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# Environmental contaminants in New Zealand's endemic, endangered Hector's dolphin (*Cephalorhynchus hectori*)

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# Abstract

Due to their coastal distribution, Hector's (*Cephalorhynchus hectori hectori*) and subspecies Māui (*C. h. maui*) dolphins are exposed to land runoff and point source inputs. However, little focus has been placed on contaminant burdens, which in the context of increased disease mortality affecting the species, is of concern. Several persistent environmental pollutants are known immunosuppressants, enhancing disease susceptibility. Here I applied generalised linear models to examine the spatiotemporal trends in contaminant burden while accounting for the effects of total body length (TBL) and sex. Polychlorinated biphenyls (PCBs, 45 congeners), multi-residue pesticides ( $\Sigma$ DDT, HCB, oxychlorodane, dieldrin and mirex) and polybrominated diphenyl ethers (PBDEs, 10 congeners) were analysed in the blubber of Hector's and Māui dolphins (n = 66; 30 males and 36 females) stranded or by-caught in New Zealand between 1997 and 2022. For both  $\Sigma$ PCB and  $\Sigma$ MRP, sex was the most important predictor, followed by total body length (TBL). In males, PCB and MRP burden increased with increasing TBL, while this trend was less pronounced in females. Dolphins from the east coast of New Zealand recorded the highest overall PCB and MRP burdens. Despite being legacy contaminants banned over 30 years ago, year was the least important predictor, with minimal to no decline observed in concentrations of  $\Sigma$ PCB,  $\Sigma$ MRP,  $\Sigma$ DDT and HCB over the 25-year study period.

For PBDEs, the most important predictor was TBL, followed by sex. Concentrations increased with TBL and were highest in males. However, no interaction between sex and TBL was observed, indicating no difference in the effects of TBL between males and females. This may signal less offloading for females during pregnancy and lactation compared to other contaminants assessed. PBDE burdens were lowest in animals from the west coast. While a marginal decline in PBDE burden over time was noted, this had almost no contribution to the overall model fit. Results show that older animals have higher concentrations than younger animals, likely due to the accumulation of contaminants in the tissues over time. Our study provides important knowledge needed to guide appropriate conservation actions, especially in the context of disease susceptibility, including but not limited to toxoplasmosis.



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## Chapter 1 - Introduction



Hector's dolphins (*Cephalorhynchus hectori hectori*), Akaroa New Zealand. Note the presence of foetal folds on the neonate at the top of the image.

Photo credit: Deborah Casano-Bally



## 1.1 The New Zealand *Cephalorhynchus*

Hector's dolphin (*Cephalorhynchus hectori*), the smallest (Dawson & Slooten, 1993) and rarest dolphin in the world (Pichler & Baker, 2000), is one of the four species belonging to the genus *Cephalorhynchus* (Dawson, 2018). Hector's dolphins are the only endemic cetacean in New Zealand and can be easily differentiated from other dolphins by their unique black rounded dorsal fin (Baker, 1978; Dawson, 1985). Since 2002, the species has been described as two separate subspecies to recognise the genetic and morphometric differences between the populations (Baker et al., 2002). Hector's dolphin (*C. h. hectori*), which is predominantly distributed around the South Island, has a population estimate of 15,000 individuals and is divided into three geographically and genetically distinct subpopulations: the west coast (WCSI), the east coast (ECSI), and the south coast (SCSI) populations (MacKenzie & Clement, 2014, 2016, 2019).

Māui dolphin (*C. h. maui*) is almost exclusively distributed around the west coast of the North Island and is estimated to consist of 54 individuals over one year of age (Constantine et al., 2021). Hector's dolphins are classified as *Endangered* by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and as *Nationally Endangered* by the New Zealand Threat Classification System (NZTCS) (Reeves et al., 2013a). Māui dolphins are classified as *Critically Endangered* by the IUCN Red List and *Nationally Critical* by the NZTCS (Reeves et al., 2013b). Both subspecies occur in shallow and coastal waters, with most sightings being within four nautical miles of shore, and are known to exhibit an inshore movement pattern during the summer austral (MacKenzie & Clement, 2014, 2016; MacKenzie & Clement, 2019; Rayment et al., 2010). As marine mammals, Hector's and Māui dolphins have a low reproductive rate, late sexual maturity and long calving interval leading to low population growth rates (Roberts et al., 2019; Slooten & Lad, 1991), making these dolphins particularly vulnerable to human-related activities.

## 1.2 Threats to Hector's and Māui dolphins

Cetaceans are subject to many natural and anthropogenic threats, such as climate change (Kebke et al., 2022; MacLeod, 2009; Sousa et al., 2019), fisheries-related activities (Anderson et al., 2020; Bearzi et al., 2006; Read et al., 2006; Tulloch et al., 2020), pollution (Baulch &

Perry, 2014; Eisfeld-Pierantonio et al., 2022; Jepson et al., 2016; Todd et al., 2015), disease (Costa-Silva et al., 2019; Gulland & Hall, 2007; Van Bresseem et al., 2009; Weiss et al., 2020), and vessel impacts (Arranz et al., 2021; Puszka et al., 2021; Ritter & Panigada, 2019; Weilgart, 2007). Such impacts can affect the reproduction, foraging, migration, growth and survival rates of cetaceans (Sanganyado et al., 2020). The exposure and type of stressors vary spatially and temporally depending on human activities (Simmonds, 2018). Species that inhabit coastal waters are often more exposed to anthropogenic impacts due to the large proportion of human activities occurring in those areas (Jefferson, 2019).

Hector's and Māui dolphins are exposed to a number of natural and human-related threats, some of which have a direct negative impact that can lead to death, while others indirectly impact the population (Roberts et al., 2019). As a coastal species, Hector's and Māui dolphins are specifically susceptible to human-related threats due to their restricted distribution overlapping with recreational and commercial activities (Roberts et al., 2019). The primary threat, which has historically been the centre point for conservation management of Hector's and Māui dolphins, is fisheries bycatch (Davies et al., 2008; Read, 2008; Slooten, 2007). However, given their highly endangered status, it is crucial to consider everything that could affect the species. The recently reviewed Threat Management Plan recognise toxoplasmosis, an infection caused by a widespread protozoan parasite (*Toxoplasma gondii*) (Roberts et al., 2020; DOC, 2020), as a significant emerging threat for this species and estimates that commercial fishing-related deaths are low compared to those from toxoplasmosis (DOC, 2021; Roberts et al., 2019). Other factors affecting Hector's and Māui dolphins include vessel strikes (Stone & Yoshinaga, 2000), whale-watch tourism (Martinez et al., 2010), diseases (Buckle et al., 2017; Roe et al., 2013a,b) and exposure to contaminants (Stockin et al., 2010).

### **1.2.1 Fisheries bycatch**

Fisheries interactions are globally recognised as a severe threat to marine wildlife (Lewison et al., 2004a; Žydelis et al., 2009). It is a crucial conservation issue for many marine organisms, especially large marine vertebrates, such as sea birds (Tasker et al., 2000; Žydelis et al., 2013), turtles (Lewison et al., 2004b; Wallace et al., 2013), sharks (Barker & Schluessel, 2005; Dulvy et al., 2008) and marine mammals (Read, 2008) due to their low reproductive rate and late sexual maturity. The biggest threat within fishery interactions is bycatch, i.e. the unintentional

catch of unwanted marine species that are later discarded, resulting in serious injury and/or mortality (Read, 2008). In the U.S., between 1990 and 1999, the annual mean of marine mammal bycatch was estimated at  $6215 \pm 448$  (SE) individuals, and most bycatches took place in gillnet fisheries (84% of cetaceans and 98% of pinnipeds) (Read et al., 2006).

However, not all bycatch is unsustainable, as some populations can withstand high loss to the populations due to their fast population growth, as observed in some pinniped populations (Underwood et al., 2008). Contrastingly, marine mammals with a small population are more vulnerable to bycatch (Read, 2008). This is particularly important for odontocetes due to their slow life history (Hamer et al., 2012; Mann, 2019). The recent extinction of the Yangtze River dolphin (*Lipotes vexillifer*) in China represented the first large vertebrate to become extinct in over 50 years and the first extinction of a cetacean due to anthropogenic activities. This species was classified as "functionally extinct" due primarily to unsustainable bycatch (Turvey et al., 2007). Another species at high risk of extinction is the vaquita (*Phocoena sinus*), a small porpoise endemic to the Upper Gulf of California. They are known to be a single relict population and highly vulnerable to bycatch and could become extinct in a few years (Rojas-Bracho et al., 2006). In New Zealand, the most common human-related mortality cause for odontocetes is due to interactions with fisheries, particularly bycatch, with the most frequently bycaught species being the common dolphin (*Delphinus delphis*) (Berkenbusch et al., 2013). Until recently, fisheries bycatch was considered the primary threat to Hector's and Māui dolphins (Roberts et al., 2019). Set nets particularly, are likely the largest fisheries-related source of mortality for both subspecies.

### **1.2.2 Vessel impacts**

Vessel interactions are known to affect marine wildlife in several ways: directly (e.g. vessel strike) and indirectly (e.g. tourism, swimming, noise pollution). Vessel strikes represent a physical impact between a water vessel and a live marine animal that cause physical trauma and often subsequent death (Peel et al., 2018). This is the case for more than 75 marine species affected (Schoeman et al., 2020), including sea birds (Cannell et al., 2016), turtles (Chaloupka et al., 2008), sharks (Towner et al., 2012) and marine mammals (Byard et al., 2012; Edwards et al., 2016; Kreuder et al., 2003). The large number of recreational and commercial vessels in the oceans, particularly in coastal areas, has led the International Whaling

Commission (IWC) Conservation Committee to identify vessel strikes as a priority to mitigate (Cates et al., 2017). There is growing evidence that many species are at risk of vessel strikes, such as sirenians (Aipanjiguly et al., 2003; Edwards et al., 2016; Groom et al., 2004), pinnipeds (Olson et al., 2021; Wilson et al., 2017) and odontocetes (Frantzis et al., 2019; Van Waerebeek et al., 2007). Many studies have reported collisions between vessels and baleen whales, including fin (*Balaenoptera physalus*), blue (*B. musculus*) and humpback whales (*Megaptera novaeangliae*) (Abrahms et al., 2019; Carretta et al., 2017; Priyadarshana et al., 2016; Redfern et al., 2019; Rockwood et al., 2017; Williams & O’Hara, 2010). In New Zealand, Bryde’s whales (*B. brydei*) are highly prone to collisions in the Hauraki Gulf (Behrens & Constantine, 2008), with on average 2.3 whales being killed by vessels strike every year (1996-2014) (Constantine et al., 2015). However, this impact was mitigated by the implementation of conservation actions initiated by the New Zealand Department of Conservation (DOC) (Ebdon et al., 2020).

Though not usually resulting in imminent mortalities, indirect impacts of vessels can have population-level consequences. Vessel-based tours are a popular way to observe cetaceans in their natural habitat. For example, whale-watching and swim-with-dolphin vessels regularly target specific species and populations for extended periods (Bejder et al., 2006a; Constantine et al., 2004; Nowacek et al., 2001). Many studies have demonstrated the indirect impacts of tourism, including short- and long-term behavioural changes in the presence of vessels, a reduction in key biological behaviours such as foraging, resting, movement, communication, habitat use, socialising and reproduction (Baş et al., 2015; Christiansen et al., 2013; Heiler et al., 2016; Marley et al., 2017; Nowacek et al., 2001; Pellegrini et al., 2021; Puszka et al., 2021; Quintana Martín-Montalvo et al., 2021). Those impacts have been observed in mysticetes (Amrein et al., 2020; Christiansen et al., 2013) and odontocetes (Bejder et al., 2006b; Nowacek et al., 2001). In some cases, animals become habituated to the presence of humans (Bach & Burton, 2017), while others will purposely avoid their presence (Lusseau, 2004) or even a particular area (Bejder et al., 2006b). Coastal animals with a limited range are more likely to regularly encounter tour vessels (Constantine et al., 2004), and the impact of such tourism could be devastating for small and endangered populations such as Hector’s and Māui dolphins. Hector’s dolphins have been observed to change their distribution in Akaroa Harbour due to increased cruise ship visits in the harbour (Carome et al., 2022) and are also known to be highly receptive to vessels and swimmers’ contact (Martinez et al., 2010).

### 1.2.3 Noise pollution

Noise pollution is another critical threat to marine mammals that use sound for communication and echolocation (Tyack & Miller, 2002). Cetaceans use sound in all biologically significant behaviours such as foraging, communicating, travelling, reproducing and parental care (Erbe, 2002; Erbe et al., 2018; Jensen et al., 2009), and are thus highly susceptible to noise disturbance. Many studies have demonstrated those effects, which include masking communication, stress, changes in behaviour, injury and loss of hearing (Clark et al., 2009; Erbe, 2002; Mann et al., 2010; Nowacek et al., 2007; Rolland et al., 2012; Wright et al., 2007), which is likely the case in Hector's dolphins (Leunissen & Dawson, 2018). Further evidence indicates that the noise from pile-driving noises can affect Hector's dolphins, such as temporary hearing loss and displacement from foraging areas (Leunissen & Dawson, 2018; Leunissen et al., 2019), although a greater understanding of prolonged exposure to noise and the accumulation of temporary responses could affect cetacean populations is still required (Erbe et al., 2018).

### 1.2.4 Diseases

Diseases can pose a severe threat to many marine animals, including birds (Olsen et al., 1995; Steele et al., 2005), turtles (Flint et al., 2009; Smyth et al., 2019), sharks (Dagleish et al., 2010; Garner, 2013) and marine mammals (Bossart, 2007; Van Bresseem et al., 2009). Morbidity can induce mortalities, reduced animal population growth, increased risk of extinction for small populations, and thus loss of biodiversity (Daszak et al., 2000; Johnson et al., 2009; Mazzariol et al., 2018). Many studies have observed a link between environmental factors, such as high sea surface temperature, low prey availability and chemical pollution, and the increased emergence and severity of diseases in marine mammals (Ross, 2002; Van Bresseem et al., 2009).

Various new diseases are emerging, known as emerging infectious diseases (EIDs) (Bengis et al., 2004), or re-emerging and evolving every year. They are usually associated with the rapid increase of human activity leading to environmental modifications from pollution, climate change or habitat degradation (Bossart, 2011; Desforges et al., 2016; Harvell et al., 2002; Van Bresseem et al., 2009) and have a potential zoonotic risk (Estrada-Peña et al., 2014). Such diseases include, for example, cetacean morbillivirus (CeMV), Brucellosis (*Brucella* spp), and

Toxoplasmosis (Buckle et al., 2017; Duignan, 2003; Roe et al., 2013a,b; Costa-Silva et al., 2019). Cetacean morbillivirus is a highly contagious disease that has caused several outbreaks with high morbidity in odontocetes worldwide (Van Bresseem et al., 2014). Brucellosis is a zoonotic disease, meaning it is capable of infecting humans. It has been described as a severe chronic disease in some species of dolphins and porpoises, presenting signs of abortions, infertility, bone and skin lesions, stranding, and even death (Guzmán-Verri et al., 2012). Toxoplasmosis is a protozoan parasite where felids serve as hosts and marine mammals as intermediate hosts. Globally, odontocetes particularly have been infected by this disease, even polar species, where felids are absent from the environment. Antimicrobial resistance is a potential growing threats for cetaceans and has been observed in various species globally (Gross et al., 2022; Park et al., 2020 Rocha et al., 2021).

Coastal species are at higher risk of diseases as polluted areas, traumatic injuries from fisheries or vessel interactions, and compromised immune systems are making these species vulnerable to pathogens (Van Bresseem et al., 2009). In particular, Hector's dolphins have been diagnosed with various diseases associated with immunosuppression (Buckle et al., 2017; Roe et al., 2013a,b). Specifically, Roe et al. (2013b) investigated the cause of death in 49 stranded Hector's dolphins between 2007 and 2012 and reported that 13 animals (27%), died of infectious disease (i.e. toxoplasmosis, brucellosis, aspergillosis (*Aspergillus fumigatus*). Toxoplasmosis was the predominant infectious agent (7 animals died from that).

### **1.2.5 Pollution**

As apex- and mesopredators, the ecology and life history of cetaceans cause them to accumulate high concentrations of pollutants. Factors such as sex, age, diet, habitat and reproductive history are known to affect the level of contaminants in individuals (Baini et al., 2020; Khairy et al., 2021; Kratofil et al., 2020; Pinzone et al., 2015). Given the prevalence and severity of diseases, it is essential to understand the effects of pollutants on the immune system.

Persistent organic pollutants (POPs) are a type of organic compounds known to bioaccumulate and persist in the environment, biomagnify in the food chains and cause adverse effects on humans and wildlife (Gong et al., 2021; Rostami & Juhasz, 2011). In 2001,

the Stockholm Convention was introduced to restrict and eventually eliminate the release of harmful contaminants in the environment known as “legacy contaminants” (Rostami & Juhasz, 2011), such as polychlorinated biphenyls (PCBs), organochlorine (OC) pesticides like dichlorodiphenyltrichloroethane (DDT) and heavy metals. Since the ban on those legacy contaminants, new compounds have been introduced known as “emerging contaminants”, such as polybrominated diphenyl ethers (PBDEs), per- and polyfluoroalkyl substances (PFAS) and microplastics. Until now, some of these contaminants are not regulated or routinely monitored, and their consequences on the environment, humans and wildlife are still unclear but have been described as emerging concerns (Pereira et al., 2015; Taheran et al., 2018).

POPs have been observed to impact many species, including birds (Burger & Gochfeld, 2001; Lodenius & Solonen, 2013), turtles (Keller et al., 2006; Komoroske et al., 2011), sharks (Boldrocchi et al., 2020; Mull et al., 2013) and marine mammals (Desforges et al., 2016; Tanabe et al., 1994). POPs have been demonstrated to impact the immune system of marine mammals (Desforges et al., 2016), increase the development of disease (Randhawa et al., 2015) and cause reproductive impairment (Murphy et al., 2018). Emerging contaminants are known to cause immunotoxicity in wildlife, but their effects on marine mammals are still unclear (Huang et al., 2020).

POPs levels vary with biological factors such as diet, body size, nutritive condition, sex and reproductive status (Aguilar et al., 1999; Khairy et al., 2021; Pinzone et al., 2015). Additionally, their high lipid content increases the chances of bioaccumulation, resulting in a high level of pollutants (Reijnders et al., 2009). Notably, many studies have focused on odontocetes, which are more likely to accumulate pollutants because of their higher trophic position and metabolic rate compared to mysticetes (Stockin et al., 2010). Pollutant concentration further varies with habitat, as coastal species are more likely to have higher pollutant levels than oceanic species due to terrestrial runoff (Jones et al., 1999). In particular, vulnerable populations, including endangered (Guo et al., 2021) and endemic (Méndez-Fernandez et al., 2018) species, are at the most significant risk of exposure due to their low population sizes and restricted distribution.

In New Zealand, a range of contaminants have historically been used or are still used, such as organochloride pesticides (e.g., DDT), PCBs, heavy metals (e.g., mercury), flame retardants (e.g., PBDEs) and PFAS. However, only a few published studies have examined those contaminants and their biological effects on New Zealand's populations, showing similar levels of contaminant across delphinid species in New Zealand and globally but increased levels of OC pesticides in Hector's and Māui dolphins than previously reported (Jones, 1998; Jones et al., 1996; Lischka et al., 2021; Stockin et al., 2007; Stockin et al., 2010; Stockin et al., 2021a; Stockin et al., 2021b).

### **1.2.5.1 Legacy contaminants**

#### **1.2.5.1.1 Polychlorinated biphenyls (PCBs)**

Many studies have demonstrated the risk and adverse effects of polychlorinated biphenyls (PCBs) in a range of mammalian taxa (Arikan et al., 2018; Bayat et al., 2014; Berninger & Tillitt, 2019; Coxon et al., 2019; Lorenzi et al., 2020; Monnolo et al., 2020; Stewart et al., 2002). This is certainly the case for marine mammals such as pinnipeds (Keogh et al., 2020; Mamontov et al., 2019), sirenians (Weijs et al., 2019), mysticetes (Muñoz-Arnanz et al., 2019; Yasunaga & Fujise, 2020) and odontocetes (Bartalini et al., 2019; Desforges et al., 2018; Jeong et al., 2020).

PCB exposure is still an elevated risk for high trophic-level predators such as marine mammals due to its high toxicity, bioaccumulation and stability in the environment (Jafarabadi et al., 2019; Pruvost-Couvreur et al., 2021; Reddy et al., 2019; Stuart-Smith & Jepson, 2017). Previous studies have investigated the biological impacts on marine mammals and have demonstrated that PCBs' toxic levels depend on species and the level of contaminant burden. For example, toxic effects in marine mammals include a lack of recruitment from reproductive impairment and immunosuppression (Jepson et al., 2005; Jepson et al., 2016; Law et al., 2012).

PCBs have historically been investigated in Hector's and Māui dolphins showing elevated levels in some animals, particularly males (Stockin et al., 2010). However, it is unclear if PCBs still persist in the environment a decade later and if those animals are still at risk from elevated levels due to new biological thresholds for adverse health effects in marine

mammals (9,000 µg/kg lipid weight) as determined by Jepson et al. (2016) based on Kannan et al. (2000) earlier findings.

#### **1.2.5.1.2 Multi-residue pesticides (MRPs)**

Many mammalian taxa have been studied to understand the level of multi-residue pesticides in the environment (Deti et al., 2014; Romanić et al., 2015). Being long-lived species, they can be used as indicators providing long-term monitoring of their environment. Specifically, marine mammals are considered bioindicators of marine ecosystem pollution (Tsygankov et al., 2018).

DDT and its metabolites have the capacity to biomagnify in the food chain, hence the important role of marine mammals that are apex predators as bioindicators (Tsygankov et al., 2018). DDT and its metabolites have been observed in many marine mammals species, including pinnipeds (Mamontov et al., 2019; Trukhin & Boyarova, 2020), sirenians (Weijs et al., 2019), mysticetes (Das et al., 2017; Winfield et al., 2020) and odontocetes (Atkinson et al., 2019; Combi et al., 2022). A range of adverse effects has been observed from exposure to DDT and its metabolites, such as immune, endocrine and reproductive impairment as well as increased susceptibility to disease and decreased population size (Desforges et al., 2016; Murphy et al., 2015; Sonne et al., 2020).

Organochlorine (OC) pesticides have previously been examined in Hector's and Māui dolphins (Stockin et al. 2010) showing significantly elevated levels above the threshold of reproductive impairment in marine mammals (8,800 µg/kg lipid weight; Barron et al. 2003), particularly in males. However, due to the limited sample size in the former study, it is unclear what spatiotemporal trends exist in the contaminant burden of this species, how sex or age class affected concentration reported and indeed, whether if OC pesticides concentrations exhibit any evidence of decline more than a decade later.

### **1.2.5.2 Emerging contaminants**

#### **1.2.5.2.1 Polybrominated diphenyl ethers (PBDE)**

In recent years, there have been increasing concerns about the risk of polybrominated diphenyl ethers (PBDE) in different mammals species (d'Havé et al., 2005; Wu et al., 2020; Zhao et al., 2022) due to their level of persistence in the environment, their toxicity and bioaccumulation (Wang et al., 2017). This is particularly the case for marine mammals, including sirenians (Weijs et al., 2019), pinnipeds (Soulen et al., 2018; Taylor et al., 2018), mysticetes (Muñoz-Arnanz et al., 2019; Simond et al., 2019) and odontocetes (Aznar-Aleman et al., 2021; Schlingermann et al., 2020; Zaccaroni et al., 2018).

PBDE has been demonstrated to have a wide range of effects on the organisms, such as endocrine-disrupting, neurotoxic, tumorigenic, DNA damaging, carcinogenic, reproductive, thyroid and cognitive effects (Eriksson et al., 2006; McDonald, 2002; Siddiqi et al., 2003; Song et al., 2022; Talsness, 2008). PBDE has also been shown to impair the immune system of marine mammals even at low concentrations (Huang et al., 2020) and to negatively affect reproduction cycles, fecundity and longevity in marine mammals (Murphy et al., 2010). This thesis is the first study to examine PBDE in Hector's and Māui dolphins.

### **1.3 Thesis rationale and objectives**

Successful conservation efforts and management are essential to protect the endangered Hector's and Māui dolphins. Many threats have been described to impact this species, with fisheries bycatch considered a primary threat. However, it is crucial to consider broader threats other than fisheries-related to ensure the successful management of this species. Contaminants are known to act as immunosuppressants, and many studies have assessed their impacts on marine mammals' health, especially in the context of disease susceptibility. To date, only one published study has examined contaminant levels in Hector's and Māui dolphins and only up until 2009. Additionally, several emerging contaminants remain unexplored. Therefore, it is crucial to further investigate the state of legacy contaminants more than a decade later and to provide the first insights into emerging contaminants for these endangered endemic dolphins.

