.05$ ) p-values are indicated. Absence of p-values indicate no statistically significant difference between the PFAS concentrations in eggs of the three marine bird species. The chicken eggs were not included in the Kruskal-Wallis analyses due to a small number of eggs analysed and are represented here only as a visual reference.

## 4. Discussion

### 4.1. Concentrations and compositional patterns

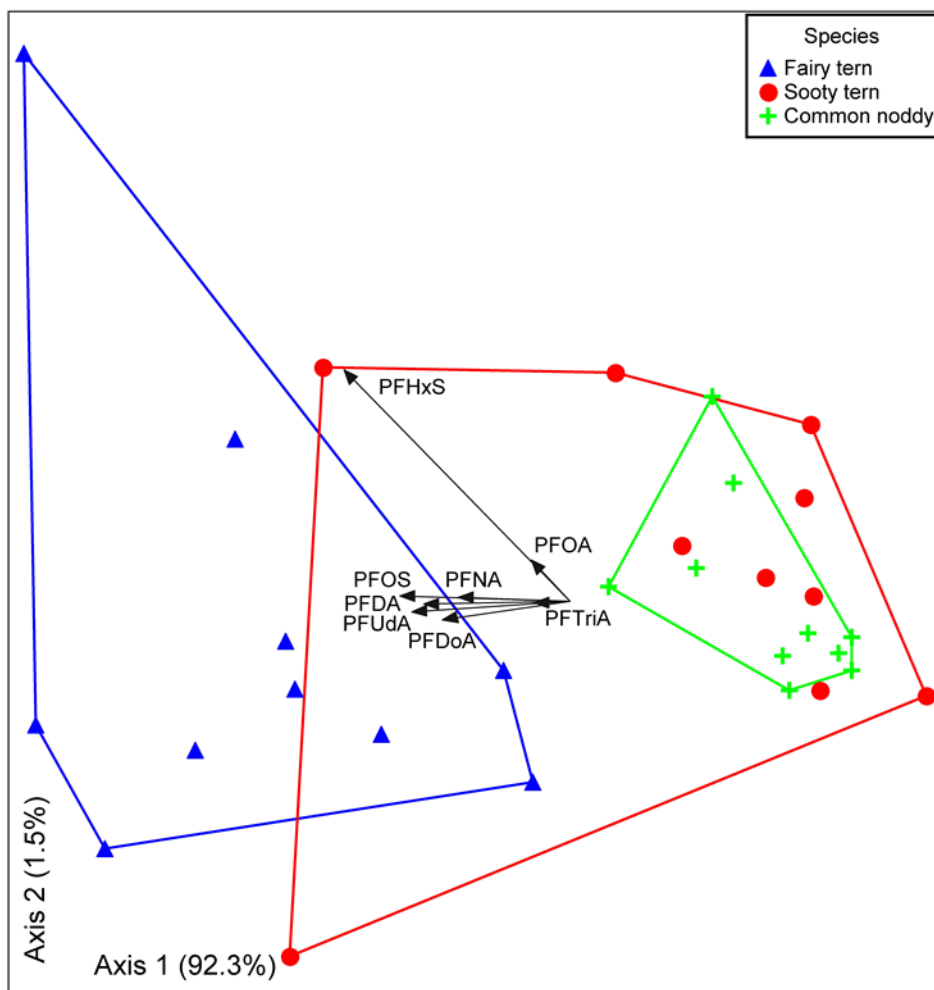
Fairy terns, sooty terns, and common noddies are all seabirds of similar size with an overlapping diet, hunting strategy, and foraging range (Shealer, 2001; Carthy *et al.*, 2009). In the Seychelles, fairy terns and sooty terns have 87% dietary overlap, while sooty terns and common noddies have 85%, and diet similarity between fairy terns and common noddies is 77% (Carthy *et al.*, 2009). It was thus expected that contaminant concentrations would overlap for all species. This was however, not the case.

The NMS graph (Fig. 3) was obtained from 84 runs and had a final stress of 6.663, which indicates a good ordination with no risk of false interference (McCune & Grace, 2002). All PFAS ordinated towards the convex hull representing fairy terns (Fig. 3). This aligns with the results shown in Table 2 and Fig. 2 where fairy terns had the highest mean concentrations of most PFAS. The convex hull for common noddy was much smaller than the other two species, suggesting a more restricted diet composition. The convex hulls representing sooty terns and common noddies overlapped greatly, possibly indicating a similar food source as a source of PFAS to both species. However, the "chemical fingerprint" of fairy terns, as seen in the NMS in Fig. 3 overlapped with sooty terns, but not with common noddies. The reason for this distribution is unknown, but indicates subtle but significant differences. One such difference might be related to their respective foraging distances from shore. Foraging patterns are known drivers of differences in PFAS concentration between different species (Munoz *et al.*, 2017; Roscales *et al.*, 2019) and individual birds (Warner *et al.*, 2019; Miller *et al.*, 2020) corresponding with what is seen in Fig. 3. Sooty terns are offshore foragers, ranging more than 300 km in search of prey, whereas common noddies tend to forage nearshore and inshore (0.5 to 50 km) (Shealer *et al.*, 2001; Carthy *et al.*, 2009; Bouwman *et al.*, 2012). The foraging distance of fairy terns can range from nearshore to offshore (0.5 to >300 km) (Shealer *et al.*, 2001). The differences in their foraging ranges and diets might be a reason for the large variability in PFAS found in the eggs of fairy terns (Table 2 and Fig. 3). Although the three species are all long-lived (the known maximum ages are 27, 36, and 37 for common noddy,

