EFFECTS OF INCREASED PCB & PBDE LEVELS ON SURVIVAL AND REPRODUCTION OF SOUTHERN RESIDENT KILLER WHALES

by

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ABSTRACT

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Southern Resident Killer Whales

Nicole L. Manteufel

Southern resident killer whales (*Orcinus orca*) (SRKW) are an iconic species that live in the coastal waters of British Columbia, Washington, and Oregon, USA. There are three pods of the SRKWs consisting of J pod, K pod and L pod. These matriline pods of SRKWs are close-knit family members who stay near each other for life, and the SRKWs have demonstrated a unique culture that involves their primary prey Chinook Salmon (*Oncorhynchus tshawytscha*). However, there are anthropogenic chemicals that have reduced fecundity and survival of the SRKWs. No other form of environmental pollution has the far-reaching detrimental effect on the population and reproductive capacity of Southern resident killer whales (SRKW) as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ether (PBDEs). PCBs and PBDEs are recognized as highly toxic and are the most detrimental of all persistent organic pollutants (POPs). One of the impacts of PCBs and PBDEs on Southern resident killer whales (SRKW) is on their reproductive processes. Partly due to the damaging effects of PCBs and PBDEs, the Southern Resident Killer Whale (SRKW) population has been declining since 1996 from a high of 98 to 75 in 2019. Even if there were no oil spills, epizootics and reduced food supply, fecundity would continue to decline, and adult mortality due to persistent organic pollutants (POPs) will continue to increase. If these trends continue, the risk of extinction of the SRKWs within 50 years is highly likely.

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Introduction

Many ecotypes of Killer whales (*Orcinus orca*) live around the world. The most studied and well-known Killer whales are an iconic species that live primarily in the Pacific Northwest, known as the Southern Resident Killer Whales (SRKWs). This ecotype of Killer whale is found in the Puget Sound around British Columbia and Washington State (Mongillo et al., 2016). The SRKWs were listed as endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and by the U.S. Endangered Species Act (ESA; 16 U.S.C.A. §§ 1531 *et seq*., as amended) in 2005 because of a steep decline in their population numbers. The population decreased from 97 whales in 1996 to just 78 whales in 2001 (Krahn et al., 2007). Another population decline of over 20% has happened in Puget Sound since 2010. In 2017, the community continued to decline to a total number of 76. In 2019, only 75 of these animals remain. Experts explain the decline of SRKWs population as the result of a combination of noise disturbance from vessel traffic, reduced prey availability and high concentrations of anthropogenic toxins from within the environment (Hickie et al., 2007; Krahn et al., 2009; Ross et al., 2000).

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are anthropogenic chemicals that remain within the environment, dissolve in fatty tissues, and thus bioaccumulate and biomagnify up the food web (Jepson et al., 2006; McHugh et al., 2007). PCBs and PBDEs can cause reproductive damage, endocrine disruption and immunotoxicity for SRKWs (Fossi & Panti, 2018; Jepson & Law, 2016; Krahn et al., 2007; Mongillo et al., 2012). Because of these concerns for this dwindling population of whales, this thesis will address the ways PCBs and PBDEs affect reproduction, pregnancy and calving in the SRKW population.

Southern Resident Killer Whale Demography

The J, K, and L pods comprise the SRKW community. The home range of these matrilineal pods includes the international waters of Puget Sound (the Salish Sea), and the Juan de Fuca and Georgia Straits (Figure 1, Taylor & Plater, 2001). However, L and K pods have been known to travel greater distances outside of the Salish Sea from Monterrey Bay, California to Vancouver Island, Canada in search of salmon (Mongillo *et al*., 2016). The SRKWs should not be confused with the mammal-eating Transient or “Biggs” Killer Whale that also frequents the Salish Sea in search of Harbor seals (*Phoca vitulina*) and California Sea lions (*Zalophus californianus*) as prey (Mongillo *et al*., 2012). Researchers now know that there is distinct genetic isolation between the SRKWs and the “Biggs” transient whales. Furthermore, there has not been any interbreeding between these two ecotypes of whales for approximately 350,000 years (Foote et al., 2013).

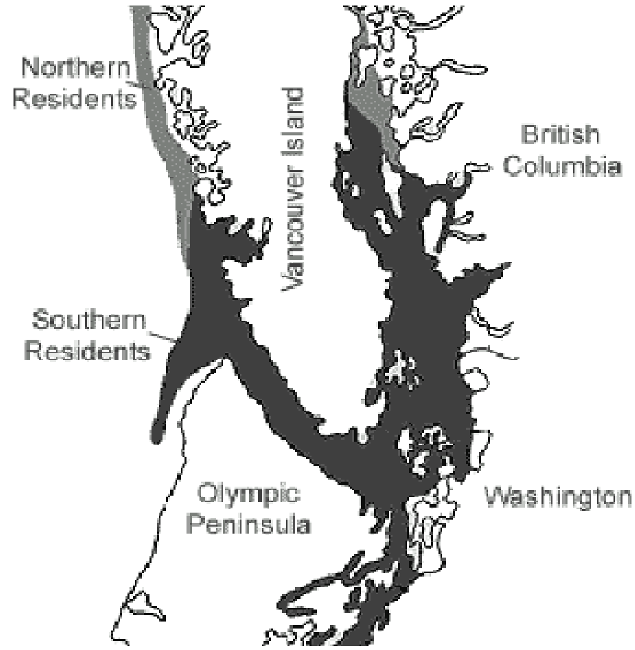

Figure 1. Range map for the Southern Resident Killer whales in Washington State waters and the closely related Northern Residents that inhabit the waters of British Columbia. Adapted from Taylor & Plater, 2001.

Knowledge Gaps

While SRKWs are among the best-studied cetaceans in the world, significant gaps in knowledge about these populations remain. While studies of SRKWs have been ongoing for nearly 50 years, the whereabouts of the whales are unknown during much of the year (Ford, 1998). Knowledge gaps that researchers have discovered include: What are the year-round distribution and behavior of SRKWs? What are the potential additional critical habitat areas required for SRKWs? What is the historical abundance of SRKWs?

Most importantly, few researchers have had the opportunity to study SRKW carcasses. Killer whale carcasses have rarely been recovered due to their negative buoyancy and tendency to sink upon death. Only eight Killer whale carcasses per year are recovered anywhere in the world (Raverty et al. 2017). As a result, scientists still lack information about the full spectrum of anthropogenic environmental contaminants on killer whale anatomy. The section that follows will further explain how PCBs and PBDEs affect SRKW fecundity and reproduction rates.

Literature Review

The Southern Resident Killer Whales (*Orcinus orca*) that live in the waters of the Salish Sea has been studied and observed for nearly 50 years. This unique ecotype of Killer whale has endured angry commercial anglers who shot them, collection for entertainment, and excessive marine vessel traffic (Fisheries and Oceans Canada, 2018). Although most harmful activity has ceased and the Southern Resident Killer Whales (SRKWs) are now protected by both the Marine Mammal Protection Act (MMPA) and the Endangered Species Act, their population numbers have still declined in recent years (C. H. Grant & Ross, 2002). The main hypotheses as to why the SRKWs numbers continue to drop are 1. lack of their preferred prey Chinook Salmon (*Oncorhynchus tshawytscha*) which is also declining rapidly, 2. marine vessel noise, and 3. anthropogenic pollution such as persistent organic pollutants (POPs) which are also considered endocrine disrupting chemicals (EDCs) (EPA, 2014 US; Fossi & Panti, 2018; Rayne et al. 2004).

Researchers have found that the SRKWs preferred prey, Chinook salmon, are declining in population numbers due to overfishing by humans and dams that block the Pacific Ocean passage for the salmon’s oceanic/river reproduction cycle (Lundin et al., 2016). Furthermore, boat traffic has been hindering the SRKWs only way of obtaining food by echolocation, and the excessive noises render the echolocation ineffective. Also, vessel noise frightens the salmon into hiding and makes looking for food for SRKWs that much more difficult. Since the SRKWs have a reduced caloric intake because of anthropogenic activity, the lack of food invites excess toxins to be released into the body (Lundin et al., 2016).

The full range of anthropogenic environmental POPs which SRKWs and their offspring endure, over time and geographic space within their habitat is not fully understood. The most toxic and long lasting anthropogenic toxins identified by researchers are polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), which are explained further below (Krahn *et al*., 2009).

Unfortunately, anthropogenic PCBs and PBDEs are spreading throughout the Salish Sea, disrupting SRKW feeding patterns by causing mental confusion (Krahn *et al*., 2007). More importantly, the impacts on the killer whale's reproduction and calving by anthropogenic toxins such as PCBs and PBDEs have been and will continue to be detrimental. PCBs and PBDEs inhibit reproduction cycles, fecundity and longevity in adult Puget Sound SRKWs, and thus will contribute to their decline until this unique ecotype of killer whale is extirpated or localized extinction from the Salish Sea.

