Assessing *Brucella ceti* Infections in Oregon and Washington Dolphins that Stranded with Histopathological Lesions Resembling Neurobrucellosis, 2006-2014

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A Thesis
Submitted in partial fulfillment
of the requirements for the degree
Master of Environmental Studies
The Evergreen State College
June 2015

This Thesis for the Master of Environmental Studies Degree

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Abstract

Assessing *Brucella ceti* Infections in Oregon and Washington Dolphins that Stranded with Histopathological Lesions Resembling Neurobrucellosis, 2006-2014

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This thesis documents the presence of *Brucella ceti* in the Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), striped dolphins (*Stenella coeruleoalba*), and short-beaked common dolphins (*Delphinus delphis*) that stranded with histopathological lesions resembling neurobrucellosis in Oregon and Washington between 2006 and 2014. These *Brucella* strandings occurred in very specific years (2006, 2012, and 2014) and seasons (winter and fall), which may have been driven by an increase in the number of overall strandings, or an environmental influence, altering their susceptibility to the disease. Further studies on the linkages between climate and disease will provide a better understanding on factors that might drive the emergence of seasonal or interannual variations seen within *Brucella* stranded individuals. Out of fifteen individuals that had histopathological lesions resembling neurobrucellosis upon histology, fourteen were sent for further *Brucella* tests and ten (71%) came back positive. The positive individuals in this study were confirmed by culture and serology. However, there were a high number of false negative PCR and IHC results, making me believe that 71% is an underestimate of the actual percentage of *Brucella* positive individuals. Demographically speaking, the striped dolphin (n=6) was the most common species to be infected with *Brucella ceti,* followed by the short-beaked common dolphin (n=3). This study is also the first, to my knowledge, to document *Brucella* in a Pacific white-sided dolphin. The observed predilections at this time include male striped dolphins, subadult individuals of all species, and short-beaked common dolphins that strand in Washington. These observed predilections are based off of a small sample size and may be subject to change if further tests are performed on prior individuals that tested negative for *Brucella* and from future strandings.

Ultimately, individuals with histopathological lesions suspicious of neurobrucellosis most often came back positive for *Brucella ceti* on further tests.

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Acknowledgements

First off, I would like to thank my thesis reader, Dr. Kevin Francis. Thank you for your guidance, encouragement, and most of all, your patience throughout this entire process. You have taught me so much these past two years and I am beyond grateful to have had you as my thesis mentor.

Second, I would like to thank Dyanna Lambourn with Washington Department of Fish and Wildlife: Marine Mammal Investigations. Thank you for all the input and advice on this thesis and for the experiences I've gained throughout my internship. Also, thank you to Jessie Huggins (Cascadia Research Collective), Jim Rice (Oregon State University Marine Mammal Institute) and Dr. Debbie Duffield (Portland State University) for providing me with data, clarifying any questions that I had, and giving valuable feedback on all my drafts.

Lastly, I would like to thank my family. To my husband, I greatly appreciate the love and support you give to me every single day. I can honestly say that I would not have kept my sanity without your help. Also, thank you to my dad, mom, and brother. Your encouragement and love have gotten me through every challenge I have faced, and I am elated to complete yet another one with each of you by my side.

Chapter One

-Introduction-

Background

Highly contagious infections from the bacterial genus, *Brucella spp.*, are found in both terrestrial and marine vertebrates and are among the most prevalent worldwide zoonotic diseases (Foster et al., 2009 and Sohn et al., 2003). *Brucella* was first isolated in marine mammals in 1994, but is now endemic in marine mammal populations worldwide (Sidor et al., 2013). There are two species of *Brucella* that are specific to marine mammals, which include *Brucella pinnipedialis* (i.e. seals) and *Brucella ceti* (i.e. cetaceans) (Cloeckaert et al., 2001). *Brucella ceti*, which this thesis focuses on, often presents with clinical manifestations that include, but are not limited to, abortion, orchitis, abscesses, muscoskeletal disorders, and neurological disorders (Cloeckaert et al., 2001; Maquart et al., 2009; Sidor et al., 2013; and Thakur et al., 2012). For this particular study, an emphasis was placed on dolphins that stranded with neurological disorders over other manifestations.

Neurobrucellosis

Neurobrucellosis occurs when there are *Brucella* caused complications within the central and/or peripheral nervous system, often presenting as meningitis or meningoencephalitis (Tuncel et al., 2008). However, inflammatory peripheral neuritis/radiculitis, inflammatory demyelinative processes, papilledema, and

meningomyelitis are also manifestations that have been documented in neurobrucellosis individuals (Tuncel et al., 2008). These specific pathological changes have been observed in humans and a small number of dolphin species, but are seldom, if at all, recorded in terrestrial hosts such as cattle, pigs, goats, and sheep (Gonzàlez-Barrientos et al., 2010).

Although neurobrucellosis is an infrequent complication in humans (roughly 5-10% of *Brucella* cases), it is recurrently observed in select dolphin species with a seemingly large predilection towards the striped dolphin (Alba et al., 2013; Ceran et al., 2011; Foster et al., 2002; Gonzàlez et al., 2002; Hernàndez-Mora et al., 2008; and Tuncel et al., 2008). *Brucella ceti* has been frequently isolated from the central nervous system of individuals that macroscopically presented with hyperemia of the meninges and brain (Hernández-Mora et al., 2013). Histologically, these animals had nonsuppurative meningoencephalomyelitis, meningoencephalitis, meningomyelitis, or meningitis (Alba et al., 2013 and Hernández-Mora et al., 2013).

Literature detailing neurobrucellosis within striped dolphins is quite common. However, documentation within other dolphin species is scarce. Other than the striped dolphin, I personally only came across two articles that discussed neurological pathologies in conjunction with a *Brucella* infection. In 2013, Davison et al. reported meningoencephalitis, along with muscoskeletal pathologies, in a short-beaked common dolphin that was associated with *Brucella ceti*. In 2009, Hernàndez-Mora et al. mentioned three bottlenose dolphins that tested positive for *Brucella* and had “neurological symptoms”. However, the authors did not specify whether these “neurological symptoms” within the bottlenose dolphins were due to neurobrucellosis explicitly.

It is important to note that nervous system disorders can also be caused by other bacterial infections (e.g. staphylococcal infections), viruses (e.g. herpesvirus and morbillivirus), parasites, and protozoa (e.g. *Toxoplasma gondii* and *Sarcocystis neurona*). For example, viral infections, such as herpesviruses and morbilliviruses, are responsible for a vast amount of neurological diseases within the striped dolphin (Gonzàlez et al., 2002). Individuals can also be co-infected with multiple infectious agents that can adversely affect the nervous system, which might make it difficult to determine the causative agent of the neural inflammation. For example, encephalitis caused by fungal origins and *Toxoplasma spp.* have been documented as secondary complications of morbillivirus within the striped dolphin (Gonzàlez et al., 2002). Inflammatory lesions caused by *Brucella ceti* have been noted to be strikingly different from encephalitis caused by other pathogens, however (Gonzàlez-Barrientos et al., 2010). According to McLean et al. (1992), meningeal infection seems to be the common pathogenic thread for *Brucella,* despite the difficulty to detect *Brucella* organisms directly in infected tissues (Seidel et al., 2003).

Significance of Research, Research Questions, and Hypotheses

It is crucial to study communicable diseases in dolphins, such as *Brucella ceti*, since they have one of the most highly social groups among mammals and are an effective sentinel for emerging and reemerging infectious diseases (Bossart, 2011 and Gaspari et al., 2007). Although there has been great insight on disease exposure and prevalence in potential vulnerable marine mammals for *Brucella,* information on transmission, pathogenicity, and susceptibility of individuals are still scarce (Sidor et al.,

2013). Since dolphins have large areas of movement that are not dependent on geographical boundaries, they can introduce *Brucella* to a wide range of new hosts and areas (Thakur et al., 2012). Also, due to the zoonotic potential of *Brucella ceti*, there are health risks to humans, domestic pets, and wild animals that may come in contact with a stranded individual. Therefore, more research needs to be conducted to prevent further terrestrial crossovers of this marine *Brucella* species.

Worldwide monitoring and research of marine *Brucella* is also necessary to better understand this disease (Hernàndez-Mora et al., 2013). There have been multiple studies conducted on *Brucella ceti* in an array of regions including, but not limited to, the UK, Costa Rica, the Mediterranean, and the U.S. East Coast (Alba et al., 2013; Davison et al., 2013; Gonzàlez et al., 2002; Gonzàlez-Barrientos et al., 2010; Hernàndez-Mora et al., 2008; Isidoro-Ayza et al., 2014; and Wu et al., 2014). Besides the very first study that detailed *Brucella* in an aborted bottlenose dolphin fetus whose mother was held in captivity in San Diego, California, I personally did not find any other studies that discussed *Brucella* in dolphins that stranded along the U.S. West Coast (Ewalt et al., 1994). For that reason, it is vital to contribute to the existing literature by looking at the occurrence of *Brucella* in dolphins that stranded along Oregon and Washington, which will also be referred to as the Pacific Northwest (PNW).

Although a variety of dolphin species have stranded in Oregon and Washington, this thesis solely focused on the Pacific white-sided dolphins (PWSD) (*Lagenorhynchus obliquidens*), striped dolphins (*Stenella coeruleoalba*), short-beaked common dolphins (*Delphinus delphis*), long-beaked common dolphins (*Delphinus capensis*), and common bottlenose dolphins (*Tursiops truncatus*) that may have stranded with histopathological

manifestations resembling neurobrucellosis. These histopathological manifestations would more specifically include nonsuppurative meningoencephalomyelitis, meningoencephalitis, meningomyelitis, or meningitis (Alba et al., 2013 and Hernández-Mora et al., 2013). The latter four species (i.e. striped, short-beaked common, long-beaked common, and bottlenose) are commonly found in warmer waters (e.g. California waters) and are considered to be unusual sightings in Oregon and Washington (Allen et al., 2011).

According to the studies of González et al. (2002), Hernàndez-Mora et al. (2008), and Xavier et al. (2009), dolphins that consistently presented with these meningeal disorders also ended up presenting with neurological ailments and tested positive for *Brucella*, more specifically on serology and immunohistochemistry (IHC). Hernàndez-Mora et al. (2009) also noted a correlation between individuals with neurological symptoms and having high titers of antibodies against *Brucella* antigens. However, along with positive serology and IHC, positive results have also been achieved via culture and polymerase chain reaction (PCR) (Sidor et al., 2013).

These findings led to the development of the research questions that this thesis seeks to address:

1) What has the stranding history looked like among these five species in Washington and Oregon from 1975-2014? From 2006-2014?
2) Out of the individuals in Oregon and Washington that stranded with histopathological lesions resembling neurobrucellosis, how many subsequently tested positive for *Brucella ceti*?

3) Out of the *Brucella ceti* positive individuals, were there any demographic predilections observed (i.e. predilection towards species, age class, stranding location, or sex)?
4) Which tests were the most commonly used and/or most successful in detecting *Brucella ceti* within this study?

Since information on this topic is scarce, it is important to note that this thesis is exploratory and attempts to provide insight on this disease specific to individuals that stranded with neurobrucellosis-like histopathological lesions in the Pacific Northwest. The goal of this thesis is to provide direction for future studies as more data is collected on impending strandings, and the conclusions drawn are based on my attempts, as a graduate student, to provide insight on *Brucella ceti* in Oregon and Washington. Further *Brucella* tests may also be conducted on multiple cases outlined in this study, so results may be subject to change.

