

The Effects of Marine Reserves on Regional Groundfish Diversity within the San
Juan Archipelago, Washington

by

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ABSTRACT

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Abstract

Groundfish populations in the greater Puget Sound region have experienced intense declines as the result of past commercial and recreational fisheries. In recent decades mitigation efforts have involved the utilization of Marine Protected Areas, such as marine reserves. Marine reserves have been shown to support previously stressed groundfish populations by prohibiting the harvest of targeted species within their boundaries. Marine reserves may also influence the population structure and biodiversity of target species outside the protected boundaries. This study investigates the regional effects of established marine reserves on the biodiversity of groundfish within the San Juan Archipelago, Washington. Fishery – Independent survey data was provided by the Washington Department of Fish and Wildlife to analyze three groundfish groups: family *Gadidae* (Cod), family *Hexagrammidae* (Lingcod and Greenlings) and the genus *Sebastes* (Rockfish). Diversity levels for the three species groups were analyzed using two diversity indices for temporal and spatial variations. Habitat and depth preference was also examined to determine what species benefit from the established marine reserves. Species normally associated with complex rocky substrate were shown to significantly prefer that habitat. Thus suggesting that established marine reserves are appropriately located for these targeted species, and the implementation of new reserves should be considered. Significant temporal variations in diversity levels were observed over the eight sampled years, with a decrease in mean diversity levels. Spatial variations in diversity were also observed in all three species groups, accurately describing changes in groundfish population structures throughout the region. Areas of significantly high or low diversity, however, had no correlation to the proximity of an established reserve. The changes in groundfish population structure were unable to be determined due to uncertainties in other variables. Due to the importance placed on marine reserves within this ecosystem the significant variations in biodiversity levels warrants continued monitoring.

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Chapter 1:

Literature Review

Marine ecosystems comprise one of the largest, most dynamic and least understood environments on the planet. Covering two-thirds of Earth's surface, oceans and seas consists of complex interactions between organisms, habitats, and external forces to form diverse ecosystems (NRC 1998). Prior to the evolution of *Homo sapiens* the biotic factors (e.g. species assemblages, predator prey interactions, and primary production) and abiotic factors (e.g. ocean currents, upwelling, and weather) were the primary governing forces (Roughgarden et al. 1998). However, humans should now be considered another factor influencing the function and structure of marine ecosystems (NRC 1998). For several millennia, human population growth, coastal development, industrialization, and more recently the indirect impacts of climate change and ocean acidification have increasingly put pressure on the marine environment and marine resources (Hilborn and Hilborn 2012, Cooley and Doney 2009). One important and widespread anthropogenic stressor is the direct exploitation and overharvest of marine organisms, namely large fish.

Humans have generally been slow to reverse the effects of overfishing, often waiting until fisheries threatened collapse (Myers and Worm 2003). Historically, due to the vast expanse of our oceans, there was a belief that the oceans represented an inexhaustible resource and source of food (Pauly and Watson 2003). Overcapitalization of global fishing efforts, and unregulated open access fisheries are only a few of the causes that have lead to the rapid decline of

global fisheries. There are, however, communities of researchers, policy makers, management agencies, and fishing industry stakeholders who have been aware of the overfishing issues for decades (NRC, 2004). Analyzing national and global fishing data has led many scientists and managers to look for ways to offset the damaging ecological effects of overfishing. Understanding the true magnitude of overfishing may not be possible, because most of the depletion occurred during early periods of exploitation, typically before data were collected on the fishery (Myers and Worm, 2003). Mitigating the effects of overfishing and maintaining a viable fishery, simultaneously, is challenging but necessary to provide support coastal communities dependent on fish and to maintain marine ecosystem function. In response to the growing concern to the state of fisheries, management agencies have begun exploring alternative management practices to restore and maintain these declining resources. The growing utilization of Marine Protected Areas (MPAs) has been cited as a strategy positively affecting populations of marine organisms (Soble and Dahlgren 2004, Palumbi 2001).