sooty tern, and fairy tern, respectively; Schreiber & Burger, 2001), the mean ages of the breeding female populations are not known, preventing further inference of this factor on concentrations.

Mean PFOA concentrations of feral chickens, fairy terns, and sooty terns were all 0.34 ng/g ww. While seabirds actively hunt marine organisms, the chickens are terrestrial where they probably mainly feed on insects and vegetation. Trace amounts of PFOA, other PFAS, and PFAS precursors might be transported aerially to remote locations such as St. Brandon's. Neutral PFAS precursors, such as FTOH and perfluoroalkane sulfonyl fluorides (PFAS) are relatively volatile and are prone to long range atmospheric transport (Ellis *et al.*, 2004; Wong *et al.*, 2015). These precursors can break down through photooxidation to PFOA and several other PFASs (Yeung *et al.*, 2017). It is possible for the compounds to be transported aerially and be deposited through precipitation (Stemmler & Lammel, 2010). Chickens are more reliant on fresh water than terns, and may be exposed to deposited PFOA in rainwater. Additionally, chickens may be exposed to PFAS and other pollutants by directly ingesting plastics (Lwanga *et al.*, 2017).

Although the concentrations of most of the PFAS compounds were lower in chicken than in tern eggs, it indicates that terrestrial exposure does occur, suggesting that further studies on terrestrial animals such as rats, mice and reptile species that occur on many of the isolated islands in the WIO (Cole *et al.*, 2005; Russel *et al.*, 2016) would be quite informative.



**Fig. 3.** NMS ordination of the distribution of PFAS in seabird eggs from St. Brandon's. The convex hulls represent eggs of different species sampled. Axis 1 explained 92.3% ordination, and Axis 2, 1.5%. Final stress was 6.663, and final instability was < 0.0001.

#### 4.2. Possible exposure pathways

Fairy terns, sooty terns, and common noddies have a similar prey composition, consisting of Mullidae (goatfish), Carangidae (mackerel), Engraulidae (anchovies), Exocoetidae (flying fish), and an assortment of fish larvae (Carty *et al.*, 2009). Fairy terns additionally supplement their diet with squid (Shealer, 2001; Carthy *et al.* 2009). Most squid species are on a higher trophic level than most small bait fish. Stomach contents of *Loligo* squid collected from East London in South Africa contained crustaceans, small fish, polychaetes, and other squid (Sauer & Lipiński, 1991). Because of the trophic difference, squid have the potential to bioaccumulate a higher concentration of pollutants than small fish (Wu *et al.*, 2019). This small difference in dietary preference could be a reason for fairy terns having higher concentration of PFAS in their eggs than the other two species (Figs. 2 and 3).

In addition to exposure through prey items, it is proposed that terns can be exposed to PFAS through direct ingestion of seawater. All birds in this study primarily hunt by means of “dipping” – picking prey from the surface whilst still in flight (Shealer, 2001). Due to the surfactant properties of PFAS, this is where most of the chemicals are concentrated. It was found that most PFAS in the ocean water column are found in the 50 µm topwater of the sea surface microlayer (Ju *et al.*, 2008), due to the composition of the PFAS molecule. The tail section of a PFAS molecule consists of a carbon chain with fluoride molecules, with a functional group head. This unique composition leads to PFAS being mostly found in the surface layer of the water column. The hydrophilic head section, comprising the terminal group, is submerged, and the hydro- and lipophobic tail section in the air (McCarthy *et al.*, 2017; ITRC, 2018; EPA, 2019). The hunting birds may be exposed to the PFAS on the water surface when they scoop up prey.

#### 4.3. Effects of PFAS and toxicity reference values of PFOS and PFOA

It is important to determine the extent of PFAS contamination in the seabirds of St. Brandon's due to the known adverse effects of PFAS. Certain PFAS are carcinogenic (Taniyasu *et al.*, 2008), and may induce oxidative stress that can disrupt cellular homeostasis, reproduction, and physiological functioning (Constantini *et al.*, 2019).