Due to the exploratory nature of this thesis, not every research question has a hypothesis. This is especially true since I used previously collected data and was able to see some of the demographics before beginning my analyses. Based on prior studies (e.g. Gonzàlez et al., 2002 and Hernàndez-Mora et al., 2008), I would suspect predilections towards the striped dolphin and subadult individuals, but may see other predilections, such as stranding location or sex, when the data is further analyzed.

Chapter Two

-Literature Review-

Brucella spp.

Brucella spp. is a genus of intracellular, gram-negative bacteria that can infect both terrestrial and marine vertebrates worldwide (Sohn et al., 2003). It does not multiply within the environment, but is usually transmitted directly from host to host (Xavier et al., 2009).

Brucella has species-specific primary reservoirs with clinical features that vary based on the host species (Sohn et al., 2003 and Xavier et al., 2009). There were traditionally six nomen species of *Brucella* that included: 1) *Brucella abortus*; 2) *Brucella melitensis*; 3) *Brucella suis*; 4) *Brucella canis*; 5) *Brucella ovis*; and 6) *Brucella neotomae* (Cloeckaert et al., 2001 and Young, n.d.). However, two more nomen species have been recently added that are specific to marine mammals: 1) *Brucella pinnipedialis* and 2) *Brucella ceti* (Cloeckaert et al., 2001) (Figure 1). DNA-DNA hybridization and other phenotypic characteristics showed that although these two marine mammal species were a part of the genus *Brucella* (more than 77% DNA relatedness), there were still distinctive characteristics that isolated them from the other terrestrial species (Cloeckaert et al., 2001; Maquart et al., 2009 and Thakur et al., 2012). All species of *Brucella* have proven to have zoonotic potential for humans except *B. ovis* and *B.neotomae* (Xavier et al., 2009).

Organism	Reservoir
B melitensis	Sheep, goats, and camels
B abortus	Buffalo, cows, and camels
B suis	Pigs
B canis	Dogs
B neotomae	Rodents
B ovis	Sheep
B pinnipediae	Marine animals
B cetaceae	Marine animals

Figure 1: Host Reservoirs for *Brucella* Species (https://online.epocrates.com/u/2924911/Brucellosis)

Marine *Brucella*

Brucella was first isolated in marine mammals in 1994, but now appears to be endemic in marine mammal populations worldwide (Ewalt et al., 1994 and Sidor et al., 2013). The name *Brucella maris* was originally suggested for all marine mammal species with three biovars (Cloeckaert et al., 2001). Biovar 1 would have included seal and otter isolates, Biovar 2 would have included cetacean isolates, and Biovar 3 would have included a particular isolate from a California bottlenose dolphin that had a contrasting dominant antigen from the previous two (Cloeckart et al., 2001). Ultimately, Biovar 3 ended up representing another serotype rather than a biovar, and Biovar 1 and 2 were distinct enough to be classified as their own species, which ended up being *Brucella pinnipedialis* and *Brucella ceti* (Cloeckaert et al., 2001). Proposals of having three nomen species of marine *Brucella* have also been made, which would include *Brucella phocae* (seals), *Brucella delphini* (dolphins), and *Brucella phoecoenae* (porpoises) (Groussaud et al., 2007). However, as of this study, this is not absolute.

Brucella ceti

Brucella ceti has been described in an array of species within the Delphinidae family including, but not limited to, the bottlenose dolphin (*Tursiops truncatus*), Atlantic white-sided dolphin (*Lagenorhynchus acutus*), short-beaked common dolphin (*Delphinus delphis*), long-beaked common dolphin (*Delphinus capensis*), dusky dolphin (*Lagenorhynchus obscurus*), striped dolphin (*Stenella coeruleoalba*), killer whale (*Orcinus orca*), and pilot whale (*Globic ephala*) (Gonzàlez et al., 2002). Positive isolations have been derived from reproductive organs of both sexes, brain, spinal cord, joints, lungs, spleen, liver, cerebrospinal fluid (CSF), fetal tissues, mammary glands, milk, multiple lymph nodes, and more (Thakur et al., 2012). Although a lot of isolates have come from symptomatic animals, *Brucella ceti* has also been isolated from normal tissues and asymptomatic animals, indicating that this bacterium can be an opportunistic invader, or even an unlikely cause of death (Thakur et al., 2012). Besides the striped dolphin, it is believed that there are low proportions of other cetacean species that show *Brucella* associated clinicopathological signs (Isidoro-Ayza et al., 2014). That would mean most infected animals remain *Brucella* carriers and shedders due to their ability to overcome the clinical disease (Isidoro-Ayza et al., 2014).

Within *Brucella ceti* there are three documented sequence types (ST) or subgroups: ST23, ST26, and ST27 (Whatmore et al., 2007 and Wu et al., 2014). ST23 is predominantly found in porpoises, ST26 is predominantly found in striped and common dolphins, and ST27 was documented in bottlenose dolphins and humans (Alba et al., 2013 and Whatmore et al., 2007). This is suggestive that ST27 has a higher zoonotic potential for human infection than the other sequence types described (Wu et al., 2014).

-Terrestrial Crossover-

Brucella isolates have the potential to infect human and non-human terrestrial animals (Xavier et al., 2009). Marine *Brucella* has been induced in cattle, sheep, and piglets through inoculations, further demonstrating that terrestrial crossovers are possible (Rhyan et al., 2001 and Thakur et al., 2012).

According to Goodwin et al. (2012), there are two drivers of zoonotic disease transmission into human populations: 1) occurrence of the disease in animals which may change due to population dynamics of hosts or vectors and alteration of habitats, and 2) variations in composition or behavior of human population, altering their susceptibility to the disease. The latter is more of a concern for transmission of marine *Brucella* into human populations due to the desire of many to live by and/or visit the beach, the curiosity to see or touch a stranded marine mammal, and the culture of some to consume marine mammal meat. No system of inspection of consumed meats and organs have been established, despite how frequently specific countries may eat this meat (Hernández-Mora et al., 2013). It is also common for people to pick up skulls, teeth, and other parts of the skeleton of a stranded marine mammal as a trophy or souvenir. This is also dangerous since these skeletal parts may serve as fomites for transmission (Hernández-Mora et al., 2013).

So far, there have been four human cases that were described to have marine *Brucella* isolates. This included one marine lab researcher and three other people who acquired the infection with no known exposure to any marine mammals (Sohn et al., 2003). Two of the individuals that acquired the infection without known marine mammal

exposure presented with neurological signs and emigrated from Peru, where they frequently ate raw shellfish and unpasteurized cheese (Sohn et al., 2003). Due to the extensive Peruvian coastlines, *Brucella ceti* could have been transmitted to domestic animals and wildlife that resided nearby (Sohn et al., 2003). The third person that acquired the infection without known marine mammal exposure developed spinal osteomyelitis and was a fisherman from New Zealand who regularly handled uncooked fish bait and raw fish (McDonald et al., 2006 and Thakur et al., 2012). The laboratory acquired case was determined to be ST23, while the remainder three cases were identified as ST27 (Whatmore et al., 2008 and Wu et al., 2014).

Brucella ceti Tests

There are a variety of tests used to diagnose a *Brucella ceti* infection. The most common tests I have come across throughout literature review included culture, serology, polymerase chain reaction (PCR), and immunohistochemistry (IHC). These were also the tests used in this particular study.

-Culture-

According to Thakur et al. (2012), the majority of the culture isolations are "done on Farrell's medium, followed by Columbia sheep blood agar, *Brucella* agar with *Brucella* selective supplement and 1.4% crystal violet and brain heart infusion agar with 5g of yeast abstract" (p.906). Farrell's medium is the most highly used medium worldwide since it inhibits the growth of most contaminants (Vicente et al., 2014). Cetacean isolates normally are visible within four days of inoculation and can grow well without increased CO_2 (Thakur et al., 2012). It is recommended that cultures be incubated

in 10% CO_2 at 37 °C (Foster et al., 2002 and Thakur et al., 2012). According to Wu et al. (2014), microbiologic culture is considered the "gold standard" for a definitive *Brucella* diagnosis. However, culturing can take up to two weeks for a definitive diagnosis, has low sensitivity, and is more hazardous to laboratory personnel (Wu et al., 2014). Poor postmortem carcasses and prolonged storage of tissues may also prevent successful isolations of *Brucella* (Sidor et al., 2013).

-Serology-

Although there are a variety of serological tests used to detect *Brucella* antibodies and agglutinins, each has its advantages and disadvantages when it comes to specificity or sensitivity (Thakur et al., 2012). Examples of commonly used serological tests include, but are not limited to, the enzyme-linked immunosorbent assays (ELISA), Rivanol, *Brucella* microagglutination test (BMAT), and Fluorescent Polarization Assays (FPA). Once again, this is not an exhaustive list.

Although serology can support evidence to *Brucella* exposure (i.e. presence of antibodies to the *Brucella* antigen), a major downfall is the inability to differentiate between a current or prior infection (Krucik, 2012). Current infections, or active infections, are based on titer levels, so serial blood draws will need to be conducted to see if the levels are rising, falling, or staying the same (Dyanna Lambourn, personal statement and Liu, 2014). Unfortunately, serial blood draws are very difficult to obtain in wild animals. Serological tests also lack validity due to the need for significant numbers of serum samples from positive infections and negative controls (Hernández-Mora et al., 2009). *Brucella* cells' immunodominant antigen is the smooth lipopolysaccharide (S-

LPS) (Thakur et al., 2012). Since other gram-negative bacterial species can also have smooth lipopolysaccharides, antibodies can cross-react, leading to false positives or misdiagnoses (Thakur et al., 2012). Along with false positives, false negatives can also occur in serology tests. For example, false negatives can occur on ELISA tests due to the presence of small amounts of agglutinating antibodies that escaped detection (Hernàndez-Mora et al., 2009).

-Polymerase Chain Reaction (PCR)/Molecular Methods-

Polymerase Chain Reaction, or PCR, can detect and identify *Brucella* at the genus, species, and biovar level. It is considered to be rapid and simple, requires little manual labor, and is reliable as long as contamination is avoided (Bricker, 2002). PCR assays can give immediate results but require more extensive sample preparation in order to remove PCR inhibiting components (Bricker, 2002). Also, additional data is needed about what is the best choice specimen and how long DNA can be detected over the course of an infection (Bricker, 2002). Since cell numbers of *Brucella* in tissues are very low, higher sensitive assays are needed to detect *Brucella* within marine mammals (Bricker, 2002 and Wu et al., 2014).

-Immunohistochemistry (IHC)-

Immunohistochemistry (IHC) is considered a useful tool at diagnosing infectious diseases in tissue samples, more commonly formalin-fixed tissue samples. According to Eyzaguirre and Haque (2008), immunohistochemistry can identify microorganisms that are present in low numbers, stain poorly, are difficult to grow, are not able to be cultured, and/or have atypical morphology. However, similar to serology, cross-reactivity can

occur since there is widespread occurrence of common antigens among bacteria (Eyzaguirre and Haque, 2008). Also, it has been recognized that IHC has lower sensitivity in identifying *Brucella* antigens in tissues compared to serology (Gonzàlez-Barrientos et al., 2010).

