

**EVALUATING SKIN DISEASE IN COMMON BOTTLENOSE DOLPHINS NEAR
BRUNSWICK AND SAPELO ISLAND, GEORGIA**

A thesis submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

in

ENVIRONMENTAL STUDIES

by

**CHELSEA ELIZABETH ACRES
APRIL 2018**

at

**THE GRADUATE SCHOOL OF THE UNIVERSITY OF CHARLESTON, SOUTH
CAROLINA AT THE COLLEGE OF CHARLESTON**

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ABSTRACT

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Common bottlenose dolphin (*Tursiops truncatus*) frequenting the areas of Brunswick and Sapelo Island, Georgia have been monitored via photo-ID, remote biopsy, radio tracking and health assessments since 2004 and have been found to contain high levels of polychlorinated biphenyls (PCBs), with a unique pattern of congeners associated with the PCB mixture Aroclor 1268. Skin lesions have been previously documented in these dolphin populations, but no long-term skin lesion monitoring has taken place. This study examines differences in lesion prevalence between sites, monthly and yearly patterns at both sites, differences in prevalence of major lesion types, correlation with environmental variables (salinity, water temperature, air temperature, and precipitation), correlation with blubber concentration of Aroclor 1268, correlation between lesions associated with the same potential causative agents, and potential spatial patterns. Results of this study show evidence for consistent prevalence and seasonal trends between BRN and SAP, with both exhibiting higher lesion prevalence in spring months, as well as significant changes in variation across years. Monthly mean max precipitation and salinity were both significantly correlated with the presence of skin lesions. Dolphins' blubber concentration of Aroclor 1268 was not found to significantly correlate with lesion presence in either male or female animals. Finally, current data do not support spatial clustering of dolphins with lesions.

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CHAPTER 1: INTRODUCTION






Common bottlenose dolphins (*Tursiops truncatus*) are a useful sentinel species for coastal and ocean ecosystem health, as they are long-lived, top-level predators who are often long-term residents of bays, sounds, and estuaries and are exposed to a variety of stressors, including chemical, environmental, and infectious (Wells et al. 2004). Marine mammals can be exposed to chemical stressors via diet; bottlenose dolphin exposure to a variety of chemical stressors, including persistent organohalogen contaminants (polychlorinated biphenyls (PCBs), chlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT), polybrominated diphenyl ethers (PBDEs), perfluoroalkyl phosphinic acids (PFPIAs), perfluoroalkyl acids (PFAAs), mercury, perfluorinated chemicals (PFCs), and polyaromatic hydrocarbons (PAHs) has been reported (Houde et al. 2005, Fair et al. 2009, Fair et al. 2010, Kucklick et al. 2011, Driscoll et al. 2013, Adams et al. 2014, De Silva et al. 2016, Reif et al. 2017). Changes in salinity and water temperature can also be a stressor for bottlenose dolphins (Hart et al. 2012). Additionally, dolphins exposed to these chemical stressors are more vulnerable to disease-causing pathogens (Schwacke et al. 2012). These pathogens include but are not limited to: cetacean morbillivirus, dolphin papillomavirus, herpesviruses, poxviruses, Methicillin-resistant *Staphylococcus aureus*, *Lacazia loboi*, and *Brucella* sp. (Bossart et al. 2017, Reif et al. 2017).






Some stressors can be directly evaluated by sampling the environment, or from the blubber of a remote-biopsied free-ranging animal, but the cumulative effects of these stressors on animal health is more difficult to assess. Currently, health status can be assessed by capture-release health assessments of free-ranging animals, or necropsy of stranded carcasses (e.g., Hart et al. 2012, Schwacke et al. 2012). During health assessments dolphins are examined on board a vessel where whole blood and surgical biopsies of skin and blubber, and a single tooth is taken for analysis (Schwacke et al. 2012). While these health assessments are informative, it is difficult

to obtain long-term data on individuals to evaluate how their health status might change in relationship to encountered stressors. Visual assessment of individual animals could be an important tool to quantify potential effects of these stressors, and one of the parameters evaluated in these visual assessments is skin disease.

1.1 Skin Disease in Marine Mammals

Skin lesions are widespread in cetaceans globally and prevalences of 0.38-1.0 have been reported for bottlenose dolphin populations worldwide (Harzen 1997, Wilson 1997, Wilson 1999, Bearzi 2009, Maldini 2010, Rowe 2010). Skin lesions have been linked to fungal, bacterial and viral infections, diatom growth, and parasites (reviewed in Wilson 1997). Skin disease in bottlenose dolphins has also been shown to be linked with environmental factors such as temperature and salinity, and anthropogenic factors such as pollution and chemical contaminants (Wilson 1999, Van Bresseem 2007, Bearzi 2009, Hart 2012). It has been suggested that skin disease might be a useful general health indicator for cetaceans (Bearzi 2009, Van Bresseem 2009). Past studies have classified various lesions as black, pale, lunar, dark-fringed spots, white-fringed spots, orange patch, tattoo-like, white velvety, lacaziosis-like, vesicular, mottled, and spotted (Wilson 1997, Van Bresseem 2007, Bearzi 2009, Maldini 2010, Hart 2012). These different variations may indicate different diseases (e.g. poxvirus, *Lacazia loboi* fungal infection), water quality issues, or different stages of a disease (Wilson 1997, Van Bresseem 2007, Bearzi 2009, Maldini 2010, Hart 2012). Table 1 summarizes each lesion type by name, image, description with reference, and potential causative agents.

Lesion Type	Image	Description <i>Reference</i>	Potential Causative Agent
Black		Uniform in color; circular or irregular; rounded edges; smooth surface; may be slightly depressed <i>Wilson et al. 1997</i>	Herpesviruses <i>Van Bressem et al 1999</i>
Dark-Fringed		Pale center with dark border; circular shape <i>Wilson et al. 1997</i>	Poxviruses <i>Maldini et al. 2010</i>
Lacaziosis-like		White/pink in color; verrucous; raised; may be ulcerated; can be localized or widespread <i>Van Bressem et al. 2009</i>	<i>Lacazia loboi</i> <i>Van Bressem et al. 2007</i>
Lunar		Black, blue-gray, or white in color; uneven surface, raised and depressed; usually large in size <i>Wilson et al. 1997</i>	Unknown
Mottled		Scattered flecks of white, pale gray, and dark gray; irregularly shaped; typically located laterally <i>Burdett Hart 2011</i>	Unknown

Orange Patch		Rusty/orange in color; distinct patches <i>Wilson et al. 1997</i>	Diatoms <i>Holmes et al. 1993</i>
Pale		White or cream in color; circular or irregular in shape; rounded or diffuse edges; may have irregular margins; smooth/flat surface <i>Wilson et al. 1997</i>	Healing from a prior trauma, ectoparasite attachment, previous viral infection, or inflammation <i>Hart et al. 2012</i>
Spotted		Paler in color than surrounding skin without dark border; circular shape; may be localized or widespread <i>Burdett Hart 2011</i>	Poxvirus <i>Hart et al. 2012</i>
Tattoo-like		Light or dark gray, black, or yellow in color; irregular or rounded shape; stippled pattern <i>Van Bresseem et al. 2009</i>	Poxvirus <i>Geraci et al. 1979</i>
Velvety		White in color; velvety appearance <i>Van Bresseem et al. 2009</i>	Unknown



Vesicular		Raised/blister-like; may be ulcerated <i>Van Bresse et al.</i> 2009	Calicivirus <i>Smith et al. 1983</i> <i>Vibrio sp.</i> <i>Schroeder et al. 1985</i>
White-Fringed		Cream/white halos surrounding dark or normally pigmented skin <i>Wilson et al. 1997</i>	Herpesvirus <i>Hart et al. 2012</i>

Table 1. Skin Lesion Summary

Several potential causative agents for skin lesions in bottlenose dolphins have been reported: *Lacazia loboi* (Migaki et al. 1971, Van Bresse et al. 2007), *Vibrio* spp. (Schroeder et al. 1985), caliciviruses (Smith et al. 1983), poxviruses (Geraci et al. 1979, Hart et al. 2012), and herpesviruses (Barr et al. 1989, Van Bresse et al. 1999, Hart et al. 2012). Infection with *Lacazia loboi*, termed lacaziosis, presents as white and/or pink in color, verrucous, raised, potentially ulcerated, and can be localized or widespread (See Table 1) (Van Bresse et al. 2009). Lacaziosis is a relatively well-documented disease that has been found only in humans, bottlenose dolphins, Guiana dolphins, and Indo-Pacific dolphins (Van Bresse et al. 2008, Reif et al. 2013). It has a relatively low prevalence among dolphin populations, and is a chronic, fungal skin disease caused by the organisms *Lacazia loboi* (Migaki et al. 1971). This disease has a long incubation and progresses slowly. Past studies have demonstrated a link between lacaziosis and impaired function of the adaptive immune system (Reif et al. 2009). The study conducted in Reif et al. (2009) showed that dolphins affected by lacaziosis presented with multiple immunological abnormalities when compared with healthy dolphins, including a depressed levels of T cells, helper T cells, B cells, circulating lymphocytes and serum albumin concentration.

Vibrio is a genus of gram-negative bacteria that are present throughout the marine environment; some can be pathogenic. Because the skin of dolphins is absent of ducts or glands, *Vibrio* spp. typically only colonize via a secondary invasion where the skin has already been broken (Van Bressem et al. 2008). *Vibrio* spp. are typically associated with vesicular skin lesions, which are raised and/or blister-like and may be ulcerated (Table 1) (Van Bressem et al. 2009).

Caliciviruses have been observed in a wide variety of terrestrial mammals, humans, and dolphins (Smith et al. 1983, Barry et al 2008). Of those demonstrated in cetaceans, most are serotypes of the San Miguel sea lion virus (SMSV) the exanthema of swine virus (VESV), and cetacean calicivirus (CCV Tur-1), which was isolated from vesicular skin lesions (reviewed in Van Bressem et al. 1999). Caliciviruses have been previously shown to be transmitted between species of cetaceans by interspecies contact, migrations routes, disease vectors such as metazoan parasites (reviewed in Van Bressem et al. 1999).

Poxvirus has been documented in a wide variety of terrestrial mammals, cetaceans and pinnipeds. Previous studies have suggested that poxvirus is associated with impaired immune function and that higher prevalence and severity of this disease is highest in contaminated marine environments, which could make it suitable as a general health indicator in cetaceans (Van Bressem et al. 2008, Van Bressem et al. 2009). Low disease prevalence in neonates and older animals suggests that immunity to poxvirus may be passed on or acquired, and the disease may resolve on its own over time (Van Bressem et al. 2008). Poxvirus has been associated with the dark-fringed, spotted, and tattoo-like skin lesion types (Table 1). Dark-fringed lesions are circular in shape with a pale center and dark border (Wilson et al. 1997). Spotted lesions are paler in color than surrounding skin without a dark border, are circular in shape, and may be localized or widespread (Burdett Hart 2011). Tattoo-like lesions are light or dark gray, black, or yellow in color, irregular or rounded in shape, and exhibit a stippled pattern (Van Bressem et al. 2009).

Herpesviruses have also been documented in beluga whales (*Delphinapterus leucas*), striped dolphins (*Stenella coeruleoalba*), harbor porpoises (*Phocoena phocoena*), and dusky dolphins (*Lagenorhynchus obscurus*) in addition to bottlenose dolphins (Barr et al. 1989). Herpesvirus is associated with black and white-fringed lesions (Table 1). Black lesions are typically uniform in color, may be circular or irregular in shape with rounded edges, a smooth surface, and may be slightly depressed (Wilson et al. 1997). White-fringed lesions are cream or white halos that surround dark or normally pigmented skin (Wilson et al. 1997).