PBDEs are a new threat to marine mammals (Mongillo, Ylitalo, Rhodes, O'Neill, & Noren, 2016; Rayne, Ikonomou, Ross, Ellis, & Barrett-Lennard, 2004; Ross, 2006). PBDEs continue to be used today as flame retardants in textile, computers, furniture and electrical equipment. PBDEs are bioaccumulating and biomagnifying in the environment and have become the contemporary endocrine disrupting chemical (EDC). PBDEs, like

PCBs, do not break down in the environment and are a health risk to humans as well (Alava, Ross, & Gobas, 2016).

SRKWs are the apex or top predator of the Salish Sea ecosystem. The SRKWs are strict pescatarians or fish eaters, and the whale's blubber layer is entirely comprised of their preferred diet of Chinook Salmon (*Oncorhynchus tshawytscha*). Chinook Salmon are high trophic level consumers, and because of their high level within the food web, Chinook Salmon also carry high levels of PCBs and PBDEs within their lipid layers (Mongillo et al., 2012).

Because of the high trophic level of the SRKWs and the high trophic level of their prey, the PCBs and PBDEs bioaccumulate within the blubber stores of the SRKWs. Ultimately, the higher the trophic level, the higher the contamination level. According to one study: "Dietary contaminant concentrations and trophic position appear to play major roles in the accumulation of PCBs in the killer whale communities" (Ross et al. 2000). Due to the PCB and PBDE contamination of the whale's prey, Chinook Salmon, SRKWs carry large toxic loads within their blubber, especially adult males and calves (Mongillo et al., 2012).

WHAT ARE PCBS AND PBDES?

Polychlorinated Biphenyls (PCBs) are synthetic, lipophilic chemicals (chemicals having an affinity for fats) and due to being misused are now deemed as a health hazard and pollutant. With the expanding electrical grid of the 1930s, there was a clear need for an inexpensive insulation fluid. PCBs were not only heat and fire resistant but could be manipulated into other forms as well. Other uses of PCBs have included, but are not limited to, caulking in elementary schools and office buildings, paint additives to reduce or prevent chipping, additives in plastics for added durability, and industrial fire retardants (C. H. Grant & Ross, 2002; McHugh et al., 2007; Mongillo, Ylitalo, Rhodes, O'Neill, & Noren, 2016; Stuart-Smith & Jepson, 2017).

Since the cessation of U.S. production of PCBs due to federal legislation passed in the late 1970s, quantities of POPs such as PCBs have plateaued as opposed to decreasing (Law, 2014). Nonpoint and point sources of pollution account for much of the PCBs found within the Salish Sea ecosystem accounting for the plateauing effect observed by scientists (US EPA, 2014). For example, damage to transformers or other electrical equipment built before the ban can spread the chemicals even today. Furthermore, abandoned electrical components can leak PCBs into the environment. For both humans and SRKWs, this has been both an unfortunate and even deadly revelation. After the cessation of the production of PCBs, a new persistent organic pollutant was developed and mass-produced for industrial purposes: Polybrominated Diphenyl Ethers (PBDEs).

PBDEs were recently added as persistent organic pollutants (POPs) by the Stockholm Convention; specifically, because of their persistence, toxicity, and bioaccumulative nature (Alava, Ross, & Gobas, 2016). PBDEs can be transported significant distances beyond the source of pollution by either wind or the ocean. As a result, these chemicals have been found in areas that have no history of PBDE use or production, such as the Antarctic and at 10,000m down in the oceans (Stuart-Smith & Jepson, 2017). Furthermore, like PCBs, PBDEs from other areas can settle into the sediment, only to be later disturbed by dredging or rough weather.

The PCBs and PBDEs enter the marine environment and are absorbed by phytoplankton and zooplankton. Then, the phytoplankton and zooplankton are eaten by another larger animal, carrying the toxin load along every step of the food web. PCBs and PBDEs accumulation continues in this fashion until the largest animals unknowingly eat the toxins, which in this instance is SRKWs. Every time contaminated salmon is consumed, the PCB and PBDE toxin becomes more concentrated within the whale's lipid or fat tissues (C. H. Grant & Ross, 2002).

PBDEs and PCBs in marine habitats can persist from decades to centuries, depending on the specific type of congener or related chemicals (C. H. Grant & Ross, 2002; Fossi & Panti, 2018). With continuous disturbance and as a result of being brought in from areas outside of the Salish Sea by wind and sea, PBDEs and PCBs will continue to cycle through the environment, causing recontamination of the food web, the SRKWs and ecosystem that they depend.

HOW DO PCBs AND PBDEs EFFECT SRKWs?

PCBs and PBDEs can affect the action of endogenous hormones and deregulate hormone balance. These toxins can mimic thyroxine and thyronine; natural hormones secreted from the thyroid during pregnancy and birth (Tavares et al., 2016) (see Figure 2).

According to one study, PBDEs have been shown to interact with PCBs, causing neurobehavioral defects when the exposure occurs during a critical stage of neonatal brain development during pregnancy in mice (Eriksson, Fischer, & Fredriksson, 2006). Neurobehavioral effects in this report in this study include defective learning and memory processes.

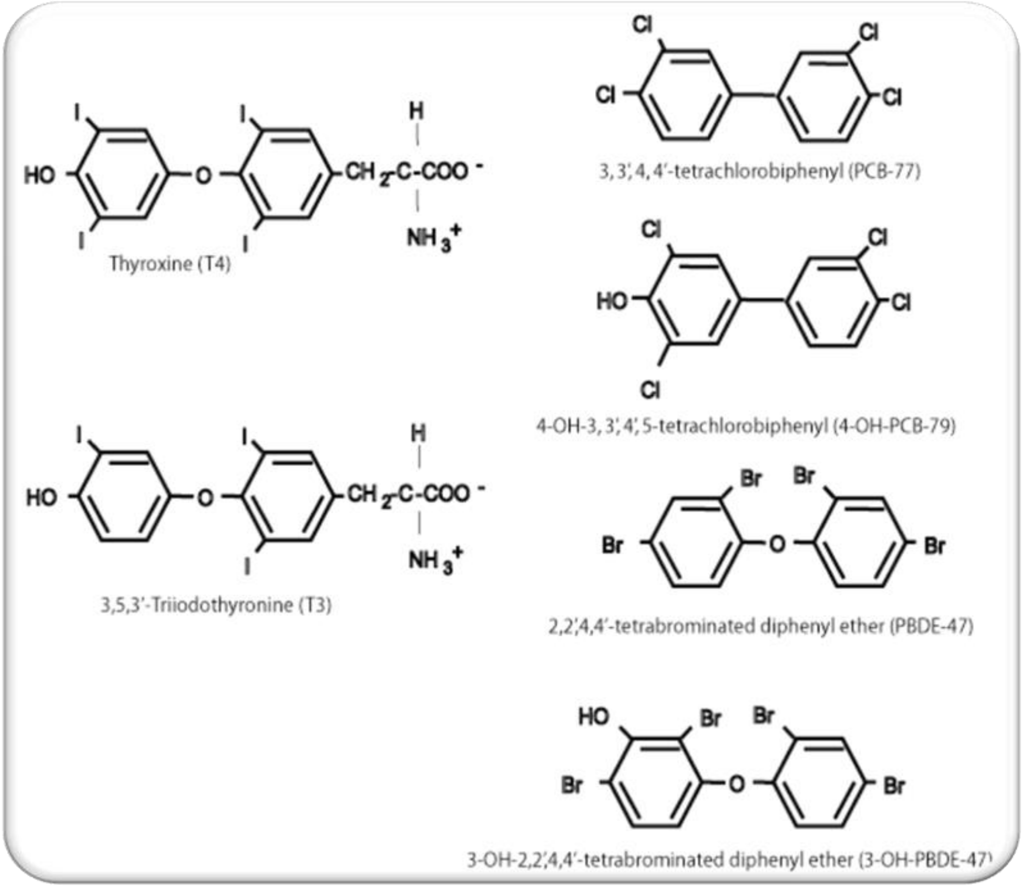

Figure 2. A comparison between natural hormones Thyroxine (T4), its metabolite Triiodothyronine (T3) and various PCBs and PBDEs.

Furthermore, the test of a PCB and PBDE mixture indicated that neurobehavioral defects appeared to advance with age in adult mice (Eriksson, Fischer, & Fredriksson, 2006). Researchers administered low doses of both PCBs and PBDEs in neonate mice; those mice had a higher rate of developmental neurotoxic effects than mice that were given very high doses of either PCBs or PBDEs later in life (Eriksson, Fischer, & Fredriksson, 2006).