Chapter Three

-Methods-

Study Area

This study looked specifically at dolphin strandings that occurred throughout Oregon and Washington. These areas included nearshore waters and shoreline of Oregon and Washington north of 42° N and south of 49°N, also including the inland waters of Washington (Norman et al., 2004).

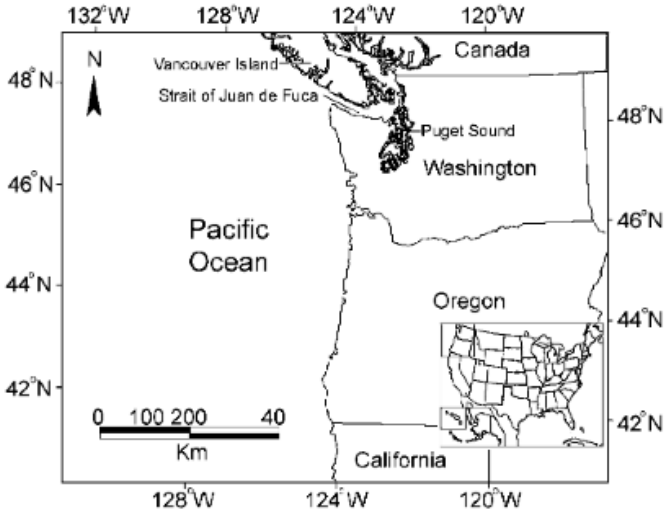

Figure 2: Area Covered by the Northwest Region Marine Mammal Stranding Network (Norman et al., 2004)

Data Collection

My data was collected from the Northwest Region Marine Mammal Stranding Network and from stranding coordinators in Washington and Oregon. The Northwest

Region Marine Mammal Stranding Network was formed in the early 1980s and is comprised of volunteers, state and federal wildlife and fisheries agencies, veterinary clinics, enforcement agencies, and other professionals (Norman et al., 2004). Stranding network activities are coordinated by the National Marine Fisheries Services, Marine Mammal Health and Stranding Response Program based in Seattle, Washington (Norman et al., 2004). For this study, stranding coordinators included, but was not limited to, Jessie Huggins (Washington; Cascadia Research Collective), Dyanna Lambourn (Washington Department of Fish and Wildlife: Marine Mammal Investigations), Jim Rice (Oregon State University Marine Mammal Institute), and Dr. Debbie Duffield (Oregon; Portland State University).

The data received included Washington and Oregon Level A records for the specific dolphin species analyzed. Histology reports and laboratory results were also obtained from the individuals that stranded with histopathological lesions resembling neurobrucellosis. Affiliated laboratories that performed histology on the tissue samples are discussed in further detail below. I did not receive histology reports on individuals that did not have neurobrucellosis-like lesions upon histology. I chose to look at the striped, short-beaked common, long-beaked common, and bottlenose dolphins since they are the most common dolphin species, according to literature review, to be infected with *Brucella ceti* that have also been documented to strand within the study area. An initial review of the data also revealed two PWSDs that stranded with histopathological lesions resembling neurobrucellosis. I decided to add the PWSD to my analyses due to this finding, as well as their prominent appearance in the Pacific Northwest (Allen et al., 2011).

Level A Data

Level A data, which is collected on marine mammal stranding responses, includes variables such as stranding date, stranding location, body measurements, body and carcass conditions, age class, sex, external injuries, etc. (Appendix A). The amount of information taken is dependent on the status of the individual (live or dead at response) as well as the level of decomposition and scavenging. The Level A data I received included individuals that had full examinations as well as non-examined individuals that only had photographs taken. Although there were an array of demographics and variables I could have analyzed, I looked specifically at the species, stranding date (year and month), stranding season, stranding location (Washington or Oregon), sex, and age class, since those were the most common categories to be assessed by other studies as well.

Histology and Lab Results

Along with Level A data, I also received histology reports and lab results for the dolphins that had histopathological lesions resembling neurobrucellosis from 2006 to 2014. To reiterate, this included individuals that had nonsuppurative meningitis, meningoencephalitis, meningomyelitis, or meningoencephalomyelitis upon histology. Once again, if a dolphin did not have these specific manifestations, I did not receive their histology reports, only their Level A data. It is important to note that 2006 was the first year that histology and lab results were available for this study. It does not mean 2006 was the first year an individual stranded with histopathological manifestations resembling neurobrucellosis within the study area. In fact, in 2006 the Oregon Marine Mammal Stranding Network began running and started having histology performed on a regular

basis. Prior to 2006, Oregon dolphins were generally not examined histologically at all (Jim Rice, personal communication).

-*Brucella* Tests-

Although each stranding examiner may perform necropsies in a slightly different manner, they generally follow the protocols outlined by Pugliares et al. (2007). Necropsies include an extensive external and internal exam, which are documented and photographed. Complete necropsies are performed on carcass conditions that are relatively fresh with minimal scavenging. Decomposition codes, which can be found on the Level A sheet attached, are described as follows: 1) Alive; 2) Fresh Dead; 3) Moderate Decomposition; 4) Advanced Decomposition; 5) Mummified/Skeletal; and 6) Condition Unknown. The more decomposed or scavenged the carcass is, the less likely they will be necropsied since tissue viability is compromised. If they are necropsied, however, it is usually considered a limited necropsy rather than complete.

Tissues collected during necropsy for histology were stored in 10% neutral buffered formalin and tissues collected for bacterial isolation and other tests were frozen between -30°C and -40°C for Washington samples (Lambourn et al., 2013) and -20°C for Oregon samples (Jim Rice, personal communication). Although there were histology results for an array of tissue samples taken during necropsy, I only focused on the comments relating to the nervous system and the final diagnosis. If histopathological lesions resembling neurobrucellosis was found during histology, further tests were conducted to assess whether the individual was infected with *Brucella ceti* and/or other pathogens. Not all dolphins had the same *Brucella* tests performed. However, the most common tests, along with which laboratories performed them, are outlined below.

Brucella cultures for this study were performed at the National Veterinary Services Laboratory (NVSL; Ames, IA), Colorado Department of Agriculture (CODAG; Denver, CO), and the Oregon State University Veterinary Diagnostic Laboratory (OSU; Corvallis, OR). The majority of the cultures were performed at NVSL, which included the following protocols previously described by Lambourn et al. (2013):

> "Tissues were dissected, mixed with approximately 2 mL of sterile phosphate buffered saline (pH 7.2), macerated, and inoculated onto tryptose agar with 5% bovine serum and antibiotics (7.5 U/mL bacitracin, 30 mg/mL cycloheximide, and 1.8 U/mL polymyxin B); tryptose agar with 5% bovine serum, antibiotics, and ethyl violet; Ewalt's media; Farrell's media; and Columbia agar with 5% blood. Plates were incubated for 14 days in 10% CO2 at 37 C and observed for growth at 7 and 14 days." (p.804)

If growth occurred after seven days, the average sized colonies consistent with *Brucella* were counted, recorded, and transferred for identification (Mayfield et al., 1990 as cited in Lambourn et al., 2013). According to Ewalt & Forbes (1987) and Lambourn et al. (2013), isolates were confirmed with the following tests:

- Growth in the presence of basic fuchsin (1:25,000 and 1:100,000), thionin (1:25,000 and 1:100,000), and thionin blue (1:500,000);
- Growth on medium containing penicillin (5 units/mL) or erythritol (1 mg/mL and 2 mg/mL plus 5% bovine serum);
- Urease activity;
- Catalase activity;
- H_2S production;

- and CO_2 dependence

Biotyping was conducted as previously described (Alton et al., 1988 as cited in Lambourn et al., 2013). An agglutination test using A and M- monospecific antisera (1:50-1:200) and R antiserum (1:25-1:100) determined the dominant antigen and isolates were tested by the phages Tbilisi (Tb), Firenze (Fi), Weybridge (Wb), S708, Me/75, D, BK2, R, R/C, and R/O for lysis susceptibility (Lambourn et al., 2013).

Serology tests were performed at the Washington Department of Agriculture (WDA; Olympia, WA) and the Washington Animal Disease Diagnostic Lab (WADDL; Pullman, WA). Serology protocols screening for antibodies were as previously outlined in Garner et al.'s (1997) article. Individuals were considered "suspect-positive" if the buffered plate agglutination test antigen (BAPA) or brucellosis card test using buffered *Brucella* antigen (BBA) detected antibodies (Lambourn et al., 2013). They were considered "positive" if they were positive on BAPA or BBA, and subsequently positive on the complement fixation (CF) and/or the Rivanol (RIV; +50 to 200) precipitation tests (Lambourn et al., 2013).

Polymerase chain reaction (PCR) was performed at the Animal Health Center (AHC; Abbotsford, British Columbia, Canada), Mystic Aquarium & Institute for Exploration (MAIE; Mystic, Connecticut), and University of Iowa (UI; Iowa City, Iowa). These laboratories "used previously described PCR techniques for *Brucella* (AHC; Bricker et al., 2000) and real-time PCR (qPCR) analysis that used primers, probes, and protocols that targeted the gene for a 31 kDa outer membrane protein bcsp31 specific to the genus *Brucella"* (MAIE, Probert et al., 2004, and Sidor et al., 2013 as cited in Lambourn et al., 2013, p. 804).

Immunohistochemistry (IHC) tests were performed at MAIE, United States Department of Agriculture (USDA; Fort Collins, Colorado), and NVSL. IHC tests were performed using previously described techniques for *Brucella* as mentioned in Lambourn et al.'s (2013) article.

> "Formalin-fixed, paraffin-embedded tissues were stained with hematoxylin and eosin and select sections were also stained with Giemsa and with Brown and Brenn. Immunohistochemistry was performed on a subset of culture-positive cases. Tissue sections were mounted on charged slides, deparaffinized, hydrated with a buffer (PBS), treated with 3% H2O2 (5 min) to quench endogenous peroxidase, incubated for 5 min at 37 C with nonimmune goat serum, rinsed, and incubated for 30 min at 37 C with a polyclonal antibody (1:10,000) prepared against B. abortus. Amplification was conducted with biotinylated, goat origin, anti-rabbit immunoglobulin (Ig), and peroxidase-labeled streptavidin; the chromagen was 3-amino-9 ethylcarbazole in N, Ndimethylformamide. Sections were counterstained with Gill II hematoxylin. Nonimmunized rabbit Ig fraction was substituted for primary antibody as a negative control (Garner et al., 1997)" (Lambourn et al., 2013, p. 804).

Data Analyses

Stranding History and Demographics

The Level A data I received went back to 1975. The first thing I wanted to do was get an overall view of the reported stranding patterns that occurred throughout the years for each analyzed species. The number of reported strandings every year between 1975

and 2014 were graphed, while simultaneously identifying how many of each species were recorded to have stranded in each specific year. Two pie charts were subsequently created detailing the number and percentage of each species that stranded between 1975-2005 and 2006-2014, providing a rank of which species were reported to be the most and least common to strand among the two year ranges. Although stranding numbers and species rank were graphed out beginning in 1975, the remainder of the demographic analyses only included data beginning in 2006, since reported strandings were considered to be more consistent and dolphins started to be routinely tested for *Brucella.*

After graphing out the number of reported strandings by species, a table was created outlining different demographic factors such as stranding location, age class, and sex for the individuals that stranded between 2006 and 2014. Stranding seasons were also graphed out to identify "high strand seasons" for each species. Spring months included March, April, and May; summer months included June, July, and August; fall months included September, October, and November; and winter months included December, January, and February. These results could suggest a more environmental cause behind the increase in strandings that may, for example, be influenced by water temperature and/or food source availability.