In addition to disease caused by the pathogens described above, diatom films may occur when microalgae attach to or penetrate the skin (Nemoto et al. 1977). It is hypothesized that diatom films do not have a significant impact on health. However, a recent study of Antarctic killer whales (*Orcinus orca*) identified that animals may have maintenance migrations that can cover over 9,400 km to slough diatoms from their skin and allow for skin regeneration (Durban and Pitman 2012).

Besides these specific causative agents, ectoparasite attachment, inflammation, and healing associated with physical trauma are also potential causes of skin disease in bottlenose dolphins (Hart et al. 2012).

1.2 Photographic-identification Analysis

Photographic-identification analysis (hereafter referred to as photo-ID) is a useful methodology for studying populations of bottlenose dolphins. Photo-ID of dolphins' dorsal fins permits identification of individuals that can be monitored over time (Wursig 1990, Melancon 2011). The dorsal fin shape is the product of a combination of many different attributes: a chopped fin (usually caused by fishery or boat interactions), notches in the upper, middle, and lower portions of the trailing edge, notches in the leading edge, notches in the apex of the fin, scars/notches in the peduncle, bend, scars, freeze brand, holes, and skin disorder (Melancon

2011). Photo-ID has been used to determine stock structure (e.g. Defran et al. 2015, Grellier et al. 2003), site fidelity (e.g. Balmer et al. 2008, de Boer et al. 2013), movement patterns (e.g. Baird et al. 2005, Labach et al. 2015), population size (e.g. Read et al. 2003, Speakman et al. 2010), reproductive patterns e.g. (e.g. Lane et al. 2015, McFee et al. 2014), and prey habits (e.g. Hanson et al. 2010, Morton 2000) in various marine mammal species. Recently, photo-ID analyses have expanded to not only focus on individual identification but also to use these data to provide insight into animal health. For example, Van Bressemer et al. (2007) utilized photo-ID methods to describe and evaluate a range of skin diseases, deformations and traumata in free-ranging dolphins with comparison to skeletal materials and dead specimens. Photo-ID has also been used to study how skin lesions might change over time in a single individual animal, as shown in the two case studies conducted by Riggins and Maldini (2010). Hart et al. (2012) also utilized photo-ID techniques to investigate skin lesions and compared lesion prevalence across three regions in the southeastern US. Although all of these photo-ID studies provided an assessment of skin disease for marine mammals, a limitation of this methodology is that photos are limited to the dorsolateral surface. Because skin lesions could be present elsewhere on the body, photo-ID may not provide a comprehensive assessment of skin lesion prevalence on an individual animal.

1.3 Western North Atlantic Common Bottlenose Dolphin Stock Structure

Research concerning stock structure of the western north Atlantic common bottlenose dolphin began following the 1987-1989 unusual mortality event (Geraci 1989). Currently, two major stock types are defined as being morphologically and genetically distinct: coastal and offshore (Hersh and Duffield 1989, Mead and Potter 1995, Curry and Smith 1997, Hayes et al. 2017, Rosel et al. 2009). These two morphotypes have been previously differentiated based upon mitochondrial and nuclear markers (Hoelzel et al. 1998, Rosel et al. 2009). However, the coastal morphotype can further be differentiated into two groups: bay, sound, and estuary stocks (BSE)

or nearshore coastal (Waring et al. 2015). Photo-id and genetic analyses conducted both in the western North Atlantic and Gulf of Mexico provide evidence to support the differentiation between resident estuarine animals and coastal animals (Wells et al 1987, Caldwell 2001, Gubbins 2002, Zolman 2002, Gubbins et al. 2003, Balmer et al 2008, Mazzoil et al. 2015, Litz et al. 2012).

This study's extent primarily covered two BSE stocks, as well as two coastal stocks the Central Georgia Estuarine System Stock (CGES; shown in Figure 1) South Georgia Estuarine System Stock (SGES; shown in Figure 2), the South Carolina/Georgia Coastal Stock, and the Southern Migratory Coastal Stock (Waring et al. 2015). The CGES extends from Ossabaw Sound in the north to Altamaha Sound in the south, and encompasses Sapelo Island and Sapelo Sound, as well as the Sapelo Island National Estuarine Research Reserve (Hayes et al. 2017). The most recent estimated average population abundance is 192, and the minimum population estimate is 185 (Hayes et al. 2017). Much of the coastline in central Georgia is privately owned, so the waters here have remained relatively unspoiled (Hayes et al. 2017). The SGES (Figure 2) extends from Altamaha Sound in the north to Cumberland Sound in the south and encompasses the Turtle/Brunswick River Estuary (TBRE) (Hayes et al. 2017). The most recent estimated average population abundance is 194, and the minimum population estimate is 184 (Hayes et al. 2017). The spatial extent and seasonal movements of the South Carolina/Georgia Coastal Stock are poorly understood, but it generally thought to extend from the North Carolina/South Carolina border to the Georgia/Florida border with no obvious borders and most animals residing in waters around 10m deep (Waring et al. 2017). The best population estimate for this stock is approximately 4,377 (Hayes et al. 2017). Spatial distribution and migratory patterns for the Southern Migratory Coastal Stock are even less well understood, but generally extends from New Jersey to North Carolina in summer months and moves as far south as Florida during winter

months (Waring et al. 2015). The best population estimate for this stock is approximately 9,173 (Hayes et al. 2017).



Figure 1. Geographic extent of the CGES stock. Waring et al. (2015).

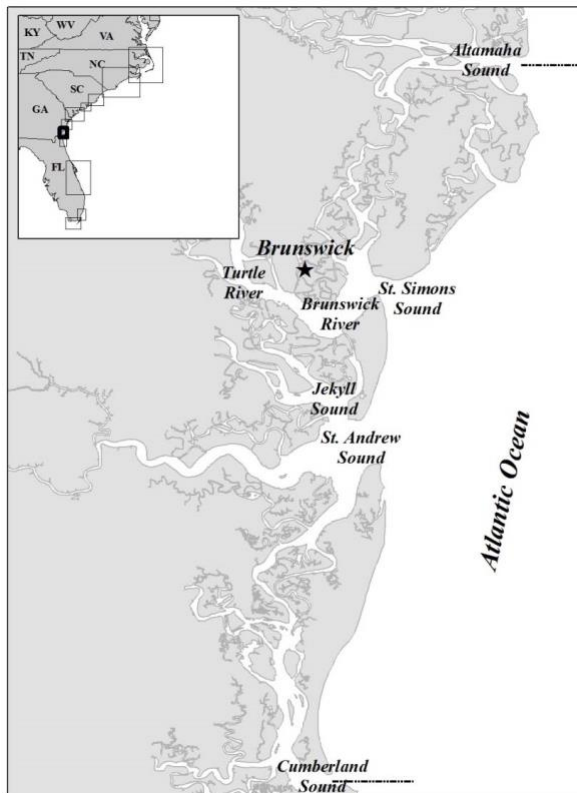


Figure 2. Geographic extent of the SGES stock. Waring et al. (2015).

Nearshore estuarine populations may have higher exposure to some anthropogenic stressors, and are very susceptible to persistent chemical pollution, acoustic pollution, and marine debris (Bearzi 2009). Since 2004, the National Oceanographic and Atmospheric Administration (NOAA) the Georgia Department of Natural Resources, and other non-governmental have been collaborating on photo-ID, remote biopsy sampling, and health assessment projects to better understand the dolphins located in the coastal and estuarine waters of central and southern Georgia (Balmer et al. 2011, 2013, 2014; Schwacke et al. 2012).

1.4 Dolphin Photo-ID Studies in Sapelo National Estuarine Research Reserve and near Brunswick, Georgia

The TBRE is home to four heavily contaminated hazardous waste sites that have been designated as National Priority List (NPL) Superfund sites by the U.S. Environmental Protection Agency, the most prominent being the LCP Chemicals site which was placed on the NPL in 1996 after closing operations in 1994 (EPA 2007). Studies published in 1997 and 1998 by Kannan et al. demonstrated extensive contamination of a specific mixture of polychlorinated biphenyl (PCB) congeners known as Aroclor 1268 in the soil and marsh sediments at the LCP Chemicals site, as well as in various species of crab, fish, terrapin and birds collected from the area. Aroclor 1268 is made up of IUPAC PCB numbers 174, 180, 183, 187, 194, 196, 199, 200, 201, 202, 206, 207, 208, and 209 (Maruya and Lee 1998). As PCBs are known to bioaccumulate and biomagnify, subsequent studies investigated higher trophic levels. Pulster and Maruya (2008) and Pulster (2009) found high levels of Aroclor 1268 contamination in resident estuarine dolphins, suggesting they were excellent sentinels for assessing coastal ecosystem health. A subsequent study by Kucklick et al. (2011) further supported bottlenose dolphins of the TBRE as indicators of persistent organic pollutants and of Aroclor 1268 specifically. Balmer et al. (2011) found that male dolphins found in the TBRE had some of the highest levels of PCB contamination of

cetaceans worldwide, despite feeding at lower trophic levels than other highly contaminated animals such as killer whales. This study also found that individuals sighted closer to the LCP Chemicals site had higher Aroclor 1268 proportions, and that Aroclor 1268 may not be offloaded from females via parturition and lactation as easily as other persistent organic pollutants (Balmer et al. 2011). Health assessments conducted in the TBRE area by Schwacke et al. (2012) showed that contaminated animals exhibited a high proportion of anemia, reduced thyroid hormone, total thyroxine, free thyroxine, triiodothyronine, T-lymphocyte proliferation levels, and that indices of innate immunity all negatively correlated with PCB concentration in blubber, all of which suggested a potential increased susceptibility to infectious disease. Distribution and sources of PCBs were examined in Wirth et al. (2014) and found that contamination was lower in Sapelo Island (SAP) than Brunswick (BRN), and that congener profiles were consistent across both sites with Aroclor 1268. This study also supported the idea that a likely source of Aroclor 1268 in these dolphins is contaminated prey fish (Wirth et al. 2012).

A study published in 2012 by Hart et al. investigated skin lesion prevalence and type in BRN and Sapelo Island (SAP) (BSG) in comparison to Sarasota Bay, FL (SSB) and Charleston, SC (CHS), examining the months of February, April, July, and October within a single year, 2009. Of the 322 animals observed in the BRN and SAP, 189 overall presented with lesions making the overall prevalence of lesions 0.587. This prevalence was significantly higher than both SSB (0.380) and CHS (0.487). The BSG region also exhibited higher disease prevalence in February (0.594) and April (0.691) than July (0.256) and October (0.296). Major lesion types were described as those with a prevalence greater than 0.1; major lesion types in BSG were black (0.201), dark-fringed (0.577), pale (0.212), tattoo (0.212), vesicular (0.302), and white-fringed (0.116) for all months. Additionally, Hart et al. (2012) found a correlation between colder water temperature and high lesion prevalence but suggested that it was not the only potential factor that

might influence skin lesion prevalence. Other suggestions included freshwater input from the Altamaha River and PCB contamination.

1.5 Objectives

This study builds on the previous work by Hart et al. (2012) adding analysis of fin images over a seven-year period to re-examine the prevalence, types, spatial distribution, and potential contributing factors to skin disease in bottlenose dolphins of BRN (SGES) and SAP (CGES), GA. The objectives of this study are to establish longer-term baseline information on skin disease within the SGES and CGES, examine correlation with environmental variables over a broader gradient of environmental conditions, and establish potential use of skin lesions as an indicator of PCB contamination. Specifically, this project wishes to examine the following hypotheses:

1. The overall prevalence of skin lesions differs between SGES and CGES
2. The prevalence of skin lesions differs by month
3. The prevalence of skin lesions differs by year
4. Prevalence of lesion types differs between SGES and CGES, and by year
5. Overall lesion prevalence is correlated with one or more of the following environmental variables: air temperature, water temperature, salinity, and precipitation
6. The prevalence of lesion types associated with herpesvirus (black and white-fringed lesions) and poxvirus (dark-fringed, tattoo-like, and spotted lesions) are correlated.
7. The presence of skin lesions is correlated with the concentration of Aroclor 1268 PCBs in blubber
8. Exploratory: Are there any spatial trends in lesion animals?