Fisheries Management

The problem of overfishing has been of interest to researchers, fisherman and governments well before the modernization of fishing fleets and modern fisheries management practices (Pinnegar and Engelhard 2008). Prior to modern fisheries and management practices, community based management was the normal means of managing a local fishery (Hilborn and Hilborn 2012). This put the fishermen in direct control of their local resource. The conservation needs of the resource were met in part through the intuition and knowledge of the local

peoples responsible for the harvest (Johannes et al. 2000). As fishing fleets have modernized, so have management practices. In recent decades an increased demand for fishery resources on areas well outside of coastal communities has led to a departure from community based fisheries management (Jentof et al. 1998). Overarching governmental agencies have adopted management roles with varying degrees of success (Scheiber 2002). This has led to the identification of modern fisheries management practices as the primary contributors to the depletion of high trophic level large fish.

Most management practices use a single species approach, focused on maintaining the harvest goal of a maximum sustainable yield (MSY) for a particular target species. The contradiction between “maximum” and “sustainable” make this concept controversial (Heneman 2002). The sustainable harvest level of a species is a biological reference point that allows for recruitment to replace the individuals of a species removed by fishing. In many fisheries, analyses of stock assessments and catch data from previous years are used in calculations for setting seasonal MSY; these calculations cannot possibly account for fluctuations inherent with a dynamic population. This approach maximizes short-term profits by assuming knowledge of a maximum sustainable harvest level. An incentive is then placed on fishing directly to the maximum yield. However, reaching MSY is not economically cost effective. Other reference points such as, Optimal Yield (OY), have been employed concurrently with MSY. The Magnuson –Stevens Fisheries Conservation Act (1976) defines “optimum” as the amount of fish that “will provide the greatest overall benefit to

the Nation... taking into account the protection of marine ecosystems” [16 U.S.C 180(28)(A)]. Optimal Yield, unlike MSY, considers ecosystem, social, and economic variables accounting for changes in fish stocks, optimizing the fishery to maximize profit (Goldberg 2002). Thus OY should be much lower than MSY. Failures with OY occur when governing agencies such as The National Marine Fisheries Service (NMFS) permit fisheries management plans that set OY at MSY, negating any positive effects of a lower catch (Goldberg 2002).

Other management strategies have focused on a “bottom up” approach that targets individual fishing communities and fishermen, similar to past fisheries management strategies (Jentof et al. 1998). In the United States, Individual Fishing Quotas (IFQs) or Community Fishing Quotas (CFQs) assign a given amount of the total allowable catch to licensed quota holders (NRC 1998). These quota-based systems are generally established after a fishery has reached an overfished status, and give exclusive rights to catch and sell (and to sell the right to catch and sell fish) to those who have been most effective at catching fish (Allison 2002). These methods do not target the cause of overfishing as limiting catches with optimal yields and IFQs/CFQs only works when the society in which the fishery is based is tolerant of the shifting management practices. Unfortunately these management practices cannot mitigate against ecosystem damaging effects caused by overfishing, nor have the capacity to be effective when they are set under an overall maximum/optimum yield quota and thus do not offer a solution (Macinko and Hennessey 2002).

Solutions to the overfishing crisis have been sought after using reinventions of current fisheries management practices, however, these do not address the root cause of the problem (Rosenberg 2003). Commonly utilized modern fisheries management practices are not without scientific support. The testing of alternative hypotheses and sensitivity analyses determine where uncertainties in parameter estimations are likely, thus influencing managing agencies decisions (NRC 2004). The problem is these same analyses now show the true state of marine fisheries as one of political and/or fisheries mismanagement and dwindling stocks. Although a solution to the overfishing seems to be simple –a reduction in fishing efforts-, the approach to this is mainly political with wide reaching ecological and socio-economic implications (Rosenberg 2003). In response, a shifting trend towards adaptive ecosystem based management has been observed through the use of alternative management strategies such as MPAs, within the fisheries. Within an ecosystem based management scheme, the science departs from analyzing a single population, and looks for changes in the environment that would affect that population of interest (Boehlert 2002). Within the fisheries, biotic and abiotic factors such as habitat structure, biodiversity, and species interactions are examined giving scientists an overwhelming amount of information to better inform alternative management needs like the justification of more conservative management practices like the use of MPAs. This information has also lead to better analysis methods that examine the ecological, biological and social consequences of the overfishing problem.

Analyzing the problems associated with overfishing begin with identifying where population exploitation is occurring. Accomplishing this for individual species requires distinguishing distinct population segments from one another and how these segments interact. These units are called unit stocks, an idealized discrete entity with its own demographics, and fate (Waldman 2005). Modern fisheries science uses several techniques in the identification of fish stocks. These vary in effectiveness, and the use of these techniques has increased as technology has advanced and population dynamics theories have become integral components of modern fisheries assessments for effective fisheries management (Begg and Waldman 1999).