Analyses of Neurobrucellosis Suspicious Individuals

A table was created detailing demographic data (stranding location, age class, and sex), the specific *Brucella* tests performed along with their results, and the histopathological diagnoses of each individual that had the neurobrucellosis-like lesions as previously described. Each individual's histopathology and lab results are discussed in greater detail in Appendix B. Outlining these variables in the table allowed for the

visualization of potential demographic predilections as well as the most, and least, successful *Brucella* tests according to this study

Each demographic (stranding location, age class, and sex) was discussed in further detail specific to each species that were found to have *Brucella*. For each demographic, a table was created comparing the total number of individuals that stranded based on the demographic, the number of *Brucella* positive individuals that stranded based on the demographic, and the total number of individuals *minus* the known *Brucella* positive individuals that stranded based on the demographic. Comparing these numbers side-by-side helped clarify whether any observed *Brucella* predilections were indeed potential predilections, or if the observed trend was simply based off the normal stranding patterns of that species. Tables were only created for the striped and short-beaked common dolphin, however, because they were the only two species to have enough positive individuals to observe a potential demographic predilection.

Chapter Four

-Results-

Overall Stranding History

Stranding Numbers

Between 1975 and 2005, there were fifty-three reported strandings of the analyzed species: thirty-four (64%) PWSDs, thirteen (24.5%) striped, four (7.5%) short-beaked common dolphins, and two (4%) bottlenose dolphins. Between 2006 and 2014, there were forty-nine reported strandings: thirteen (27%) PWSDs, twenty-one (43%) striped, ten (20%) short-beaked common, three (6%) bottlenose, and two (4%) long-beaked common dolphins. As illustrated in Figure 3, an increase in strandings was noted from 2006, with the highest years in 2006 (n=9), 2012 (n=10), and 2014 (n=13). Besides 2006, 2012, and 2014, the number of reported strandings per year fluctuated between 0-5 individuals.

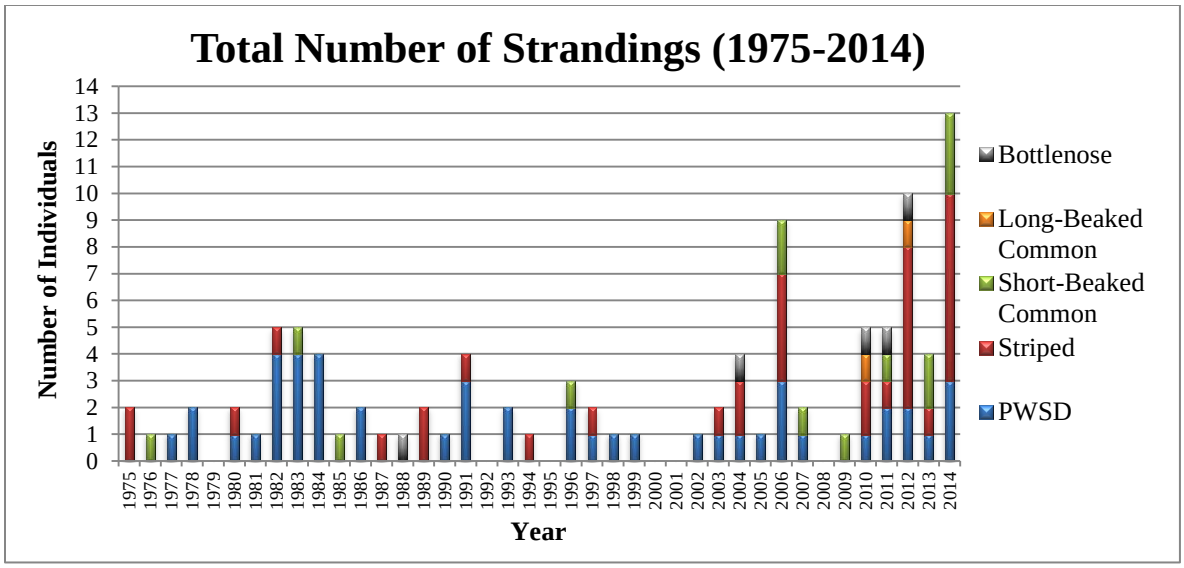

Figure 3: Total Number of Strandings (1975-2014)

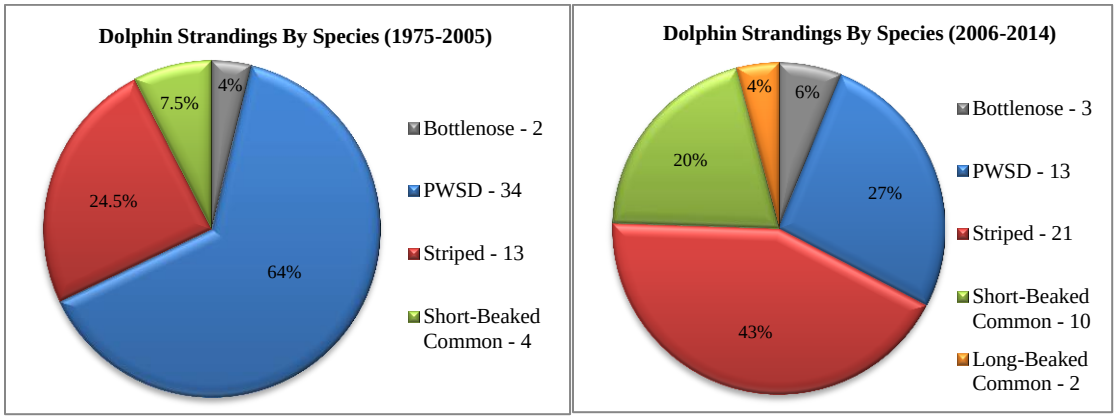

Figure 4: Reported Dolphin Strandings by Species (1975-2005) and (2006-2014)

Stranding Demographics

-Stranding Location-

Since 2006 there were thirty-five (71%) reported strandings in Oregon and fourteen (29%) reported strandings in Washington. The PWSD had eleven (85%) reported strandings in Oregon and two (15%) in Washington. The striped dolphin had sixteen (76%) reported strandings in Oregon and five (24%) in Washington. The short-beaked common dolphin had seven (70%) reported strandings in Oregon and three (30%)

in Washington. There were only two reported long-beaked common dolphin strandings and both occurred in Washington. Finally, the bottlenose dolphin had one (33%) reported stranding in Oregon and two (67%) in Washington.

-Age Class-

Since 2006 there were twenty-four (49%) reported subadult strandings, twenty-two (45%) adults, and three (6%) unknowns. The PWSD had six (46%) reported subadult strandings and seven (54%) adult strandings. The striped dolphin had eleven (52%) reported subadult strandings, eight (38%) adult strandings, and two (10%) unknown age class strandings. The short-beaked common dolphin had five (50%) reported subadult strandings and five (50%) adult strandings. The long-beaked common dolphin had one (50%) reported subadult stranding and one (50%) unknown age class stranding. Finally, the bottlenose dolphin had one (33%) reported subadult stranding and two (67%) adult strandings.

-Sex-

Since 2006 there were twenty-three (47%) reported female strandings, eighteen (37%) male strandings, and eight (16%) unknowns. The PWSD had eight (62%) reported female strandings, three (23%) male strandings, and two (15%) unknowns. The striped dolphin had seven (33%) reported female strandings, ten (48%) male strandings, and four (19%) unknowns. The short-beaked common dolphin had five (50%) reported female strandings, four (40%) male strandings, and one (10%) unknown. The long-beaked common dolphin had one (50%) reported female stranding and one (50%) unknown.

Finally, the bottlenose dolphin had two (67%) reported female strandings and one (33%) unknown stranding.

Species	Total Number of Strandings (2006-2014)	Location		Age Class			Sex		
		OR	WA	SA	A	U	F	M	U
L.o.	13	11	2	6	7	0	8	3	2
S.c.	21	16	5	11	8	2	7	10	4
D.d.	10	7	3	5	5	0	5	4	1
D.c.	2	0	2	1	0	1	1	0	1
T.t.	3	1	2	1	2	0	2	1	0
Total	**49**	**35**	**14**	**24**	**22**	**3**	**23**	**18**	**8**

Table 1: Overall Stranding Demographics (2006-2014)

**L.o.* = PWSD; *S.c.* = Striped; *D.d.* = Short-Beaked Common; *D.c.* = Long-Beaked Common; *T.t.* = Bottlenose

* OR = Oregon; WA= Washington

* SA = Subadult; A= Adult; U = Unknown

* F= Female; M = Male; U= Unknown

Stranding Seasons

Total, there were six (12%) reported strandings in the spring, seven (14%) in the summer, fifteen (31%) in the fall, and twenty-one (43%) in the winter between 2006 and 2014. The majority of the fall strandings was comprised of the short-beaked common dolphin and the majority of the winter strandings was comprised of the striped dolphin.

Between 2006 and 2014 there was no observed temporal trend in PWSD strandings besides a slight increase in the winter season. There were three (23%) strandings in the spring, two (15%) in the summer, three (23%) in the fall, and five (39%) in the winter.

Between 2006 and 2014, the striped dolphin stranded between January-March, July, and October-December. There seems to be an observed temporal trend for winter strandings since there were only two (10%) reported strandings in the spring, three (14%) in the summer, three (14%) in the fall, but thirteen (62%) in the winter.

Between 2006 and 2014, the common dolphin stranded in January (one short-beaked), March (one long-beaked), and every month between September-December (one long-beaked in November and the remainder were short-beaked common). Season wise, the short-beaked common dolphin has an observed temporal trend to strand during the fall, since there were eight (80%) strandings in the fall and two (20%) strandings in the winter. The long-beaked common dolphin had one (50%) stranding in the spring and one (50%) in the fall. Since there were only two individuals, there was no observed temporal trend for the long-beaked common dolphin.

Between 2006 and 2014, there was one reported bottlenose stranding in January and two in July. Season wise, there were two (67%) reported strandings in the summer and one (33%) in the winter. Since there were only three individuals, there was no observed temporal trend.