CHAPTER 2: METHODS

2.1 Surveys

This project utilized data previously collected from on-going photo-ID, biopsy, radio-tracking, and capture-release surveys conducted by NOAA, the Georgia Department of Natural Resources in Brunswick, GA, and other partners. Photo-ID surveys and analysis protocols used are described by Melancon et al. (2011).

Boat surveys were completed under NOAA photo-ID survey protocols for roughly ½ of the total area of both CGES and SGES stocks, which dictated that 1-2 small boats survey along specific track lines in the designated survey area (Melancon 2011). Each boat had one boat operator and generally two to three observers who were responsible for visually searching for the presence of dolphins in the 180-degree area centered around the bow of the boat. Once dolphins were sighted, observers attempted to capture high-quality photographs for both the left and right side of the dorsal fin for each animal present. A given sighting was considered completed once all individual dolphins had been photographed, the group was lost, or the dolphins were uncooperative with photo-ID efforts after 40 minutes had elapsed (Melancon 2011). Data sheets were completed for each sighting. Data sheets included information on date/time, location, number of dolphins sighted, behaviors, and other relevant information (Melancon 2011). In addition, a YSI water monitoring device was used at the sighting location to measure salinity and water temperature. FinBase Photo-ID Database system, a customized Microsoft Access database, was used to organize, store and analyze photo-ID survey data (Adams et al. 2006). Survey and sighting data were entered into FinBase from the datasheets, corresponding photos for each sighting were added, and the photos were matched to unique individuals with a unique catalog ID number (Melancon 2011). When initially cataloged, images in FinBase were scored on quality (good, average, and poor) and fin distinctiveness (high, average, low, and not distinct). Only images of good or average quality and fin distinctiveness of high or average were used for

analysis. Images with poor quality or fin distinctiveness of low or not distinct were excluded from analysis.

Data exist for 340 surveys from 2004-2016, but only a subset of these data were selected for this study. Data from surveys completed 2007-2014 in the months of March, April, August, and September were chosen to ensure an even coverage of data and for feasibility of lesion classification. The survey data and photographs were managed and analyzed using Darwin and FinBase software applications.

2.2 Lesion classification

Within each sighting, the highest quality image of each individual, based upon image resolution, lighting, and overall skin exposure, was selected to evaluate the presence of skin lesions. Lesion types were classified based on parameters discussed in the introduction and description in the literature into the following categories: black, pale, lunar, dark-fringed spots, white-fringed spots, orange patch, tattoo-like, white velvety, lacaziosis-like, vesicular, mottled, spotted (Bearzi 2009, Hart 2012, Maldini 2010, Wilson 1997). (See Table 1.). The presence (“1”) or absence (“0”) of skin lesions was recorded for each dolphin within each sighting; this was done for each individual lesion type as well as for the presence or absence of any skin lesion. Lesion prevalence was calculated as the proportion of animals that presented with a lesion; prevalence was calculated for the presence of any skin lesions overall and by each individual lesion type. Prevalence was then compared with results from Hart et al. 2012 to test to consistency and accuracy in lesion classification. Tables 2 and 3 show the number of overall, yearly, and monthly number of observations in each study site.

	Overall	2007	2008	2009	2010	2011	2012	2013	2014
<i>All Months</i>	1130	198	171	110	54	140	160	159	138
<i>March</i>	326	198					27	52	49
<i>April</i>	502		60	83	54	110	96	40	59
<i>August</i>	129		111	18					
<i>September</i>	173			9		30	37	67	30

Table 2. Number of observations of individual animals stratified by year and month (BRN)

	Overall	2007	2008	2009	2010	2011	2012	2013	2014
<i>All Months</i>	1206	225	561	324	96				
<i>March</i>	139		111	28					
<i>April</i>	327		134	97	96				
<i>August</i>	634	225	316	93					
<i>September</i>	106			106					

Table 3. Number of observations of individual animals stratified by year and month (SAP)

2.3 Other Data Collection

To determine if contaminant levels correlated with skin lesion prevalence, data on PCB concentrations in blubber of dolphins sighted in 2007-2008 were obtained from Balmer et al. 2011. In that study, biopsy samples were collected via remote biopsy sampling survey. The remote biopsy sampling survey used a “0.3m long carbon fiber dart with a 25 mm stainless steel cutterhead, propelled from a 0.22 blank charge dart from a modified 0.22 caliber rifle...equipped with a holosight to improve sampling accuracy” approximately 2-6m from a vessel in combination with digital video and still camera observance to ensure accuracy of animal ID (Balmer et al. 2011). Samples were typically taken from the animal’s flank area below the caudal insertion of the dorsal fin, using a biopsy dart which was 10 mm in diameter. Blubber samples typically weighted 0.5-1.0g, not including the superficial epidermis layer which was removed (Balmer et al. 2011). Molecular methods were used on the epidermis layer to identify the sex of the animal (Balmer et al. 2011). Gas chromatography was utilized to determine PCB concentration for each sample (Balmer et al. 2011). These samples were analyzed for a suite of persistent contaminants, including 54 PCB congeners, but only the 12 PCB congeners comprising the mixture of Aroclor 1268 were examined in the present study.

To assess if environmental parameters correlated with skin lesion prevalence, monthly mean/mean maximum air temperature and monthly mean/mean maximum precipitation levels for each study site were obtained from the NOAA National Weather Service website (National Weather Service)

2.4 Statistical Analysis

Statistical analysis was conducted in the R statistical software package. Prevalence was calculated as the proportion of animals that presented with lesions (overall and by type); these proportions were stratified by region, year and month and tested for significance using chi-squared tests and pairwise t-tests with Bonferroni correction. All anthropogenic and environmental factors were standardized using the R function “scale()” for comparison between varying magnitudes. This function calculates the mean and standard deviation of the entire vector, then scales each element by those values by subtracting the mean and dividing by the standard deviation. A correlation matrix was utilized to test for correlation between environmental factors. Monthly mean maximum air temperature and monthly mean average air temperature were perfectly correlated with water temperature, and monthly mean precipitation was highly correlated (>0.95) with monthly mean maximum precipitation, as such monthly mean maximum air temperature, monthly mean average air temperature, and monthly mean average precipitation were eliminated from further analyses.

A generalized linear mixed model (GLMM) with a binary response variable was used to assess the association of skin lesions (presence/absence) with environmental variables: season(fall/spring), water temperature, salinity, and monthly mean maximum precipitation. Individual dolphin ID was included as a random effect due to the presence of multiple sightings over time for some dolphins.

Chi-squared tests examined correlation between black and white-fringed lesions (associated with herpesvirus) and dark-fringed, spotted, and tattoo-like lesions (associated with poxvirus).

To evaluate association of lesion presence and blubber PCB concentration, a GLMM with a binary response variable was also utilized with individual dolphin ID included as a random effect. Previous studies have shown that PCB concentrations in female dolphins are influenced by reproductive state, as females depurate lipophilic contaminants during gestation and lactation (Aguilar et al. 1999, Wells et al. 2005). Therefore, data were stratified by sex for analysis. The PCB sum associated with the Aroclor 1268 was compared in either sex with general lesion presence and with each individual lesion type.

2.5 Spatial Analysis

Detection of spatial signal was also conducted in R. Specifically, I examined distance-based correlations between lesion status (presence / absence of a lesion) and geographic coordinates, also known as a Mantel analysis (Legendre and Legendre 1998). Clustering will result in a positive correlation in which spatially proximate sightings are more similar, but more distance samples are more dissimilar. If there is no spatial pattern in the presence of lesions, then this correlation will be zero.

CHAPTER 3: RESULTS

3.1 Lesion type and prevalence

Because no surveys took place in SAP in 2007 and 2011-2014, only years 2008-2010 were used for comparison in seasonal lesion prevalence between field sites. In comparing overall lesions prevalence, only March and April were considered to account for seasonal changes in lesion prevalence. Overall prevalence for BRN was 0.618 (N=207), while overall prevalence for SAP was 0.563 (N=552), which were not significantly different (χ^2 p-value=0.0887) (See Table 4 and Figure 3).

Site	Total Number of Animals Observed	Number of Animals Observed with Lesions	Prevalence	Confidence Interval (95%)
BRN	335	207	0.618	0.563-0.670
SAP	981	552	0.563	0.531-0.594

Table 4. Overall Lesion Prevalence in BRN and SAP (April and March of 2008-2010.

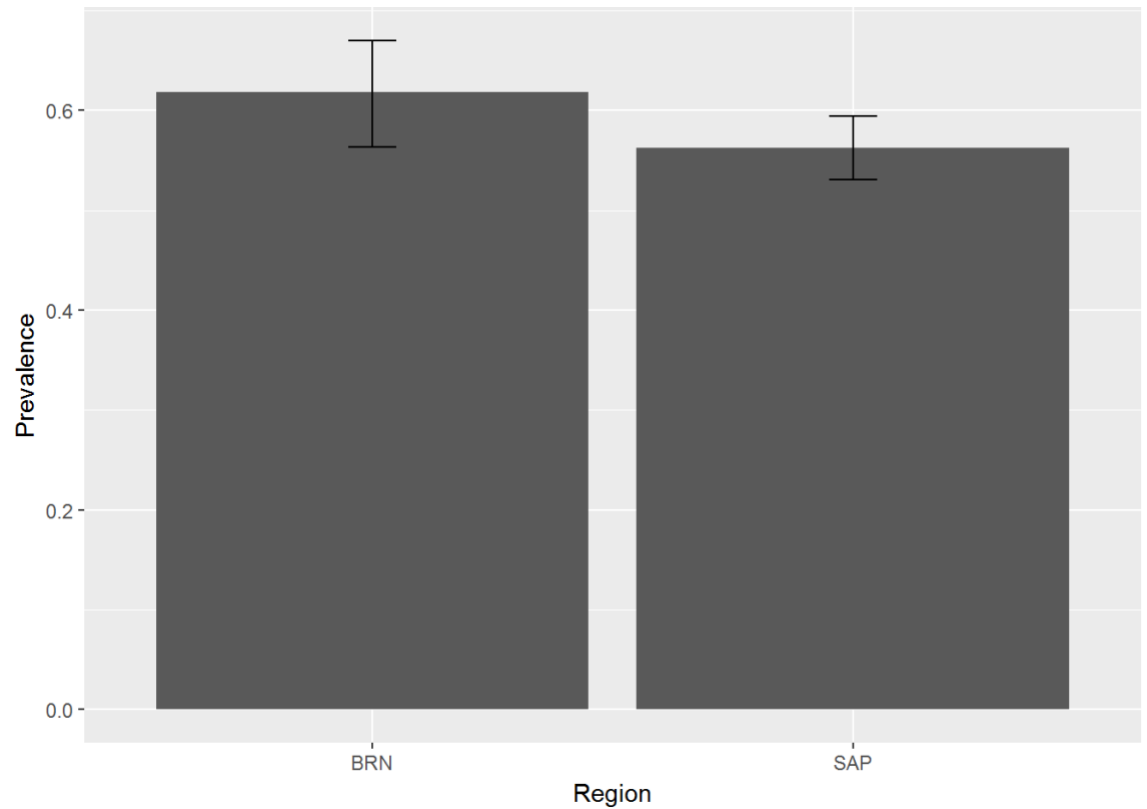


Figure 3. Overall Lesion Prevalence Compared Between BRN and SAP for April of 2008-2010. Bars represent prevalence, whiskers represent 95% CI.