This thesis aims to quantify the level of legacy and emerging contaminants in Hector's and Māui dolphins. Specifically, I examine the spatiotemporal trends while considering the effects of TBL and sex for (1) legacy contaminants (PCBs and MRPs) between 1997 and 2022, and (2) emerging contaminants (PBDEs) between 2010 and 2022. Specifically, my thesis addresses the following;

**Chapter I – Introduction:** Provides background context to the conservation status and threats faced by Hector's and Māui dolphins.

**Chapter II – Materials & Methods:** Outlines the full methods for the study, including sample collection, chemical methods and statistical analysis used to determine contaminants load in Hector's and Māui dolphins.

**Chapter III – Results:** Presents the results of this study. It quantifies the legacy and emerging contaminant levels in Hector's and Māui dolphins between 1997 and 2022.

**Chapter IV – Discussion:** Concludes by summarising the research chapters and investigating future research priorities.



## Chapter 2 - Materials & Methods



Hector's dolphin surfacing off the coast of Christchurch, New Zealand.

Photo credit: Steve Dawson



## 2.1 Sampling and biological data

### 2.1.1 Sample collection

Blubber samples were collected from 66 Hector's (*Cephalorhynchus hectori hectori*) and Māui (*C. h. maui*) dolphins recovered from stranding or bycatch events along the New Zealand coastline between 1997 and 2022 (Table A.1 & A.2). The majority of samples comprised Hector's dolphins (n = 62; female = 33 and male = 29), recovered along the east coast of the South Island (ECSI; between Farewell Spit and Nugget Point), the west coast of the South Island (WCSI; between Farewell Spit and Milford Sound) and the south coast of the South Island (SCSI; between Nugget Point to Long Point) (Figure 2.1), of which 44 were stranded, and 17 were known bycatch or suspected as bycatch following post-mortem examinations. A further subset of Māui dolphins (n = 4; female = 3 and male = 1) was also included, all recovered from stranding along the west coast of the North Island (WCNI) (Figure 2.1). This research was conducted under marine mammal 39239-MAR issued to Massey University.

All samples originated from stranded and bycaught animals submitted to Massey University for post-mortem examination by the Department of Conservation. Tissue samples were taken in a standardised manner for PCBs, multi-residue pesticides (MRP) and flame retardants (PBDE). PCBs and MRPs were tested for the animals across the entire study period (n = 66), while as an emerging contaminant, PBDE was tested from 2010 (n = 36). Cross-sectional full blubber depth samples excised adjacent to the dorsal fin were sampled from each carcass using a stainless-steel knife, following standardised protocols (e.g. Kuiken et al., 1994). Samples were placed in whirl pack bags and stored at -20° until subsequent chemical analyses.

### 2.1.2 Biological data

Information on subspecies, sex, total body length (TBL), date and location of stranding or bycatch (Table A.1 & A.2) were obtained from pathology reports and the Hector's and Māui dolphin incident database publicly available from the Department of Conservation website (DOC, 2022b). This database includes information on beachcast carcasses, dolphins found entangled in fishing gear, recovered at sea and other types of mortalities since 1921. Veterinary pathologists determined sex by anatomical examination of the genitals and gross and histological examination of reproductive organs during post-mortem examinations or by

DNA analysis when sex could not be inferred from anatomy (Jayasankar et al., 2008). I separated animals into yearlings vs non-yearlings categories based on their TBL: yearlings = <99 cm, non-yearlings = >99cm, following Slooten (1991).

For the purpose of this study, the term “Hector’s dolphins” will be inclusive of both subspecies. All samples recovered from the west coast of both the North and South Island were classified as “west coast” when using Generalized linear models (GLMs) due to the small sample size of Māui dolphins.

## 2.2 Chemical analyses

### 2.2.1 Polychlorinated biphenyls (PCBs)

PCBs were determined in blubber by high-resolution gas chromatography–high-resolution mass spectrometry (HRGC–HRMS). Extraction and quantification of 45 polychlorinated biphenyls congeners were examined alongside  $\Sigma$ 7PCBs (Table 2.1) in each animal.

**Table 2.1:** PCB congeners assessed in Hector’s dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. Note: Bold congeners represent the ICES 7CBs used for comparison across the entire study period.

1997-2009 (Stockin et al. 2010)					2010-2022 (Present Study)		
n = 45					n = 18		
<b>CB1</b>	CB3	CB4/10	CB15	CB19	<b>CB28</b>	<b>CB52</b>	CB77
<b>CB28</b>	CB37	CB44	CB49	<b>CB52</b>	CB81	<b>CB101</b>	CB105
<b>CB54</b>	CB70	CB74	CB77	CB81	CB114	<b>CB118</b>	CB123
<b>CB99</b>	<b>CB101</b>	CB104	CB105	CB110	CB126	<b>CB138</b>	<b>CB153</b>
<b>CB114</b>	<b>CB118</b>	CB123	CB126	<b>CB138</b>	CB156	CB157	CB167
<b>CB153</b>	CB155	CB156	CB157	CB167	CB169	<b>CB180</b>	CB189
<b>CB169</b>	CB170	<b>CB180</b>	CB183	CB187			
<b>CB188</b>	CB189	CB194	CB196/203	CB200			
<b>CB202</b>	CB205	CB206	CB208	CB209			

Prior to taking test portions, test samples were homogenised using a blender. Test portions (2.5 g) were further blended with sodium sulfate then Soxhlet extracted with dichloromethane:hexane (1:1, v:v). A split of the extract (0.4%) was taken for analysis and spiked with  $^{13}\text{C}$ -labelled internal standard solution. Extracts were cleaned using acidic/basic silica gel chromatography, and then evaporated to a final volume of 0.1 mL (nonane). Final extracts were analysed by GC-MS/MS with detection performed in MRM mode. Individual analyte concentrations were calculated from their relative response to an internal standard against the slope of a multi-point calibration curve. The laboratory method was an in-house validated method and accredited under International Accreditation New Zealand (AINZ) (131). A reagent blank, a QC spike and a sample duplicate were analysed with the batch of samples.

### 2.2.2 Multi-residue pesticides (MRPs)

Multi-residue pesticides were determined in blubber by high-resolution gas chromatography–high-resolution mass spectrometry (HRGC-HRMS) with detection performed in MRM mode. Extraction and quantification of MRP (Table 2.2) were examined in each animal.

**Table 2.2:** MRPs assessed in Hector’s dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. Note: bold represents the MRPs analysed throughout the entire study period.

1997-2009 (Stockin et al 2010) n = 6		2010-2022 - Present study n = 10		
<b>Dieldrin</b>	<b>HCB</b>	<b>Dieldrin</b>	<b>HCB</b>	<b>ΣDDT</b>
<b>p,p'DDD</b>	<b>p,p'DDE</b>	<b>p,p'DDD</b>	<b>o,p'DDD</b>	Mirex
<b>o,p'DDT</b>	<b>p,p'DDT</b>	<b>p,p'DDE</b>	<b>o,p'DDE</b>	Oxychlorthane
<b>ΣDDT</b>		<b>p,p'DDT</b>	<b>o,p'DDT</b>	

Test portions (1.5 g) were dissolved in acetonitrile by heating in a water bath. The acetonitrile solution was then placed in a freezer overnight. After filtration, an aliquot was taken for

cleanup by dispersive solid-phase extraction. A split of the extract was taken and evaporated to a volume of 0.1 ml. An additional 0.3 ml of toluene was added to the extract to give a final volume of 0.4. Individual analyte concentrations were calculated from their relative response to an internal standard against the slope of a multi-point calibration curve.

### 2.2.3 Polybrominated diphenyl ethers (PBDEs)

Selected polybrominated diphenyl ethers were determined in blubber by gas chromatography–high-resolution mass spectrometry (GC-MS/MS). Extraction and quantification of flame retardants (Table 2.3) were considered in isolation and used to calculate the total PBDE.

**Table 2.3:** PBDE congeners assessed in Hector’s dolphins (*Cephalorhynchus hectori*) in New Zealand between 2010-2022.

2010-2022 (n = 10)				
BDE-28	BDE-47	BDE-66	BDE-85	BDE-99
BDE-100	BDE-153	BDE-154	BDE-183	BDE-209

Test samples were homogenized using a blender prior to taking test portions. Test portions (5 g) were further blended with sodium sulphate, spiked with <sup>13</sup>C-labelled internal standard solution, then Soxhlet-extracted with dichloromethane:hexane (1:1, v:v). The extracts were cleaned using dispersive SPE (acidic silica gel), followed by acidic/basic silica gel and Florisil column chromatography, then evaporated to a final volume of 2 mL (toluene). Final extracts were analysed by GC-MS/MS, with detection performed in MRM mode. Individual analyte concentrations were calculated from their relative response to an internal standard against the slope of a multi-point curve. While this laboratory method has not been validated or accredited, it employs well-established principles and procedures. A reagent blank, three QC spikes and a sample duplicate were analysed with the batch of samples.

### 2.2.4 Instrumental

The HRGC–HRMS analyses were performed on an Agilent 6890 gas chromatograph equipped with a Phenomenex Zebron ZB5 60 m x 0.25 mm id x 0.25 µm phase thickness column (for

multi-residue pesticides) or an Agilent J&W DB1 30 m x 0.25 mm id x 0.25  $\mu$ m phase thickness column (for polychlorinated biphenyls) using splitless injection, coupled to a Micromass AutoSpec Ultima high-resolution mass spectrometer. All analyses were performed under the laboratory's IANZ accreditation (No. 131) based on Stockin et al. (2007) and Stockin et al. (2010).

### 2.2.5 Quality assurance

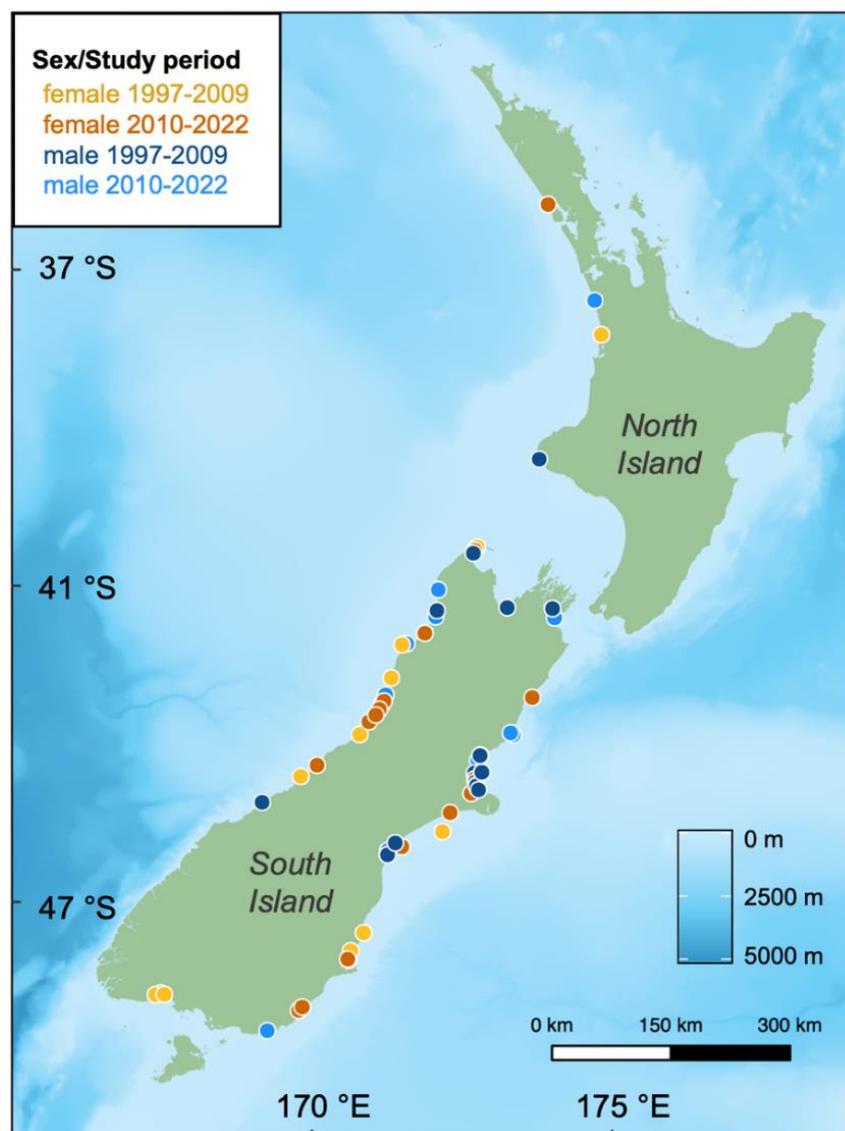
The analyses were based on USEPA Methods 1668A (PCBs) and 1699 (Organochlorine pesticides). Following methods described in the USEPA, all QC/QC procedures including the analysis of a laboratory method blank and spiked control sample with every batch of 15 samples were performed. The criteria shown in the USEPA methods were used to assess the results of these QC samples. Additionally, each sample was fortified with a range of isotopically labelled internal standards, and the recovery of the standards was also evaluated against criteria presented in the USEPA methods.

### 2.3 Data analysis

Each sample was categorised based on the sex (male or female), yearling proxy (yearling or non-yearling based on TBL), study period (1997-2009 or 2010-2022) and coastline (west, east and south). The total PCBs was used as a reference and the ICES list of seven congeners (Table 2.1) was used to compare with different datasets, which generally use different suites of congeners.  $\Sigma$ DDT concentrations were calculated based on o,p'-DDD + p,p'-DDD + o,p'-DDE + p,p'-DDE + o,p' DDT + p,p' DDT. Ten different PBDE congeners were analysed (Table 2.3).

To analyse multiple variables together, Generalized linear models (GLMs) were used in R version 4.1.0 (R Core Team, 2022). This method has been proven useful in interpreting spatiotemporal trends of contaminants in cetaceans (Gui et al., 2017; Jepson et al. 2016; Law et al. 2012). Dependent variables were allPCB, 7PCBs, MRP and PBDE, independent variables were TBL, sex, coast and year. The dependent variables were log-transformed to stabilize the variance. For each dependent variable, two GLMs with a gaussian error distribution and log link function were built: one including all independent variables, and one excluding the independent variable year. These models were also built both with and without the

interaction between sex and TBL. For all independent variables except MRP, the interaction between sex and TBL improved model fit and was retained. The model fit was assessed using AICc. Models were checked for normality, heteroskedasticity and potential patterns in the residuals and were then averaged based on their corrected Akaike's information criterion weights adjusted for small sample size (wAICc) (Burnham et al., 2011) using the MuMIn R package (Barton, 2009). For the final averaged models, the importance of each independent variable was evaluated by calculating the change in goodness of fit (i.e., the adjusted amount of deviance accounted for by the GLM) when the respective variable is excluded from the final model.



**Figure 2.1:** Distribution of Hector's dolphins (*Cephalorhynchus hectori*) stranded and by-caught between 1997 and 2022 in New Zealand. Notes: light orange = female 1997-2009, dark orange = female 2010-2022, dark blue = male 1997-2009, light blue = male 2010-2022.





## Chapter 3 - Results



Hector's dolphin (*Cephalorhynchus hectori hectori*), Banks Peninsula, New Zealand.  
Photo credit: Steve Dawson



### 3.1 Polychlorinated biphenyls - PCBs

#### 3.1.1 Sample summary

PCBs were analysed in 66 animals, including 36 females (8 yearlings and 28 non-yearlings) and 30 males (7 yearlings and 23 non-yearlings) between 1997 and 2022.

#### 3.1.2 PCB concentration and multivariate analysis

A total of 45 congeners was analysed (Table 2.1). Overall, the  $\sum$ allPCBs concentration ranged from 8.4 to 11,375 (mean = 1,108; SD = 1,726; n = 66)  $\mu\text{g}/\text{kg}$  lipid weight.  $\sum$ allPCBs concentrations ranged from 16.2 to 5,574 (mean = 1,536; SD = 1,409; n = 30) and 8.4 to 11,375 (mean = 751; SD = 1,896; n = 36)  $\mu\text{g}/\text{kg}$  lipid weight for males and females, respectively. When excluding yearlings from the total sample,  $\sum$ allPCBs concentrations ranged from 45.5 to 11,375 (mean = 1,225; SD = 1,821; n = 51)  $\mu\text{g}/\text{kg}$  lipid weight. Still excluding yearlings by sex,  $\sum$ allPCBs concentrations varied from 299 to 5,574 (mean = 1,688; SD = 1,289; n = 23) and 45.5 to 11,375 (mean = 845; SD = 2,111; n = 28)  $\mu\text{g}/\text{kg}$  lipid weight for males and females, respectively.

Stockin et al. (2010) examined a total of 45 congeners in Hector's dolphins between 1997 and 2009, of which 18 congeners (including the ICES 7CBs CB28, CB52, CB101, CB138, CB153, CB180) were replicated during the present study period (2010-2022; Table 2.1). To compare historical concentrations from Stockin et al. 2010 and draw meaningful comparisons between the studies over time, here in I assess only 7CBs to allow temporal comparison across the entire study period.

$\sum$ 7CBs ranged from 7.7 to 10,746 (mean = 946; SD = 1,558; n = 66)  $\mu\text{g}/\text{kg}$  lipid weight across 1997-2022. In males and females, the sum concentration ranged from 14.9 to 4,499 (mean = 1,285.4; SD = 1,156; n = 30) and 7.7 to 10,746 (mean = 663; SD = 1,794; n = 36)  $\mu\text{g}/\text{kg}$  lipid weight, respectively. When excluding yearlings,  $\sum$ 7CBs ranged from 28.9 to 10,746 (mean = 1,039; SD = 1,643; n = 51)  $\mu\text{g}/\text{kg}$  lipid weight. Still excluding yearling by sex, the  $\sum$ 7CBs concentration ranged from 217 to 4,276 (mean = 1,396, SD = 991; n = 23) and 28.9 to 10,746 (mean = 747; SD = 2,000; n = 28)  $\mu\text{g}/\text{kg}$  lipid weight, in females and males, respectively.

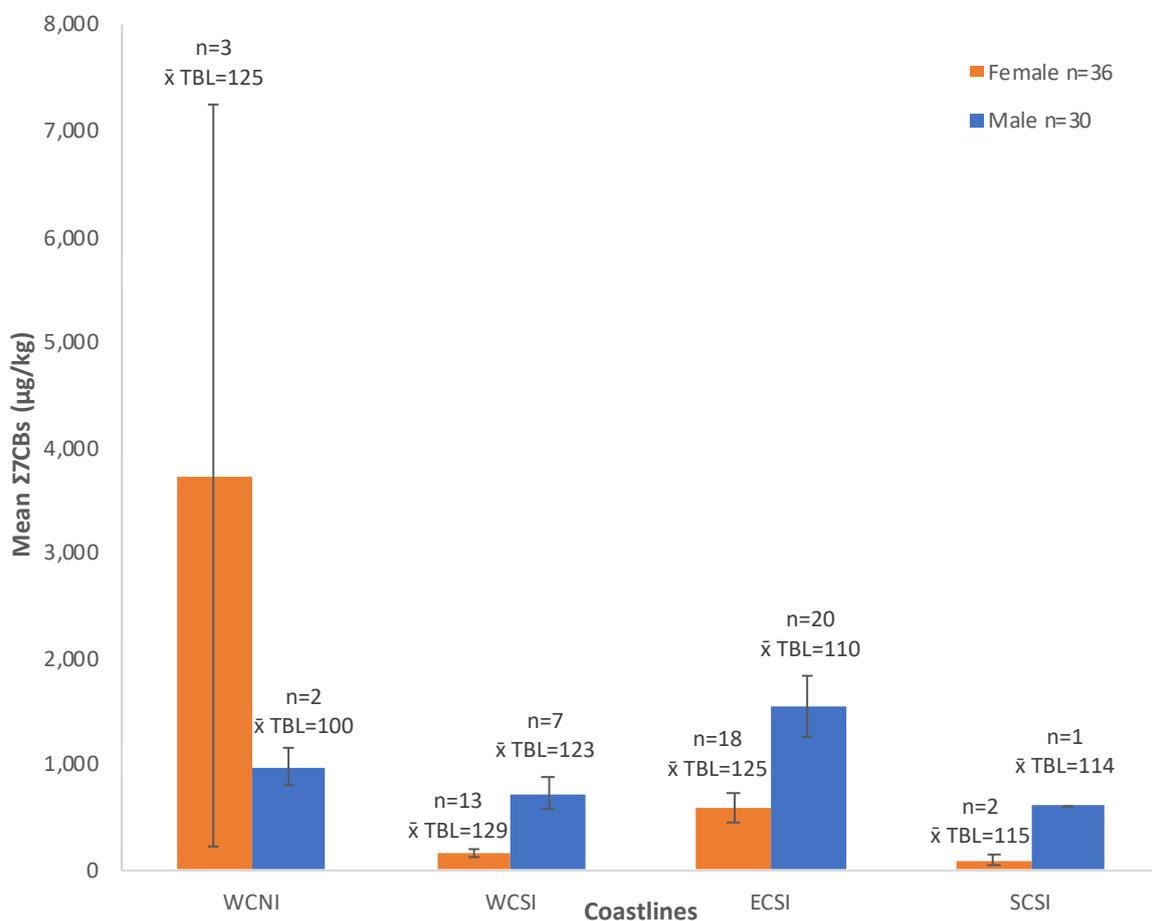
Spatially, a female (H243, 151 cm mature Māui dolphin from WCNI) showed the highest level of  $\Sigma$ 7CBs on the WCNI (up to 10,746  $\mu\text{g}/\text{kg}$  lw; mean = 3,735; SD = 6,072) compared to their females Hector counterparts from the South Island, with the highest contaminant burden recorded on the ECSI (2,171  $\mu\text{g}/\text{kg}$  lw; mean = 581; SD = 611), despite similar mean body lengths reported between the four regions (Fig 3). WCSI presented the lowest  $\Sigma$ PCBs of the 4 regions with 7.7  $\mu\text{g}/\text{kg}$  lw (mean = 156; SD = 102) (Fig 3.1). For males, the ECSI recorded the highest level of  $\Sigma$ 7CBs with up to 4,499  $\mu\text{g}/\text{kg}$  lw (mean = 1,549; SD = 1,328) out of the four regions. The highest  $\Sigma$ 7CBs burden present in WCNI and WCSI animals were similar (1,164  $\mu\text{g}/\text{kg}$  lw; mean = 977; SD = 265 and 1,144  $\mu\text{g}/\text{kg}$  lw; mean = 719; SD = 396, respectively). Mean TBL was also similar between the four regions for males. Males reported the highest mean for  $\Sigma$ 7CBs across the South Island (ECSI, SCSi and WCSI), whereas females demonstrated the highest mean  $\Sigma$ 7CBs across the WCNI (Fig 3.1).

Throughout the entire study period, mean  $\Sigma$ 7CBs has increased around the WCNI from 229 (1997-2009; n = 17) to 10,746 (2010-2022; n = 19)  $\mu\text{g}/\text{kg}$  lw, and almost doubling from 373 (1997-2009; n = 17) to 686 (2010-2022; n = 19)  $\mu\text{g}/\text{kg}$  lw in the ECSI, for females. Notably, for WCNI males, mean  $\Sigma$ 7CBs declined from 1,164 to 790  $\mu\text{g}/\text{kg}$  lw across the same two study periods respectively. Males further contradicted female trends in the ECSI (2,055 to 1,331  $\mu\text{g}/\text{kg}$  lw) and WCSI (783 to 559  $\mu\text{g}/\text{kg}$  lw) between 1997-2009 (n = 13) and 2010-2022 (n = 17), respectively.

When excluding yearlings,  $\Sigma$ 7CBs ranged from 71 to 1,691 (mean = 565; SD = 496), from 29 to 353 (mean = 565; SD = 496), from 318 to 10,746 (mean = 5,532; SD = 7,374) and was equal to 42  $\mu\text{g}/\text{kg}$  lipid weight, in ECSI, WCSI, WCNI and SCSi respectively for females. Still excluding yearlings,  $\Sigma$ 7CBs ranged from 217 to 4,276 (mean = 1,696; SD = 1,034), from 439 to 1,144 (mean = 836; SD = 269), and was equal to 790 and 605  $\mu\text{g}/\text{kg}$  lipid weight, in ECSI, WCSI, WCNI and SCSi, respectively for males.

For both  $\Sigma$ allPCBs and  $\Sigma$ 7PCBs, the two models with and without year were similar in the amount of deviance they explained as well as their AICc weights (Table 3.2).