SRKWs could be suffering similar neurobiological effects of PCBs and PBDEs as witnessed by Eriksson, Fischer, & Fredriksson. The mixing of these toxins reduces the cognitive ability and memory of adult mice and humans, especially as the adults age (Eriksson, Fischer, & Fredriksson, 2006).

Lipid layers are essential for a pregnant SRKW. The female needs extra blubber to assist in the production of milk for her calf and the formation of the placenta (Mongillo et al., 2012). When a pregnant female SRKW begins to produce milk or cannot find enough salmon to eat, she begins to burn her blubber stores, releasing PCBs and PBDEs into her bloodstream and the bloodstream of her unborn calf.

PCBs and PBDEs released from lipids during gestation and lactation can cause adverse health effects like neurological effects and immune disruption in mature female SRKWs (Mongillo et al., 2012; Eriksson, Fischer, & Fredriksson, 2006). Also, PCBs and PBDEs have been determined to depress thyroid hormones, which assist *en utero* development of neonate calves (Fossi & Panti, 2018; Stuart-Smith & Jepson, 2017; Wasser et al., 2017). Furthermore, if a female SRKW become pregnant, the fetus becomes poisoned from PCB/PBDE lipid concentrations and is typically miscarried during late pregnancy or dies soon after birth (Rayne, Ikonomou, Ross, Ellis, & Barrett-Lennard, 2004; Wasser et al., 2017).

Additionally, since late-term miscarriages are becoming more frequent than in the past, a fetus can become lodged within the birth canal, and the adult female can succumb to sepsis or poisoning of the bloodstream (Krahn et al., 2009; Krahn et al., 2007). In sum, PCBs and PBDEs are hindering the reproductivity of the SRKWs. With this hindrance of reproductivity and loss of mature breeding adults due to these anthropogenic toxins, the SRKW population is likely to continue its downward trend.

In mother/calf pairs, the calves had 2-3 times the PCB and PBDE concentrations in their blubber than their lactating mother did at the time (Haraguchi et al., 2009). The researchers suggest that females were transferring toxic chemicals during lactation (Haraguchi et al., 2009; Jepson et al., 2016). The female whale transfers approximately 70%, of her initial toxicity load to her calf, allowing the female whale to maintain relatively low PCB levels in her blubber after the birth of her calf (Wasser et al., 2017; Haraguchi et al., 2009).

Additionally, PCB levels in reproductive adult female whales affect calf mortality: the probability of a calf surviving within the first six months decreased as the mother's lipid toxicity rate increased. For example, every 5% increase of toxins in the mother's lipid layer, the calf's survival dropped by 10% (Mongillo et al., 2012; Wasser et al., 2017). Scientists have found that the milk produced for the calf has a high toxicity content and can poison the calf well after its birth (C. H. Grant & Ross, 2002; Haraguchi et al., 2009; Mongillo et al., 2012; Wasser et al., 2017).

The SRKWs inner blubber layer is critical to the health of each animal and is needed for many natural bodily functions, especially the production of reproductive gametes such as male killer whales' sperm and female whale's ova (Pedro et al., 2017). Ongoing studies have identified that male SRKWs have complications from PCB and PBDEs as well (Krahn et al., 2007; Krahn et al., 2009).

In a study conducted by Ross et al., a computer statistical model was constructed to examine the on future population levels if PCBs and other toxins were to persist in the Salish sea environment. According to the study, population decline was observed near 9.0 mg per kg lipid weight (Ross et al., 2000). This level is referred to as the "Ross

Threshold.” To observe at what level the PCB and PBDE levels were in the SRKW population, researchers took 21 biopsy samples from individuals from the three different wild pods of SRKWs (Ross et al., 2000).

Male SRKW had exceptionally high PCB/PBDE concentrations in their inner blubber layer of over 100 mg/kg. The PCB/PBDE levels in male calves increased steadily over time and continued to increase with age because males have no outlets, such as calving or lactation, to transfer some of their PCB load (Ross, 2006).

Mongillo et al. found that PCB and PBDE levels were approximately double the predicted amount in male animals between 12 and 18 years old than it was in female animals of the same age (Mongillo et al., 2012). With increasing levels of PCBs and PBDEs in their blubber, male SRKWs will develop cognitive impairment, which reduces their ability to catch their fast-moving Chinook Salmon prey (Wasser et al., 2017). Furthermore, male mammals exposed to PCBs and PBDEs throughout their lives have been found to have spermatozoa that are deformed and that have a reduced motility ability (Tavares *et al*., 2016). The deformation of spermatozoa impairs their ability to reproduce. Killer whales have the same basic anatomy, hormones, anabolism, catabolism and metabolic processes as humans, meaning that humans and SRKWs likely will develop the same physical anomalies when exposed to endocrine disrupting chemicals (EDCs) like PCBs and PBDEs (Kannan, Blankenship, Jones, & Giesy, 2000).

PCB concentrations above the Ross threshold of 9.0 mg per kg have been found in the SRKWs, which is a similar result to those samples taken in other parts of the world (Ross et al., 2000). Krahn et al., 2008, 9.0 mg per kg are observed in all whales tested, reaffirming the 9.0 mg per kg is the minimum for toxicity burdens of cetaceans that were first observed in the study of Hall et al., 2006. This research indicates that the entire population of SRKWs could be suffering from PCB and PBDE poisoning.

ANATOMICAL PROBLEMS OF PCBS AND PBDES

High PBDE sickness or 'bromism' is described as a neurological disturbance which results in somnolence, psychosis, seizures and delirium within lab rats. The condition of bromism to an already sick animal can lead to an early death through starvation due to the inability to hunt for food (Boas, Feldt-Rasmussen, Skakkebæk, & Main, 2006). Brominism could explain male physical declines due to their high toxicity levels (Ross, 2006).

These toxins negatively affect the formation of sperm in male SRKWs, and what sperm is produced may be deformed or non-viable. Furthermore, the relationship between PCBs and PBDEs and SRKW reproduction leads researchers to hypothesize that there are only about five years left until the current SRKWs lose their reproductive abilities altogether (Cossaboon et al., 2019).

About 16% of the population of SRKWs have adverse effects from PBDEs (C. H. Grant & Ross, 2002). PBDEs have not yet had the decades of accumulation in the tissues of the whales as the PCBs had due to their relative newness in the environment. However, early peer-reviewed research indicates that PBDEs are behaving similarly to PCBs (they have bioaccumulative properties, do not break down in the environment and causes endocrine disruption). PCBs and PBDEs also can interact with each other to compound the health effects in SRKWs (Erriksson, Fischer, & Fredriksson, 2006). The full spectrum of health effects due to the chemical reactions between PCBs and PBDEs within the bodies of SRKW is still unknown.

Most researchers have focused on why PCBs and PBDEs are affecting SRKWs, while very few researchers have done work on how these toxins affect the bodies of SRKWs and ultimately fecundity. This discussion section will explain the pathway by which PCBs and PBDEs are negatively affecting the SRKWs. Few killer whales have been found to do necropsies of the physical effects of PCBs and PBDEs (Krahn et al., 2009). However, evidence collected from toxin studies conducted on Common Bottlenosed Dolphins (*Tursiops truncates*), Sperm Whales (*Physeter macrocephalus*), Atlantic Spotted Dolphin (*Stenella frontalis*) and Striped Dolphins (*Stenella coeruleoalba*) could explain what researchers see in the SRKWs (Mongillo et al., 2016).

The chemical pathways which are shown in Figure 10 show the thyroid feedback loop. This illustration tracks the primary hormone produced by the body, thyroxine (T4) and its metabolite triiodothyronine (T3). The thyroid hormones influence the metabolic rate and protein synthesis within the body (Ridgway & Patton, 1971). Thyroid hormones have many other essential roles, including ensuring proper neural development of the fetus during pregnancy (Fossi & Panti, 2018).

High PCB and PBDE levels within the lipid layer creates a dangerous feedback loop (Boas, Feldt-Rasmussen, Skakkebæk, & Main, 2006). The female SRKWs rely on their blubber reserves during pregnancy. Pregnancy and high activity levels release PCBs and PBDEs into their bloodstream (Hall et al., 2018; Wasser et al., 2017). PCBs and PBDEs bind to receptors much more quickly than the body’s natural hormone, thyroxine. The toxins are readily taken up by the body, and natural hormones are quickly replaced by PCBs and PBDEs (Jepson et al., 2016).