There was evidence of a significant seasonal difference in lesion prevalence at both sites (BRN: χ^2 p-value<2.2e-16; SAP: χ^2 p-value<2.2e-16). In both regions, lesions occurred at a higher rate in March and April, somewhat lower in August, and at a much lower rate in September (see Tables 5 & 7; Figures 4 & 5). Pairwise comparisons with Bonferroni correction in BRN show that significant differences between all sampled (see Table 6), while comparisons done in SAP show significant differences between all sampled months except for April and March (see Table 8).

Month	Total Number of Animals Observed	Number of Animals Observed with Lesions	Prevalence	Confidence Interval (95%)
<i>March</i>	326	252	0.773	0.723-0.817
<i>April</i>	502	321	0.639	0.560-0.681
<i>August</i>	129	63	0.488	0.400-0.577
<i>September</i>	173	42	0.243	0.182-0.315

Table 5. Monthly Overall Lesion Prevalence in BRN 2007-2014

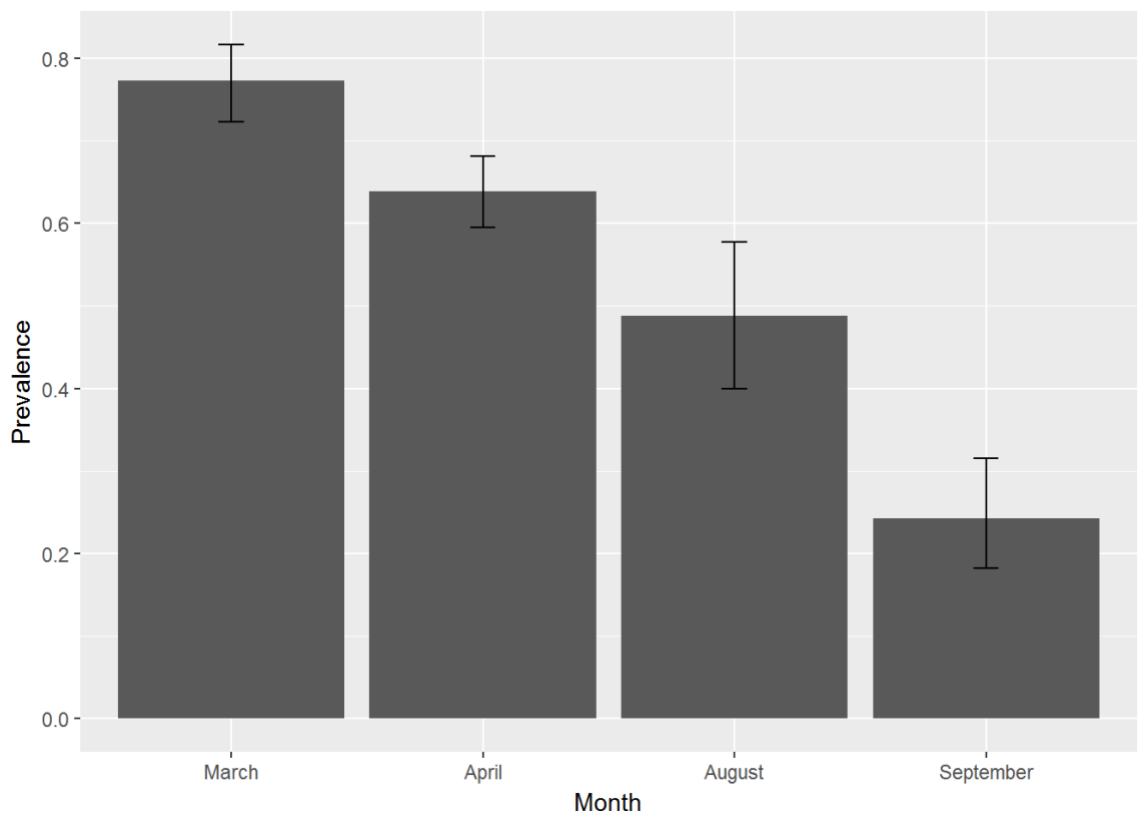


Figure 4. Monthly Overall Lesion Prevalence in BRN 2007-2014. Bars represent prevalence, whiskers represent 95% CI.

	March	April	August
<i>March</i>	-	0.00027*	-
<i>August</i>	2.0e-08*	0.00527*	-
<i>September</i>	< 2e-16*	< 2e-16*	2.8e-05*

Table 6. P-values from pairwise t-tests between months with Bonferroni correction (BRN 2007-2014)

Month	Total Number of Animals Observed	Number of Animals Observed with Lesions	Prevalence	Confidence Interval (95%)
<i>March</i>	139	103	0.741	0.659-0.810
<i>April</i>	327	248	0.758	0.708-0.803
<i>August</i>	634	348	0.549	0.509-0.588
<i>September</i>	106	15	0.142	0.084-0.226

Table 7. Monthly Overall Lesion Prevalence for SAP 2008-2010

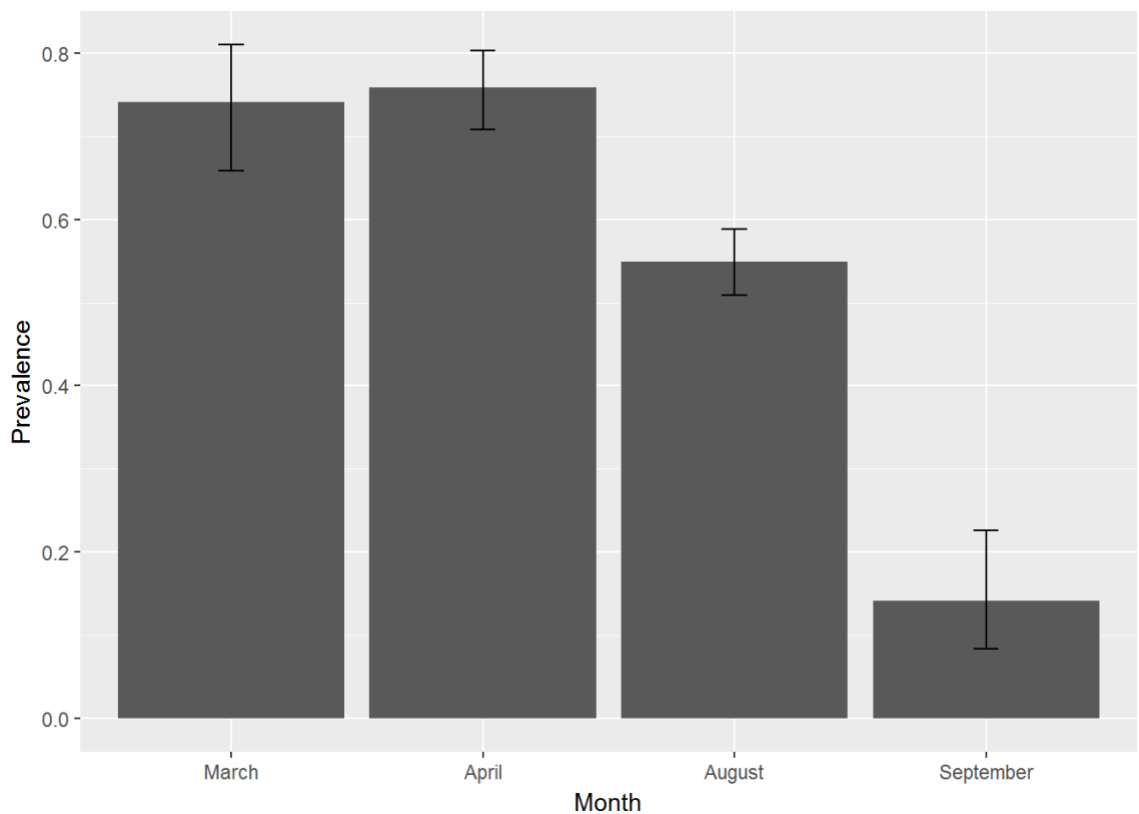


Figure 5. Monthly Overall Lesion Prevalence for SAP 2008-2010. Bars represent prevalence, whiskers represent 95% CI.

	March	April	August
<i>March</i>	-	1	-
<i>August</i>	5.9e-05*	2.5e-10*	-
<i>September</i>	< 2e-16*	< 2e-16*	7.2e-16*

Table 8. P-values from pairwise t-tests between months with Bonferroni correction (SAP 2008-2010)

Skin lesion prevalence also varied across years. In BRN, skin lesion prevalence was highest in 2008(0.900, N =54), before dropping in 2009 (0.663, N = 55) and remaining between 0.500-0.687 for 2010-2014 (See Table 9 and Figure 6). There was a statistically significant difference in overall lesion prevalence between years (χ^2 p-value=6.9e-05). Further pairwise comparisons with Bonferroni correction show significant difference between 2008 and 2010 (p-value=0.0474), 2008 and 2012 (p-value=6.9e-06), and 2008 and 2013 (p-value=0.0022) (See Table 10). Although a smaller dataset, SAP had a similar temporal trend, with highest lesion prevalence in 2008 (0.843, N=113), followed by a decrease in 2009 and 2010 (0.701, N=68 and 0.698, N=67, respectively) (See Table 11 and Figure 7). There was a statistically significant difference in overall lesion prevalence between years (χ^2 p-value=0.012), and additional pairwise comparisons with Bonferroni correction showed significant difference between 2008 and 2009 (p-value=0.037), and 2008 and 2010 (p-value=0.032) (See Table 12).

Year	Total Number of Animals Observed	Number of Animals Observed with Lesions	Prevalence	Confidence Interval (95%)
2008	60	54	0.900	0.788-0.959
2009	83	55	0.663	0.550-0.760
2010	54	34	0.630	0.487-0.754
2011	110	69	0.627	0.529-0.716
2012	96	48	0.500	0.402-0.598
2013	40	21	0.525	0.363-0.682
2014	59	40	0.678	0.542-0.790

Table 9. Yearly Overall Lesion Prevalence in BRN (April only)

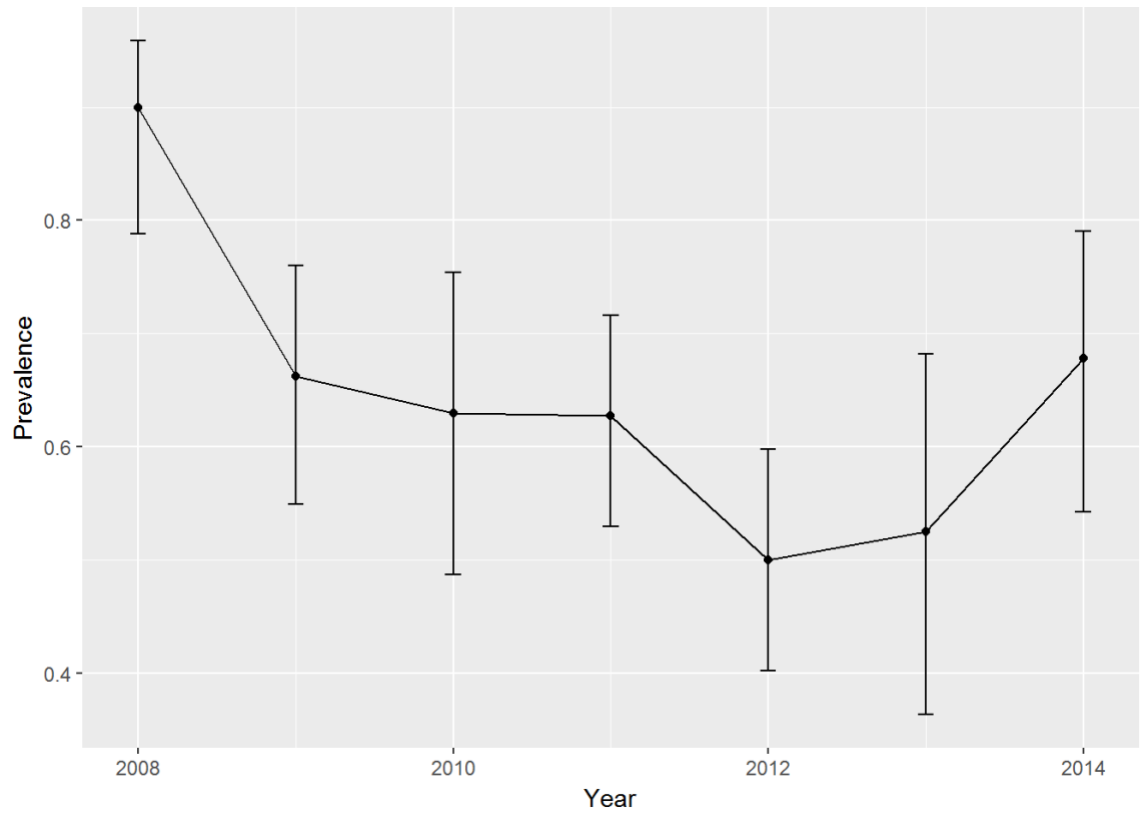


Figure 6. Yearly Overall Lesion Prevalence in BRN (April only). Points represent prevalence, whiskers represent 95% CI.