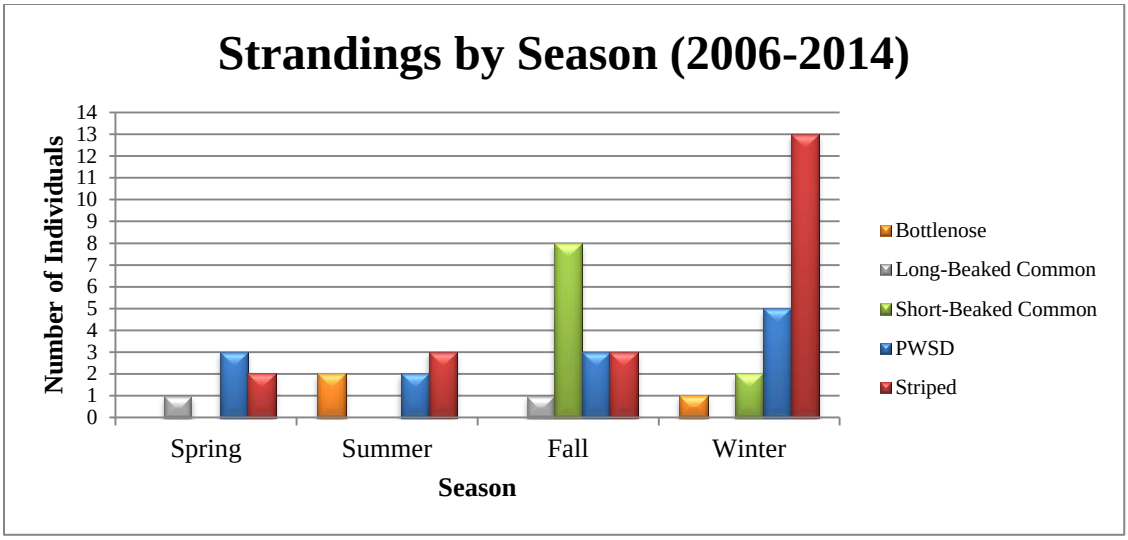

Figure 5: Strandings by Season and Species (2006-2014)

Nervous System Disorders

General Findings

To my knowledge and request, fifteen individuals came back with histopathological lesions suspicious of neurobrucellosis out of the individuals that had histology performed between 2006 and 2014. Out of the fifteen individuals, there were two (13%) PWSDs, nine striped dolphins (60%), three short-beaked common dolphins (20%), and one long-beaked common dolphin (7%). Two (13%) of these strandings occurred in 2006, six (40%) occurred in 2012, and seven (47%) occurred in 2014. I did not receive any bottlenose histology reports that detailed histopathological manifestations resembling neurobrucellosis. It is known, however, that at least two of the individuals were too decomposed to be able to perform a complete necropsy and obtain viable tissue for histology. As previously mentioned, I did not receive any histology reports on individuals that did not have neurobrucellosis-like lesions, so the total number of dolphins receiving histology is unknown for this study.

Brucella ceti Analyses

Out of the fifteen individuals that had histopathological manifestations resembling neurobrucellosis, ten (71%) tested positive for *Brucella*, four (29%) were negative, and one did not have any specific *Brucella* tests performed at the time of this study. *Brucella* positive individuals included 6/8 (75%) striped dolphins (the ninth was not tested specifically for *Brucella*), 3/3 (100%) short-beaked common dolphins, 1/2 (50%) PWSDs, and 0/1(0%) long-beaked common dolphin. Although different individuals were tested by culture, PCR, IHC, and/or serology, positive results were only received via culture and serology (Table 2). All positive cases that received a sequence type (n=6) were identified as ST26. These included Individuals 6, 7, 9, 10, 11, and 12.

Individual	Species	Date	State	Age	Sex	Culture	PCR	Serology	IHC	Histopathological Diagnosis
1	L.o.	Jan 16, 2006	WA	A	F	NA	- (AHC)	NA	NA	Marked, multifocal, necrotising, nonsuppurative encephalitis with scattered microgliosis with clusters of intra and extracellular oblong basophilic deposits
2	D.d.	Nov 29, 2006	WA	SA	F	NA	- (AHC) - Mes. LN (MAIE)	+ (WDA)	Pending (MAIE)	Severe, multifocal to coalescing, nonsuppurative meningoencephalitis with prominent perivascular lymphoplasmacytic cuffing, satellitosis and acute subcortical hemorrhage
3	D.c.	Mar 28, 2012	WA	SA	F	- (CODAG)	- (AHC)	NA	NA	Marked, focally extensive, necrotising meningoencephalitis with variably extensive meningeal fibrosis, numerous acicular clefts, and multifocal lymphoplasmacytic perivascular cuffing
4	S.c.	July 14, 2012	WA	A	M	- (NVSL)	- (AHC)	NA	NA	Marked, diffuse, nonsuppurative meningitis with circumferential, peripheral myelin vacuolation and occasional malacia (spinal cord; 5-6 cervical vertebrae)
5	S.c.	July 23, 2012	WA	SA	M	NA	- (UI)	NA	NA	Severe, chronic, nonsuppurative meningoencephalomyelitis
6	S.c.	Dec 5, 2012	OR	SA	M	+ (NVSL)	- (UI)	NA	NA	Moderate to severe, nonsuppurative meningoencephalomyelitis
7	L.o.	Dec 10, 2012	OR	SA	F	+ (NVSL)	- (UI)	NA	NA	Lymphoplasmacytic meningoencephalitis
8	S.c.	Dec 10, 2012	OR	SA	M	+ (OSU)	- (UI)	NA	NA	Lymphoplasmacytic meningoencephalitis
9	S.c.	Feb 19, 2014	OR	A	M	+ (NVSL)	NA	NA	NA	Severe, nonsuppurative meningitis, choroid plexitis, and perivasculitis
10	S.c.	Feb 20, 2014	OR	SA	F	+ (NVSL)	NA	NA	NA	Severe, lymphocytic meningitis, encephalitis, myelitis, and radiculoneuritis
11	S.c.	Feb 21, 2014	OR	SA	M	+ (NVSL)	NA	NA	NA	Severe, lymphocytic meningitis (brain and spinal cord)
12	S.c.	Mar 17, 2014	WA	A	M	+ (NVSL)	NA	NA	- (USDA)	Marked, nonsuppurative meningoencephalomyelitis and root ganglioneuritis
13	D.d.	Oct 25, 2014	WA	SA	F	+ (NVSL)	NA	NA	NA	Severe, nonsuppurative meningomyelitis and root ganglioneuritis
14	D.d.	Nov 9, 2014	WA	SA	M	+ (NVSL)	- (AHC)	+ (WADDL)	- (NVSL)	Severe, nonsuppurative meningoencephalomyelitis
15	S.c.	Dec 27, 2014	OR	A	F	NA	NA	NA	NA	Severe, diffuse, lymphocytic meningitis; Mild, multifocal lymphocytic encephalitis; Focal lymph node pyogranuloma

Table 2: Overview of Stranded Individuals Suspicious of Neurobrucellosis

*L.o. = PWSD; S.c. = Striped; D.d. = Short-Beaked Common; D.c. = Long-Beaked Common

* NVSL= National Veterinary Services Laboratory; AHC= Animal Health Center; WADDL= Washington Animal Disease Diagnostic Lab; WDA = Washington Department of Agriculture; NWZP = Northwest ZooPath; MAIE = Mystic Aquarium & Institute for

Exploration; UI = University of Iowa; CODAG = Colorado Department of Agriculture; USDA = United States Department of Agriculture

* A = Adult; SA = Subadult

* F= Female; M = Male

Individual	Animal I.D.
1	CRC-702
2	CRC-779
3	CRC-1200
4	MKH2012-025
5	PSU-12-07-23Sc
6	HMSC-12-12-05Sc
7	HMSC-12-12-10Lo
8	HMSC-12-12-10Sc
9	PSU-14-02-19Sc
10	HMSC-14-02-20Sc
11	HMSC-14-02-21Sc
12	MKH2014-002
13	CRC-1462
14	MKH2014-29
15	HMSC-14-12-27Sc

Table 3: Identification Key

-Stranding Location-

Out of all the positive *Brucella* individuals, there were six (60%) strandings in Oregon and four (40%) in Washington. There were five (83%) striped dolphins that stranded in Oregon and one (17%) that stranded in Washington. Three out of three (100%) positive short-beaked common dolphins stranded in Washington and the single positive PWSD stranded in Oregon. As seen from Table 2, multiple individuals would strand within the same month in the same state. For example, the three positive 2012 cases all occurred in December and all occurred in Oregon. Also, three individuals stranded in February of 2014, which also all occurred in Oregon. Contrarily, the remainder of the 2014 *Brucella* cases all occurred in Washington.

Location	All Strandings	*Brucella* Positive	All Strandings Excluding *Brucella* Individuals
Oregon	76%	83%	73%
Washington	24%	17%	27%

Table 4: Stranding Location Comparisons of the Striped Dolphin (2006-2014)

Location	All Strandings	*Brucella* Positive	All Strandings Excluding *Brucella* Individuals
Oregon	70%	0%	100%
Washington	30%	100%	0%

Table 5: Stranding Location Comparisons of the Short-Beaked Common Dolphin (2006-2014)

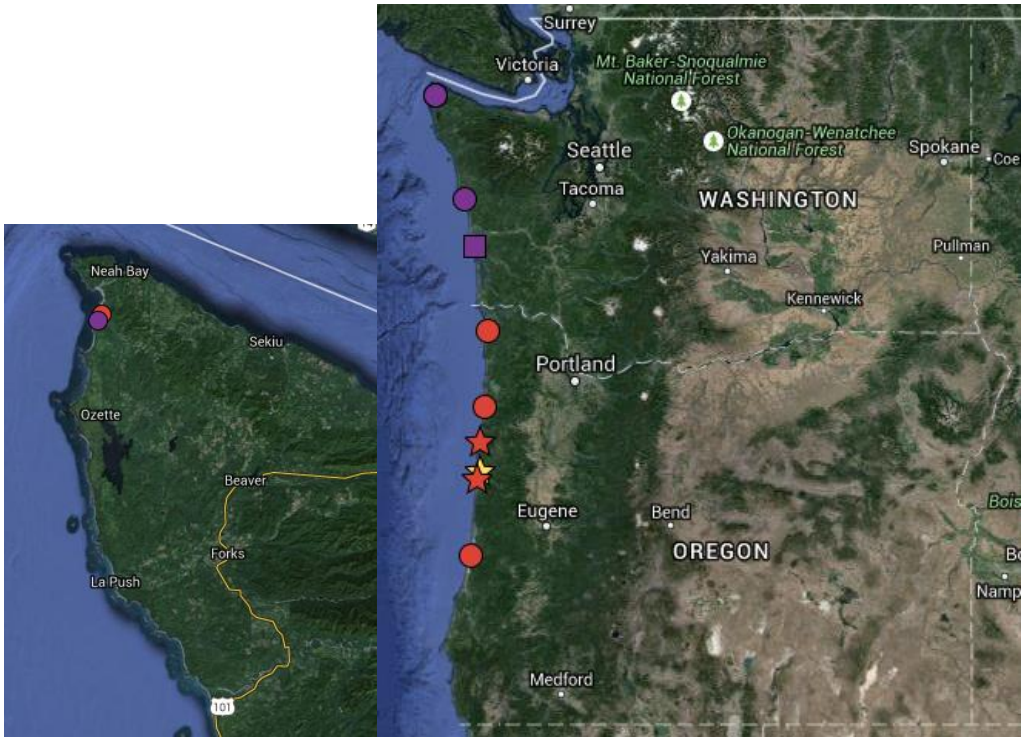

Figure 6: Stranding Location of Positive *Brucella* Cases

* Yellow= PWSD; Red = Striped; Purple = Short-Beaked Common Dolphin

* Square = 2006; Star = 2012; Circle = 2014

Age Class

Overall, 80% of the *Brucella* positive individuals were reported to be subadults. This included 3/3 (100%) short-beaked common dolphins, the single PWSD, and 4/6 (67%) striped dolphins.