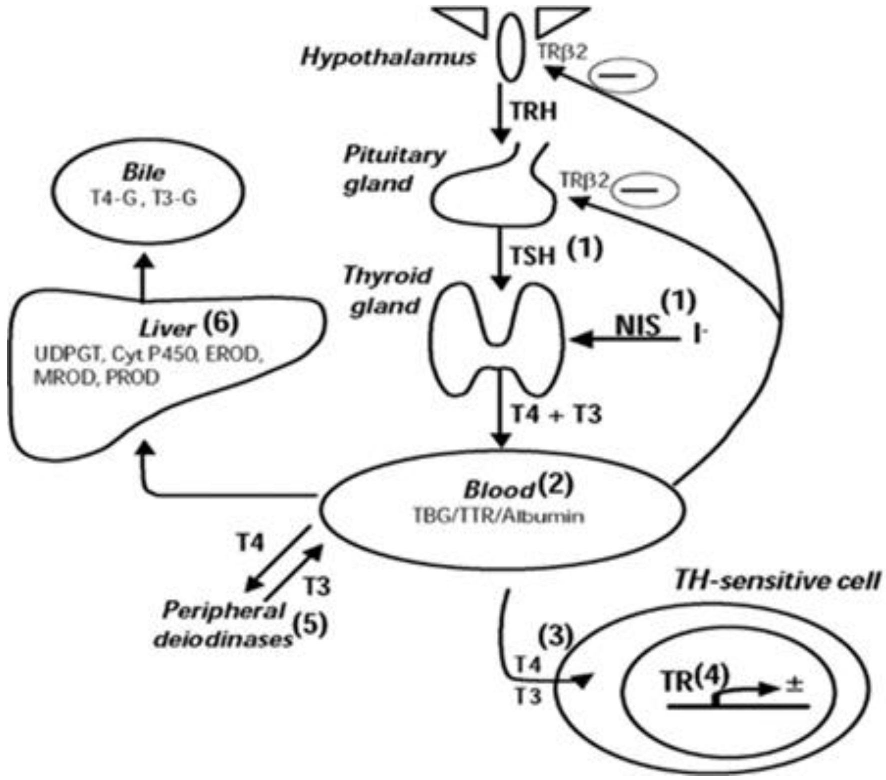

Figure 3. Thyroxine (T3) and triiodothyronine (T3) feedback loop.
Adapted from (Boas et al., 2006)

The natural process is vital for pregnancy: T4 and T3 hormones are tripled within the body for the increased cell production of the endometrium within the uterus in adult females SRKWs (Fossi & Panti, 2018; Mongillo et al., 2016). When PCBs and PBDEs have replaced thyroxine and are the primary 'hormone', the female SRKW develops a high toxicity load during pregnancy, and she has a higher risk of late-term miscarriage (Desforges et al., 2016).

PCBs and PBDEs reduce T4 and T3 hormone production by deceiving the body into thinking that enough thyroid hormone has been made. Therefore, the toxins that are circulating within the body alongside the T3 and T4, passing through the blood/brain barrier (BBB), through the uterus to the fetus, and passing to the neonate during feeding

(Fossi & Panti, 2018). Thyroxine shifts during the birth of the calf into lactation and mobilizes lipids and any lipid-soluble toxins within the blubber layer (Fossi & Panti, 2018).

Female Atlantic Spotted dolphins (*Stenella frontalis*) can offload as much as 90% of their toxicity burdens to their calves (Fossi & Panti, 2018; Krahn et al., 2009; Lavandier et al., 2019). Toxin offloading means that SRKWs can be transferring PCBs and PBDEs to their calves. Toxin offloading can have devastating results: consider the death of Tahlequah's (J35) calf during the summer of 2018, who died 30 minutes after being born.

PCB and PBDE offloading to neonate and calves, and their subsequent deaths are what the researchers have seen with SRKWs (Fossi & Panti, 2018). Toxic contamination levels depend on the age and sex of the individual whale (Fossi & Panti, 2018; Ross, 2006) and are also influenced by recruitment, explicitly referring to the birth order of the calves (Fossi & Panti, 2018; Ylitalo et al., 2001). In other words, the first calf born to a new mother will receive the highest amount of toxins, then to the second, then the third calf. It may take the female SRKW 2 to 3 calves before a viable calf is born. This observation supports what is seen in both my statistics and the statistics of other researchers for the lack of fecundity. Also, this has been observed by researchers as recently as 2019, when three SRKW females were pregnant, but only one calf was observed to have survived.

More individuals are dying than are being replaced in the SRKW population. PCBs and PBDEs can cause spontaneous miscarriage (as seen in Princess Angeline (J17) and Deadhead (K27) in late 2018), disorders of lactation and ovulation, ovarian failure, ovarian tumors, and hermaphroditism (Fossi & Panti, 2018).

Furthermore, with the addition of other anthropogenic challenges, the SRKWs have to face, such as lack of food, the PCBs and PBDEs affect the whales by hindering their ability to be resilient to minor setbacks. For example, small abrasions can cause serious infections that cannot be overcome in males and pregnancy in females. After the birth or miscarriage in the female, the PCBs and PBDEs have taken their toll on the body of the mother SRKW, and body homeostasis (steady internal physical and chemical systems) has been permanently compromised until the female dies (Rayne et al., 2004).

Researchers should expand their research areas to understand the full extent of the SRKWs population dynamics. Next, we need to oppose any weakening of the Endangered Species Act and the Marine Mammal Protection Act, which provide critical protections to the SRKWs. Everyone must take steps to reduce pollutants that potentially contaminate watersheds. Also, for meals, please choose salmon species other than Chinook salmon (*Oncorhynchus tshawytscha*), the SRKWs primary prey. Pink salmon (*Oncorhynchus gorbuscha*) and Chum salmon (*Oncorhynchus keta*) are more abundant than the Chinook salmon.

POPULATION DECLINE OF THE SRKWS

In April of 2018, researchers conducted new genetic testing on the SRKWs in the Salish Sea. The results confirmed what scientists had dreaded, that only two males, ‘Mega’ (L41) and ‘Ruffles’(J1), had fathered nearly 52% of the calves since 1990 and 80% of all calves (Ford et al., 2018). This study has revealed that SRKW genetic diversity is dangerously low and reduced sperm counts, deformed sperm, which are caused by PCBs and PBDEs may contribute to population numbers dwindling even further.

Ruffles died in 2010 of old age; he was believed to be approximately 70 years old. Although Mega is still alive today, he is believed to be approximately 41 years of age, which is past reproductive age of SRKWs. With the last bull ageing out of reproduction, there are very few bulls to replace genetic input (Ford et al., 2018). Due to PCBs and PBDEs, most of the males in any of the pods in Puget Sound have no sperm count or deformed sperm and possibly underdeveloped genitals. Anthropogenic toxins can result in no libido and the lack of sexual behaviors in males (Ford et al., 2018). Further genetic studies are ongoing to conclude who is and who is not fathering calves of the SRKWs.

According to Ford et al., only 10 of 27 females are currently of reproductive age, and the other females are older than 40 when reproductive senescence is observed (Ford et al., 2018). In the presence of PCBs, those females have not produced any calves. Births also occur at longer intervals of one calf nearly every ten years, instead of every five years as generally seen in the past (Ford et al., 2018). This new information suggests that SRKWs could become extinct within the next 50 years (Ford et al., 2018).

Researchers have tracked the Southern Resident Killer Whales (SRKW) since the 1960s. The Center for Whale Research in Friday Harbor, Washington has been conducting a formal annual census since the 1970s. Since the beginning of these research observations, three significant declines have been observed of the SRKWs (see Figure 3). Steady recruitment had increased after the live capture incidents for aquarium purposes in the early 1970s but a second decline occurred between 1980 and 1984 with an annual rate of decline of 3% (Taylor & Plater, 2001). The overall population peaked in 1995 with 98 individuals. In 2019, three females were confirmed pregnant. However, of these three pregnancies, only one birth was confirmed. The new calf, named Lucky, has brought the population number to a precarious 75.