	2008	2009	2010	2011	2012	2013
2009	0.0629	-	-	-	-	-
2010	0.0474*	1.000	-	-	-	-
2011	0.068	1.0000	1.0000	-	-	-
2012	6.9e-06*	0.4458	1.0000	1.0000	-	-
2013	0.0022*	1.0000	1.0000	1.0000	1.0000	-
2014	0.2140	1.0000	1.0000	1.0000	0.4697	1.0000

Table 10. P-values from pairwise t-tests between years with Bonferroni correction (BRN April Only)

Year	Total Number of Animals Observed	Number of Animals Observed with Lesions	Prevalence	Confidence Interval (95%)
2008	134	113	0.843	0.768-0.898
2009	97	68	0.701	0.598-0.788
2010	96	67	0.698	0.594-0.785

Table 11. Yearly Overall Lesion Prevalence in SAP (April only)

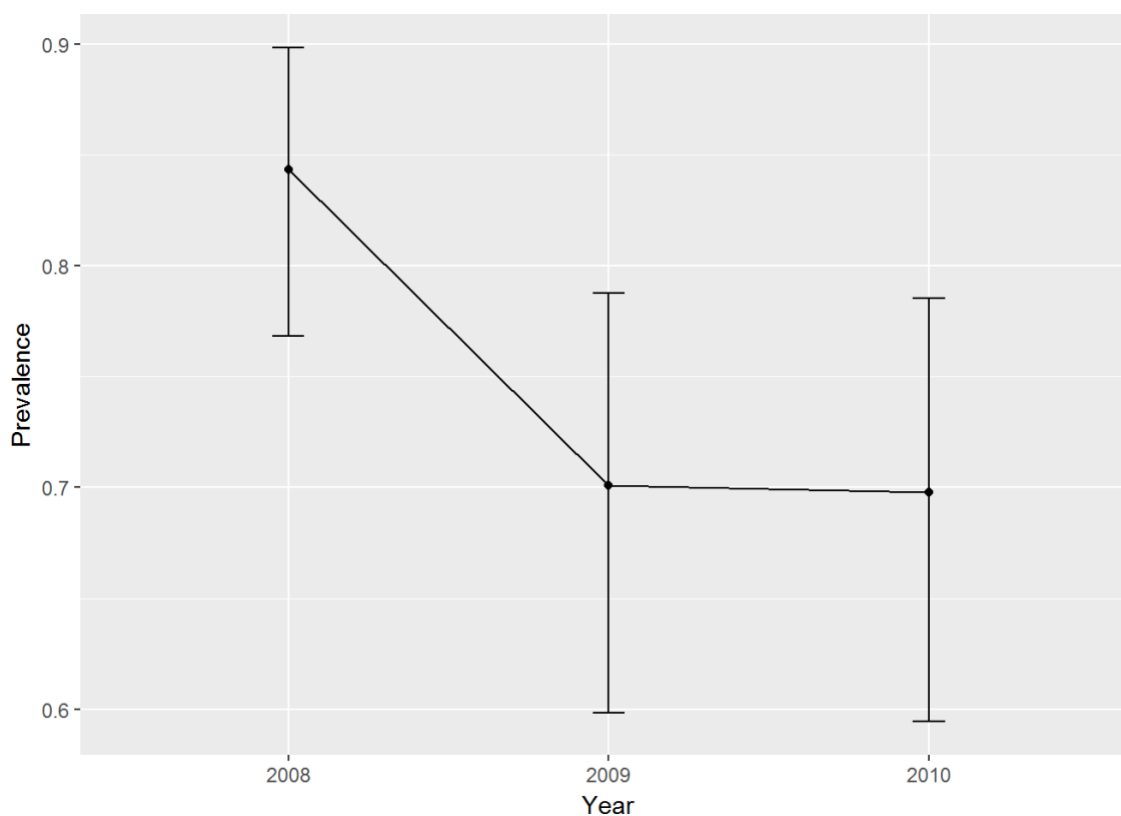


Figure 7. Overall Yearly Lesion Prevalence in SAP (April only). Points represent prevalence, whiskers represent 95% CI.

	2008	2009
2009	0.037*	-
2010	0.032*	1.000

Table 12. P-values from pairwise t-tests between years with Bonferroni correction (SAP April Only)

Because of these observed seasonal differences and lack of data in some months/years in either BRN or SAP (See Tables 2 and 3), only April of years 2008-2010 were used for further comparisons in lesion prevalence by type between sites.

Prevalence of each individual lesion type (see Table 1) were also evaluated (Table 13 and Figure 8). Pale lesions were the most commonly observed (BRN: 0.340, N=67; SAP: 0.327, N=107), followed by dark-fringed (BRN: 0.294, N=58; SAP: 0.269, N=88). Because pale, dark-fringed, black, and mottled lesions showed a prevalence of >0.100, they were considered major lesion types (See Figure 9). Pairwise t-tests with Bonferroni corrections showed that only mottled lesions had significantly difference prevalence between field sites (p-value=0.004).

Type	BRN (n=197)	SAP (n=327)	P-value from Pairwise T-Test with Bonferroni Correction
<i>Any</i>	0.726	0.758	0.46
<i>Black</i>	0.188	0.208	0.82
<i>Dark-fringed</i>	0.294	0.269	0.16
<i>Lacaziosis-like</i>	0.005	0.003	0.53
<i>Lunar</i>	0.015	0.027	0.33
<i>Mottled</i>	0.137	0.211	0.004*
<i>Orange</i>	0.071	0.040	0.38
<i>Pale</i>	0.340	0.327	0.4
<i>Spotted</i>	0.005	0.012	0.22
<i>Tattoo-like</i>	0.076	0.055	0.22
<i>Velvety</i>	0.000	0.012	0.19
<i>Vesicular</i>	0.030	0.034	0.23
<i>White-fringed</i>	0.081	0.028	0.18*

Table 13. Prevalence of Each Lesion Type Between BRN and SAP for April of Years 2008-2010

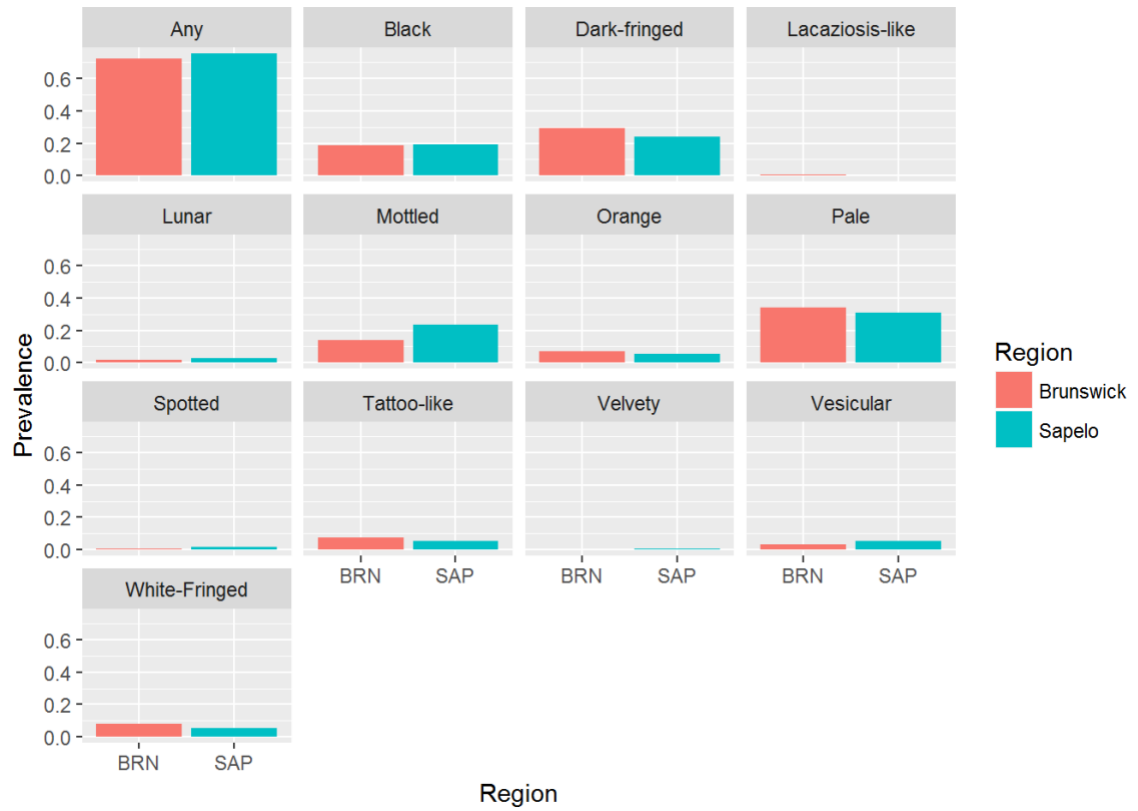


Figure 8. Prevalence of Each Lesion Type Between BRN and SAP for March and April of Years 2008-2010.

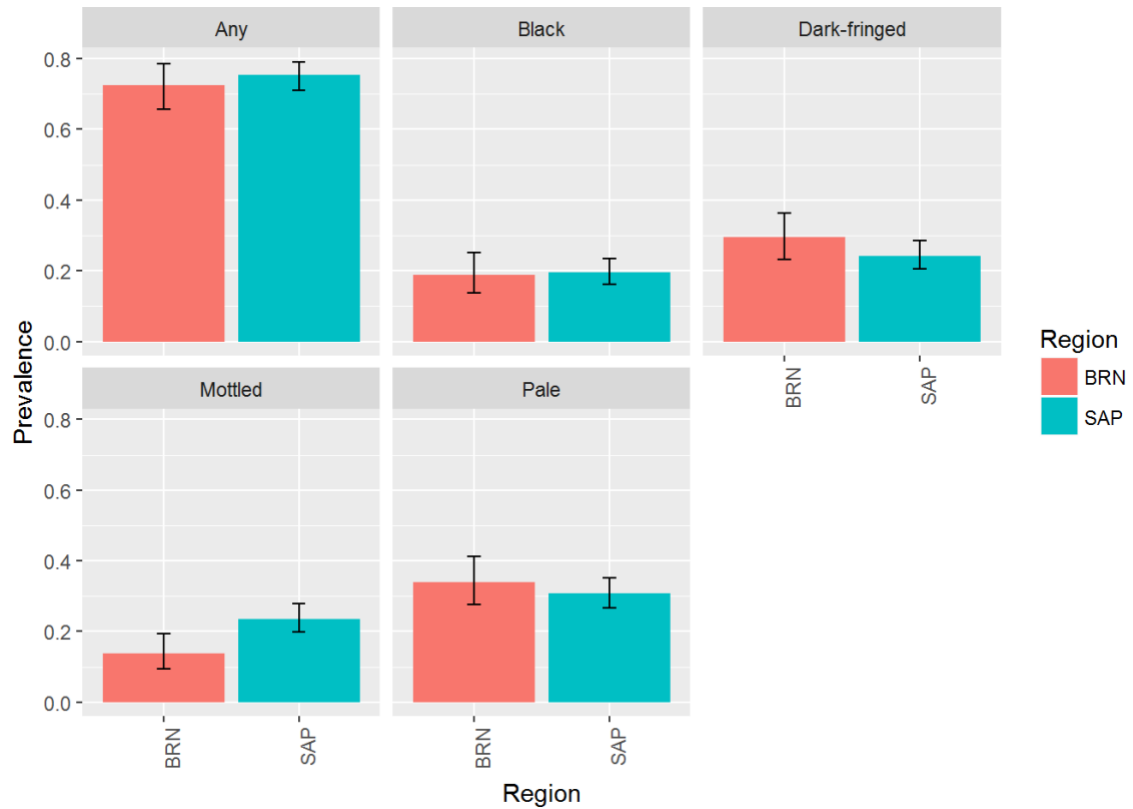


Figure 9. Prevalence of Major Lesion Types Between BRN and SAP for April of Years 2008-2010. Bars represent prevalence, whiskers represent 95% CI.