The averaged models for  $\Sigma$ allPCBs and  $\Sigma$ 7PCBs show that PCB burdens were lower for animals from the west and the south coast compared to the east coast (Table 3.3). While males increased in contaminant burden with increasing total body length, this increase was less pronounced in females (Fig 3.2 A&C). PCB burdens declined minimally with time (Fig 3.2 B&D). Of all included variables, sex was the most important predictor, followed by total body length and coast, with the interaction between total body length, sex, and year only contributing minimally to model fit (Fig 3.3).



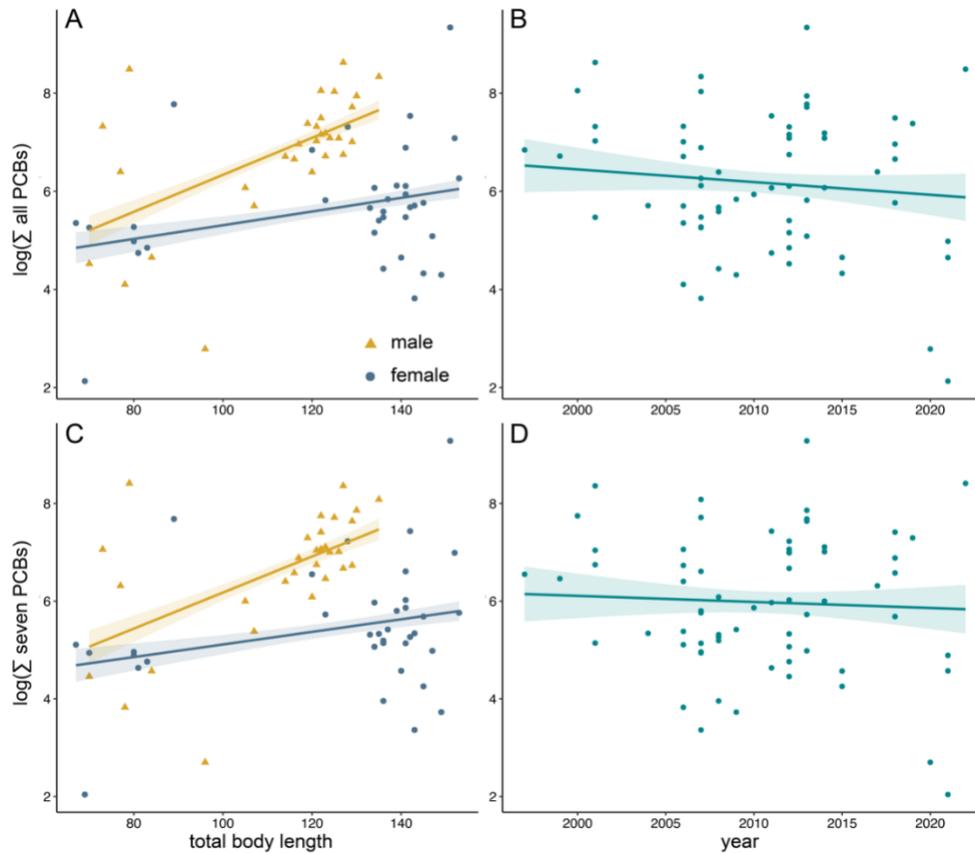
**Figure 3.1:** Mean  $\Sigma$ 7CBs in Hector's dolphins (*Cephalorhynchus hectori*) by coastline between 1997 and 2022. Note: n = sample size,  $\bar{x}$  = mean, TBL = total body length.

**Table 3.1:** Generalised linear models (GLMs) for  $\sum$ allPCBs (A) and  $\sum$ 7PCBs (B) in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. Models were averaged based on their AICc weights. Note: K = number of parameters, logLik = log-likelihood, % DE = % deviance explained,  $\Delta$ AICc = difference in Akaike's information criterion corrected for small sample size (AICc) of the current and top-ranked model, wAICc = AICc weight.

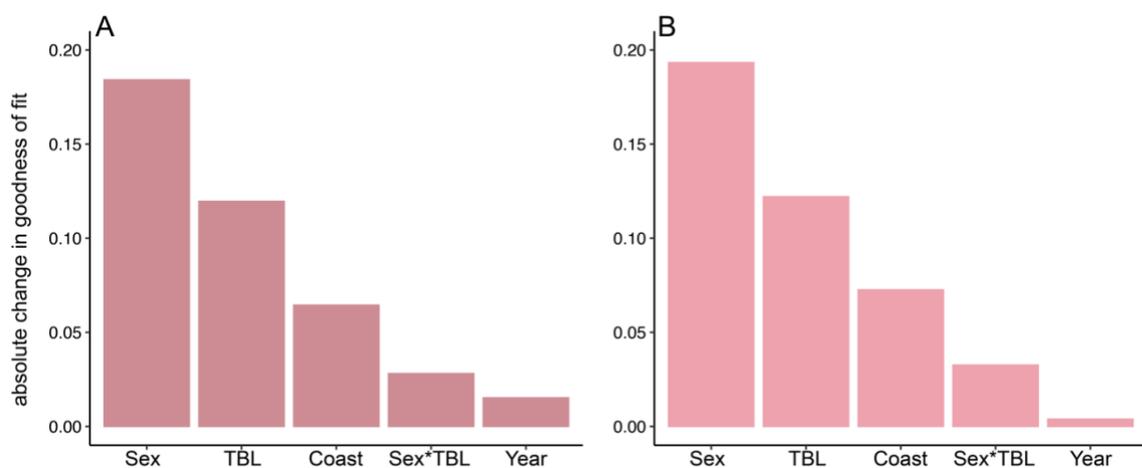
Model	K	logLik	AICc	$\Delta$ AICc	wAICc	% DE
<b>A) <math>\sum</math>allPCBs</b>						
log( $\sum$ allPCBs) ~ TBL * Sex + Coast	7	-98.94	213.82	0	0.51	41.27
log( $\sum$ allPCBs) ~ TBL * Sex + Coast + Year	8	-97.68	213.88	0.06	0.49	38.97
<b>B) <math>\sum</math>7PCBs</b>						
log( $\sum$ 7PCBs) ~ TBL * Sex + Coast	7	-99.68	215.29	0	0.7	40.67
log( $\sum$ 7PCBs) ~ TBL * Sex + Coast + Year	8	-99.22	216.97	1.68	0.3	39.84

**Table 3.2:** Averaged generalised linear model (GLM) results for  $\sum$ allPCBs (A) and  $\sum$ 7PCBs (B) in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. Note: SE = standard error, TBL = total body length.

Term	Estimate	SE	Adjusted SE	Statistic	P-value
<b>A) <math>\sum</math>allPCBs</b>					
(Intercept)	42.53	52.99	53.5	0.8	0.43
TBL	0.01	0.01	0.01	1.91	0.06
Sex_male	-1.52	1.53	1.56	0.97	0.33
Coast: south	-0.93	0.69	0.7	1.32	0.19
Coast: west	-0.73	0.3	0.31	2.39	0.02
Sex_male*TBL	0.02	0.01	0.01	1.83	0.07
Year	-0.02	0.03	0.03	0.72	0.47
<b>B) <math>\sum</math>7PCBs</b>					
(Intercept)	18.55	36.16	36.65	0.51	0.61
TBL	0.01	0.01	0.01	1.76	0.08
Sex_male	-1.63	1.55	1.58	1.03	0.3
Coast: south	-1.12	0.7	0.71	1.57	0.12
Coast: west	-0.78	0.3	0.31	2.53	0.01
Sex_male*TBL	0.03	0.01	0.01	1.91	0.06
Year	-0.01	0.02	0.02	0.39	0.7



**Figure 3.2:** Averaged generalised linear model (GLMs) for  $\sum_{\text{all}} \text{PCBs}$  (A&B) and  $\sum_7 \text{PCBs}$  (C&D) showing the effect of the interaction between total body length and sex (A&C) and the effect of year (B&D) on Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. The averaged models explained 40.1 and 40.4% of the deviance, respectively.



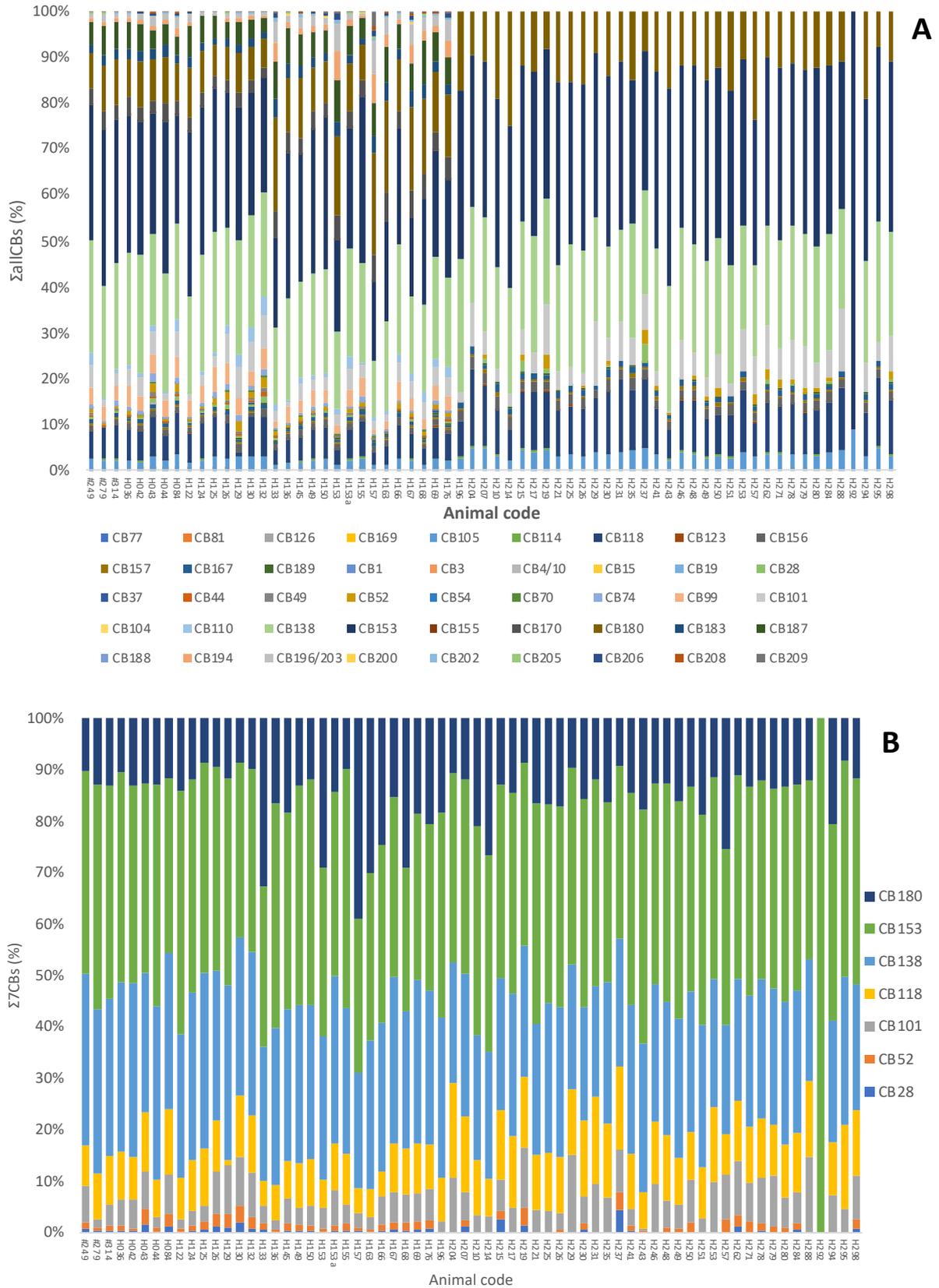
**Figure 3.3:** Variable importance for averaged generalised linear models (GLMs) shown as absolute change in goodness of fit for  $\sum_{\text{all}} \text{PCBs}$  (A) and  $\sum_7 \text{PCBs}$  (B) in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022.

### 3.1.3 PCB profile

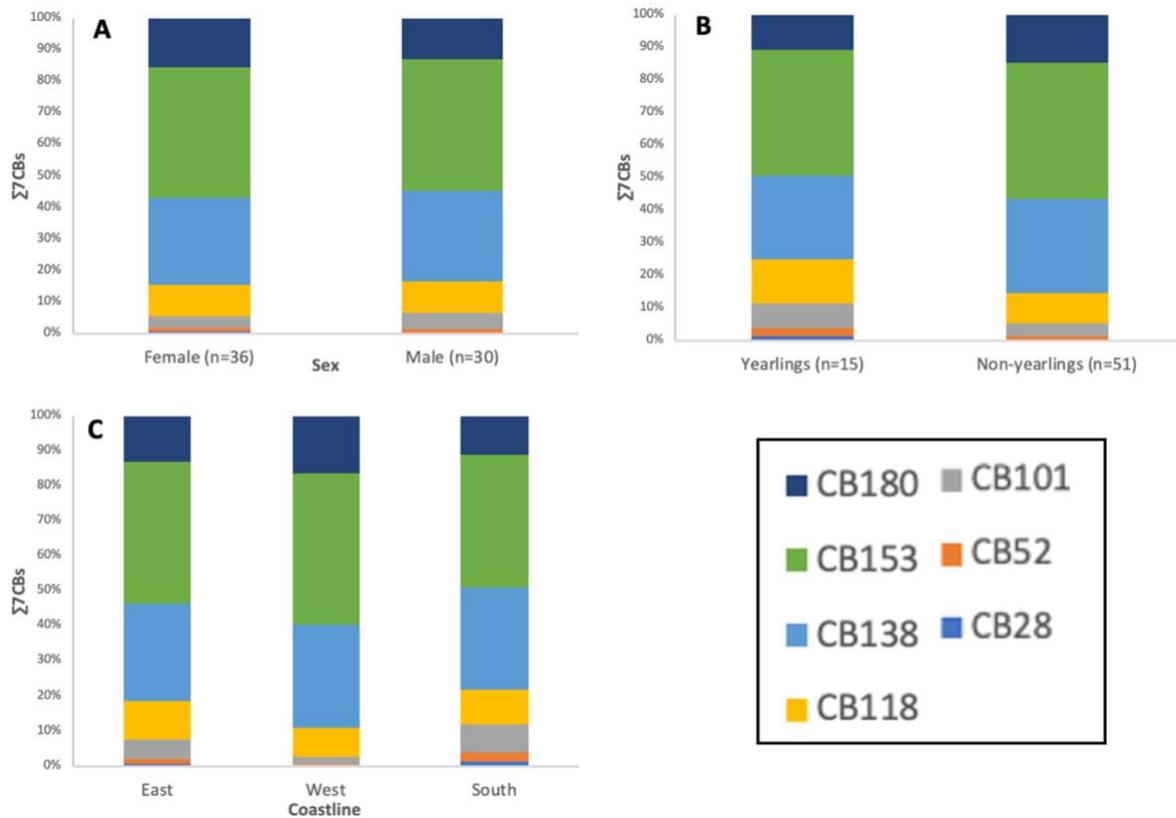
The PCB profile appears relatively consistent over time, with CB180, CB153 and CB138 the primary congeners present across all animals, except for H292 (a male neonatal Hector's dolphin that stranded on WCSI in 2021, which only comprised of CB153 and CB105; Fig 3.4 A). Several congeners were not in the profile of dolphins examined from 2010 onwards since these congeners were not analysed as part of standard assessment within AsureQuality.

The profile of the 7CBs was temporally consistent, with CB180, CB153 and CB138 the dominant congeners throughout the entire study period, comprising >80% of the total PCB profile, except H292 (a neonate male Hector's dolphin that stranded on WCSI in 2021), which comprised only CB153 (Fig 3.4 B).

The 7CBs profile was consistent between males and females, with three primary congeners (CB153, CB138 and CB180) comprising more than 80% of the total profile (Fig 3.5 A). The three primary 7CB congeners were present in a relatively similar concentration between yearlings (>75% of the total profile) and non-yearlings (>85% of the total profile; Fig 3.5 B). The 7CBs profile was relatively similar across the different coasts, with CB153, CB130 and CB180 as the dominant congeners (Fig 3.5 C).



**Figure 3.4:** All CBs (A) and 7CBs (B) profile in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022.



**Figure 3.5:** 7CBs profile in Hector's dolphins (*Cephalorhynchus hectori*) between sex (A), yearling vs non-yearling (B) and coastline (C) in New Zealand between 1997 and 2022.

## 3.2 Multi-residue pesticides - MRPs

### 3.2.1 Sample summary

MRPs were analysed in a total of 66 animals, including 36 females (8 yearlings and 28 non-yearlings) and 30 males (7 yearlings and 23 non-yearlings) across the entire study period (1997-2022).

### 3.2.2 MRP concentration and multivariate analysis

A total of 10 multi-residue pesticides (MRPs) were analysed (Table 2.2). Of the various MRPs,  $\Sigma$ DDT (up to 57,390  $\mu\text{g}/\text{kg lw}$ ), p,p'DDE (up to 53,000  $\mu\text{g}/\text{kg lw}$ ), p,p'DDT (up to 2,400  $\mu\text{g}/\text{kg lw}$ ), o,p'DDT (2,100  $\mu\text{g}/\text{kg lw}$ ) and p,p'DDD (up to 1,200  $\mu\text{g}/\text{kg lw}$ ) were present at the highest concentration. Dieldrin (up to 490  $\mu\text{g}/\text{kg lw}$ ), mirex (up to 240  $\mu\text{g}/\text{kg lw}$ ), HCB (up to 130  $\mu\text{g}/\text{kg lw}$ ) and oxychlorodane (up to 130  $\mu\text{g}/\text{kg lw}$ ) were detected at lower concentrations, and

o,p'DDD (up to 56 µg/kg lw) and o,p'DDE (38 µg/kg lw) were detected at a considerably lower concentration than all multi-residue pesticides.

Throughout the study period, dieldrin ranged from 6 to 490 (mean = 125; SD = 103) and 5 to 300 (mean = 58; SD = 70) µg/kg lipid weight in males and females, respectively. In males, dieldrin concentration ranged from 6 to 490 (mean = 170; SD = 128) and 19 to 220 (mean = 88; SD = 58) µg/kg lipid weight between 1997-2009 and 2010-2022, respectively, indicating a decline. In females, dieldrin concentration increased from 5 to 140 (mean = 35; SD = 35) and 12 to 300 (mean = 86; SD = 90) µg/kg lipid weight between the same sampling periods, respectively. When excluding yearlings from the total sample, dieldrin concentration ranged from 5.3 to 490 (mean = 97; SD = 96; n = 51) µg/kg lipid weight. Still excluding yearlings by sex, dieldrin concentrations varied from 31 to 490 (mean = 146; SD = 104; n = 23) and 5.3 to 300 (mean = 54; SD = 64; n = 28) µg/kg lipid weight for males and females, respectively.

Throughout the study period, HCB ranged from 7 to 130 (mean = 56; SD = 28; n = 30) and 3 to 110 (mean = 34; SD = 28; n = 36) µg/kg lipid weight in males and females, respectively. In males, HCB concentration increased from 7 to 90 (mean = 53; SD = 24, n = 17) to 26 to 130 (mean = 59; SD = 31; n = 19) µg/kg lipid weight between 1997-2009 and 2010-2022, respectively. In females, HCB concentration increased from 3 to 61 (mean = 20; SD = 18; n = 13) to 26 to 130 (mean = 59; SD = 31; n = 17) µg/kg lipid weight between the same sampling periods, respectively. When excluding yearlings from the total sample, HCB concentration ranged from 2.7 to 130 (mean = 48; SD = 30; n = 51) µg/kg lipid weight. Still excluding yearlings by sex, HCB concentrations varied from 26 to 130 (mean = 61; SD = 26) and 2.7 to 110 (mean = 58; SD = 82) µg/kg lipid weight for males and females, respectively.

Mirex and oxychlorane were only screened from 2010 to 2022. In that timescale, mirex ranged from 12 to 110 (mean = 40; SD = 34, n = 17) and 12 to 240 (mean = 58; SD = 82; n = 19) µg/kg lipid weight in males and females, respectively. When excluding yearlings, mirex concentration did not change for either sex because all yearlings were detected lower than the limit of detection (LOD). Oxychlorane ranged from 10 to 52 (mean = 27; SD = 16; n = 17) and 10 to 130 (mean = 38; SD = 52; n = 19) µg/kg lipid weight in males and females, respectively. This range only changed for females from 11 to 130 (mean = 45; SD = 57; n = 14)

$\mu\text{g}/\text{kg}$  lipid weight, when excluding yearlings. This is because all male yearlings were detected lower than the LOD.

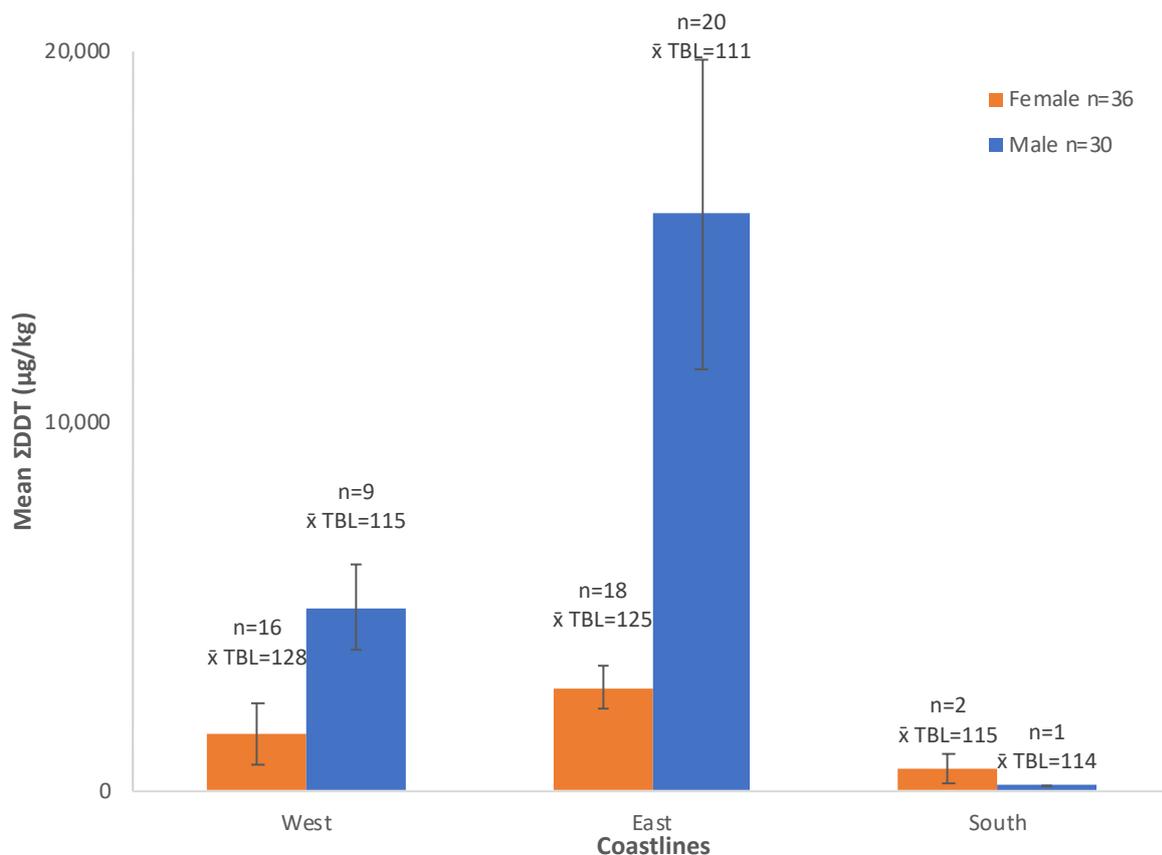
$\Sigma\text{DDT}$  (determined as the sum of p,p'DDD; p,p'DDE; o,p'DDT; p,p'DDT between 1997-2009 and the sum of o,p'DDD; p,p'DDD; o,p'DDE; p,p'DDE; o,p'DDT; p,p'DDT between 2010-2022) had the highest concentration out of those five multi-residue pesticides. Throughout the entire study period,  $\Sigma\text{DDT}$  ranged from 44 to 57,390 (mean = 12,061; SD = 16,010) and 31 to 12,860 (mean 2,076, SD = 2,743)  $\mu\text{g}/\text{kg}$  lipid weight in males and females, respectively. Males recorded a slight decrease, with mean  $\Sigma\text{DDT}$  of 12,398 (SD = 18,161) compared to 11,809 (SD = 14,732)  $\mu\text{g}/\text{kg}$  lipid weight, between 1997-2009 and 2010-2022, respectively. In females, mean  $\Sigma\text{DDT}$  increased from 1,358 (SD = 1,974) to 2,718 (SD = 3,201)  $\mu\text{g}/\text{kg}$  lipid weight between the same study periods, respectively.