Most of the laboratory studies that focussed on the effects of PFAS used only PFOS, and some PFOA, as study compounds. Peden-Adams *et al.* (2009) determined that PFOS in the eggs of white leghorn chickens could negatively affect the chick's immune system, cause liver and spleen enlargement, and an asymmetry in the chick's wings- and brain development. Similarly Yanai *et al.* (2008) found that elevated PFOA concentration increased the incidence of splayed legs in domestic chickens and inhibited the development of yellow plumage. However, these negative effects only emerged at a concentration of 5 mg/kg (5000 ng/g) (Peden-Adams *et al.*, 2009; Yanai *et al.*, 2008), which is three orders of magnitude higher than the highest maximum concentration found in fairy terns (0.58 ng/g PFOA and 3.8 ng/g w/w PFOS).

**Table 3.** PFAS concentrations (ng/g wm) in piscivorous bird eggs. The first column shows the location numbers used in Fig. 4. The results from this study are indicated in bold.

Nr.	Species	Location	Year	n	PFOS	PFHxS	PFTriA	PFDoA	PFDA	PFNA	PFUdA	Reference
1	Ring-billed gull	Lake Huron. USA	1995	3	67							Kannan <i>et al.</i> (2001)
2	Double crested cormorant	Lake Winnipegosis. Canada	1995	4	157							Kannan <i>et al.</i> (2001)
3	Osprey	Chesapeake Bay. USA	2000	16	291				10.5		18.60	Rattner <i>et al.</i> (2004)
4	Osprey	Elizabeth River. Chesapeake Bay. USA	2001	15	149							Rattner <i>et al.</i> (2004)
5	Skuas	Shetland	2008	20	22	0.123	6.77	2.76	1.22	0.488		Leat <i>et al.</i> (2013)
6	Black guillemot	Prince Leopold Island. Canada	2008	9	39.8	0.11						Braune & Letcher (2013)
7	Thick billed murre	Prince Leopold Island. Canada	2008	15	30.7	0.13						Braune & Letcher (2013)
8	Northern fulmar	Prince Leopold Island. Canada	2008	15	36	0.2						Braune & Letcher (2013)
9	Glaucous gull	Prince Leopold Island. Canada	2008	9	20	0.39						Braune & Letcher (2013)
10	Black-legged kittiwake	Prince Leopold Island. Canada	2008	15	9.58	<0.11						Braune & Letcher (2013)
11	Northern fulmar	Prince Leopold Island. Canada	2011	15	19.8	<0.1						Braune & Letcher (2013)
12	Thick billed murre	Prince Leopold Island. Canada	2011	15	23.8	<0.1						Braune & Letcher (2013)
13	Great blue heron	Pig's eye. Minesota. USA	2011	10	396	1			27	2.4		Custer <i>et al.</i> (2013)
14	Great blue heron	Brainerd. Minesota. USA	2011	10	75	n.d			4.8	2		Custer <i>et al.</i> (2013)
15	Caspian tern	St. Mary's River. Michigan. USA	2013	10	387	0.59		11.1	27.9	11.5	35.8	Su <i>et al.</i> (2017)
16	Caspian tern	Saginaw Bay. Michigan. USA.	2014	10	1395	0.66		22.3	69.4	11.5	61.6	Su <i>et al.</i> (2017)
17	Herring gull	St. Mary's River. Michigan. USA	2014	10	165	0.38		4.09	6.62	4.19	9.59	Su <i>et al.</i> (2017)
18	Herring gull	Saginaw Bay. Michigan. USA.	2012/13	20	170	0.84		3.39	4.7	1.94	7.81	Su <i>et al.</i> (2017)
19	African penguin	Robben and Bird islands, South Africa	2011/12	20	5.4	5			0.71		2.4	Bouwman <i>et al.</i> , (2015)
20	Fairy tern	St. Brandon's	2010	10	<b>2.03</b>	<b>0.13</b>	<b>0.46</b>	<b>0.24</b>	<b>0.7</b>	<b>0.93</b>	<b>2.3</b>	<b>This study</b>
21	Sooty tern	St. Brandon's	2010	10	<b>1.11</b>	<b>0.06</b>	<b>0.41</b>	<b>0.33</b>	<b>0.13</b>	<b>0.4</b>	<b>1.03</b>	<b>This study</b>
22	Common noddy	St. Brandon's	2010	10	<b>0.46</b>	<b>0.019</b>	<b>0.41</b>	<b>0.27</b>	<b>0.11</b>	<b>0.44</b>	<b>0.8</b>	<b>This study</b>

The lowest-observed-adverse-effect-level (LOAEL) of PFOS in chicken eggs was calculated as 0.1 µg/g (100 ng/g) (Molina *et al.*, 2006). Chicken eggs showed reduced hatchability at 100 ng/g. The only TRVs pertaining to PFAS in birds is a study on PFOS in mallard ducks and bobtail quail (Newsted *et al.* 2005; Newsted *et al.*, 2007). However, Newsted *et al.*, (2005) extrapolated TRV values for PFOS so that it would be relatable to predatory birds, such as seabirds. The derived TRV value for PFOS was 1.7 µg/ml (1700 ng/g) and the predicted-no-effect-concentration (PNEC) value for eggs was 1 µg/ml (1000 ng/g). All PFOS concentrations in the seabird eggs collected from St. Brandon's in 2010 were far lower than the reported TRV and PNEC referred to by Newsted *et al.*, (2005).