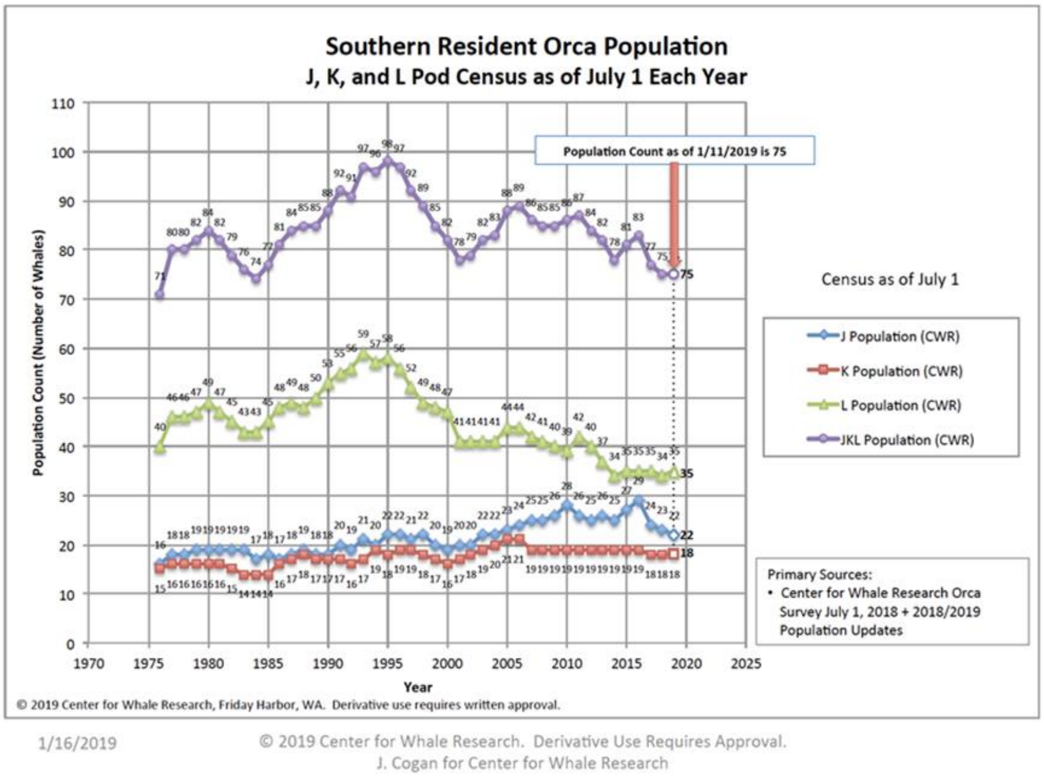

Figure 4. Population chart of the SRKWs from 1975 to 2019. Used with permission from the Center of Whale Research in Friday Harbor, WA.

The population went from 97 whales in 1996 to just 78 whales in 2001 (Krahn et al., 2007). In 2019, the community continued to decline to a total number of 75 whales (Center for Whale Research, 2019). Unfortunately, all the calves that were born in the 'baby boom' of 2016 have since died. In the presence of the persistent and slow toxicity of PCBs and PBDEs and other stresses within the Salish Sea ecosystem, no calves survived the year.

There is evidence that PCBs and PBDEs have direct effects on the decline of SRKWs in the Salish Sea. If this decline persists, the SRKWs are likely to be extirpated (a localized extinction) from the area (Rayne et al., 2004). This iconic marine mammal is on the verge of extinction because PCBs, PBDEs and other anthropogenic toxins are impacting their reproductive rates and fecundity is nearly 0% per year. If these animals disappear from the Salish Sea, tourism from whale watching will decline, and First Nations in the Washington State and Oregon, Alaska and British Columbia coast will lose a vital part of their heritage. The SRKWs are also an important indicator species that that could be lost to science.

Researchers are attempting to understand what anthropogenic PCBs and PBDEs are doing to the Salish Sea ecosystem, and ultimately, the risks are to humans themselves if we allow this problem to persist. PCBs and PBDEs have infiltrated the marine food web of the Salish Sea. We as humans will do a great injustice to the SRKWs and the environment as a whole if we allow this unique ecotype of killer whale to disappear from the rare ecosystem of what is called the Salish Sea.

Desforges et al., states eloquently “Although killer whale populations face other anthropogenic stressors such as prey limitation and underwater noise, our assessment demonstrates the high risk of collapse for many killer whale populations as a consequence of their PCB exposures alone.” - J.P. Desforges

Methods

In order to gather toxicology information on the Southern Resident Killer Whale (SRKW) population, scientists have taken various data on individual whales since 1976. Sex determination, reproductive history, birth order and blubber samples have been obtained for toxicology purposes from photo-identified individuals. These samples have been recorded and maintained by the Center for Whale Research in Friday Harbor, Washington State and National Oceanic and Atmospheric Administration (NOAA). I acquired all toxicology data through the Center for Whale Research, NOAA, Mote Marine Laboratory, Aquarium and Fisheries and Oceans Canada, The University of Nebraska and The University of Hokkaido in Japan.

All univariate, bivariate and multivariate and analyses were conducted using JMP Statistical Software (PC professional edition, version 12.1). All comparisons I did were significance tested using a two-sample t-test assuming unequal variances. Significant differences among multiple groups were evaluated using a Tukey test (α =0.05). Differences in polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) levels were examined by comparing concentrations expressed on standardized lipid totals (mg/kg total lipids).

Only females of reproductive age are used in my statistics. We consider reproductive age in females between the ages of 16 and 40. After the age of 40, reproductive senescence is observed in SRKW females (C. H. Grant & Ross, 2002). Reproductive senescence is considered the age after 40 and above in females that no longer bear calves. Calves are defined as from birth to one year of age. This thesis utilized previously gathered data and the data that was collected from other researchers that did not specify which actual individual the sample was obtained from; females of reproductive age were assigned a PCB and a PBDE level based on age and the number of calves delivered, whether the calves were born deceased or viable. Male PCB and PBDE levels were assigned based on the age of the male.

Beyond the difference in anatomical size, the functional or structural proteins of cetaceans differ little than those of terrestrial mammals, even though published DNA sequencing may reflect a difference in the nucleotide sequence as much as 30% between species (Jepson & Law, 2016). Evolutionarily homologous genes are the result of a speciation event that indicates that much of the research done for humans and how PBDEs and PCBs affect humans can also be generally applied to marine mammals as well (Dachs et al., 2002). Homologous genes mean genes that give rise to structures that are similar to other species, for example, finger bones in humans to pectoral fin bones in cetaceans. For this research, general comparisons are made based on similar protein structures of dolphins and SRKWs.

Results

SRKWs encounter many unfavorable conditions during their day to day lives (noise, boat traffic, declines in food stocks) on the population of these whales. This thesis focuses only on the impact of toxic PCBs and PBDEs on the whale population.

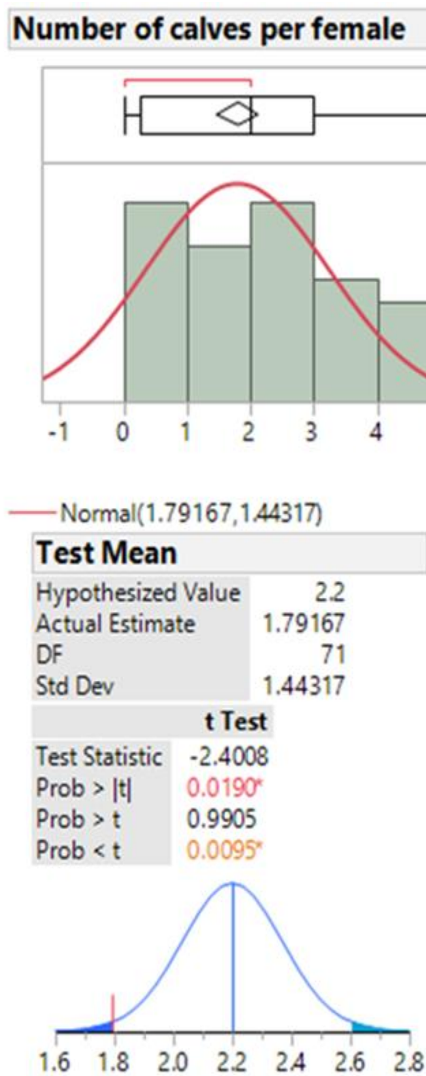

Figure 5. The number of calves per female. Matriline data from Taylor & Plater, & 2001[1] and the Whale Museum Friday harbor, WA.

[1] Taylor, M. F. J., & Plater, B. (2001). Population viability analysis for the southern resident population of the killer whale (*Orcinus orca*).

The histogram in Figure 5 illustrates the total number of calves in J, K, and L pod females. All births were counted: stillborn calves, calves that died soon after birth, calves that died years later, and those that are still alive were counted as a birth. Each calf represents a potential decrease of PCB and PBDE toxicity levels in the mature female whale’s body as those toxins transfer to the newborn. The calculated value of 2.2 in the t-test is the mean rate of fecundity of the Northern Resident Killer Whales in British Columbia, Canada (Mongillo et al., 2016). This population of killer whales has a positive annual fecundity rate of 2.2 calves per year. The SRKWs need a rate of fecundity of at least 2.2 to strengthen the population and increase recruitment. The data reveals that the SRKWs are reproductive rates are under the hypothesized 2.2 calves per year to increase their population numbers. At 1.8 calves per year for the SRKWs, and the calves that are born die before sexual maturity, fecundity and recruitment will continue to decline (McHugh et al., 2007).

Figure 6 on the next page shows the total PBDEs and PCBs in the SRKW population. The data includes both males and females. The hypothesized value of 9 mg/kg lipid weight was the Ross threshold for PCB toxicity. The Ross threshold was used for PBDEs as well because there is no known threshold for PBDEs at this time. From the 2 sample t-test of the PBDEs, the SRKWs have 16 mg/kg lipid weight of PBDEs in their blubber layer, far above the 9 mg/kg.