Between 1997 and 2009,  $\Sigma\text{DDT}$  ranged from 252.4 to 57,390 (mean = 12,390; SD = 18,161) and 93.7 to 8,210 (mean = 1,358; SD = 1,974)  $\mu\text{g}/\text{kg}$  lipid weight in males and females, respectively. During the same study period, the  $\Sigma\text{DDT}$  concentration was relatively low apart from 2 males (H36, H44; Table 2.2), which clearly showed higher  $\Sigma\text{DDT}$  concentration than the rest of the animals. Between 2010 and 2022,  $\Sigma\text{DDT}$  ranged from 44 to 50,698 (mean = 11,809; SD = 14,732) and 31 to 12,860 (mean = 3,098; SD = 3,550)  $\mu\text{g}/\text{kg}$  lipid weight in males and females, respectively. Two male Hector's dolphins (H241 and H284, Table 2.2) also presented higher  $\Sigma\text{DDT}$  concentrations than the rest of the animals. When excluding yearlings from the total sample,  $\Sigma\text{DDT}$  concentration ranged from 93.7 to 57,390 (mean = 8,223; SD = 13,206; n = 51)  $\mu\text{g}/\text{kg}$  lipid weight. Still excluding yearlings by sex,  $\Sigma\text{DDT}$  concentrations varied from 569 to 57,390 (mean = 15,194; SD = 17,122; n = 23) and 93.7 to 12,860 (mean = 2,497; SD = 2,946; n = 28)  $\mu\text{g}/\text{kg}$  lipid weight for males and females, respectively.

When assessing contaminant burdens for females in a spatial context, WCNI showed the highest level of  $\Sigma\text{DDT}$  (up to 12,860  $\mu\text{g}/\text{kg}$  lw; mean = 4,530; SD = 7,215) compared to the dolphins from the South Island, with the highest level present in the ECSI with up to 8,210  $\mu\text{g}/\text{kg}$  lw (mean = 2,811; SD = 2,408; Fig 3.6). Apparent differences occurred despite similar mean TBL between the 4 regions. Conversely, WCSI presented the lowest  $\Sigma\text{DDT}$  out of the four regions, with 31  $\mu\text{g}/\text{kg}$  lw (mean = 851; SD = 721; Fig 3.6). The ECSI had the highest level

of  $\Sigma$ DDT within males, up to 57,390  $\mu\text{g}/\text{kg}$  lw (mean = 15,625; SD = 18,614), despite similar mean TBL between the 4 regions (Fig 3.6). The SCSi (mean = 3,597; SD = 3,597), WCSi (mean = 5,217; SD = 3,690) and WCNI (mean = 4,121; SD = 2,787) had significantly lower  $\Sigma$ DDT compared to the ECSi (Fig 3.8). Males demonstrated higher mean  $\Sigma$ DDT across the south island (ECSi, SCSi and WCSi), whereas females had higher mean  $\Sigma$ DDT across the WCNI (Fig 3.6).

Throughout the entire study period, mean  $\Sigma$ DDT increased in WCNI males from 2,151 to 6,092  $\mu\text{g}/\text{kg}$  lw, respectively. For ECSi and WCSi males, mean  $\Sigma$ DDT has decreased from 20,983 (1997-2009) to 13,281 (2010-2022)  $\mu\text{g}/\text{kg}$  lw and from 5,884 (1997-2009) to 4,364 (2010-2022)  $\mu\text{g}/\text{kg}$  lw, respectively. For females, mean  $\Sigma$ DDT has increased from 2,671 (1997-2009) to 2,912 (2010-2022)  $\mu\text{g}/\text{kg}$  lw for the ECSi and from 365 (1997-2009) to 12,860 (2010-2022)  $\mu\text{g}/\text{kg}$  lw for the WCNI. However, in WCSi, mean  $\Sigma$ DDT has declined from 744 to 640  $\mu\text{g}/\text{kg}$  lw across the consecutive study periods.



**Figure 3.6:** Mean  $\Sigma$ MRP in Hector's dolphins (*Cephalorhynchus hectori*) across the different New Zealand coasts between 1997 and 2022. Note: n = sample size,  $\bar{x}$  = mean, TBL = total body length.

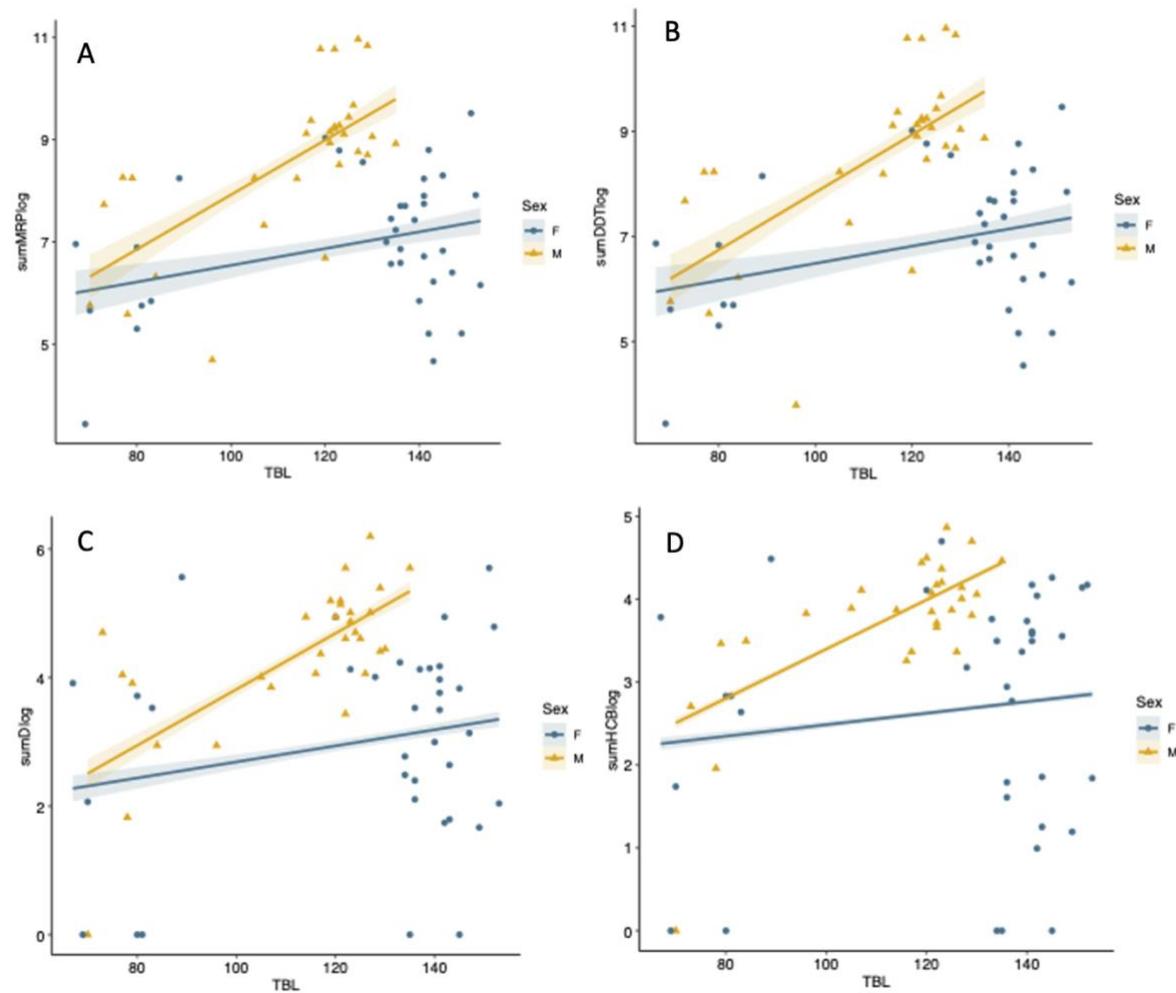
For both  $\sum$ MRP and  $\sum$ DDT, the two models with and without year were similar in deviance explained and AICc weight. Whereas, for dieldrin and HCB, the two models with and without the year were slightly different from all the other sets of MRP (Table 3.5). The averaged models showed that  $\sum$ MRP,  $\sum$ DDT, dieldrin and HCB burdens were lower for animals from the west and south coasts compared to the east coast (Table 3.6). The contaminant burden increased with total body length for males, though this increase was less notable for females (Figure 3.7 A, B, C & D).  $\sum$ MRP,  $\sum$ DDT and HCB burdens declined marginally with time, although this decline was more pronounced for dieldrin (Figure 3.8 A, B, C & D). Of all variables included in this model, the most important predictor was sex, followed by total body length and coast, with the interaction between total body length and sex, and year only contributing little to model fit (Fig 3.9).

**Table 3.3:** Generalised linear models (GLMs) for  $\sum$ MRP (A),  $\sum$ DDT (B), dieldrin (C) and HCB (D) in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. Models were averaged based on their AICc weights. Note: K = number of parameters, logLik = log-likelihood, % DE = % deviance explained.

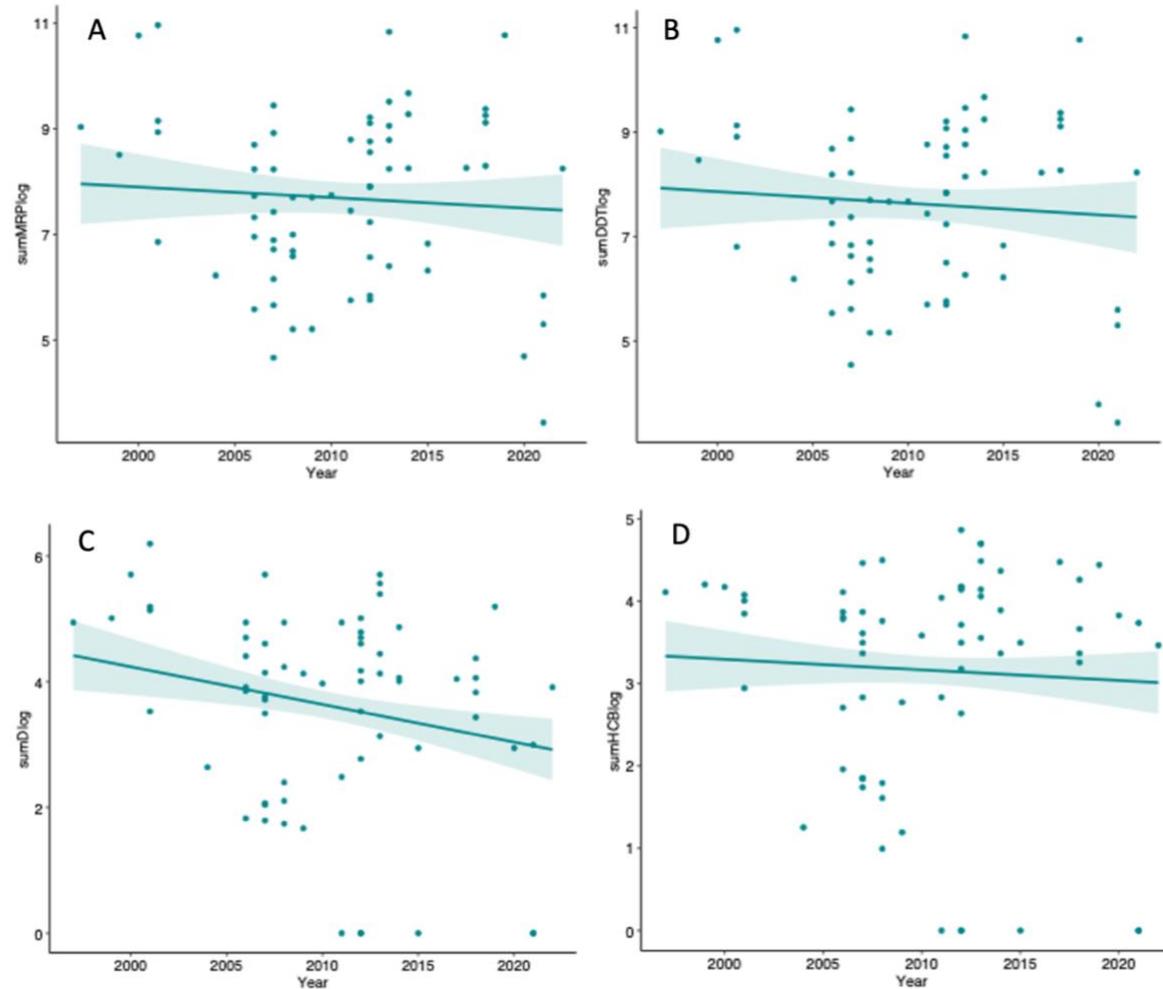
Model	K	logLik	AICc	$\Delta$ AICc	wAICc	% DE
<b>A) <math>\sum</math>MRP</b>						
$\log(\sum\text{MRP}) \sim \text{TBL} * \text{Sex} + \text{Coast}$	7	-98.52	212.98	0	0.65	57.15
$\log(\sum\text{MRP}) \sim \text{TBL} * \text{Sex} + \text{Coast} + \text{Year}$	8	-97.83	214.19	1.21	0.35	56.24
<b>B) <math>\sum</math>DDT</b>						
$\log(\sum\text{DDT}) \sim \text{TBL} * \text{Sex} + \text{Coast}$	7	-101.27	218.48	0	0.61	56.18
$\log(\sum\text{DDT}) \sim \text{TBL} * \text{Sex} + \text{Coast} + \text{Year}$	8	-100.43	219.39	0.91	0.39	55.04
<b>C) Dieldrin</b>						
$\log(\text{Dieldrin}) \sim \text{TBL} * \text{Sex} + \text{Coast}$	8	-107.52	233.57	0	0.73	38.08
$\log(\text{Dieldrin}) \sim \text{TBL} * \text{Sex} + \text{Coast} + \text{Year}$	7	-109.81	235.55	1.98	0.27	33.63
<b>D) HCB</b>						
$\log(\text{HCB}) \sim \text{TBL} * \text{Sex} + \text{Coast}$	7	-103.45	222.83	0	0.77	26.07
$\log(\text{HCB}) \sim \text{TBL} * \text{Sex} + \text{Coast} + \text{Year}$	8	-103.37	225.28	2.45	0.23	25.91

**Table 3.4:** Averaged generalised linear model (GLM) for  $\Sigma$ MRP (A),  $\Sigma$ DDT (B), dieldrin (C) and HCB (D) in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. Note: SE = standard error, TBL = total body length, DE = deviance explained.

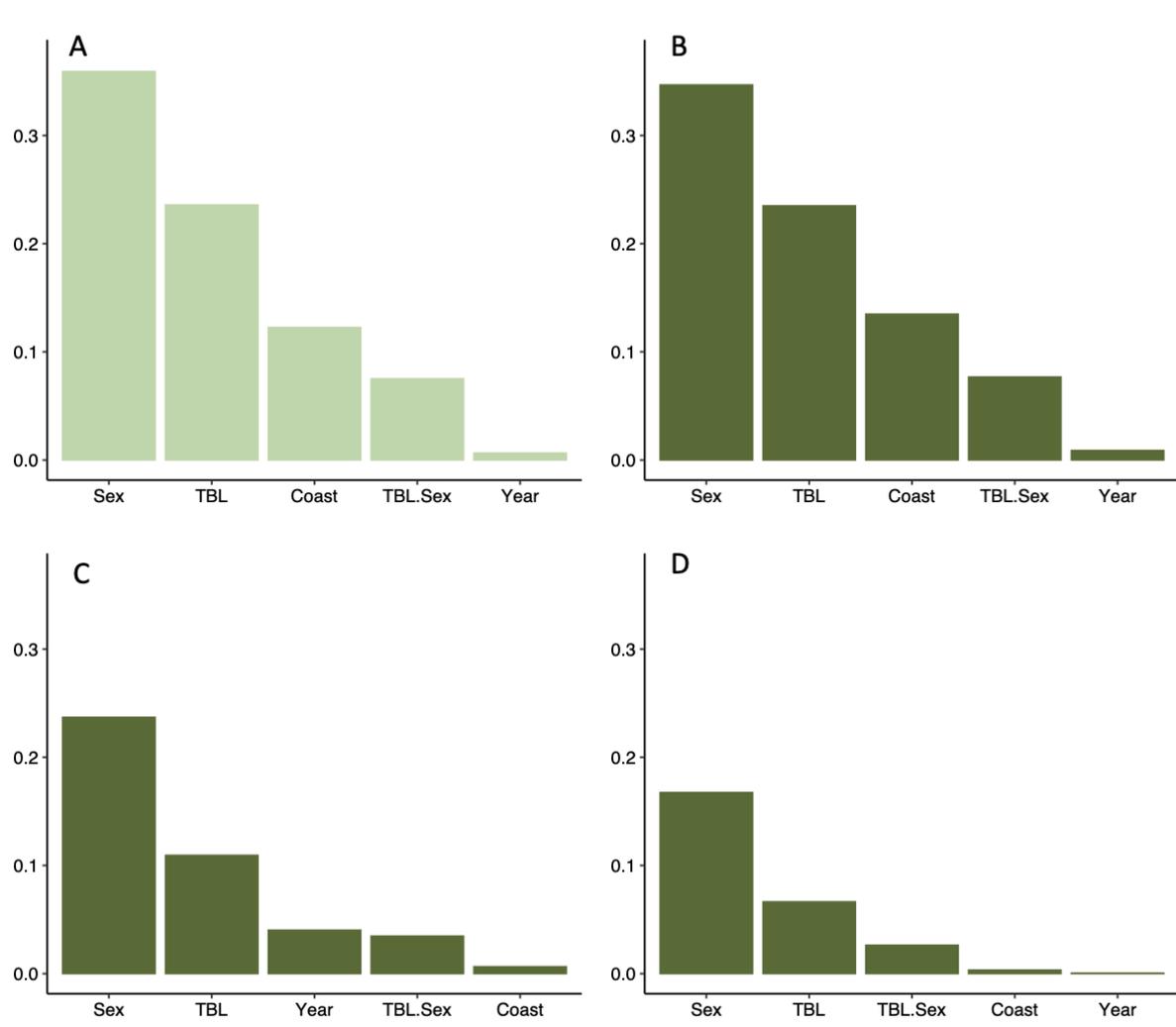
Term	Estimate	SE	Adjusted SE	Statistic	P-value
<b>A) <math>\Sigma</math>MRP</b>					
(intercept)	25.675618	41.090945	41.568641	0.618	0.536794
TBL	0.016661	0.007029	0.007175	2.322	0.020223
Sex_male	-2.668625	1.521891	1.553314	1.718	0.085793
Coast: south	-1.13888	0.685804	0.699914	1.627	0.103701
Coast: west	-1.071227	0.297237	0.303356	3.531	0.000414
Sex_male*TBL	0.038586	0.01285	0.013115	2.942	0.00326
Year	-0.010083	0.020393	0.020629	0.489	0.625024
DE	0.57				
<b>B) <math>\Sigma</math>DDT</b>					
(intercept)	30.953324	46.306043	46.810107	0.661	0.50845
TBL	0.016682	0.007325	0.007477	2.231	0.025664
Sex_male	-2.842854	1.586025	1.618768	1.756	0.079057
Coast: south	-1.173682	0.715199	0.729898	1.608	0.107833
Coast: west	-1.15276	0.309941	0.316316	3.644	0.000268
Sex_male*TBL	0.039931	0.013394	0.01367	2.921	0.003488
Year	-0.012719	0.022981	0.023232	0.547	0.584052
DE	0.56				
<b>C) dieldrin</b>					
(intercept)	90.783192	74.461037	75.191219	1.207	0.2273
TBL	0.011571	0.008223	0.008393	1.379	0.168
Sex_male	-1.754325	1.780925	1.817736	0.965	0.3345
Coast: south	-0.186994	0.807107	0.82362	0.227	0.8204
Coast: west	-0.231376	0.349476	0.356638	0.649	0.5165
Year	-0.04433	0.036959	0.037321	1.188	0.2349
Sex_male*TBL	0.028733	0.015055	0.015366	1.87	0.0615
DE	0.37				
<b>D) HCB</b>					
(intercept)	6.454971	27.952811	28.48502	0.227	0.821
TBL	0.006633	0.007579	0.007736	0.857	0.391
Sex_male	-1.424764	1.64062	1.67451	0.851	0.395
Coast: south	-0.420296	0.737484	0.752714	0.558	0.577
Coast: west	-0.066551	0.319771	0.326375	0.204	0.838
Sex_male*TBL	0.023108	0.013846	0.014132	1.635	0.102
Year	-0.002273	0.013867	0.014131	0.161	0.872
DE	0.26				



**Figure 3.7:** Averaged generalised linear models (GLMs) showing the effect of the interaction between total body length and sex for  $\Sigma$ MRP (A),  $\Sigma$ DDT (B), dieldrin (C), and HCB (D) on Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. The averaged models explained 57, 56, 37 and 26% of the deviance, respectively.



**Figure 3.8:** Averaged generalised linear models (GLMs) showing the effect of year for  $\Sigma$ MRP (A),  $\Sigma$ DDT (B), dieldrin (C), and HCB (D) on Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022. The averaged models explained 57, 56, 37 and 26% of the deviance, respectively.



**Figure 3.9:** Variable importance for averaged generalised linear models (GLMs) shown as absolute change in goodness of fit for  $\Sigma$ MRP (A),  $\Sigma$ DDT (B), dieldrin (C) and HCB (D) in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 1997 and 2022.

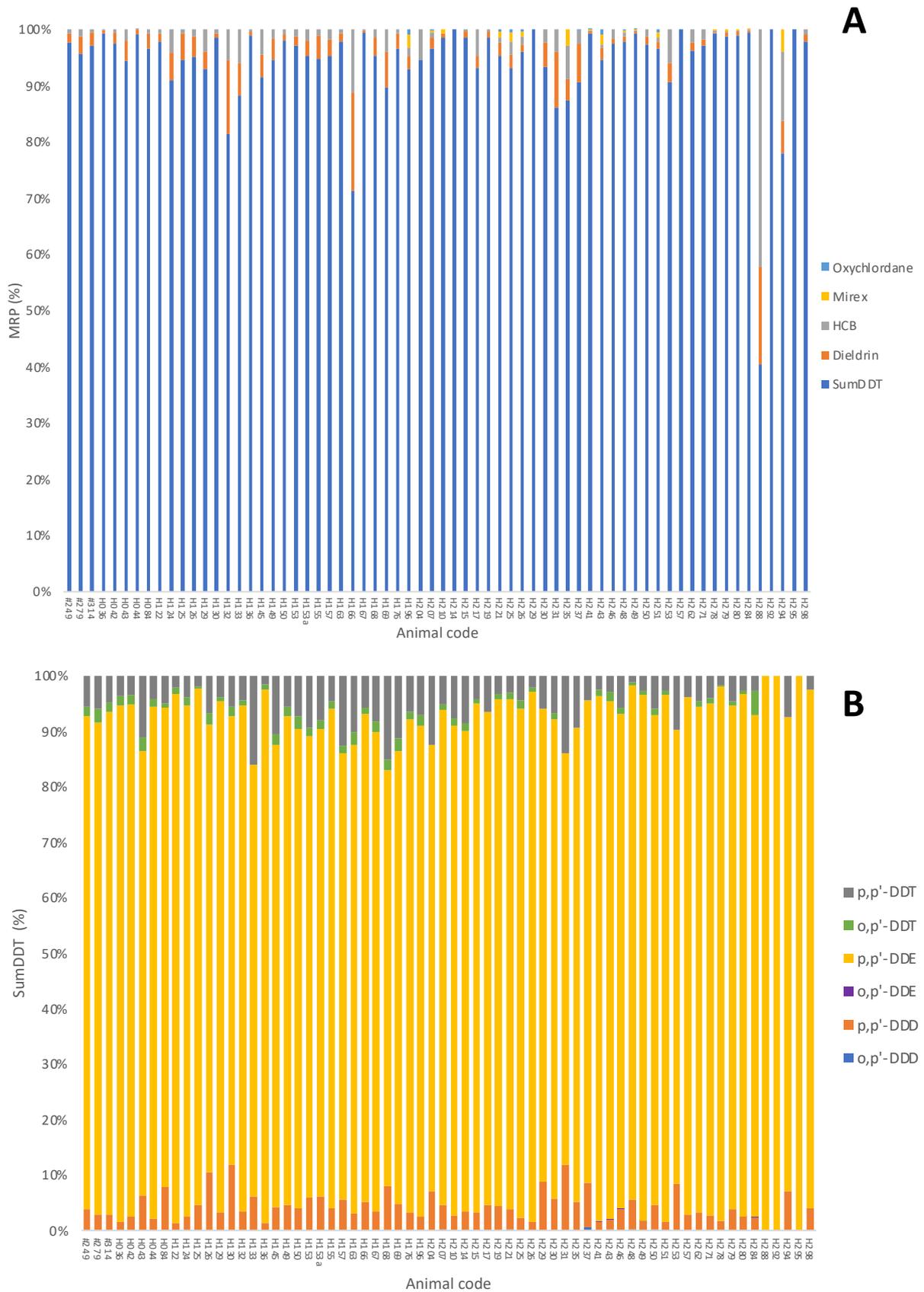
### 3.2.3 MRP profile

The MRP profile was primarily dominated by  $\Sigma$ DDT, comprising more than 90% of the total profile in most individuals (Fig 3.10 A). Individual H288 (96 cm male neonate from WCSI) was the only animal that exhibited a higher concentration of HCB than  $\Sigma$ DDT. Sum DDT was predominantly comprised of p,p'-DDE, a dominant breakdown product of DDT present across all animals, accounting for >92% of the total  $\Sigma$ DDT profile (Fig 3.10 B). Indeed, H288, H292 (69 cm female neonate from WCSI) and H295 (80cm female neonate from WCSI) were only composed of p,p'-DDE as the other DDT breakdown was detected lower than the LOD. o,p'-DDD and o,p'-DDE weren't analysed in the animals from 1997-2009.