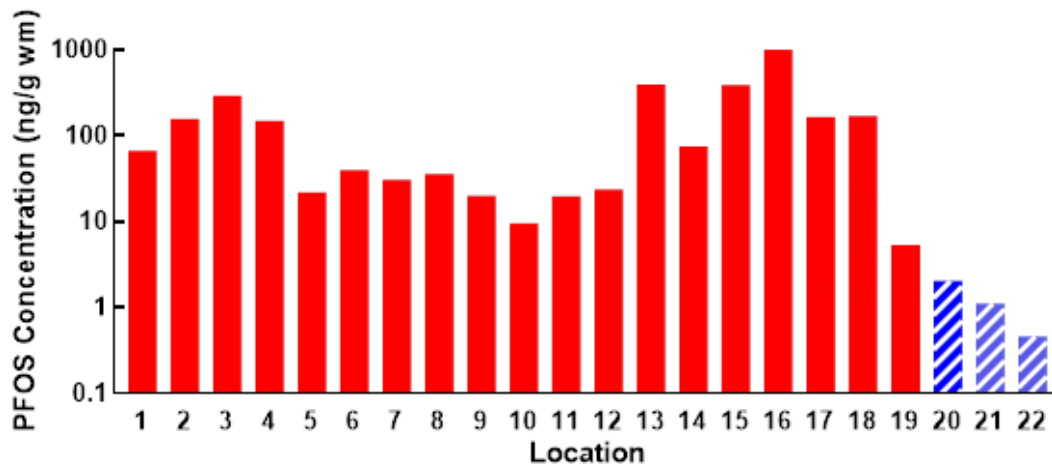
Based on the present toxicity assessment, we do not expect PFAS to have a significant impact on embryo development of the birds studied from St. Brandon's.

#### 4.4. Comparison with other studies

Very few studies have been conducted on the concentrations of PFAS in the eggs of piscivorous birds from various locations, providing comparable data (Table 3). These studies provide a backdrop against which the results of the current study can be contextualized. Even though the analytical methods differ, the concentrations are reliably presented and are comparable with this study.

PFAS concentrations in seabird eggs from St. Brandon's are lower than the PFAS concentrations in most of the studies in Table 3. The PFOS concentrations were the lowest compared with any other study (Fig. 4). This is likely due to the isolated nature of St. Brandon's and the lack of industrial activities on the atoll. This is in stark contrast to PFAS concentrations found by Su *et al.* (2017) who quantified 1395 ng/g w/m PFOS in Caspian terns from Michigan, which were two orders of magnitude higher than our results. Caspian terns are the most comparable to common noddies, fairy- and sooty terns in terms of taxonomy, size, and diet, but contained the highest concentrations of PFOS. Most of the bird species listed in Table 3 are larger than the terns and noddies from this study, with the potential of preying on larger prey at higher trophic levels.

Other studies have found that the odd-chained compounds, PFNA (C9), PFUdA (C11), and PFTriA (C13) were present in higher concentrations than even numbered compounds in blood and plasma in seabirds from Southern Ocean (Bossi *et al.*, 2015; Munoz *et al.*, 2017). This corresponds to the data collected in this study, and to some of the studies noted in Table 3, but not to all. PFOS is the compound found most often at higher concentration, followed by long chained PFCA compounds.



**Fig. 4.** Comparison of PFOS concentrations in tern eggs from St. Brandon's (20-22, diagonal, blue) with results from elsewhere (solid, red). Y-axis is on a log scale. Location numbers correspond with Table 3.

#### 4.5. Possible sources of PFAS in St. Brandon's

PFAS compounds have been quantified in various media from remote locations worldwide (Jahnke, 2007; Huber *et al.*, 2015; Raubert *et al.*, 2018). However, the physico-chemical nature of long chained PFAS does not lend itself to direct aerial transport in the same manner as FTOH (Haukás *et al.*, 2007; Schenker *et al.*, 2008). After photooxidation of FTOH occurs, the PFCA compounds are removed from the atmosphere via wet deposition (Ellis *et al.*, 2004). Long chain compounds are more bioaccumulative than short chain compounds (Munoz *et al.*, 2017), therefore being a possible reason for compounds such as PFUdA and PFNA being prevalent in creatures in higher trophic levels, such as seabirds in remote locations.