Major lesion types also showed temporal variation throughout the sampled years (Figure 10), though no pattern is obvious with such a brief time period. However, prevalence of pale and dark-fringe lesions seems to drive overall temporal patterns of lesions. This is not surprising, as pale and dark-fringed lesions were observed most often of all 12 lesion types.

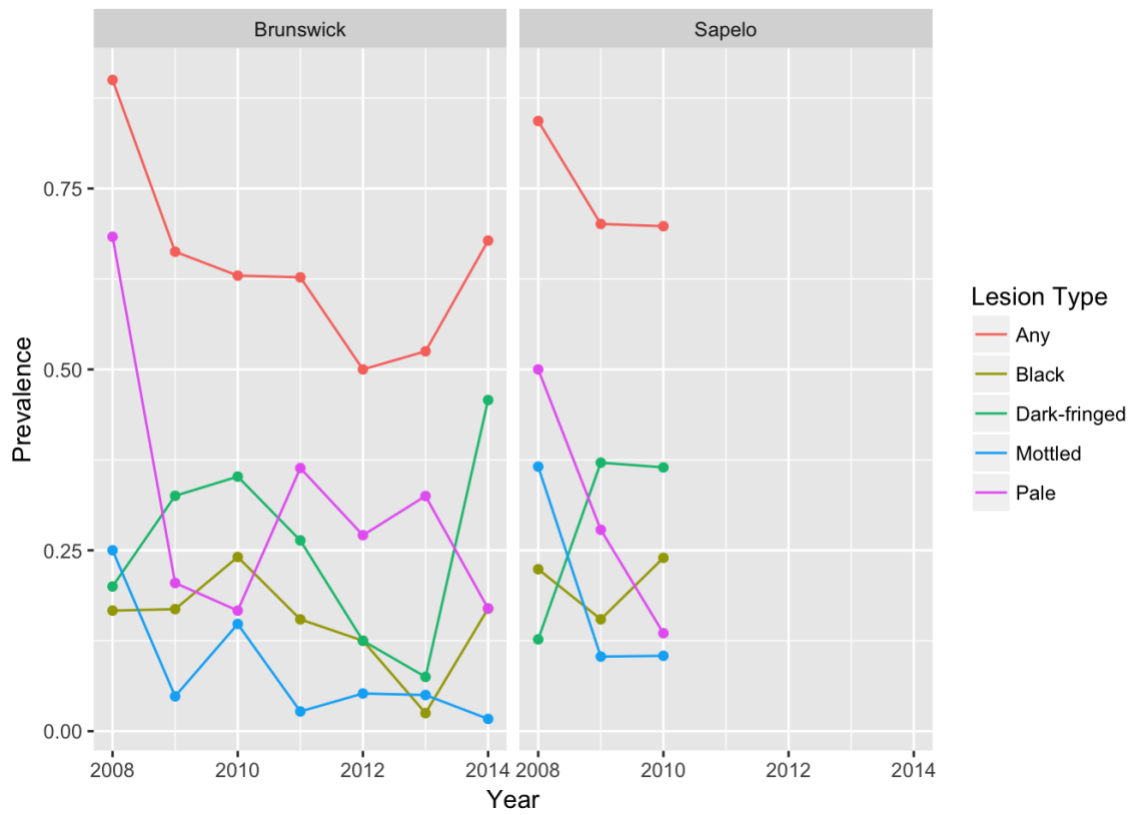


Figure 10. Temporal Variation in Prevalence of Major Lesion Types Between BRN and SAP for April.

3.2 Relationship to Environmental Factors

	Min	Median	Mean	Max
<i>Sighting water temperature (°C)</i>	16.70	19.85	22.35	32.00
<i>Salinity (ppt)</i>	0.20	25.04	24.21	35.30
<i>Max Monthly Air Temperature (°C)</i>	74	77.3	80.17	90
<i>Mean Monthly Air Temperature (°C)</i>	63.5	67.3	70.68	82.40
<i>Max Monthly Precipitation (cm)</i>	0.508	2.667	3.753	7.874
<i>Mean Monthly Precipitation (cm)</i>	0.0508	0.230	0.349	1.016

Table 14. BRN Environmental Gradient

	Min	Median	Mean	Max
<i>Sighting water temperature (°C)</i>	9.50	27.80	24.94	32.70
<i>Salinity (ppt)</i>	0.10	29.80	27.26	35.60
<i>Max Monthly Air Temperature (°C)</i>	-	-	-	-
<i>Mean Monthly Air Temperature (°C)</i>	60.1	67	72.66	82.1
<i>Max Monthly Precipitation (cm)</i>	0.533	3.531	4.8120	9.246
<i>Mean Monthly Precipitation (cm)</i>	0.102	0.305	0.422	0.787

Table 15. SAP Environmental Gradient

Because of similarities in environmental gradients and the need for higher power for the analysis, sites were combined for modeling. The GLMM showed a significant correlation ($p < 0.05$) between the presence of any lesion and the monthly mean maximum precipitation, and the presence of any skin lesions and the mean monthly air temperature. The model's coefficient estimate of the monthly mean maximum precipitation was -1.161, with a p-value of 0.0140, while

the model's coefficient estimate of salinity was 0.385 with a p-value of 0.0190. Figure 11 shows the regression curves for both factors in the model.

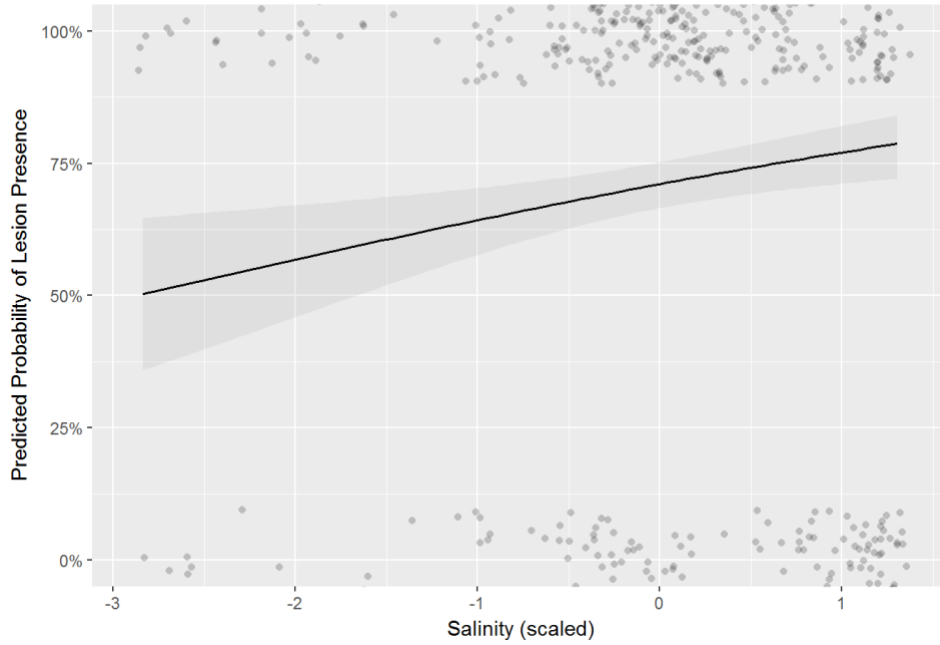


Figure 11. Regression curve for GLMM of the predicted probability of skin lesion presence using salinity (ppt) as an independent variable.

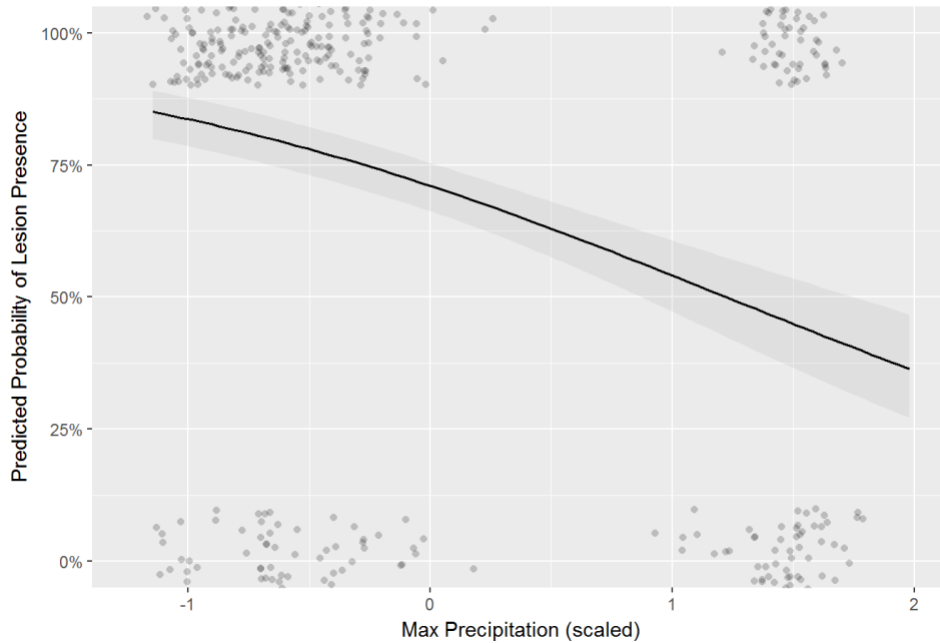


Figure 12. Regression curve for GLMM of the predicted probability of skin lesion presence using monthly mean max precipitation (cm) as an independent variable.

3.3 Relationships with Lesion Types Associated with Herpesvirus and Poxvirus

χ^2 test ($p=0.230$) between presence of black and white-fringed lesions revealed no evidence for correlation between lesion types associated with herpesvirus was found. Similarly, no evidence was found for correlation between lesion types associated poxvirus, with comparisons of dark-fringed and tattoo-like (χ^2 $p=0.107$), dark-fringed and spotted (χ^2 $p=0.380$), and tattoo-like and spotted (χ^2 $p=0.421$) showing no significance.