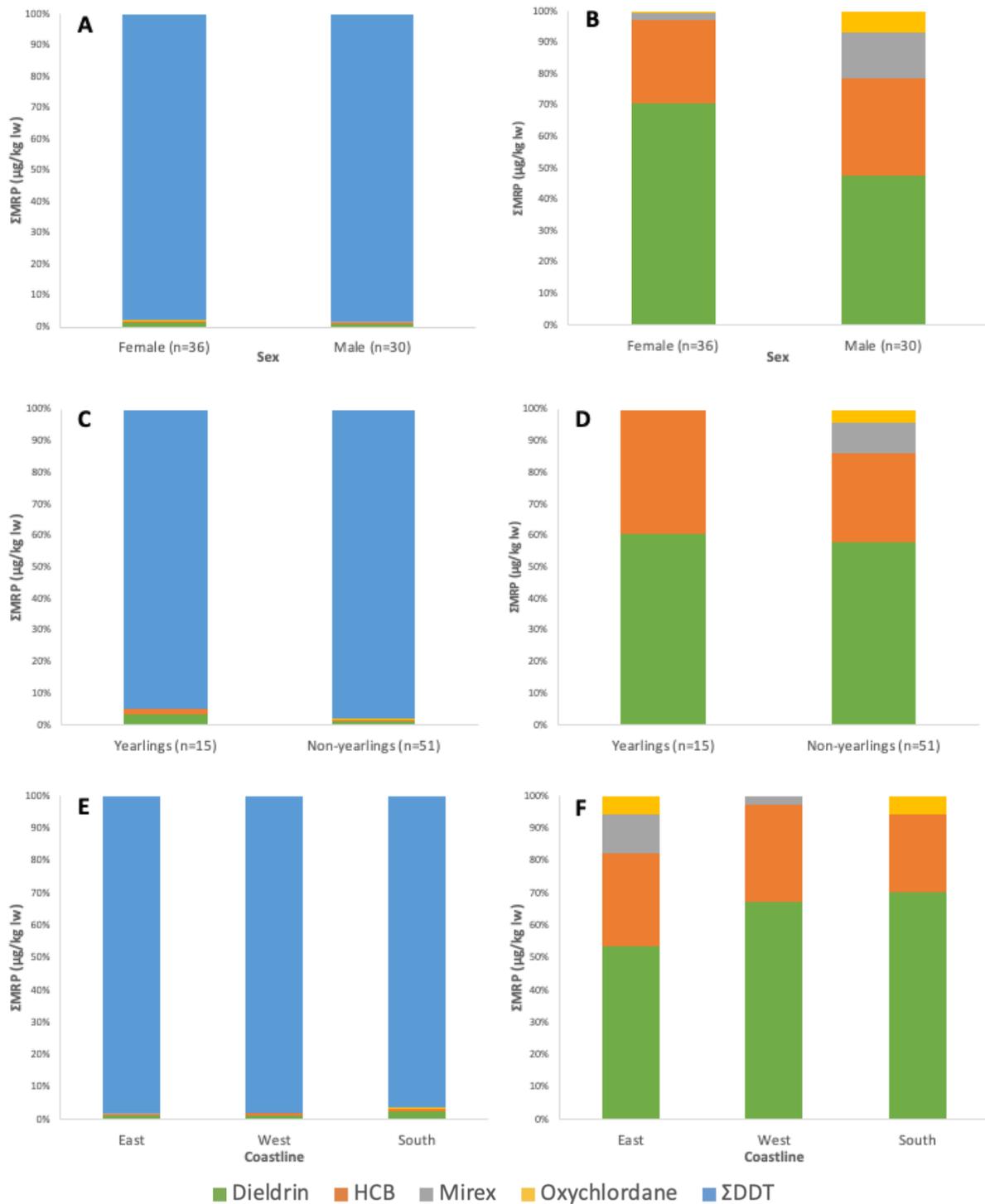
While  $\Sigma$ DDT made up the majority of  $\Sigma$ MRP profile in both males and females, the rest of the MRP profile differed between the sexes (Fig 3.11 A). When excluding  $\Sigma$ DDT, the females' MRP profile was primarily composed of dieldrin and HCB, with a very low concentration of mirex and oxychlorane. Conversely, the males' MRP profile was primarily composed of dieldrin (though at a lower concentration than females) and HCB, and recorded higher concentrations of mirex and oxychlorane compared to their female counterparts (Fig 3.11 B).

Yearling and non-yearling profiles were predominantly composed of  $\Sigma$ DDT (Fig 3.11 C). When excluding  $\Sigma$ DDT, the yearlings' profile was only made of dieldrin and HCB, whereas the non-yearling profile comprised all four MRPs (dieldrin, HCB, oxychlorane and mirex). Dieldrin was present in similar concentrations between yearlings and non-yearlings, while HCB was the second most present MRP detected in non-yearlings (Fig 3.11 D).

Sum DDT comprised most of  $\Sigma$ MRP profile around all the coasts (Fig 3.11 E). When excluding  $\Sigma$ DDT, dieldrin was the predominant MRP with the highest concentration on the south coast, followed by HCB, mirex and oxychlorane. Mirex was absent from the south coast whereas oxychlorane was absent from the west coast (Fig 3.11 F).



**Figure 3.10:** MRP (A) and  $\Sigma$ DDT (B) profile in Hector's dolphins (*Cephalorhynchus hectori*) between 1997 and 2022.



**Figure 3.11:** ΣMRP profile of Hector's dolphin (*Cephalorhynchus hectori*) by sex, yearlings vs non-yearlings and coastline including ΣDDT (A, C, E) and excluding ΣDDT (B, D, F) between 1997 and 2022.

### 3.3 Polybrominated diphenyl ethers - PBDEs

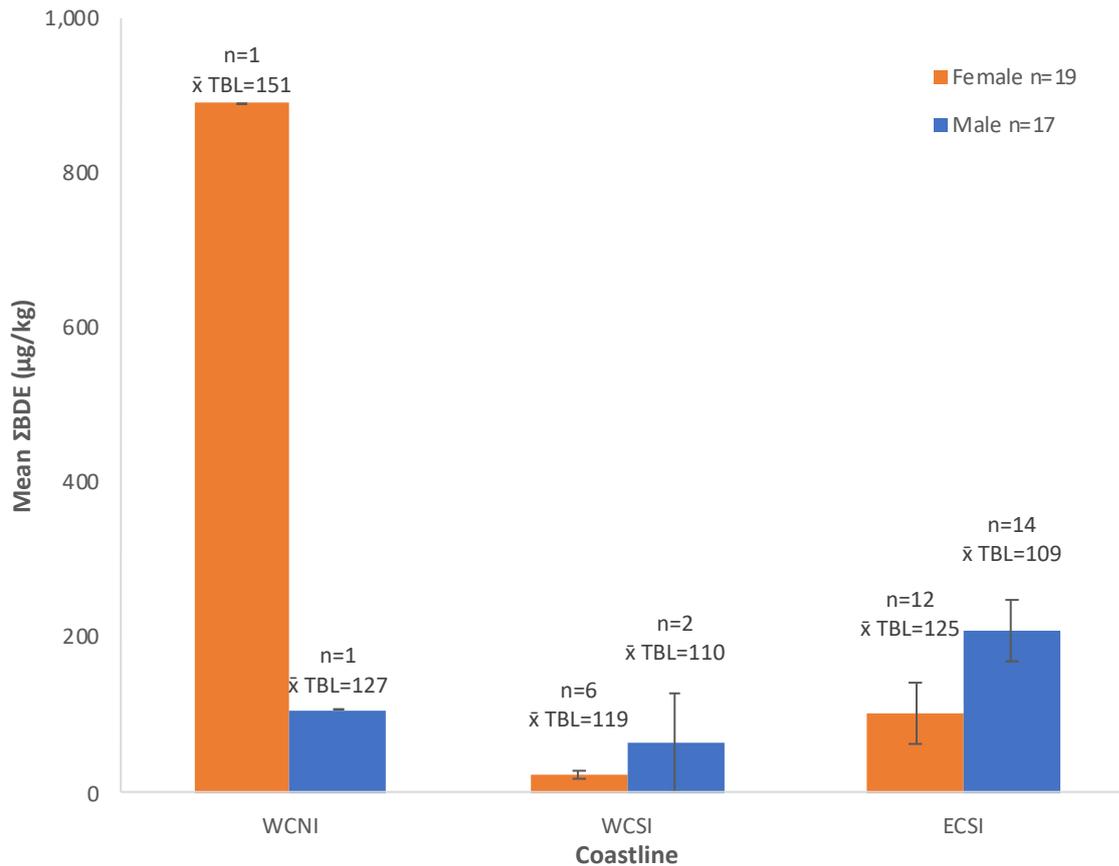
#### 3.3.1 Sample summary

A total of 36 animals, including 19 females (5 yearlings, 14 non-yearlings) and 17 males (5 yearlings and 12 non-yearlings), were examined for PBDE between 2010 and 2022.

#### 3.3.2 PBDE concentration and multivariate analysis

Between 2010 and 2022, a total of 10 PBDE congeners were examined (Table 2.3).  $\Sigma$ BDE concentration was relatively low compared to the other contaminants analysed, ranging from 1.6 to 890 (mean = 151; SD = 186)  $\mu\text{g}/\text{kg}$  lipid weight, apart from the female Māui dolphin (H243, 151cm mature from WCNI), which was largely above all the other animals ( $\Sigma$ BDE = 890  $\mu\text{g}/\text{kg}$  lipid weight). Specifically,  $\Sigma$ BDE concentration ranged from 2 to 424.4 (mean = 186; SD = 143) and 1.6 to 890 (mean = 119; SD = 217)  $\mu\text{g}/\text{kg}$  lipid weight in males and females, respectively.

From a spatial perspective, females presented the highest  $\Sigma$ BDE concentration in the WCNI from the female Māui dolphin (mean = 890  $\mu\text{g}/\text{kg}$  lipid weight), with the lowest  $\Sigma$ BDE present in WCSI animals (range 1.6 to 36.8; mean = 23.1; SD = 13.2)  $\mu\text{g}/\text{kg}$  lipid weight, despite relatively similar TBL between the different coasts (Fig 3.12). For males, the highest  $\Sigma$ BDE concentration occurred off ECSI, with up to 424.5 (mean = 209.2; SD = 145.5)  $\mu\text{g}/\text{kg}$  lipid weight. Lowest concentrations were present off the WCSI (mean = 65.8; SD = 90.2), despite similar TBL (Fig 3.12). Males typically exhibited higher  $\Sigma$ BDE concentrations around the ECSI and WCSI, whereas females displayed higher burdens off the WCNI (Fig 3.12).



**Figure 3.12:** Mean  $\Sigma$ PBDE by coast in Hector's dolphins (*Cephalorhynchus hectori*) between 2010 and 2022. Note: n = sample size,  $\bar{x}$  = mean.

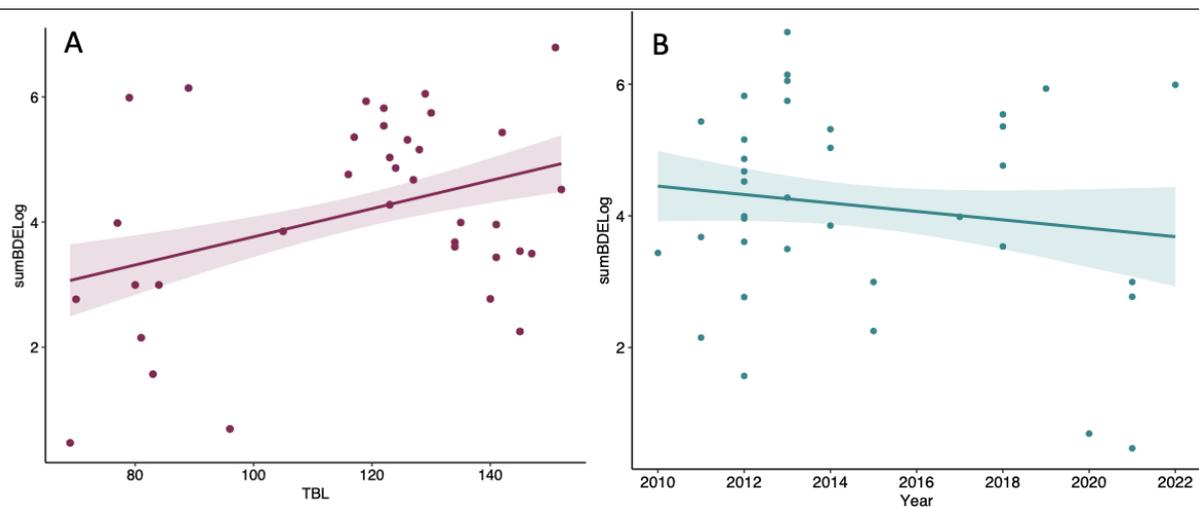
For  $\Sigma$ BDE, the two models with and without year were similar in deviance explained and AICc weight (Table 3.8). The averaged models revealed that PBDE burdens were lower for animals from the west compared to the east coast (Table 3.9). Contaminant burdens increased with total body length, meaning that older animals (using TBL as a proxy of age) exhibited higher concentrations than presumed younger animals. The interaction between sex and TBL was not retained in the final models, indicating no difference in the effects of TBL between males and females (Figure 3.13 A). A marginal decrease in PBDE burden over time (Figure 3.13 B) was detected. However, of all included variables, TBL was the most important predictor of  $\Sigma$ BDE, followed by sex and coast, while year contributed almost nothing to the model fit (Figure 3.14).

**Table 3.5:** Generalised linear models (GLMs) for  $\sum$ BDE in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 2010 and 2022. Models were averaged based on their AICc weights. K = number of parameters, logLik = log-likelihood, % DE = % deviance explained,  $\Delta$ AICc = difference in Akaike's information criterion (AICc) of the current and top-ranked model, wAICc = AICc weight.

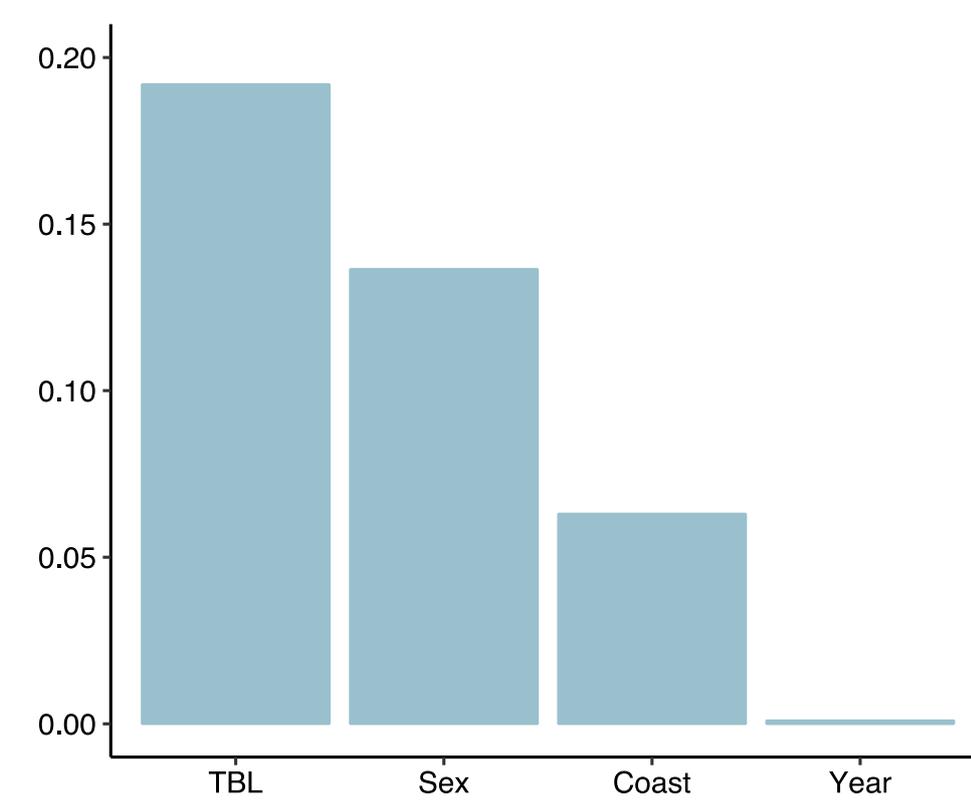
Model	K	logLik	AICc	$\Delta$ AICc	wAICc	% DE
$\log(\sum\text{BDE}) \sim \text{TBL} + \text{Sex} + \text{Coast}$	5	-57.11	126.21	0	0.8	40.52
$\log(\sum\text{BDE}) \sim \text{TBL} + \text{Sex} + \text{Coast} + \text{Year}$	6	-57.03	128.95	2.74	0.2	40.25

**Table 3.6:** Generalised linear model (GLM) for  $\sum$ BDE in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 2010 and 2022. Note: SE = standard error, TBL = total body length, DE = deviance explained.

Term	Estimate	SE	Adjusted SE	Statistic	P-value
(Intercept)	10.79	66.45	68.88	0.16	0.88
TBL	0.03	0.01	0.01	3.35	0
Sex_male	1.25	0.45	0.47	2.68	0.01
Coast: west	-0.92	0.48	0.5	1.83	0.07
Year	-0.01	0.03	0.03	0.15	0.88
DE	0.4				



**Figure 3.13:** Averaged generalised linear model (GLMs) for  $\sum$ BDE (A&B) showing the effect of the total body length (A) and the effect of year (B) on Hector's dolphins (*Cephalorhynchus hectori*) between 2010 and 2022 in New Zealand. The averaged models explained 40.4% of the deviance.

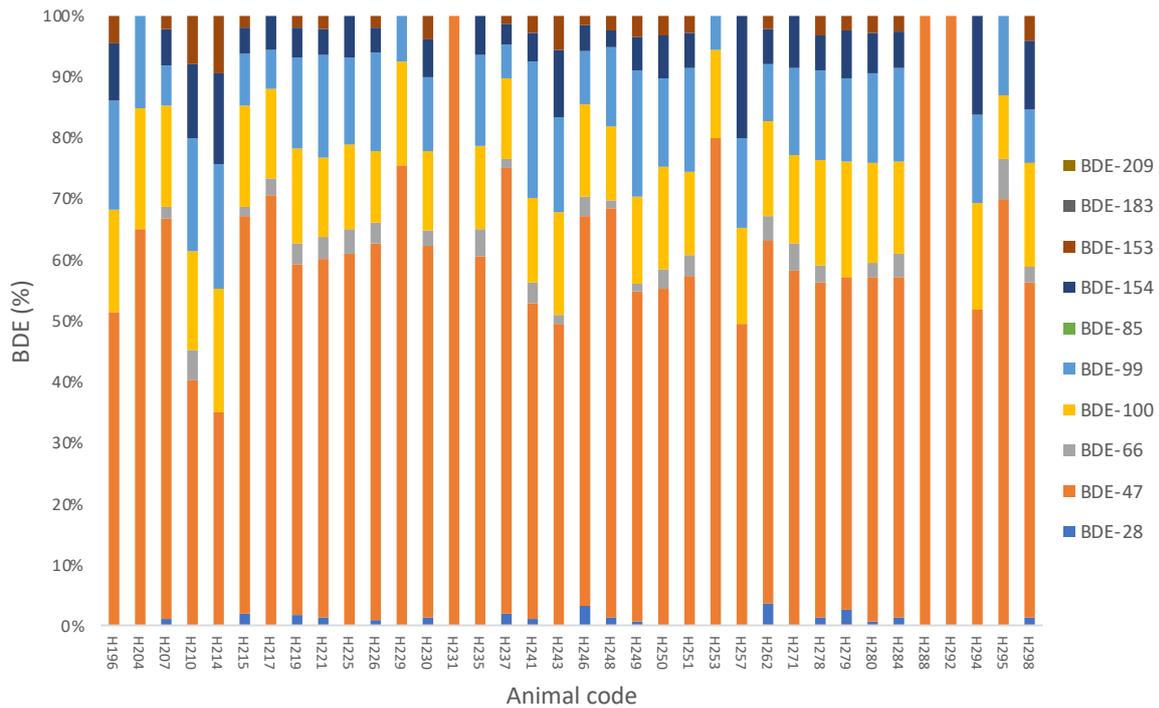


**Figure 3.14:** Variable importance for averaged generalised linear models (GLMs) shown as absolute change in goodness of fit for  $\Sigma$ BDE in Hector's dolphins (*Cephalorhynchus hectori*) between 2010 and 2022 in New Zealand.

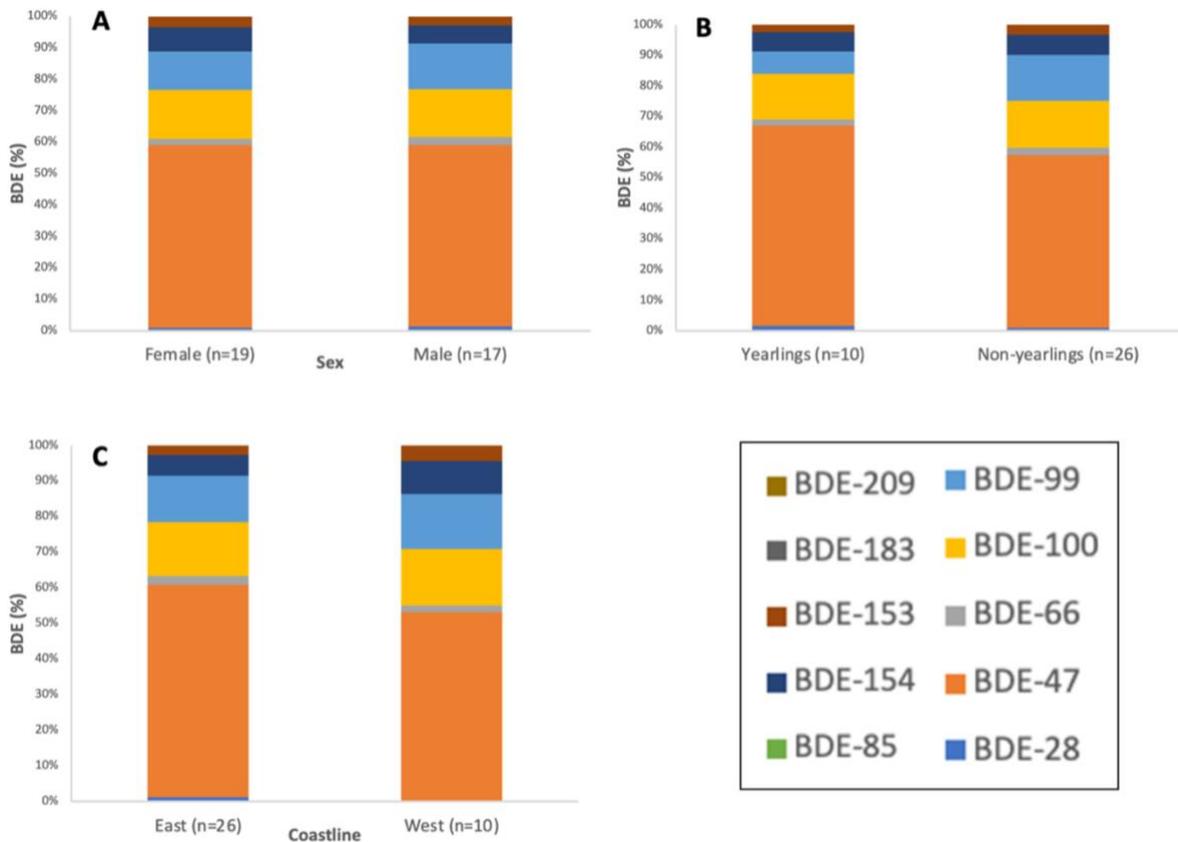
### 3.3.3 PBDE profile

BDE-47, -99 and -100 were the dominant PBDE congeners, comprising more than 86% of the total PBDE profile (Fig 3.15). Two animals, H288 (96 cm male neonate from WCSI) and H292 (69 cm female neonate from WCSI) only composed BDE-47, with all other BDEs present lower than the LOD (Fig 3.15).