Another transport route of PFAS to St. Brandon's is via direct ocean transport (Prevedouros *et al.*, 2006; Yamashita *et al.*, 2008). PFAS compounds are also known to adsorb to plastic particles (Llorca *et al.*, 2014; Sanchez-Vidal, 2015). Long-range transfer of plastics and associated adhered pollutants occurs when the plastic particle is transported through ocean currents to remote island settings (Bouwman *et al.*, 2016; Jambeck *et al.*, 2018). In 2010, an estimated 50 000 anthropogenic debris items were present on the beaches of St. Brandon's (Bouwman *et al.*, 2016). Some of the debris was clothing and food packaging, which are known to include PFAS as a production component (Buck *et al.*, 2011). In addition to long-range transported debris items, shipwrecks on the atoll could be a possible contamination source (van der Schyff *et al.*, 2020b). According to the International Convention for the Safety of Life at Sea (SOLAS), all commercial vessels are required to carry foam firefighting equipment at all times in the boiler rooms (SOLAS, 2018). Because no replacement to AFFF with the same efficiency has yet developed, AFFF are most often used on aeroplanes



and ships to contain chemical fires with the least risk to human lives (Paley, 2019). It is speculated that the AFFF and electronic wires in the shipwrecks on the reef crest of St. Brandon's could be a potential source of PFAS pollution. Another potential source of pollution could be fish and other prey items that encountered pollution elsewhere before migrating to St. Brandon's.

## **5. Conclusions**

In Saint Brandon's Atoll, fairy tern eggs contained the highest concentrations of most PFAS, followed by sooty tern eggs, and eggs of common noddies. It is proposed that the difference in PFAS concentrations in the eggs can be ascribed to small dietary differences and different foraging patterns of the different species. PFAS compounds with long carbon chains were prevalent. PFUdA was the compound that was found in the highest mean concentrations (2.3 ng/g wm), followed by PFOS (2.03 ng/g wm), and PFNA (0.93 ng/g wm). Concentrations of all PFAS were lower than values found in literature. The PFOS and PFOA concentrations in all eggs were lower than TRVs and LOAEL concentrations, suggesting no hazard to the embryos. The isolated nature of St. Brandon's might protect biota from toxicological impacts; however, shipwrecks and long-range transported marine debris could potentially act as vectors for PFAS pollution to isolated island systems. Further studies are recommended to determine whether PFAS concentrations in seabird eggs from St. Brandon's have increased since 2010. The possible modes of transportation of PFAS to St. Brandon's and other islands also require further thorough research in order to mitigate the inflow of pollutants to these remote systems. The low concentrations suggest that St. Brandon's would be useful as a long-term monitoring site to determine background changes on a regional and perhaps global scale.

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## Chapter 6: Conclusion and recommendations

### 6.1 Aims and Hypotheses

In Section 1.1 of this thesis (State of the science in the western Indian Ocean), I concluded that it is disconcerting that only a few toxicological surveys have been conducted in the Mauritian EEZ. The resultant lack of scientific data indicated the need for more ecotoxicological studies in the region and led to the following research questions:

- At what concentrations and compositional patterns are anthropogenic pollutants present in coral, fish, and sea birds from the Mauritian Outer Islands in the Mascarene Basin?
- Which factors are involved that may explain the concentrations and patterns of pollutants observed?
- What role could remote coral islands play in monitoring pollution?

In order to answer these research questions, one general objective and five specific objectives were formulated. Seven hypotheses, or predictions, listed in Chapter 1 of this thesis, were interlinked with the respective objectives.

#### General objective

The general objective of this study was to provide a comprehensive overview of the ecotoxicological state of knowledge of three islands in the Mascarene Basin with a specific focus on quantifying pollutants in marine biota from coral reefs of the Mauritian Outer Islands. In this thesis, I reported the results of a comprehensive toxicological study conducted in the Mascarene Basin. Metals, POPs, NBFRs, and PFAS were quantified in biota from the Mascarene Basin. The biota that were examined included algae, coral, fish, and sea birds. The hypotheses that were presented in Section 2.2 of Chapter 1 (Aims and hypotheses) will either be accepted or rejected based on the body of knowledge that was presented in the subsequent chapters.

This is the first study to quantify PFAS in seabirds in the WIO, and POPs and NBFRs in coral and coral reef fish in the Mascarene Basin. To the best of my knowledge, this was the first time that brominated compounds (PBDEs and NBFRs) were quantified in coral in any study.

*Hypothesis 1: Pollutants will be present in all biota from the Mascarene Basin.*

This hypothesis is accepted.

Various pollutants were quantified in algae, hard coral, soft coral, coral reef fish, and bird species from St. Brandon's Atoll. These pollutants were also quantified in coral and fish from Agalega and Rodrigues. All biological samples or sample pools contained quantifiable concentrations of anthropogenic contaminants. The concentrations varied between taxa and per contaminant, but concentrations of pollutants in biota that were sampled from the Mascarene Basin were lower than the concentrations reported in similar studies from elsewhere.

Specific objectives:

Five specific objectives were formulated to address the respective hypotheses.

The first specific objective was to investigate shipwrecks as a vector of pollutants to remote islands by determining the ecological and toxicological effects of a shallow shipwreck.

*Hypothesis 2: Shallow shipwrecks cause quantifiable ecological damage to the reef of isolated tropical islands and serve as a vector for pollutants to these islands.*

This hypothesis is accepted.