PCBs have far exceeded the Ross threshold with 23 mg/kg lipid weight in the SRKW blubber. With toxin levels as shown here, females will struggle to produce calves and males are unlikely to have enough viable sperm fertilize ova. The outliers in the histogram are all male SRKWs that have no way to offload their toxicity levels.

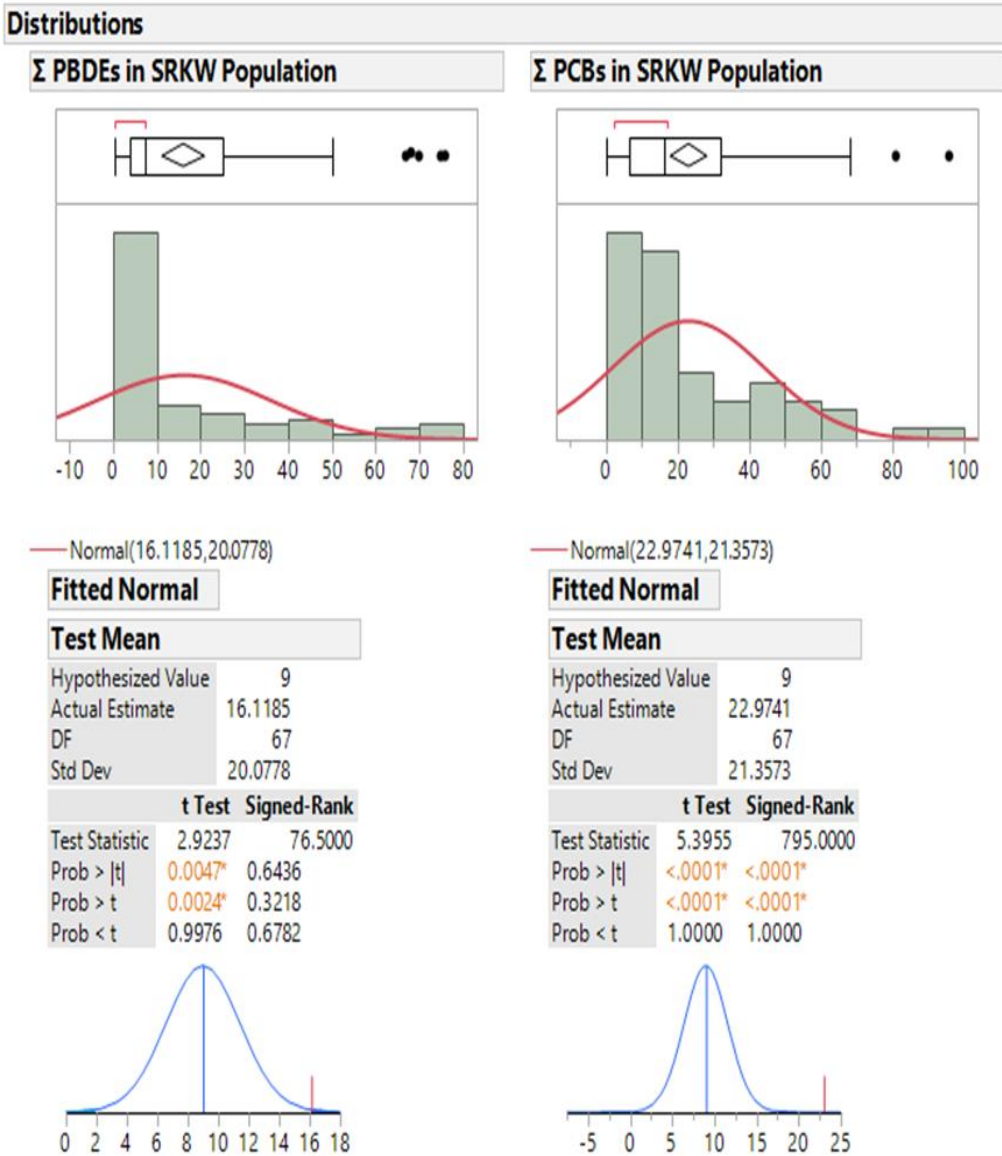

Figure 6. The PBDEs and PCBs in the SRKW population. Toxicity data from NOAA technical memorandum[2].

[2] NOAA Technical Memorandum NMFS-NWFSC. https://doi.org/10.7289/v5/tm-nwfsc-135

Figure 7 below is a fit curve that illustrates that as the level of PCBs (in mg/kg lipid weight) goes up, the number of calves a female has goes down. Also, the R^2 value indicates that there is a strong correlation between PCBs and the lack of calves seen in the Salish Sea.

Fit Curve

Model Comparison

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Logistic 3P	30.016581	38.259691	5.4524597	0.083884	0.2896273	0.9588904

Plot

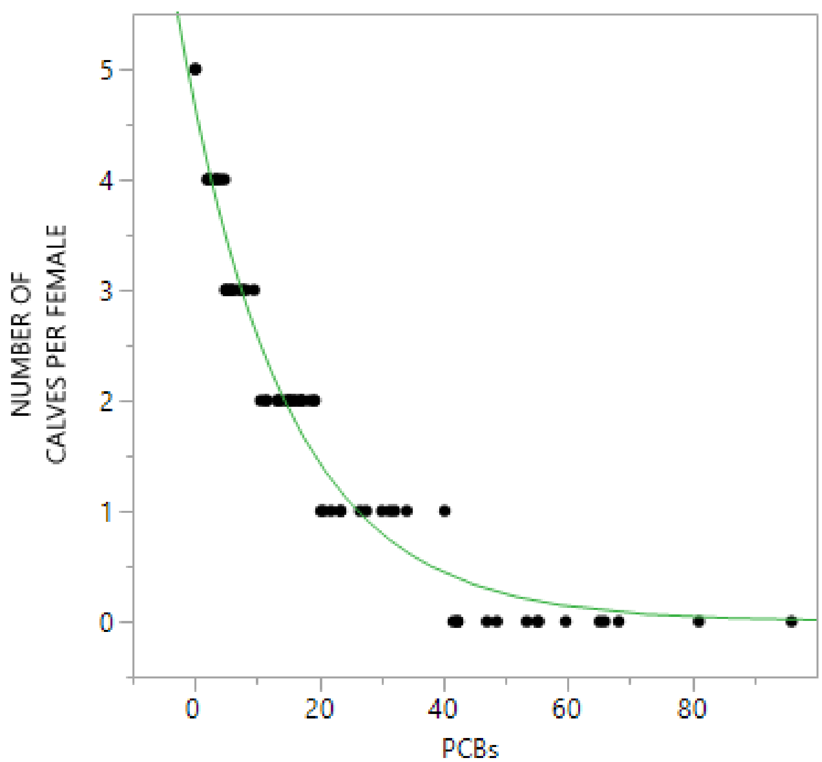

Figure 7. Fit curve comparison of total calves per female of the SRKW population. Toxicity level data is from NOAA technical memorandum[2] and the Recovery Strategy from Fisheries and Oceans Canada[3].

[3] Fisheries and Oceans Canada. (2018). Recovery Strategy for the Northern and Southern Resident Killer Whales (*Orcinus Orca*) in Canada.

As shown in Figure 7, of whales within the population have PCB concentrations above the Ross threshold of 9 mg/kg lipid weight (the concentration at which adverse health effects begin to rake their toll in both sexually immature and sexually mature animals). Figure 6 displays the relationship between SRKW fecundity and (PCBs). The Ross threshold of 9 mg/kg lipid weight minimum of PCBs is seen here as the tipping point at which there is a steep decline in calves per female. At 20 mg/kg lipid weight, there is a complete drop off of calves per female.

The figure 8 fit curve model compares the amounts of PBDEs per mg/kg lipid weight to the number of calves a female has delivered. Take note of the R^2 value of 0.85, and this indicates a strong correlation between the higher amount of PBDEs that are in the SRKWs blubber the lower number of calves that are observed in the population.