The PBDE profile was similar between males and females, with BDE-47, -99 and -100 consistently the dominant PBDE congeners detected (Fig 3.16 A). Yearlings and non-yearlings were mostly similar in PBDE profile though except for BDE-47 which was present in higher concentrations in yearlings compared to non-yearlings, which reported higher concentrations of BDE-99 (Fig 3.16 B). The PBDE profile between coastlines is consistent, however, BDE-28 and -47 were present in higher concentrations on the east coast and BDE-99, -154 and -153 were present in higher concentrations on the west coast (Fig 3.16 C).



**Figure 3.15:**  $\Sigma$ BDE profile in Hector's dolphins (*Cephalorhynchus hectori*) in New Zealand between 2010 and 2022.



**Figure 3.16:** PBDE profile in Hector's dolphins (*Cephalorhynchus hectori*) by sex (A), yearling vs non-yearling (B) and by coastline (C) between 2010 and 2022..





## Chapter 4 - Discussion



Hector's dolphins (*Cephalorhynchus hectori hectori*), New Zealand

Photo credit: Steve Dawson



The endangered Hector's dolphins (*Cephalorhynchus hectori hectori*), and critically endangered Māui dolphins (*C. h. maui*) are endemic, coastal species (Bräger et al., 2003; Reeves et al., 2013a,b) of New Zealand. Accordingly, their close proximity to land places them at elevated risk from inshore threats, including fisheries bycatch (Dawson, 1991; Pichler & Baker, 2000; Rayment & Webster, 2009), vessel impacts (Carome et al., 2022; Stone & Yoshinaga, 2000), tourism (Martinez et al., 2010), disease (Buckle et al., 2017; Duignan, 2003; Roe et al., 2013a,b) and environmental contaminants (Stockin et al., 2010). Indeed, the recent increase in Hector's dolphin mortality attributable to toxoplasmosis (Buckle et al., 2017; Duignan, 2003; Roe et al., 2013a,b) warrants questions about the underlying health status of the species and specifically, why increased susceptibility to toxoplasma has become an issue for the species only in more recent years.

Toxoplasmosis is typically a secondary disease in mammals since it predominantly occurs with a primary, often immune-compromising diseases such as HIV (Gibson et al., 2011; Robert-Gangneux & Dardé, 2012) and cancer (Bajnok et al., 2019; Daher et al., 2021). Toxoplasmosis is also noted in mammals that are otherwise immunocompromised, either during pregnancy (Chaudhry et al., 2014; Paquet et al., 2013) and in the case of humans, in patients with a history of known substance abuse (Elmorsy et al., 2018; Samońłowicz et al., 2017). In wildlife, immune suppression has further been noted in the context of environmental contaminants (Bossart, 2011; Desforges et al., 2016; Letcher et al., 2010).

Here I investigated environmental contaminant burdens in Hector's dolphins across a 25-year period. Specifically, this thesis explored ontogeny and spatiotemporal effects on contaminant burden in New Zealand Hector's dolphins. Exploring trends in PCBs, MRPs (including banned DDT) and PBDEs, I present findings on the ontogenetic and spatiotemporal trends of persistent environmental contaminants in an endangered, endemic species and discuss the conservation implications of these results.

## 4.1 Polychlorinated biphenyls - PCBs

### 4.1.1 PCB concentration and profile

PCB concentrations in New Zealand's Hector's dolphins ( $\Sigma 45\text{CB}$ : males: mean = 1,675, SD = 1,409, range = 16.2-5,574; females: mean = 751, SD = 1,896, range = 8.4-11,375) were lower than reported in international species of similar body size and coastal range, such as harbour porpoise (*Phocoena phocoena*) in the UK (males: 440-150,470  $\mu\text{g}/\text{kg}$  lw and females: 13,490-159,680; 1990-2012; (Jepson et al., 2016)), in the French and Belgian North Sea (All: 1,900-404,000  $\mu\text{g}/\text{kg}$  lw; 1997-2001; n = 21 (Covaci et al., 2002)), Indo-Pacific finless porpoise (*Neophocaena phocaenoides*) in the Pearl River estuary, China (Adult males: 2,319-27,852  $\mu\text{g}/\text{kg}$  lw, n = 9 and adult females: 457-5,456  $\mu\text{g}/\text{kg}$  lw, n = 6; 2007-2019; (Xie et al., 2021)); and in larger delphinids such as Franciscana dolphins (*Pontoporia blainvillei*) in Southeastern Brazil (All: 6,107-26,199  $\mu\text{g}/\text{kg}$  lw; 2011-2012; n = 9; (Lavandier et al., 2016)) and Indo-Pacific humpback dolphins (*Sousa chinensis*) in Pearl River estuary, China (Adult males: 1,606-96,233  $\mu\text{g}/\text{kg}$  lw, n = 11 and adult females: 323-30,682  $\mu\text{g}/\text{kg}$  lw, n = 32; 2004-2020; (Xie et al., 2021))

Differences detected are most likely due to the dissimilarity in the level of production and use between the countries before PCBs were banned. Historically, there has been a wider use of PCBs in Europe, Asia and America, where human population size and, therefore, the use of PCBs is greater compared to New Zealand. In New Zealand, PCBs were never produced nationally but imported prior to restrictions and ban on their use in 1988 and 1994, respectively (MfE, 1998). This ban was comparatively late with respect to most other countries which banned PCBs in the 1970s (Denison, 2013).

Another explanation relates to the diet which may also play a role in the temporal accumulation of contaminants. For example, the diet of apex predators such as dolphins contributes to the biomagnification of PCBs (Johnson-Restrepo et al., 2005). Xie et al. (2021) reported a difference in PCB concentration between odontocetes and mysticetes that might be best explained by diet, as odontocetes are known to feed at higher trophic levels and therefore, more prone to accumulate higher concentration. Hector's dolphins are known to feed on either small and/or juvenile fish species, such as red cod (*Pseudophycis bachus*) and ahuru (*Auchenoceros punctatus*) (Miller et al., 2013). While no studies have investigated the

level of PCBs in red cod and ahuru, red cod is reported with moderate levels of MeHg (Sadhu et al., 2015), suggesting that bioaccumulation of contaminants does occur for this species. Previous studies have also noted that species inhabiting cold and temperate waters have higher PCB concentrations than species from tropical waters (Chou et al., 2004; Minh et al., 2000a). It has also been described that coastal species, such as Hector's dolphins, generally have higher contaminant concentrations than offshore species, potentially due to their nearshore proximity to coasts and estuaries (Xie et al., 2021).

In the South Pacific, PCB concentrations in Hector's dolphins are low compared to larger bottlenose dolphins (*Tursiops aduncus*) from South Australia (1989-1995: n = 17; 97-25,000 µg/kg lw; 2009-2014: n = 26; 67-50,000 µg/kg lw; (Weijs et al., 2020)), Australian humpback dolphins (*S. sahuensis*) from south-east Queensland, Australia (n = 6; 1,600-370,000 µg/kg lw; (Weijs et al., 2016)), and central and southern Great Barrier Reef, Australia (n = 18; 776-93,522 µg/kg lw (Cagnazzi et al., 2013)), as well as from Australian snubfin dolphins (*Orcaella heinsohni*) from central and southern Great Barrier Reef, Australia (n = 17; 168-21,424 µg/kg lw (Cagnazzi et al., 2013)). Physiological and size differences between these larger species (up to 2.7 m) compared to smaller Hector's dolphins (up to 1.7 m) may be implicated (Yordy et al., 2010a). Notably, PCBs contamination has previously been linked to more industrialised countries (Van Den Berg et al., 2017). Accordingly, differences may further be explained by the greater level of industrial activities and higher population size internationally compared to that of New Zealand.

In New Zealand, the  $\Sigma$ PCB concentrations reported in Hector's dolphins ( **$\Sigma$ 45CB**: *males*: range = 16.2-5,574, mean = 1,675, SD = 1,409; *females*: range = 8.4-11,375, mean = 751, SD = 1,896;  **$\Sigma$ 7CBs**: *males*: range = 14.9-4,499, mean = 1,285.4, SD = 1,156; *females*: range = 7.7-10,746, mean = 663, SD = 1,794) was considerably higher than previously reported in New Zealand common dolphins (*D. delphis*) between 1995 and 2005 ( **$\Sigma$ 45CB**: *males*: 268-1,634 µg/kg lw and *females*: 49-386 µg/kg lw;  **$\Sigma$ 7CBs**: *males*, n = 12: 192-1,183 µg/kg lw and *females*, n = 7: 29-289 µg/kg lw; (Stockin et al., 2007)). Jones et al. (1999), previously reported PCB concentrations in six Hector's dolphins stranded on the WCSI and ECSI between 1985-1987. The  $\Sigma$ 7CBs was significantly lower and ranged from 319 to 1,916 (mean = 887; SD = 355) and 447 to 706 (mean = 577; SD = 130) µg/kg ww in males and females, respectively. The PCB

levels in Hector's dolphins were also significantly higher than levels reported in long-finned pilot whales (*Globicephala melas edwardii*) from New Zealand (range = 33-931 µg/kg, mean = 311, SD = 26; n = 61 (Schröder & Castle, 1998)).

The PCBs congeners predominantly observed in Hector's dolphins (CB153, 138 and 180) here are consistent with what has been reported for wider marine mammal species (Jepson et al., 2016; Lavandier et al., 2016; Ramu et al., 2006; Santos-Neto et al., 2014; Xie et al., 2021), demonstrating the extensive use, persistence and accumulation of these congeners. For example, CB153 is the primary congener present in Hector's dolphins, contributing 12% of total PCBs and 41% of Σ7CB. This aligns with the 20 to 30% of total PCBs noted in all cetacean species from Taiwan waters (Chou et al., 2004). Indeed, out of the ten most abundant congeners for the species in Taiwanese waters, Hector's dolphins shared seven (CB153, CB138, CB180, CB118, CB101, CB105, CB187), though concentrations observed were not reported to the same magnitude. In Franciscana dolphins, Guiana dolphins (*Stotalia guianensis*) and rough-toothed dolphins (*Steno bredanensis*) (Dorneles et al., 2013) primary PCB congener CB118 (Dorneles et al., 2013), was reported as the fourth most abundant congener in Hector's dolphins. In bottlenose dolphins (*T. truncatus*) from the Mediterranean Sea (Storelli & Marcotrigiano, 2003), the PCB profile predominantly comprised CB153 and CB138 (Storelli & Marcotrigiano, 2003) which are also the two main congeners observed in Hector's dolphins.

In the South Pacific, the PCB profile of bottlenose dolphins (*T. aduncus*) from Australia is also primarily comprised of CB153, followed by CB99, 118, 138, 187 and 180 (Weijs et al., 2019), which also aligns with the top ten dominant congeners reported for Hector's dolphins. In New Zealand specifically, CB153, CB138 and CB180 were the three predominant congeners with the highest concentration in Hector's dolphins, which aligns with further Hector's dolphins (n = 6) stranded on the WCSI and ECSI between 1985-1987 (Jones et al., 1999) and New Zealand's common dolphins (n = 19) between 1995-2005 (Stockin et al., 2007). Notably, the same top three congeners were also reported in human breast milk (Mannetje et al., 2013), in paradise shelducks (*Tadorna variegata*) and in southern black-backed gulls (*Larus dominicanus*) (Numata et al., 2008), and CB180 and 153 were the top congeners in long finned eels (*Anguilla*

*dieffenbachia*) (Holmqvist et al. 2006) . Those congeners were historically used as coolants and lubricants in electrical transformers, capacitors, and other various electrical equipment.

It is also important to note that some PCB congeners were not routinely screened for in New Zealand from 2010 onwards (n = 27). Accordingly, the results for those congeners will be underrepresented since they were not assessed in animals examined beyond 2010.

#### **4.1.2 Influence of sex and TBL**

A sex-specific difference in PCB concentrations was noted, as observed in white-sided dolphins (*Lagenorhynchus acutus*) from the northwest Atlantic (Weisbrod et al., 2001), harbour porpoises and harbour seals (*Phoca vitulina*) from the Southern North Sea (Weijs et al., 2009) and in blue whales (*Balaenoptera musculus*) from the Gulf of St. Lawrence, Canada (Metcalf et al., 2004). In males, contaminants burden increased continuously with TBL, whereas in females, contaminants burden increased at a slower rate with TBL. Females typically display lower concentrations compared to their male counterparts at any given body length. Such differences between the sexes may be explained by variations in stages of sexual maturation. Females are known to offload contaminants to their offspring via gestation and lactation (Noren et al., 2018; Weijs et al., 2013; Wells et al., 2005; Yordy et al., 2010b), whereas continuous accumulation in males results in higher contaminant overall compared to their female counterparts (Fair et al., 2010; Weijs et al., 2013). However, in some cases, reproductive failure in females means that high concentrations can still be present in mature females (Kannan et al., 2000).

Stockin et al. (2010) previously examined the maternal transfer of PCBs from a female Māui dolphin to her unborn calf. The concentration of PCBs (both all CBs and  $\Sigma$ 7CBs) was approximately half the concentration (0.45x lower) of the mother, with a mean transfer of 4.3% between mother and offspring. Beyond the offloading of females to their calves, TBL alone (without the interaction of sex) influenced the PCB concentration. For males, their concentration is predominantly age-dependent (Ylitalo et al., 2001). In this case, the PCB concentration increase with TBL, meaning that older (and thus typically larger) animals have higher loads than younger, thus smaller counterparts, as they had more time to accumulate contaminants in their tissues (Gui et al., 2014).

### 4.1.3 Spatiotemporal trend

PCBs were banned internationally in the late 1970 to 1980s, though in New Zealand, their use became illegal only in 1995 (MfE, 1998). In 2004, New Zealand ratified the Stockholm Convention, where measures to eliminate and reduce the release of POPs in the environment occurred (Denison, 2013; Marnett et al., 2013). Although PCBs have never been manufactured in New Zealand, electrical equipment containing PCBs, such as capacitors and transformers, were historically imported and heavily used here. In the current study, “Coast” was identified by the model to be a key driver of PCB concentration in Hector’s dolphins, with animals from the east coast exhibiting higher PCB concentration than counterparts from either the west or southern coasts. The east coast of New Zealand is well known for both its agricultural activities, such as in the Canterbury Plains and the Canterbury region, in addition to its various industries. Indeed, Canterbury is the second most populated region in New Zealand. The west coast was the second coast with the highest PCB concentration, which is well known in the South Island for its mining activity, and in the North Island is located close to the biggest city in New Zealand, Auckland (36.8509° S, 174.7645° E). Because of the small sample size of the subspecies Māui dolphins (n=4), all individuals were pooled as Hector’s dolphins from the WCSI as part of “west” for the GLM analysis. However, it is clear that the 3 female Māui dolphins from the WCNI presented much higher concentrations of PCB than their Hector’s dolphins from the WCSI.

The stable temporal trend noted in Hector’s dolphin PCB burdens aligns with similar stable trends observed in European cetaceans (Jepson et al., 2016), but differs from what has been observed in killer whales (*Orcinus orca*) (Houde et al., 2005; Ross et al., 2000) and bottlenose dolphin populations (*T. truncatus*) (Wells et al., 2005) in North America, and from humpback dolphins (*S. chinensis*) and finless porpoises (*N. phocaenoides*) in the Pearl River estuary, China (Xie et al., 2021). This potentially can be explained by the somewhat delayed restrictions posed by the UK in 1981 (Law et al., 2012), Europe in 1987 (Borrell & Aguilar, 2007) and New Zealand in 1995, compared to the US (Aguilar et al., 2002) and China (Wong & Poon, 2003) which banned PCBs in 1979 and 1974, respectively.

## 4.2 Multi-residue pesticides - MRPs

### 4.2.1 MRP concentration and profile

#### 4.2.1.1 DDT

$\Sigma$ DDT was by far the most prominent multi-residue pesticide detected. This reflects the extensive historical use of pesticides in agriculture in New Zealand, which was only banned in 1989. In Hector's dolphins,  $\Sigma$ DDT concentration varied by sex ( $\Sigma$ DDT: males: mean = 12,061; SD = 16,010; range = 44-57,390; n = 30; females: mean = 2,076, SD = 2,743, range = 31-12,860; n = 36) and was lower than reported for bottlenose dolphins (*T. truncatus*) from the Canary Islands, Spain (all:  $104,739 \pm 202,926$   $\mu\text{g}/\text{kg}$  lw, range = 189-1,252,210, 2003-2011, n = 64; (García-Alvarez et al., 2014)), white-sided dolphins (*Lagenorhynchus acutus*) from Cape Cod Bay, US (all:  $14,049 \pm 9,599$   $\mu\text{g}/\text{kg}$  lw, 1994-1996, n = 6; (Weisbrod et al., 2001)), and Indo-Pacific humpback dolphins (*S. chinensis*) from the Pearl River Estuary, China ( $64,200$   $\mu\text{g}/\text{kg}$  lw, 845-179,000, 2004-2009, n = 15; (Wu et al., 2013)). Comparable  $\Sigma$ DDT concentrations were detected in common dolphins (*D. delphis*) from England and Wales (females only: 200-16,100  $\mu\text{g}/\text{kg}$  lw, 1992-2006, n = 43; (Law et al., 2013)) and in harbour porpoise from the southern North Sea (all:  $15,000 \pm 20,000$   $\mu\text{g}/\text{kg}$  lw, range = 700-96,000, 2010-2013, n = 20; (Mahfouz et al., 2014)).

Differences in reported concentrations, to some extent, reflect geographical variation in the contaminant's concentration of species (Durante et al., 2016). For example, fish-eating cetaceans from the northern hemisphere, especially from the mid-latitudes of Europe and North America, exhibit the greatest concentration of DDTs, while species from the southern hemisphere typically presented the lowest concentrations (Aguilar et al., 2002). Those differences in concentration between countries are related to different levels of production and use of DDT. For example, Europe, the US and Asia, have large population densities and, therefore, higher usage of DDT compared to New Zealand. Another explanation for the difference in contaminants between species could be the role of feeding habits and habitats of prey, for example, benthopelagic and oceanodromous fish have been observed to have higher DDT concentrations than pelagic-neritic fish (Yu et al., 2020). Marine mammals showing higher concentrations of DDT are more likely to feed at higher trophic levels and, thus, accumulate greater concentrations (Borrell & Aguilar, 2005).

Another explanation for the differences detected internationally is most likely due to the dissimilarity in the level of production and use between the countries before DDT was banned. Historically, DDT was widely used in the US, Europe and Asia, prior to bans in the US in 1972 (Whitney, 2012), in Europe in 1978 (Levain et al., 2015) and in China in 1983 (Cheng, 1990). Since 1955, DDT is still produced in certain countries such as China and used in public health for the control of vector-borne disease (i.e. malaria) in China, India, and South Africa (Mansouri et al., 2017; Van den Berg et al., 2017; WHO, 2011). In New Zealand, DDT was historically used in agriculture as an insecticide to control grass grub (*Costelytra zealandica*) and porina caterpillars (*Wiseana cervinata*) (MfE, 1998). Restrictions on DDT use in New Zealand began in the 1960s, with full deregistration occurring in 1989 (Buckland et al., 1998).

In the South Pacific,  $\Sigma$ DDT concentrations of humpback dolphins (*S. sahulensis*) from Moreton Bay in Queensland, Australia (all: range = 1,800-17,000  $\mu\text{g}/\text{kg}$  lw, 2002-2014, n = 6; (Weijs et al., 2016)) and from Port Curtis and Fitzroy River in Queensland, Australia (all: range = 310-6,200  $\mu\text{g}/\text{kg}$  lw, 2009-2010, n = 18; (Cagnazzi et al., 2013)) were lower comparatively to Hector's dolphins in New Zealand. Conversely, humpback dolphins (*S. sahulensis*) from Fitzroy River in Queensland, Australia (male: 13,803, n = 1; female:  $16,209 \pm 2,501$   $\mu\text{g}/\text{kg}$  lw, range = 516-32,527, n = 5, 2014-2016; (Cagnazzi et al., 2020)), and from Port Curtis in Queensland, Australia (male: 21,241, n = 1; female:  $15,705 \pm 2,709$   $\mu\text{g}/\text{kg}$  lw, range = 516-32,527, n = 10; 2014-2016; (Cagnazzi et al., 2020)), and snubfin dolphin in Fitzroy River in Queensland, Australia (male:  $15,975 \pm 2,530$   $\mu\text{g}/\text{kg}$  lw, range = 8,059-29,662, n = 9; female:  $16,458 \pm 2,783$   $\mu\text{g}/\text{kg}$  lw, range = 7,465-33,886, n = 9; 2014-2016; (Cagnazzi et al., 2020)) have higher concentrations of  $\Sigma$ DDT than Hector's dolphins. An explanation for the higher levels observed in Queensland, Australia is the intensive agricultural sector along the Queensland coast (Haynes et al., 2000). There is also a clear increase in the  $\Sigma$ DDT concentrations among dolphins from 2009-2010 and 2014-2016 from the same locations. The most likely explanation for this increase is due to extensive flooding near catchments and port development that took place during the same period (Cagnazzi et al., 2020).

In New Zealand, the  $\Sigma$ DDT concentrations reported in Hector's dolphins were considerably higher than what has previously been reported in New Zealand common dolphins (*D. delphis*) between 1999 and 2005 (male: range = 654-4,430, n = 12; female: range = 17-337, n = 7;

(Stockin et al., 2007)). In part, this could be explained by the difference in study periods and sample sizes between both studies, with a 7-year study period and sample size of 19 animals for the common dolphin study, compared to the present 26-year study period and sample size of 66 animals. The proportion of adult males is significantly greater in this study ( $n = 17$ ) than in Stockin et al. (2007) ( $n = 6$ ), which males can't offload to their offspring (Fair et al., 2010; Weijs et al., 2013), could further explain why Hector's dolphins present higher  $\Sigma$ DDT concentrations. Hector's dolphins are a coastal species and therefore, distributed close to populated areas with major anthropogenic activities, thus are more prone to contaminants from coastal pollution (Davidson et al., 2012), whereas common dolphins are typically mesopelagic (Gaskin, 1992), and considered thus less prone to anthropogenic stressors.

The  $\Sigma$ DDT profile reported here is similar to what has been observed in other species globally, such as in Guiana dolphins from Brazil (Lailson-Brito et al., 2010; Santos-Neto et al., 2014), common dolphins (*D. delphis*) and Fraser's dolphins (*Lagenodelphis hosei*) from Argentina (Durante et al., 2016), striped dolphins from western Mediterranean (Aguilar & Borrell, 2005) and in humpback (*S. sahulensis*) and snubfin dolphins from Fitzroy River and Port Curtis in Queensland, Australia (Cagnazzi et al., 2020), with p,p'DDE also being the most abundant isomer across studies. The large proportion of p,p'DDE represents historical DDT in the environment since p,p'DDT degrades into p,p'DDD first, and then into p,p'DDE (Aguilar, 1984). Thus, the large proportion of p,p'DDE isomer shows a decline and historical use of this pesticide. The p,p'DDE/ $\Sigma$ DDT ratio, equal to 0.92, further indicates the lack of new DDT sources entering the environment (Tanabe et al., 1997).

#### 4.2.1.2 Dieldrin

Dieldrin has been widely used worldwide since 1950 to control various agricultural pests, as well as in public health to control insect-borne diseases (WHO, 1989). It was banned globally by the 1980s, and in New Zealand restrictions on the use of dieldrin started in the late 1960s, though complete deregistration only occurred in 1989 (Buckland et al., 1998). In New Zealand, dieldrin was used to control sheep ectoparasites, carrot rust fly (*Psila rosae*), crickets (*Grylloidea* sp.) and armyworm (*Spodoptera frugiperda*), as well as for timber conservation and to mothproof carpets (MfE, 2018).