3.4 Relationship to PCB Concentrations

All models showed no evidence for correlation between the Aroclor 1268 PCB sum and overall or any type of lesion in either males or females. Examples of regression curves generated from these models can be found in the appendices. These findings are further supported by boxplots comparing the presence/absence of lesions with the scaled PCB sum. (See Figures 12 and 13)

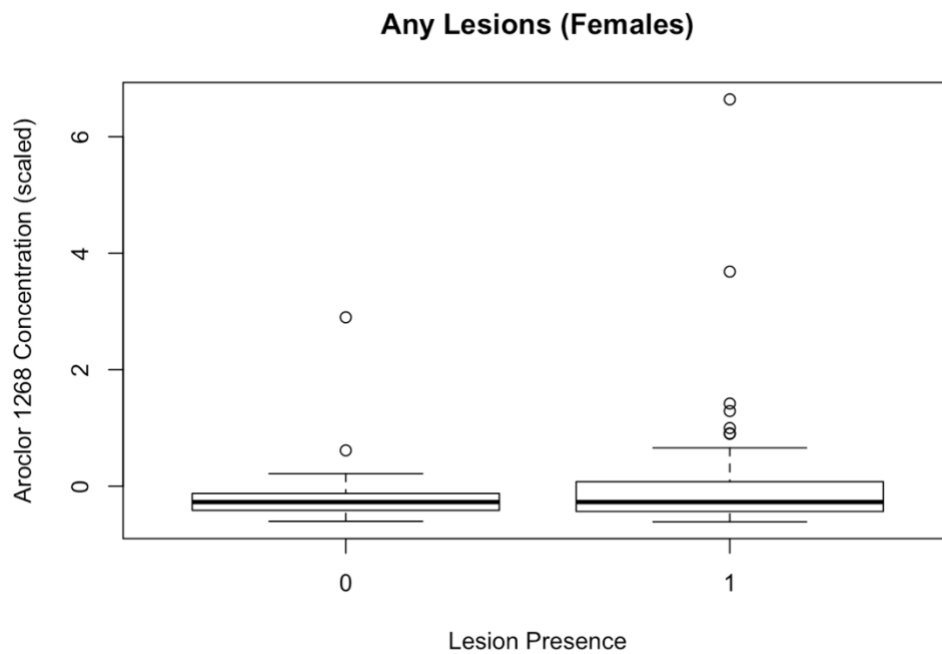


Figure 13. Boxplot of Lesion Presence/Absence in Females vs. Aroclor 1268. Dark black center line denotes median value, box denotes upper and low quartile, interval lines denote maximum and minimum, dots denote outliers.

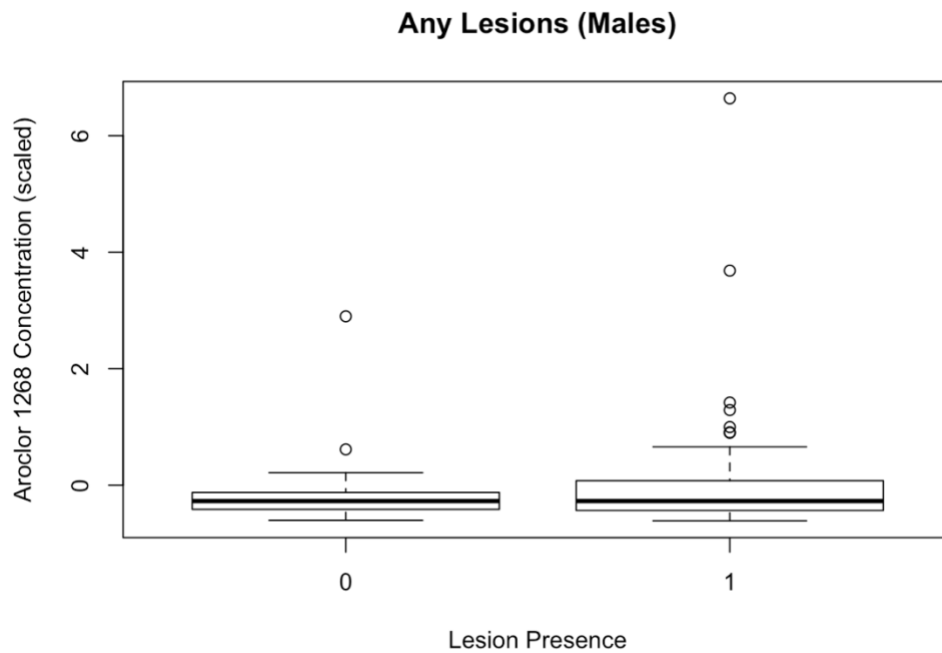
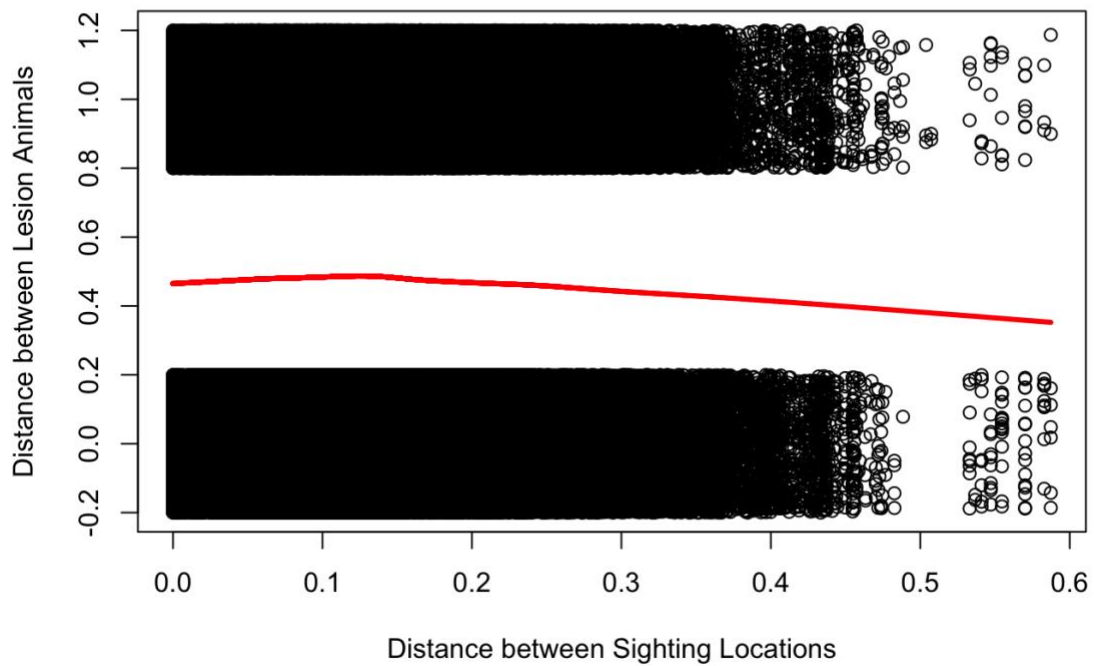


Figure 14. Boxplot of Lesion Presence/Absence in Males vs. Aroclor 1268. Dark black center line denotes median value, box denotes upper and low quartile, interval lines denote maximum and minimum, dots denote outliers

3.5 Spatial patterns

Mantel analyses conducted for both sites revealed no evidence for a significant spatial relationship in animals presenting with lesions (See Figure 14). The lowess line in both analyses were flat, which indicated that there was no spatial trend in lesion status. Mantel analysis for SAP can be found in the appendices.



*Figure 15. Mantel Analysis of BRN Animals Exhibiting Any Lesion Type.
Flat lowess line denotes lack of evidence for a spatial trend in lesion presence. Circles indicate jittered data points.*

CHAPTER 4: DISCUSSION

Results of this study show evidence for consistent prevalence trends between BRN and SAP, with both exhibiting significantly higher prevalence in spring months, as well as significant changes in variation across years. Monthly mean max precipitation and salinity were both significantly correlated with the presence of skin lesions, while no evidence for correlation with other environmental variables was found. The blubber concentration of Aroclor 1268 was also not found to have significant correlation between lesion presence in either male or female animals. Lesion types associated with herpesviruses (black and white-fringed) and poxviruses (dark-fringed, spotted, and tattoo) were also found to have no significant correlation. Finally, no evidence for any spatial pattern was found in lesion animals.

Results of this study show that the majority of photographed animals in both BRN and SAP present with some type of skin lesion, lesions are more prevalent in spring months, and that the most common types observed were pale, dark-fringed, black, and mottled lesions, which are consistent with results from Hart et al. (2012). However, the BSG group from that study (animals sampled in BRN and SAP combined into a single group) has been further separated out in this study to compare BRN and SAP to one another rather than combining them. Both sites also showed yearly significant variation in prevalence. Because pale lesions can be associated with general trauma, ectoparasite attachment, and wound healing, it makes sense that they are the most prevalent skin lesion as there are more potential causes that can happen often rather than one pathogen type. The next two most numerous lesion types, dark-fringed and black, can be associated with Poxvirus and Herpesvirus, respectively, and thus denote a potential prominence of those diseases in both portions of CGES and SGES stock boundaries (surveys sampled approximately $\frac{1}{2}$ of total area). While mottled lesions were observed in higher numbers in SAP than BRN, their potential causative agent(s) is currently unknown so what this could mean for these stocks is unclear. It is important to note the difference in land use between the two sites:

BRN is more urbanized and industrial, while SAP is a part of the National Estuarine Research Reserve system and seems relatively pristine. However, Aroclor 1268 concentrations in dolphins in SAP are still relatively high, and a past study by Stewart et al. (2014) has also shown similar resistant bacteria found in fecal and blowhole swabs taken from dolphins in both BRN and SAP, which could be due to the agriculture and livestock that takes place on the island (Balmer et al. 2013). These land use differences could potentially explain the differences in lesion prevalence and type between sites by affecting the distribution of potential causative agents.

It is important to note that both CGES and SGES stock exhibit high site fidelity (Waring et al. 2015). This study could be improved upon in the future with larger datasets of photo-ID, remote biopsy, and health assessments with a greater variety of location sampling would be useful in determining patterns of lesion prevalence. Additionally, full body visual assessments completed with health assessments would be a useful way to discern more than a minimum lesions prevalence as both this study and Hart et al. 2011 did, as only a portion of the dorsolateral surface of the animal is visible from photo-ID surveys. Drones might also be a future potential option, as surveys could be completed more frequently and be able to photograph a larger portion of an individual's body surface from multiple angles.

Yearly trends seem to be driven by the prevalence of the two most numerous lesion types, pale and dark-fringed, and show an overall falling of lesion prevalence 2008-2012, with a rise 2013-2014. An unusual mortality event (UME) was reported from NOAA NMFS for common bottlenose dolphins in the Mid-Atlantic during 2013-2015 caused by morbillivirus (NOAA Fisheries 2015). In this time-period, 103 strandings were reported in Georgia, while strandings in 2007-2012 totaled only 18. Lesion prevalence did increase in 2014 as compared to previous years, so it is possible that some interaction from this pathogen could be effecting lesions presence. Additionally, a definite seasonal trend is present in lesion prevalence. Spring months

show a much higher incidence of lesion, while lesions were observed much less frequently in August and September.

The GLMM used to explore correlations between environmental factors included model terms for season (fall/spring), water temperature, salinity, and monthly mean maximum precipitation and included individual dolphin catalog ID as a random effect. Because the environment an individual is present in may vary before sighting factors are measured (e.g., water temperature and salinity), additional meteorological factors were included for the entire month to compensate for this; however, all but monthly mean max precipitation were highly correlated with sighting conditions. Because water temperature and salinity have both been previously shown to be correlated with the presence of skin lesions in dolphins from BRN and SAP, they were both included in this model (Hart et al. 2012). Results from this study have shown significant differences in prevalence by months (see Tables 6 and 8 and Figures 4 and 5), so season (fall/spring) was also included as a model term. For the sampled months/years, sightings of individual animals ranged from 24 instances to 1 instance. Because sighting instances varied and there was potential for differences in skin exposure for lesion classification, dolphin catalog ID was included as a random effect to compensate for this.