In Chapter 2, comprising of the article titled *Impacts of a shallow shipwreck on a coral reef: A case study from St. Brandon's Atoll, Indian Ocean*, (van der Schyff *et al.*, 2020b) published in *Marine Environmental Research*, it was indicated that all coral around the wreck fragments were dead and blackened. Downstream from the wreck-site, a 40-hectare macroalgae bloom smothered and ripped coral colonies from the substrate, causing ecological damage. The coral fragments in the direct vicinity of the wreck contained significantly elevated concentrations of metals such as As, Cd, Cr, Cu, Pb, U, and Zn, which indicated that the wreck is a vector of pollutants to the surrounding reef system.

The second specific objective was to determine POPs and NBFR concentrations in the biota from lower positions of the coral reef food web by quantifying POPs and NBFRs in corals and coral reef fish from the Mascarene region.

*Hypothesis 3: Fish will contain higher POPs concentrations than corals due to biomagnification at a higher trophic level.*

This hypothesis is rejected.

In Chapter 3, comprising of the article titled *Differences in concentrations and compositions of chlorinated and brominated POPs and novel brominated flame retardants in hard coral, soft coral, and reef fish*, submitted to *Chemosphere*, it is indicated that fish consistently contained higher concentrations of POPs and NBFRs than hard coral. Soft coral, however, contained higher concentrations of  $\Sigma$ PBDE,  $\Sigma$ HCH, and HCB than fish tissue. There were no statistically significant differences between the concentrations of pollutants in soft coral and fish tissue from the Mauritian Outer Islands in the Mascarene Basin.

The third specific objective was to determine pollutant concentrations in biota from higher positions in the tropical island food web by quantifying POPs and PFAS in the eggs of seabirds from the Mascarene region.

*Hypothesis 4: Seabird eggs will contain quantifiable, but low concentrations of POPs and PFAs*

This hypothesis is accepted.

This hypothesis was explored over two chapters, in two articles, one published and one submitted. Chapter 4, comprising of the article, *Persistent Organic Pollutants in sea birds from the Indian Ocean's Mascarene Basin*, submitted to *Science of the Total Environment* reported the lowest concentrations of POPs in seabird eggs worldwide. Chapter 5, comprising of the article *Perfluoroalkyl substances (PFAS) in tern eggs from St. Brandon's Atoll, Indian Ocean*, published in *Marine Pollution Bulletin* (van der Schyff, 2020c), indicted a similar trend, where the eggs from seabirds nesting on St. Brandon's Atoll contained the lowest concentrations of PFAS compared with other studies. All the eggs of fairy terns, sooty terns, and common noddies collected from St. Brandon's Atoll contained quantifiable concentrations of all classes of POPs and PFAS that were analysed.

The fourth specific objective was to determine the factors that may explain the concentrations and patterns of pollutants.

*Hypothesis 5: Pollutants are transported to remote islands in the WIO through multiple pathways.*

This hypothesis is accepted.

This hypothesis was explored over all four articles, through data analyses and literature reviews. In Chapter 2 (*Impacts of a shallow shipwreck on a coral reef: A case study from St. Brandon's Atoll, Indian Ocean*), indicated significantly elevated concentrations of metals around the wreck-affected areas, compared with the reference reef (as stated in Hypothesis 2). Aerial transfer and wet deposition of pollutants are confirmed in both Chapters 4 (*Persistent Organic Pollutants in sea birds from the Indian Ocean's Mascarene Basin*) and 5 (*Perfluoroalkyl substances (PFAS) in tern eggs from St. Brandon's Atoll, Indian Ocean*), as POPs and PFAs were quantified in chicken eggs from St. Brandon's Atoll. These two pathways were specifically indicated by the studies done. Confirmation is also found in the literature review where it is indicated in other studies that pathways of pollutant transfer to remote islands include marine debris transported by oceanic currents and redistribution of pollutants through seabird excreta.

*Hypothesis 6: Different islands will have different concentrations and patterns of pollutants.*

This hypothesis is accepted.

In Chapter 3 (*Differences in concentrations and compositions of chlorinated and brominated POPs and novel brominated flame retardants in hard coral, soft coral, and reef fish*), brominated and chlorinated pollutants were quantified in biota from Agalega, St. Brandon's Atoll, and Rodrigues. The pollutant compositional pattern differed between the islands. PBDEs were the most prominent pollutant class for all three island systems, but the proportional composition of the class ranged from 49.3% of the total pollutant load in Agalega to 26.5% of the total pollutant load in Rodrigues. PCB was the second most abundant pollutant for Agalega and St. Brandon's atoll, while HCB was the second most prominent compound in biota from Rodrigues. Rodrigues also contained the highest proportion of HCH between the three islands. In Chapter 4 (*Persistent Organic Pollutants in sea bird eggs from the Indian Ocean's Mascarene Basin*), seabird eggs from St. Brandon's Atoll contained lower concentrations of POPs than eggs from the same species that were collected from Rodrigues. The fact that

Rodrigues has a larger human population and is more industrialised than either St. Brandon's or Agalega is the main difference in the concentrations and patterns of pollutants in the island.