Age Class	All Strandings	*Brucella* Positive	All Strandings Excluding *Brucella* Individuals
Subadult	52%	67%	40%
Adult	38%	33%	47%
Unknown	10%	0%	13%

Table 6: Age Class Comparisons of the Striped Dolphin (2006-2014)

Age Class	All Strandings	*Brucella* Positive	All Strandings Excluding *Brucella* Individuals
Subadult	50%	100%	29%
Adult	50%	0%	71%
Unknown	0%	0%	0%

Table 7: Age Class Comparisons of the Short-Beaked Common Dolphin (2006-2014)

Sex

Out of the *Brucella* positive individuals, there were six (60%) males and four (40%) females. Out of the striped dolphin, there were five (83%) males and one female (17%). There were two (67%) female short-beaked common dolphins and one (33%) male. The sole PWSD was female.

Sex	All Strandings	*Brucella* Positive	All Strandings Excluding *Brucella*
Male	48%	83%	33%
Female	33%	17%	40%
Unknown	19%	0%	27%

Table 8: Sex Comparisons of the Striped Dolphin (2006-2014)

Sex	All Strandings	*Brucella* Positive	All Strandings Excluding *Brucella*
Male	40%	33%	43%
Female	50%	67%	43%
Unknown	10%	0%	14%

Table 9: Sex Comparisons of the Short-Beaked Common Dolphin (2006-2014)

Chapter Five

-Discussion-

Overall Stranding History

Stranding Numbers

Reported strandings were inconsistent prior to 2002, but funding from stranding networks through the "John H. Prescott Marine Mammal Rescue Assistance Grant Program" has significantly improved stranding responses since then (Jim Rice, personal communication). Although this could have contributed to the overall increases seen within the data, the number of strandings in 2006, 2012, and 2014 still seem suspiciously high, and may have been influenced by an environmental factor and/or an influx of disease, which is further discussed in the subsequent sections below.

-Strandings by Species-

Between 2006 and 2014 the striped dolphin was the most common species to strand and the short-beaked common dolphin only had three fewer strandings than the PWSD. Since the striped dolphin and the short-beaked common dolphin do not normally inhabit Oregon and Washington waters, the amount of strandings as well as their stranding rank is suspicious. It is important to keep in mind, however, that advancements in technology (i.e. cell phones) could have contributed to the rise in strandings seen within the uncommon species. Since high-quality photos of a stranded individual can now be taken, sent, and received in a matter of seconds, the accuracy of identifying the

species of the individual vastly increases or is even absolute, compared to solely relying on a verbal description from a passerby.

We may also be seeing a high number of striped dolphin strandings in the PNW due to the possibility of their range expanding northward from warming sea temperatures (Allen et al., 2011). Since the striped dolphin is associated with convergence zones of warm and colder waters, they may be on the leading edge of the shift in ranges that is being seen with warmer water species (Allen et al., 2011). Because of this, we may also see an increase in strandings for the remainder of the uncommon species, as well as more strandings in Washington and farther north into Canada due to predicted warmer sea temperatures and climate change.

-Stranding Seasons-

As discussed in the results, the majority of the strandings occurred in winter (December-February) and was closely followed by fall (September-November). The winter strandings were primarily comprised of the striped dolphin, and the fall was primarily comprised of the short-beaked common dolphin. Environmental factors, such as changes in sea surface temperature and/or the movement of the dolphins' preferred food sources, could potentially be a reason as to why there has been an increase in reported strandings of the uncommon species, as well as why we are seeing more of one species strand in a specific season over others.

Research has, and will continue to be conducted on relationships between environmental variability and recruitment of an array of fish species, cephalopods, copepods, etc., focusing on the roles of local upwelling vs. large basin-scale climate

cycles (Peterson et al., 2014). These cycles include the El Niño Southern Oscillation (ENSO), the Pacific Decadal Oscillation (PDO), and the North Pacific Gyre Oscillation (NPGO) (Peterson et al., 2014). Looking more closely at seasonal and interannual changes in coastal upwelling and food chain structures of these dolphin species could provide more insight as to whether environmental factors have influenced the increases in strandings seen in the data. Unfortunately, due to time constraints, this was not further assessed within this thesis, but should be in future studies.

Nervous System Disorders

General Findings

As seen in the results, individuals that stranded with neurobrucellosis-like histopathological lesions only stranded in 2006, 2012, and 2014. Each subsequent year had more individuals strand with these lesions and more individuals test positive for *Brucella* than the previous. However, 2006, 2012, and 2014 also had the highest number of reported strandings since 1975 as well. Without seeing the other histology reports, it is difficult to determine whether this is a meaningful increase in *Brucella* disorders, or if the increase is driven by more stranded individuals overall. The increase could have also been due to more performed necropsies and histology reports. It is suspicious, however, that the *Brucella* strandings did not occur sporadically throughout each stranding year, but rather within the same season, and many times within the same month.

Gonzàlez et al.'s (2002) study detailed three striped dolphins that stranded in a period of a month. Their rationale behind this "indicate[d] the contribution of an unrecognized, perhaps environmental, influence at a given time" (p. 151). As previously

discussed, environmental influences can include oil spills or a change in water temperature and food chain structures, which can alter the susceptibility of individuals to infection, leading them to strand. It has been known since the beginning of medical science that a change in weather can lead to the emergence of epidemic diseases (National Resource Council, Committee on Climate, Ecosystems, Infectious Disease, and Human Health, 2001). Factors such as temperature, precipitation, and humidity can all affect the life cycle of pathogens, potentially altering the timing and intensities of disease outbreaks, and can also increase the introduction of vectors and animal reservoirs (National Resource Council, Committee on Climate, Ecosystems, Infectious Disease, and Human Health, 2001; Sachan and Singh, 2010). Since the majority of the striped and short-beaked common dolphins in this thesis stranded when the water temperatures were colder, there is a possibility that these individuals swam farther north to the PNW, thriving during the warmer months, but became more susceptible to infection when the water temperatures dropped below a certain threshold. Although individuals may be more susceptible to infection when the water is colder, incubation periods of *Brucella ceti* is still unknown and may vary between different individuals and/or species, hence why multiple individuals strand within a few months, and even days, of each other in very specific years. However, more data would be needed to support or refute this hypothesis.

Another interesting discovery was that Individuals 2, 3, and 13 all had a previous shark bite wound and were all common dolphins (two short-beaked and one long-beaked). Besides these three, the only other individual to have a noted shark bite wound in the Level A database was a PWSD that was too decomposed to perform a complete necropsy. Shark bite wounds can cause substantial injury and could alter the individual's

susceptibility to disease or capability to fight off disease. Also, if an individual is experiencing altered swimming behaviors due to nervous system ailments from an infectious agent, this could also increase their susceptibility to a shark attack.

Brucella ceti Analyses

-General Findings-

If neurobrucellosis was suspected after histology, then verification of *Brucella* by laboratory tests most often came back positive.

-*Brucella ceti* Tests-

Positive *Brucella* cases for this study were reported via culture and serology only. Culture is considered to be the "gold standard" in testing (Wu et al., 2014), but serology is more difficult to decipher. Two individuals, 2 and 14, were tested via serology in this study. Individual 2 was only tested by serology but Individual 14 had a culture and IHC performed in addition to serology. Both individuals are considered positive rather than suspect-positive since Individual 2 came back as "Brucella-RAP positive and Rivinol positive +200" and Individual 14 had positive culture isolates.

Within this study there was a high rate of false negative results, particularly with PCR. There was not a single PCR test that came back positive, making it the least effective test out of all four, in my opinion. The AHC performs PCR on pooled tissue samples, which typically include brain, lung, liver, spleen, and lymph node (Lambourn et al., 2013). Although pooling techniques can increase analytical efficiency and promote cost savings, sensitivity is compromised because it is inversely proportional to the

number of samples in the pool, and a significant portion of the detectable microbial community could be masked (Manter et al., 2010 and Sun et al., n.d.). Although not specific to *Brucella,* other studies have also noted decreases in sensitivity of detection when compared to testing the tissues separately (Grmek-Kosnik et al., 2006 and Manter et al., 2010). IHC also did not provide any positive results despite one individual coming back positive via serology and the other coming back positive on culture. As previously mentioned, it has been recognized that IHC has lower sensitivity in identifying *Brucella* compared to serology (Gonzàlez-Barrientos et al., 2010).

The amount of false negatives is indicative that the success rate of these tests are based on how sensitive a particular pathologist's or lab's tests are, what part/which tissues are tested (e.g. was the affected part of the nervous system tested or was a sample taken from an unaffected section?), and how viable the tissue samples are (e.g. was the tissue frozen and thawed multiple times?). Due to all the factors that can provide a false negative result, I believe that 71% is an underestimate of how many individuals are truly *Brucella* positive. Again, this statistic may be subject to change after further tests are performed.

-Species-

It is believed that there are low proportions of other cetacean species that show *Brucella* associated clinicopathological signs, besides the striped dolphin (Isidoro-Ayza et al., 2014). However, the results of this paper details at least two other species, the short-beaked common dolphin and PWSD, that stranded with neurobrucellosis-like

manifestations and tested positive for *Brucella.* To my knowledge, this is the first study to publish confirmation of *Brucella* within a PWSD.

This study also reconfirmed the well-established fact that *Brucella* infections are most prominent in striped dolphin species and supports Hernàndez-Mora et al.'s (2008) study that the striped dolphin is a highly susceptible host and even a potential reservoir for the transmission of *Brucella ceti.* According to Allen et al. (2011), short-beaked common dolphins have been periodically observed in schools of striped dolphins. If the striped dolphin is a reservoir for *Brucella ceti* and periodically associates with the short-beaked common dolphin, that could explain why the short-beaked common dolphin is the second most susceptible dolphin species to *Brucella* in the PNW. More information on vertical and horizontal transmission between species would be needed, however, to make any further claims.

-Location-

Although the majority (83%) of the positive striped dolphins stranded in Oregon, the observed trend seems to be influenced by the normal stranding pattern of the species, and would not be considered a predilection at this time. However, there seems to be an observed predilection for positive short-beaked common dolphins to strand in Washington, since 100% of the short-beaked strandings in Washington ended up being *Brucella* positive. Although there were only three positive short-beaked dolphins at this time, the observed predilection should be taken into consideration on impending strandings.

It is important to keep in mind that a carcass might strand hundreds of kilometers from their normal range and/or from where they actually died, since carcass movement can be affected by wind and water currents, the height of the carcass above the water line, upwelling, and downwelling (Norman et al., 2004). However, a carcass that drifts that far is not normally fresh enough for a complete necropsy or meaningful histopathology results. Dolphins typically sink when they die, and re-float once gasses build up inside them from decomposition (Jim Rice, personal communication). Because of this, the majority of dolphin carcasses never come close to shore and those that do, are normally found freshly dead and in good enough condition for histopathology (Jim Rice, personal communication).

Also, although the PWSD and short-beaked common dolphin can generally be seen in coastal and oceanic waters, the striped dolphin is mainly pelagic (Allen et al., 2001). Therefore, the presence of them is suspicious and can indicate that they were neurologically debilitated, venturing into waters that they normally would not venture into if they were lucid.