Fit Curve

Model Comparison

Model	AICc	BIC	SSE	MSE	RMSE	R-Square
Logistic 3P	371.02424	379.26735	821.29749	12.635346	3.5546232	0.8512589

Plot

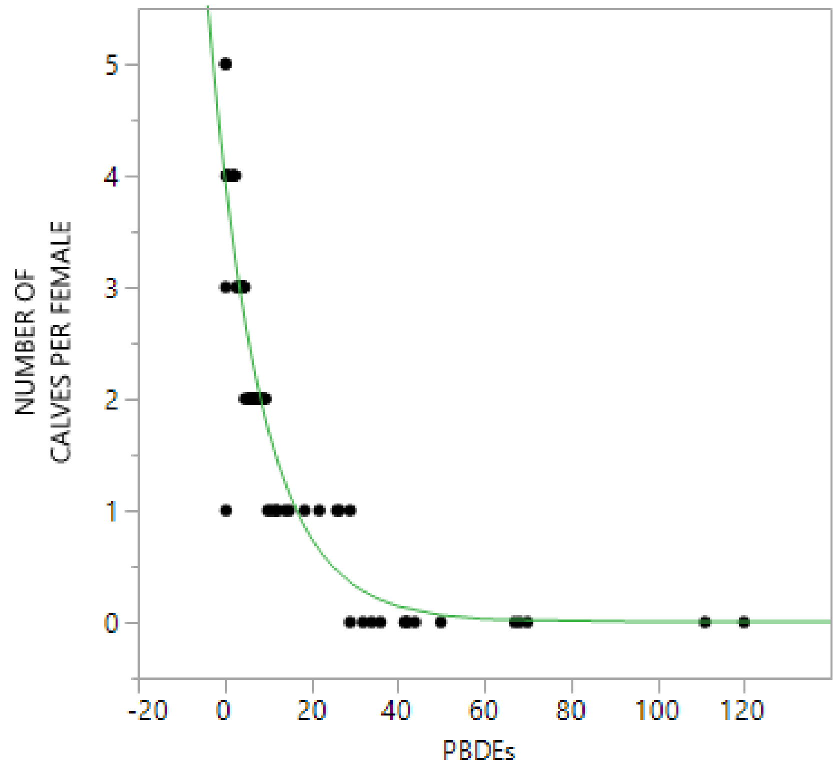

Figure 8. Fit curve comparison of the total number of calves per female with PBDEs as the variable in the SRKW population. Toxicity level data is from NOAA technical memorandum[2] and the Recovery Strategy from Fisheries and Oceans Canada[3].

As shown in figure 8, the number of calves that are observed has declined more sharply than PCBs at approximately 5 mg/kg lipid weight. PBDEs may impact SRKWs more acutely because of their continued production, use and transportation in the U.S. and around the world. The curves for both PCBs and PBDEs support the idea that high loads of these toxic chemicals interfere with the female whale's ability to reproduce (Rayne et al., 2004).

Figure 9 includes age as a variable. Even in females, age affects how high or how low the toxin levels could be. For example, a 47-year-old female whale that has had one calf can still have a higher rate of toxins as opposed to an older female that has had many more calves.

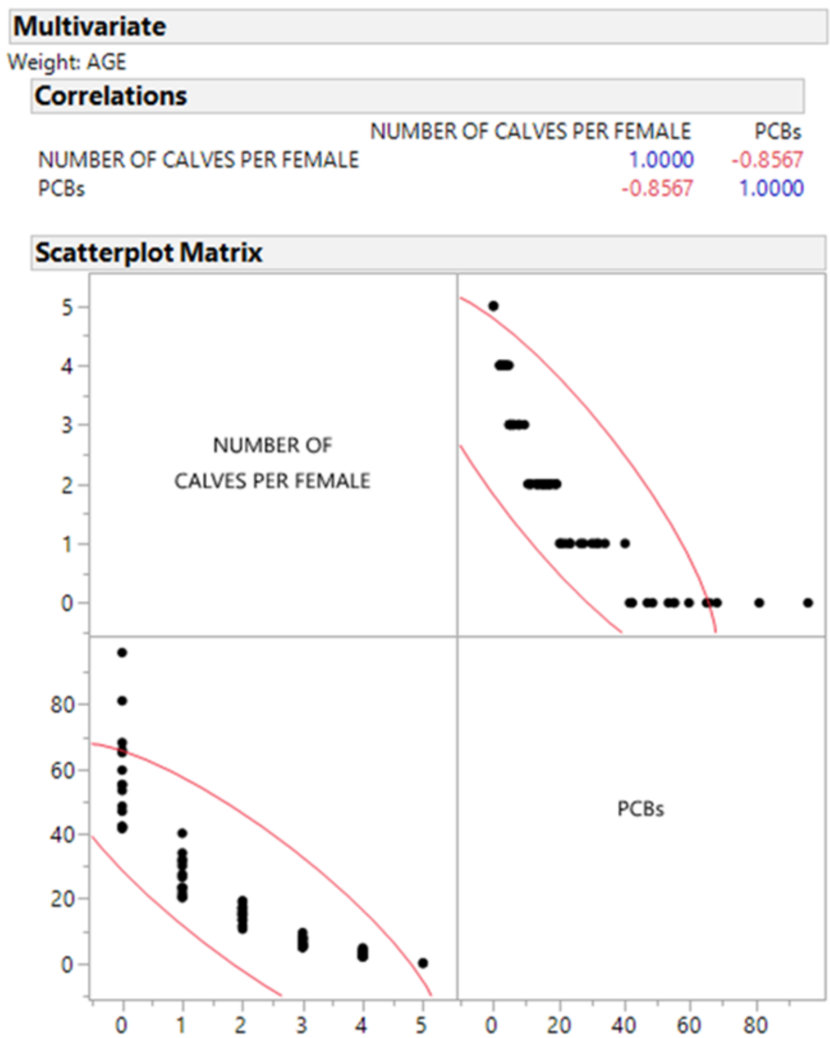

Figure 9. Multivariate Correlation between age, number of calves birthed and PCB levels of the females in J, K, and L pods in Puget Sound. Toxicity data is used with permission Toxicity level data is from NOAA technical memorandum[2] and the Recovery Strategy from Fisheries and Oceans Canada[3].

Figure 9 is a multivariate correlation that illustrates a strong correlation between the number of calves a female SRKW has given birth to and the amount of PCBs in her system, assigned to her based on her age and individual fecundity rate. The correlation rate is -0.85. This indicates a robust negative relationship: as the level of PCBs increases, the number of calves decreases.

Figure 10 on the next page illustrates another multivariate correlation. This multivariate correlation uses age as a third variable. Since age is a deciding factor on the levels of PBDEs, age is essential in understanding how PBDEs affect the SRKW community. The negative correlation rate of -0.74 indicates that higher PBDE levels are associated with a reduced number of calves per female SRKW.

Multivariate

Weight: AGE

Correlations

	Number of calves per female	PBDEs
Number of calves per female	1.0000	-0.7464
PBDEs	-0.7464	1.0000

Scatterplot Matrix

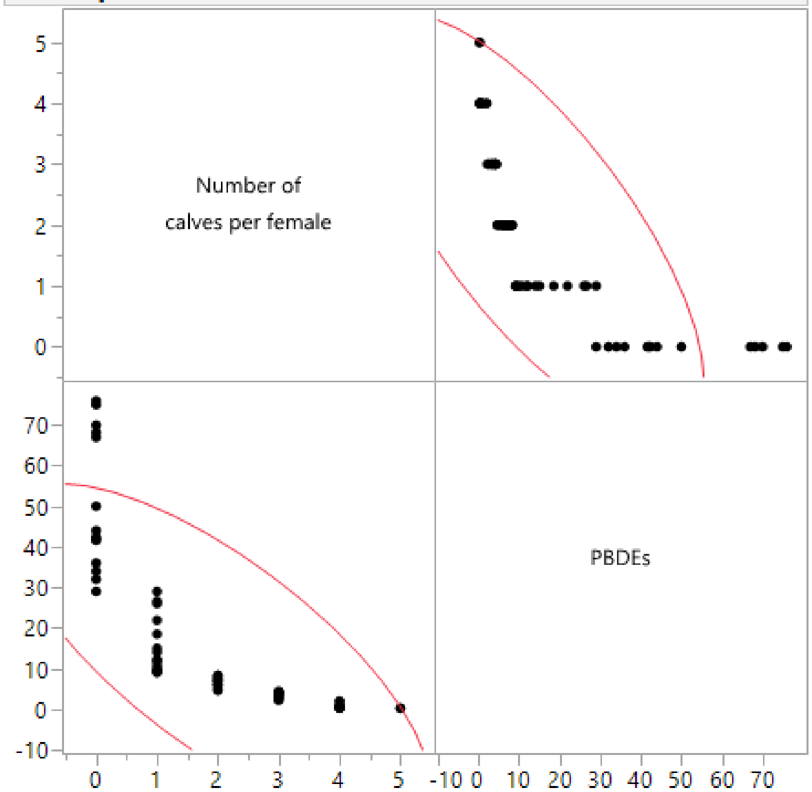

Figure 10. A multivariate correlation between PBDEs and the fecundity rates of the females of SRKWs. Toxicity level data is from NOAA technical memorandum[2] and the Recovery Strategy from Fisheries and Oceans Canada[3].

Figure 10 demonstrates that the SRKW females exhibit a lower pregnancy rate and longer calving interval than other conspecific populations, which could be resulting from PBDE mediated effects. Based on these data, I recommend that areas that may contain PCBs or PBDEs are identified and proper containment and disposal of PCBs and PBDEs are examined and issued.