Dieldrin concentrations reported here for Hector's dolphins (males: mean = 125, SD = 103, range = 6-490, n = 30; females: mean = 58, SD = 70, range = 5-300, n = 36) were lower compared to harbour porpoises in Moray Firth, Scotland (male: mean = 1,700, range = 790-3,430, n = 21; female: mean = 540, range = 730-2,300, n = 15; 1988-1991; (Wells et al., 1994)) which are similar in size and range, and to larger *Delphinus* such as in white-sided dolphins (*L. acutus*) in Cape Cod Bay, US (all:  $1,019 \pm 949$   $\mu\text{g}/\text{kg}$  lw, 1994-1996, n = 6; (Weisbrod et al., 2001)). Comparable concentrations to those in Hector's dolphins were found in species such as common dolphins (*D. delphis*) from England and Wales (females only: 10-730  $\mu\text{g}/\text{kg}$  lw, 1992-2006, n = 43; (Law et al., 2013)), Indo-Pacific humpback dolphins (*S. chinensis*) from the Pearl River Estuary, China ( $58.8 \pm 55.2$   $\mu\text{g}/\text{kg}$  lw, 2004-2013, n = 45; (Gui et al., 2014)) and humpback dolphins (*S. sahulensis*) from south-east Queensland, Australia (all: range = 87-700  $\mu\text{g}/\text{kg}$  lw, 2002-2014, n = 6; (Weijs et al., 2016)). In New Zealand, dieldrin concentrations were higher in Hector's dolphins than previously reported for common dolphins (*D. delphis*) (male:  $47 \pm 26$   $\mu\text{g}/\text{kg}$  lw, range = 19-87, n = 6; female:  $26 \pm 37$   $\mu\text{g}/\text{kg}$  lw, range = 4.2-100, n = 11; 1999-2005; (Stockin et al., 2007)) and from eels (*Anguilla* sp.), brown trout (*Salmo trutta*) and black flounder (*Rhonbosolea retiaria*) (eels: <0.05-16.3  $\mu\text{g}/\text{kg}$  ww, trout and flounder: 0.08-0.65  $\mu\text{g}/\text{kg}$ ; (Stewart et al., 2011)).

One explanation for the differences between the species and studies reported relates to the temporal span of samples examined in each study. For example, the highest concentrations of dieldrin occurred in studies from the 1990s, whereas comparable concentrations observed here for Hector's dolphins align with more recent studies. Country of origin also plays a significant role in the different concentrations observed, with the US (Jorgenson, 2001) and Europe (Lipnick & Muir, 2000) reporting highest use and production of dieldrin globally.

#### 4.2.1.3 HCB

HCB has historically been used as an agricultural pesticide since 1945 to control for fungi that can affect food crops (Thakur & Pathania, 2020). HCB also currently exists as a trace contaminant in some pesticides, as well as a by-product of various industrial chemicals (Menzie, 1986; Tobin, 1986). HCB production has since decreased following restrictions on its use, with most countries instigating a ban from the 1970s to 1980s (Eastmond & Balakrishnan,

2010). In New Zealand, HCB was used as an experimental seed-dressing fungicide for cereal grain between 1970-1972 before becoming banned in 1972 (Buckland et al., 1998).

HCB concentrations reported here for New Zealand's Hector's dolphins (males: mean = 56, SD = 28, range = 7-130, n = 30; females: mean = 34, SD = 28, range = 3-110, n = 36) were lower compared to common dolphins (*D. delphis*) from England and Wales (females only: 10-270 µg/kg lw, 1992-2006, n = 43; Law et al., 2013) and white-sided dolphins (*L. acutus*) from Cape Cod Bay, US (all:  $175 \pm 164$  µg/kg lw, 1994-1996, n = 6; Weisbrod et al., 2001). However, similar concentrations to those observed in Hector's dolphins were reported in Guiana dolphins (*S. guianensis*) from the coast of São Paulo, Brazil (range = 70-170 µg/kg lw, 2004-2005, n = 6, (Alonso et al., 2010), while Hector's dolphins presented higher HCB concentration than Indo-Pacific humpback dolphins (*S. chinensis*) from Pearl River estuary, China (10 µg/kg lw, range = nd-60, 2004-2009, n = 15; (Wu et al., 2013)) and humpback dolphins (*S. sahulensis*) from south-east Queensland, Australia (all: range = 9.4-17 µg/kg lw, 2002-2014, n = 6; (Weijs et al., 2016)). The differences in concentrations between species likely represent differences in the use and production of HCB between countries.

New Zealand had only limited use of HCB before it became deregistered; therefore, lower concentrations were able to accumulate and persist in New Zealand's environment compared to countries like Europe (Pacyna et al., 2003) and the US (Bradman et al., 2007), where HCB was established for considerably longer. Another explanation for the differences in HCB concentration may be the timeframe of the studies, as studies with higher concentrations occurred from the 1990s, meaning animals examined lived pre and post-bans of HCB, when concentrations were likely high in the environment. Species that presented lower concentrations than Hector's dolphins were from the most recent study periods, indicating that environmental levels have temporally declined. Water temperature is another factor known to influence HCB concentrations, with higher levels found in temperate waters and moderate to high levels typically more common in tropical and cold waters (Minh et al., 1999; Minh et al., 2000b).

#### 4.2.1.4 Mirex

Mirex is an insecticide used primarily to control fire ants (*Solenopsis invicta*) and is also used as a fire retardant in plastics, rubber and electrical goods (Kaiser, 1978). In New Zealand, only small quantities of mirex were used to control public health pests, and its use was ratified by the Stockholm Convention in 2004 (Denison, 2013).

Mirex concentrations observed in Hector's dolphins (males: mean = 40, SD = 34, range = 12-110, n = 17; females: mean = 58, SD = 82, range = 12-240, n = 19) were lower than what has been reported in Guiana dolphins (*S. guianensis*) in São Paulo, Brazil (range = 240-1,870 µg/kg lw, 2004-2005, n = 6, (Alonso et al., 2010)), similar to white-sided dolphins (*L. acutus*) in Cape Cod Bay, US (all:  $44.7 \pm 20.4$  µg/kg lw, 1994-1996, n = 6; (Weisbrod et al., 2001)) and Franciscana dolphins (*P. blainvillei*) in Southern Brazil waters ( $61.8 \pm 21.8$  µg/kg lw, range = 34-106, 1994-2004, n = 73; (Leonel et al., 2014)) and marginally higher than mirex concentration reported in humpback dolphins (*S. sahulensis*) in south-east Queensland, Australia (all: range = 4.1-84 µg/kg lw, 2002-2014, n = 6; (Weijs et al., 2016)). This is consistent with earlier findings, where species from the Southern Hemisphere generally present lower levels of contaminants compared to their Northern Hemisphere counterpart species (Borrell & Aguilar, 1999; Cockcroft, 1999; Lavandier et al., 2015; Lavandier et al., 2016). However, there are notably a limited number of studies for the Pacific region, so meaningful comparisons are problematic. The high levels in Guiana dolphins from Brazil might result from the high stability of mirex in the environment since mirex has been banned in that region since 1992 (Taniguchi et al. 2016).

#### 4.2.1.5 Oxychlorane

Oxychlorane is a breakdown product of chlordane, a broad-spectrum pesticide used in agricultural crops to control for termites (Eisler, 1990). Oxychlorane has been observed to be more toxic than the chlordane mixture and its other compounds (Bondy et al., 2003). Chlordane was banned in 1988 in the US (USEPA, 1985), though used in New Zealand until 1989, predominantly as an insecticide in agriculture and glues and as a termiticide in forestry (Buckland et al., 1998). The first regulations on the use, sales, transport and storage first started in the 1960s, followed by the deregistration of chlordane in 1989 by the Pesticides Board (Buckland et al., 1998).

In this study, Hector's dolphins had lower levels of oxychlordanes (males: mean = 27, SD = 16, range = 10-52, n = 17; females: mean = 38, SD = 52, range = 10-130, n = 19) compared to Franciscana dolphins from the coasts of São Paulo and Paraná, Brazil ( $\Sigma$ CHL: mature male: mean = 64  $\mu\text{g}/\text{kg}$  lw, range = 38-110, n = 5; mature female: mean = 39  $\mu\text{g}/\text{kg}$  lw, range = 31-47, n = 2; 1997-1999; (Kajiwara et al., 2004), bottlenose dolphins (*T. truncatus*) in Queensland, Australia (male: mean = 305, n = 2; female: mean = 9, n = 2; 1996-1999; (Vetter et al., 2001)), and in South Australia (1989-1995: adult male: 1,300  $\pm$  2,300, n = 5; adult female: 180  $\pm$  160, n = 2; 2009-2014: adult male: 1,100  $\pm$  1,200; adult female: 700  $\pm$  1,100, n = 5; (Weijs et al., 2020)). These differences may be due to the difference in the use and production of chlordanes between countries. It has been documented that species inhabiting areas close to where oxychlordanes were used to control for termites usually show higher concentrations (Eisler, 1990). Typically, species presenting high levels of oxychlordanes have a higher ability to metabolise chlordanes compounds, as noted in Franciscana dolphins from Brazilian coastal waters (Kajiwara et al., 2004). However, it is important to note that the levels reported for Franciscana dolphins in Brazil comprise of  $\Sigma$ CHL (5 chlordanes compounds including oxychlordanes) and not just oxychlordanes.

#### 4.2.2 Influence of sex and TBL

Notable sex-specific differences in concentration of MRPs were recorded, as observed in Indo-Pacific humpback dolphins (*S. chinensis*) from the Pearl River estuary, China (Gui et al., 2014), Atlantic bottlenose dolphins (*T. truncatus*) from southeastern US estuaries (Fair et al., 2010) and in finless porpoises (*N. asiaeorientalis*) from Korean coastal waters (Jeong et al., 2020). The concentrations of  $\Sigma$ DDT, dieldrin, HCB, mirex and oxychlordanes significantly increased with TBL in males, whereas, for females, the bioaccumulation trend with TBL was less pronounced. Multiple studies have shown that males have much higher concentrations than females (Jeong et al., 2020). This is due to a variation in concentration between sexes during sexual maturation, where the maternal offloading of females via gestation and lactation to their offspring is known to affect the concentration of MRP in mature females (Borrell & Aguilar, 2005; Yordy et al., 2010). The maternal transfer of  $\Sigma$ DDT between a female Māui dolphin and her unborn calf was previously examined by Stockin et al. (2010). Stockin and colleagues found that the concentration was approximately half ( $\Sigma$ DDT 0.6x lower) of that in the mother, with a mean transfer of 5.7%. Whereas the MRP concentration in males is mostly

age-dependent (Ylitalo et al., 2001). In this case, for males, the MRP concentration increases with TBL, meaning that older, larger animals have higher loads than their smaller/younger counterparts due to a more extended period of accumulation of contaminants in their tissues (Gui et al., 2014).

#### **4.2.3 Spatiotemporal trend**

During the 1970s-1980s, MRPs were banned from most developed countries globally. However, because of their low price and easy availability, MRPs are still used in most third world countries (Ali et al., 2014). In 2001, the Stockholm Convention included the ban on production and restriction in industry and agricultural use of DDT, dieldrin, HCB, mirex and chlordane (UNEP, 2001). In New Zealand, MRPs were used predominantly for agriculture, horticulture and timber treatment.

Stockin et al. (2010) previously reported that DDT and its breakdown products in Hector's dolphins resulted from the historical use of DDT in agriculture in New Zealand. In New Zealand, agricultural soil has higher DDT concentrations than background soils (Jones & Geisy, 2000). Animals from the east coast presented elevated levels of MRP compared to animals examined from the west and south coasts. The Canterbury Plains, on the east coast of the South Island, are known as one of the most agricultural regions in New Zealand, causing important input of contaminants in the marine environment. Significantly highest concentrations of  $\Sigma$ DDT in sediment across 12 estuaries around New Zealand was reported in Moutere Inlet, located at the top of the South Island and Avon-Heathcote estuary located in Canterbury (MfE, 1998). In the case of this study, both locations would be considered part of the ECSI based on the distribution of Hector's dolphins detailed in MacKenzie and Clement (2014).

### **4.3 Polybrominated diphenyl ethers - PBDEs**

#### **4.3.1 PBDE concentration and profile**

PBDE concentrations in New Zealand's Hector's dolphins (males: range = 2-424, mean = 186, SD = 143, n = 17; females: range = 1.6-890, mean = 119, SD = 217, n = 19) were relatively similar to species of similar size and range. For example, finless porpoise (*N. phocaenoides*)

from the South China sea (1990-2001: ranged from 84 to 980  $\mu\text{g}/\text{kg}$  lw;  $n = 12$ ; (Ramu et al., 2006)) and Franciscana dolphins from Southern Brazil (2002-2005: males: 67.8-764; females:  $<0.65$ -228; (Leonel et al., 2014)), though lower than for harbour porpoise from the North Sea (mean = 1,540, SD = 960  $\mu\text{g}/\text{kg}$  lw; 1999-2004;  $n = 35$ ; (Weijs et al., 2009)).

PBDE concentration reported in Hector's dolphins were considerably higher than in larger spinner dolphins (*Stenella longirostris*) from India (6.8  $\mu\text{g}/\text{kg}$  lw) and from the Philippines (36  $\mu\text{g}/\text{kg}$  lw), and Indo-Pacific humpback dolphins (*S. chinensis*) from India (11  $\mu\text{g}/\text{kg}$  lw) ((Kajiwara et al., 2006), though lower than reported common dolphins (*D. capensis*) from Korean coastal waters (mean = 1,700, SD = 720  $\mu\text{g}/\text{kg}$  lw, range: 140-3,100  $\mu\text{g}/\text{kg}$  lw; 2006;  $n = 22$ ; (Moon et al., 2010)), Indo-Pacific humpback dolphins (*S. chinensis*) from Hong Kong (mean = 1,900, SD = 2,000  $\mu\text{g}/\text{kg}$  lw; 1995-2001;  $n = 15$ ; (Ramu et al., 2005)), and bottlenose dolphins (*T. truncatus*) from the UK (mean = 7,700, SD = 5,300  $\mu\text{g}/\text{kg}$  lw; 1995-2001;  $n = 9$ ; (Law et al., 2005), from Charleston harbour, US (mean = 5,860, SD = 4,290, range = 4,680-7,050, 2003-2004,  $n = 53$ ; (Fair et al., 2007)), and from Indian River lagoon, US (mean = 1,260, SD = 979, range = 1,050-1,470, 2003-2004,  $n = 58$ ; (Fair et al., 2007) and Southern Resident killer whales from Canada (mean = 7,900  $\mu\text{g}/\text{kg}$  lw; Noel et al., 2018)) which is the highest PBDEs concentration in cetacean's blubber reported to date.

Differences noted likely reflect the level of industrialisation between countries, with the Philippines and India classified as developing countries (Kajiwara et al., 2006) and therefore, with less industrialisation, coastal development, sewage or shipping (Leonel et al., 2014) compared to countries like Korea, the UK or Hong Kong. This has previously been observed in the PBDE levels of humpback dolphins (*S. chinensis*) from the Pearl River in China, known as one of the most industrialised and urbanised area (Xie et al., 2022), and Atlantic spotted dolphins (*Stenella frontalis*) from the coasts of São Paulo, which is one of the most industrialised regions in the southwest Atlantic (Méndez-Fernandez et al., 2018). Another explanation could be the difference in the level of production and uses of PBDE between those countries. For example, Europe has historically experienced extensive use of PBDE (Prevedouros et al., 2004), akin to China, where the shipment and disposal of electronic waste are thought to be a major source of contamination (Martin et al., 2004).

In the South Pacific, limited studies observing the levels of PBDE in odontocetes exist. Indo-Pacific bottlenose dolphins (*T. aduncus*) sampled from South Australia between 1989 and 1995 reported lower PBDE concentrations (0.8-91 µg/kg lw), though individuals more recently sampled from 2009 to 2014 have recorded concentrations (23-1,900 µg/kg lw; (Weijs et al., 2020)) higher than Hector's dolphins. Humpback dolphins (*S. sahulensis*) from southeast Queensland, Australia, demonstrate a similar concentration range (150-440 µg/kg lw; (Weijs et al., 2016)) to Hector's dolphins presented here. Although PBDEs have never been manufactured in Australia, their levels have increased in recent years, and this may be due to the continuous import of products containing PBDEs (Bartalini et al., 2022).

This is the first study investigating PBDE levels in marine mammals in New Zealand. PBDEs levels have been examined and detected in blue cod (*Paraperca colias*) and spotted wrass (*Notolabrus celidotus*) livers from farming sites in the Malborough Sounds, where salmon feed imported from Chile is used (9.4-57.74 and 5.85-19.60 µg/kg lw, respectively; (McMullin et al., 2022)). Estuarine sediments have also been analysed around New Zealand's biggest city, Auckland (36.8509° S, 174.7645° E), with PBDE concentration ranging from 0.55 to 573 µg/kg dw (Stewart et al., 2014). This demonstrates that although PBDEs have never been manufactured nor directly imported as commercial chemicals in New Zealand (Harrad & Prter, 2007), they are still present in the environment. The most likely source of contamination for New Zealand is through indirect importation from consumer goods treated with PBDE (Harrad & Prter, 2007).

The main PBDE congeners recorded in Hector's dolphins (BDE-47, -99, -100) are noted in commercial formulations used as additives in polyurethane foams (Mannetje et al., 2013) and are part of pentabromodiphenylether (pentaBDE), which, along with octabromodiphenylether (octaBDE), have been listed in the Stockholm Convention in 2004. Decabromodiphenylether (decaBDE) was listed in 2019 (Sharkey et al., 2020). Those primary congeners are consistent with what has been reported in other odontocetes species worldwide, such as in Franciscana dolphins from Southern Brazil, where those congeners were the only ones detected (Lavandier et al., 2016; Leonel et al., 2014), in harbour porpoise from England and Wales (Law et al., 2002), in common dolphins (*D. capensis*) from Korean

waters (Moon et al., 2010), and striped dolphins from the Mediterranean Sea (Aznar-Aleman et al., 2021).

#### **4.3.2 Influence of sex and TBL**

The PBDE levels increased with TBL, meaning older animals presented higher concentrations than younger animals. Males showed higher PBDE concentrations than females; however, there was no difference in the effects of TBL between males and females. This may be due to some individuals (15 out of 36) having not reached sexual maturity; therefore could be expected that both males and females have similar concentrations since the females haven't been able to offload to their offspring yet. An alternative hypothesis is that PBDEs are not as readily mobilised and transferable between mothers and their offspring due to their high lipophilic properties (Barbosa et al., 2018; Kajiwara et al., 2008). Here, the two highest PBDE concentrations were from a mature female Māui dolphin (H243) and an immature female Hector's dolphin (H237); this could be explained by failed pregnancies from the mature Māui dolphin as previously noted in harbour porpoise (Murphy et al., 2015) and first-born calf mortality as seen in bottlenose dolphins (*T. truncatus*; Wells et al., 2005). Calf mortality has been common in recent years in Hector's dolphins, as seen with eight calves out of fifteen animals found dead at the end of 2021 (DOC, 2022a).

#### **4.3.3 Spatiotemporal trend**

Multiple studies have shown that POPs such as PBDEs are found in higher concentrations close to industrialised and highly populated areas (Lebeuf et al., 2004; Leonel et al., 2014). PBDEs have never been produced nor used directly as commercial formulations in New Zealand, indicating that the main source of exposure for humans is through imported consumer goods (Harrad & Porter, 2007). In New Zealand, women from urban areas have higher PBDE concentrations in their breast milk compared to women from rural areas (Mannetje et al., 2013), suggesting that urban areas have more sources of PBDEs. The same observation has been made for cetaceans from industrialised countries with high economic growth rates, such as Hong Kong and Japan (Tanabe & Ramu, 2012). In this study, Hector's dolphins are showing higher levels on the east coast, which has higher urbanisation and industrialisation compared to the west coast.

Although most legacy contaminants show a decline in concentration over time due to bans and restrictions on their production and use, the emerging contaminant PBDE shows a continuous increase in its level in different regions of the world, such as in North America (Norstrom et al., 2002), Canadian Arctic (Ikonomou et al., 2002), Japan (Akutsu et al., 2003), and Sweden (Norén & Meironyté, 2000). This trend can be explained by the large-scale production of PBDEs that started in the early 1970s. PBDEs have been listed as POPs under the Stockholm Convention in 2009 (UNEP, 2017) but are still produced and used internationally (Alonso et al., 2014). Due to the continued increase in the level of PBDEs worldwide, it is believed that their levels might surpass those of PCBs, which have decreased over time (Breivik et al., 2011). PBDEs are known to affect the environment as well as animals; however, it is still unknown what the toxicity threshold for marine mammals is (Shaw et al., 2008). In Hector's dolphins, the concentration of PBDEs slightly decreased between 2010 and 2022; however, the variable "year" was contributing least to the overall model fit. It is also important to note the spread of the data over time, with most data spreading from 2010 to 2014.

#### **4.4 Summary**

The primary aim of this research was to explore spatiotemporal trends of PCB and MRP levels reported in Hector's dolphins over the previous quarter of a century. A second objective was to gain first insights into PBDE concentrations in Hector's dolphins in New Zealand. As a general trend,  $\Sigma$ DDT and PCB concentrations reported the highest burden in Hector's dolphins, followed by PBDEs, dieldrin, HCB, mirex and oxychlorane. Multiple factors are known to influence the concentration of contaminants in a species, including sex, age and distribution. Additionally, the prolonged historical use of legacy contaminants, such as DDT and PCBs in New Zealand compared to more recent, emerging contaminants, such as PBDE, do reflects at different magnitudes within tissues analysed as part of this study.

In general, the environmental contaminants reported in Hector's dolphins here were higher than in any other species assessed from New Zealand to date. This, in part can be explained by the coastal habitat of Hector's dolphins. However, it should be noted that environmental contaminants explored here have only been assessed in a small number of New Zealand

marine mammals, all of which are predominantly offshore oceanic species. Accordingly, it is hard to fully evaluate reported burdens here for Hector's in the absence of comparable coastal species, for example, bottlenose dolphins. When comparing internationally, most contaminants reported for Hector's dolphins were at lower concentrations than many overseas populations. However, certain populations internationally showed similar concentrations to Hector's dolphins for contaminants, including  $\Sigma$ DDT (*D. delphis*, Law et al., 2013; *P. phocoena*, Mahfouz et al., 2014), dieldrin (*D. delphis*, Law et al., 2013; *S. chinensis*, Gui et al., 2014; *S. sahalensis*, Weijs et al., 2016), HCB (*S. guianensis*, Alonso et al., 2010), mirex (*L. acutus*, Weisbrod et al., 2001; *P. blainvillei*, Leonel et al., 2014) and PBDEs (*N. phocaenoides*, Ramu et al., 2006; *P. blainvillei*, Leonel et al., 2014). In some cases, Hector's dolphins even showed elevated concentrations globally for  $\Sigma$ DDT (*S. sahalensis*, Weijs et al., 2016; Cagnazzi et al., 2013), HCB (*S. chinensis*, Wu et al., 2013; *S. sahalensis*, Weijs et al., 2016), mirex (*S. sahalensis*, Weijs et al., 2016) and PBDEs (*S. longirostris* and *S. chinensis*, Kajiwara et al., 2006), particularly so for flame retardants.

This thesis examined the spatiotemporal trends of legacy contaminants (PCBs and MRPs) while considering the effects of TBL and sex between 1997 and 2022. For both PCBs ( $\Sigma$ PCB) and MRPs ( $\Sigma$ MRP,  $\Sigma$ DDT, HCB and dieldrin), sex was the most important predictor, followed by TBL. In males, PCBs and MRPs burden increased with increasing TBL, while this trend was less pronounced in females due to reproductive offloading. Dolphins examined from the east coast of New Zealand exhibited higher PCB and MRP burdens compared to animals from other regions. Despite being legacy contaminants, "year" was the least important predictor, with minimal to no decline observed in concentrations of  $\Sigma$ PCBs,  $\Sigma$ MRP,  $\Sigma$ DDT and HCB over time. Only dieldrin showed evidence of temporal decline, which importantly demonstrates how despite bans, many legacy contaminants are not as yet observing declines in the marine environment.

This thesis also quantified the levels of an emerging contaminant (PBDE) in Hector's dolphins for the first time and examined the spatiotemporal trend of PBDEs in the context of ontogeny between 2010 and 2022. For PBDEs, the most important predictor was TBL, followed by sex. This may indicate that reproductive transfer is less of a factor in PBDEs compared with other lipophilic contaminants. Concentrations increased with TBL, meaning that potentially older

animals (as inferred from TBL) exhibited higher levels than presumed younger animals. This was most evident in males, and likely due to the accumulation of contaminants in the tissues over time. We found no interaction between sex and TBL; indicating no difference in the effects of TBL for males and females. PBDE levels were lowest in animals examined from the west coast, likely due to the higher level of urbanisation along the east coast. A marginal decrease in PBDE burden over time was noted, however, the variable year had almost no effect on the overall model fit.

#### 4.5 Conservation significance

Successful conservation management of wild populations relies on a detailed understanding of the species through targeted and up-to-date information. However, very few biology studies on Hector's and Māui dolphins have been conducted (Dawson & Slooten, 1993; Pichler & Baker, 2000; Slooten, 1991; Slooten & Lad, 1991; Webster et al., 2009), with most dating from the 1990s. Specifically, aspects of biology and overall health, including environmental contaminants burden of the species, remain lacking.