-Age Class-

The findings of age class supports this thesis' hypothesis as well as Gonzàlez et al.'s (2002) study noting the greater probability of subadults to develop neurobrucellosis compared to adults. Although there were a couple adult striped dolphins that came back *Brucella* positive, the remainder were subadult individuals. This does not fit the "normal" stranding trends observed from the historical data, which showed roughly half of the strandings being subadult and half being adult. Why we are seeing *Brucella* more in

subadult individuals than adults is still to be determined. There were a decent amount of unknown age classes, however, which may have changed the results of this study if they were known.

Lambourn et al. (2013) noted the possibility of subadults to cease producing *Brucella* antibodies or even clearing infection, subsequently coming back negative on serology tests. Although this was pertaining to *Brucella pinnipedialis* in harbor seals, it could possibly be applicable to *Brucella ceti* as well. It is also not known if individuals can be reinfected and if so, the consequent antibody response to that reinfection (Lambourn et al., 2013). Although the serology tests came back positive for this study, this should be taken into consideration in future studies if negative serology tests are obtained.

-Sex-

There seems to be an observed predilection towards striped male dolphins. Striped dolphins have complex systems of individuals organized by age, sex, and breeding status (Allen et al., 2011). According to Gaspari et al.'s (2007) study, female striped dolphins have higher average kinship within groups rather than between groups. Females were also found to associate more with adult kin than males. Assuming that *Brucella ceti* can be passed horizontally, that would make sense why we are seeing more males with *Brucella* than females, since the males associate more between different groups and are not preferring to associate only with adult kin. There did not seem to be an observed predilection for the short-beaked common dolphin at this time.

Chapter Six

-Conclusion-

Overall Findings

This thesis documents the presence of *Brucella ceti* in dolphin species along the Oregon and Washington coast. According to the results, there were subsequent increases in dolphins that stranded with neurobrucellosis-like manifestations in 2006, 2012, and 2014. Since they occurred in very specific years and seasons, the increases may have been due to an unknown environmental influence, altering their susceptibility to the disease. Or, the increases may have been driven by an overall rise in strandings, which could have also occurred due to an unknown environmental influence. This thesis also outlines the high susceptibility of the striped dolphin to be infected with *Brucella ceti* followed by the short-beaked common dolphin. This study is also the first, to my knowledge, to document *Brucella* in a PWSD. Other observed predilections at this time include male striped dolphins, subadult individuals of all species, and short-beaked common dolphins that strand in Washington. Once again, these observed trends are based on a small sample size and the results may be subject to change based on re-testing some negative individuals as well as future strandings.

The high rate of false negative tests (i.e. PCR and IHC tests) reinforces the suspicion that there is an underestimate in positive *Brucella* cases among the individuals that stranded with histopathological manifestations resembling neurobrucellosis. Further

tests will be conducted on individuals with available and viable samples, so the current results may be subject to change. Determining whether or not neurobrucellosis was the cause of these neurological disorders can also be difficult to conclude, as an array of other infectious agents can cause similar manifestations. For example, as previously stated, viral infections are responsible for a vast amount of neurological diseases within the striped dolphin, and encephalitis caused by fungal origins and *Toxoplasma spp.* have been documented as secondary complications of morbillivirus within the striped dolphin as well (Gonzàlez et al., 2002). This is especially true since it can be difficult to detect *Brucella* organisms (as well as other organisms) directly in infected tissues (Seidel et al., 2003). This thesis does conclude, however, that individuals with histopathological manifestations suspicious of neurobrucellosis most often come back positive for *Brucella* on further tests. Because of this, any individual remotely suspicious for neurobrucellosis (i.e. strandings of the demographics discussed or suspicion of a debilitated nervous system) should be deemed high risk for having *Brucella* and appropriate precautionary measures should be implemented to avoid zoonotic exposure.

Future Studies and Suggestions

Unknown Cases

Although this study only went back to 2006, there were two 2003 individuals found in older records that were suspicious for neurobrucellosis and subsequently tested positive for *Brucella* via serology. One case was an adult, male, Northern right whale dolphin that stranded in Seattle, Washington in March of 2003. If more time was

available for this study, I would have liked to have included the Northern right whale dolphin in my list of analyzed species. The other individual stranded in February of 2003 and was an adult, female, common dolphin. This individual stranded in Long Beach, but it was unable to be determined if it was Long Beach, Washington or Long Beach, British Columbia, Canada. There was no record of this individual in my Level A data and I only received a small excerpt, detailing the histopathology results. Knowing more information about this individual will be helpful in any future studies.

Expected Sea Temperature Changes

The region of the North Pacific Ocean has been the warmest on record due to what has been nicknamed by climate scientist, Nick Bond, to be "the blob" (Hickey, 2015 and Milstein, 2014). One factor that has been impacting the California coast is a low-pressure trough between California and Hawaii (Milstein, 2014). The winds that typically drive upwelling of deep, cold water has been weakened by the low-pressure trough, resulting in warmer waters that have been persisting longer than usual (Milstein, 2014). Besides a narrow strip of cold water along the PNW coast that is being fed by upwelling from the deep ocean, the North Pacific Ocean sea surface temperature has increased as much as 3°C (Milstein, 2014). This change in water temperature has and will continue to favor warmer water species, such as the dolphins in this study, and will have detrimental impacts on marine populations preferring a colder, more productive ocean (Milstein, 2014 and Profita, 2015). A survey of whales and dolphins off the West coast revealed marine mammals, as well as other marine fauna, farther north from their normal ranges due to the unusually warm waters (Profita, 2015). In fact, one hundred common dolphins

were documented in an area not normally seen due to this warm water surge (CBS SF, 2014).

There is also an estimated 65% chance that El Niño will arrive later in 2015, which is a separate warming event from the blob (Milstein, 2014). ENSO events can impact sea surface temperature and current patterns, which can lead to warmer water temperature and a change in cetacean species distributions (Norman et al., 2004). This will only reinforce the warming seen from the blob and will further have an impact on marine ecosystems.

Studying linkages between climate and disease can provide understanding on factors that might drive the emergence of seasonal or interannual variations in diseases such as *Brucella* (National Resource Council, Committee on Climate, Ecosystems, Infectious Disease, and Human Health, 2001). Because of this, stranding data from this year and the next couple years will be vital in order to assess stranding patterns based on this change in water temperature and its potential impacts on the dolphins' susceptibility to infection.

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Appendix A: Level A Data Sheet

MARINE MAMMAL STRANDING REPORT - LEVEL A DATA

FIELD #: _____ **NMFS REGIONAL #:** _____ (NMFS USE) **NATIONAL DATABASE#:** _____ (NMFS USE)

COMMON NAME: _____ **GENUS:** _____ **SPECIES:** _____

EXAMINER Name: _____ Affiliation: _____

Address: _____ Phone: _____

Stranding Agreement or Authority: _____

LOCATION OF INITIAL OBSERVATION

State: _____ County: _____

City: _____

Body of Water: _____

Locality Details: _____

_____

Lat (DD): _____ . _____ N

Long (DD): _____ . _____ W

☐ Actual ☐ Estimated

How Determined: (check ONE)

☐ GPS ☐ Map ☐ Internet/Software

OCCURRENCE DETAILS ☐ Restrand GE# _____ (NMFS Use)

Group Event: ☐ YES ☐ NO

If Yes, Type: ☐ Cow/Calf Pair ☐ Mass Stranding # Animals: _____ ☐ Actual ☐ Estimated

Findings of Human Interaction: ☐ YES ☐ NO ☐ Could Not Be Determined (CBD)

If Yes, Choose one or more: ☐ 1. Boat Collision ☐ 2. Shot ☐ 3. Fishery Interaction

☐ 4. Other Human Interaction: _____

How Determined (Check one or more): ☐ External Exam ☐ Internal Exam ☐ Necropsy

☐ Other: _____

Gear Collected? ☐ YES ☐ NO Gear Disposition: _____

Other Findings Upon Level A: ☐ YES ☐ NO ☐ Could Not Be Determined (CBD)

If Yes, Choose one or more: ☐ 1. Illness ☐ 2. Injury ☐ 3. Pregnant ☐ 4.Other: _____

How Determined (Check one or more): ☐ External Exam ☐ Internal Exam ☐ Necropsy

☐ Other: _____

INITIAL OBSERVATION

Date: Year: _____ Month: _____ Day: _____

First Observed: ☐ Beach or Land ☐ Floating ☐ Swimming

CONDITION AT INITIAL OBSERVATION (Check ONE)

☐ 1. Alive ☐ 4. Advanced Decomposition

☐ 2. Fresh dead ☐ 5. Mummified/Skeletal

☐ 3. Moderate decomposition ☐ 6. Condition Unknown

LEVEL A EXAMINATION ☐ Not Able to Examine

Date: Year: _____ Month: _____ Day: _____

CONDITION AT EXAMINATION (Check ONE)

☐ 1. Alive ☐ 4. Advanced Decomposition

☐ 2. Fresh dead ☐ 5. Mummified/Skeletal

☐ 3. Moderate decomposition ☐ 6. Unknown

INITIAL LIVE ANIMAL DISPOSITION (Check one or more)

☐ 1. Left at Site ☐ 6. Euthanized at Site

☐ 2. Immediate Release at Site ☐ 7. Transferred to Rehabilitation:

☐ 3. Relocated Date: Year:_____ Month:_____Day:___ Facility:_____

☐ 4. Disentangled ☐ 8. Died during Transport

☐ 5. Died at Site ☐ 9. Euthanized during Transport

☐ 10. Other: _____

CONDITION/DETERMINATION (Check one or more)

☐ 1. Sick 7. Location Hazardous

☐ 2. Injured ☐ a. To animal

☐ 3. Out of Habitat ☐ b. To public

☐ 4. Deemed Releasable ☐ 8. Unknown/CBD

☐ 5. Abandoned/Orphaned ☐ 9.Other_____

☐ 6. Inaccessible _____

MORPHOLOGICAL DATA

SEX (Check ONE)

☐ 1. Male

☐ 2. Female

☐ 3. Unknown

AGE CLASS (Check ONE)

☐ 1. Adult ☐ 4. Pup/Calf

☐ 2. Subadult ☐ 5. Unknown

☐ 3. Yearling

☐ Whole Carcass ☐ Partial Carcass

Straight length:_____ ☐ cm ☐ in ☐ actual ☐ estimated

Weight:_____ ☐ kg ☐ lb ☐ actual ☐ estimated

PHOTOS/VIDEOS TAKEN: ☐ YES ☐ NO

Photo/Video Disposition: _____

_____

TAG DATA Tags Were:

Present at Time of Stranding (Pre-existing): ☐ YES ☐ NO

Applied during Stranding Response: ☐ YES ☐ NO

ID#	Color	Type	Placement* (Circle ONE)	Applied	Present
			D DF L LF LR RF RR	☐	☐
			D DF L LF LR RF RR	☐	☐
			D DF L LF LR RF RR	☐	☐

* D= Dorsal; DF= Dorsal Fin; L= Lateral Body
LF= Left Front; LR= Left Rear; RF= Right Front; RR= Right Rear

CARCASS STATUS (Check one or more)

☐ 1. Left at Site ☐ 4. Towed: Lat_____Long_____ ☐ 7. Landfill

☐ 2. Buried ☐ 5. Sunk: Lat_____Long_____ ☐ 8. Unknown

☐ 3. Rendered ☐ 6. Frozen for Later Examination ☐ 9. Other_____

SPECIMEN DISPOSITION (Check one or more)

☐ 1. Scientific collection ☐ 2. Educational collection

☐ 3. Other: _____

Comments: _____

_____

NECROPSIED ☐ NO ☐ YES ☐ Limited ☐ Complete

☐ Carcass Fresh ☐ Carcass Frozen/Thawed

NECROPSIED BY: _____

Date: Year: _____ **Month:** _____ **Day:** _____

NOAA Form 89-864; OMB Control No.0648-0178; Expiration Date 01/31/2017 PLEASE USE THE BACK SIDE OF THIS FORM FOR ADDITIONAL REMARKS

ADDITIONAL REMARKS

ADDITIONAL IDENTIFIER: _____ (If animal is restranded, please indicate any previous field numbers here)

DISCLAIMER

THESE DATA SHOULD NOT BE USED OUT OF CONTEXT OR WITHOUT VERIFICATION. THIS SHOULD BE STRICTLY ENFORCED WHEN REPORTING SIGNS OF HUMAN INTERACTION DATA.