The GLMM found mean maximum monthly precipitation and sighting salinity were both correlated with the presence of any lesion type, negatively and positively respectively. The positive correlation with salinity is opposite of findings by Hart et al. (2012) which also found a significant correlation negative between salinity and lesion presence in the observed Georgia dolphins. Precipitation and salinity could be driving the seasonal patterns that were observed. It is also important to note that the water temperature and salinity are measured by YSI at the time and place of a sighting; dolphins can be very mobile within their range, and changes in temperature before the time of sighting cannot be taken into account. This is also true of salinity, which can vary within an estuary system. It is possible that water temperature and salinity factor more

heavily into lesion presence, and this should be explored in future studies to examine time lagged effects in time-series models.

Both coastal stocks present in this area (South Carolina/Georgia and Southern Migratory) are present in the area of the field sites in winter and early spring and could potentially be bringing disease agents with them during their seasonal movements (Waring et al.2015). It is also possible that any potential causative agents may spread and thrive more easily in colder temperatures found in March and April compared to August and September. Future studies to investigate seasonal and temporal variation should sample from all months across multiple years to further validate and examine these findings and should include more remote biopsy sampling or comprehensive health assessments for histological and microbial analysis. Additionally, surveys that investigate movements of both coastal stocks and interactions with the resident populations should be implemented to investigate the potential for disease transfer.

Lesion types associated with Herpesvirus and Poxvirus were not found to be correlated with one another. These findings lend evidence to the idea that different types of lesions associated with one virus (black and white-fringed for Herpesvirus and dark-fringed, tattoo-like, and spotted for Poxvirus) may indicate different stages of the given disease. Possible alternative explanations are that viruses might not always present with lesions, or that certain types of each virus might present with different types of lesions. Hart et al. (2012) suggested that different lesion types are not always associated with different disease types, as two distinct lesion types sampled were found by PCR to be infected with identical strains of herpesvirus. In the future, more stranding samples should be tested for disease identification in combination with full body visual assessments.

No evidence for correlation between the PCB mixture associated with Aroclor 1268 and either female or male animals was found. Though correlation between high doses of PCBs and

skin disease has been previously demonstrated in mice, guinea pigs, rats, and rabbits, these findings suggest that skin lesions may not be a useful indicator for PCB contamination of dolphins, or as a useful health status indicator for health issues related to PCB contamination in these two stocks (Vos 1972, Zinkl 1977, Kimbrough 1978). Alternatively, it may be possible that photo-ID alone might be a poor methodology for examining correlation, and that full body visual assessments in conjunction with health assessments and remote biopsy sampling should be explored in the future. However, other health parameters should be examined in the future for potential correlation with skin lesion prevalence.

Finally, no spatial pattern was found for overall lesion prevalence, or for the prevalence of any particular lesion type. Animals in this region typically have high site fidelities to either BRN or SAP and are thus exposed to different stressors in each region in terms of PCB exposure, surrounding land use, and vessel activity (Balmer et al. 2013, Waring et al. 2015). Potential causative agents at either field site are currently poorly understood, so more stranding sampling, remote biopsy sampling, and health assessments are needed to better understand lesion causes. It is possible that potential causative agents are uniformly distributed throughout the marine environment at each field site, but still differ from one another. Alternatively, the methodology for examining spatial patterns in this study maybe be unsuitable. Future studies should build upon Balmer et al. (2013) to determine associations between lesion prevalence and Strahler stream order and individual ranging pattern.

This study built upon the previous done by Hart et al. (2012) by examining prevalence by separating sites, adding additional environmental parameters for correlation testing, and by examining lesions over multiple years. Despite both sites being mainly populated by separate stocks (SGES and CGES), lesion prevalence was not significantly different between sites which supports the previous study not separating the sites. Further supporting Hart et al. (2012), lesion prevalence was found to be higher in spring months when compared with fall. However, unlike

the previous study significant yearly variation was also found and environmental correlations to lesions prevalence were found to differ. Hart et al. (2012) showed significant correlation with water temperature and salinity in animals from BRN and SAP; however, this study found significant correlation with salinity and precipitation.

This study gives a more comprehensive base of information concerning the overall knowledge of skin lesions in Brunswick and Sapelo Island, Georgia compared to previous knowledge. While this study cannot confirm the use of skin lesions in long-term health monitoring for PCB exposure, there is still potential that other measured health parameters covered in comprehensive health assessments are related to skin lesion presence and should be explored. Because few clear conclusions could be drawn about causes and factors that influence skin disease, this study highlights the needs for a more systematic approach to the use of photo-ID as a method to evaluate long-term health impacts to common bottlenose dolphins.

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APPENDIX

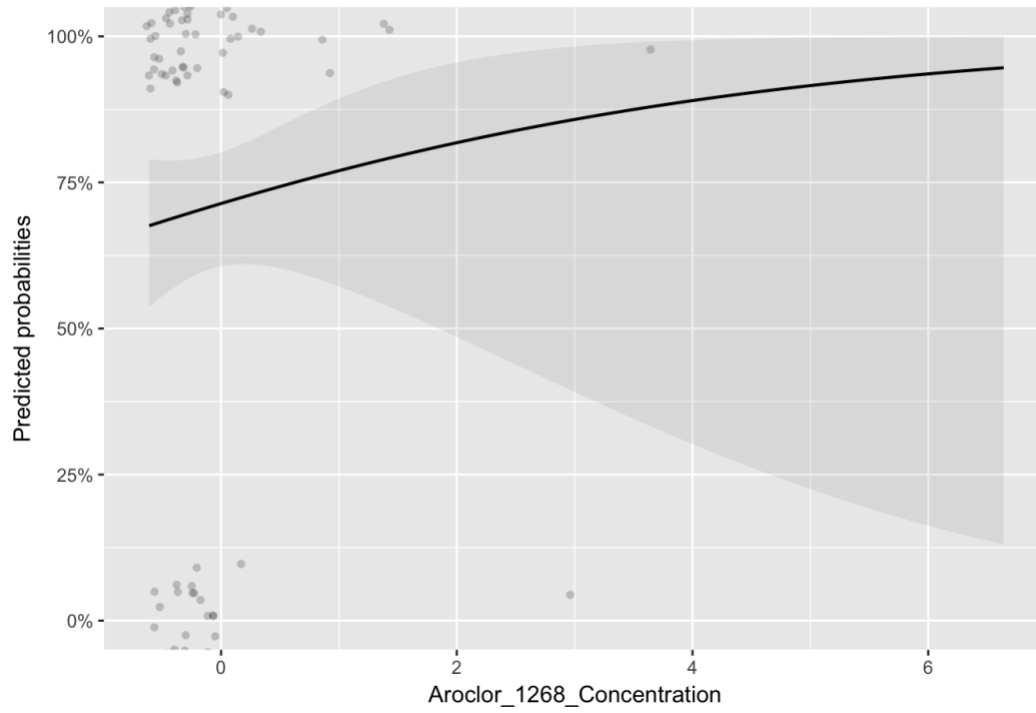


Figure 16. Regression Curve for the GLMM in Females

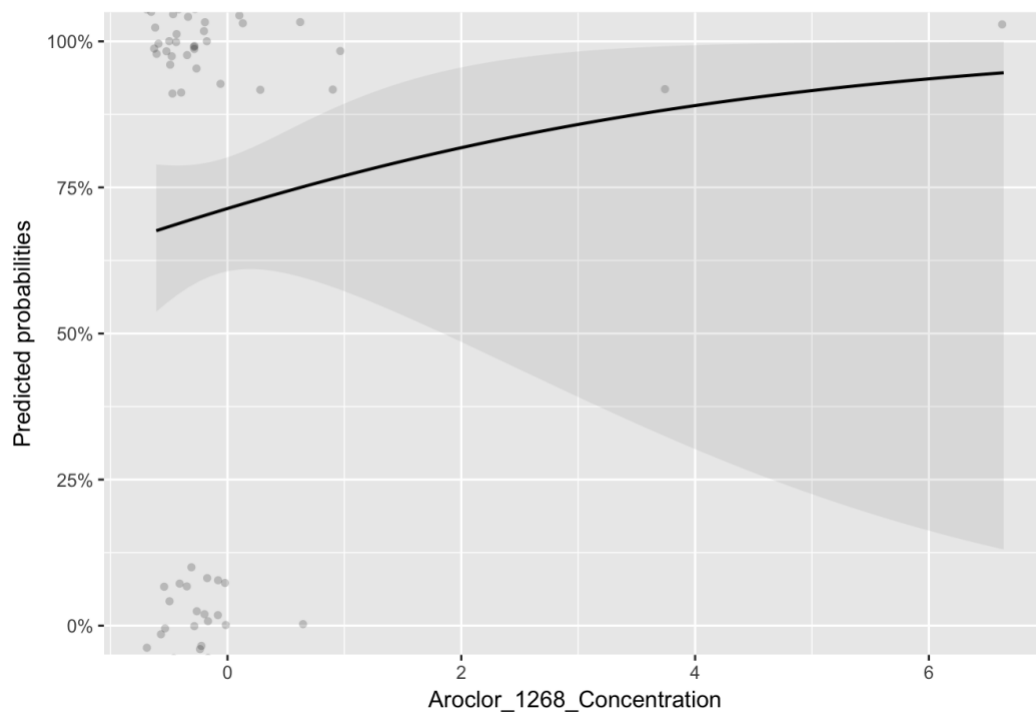
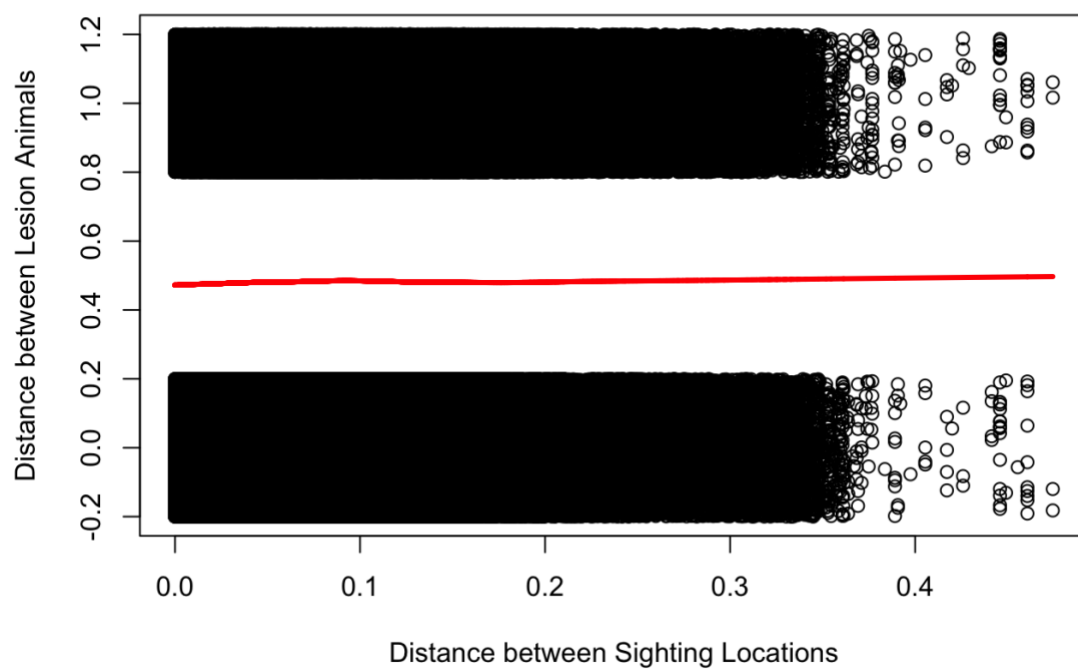


Figure 17. Regression Curve for the GLMM for Males



*Figure 18. Mantel Analysis for SAP Animals Exhibiting Any Lesions.
Flat lowess line denotes lack of evidence for a spatial trend in lesion presence. Circles indicate jittered data points.*