Discussion

The purpose of this thesis was to examine if there was any correlation to the anthropogenic toxins PCBs and PBDEs. I have found that there is a strong correlation between these specific toxins and the fall of fecundity in recent years. The Ross threshold of 9 mg/kg lipid weight is consistent with SRKW data as the level where fecundity is declining. As PCBs and PBDEs increase, SRKWs decrease their calving and their recruitment. The decline in the number of calves per female is steeper for PBDEs than PCBs. This means that PBDEs could be impacting SRKW fecundity more than PCBs due to the contemporary and continued use of those toxins, or because they have a stronger biological effect.

The histogram of figure 5 shows how far behind the SRKWs are to the more productive Northern Resident Killer Whales (NRKWs) of Canadian waters. The NRKWs population fecundity rate was 2.2 calves per year, and the SRKWs population fecundity rate is only 1.8. The low fecundity rate has also been observed where three females were pregnant, but only one female produced a calf.

The two histograms contained in Figure 6 showed that there are very high toxin levels in the SRKW population in the Salish Sea. The outliers of those histograms came from male individuals that could not transfer their toxin loads by calving. Therefore for male SRKWs, their PCB and PBDE toxin levels continue to rise above safe levels. With toxin levels as high as seen in this thesis of above 80 mg/kg of lipid weight for PBDEs and above 100 mg/kg of lipid weight in PCBs, males are at risk of bromine poisoning (Tavares et al., 2016).

In the model fit curves (Figures 7 and 8), the drastic declines of the SRKWs females calving may be due to their blubber or lipid toxicity. PBDEs appear to have a more detrimental impact on female's ability to reproduce than the PCBs. Fecundity drops sharply at a PBDE concentration of only 5 mg/kg and at a PCB concentration of 9 mg/kg, which means that PBDEs could have a more detrimental impact on the SRKWs than PCBs.

The last two statistical figures 9 and 10 are multivariate correlations to determine how much of a relationship there is between the toxins and fecundity of the SRKWs. These suggest a high probability that PCBs and PBDEs are affecting SRKW females to produce less healthy calves. With the strong correlations of -0.85 for PCBs and -0.74 for PBDEs, there is much more to this story than lack of food for the SRKWs.

Conclusion and Recommendations

This thesis touches on only one of many anthropogenic environmental stressors that plague the SRKWs. Currently, the population is declining at a rapid rate. As of September of 2018, three female SRKWs were identified as pregnant: Deadhead (K27), Matia (L77), and Princess Angeline (J17). However, of all three confirmed pregnant, only one, Matia (L77), produced a viable calf later to be named Lucky (L124). These pregnancy failures are one indication of PCB and PBDE intoxication, where observations show 2 of 3 pregnancies fail in the SRKW pods, that statistics in this thesis support.

My thesis has reinforced the Ross threshold and that PCBs and PBDEs are detrimental to SRKW reproductive health. The high R^2 correlations of the statistics have further strengthened that PCBs and PBDEs are both contributing to the decline of SRKWs. The internal impacts of PCBs and PBDEs can cause damage to the reproductive organs and cognition of SRKWs. These problems, combined with other anthropogenic stressors, are causing the decline of the SRKWs.

As of May 2019, Princess Angeline (J17) and her four-year-old daughter Kiki (J53), both have been observed with emaciation and poor body condition. At the time of this writing, their survival is still precarious. A male SRKW named Scoter (K25) has been ailing in 2019. At 27 years old, this whale is in the prime of his life and should be contributing to the population. Three whales may die within the next six months with only one calf that has been born to the L pod, which statistically the calf will not survive past the first five years of life. Along with a scarce food supply, PCBs and PBDEs only exacerbate other challenges and compound the SRKWs decline.

Even though PCBs have not been manufactured in the U.S. since the 1970s, there are still areas that contain PCBs. February of 2019 an electrical transformer failed the Olympia Brewery and up to 677 gallons of PCBs into a nearby park and the banks of the Deschutes River. The hazardous clean up of this area could take years, and irreparable harm to the park and surrounding wildlife can occur. Furthermore, the PCBs in the transfer fluid could contaminate South Puget Sound downstream.

RECOMMENDATIONS

A recommendation to alleviate issues with storing or transporting PBDEs could be the continuous Grignard reaction (Dachs et al., 2002). This chemical reaction persists of heating and pressurizing PBDEs. Combining the toxin with magnesium (Mg) and converting PBDEs into more manageable chemical alcohols or hydrocarbons (Dachs et al., 2002). Furthermore, hydrocarbons can be further broken down by hydrocarbon consuming bacteria. Since PBDEs are still being produced and used, this may be a viable option for safe disposal for PBDEs. However, after PBDE toxins are released into the environment; before the Grignard reaction is applied, nothing can be done besides a toxic spill cleanup (Dachs et al., 2002).

The chlorine in PCBs molecules are very stable and would not be affected by the Grignard reaction. However, there is a way to destroy PCBs. The PCBs need to be identified and taken to a facility that is equipped to handle these toxins. Next, the PCBs need to be heated to 649°C (1,200°F) in an enriched oxygen mixture, unfortunately, if this process is not done correctly, the PCBs breakdown into dioxins (Dachs et al., 2002). Dioxins are more hazardous than PCBs. Furthermore, this is an expensive process, and very few facilities are equipped for such high temperatures. However, something needs to be done quickly.

The SRKWs are on the cusp of extinction. All stakeholders need to come together and make a definitive ban regarding these chemicals. If humans continue to conduct 'business as usual' legislative laws and polluting, The SRKWs will decline until there are none. Researchers have already seen the outcome of other Resident Killer whales near the Coast of England and Scotland. There are eight left, of those eight, none are females. Time is running out for the SRKWs of the Salish Sea. The famed oceanographer, Dr. Sylvia Earle said it best: "We need to respect the oceans and take care of them like our lives depend on it. Because they do."

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Appendix

Current Southern Resident Killer Whales that inhabit the Salish Sea. Age is in years unless otherwise noted.

J Pod	Sex/Age
J16 (Slick)	F 47
J17 (Princess Angeline)	F 42
J19 (Shachi)	F 40
J22 (Oreo)	F 34
J26 (Mike)	M 28
J27 (Blackberry)	M 28
J 31 (Tsuchi)	F 24
J35 (Tahlequah)	F 21
J36 (Alki)	F 20
J 37 (Hy'Shqa)	F 18
J 38 (Cookie)	M 16
J39 (Mako)	M 16
J40 (Suttles)	F 15
J 41 (Eclipse)	F 14
J 42 (Echo)	F 12
J 44 (Moby)	M 10
J45 (Se-Yi'-Chn)	M 10
J 46 (Star)	F 10
J47 (Notch)	M 9
J49 (T'ilem I'nges)	M 7
J51 (Nova)	M 4
J53 (Kiki)	F 4

K Pod	Sex/Age
K12 (Sequim)	F 49
K14 (Lea)	F 42
K16 (Opus)	F 34
K20 (Spock)	F 33
K21 (Cappuccino)	M 33
K22 (Sekiu)	F 32
K25 (Scoter)	M 28
K26 (Lobo)	M 26
K27 (Deadhead)	F 25
K33 (Tika)	F 18
K34 (Cali)	M 18
K35 (Sonata)	M 17
K36 (Yoda)	F 16
K 37 (Rainshadow)	M 15
K38 (Comet)	M 15
K42 (Kelp)	M 11
K43 (Saturna)	F 9
K44 (Ripple)	M 8

L Pod	Sex/Age
L22 (Spirit)	F 48
L25 (Ocean Sun)	F 91
L41 (Mega)	M 42
L47 (Marina)	F 45
L54 (Ino)	F 42
L55 (Nugget)	F 42
L72 (Racer)	F 33
L77 (Matia)	F 32
L82 (Kasatka)	F 29
L83 (Moonlight)	F 29
L84 (Nyssa)	M 29
L85 (Mystery)	M 28
L86 (Surprise!)	F 28
L87 (Onyx)	M 27
L88 (Wave Walker)	F 26
L89 (Solstice)	M 26
L90 (Ballena)	F 26
L91 (Muncher)	F 24
L94 (Calypso)	F 24
L103 (Lapis)	F 16
L105 (Fluke)	M 15
L106 (Pooka)	M 14
L108 (Coho)	M 13
L109 (Takoda)	M 12
L110 (Midnight)	M 12
L113 (Cousteu)	F 10
L115 (Mystic)	M 9
L116 (Finn)	M 9
L117 (Keta)	M 9
L118 (Jade)	F 8
L119 (Joy)	F 7
L121 (Windsong)	M 4
L122 (Magic)	M 4
L123 (Lazuli)	M 4
L124 (Lucky)	*5 months
	* Sex Unknown