DATA ACCESS FOR LEVEL A DATA

UPON WRITTEN REQUEST, CERTAIN FIELDS OF THE LEVEL A DATA SHEET WILL BE RELEASED TO THE REQUESTOR PROVIDED THAT THE REQUESTOR CREDIT THE STRANDING NETWORK AND THE NATIONAL MARINE FISHERIES SERVICE. THE NATIONAL MARINE FISHERIES SERVICE WILL NOTIFY THE CONTRIBUTING STRANDING NETWORK MEMBERS THAT THESE DATA HAVE BEEN REQUESTED AND THE INTENT OF USE. ALL OTHER DATA WILL BE RELEASED TO THE REQUESTOR PROVIDED THAT THE REQUESTOR OBTAIN PERMISSION FROM THE CONTRIBUTING STRANDING NETWORK AND THE NATIONAL MARINE FISHERIES SERVICE.

PAPERWORK REDUCTION ACT INFORMATION

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NOAA Form 89-864; OMB Control No.0648-0178; Expiration Date 01/31/2017

Appendix B: Histopathology Results

Individual	Animal I.D.
1	CRC-702
2	CRC-779
3	CRC-1200
4	MKH2012-025
5	PSU-12-07-23Sc
6	HMSC-12-12-05Sc
7	HMSC-12-12-10Lo
8	HMSC-12-12-10Sc
9	PSU-14-02-19Sc
10	HMSC-14-02-20Sc
11	HMSC-14-02-21Sc
12	MKH2014-002
13	CRC-1462
14	MKH2014-29
15	HMSC-14-12-27Sc

Individual one was an adult, female, PWSD that had marked, multifocal, necrotising, nonsuppurative encephalitis which was deemed to be severe enough to cause antemortem morbidity and the death of this individual. The brain also had scattered microgliosis with intracellular and extracellular clusters of oblong basophilic deposits. This was the only individual to have encephalitis and not have a meningeal predilection that is commonly seen in other *Brucella* cases. According to the pathologist, the intralesional structures were suggestive of a protozoal infection, which, after a protozoal PCR test, came back positive for *Sarcocystis neurona*. PCR was negative for morbillivirus and negative for *Brucella*. An aerobic culture was performed on the brain, lung, lymph nodes, and small intestines of the animal, but the only bacteria to be isolated were a light to moderate mix of *Aeromonas hydrophila, Enterobacter spp, Enterococcus spp.*, and *Rahnella aquatilis.* According to the pathologist, these bacterial isolates most likely occurred postmortem. A specific *Brucella* culture was not performed.

Individual two was a subadult, female, short-beaked common dolphin that had severe, multifocal to coalescing, nonsuppurative meningoencephaltis with prominent

periviascular lymphoplasmacytic cuffing, satellitosis, and acute subcortical hemorrhage. According to the pathologist, the meningitis and acute multifocal hemorrhaging attributed to the death of this animal. PCR attempts came back negative for *Brucella* and morbillivirus and there were no bacterial growths seen from the lung, lymph node, brain, spleen, uterine, or small intestines of the animal. It is important to note that a specific *Brucella* culture was not done and PCR was performed on a mesenteric lymph node and not on nervous system tissue. Virology tests also came back negative for this individual. This animal did come back *Brucella* positive via serology (RAP positive and Rivinol positive +200), however, and IHC tests for *Brucella* are currently pending as well.

Individual three was a subadult, female, long-beaked common dolphin that had marked, focally extensive, necrotising meningoencephalitis with variably extensive meningeal fibrosis, numerous acicular clefts, and multifocal lymphoplasmacytic perivascular cuffing. The skull had moderate meningeal to periosteal adhesions on gross findings with an accumulation of clear fluid. According to the pathologist, the meningeal fibrosis and presumptive adhesion to the skull were severe enough to account for the cerebrospinal fluid accumulation and the death of this animal. This animal did not come back positive for *Brucella* via culture or PCR. IHC and PCR came back negative for protozoa (*Toxoplasma gondii, Sarcocystis neurona,* and *Neospora caninum*), PCR came back negative for Apicomplexa, and PCR came back negative as well for morbillivrus. Although no significant bacteria were recovered from sampled tissues, the pathologist still believes these ailments were due to a bacterial infection.

Individual four was an adult, male, striped dolphin that had marked, diffuse, nonsuppurative meningitis of the spinal cord at the 5-6 cervical vertebrae level with

circumferential, peripheral myelin vacuolation and occasional malacia. According to the pathologist, the cervical meningitis likely contributed to the antemortem morbidity and death of this animal and an infectious agent is a prime consideration. IHC came back negative for *Toxoplasma gondii*, *Neospora caninum,* and *Sarcocystis neurona.* Special histochemical stains did not come back positive for any acid fast bacilli, fungal elements, or bacteria. No bacteria were recovered from the brain and PCR of the brain came back negative for *Brucella* and canine distemper virus. Morbillivirus also came back negative and further protozoa PCR is pending. Although the brain did not come back positive with any pathogens, the spinal cord, which was the location of the meningitis, was not tested. This individual's tissues were also submitted for testing after two years of being frozen and thawed multiple times, which can damage cell structures and denature proteins, leading to false negatives.

Individual five was a subadult, male, striped dolphin that had severe, chronic, nonsuppurative meningoencephalomyelitis. PCR came back negative for *Brucella* but culture, serology, and IHC were not performed. Although *Brucella* was not confirmed by PCR, the pathologist's statement was in support of neurobrucellosis since the lesions in this individual strongly resembled the lesions in other striped dolphins described by the CDC that did have neurobrucellosis. Other pathogen types were not strong contenders due to the presence and/or absence of specific microscopic findings subsequently listed: 1) The meningeal predilection was unusually strong for a viral pathogen; 2) there were no microglial nodules within the lymphocytic infiltrate commonly accompanied by a protozoal infection; 3) there was not enough mitotic activity to indicate lymphoma; and 4) the presence of mixed character of cells excluded neoplasia.

Individual six was a subadult, male, striped dolphin that had moderate to severe, nonsuppurative meningoencephalomyelitis. According to the pathologist, the cervical spinal cord section showed that this was not merely a cellular residue from a contained exposure, but ongoing due to the damage seen in the grey and white matter tracts of the spinal cord. Protozoal infection is less likely since there were no microglial nodules presenting with the encephalitis and a virus did not seem likely due to the meningeal predilection over an encephalitis predilection. This individual came back *Brucella* negative by PCR, but positive (ST 26) via *Brucella* culture. Therefore, this individual would be considered to be *Brucella* positive despite the false negative PCR result, which is commonly seen in this study. The pathologist also noted that this individual had a lot of similarities to individual five, which tested negative for *Brucella* by PCR. Since this individual also tested negative by PCR then subsequently tested positive via culture, it only further supports the belief that individual five will be *Brucella* positive if culture or serology is to be conducted in the future.

Individual 7 was a subadult, female, PWSD that had lymphoplasmacytic meningoencephalitis, which according to the pathologist, was suggestive of neurobrucellosis and likely accounted for the stranding and contribution to the death of this animal. An aerobic brain culture came back with 2+ mixed Gram-positive and Gram-negative organisms, which included *Aeromonas spp.* and *Staphylococcus spp.* A specific *Brucella* culture isolated *Brucella* in the brain tissue (ST 26), but a PCR came back negative. Since there were isolates via culture, this animal is considered to be *Brucella* positive.

Individual eight was a subadult, male, striped dolphin that had lymphoplasmacytic meningoencephalitis. According to the pathologists, the lesions in the brain were consistent with neurobrucellosis and were severe enough to have led to the stranding and/or the death of this animal. *Brucella* was cultured in brain tissue despite a negative PCR, and was sent to the CDC for sequence typing. This individual also had negative tests for *Leptospira* and canine distemper virus.

Individual nine was an adult, male, striped dolphin that had severe, nonsuppurative meningitis, choroid plexitis, and perivasculitis. According to the pathologist, these lesions are consistent with those caused by *Brucella. Brucella* was isolated (ST 26) in the individual's cerebrospinal fluid, lung, and pulmonary lymph node. This was the only individual in my dataset to have a positive result from an area other than the nervous system.

Individual ten was a subadult, female, striped dolphin that had severe lymphocytic meningitis, encephalitis, myelitis, and radiculoneuritis. The pathologist noted that although some of the changes were consistent with *Brucella,* a viral infection was also a consideration. This individual came back positive for *Brucella* (ST 26) via culture of the brain but no inclusion bodies typical of Morbillivirus were observed.

Individual eleven was a subadult, male, striped dolphin that had severe lymphocytic meningitis of the brain and spinal cord, which were noted to be typical of the changes observed in *Brucella* infections. This individual came back *Brucella* positive (ST 26) via culture in both the brain and spinal cord.

Individual twelve was an adult, male, striped dolphin that had marked, nonsuppurative meningoencephalomyelitis and root ganglioneuritis. This individual had a negative herpesvirus PCR of the spinal cord and was also negative for morbillivirus testing, despite the pathologist's statement that the lesions were suggestive of a viral infection. According to the pathologist, this individual lacked the microglial nodule formation typically associated with protozoan infection and the suppurative components typically associated with most bacterial infections. *Brucella* came back negative on IHC for this individual, but did come back with positive isolates in the brain via culture.

Individual thirteen was a female, subadult, short-beaked common dolphin that had severe, nonsuppurative meningomyelitis and root ganglioneuritis. Lesions were also found in the meninges and neuropil of the brain, but were much more mild. A small focus of lymphocytic inflammation was also noted in the meninges of the optic nerve. This individual came back positive via culture of the brain with a pending sequence type.

Individual fourteen was a subadult, male, short-beaked common dolphin that had severe, nonsuppurative meningoencephalomyelitis. This individual had negative PCR and IHC results for *Brucella*, but came back positive via serology and culture with a pending sequence type. This indiviudal was also negative for morbillivirus and herpesvirus but has a pending protozal PCR test.

Individual fifteen was an adult, female, striped dolphin that had severe, diffuse, lymphocytic meningitis and mild, multifocal lymphocytic encephalitis. Although no bacterial isolates were recovered from a general aerobic brain culture, a bacterial etiology is still more likely than viral due to the severe meningeal predilection. Sending brain

tissue for a specific *Brucella* culture would be suggested, but no frozen brain was kept from necropsy.