*Hypothesis 7: Different coral reef biota will have different compositional patterns of pollutants.*

This hypothesis is accepted.

In Chapters 3, and 4 it was observed that physiological and behavioural differences in biota cause different pollutant accumulation and composition patterns. In Chapter 3 (*Differences in concentrations and compositions of chlorinated and brominated POPs and novel brominated flame retardants in hard coral, soft coral, and reef fish*), hard- and soft coral had different pollutant concentrations and patterns. In Chapter 4 (*Persistent Organic Pollutants in sea birds from the Indian Ocean's Mascarene Basin*), the foraging behaviour of seabirds also play a role in their pollutant concentrations and patterns. The eggs of species that forage further offshore—sooty- and fairy terns—had higher concentrations of pollutants than the nearshore-foraging common noddies.

The final specific objective was to establish the role that remote coral islands could play in monitoring pollution in the WIO.

*Hypothesis 8: Remote oceanic islands can be used as reference sites to determine regional background concentrations of pollutants against which concentrations of marine pollution elsewhere can be compared.*

This hypothesis is accepted.

It was concluded in Chapters 3, 4, and 5 that the remoteness of the Mauritian Outer Islands makes them ideal reference sites to study the background concentrations of pollutants in the WIO. Agalega and St. Brandon's Atoll, in particular, are prime locations, not only because of their remote geographical locations but also because of their lack of industry and small human settlement. The concentrations of POPs and NBFs in coral and fish and PFAs concentrations in seabird eggs from these islands were lower than any concentrations found in literature. Although the islands are not entirely uncontaminated, it does offer valuable insight into the background concentrations of pollutants in the WIO. Remote study sites are less affected by

variations in releases from various sources than those located close by. Contaminants reaching remote areas are often diluted, compared with the source of exposure and nearby areas. These remote reference sites in the WIO can be used to determine the increase of pollution elsewhere and revisited in future studies.

## 6.2 General conclusions

The Indian Ocean is the third-largest body of water on earth, but also one of the least studied in an ecotoxicological context. The fact that pollutants could be quantified in one of the most remote tropical islands in the WIO shows the extent of how ubiquitous pollutants are distributed in the environment. Most pollutants are transported to remote locations where no or limited local emission is present, through long-range transfer. Certain shipwrecks are confirmed to cause ecological harm by physically damaging a reef system when grounding and long-term toxicological harm when metals and other pollutants leech from the wreck to the surrounding reef system (Chapter 2). This has the potential to kill corals, destabilising the base of the coral reef ecosystem. Aside from one study quantifying metals in coral from the Mascarene Basin (van der Schyff *et al.*, 2020a), no pollutants have been quantified in coral reef biota from this basin. For the first time, brominated and chlorinated pollutants were quantified in coral and coral reef fish from the Mascarene Basin (Chapter 3). A distinct difference could be seen in the pollution accumulation pattern of different functional groups of coral reef organism from the Mascarene Basin. Soft coral contained higher concentrations than hard coral for all POPs, but hard coral contained higher concentrations of NBFRs than soft coral. Coral reef fish tissue consistently contained higher concentrations of pollutants than hard coral. The concentrations of pollutants in soft coral and fish did not differ significantly for any compound class. This study indicated the widespread occurrence of the NBFRs, pentabromotoluene (PBT) when it was quantified in all 21 sample pools from across three islands. As far as I am aware, it also indicated the accumulation of brominated compounds in coral for the first time.

The concentrations of all pollutants in coral and coral reef fish from the Mascarene Basin were very low. The highest concentration that was quantified was 1.56 ng/g wm of BDE-209 in *Sinularia* soft coral from Agalega Island. A previous study has quantified POPs in seabirds from Rodrigues island (Bouwman *et al.*, 2012), but no recent studies quantified POPs in seabirds from St. Brandon's Atoll. POPs were determined in the eggs of fairy terns (*Gygis alba*), sooty terns (*Onychoprion fuscatus*), and common noddies (*Anous stolidus*) (Chapter 4). This study found that concentrations of all compounds were lower in the eggs from birds from St. Brandon's Atoll than from anywhere else worldwide. The differences between species were

ascribed to the differences in their foraging ranges. Sooty- and fairy terns forage further offshore and were seemingly exposed to more pollutants than common noddies, who are nearshore foragers. This study also reported POPs in the eggs of a terrestrial bird species, chicken (*Gallus gallus*), from the Mascarene Basin for the first time. Although the concentrations of POPs in chicken eggs were low, some compounds were found at higher concentrations than in the eggs of seabirds. This likely indicates aerial transport of pollutants to the WIO. Because no study has studied PFAS in bird eggs from the WIO before, we also quantified PFAS in the eggs of the terns from St. Brandon's Atoll (Chapter 5). Similar to POPs, the concentrations of PFAS in the eggs from seabirds from St. Brandon's Atoll were lower than any other study. Fairy tern eggs contained the highest concentrations of PFAS, followed by sooty terns, and common noddies. Long chained PFAS compounds were prevalent over short chains. PFUdA was the compound found at the highest concentrations (2.3 ng/g ww). Chicken eggs were also analysed to obtain a terrestrial reference of PFAS in biota. All chicken eggs contained quantifiable concentrations of PFAS compounds.