The primary objective of this study was to analyse the contaminant concentrations in Hector's dolphins in order to provide insight into if and how ontogenic and spatiotemporal variables may impact burden and potential associated health. From a conservation perspective, this is critical since Hector's dolphins are an endemic species, listed as *Endangered* by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species and as *Nationally Endangered* by the New Zealand Threat Classification System (NZTCS) (Reeves et al., 2013a). The Māui dolphin subspecies is further classified as *Critically Endangered* by the IUCN Red List and as *Nationally Critical* by the NZTCS (Reeves et al., 2013b). The recent increase in mortality in both taxa attributable to disease, in particular, toxoplasmosis (Roe et al., 2013a,b), warrants concerns about the potential for immune suppression as a consequence of environmental contaminants of concern.

The coastal distribution of Hector's and Māui dolphins places them at elevated risk from inshore threats, including but not limited to environmental contaminants (Buckle et al., 2017; Carome et al., 2022; Dawson, 1991; Duignan, 2003; Martinez et al., 2010; Pichler & Baker, 2000; Rayment & Webster, 2009; Roe et al., 2013a,b; Stone & Yoshinaga, 2000). In recent

years, there has been increasing concern about Hector's and Māui dolphin deaths, particularly those related to human-induced impacts. As coastal species, Hector's and Māui dolphins are at increased risk from anthropogenic impacts; therefore, there is a real need to manage human-induced threats to these dolphins. To safeguard the long-term survival of this species, the mitigation of those threats is necessary. Toxicological studies are important, as recognised by the toxoplasmosis action plan for Hector's and Māui dolphins (DOC 2020), to protect species and understand how their health is impacted by contaminants.

The results of this study show that one female Māui dolphin exceeded the threshold for onset of physiological effects for PCBs in marine mammals (9,000 µg/kg), while thirteen individuals, including the same female Māui dolphin, further exceeded the threshold for onset of reproductive impairment for DDT in marine mammals (8,000 µg/kg). Accordingly, it is possible that contaminants may impact on the physiology and reproduction of Hector's and Māui dolphins in Aotearoa, New Zealand.

With the Māui dolphins' population in decline since 2000 (Constantine et al., 2021) and Hector's dolphins' population declining by over 50% in the last three generations (Roberts et al., 2019), successful conservation is vulnerable to any level of mortality above zero. Accordingly, it is important to understand and investigate if and how contaminants, known to be immunosuppressants, may potentially impact this species in the context of disease susceptibility.

#### **4.6 Study limitations**

This thesis relies upon stranded or bycaught animals, which are constrained by a number of factors. Firstly, the opportunistic nature of sample acquisition, for example, samples available may be biased both in a temporal and spatial context. Another limitation of stranded animals is the state of preservation, i.e. samples can be decomposed which may impact accurate contaminant assessment. The use of stranded animals can also result in a positive bias towards animals with underlying health or disease issues. However, the addition of bycaught animals goes some way to mitigating this bias.

Due to limited sample sizes, especially for Māui dolphins, all WCNI samples were pooled with Hector's dolphins from the WCSI. Accordingly, these results are not representative of the subspecies Māui dolphins and future efforts should ensure this is rectified by adequate post-mortem sampling of all available carcasses.

As aging was only possible for subset of the animals for which contaminants were assessed, I used TBL as a proxy of age. However, it is recognized that TBL is only a partial proxy of age since other factors beyond age alone can affect body size. To mitigate this, my dataset used TBL to instead differentiate between yearlings and non-yearlings, so as to identify the potential neonates which would have received reproductive offloading via gestation and lactation. In this case, yearlings were defined as anything less than 99cm, as defined by Slooten (1991).

#### **4.7 Future research**

Long-term studies are necessary for gathering data, specifically for effective conservation management of endangered and endemic species. This study has highlighted the need for further investigations into environmental contaminants and disease susceptibility in an endangered endemic species. Specifically, contaminant burdens presented here should be expanded, especially in the context of there being (1) little evidence of a decline in banned legacy contaminants (except dieldrin) and (2) an urgent need to assess emerging contaminants, especially those such as per- and polyfluorinated substances (PFAS), known as forever chemicals. Such toxicological assessments should further be assessed in the context of pathological findings, as revealed at post-mortem. Accordingly, it is recommended that systematic sampling of liver, kidney, muscle and blubber be sampled in every necropsied in the future. Furthermore, it is vital that tissue preservation extends beyond formalin-fixed tissues alone in order to investigate not only trends in contaminants, but importantly, detect toxicity thresholds (both lethal and sublethal) via bioassays.

The connection between the health of the environment, animals and humans (otherwise termed "One Health") is crucial to successful conservation of threatened and endemic species. Our current understanding of the effects of contaminants in diseased animals particularly remains within its infancy. Future studies investigating the effects of

contaminants on cetaceans using “omics” methods (Mancia, 2018) offer the best promise to investigate these complex relationships. Omics technologies (i.e. epigenomics, transcriptomics, metabolomics, proteomics), require only a small amount of tissue and can be used for various analyses. Use of such emerging omics technologies will in the future allow us to better understand the links between ocean health and what implications that has not only for marine mammals but also human health.



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# Appendices

**Table A.1:** Specimen details for females Hector's (*Cephalorhynchus hectori hectori*) and Māui (*C. h. maui*) dolphins stranded and bycaught between 1997 and 2022 in New Zealand waters. Note: B = bycaught, S = stranded/beachcast, TBL = total body length, bolding = Māui dolphins.

PM code ID	DOC ID	Subspecies	Yearling vs non yearling	TBL (cm)	Date	Source	Location	Coast
31309	#249	Hector's dolphin	Non-yearling	120	27/11/97	B	North Canterbury	East
32950	H043	Hector's dolphin	Non-yearling	136	22/12/01	B	Paroa Beach, Greymouth, West Coast	West
36528	H084	Hector's dolphin	Non-yearling	143	9/10/04	S	New Brighton Beach, Christchurch, Canterbury	East
39484	H124	Hector's dolphin	Yearling	67	10/11/06	S	Farewell spit, Golden Bay, Tasman	East
39794	H132	Hector's dolphin	Yearling	80	6/01/07	B	Port Craig and Flat Beach, Te waewae Bay, Soutland	South
39822	H133	Hector's dolphin	Non-yearling	143	6/01/07	B	Gillespie Beach, South Westland, West Coast	West
40256	H145	Hector's dolphin	Non-yearling	141	29/04/07	S	Punakaiki, Greymouth, West Coast	West
40664	H149	Hector's dolphin	Non-yearling	139	27/07/07	S	Buller Kawatiri, West Coast	West
40850	H150	Hector's dolphin	Non-yearling	141	16/09/07	S	Waikouaiti River, Dunedin, Otago	East
<b>41043</b>	<b>H153</b>	<b>Māui dophin</b>	<b>Non-yearling</b>	<b>153</b>	<b>10/11/07</b>	<b>S</b>	<b>Manu Bay, Raglan, Waikato</b>	<b>West</b>
<b>41043</b>	<b>H153(a)</b>	<b>Māui dophin</b>	<b>Yearling</b>	<b>70</b>	<b>10/11/07</b>	<b>S</b>	<b>Manu Bay, Raglan, Waikato</b>	<b>West</b>
41223	H157	Hector's dolphin	Non-yearling	149	8/01/09	S	Te Wae Wae Bay, Southland	South
42764	H163	Hector's dolphin	Non-yearling	136	2/08/08	S	Paroa Beach, Greymouth, West Coast	West
42649	H167	Hector's dolphin	Non-yearling	136	3/10/08	S	Ross Beach, Totara, Hokitika, West Coast	West
42366	H168	Hector's dolphin	Non-yearling	142	7/10/08	S	Waipara Rocks, Canterbury	East
42644	H169	Hector's dolphin	Non-yearling	133	7/11/08	S	North of Hector Beach, Ngakawau, West Coast	West
42749	H176	Hector's dolphin	Non-yearling	137	21/01/09	S	Keepers Beach, North Otago, Otago	East
44666	H196	Hector's dolphin	Non-yearling	141	28/02/10	S	Greymouth beach, West Coast	West
	H204	Hector's dolphin	Yearling	81	24/01/11	S	Kaka Point, Otago	East
	H207	Hector's dolphin	Non-yearling	142	9/03/11	S	Governer's Bay, Canterbury	East
	H210	Hector's dolphin	Non-yearling	134	17/10/11	S	Matau Branch, Clutha River mouth, Otago	East

47189	H214	Hector's dolphin	Non-yearling	135	22/02/12	B	2km north of Kairaki Beach past Pines Beach, North Canterbury	East
47298	H217	Hector's dolphin	Non-yearling	134	23/03/12	S	Hokitika Rivermouth, Hokitika, West Coast	West
47317	H219	Hector's dolphin	Non-yearling	128	27/03/12	S	1km south of Spencer Park, Canterbury	East
48721	H225	Hector's dolphin	Non-yearling	141	24/08/12	S	between Taupata and Billy King Creek, Tasman	East
49092	H230	Hector's dolphin	Non-yearling	152	16/12/12	S	Mapoutahi beach(?) Otago Doctors point near Blueskin bay, Otago	East
49091	H231	Hector's dolphin	Yearling	83	16/12/12	S	Warrington, Otago	East
49196	H235	Hector's dolphin	Non-yearling	147	7/02/13	S	Taramakau River mouth, West Coast	West
49284	H237	Hector's dolphin	Yearling	89	28/02/13	S	South Brighton Beach, Pegasus Bay, Canterbury	East
<b>50066</b>	<b>H243</b>	<b>Māui dophin</b>	<b>Non-yearling</b>	<b>151</b>	<b>13/09/13</b>	<b>S</b>	<b>Ripiro Beach, South of Glinks Gully, Northland</b>	<b>West</b>
50532	H246	Hector's dolphin	Non-yearling	123	2/12/13	B	Somewhere off Timaru, Canterbury	East
52593	H257	Hector's dolphin	Non-yearling	145	3/10/15	S	Coopers beach, Canterbury	East
55810	H271	Hector's dolphin	Non-yearling	145	22/04/18	S	South Bay, Canterbury	East
60375	H292	Hector's dolphin	Yearling	69	16/11/21	S	Hector, Buller	West
60336	H294	Hector's dolphin	Non-yearling	140	28/11/21	S	Awatuna, Hokitika, West Coast	West
60311	H295	Hector's dolphin	Yearling	80	30/11/21	S	Okarito, West Coast	West

**Table A.2:** Specimen details for males Hector's (*Cephalorhynchus hectori hectori*) and Māui (*C. h. maui*) dolphins stranded and bycaught between 1997 and 2022 in New Zealand waters. Note: B = bycaught, S = stranded/beachcast, TBL = total body length, bolding = Māui dolphins.

PM code	DOC ID	Subspecies	Yearling vs non-yearling	TBL (cm)	Date	Source	Location	Coast
31311	#279	Hector's dolphin	Non-yearling	123	5/02/99	B	Little Wanganui River, West Coast	West
32211	#314	Hector's dolphin	Non-yearling	121	25/02/01	B	North Beach, Westport, West Coast	West
32208	H036	Hector's dolphin	Non-yearling	122	19/12/00	B	Leithfield Beach, Canterbury	East
32949	H042	Hector's dolphin	Non-yearling	121	1/12/01	B	Karoro Beach, Greymouth, West Coast	West
33351	H044	Hector's dolphin	Non-yearling	127	24/12/01	B	Wasdyke, Timaru, Canterbury	East
39380	H122	Hector's dolphin	Non-yearling	129	4/09/06	S	Twin Beach, Buller, West Coast	East

39485	H125	Māui dolphin	Yearling	73	13/11/06	S	Sunset Beach, Port Waikato, Waikato	West
39548	H126	Hector's dolphin	Non-yearling	114	30/11/06	S	Te Wae Wae Bay, Southland	South
39587	H129	Hector's dolphin	Non-yearling	107	6/12/06	S	Kaikoura, Canterbury	East
39824	H130	Hector's dolphin	Yearling	78	7/12/06	S	Gore Bay, Canterbury	East
39860	H136	Hector's dolphin	Non-yearling	125	20/02/07	S	Rarangi Beach, Blenheim, Marlborough	East
41362	H155	Hector's dolphin	Non-yearling	135	14/12/07	S	Long Beach, Otago, Southland	East
42428	H166	Hector's dolphin	Non-yearling	120	2/10/08	S	Rapahoe Beach, Greymouth, West Coast	West
47190	H215	Hector's dolphin	Non-yearling	122	22/02/12	B	2km north of Kairaki Beach past Pines Beach, Canterbury	East
47731	H221	Hector's dolphin	Non-yearling	127	25/04/12	S	330m south of river mouth, Kina Road, Opunake, Taranaki	West
48846	H226	Hector's dolphin	Non-yearling	124	19/09/12	S	between Karamea and Little Wanganui, West Coast	West
49080	H229	Hector's dolphin	Yearling	69.5	14/12/12	S	Waimairi Beach North of Christchurch, Canterbury	East
49657	H241	Hector's dolphin	Non-yearling	129	30/05/13	S	South bay, Timaru, Canterbury	East
50404	H248	Hector's dolphin	Non-yearling	130	15/12/13	S	Christy's Bay, Picton, Marlborough	East
51147	H249	Hector's dolphin	Non-yearling	123	11/08/14	S	Scarborough Beach, Christchurch, Canterbury	East
51361	H250	Hector's dolphin	Non-yearling	105	2/10/14	S	South Brighton, Canterbury	East
51492	H251	Hector's dolphin	Non-yearling	123	30/10/14	S	Golden Bay, Tasman	East
51635	H253	Hector's dolphin	Yearling	83.5	10/01/15	B	Bolton's hole in shallow water, Nelson	East
54222	H262	Hector's dolphin	Yearling	77	10/02/17	S	Taylor's Mistake, Canterbury	East
56797	H278	Hector's dolphin	Non-yearling	122	20/12/18	B	Pegasus Bay, Christchurch, Canterbury	East
56798	H279	Hector's dolphin	Non-yearling	116	20/12/18	B	Pegasus Bay, Christchurch, Canterbury	East
56799	H280	Hector's dolphin	Non-yearling	117	20/12/18	B	Pegasus Bay, Christchurch, Canterbury	East
58327	H284	Hector's dolphin	Non-yearling	119	28/11/19	S	Northward of Macaulay Road. Temuka, Canterbury	East
58833	H288	Hector's dolphin	Yearling	96	1/01/20	S	Cole Creek Beach, West Coast	West
60319	H298	Hector's dolphin	Yearling	79	14/01/22	S	Waipara river, Canterbury	East

**Table A.3:** Summary table of ΣPCBs by species globally (µg/kg). Note: *n* = sample size, SD = standard deviation, M = male, F = female, Ad = Adult.

Species	Location	Year	Sex	<i>n</i>	Mean	SD	Range	Reference
Hector's dolphin ( <i>C. hectori</i> )	New Zealand	1997-2022	M	30	1,675	1,409	16.2-5,574	This study
			F	36	751	1,896	8.4-11,375	This study
Harbour porpoise ( <i>P. phocoena</i> )	UK	1990-2012	M	388	19,410		440-150,470	Jepson et al., 2016
			F	318	13,490		13,490-159,680	Jepson et al., 2016
	French and Belgian North Sea	1997-2001	All	21	36,400	26,400	1,900-404,000	Covaci et al., 2002
Indo-pacific finless porpoise ( <i>N. phocaenoides</i> )	Pearl River estuary, China	2007-2018	AdM	9	12,002	6726	2,319-27,852	Xie et al., 2021
		2015-2019	AdF	6	3342	1647	457-5,456	Xie et al., 2021
Franciscana dolphins ( <i>P. blainvillei</i> )	Southeastern Brazil	2011-2012	All	9	208-5,543	121-3,587	6,107-26,199	Lavandier et al., 2016
Indo-Pacific humpback dolphins ( <i>S. chinensis</i> )	Pearl River estuary, China	2004-2020	AdM	11	31,686	20,416	1,606-96,233	Xie et al., 2021

		2004-2019	AdF	32	10,812	4,936	323-30,682	Xie et al., 2021
Bottlenose dolphins ( <i>T. aduncus</i> )	South Australia	1989-1995		17			97-25,000	Weijs et al., 2020
		2009-2014		26			67-50,000	Weijs et al., 2020
Australian humpback dolphins ( <i>S. sahalensis</i> )	south-east Queensland, Australia			6			1,600-370,000	Weijs et al., 2016
	Great Barrier Reef, Australia			18			776-93,522	Cagnazzi et al., 2013
Common dolphins ( <i>D. delphis</i> )	New Zealand	1995-2005	M				268-1,634	Stockin et al. 2007
			F				49-386	Stockin et al. 2007
Hector's dolphins ( <i>C. hectori</i> )	New Zealand	1985-1987	M		887	355	319-1,916	Jones et al. 1999
			F		577	130	447-706	
Long-finned pilot whales ( <i>G. m. edwardii</i> )	New Zealand			61	311	26	33-931	Schröder & Castle, 1998

**Table A.4:** Summary table of MRP by species globally ( $\mu\text{g}/\text{kg}$ ). Note:  $n$  = sample size, SD = standard deviation, M = male, F = female, Ad = Adult.

Species	Location	Year	Sex	$n$	Mean	SD	Range	Reference
<b><math>\Sigma</math>DDT</b>								
Hector's dolphin ( <i>C. hectori</i> )	New Zealand	1997-2022	M	30	12,061	16,010	44-57,390	This study
			F	36	2,076	2,743	31-12,860	This study
Bottlenose dolphins ( <i>T. truncatus</i> )	Canary Islands, Spain	2003-2011	All	64	104,739	202,926	189-1,252,210	García-Alvarez et al., 2014
White-sided dolphins ( <i>L. acutus</i> )	Cape Cod Bay, US	1994-1996	All	6	14,049	9,599		Weisbrod et al., 2001
Indo-pacific humpback dolphins ( <i>S. chinensis</i> )	Pearl river estuary, China	2004-2009	All	15	64,200		845-179,000	Wu et al., 2013
Common dolphins ( <i>D. delphis</i> )	England & Wales	1992-2006	F	43			200-16,100	Law et al., 2013
Harbour porpoise ( <i>P. phocoena</i> )	Southern North Sea	2010-2013	All	20	15,000	20,000	700-96,000	Mahfouz et al., 2014
Humpback dolphins ( <i>S. sahalensis</i> )	Moreton Bay, Queensland	2002-2014	All	6			1,800-17,000	Weijs et al., 2016

	Port Curtis and Fitzroy River, Queensland	2009-2010	All	18			310-6,200	Cagnazzi et al., 2013
	Fitzroy River, Queensland	2014-2016	M	1	13,803			Cagnazzi et al., 2013
			F	5	16,209	2,501	516-32,527	Cagnazzi et al., 2013
	Port Curtis, Queensland	2014-2016	M	1	21,241			Cagnazzi et al., 2020
			F	10	15,705	2,709	516-32,527	Cagnazzi et al., 2020
Snubfin dolphin ( <i>O. heinsohni</i> )	Fitzroy River, Queensland	2014-2016	M	9	15,975	2,530	8,059-29,662	Cagnazzi et al., 2020
			F	9	16,458	2,783	7,465-33,886	Cagnazzi et al., 2020
Common dolphins ( <i>D. delphis</i> )	New Zealand	1999-2005	M	12			654-4,430	Stockin et al. 2007
			F	7			17-337	Stockin et al. 2007
<b>Dieldrin</b>								
Hector's dolphin ( <i>C. hectori</i> )	New Zealand	1997-2022	M	30	125	103	6-490	This study
			F	36	58	70	5-300	This study

Harbour porpoises ( <i>P. phocoena</i> )	Moray Firth, Scotland	1988-1991	M	21	1,700		790-3,430	Wells et al., 1994
			F	15	540		730-2,300	Wells et al., 1994
White-sided dolphins ( <i>L. acutus</i> )	Cape Cod Bay, US	1994-1996	All	6	1,019	949		Weisbrod et al., 2001
Common dolphins ( <i>D. delphis</i> )	England & Wales	1992-2006	F	43			10-730	Law et al., 2013
Indo-pacific humpback dolphins ( <i>S. chinensis</i> )	Pearl river estuary, China	2004-2013	All	45	58.8	55.2		Gui et al., 2014
Humpback dolphins ( <i>S. sahalensis</i> )	South-east Queensland, Australia	2002-2014	All	6			87-700	Weijs et al., 2016
Common dolphins ( <i>D. delphis</i> )	New Zealand	1999-2005	M	6	47	26	19-87	Stockin et al., 2007
			F	11	26	37	4.2-100	Stockin et al., 2007
<b>HCB</b>								
Hector's dolphin ( <i>C. hectori</i> )	New Zealand	1997-2022	M	30	56	28	7-130	This study
			F	36	34	28	3-110	This study

Common dolphins ( <i>D. delphis</i> )	England & Wales	1992-2006	F	43			10-270	Law et al., 2013
White-sided dolphins ( <i>L. acutus</i> )	Cape Cod Bay, US	1994-1996	All	6	175	164		Weisbrod et al., 2001
Guiana dolphins ( <i>S. guianensis</i> )	São Paulo, Brazil	2004-2005	All	6			70-170	Alonso et al., 2010
Indo-pacific humpback dolphins ( <i>S. chinensis</i> )	Pearl river eastuary, China	2004-2009	All	15	10		nd-60	Wu et al., 2013
Humpback dolphins ( <i>S. sahalensis</i> )	South-east Queensland, Australia	2002-2014	All	6			9.4-17	Weijs et al., 2016
<b>Mirex</b>								
Hector's dolphin ( <i>C. hectori</i> )	New Zealand	1997-2022	M	17	40	34	12-110	This study
			F	19	58	82	12-240	This study
Guiana dolphins ( <i>S. guianensis</i> )	São Paulo, Brazil	2004-2005	All	6			240-1,870	Alonso et al., 2010
White-sided dolphins ( <i>L. acutus</i> )	Cape Cod Bay, US	1994-1996	All	6	44.7	20.4		Weisbrod et al., 2001

Franciscana dolphins ( <i>P. blainvillei</i> )	Southern Brazil waters	1004-2004	All	73	61.8	21.8	34-106	Leonel et al., 2014
Humpback dolphins ( <i>S. sahuensis</i> )	South-east Queensland	2002-2014	All				4.1-84	Weijs et al., 2016
<b>Oxychordane</b>								
Hector's dolphin ( <i>C. hectori</i> )	New Zealand	1997-2022	M	17	27	16	10-52	This study
			F	19	38	52	10-130	This study
Franciscana dolphins ( <i>P. blainvillei</i> )	Saõ Paulo, Brazil	1997-1999	AdM	5	64		38-110	Kajiwara et al., 2004
			AdF	1	39		31-47	Kajiwara et al., 2004
Bottlenose dolphins ( <i>T. truncatus</i> )	Queensland, Australia	1996-1999	M	2	305			Vetter et al., 2001
			F	2	9			Vetter et al., 2001
	South Australia	1989-1995	AdM	5	1,300	2,300		Weijs et al., 2020
			AdF	5	180	160		Weijs et al., 2020
		2009-2014	AdM	5	1,100	1,200		Weijs et al., 2020
			AdF	5	700	1,100		Weijs et al., 2020

**Table A.5:** Summary table of PBDE by species globally ( $\mu\text{g}/\text{kg}$ ). Note:  $n$  = sample size, SD = standard deviation, M = male, F = female, Ad = Adult.

Species	Location	Year	Sex	$n$	Mean	SD	Range	Reference
Hector's dolphin ( <i>C. hectori</i> )	New Zealand	1997-2022	M	17	186	143	2-424	This study
			F	19	119	217	1.6-890	This study
Finless porpoise ( <i>N. phocaenoides</i> )	South China sea	1990-2001	AdM	12			84-980	Ramu et al., 2006
Franciscana dolphins ( <i>P. blainvillei</i> )	Southern Brazil	2002-2005	AdM	11			67.8-764	Leonel et al., 2014
			AdF	10			<0.65-228	Leonel et al., 2014
Harbour porpoise ( <i>P. phocoena</i> )	North Sea	1999-2004	All	35	1,540	960		Weijs et al., 2009
Common dolphins ( <i>D. capensis</i> )	Korean coastal waters	2006	All	22	1,700	720	140-3,100	Moon et al., 2010
Indo-Pacific humpback dolphins ( <i>S. chinensis</i> )	Hong Kong	1995-2001	All	15	1,900	2,000		Ramu et al., 2005
Bottlenose dolphins ( <i>T. truncatus</i> )	UK	1995-2001	All	9	7,700	5,300		Law et al., 2005
	Charleston, US	2003-2004	All	53	5,860	4,290	4,680-7,050	Fair et al., 2007
	Indian River, US	2003-2003	All	58	1,260	979	1,050-1,470	Fair et al., 2007