In all studies, it was concluded that the isolated nature and lack of industry of the Mascarene Basin was the factor that contributed most to the low concentrations of pollutants in biota. The Islands of the Mascarene Basin could be ideal reference sites to monitor the background concentrations of pollutants in the WIO.

### **6.3 Recommendations**

Through the course of this study, several gaps in our knowledge pertaining to the distribution of pollutants in the Mascarene Basin were identified. The following aspects warrant further study to ensure that toxicological knowledge of the Mascarene Basin and coral reef toxicology is optimised.

- Follow-up studies to the Mascarene Basin to determine whether concentrations of compounds have increased since 2014;
- Future studies quantifying pollutants in coral should include brominated compounds in the screening to determine brominated compounds in coral from worldwide;
- Laboratory studies should be conducted to determine the effects of brominated compound on coral physiology;
- It should be determined whether pollutants are transferred from a coral colony to gametes during spawning, and the implication thereof to coral reef conservation should be investigated;

- More studies need to be conducted on the dangers of shipwrecks to coral reefs. The specifics of the black reefs phenomena should be studied further to aid in mitigation efforts;
- The role of coral mucus in either buffering the colony from pollutants or aiding in pollutant accumulation warrants further studies;
- PBT should be investigated as a new candidate POP;
- Future studies should include quantifying NBFRs in bird eggs from remote locations.
- It should be studied further why hard corals have a greater affinity for NBFRs than soft coral;
- Future studies should quantify natural brominated compounds in WIO coral reef biota;
- Future studies in the Mascarene Basin should quantify BDE-209 in bird eggs;
- If the study is to be repeated in the future, the same pollutants should be analysed in all biological samples;
- The toxicological effects of the oil spill from the grounding of the bulk carrier *MV Wakasio* should be studied in detail. Time scales of the effects of the wreck should be undertaken to better predict and mitigate the effects of future wrecks in coral reef environments.

In the light of the recent environmental disaster caused in August 2020 by the oil spill from the *MV Wakasio* bulk carrier, ecotoxicological knowledge is now more important than ever to facilitate the conservation of coral reefs in Mauritius. This thesis contains vital information on coral reef ecotoxicology in the Mauritian EEZ that will aid in the mitigation of the environmental disaster and subsequent studies. Understanding that different biota do not interact with pollutants in the same manner will guide future researchers in choosing appropriate bio-indicator organisms to monitor the consequences following on the oil spill. Research on the long-term changes in the concentrations of pollutants resulting from the current shipwreck may be streamlined by having background concentrations from comparable remote, near-pristine, reefs of the Mauritian Outer Islands.

The need to establish and conserve marine protected areas in Mauritius are highlighted by the disaster. To provide biota from the Mauritian reef with the greatest chance of recovery, another marine reserve should be established—preferably up-current from the shipwreck.

The coral reefs of the western Indian Ocean are vibrant, yet delicate, ecological systems. They are often the only source of income and food security for the surrounding communities. Human activities pose the greatest threats to these economical and ecologically important heartlands, and human intervention and conservation are their only hope to persist and to thrive.



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## Appendix. Authorship statements

The authorship statements are presented as submitted with the respective articles in accordance with the publisher's requirements.

### AUTHORSHIP STATEMENT

Manuscript title: **Impacts of a shallow shipwreck on a coral reef: A case study from St. Brandon's Atoll, Mauritius, Indian Ocean.**

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Marine Environmental Research*.

#### Authorship contributions

Please indicate the specific contributions made by each author (list the authors' initials followed by their surnames, e.g., Y.L. Cheung). The name of each author must appear at least once in each of the three categories below.

#### **Category 1**

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All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the journal, *Chemosphere*.

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## AUTHORSHIP STATEMENT

Manuscript title: **Persistent Organic Pollutants in sea bird eggs from the western Indian Ocean's Mascarene Basin**

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Science of the Total Environment*.

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Please indicate the specific contributions made by each author (list the authors' initials followed by their surnames, e.g., Y.L. Cheung). The name of each author must appear at least once in each of the three categories below.

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Manuscript title: **Perfluoroalkyl substances (PFAS) in tern eggs from St. Brandon's Atoll, Indian Ocean**

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in the *Marine Pollution Bulletin*.

### Authorship contributions

Please indicate the specific contributions made by each author (list the authors' initials followed by their surnames, e.g., Y.L. Cheung). The name of each author must appear at least once in each of the three categories below.

#### Category 1

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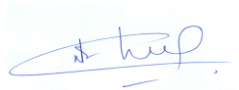

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