Fisheries managers often examine an exploited fishery as a simple system of inflow, stock, and outflow. When the inflow (recruitment rate) is greatly less than the outflow (harvest rate), the stock (harvestable fish) cannot be sustained and will decrease over time. In this simple model, for a fishery to be sustainable, the harvest rate must be small enough to allow for fluctuations in the recruitment rate, and recruitment may be influenced by multiple factors, including mortality, ENSO (El Nino Southern Oscillation), and other changing ocean conditions (Bakun and Broad 2003). This would mean a comparatively small harvest rate. Such a rate would likely not meet current economical demands of a fishery, even if that rate were more ecologically sustainable.

The Ecological Consequences of Fishing

Marine ecosystems are as complex in function and structure as they are diverse. In addition to proper stock identification, the simplest way to describe

the biota in such a diverse ecosystem is by assessing the feeding interactions among the inhabiting organisms (Pauly et al. 2002). All organisms in a marine ecosystem, ranging from benthic invertebrates to large apex predators, can be represented by their trophic level. Trophic levels represent the number of steps an organism is removed from primary production organisms such as algae and bacteria (TL=1), and generally higher trophic levels are characterized by an increase in body size, especially for piscivorous (fish eating) species (Pauly et al. 2002). Larger commercially important fish have mean trophic levels that range from 3.0 to 4.5 (Pauly et al. 2002). By examining changes in mean trophic level, it is possible to analyze the population structure for a given locality or habitat. Other means of assessing population structure can be achieved through measuring and monitoring the diversity including species richness and evenness of an area (Magurran 2004). Both means of addressing population structure become important when considering the effect fishing has on an ecosystem.

Fundamental causes for many of the changes in global marine ecosystems have been attributed to overexploitation of several different fisheries (Tetreault and Ambrose 2007). The act of harvesting fish via current fishing methods removes a desired size or specific species (target species) from a local ecosystem, thus effectively removing them from the food web. Most fisheries generally target large, predatory high-trophic level fish species, as these are the species most desired for human consumption (Myers and Worm 2003). These species are generally long-lived slow growing fish that once mature, play intricate roles as predators in their marine habitats. However, large fish are not always large.

Throughout various life stages these high trophic level organisms are preyed upon by a vast variety of organisms (Pauly et al. 2002). So, the removal of high trophic level fish not only alters predator-prey relationships changing the fish community structure, but also the feeding ecology and mean trophic level of an entire ecosystem (Sumaila et al. 2000, Pauly et al. 2002). What has been observed is a global decline in mean trophic levels, correlating to the removal of large fish from marine ecosystems (Pauly et al. 2002).

Other ecosystem damaging effects come from the gear used in certain fisheries. A common method used to fish demersal species is bottom trawling. Bottom trawling techniques consist of dragging a large net along the sea floor, thus indiscriminately catching any organisms in the path of the net. The first problem with these techniques is the incidental catch of non-target species, or by-catch. Trawling for one species often impacts many other species; however, by-catch is produced in nearly all forms of fishing (Palumbi 2001). Perhaps the most devastating, long-lasting effect of bottom trawling is the plowing of the substrate by the net. This action destroys critical habitat necessary for healthy benthic organism communities by reducing the complexity of the sea floor, thus eliminating microhabitats utilized by benthic organisms (including juvenile fish) (Sumaila et al. 2000). These benthic communities are often highly productive, comprised of low trophic level organisms, and form the base of marine ecosystem food webs (Pauly et al. 2002).

The effects of fish harvesting can be observed at multiple scales, from the individual species level, to impacts on populations, and at the ecosystem level.

Often, changes in community structure are the result of overfishing targeted fisheries. Each species within the ecosystem may respond differently to changes in population structure. One common effect seen is an increase in non-target species populations, due to the lack of predation or competition (Myers and Worm 2003). This may seem beneficial for those non-target species, but in reality it can be detrimental. Because the food webs of a marine ecosystem are so interwoven, a population increase of a previously suppressed species may lead to a sudden crash of that population when the ecosystem cannot support increased numbers (Pauly et al. 2002). Examples of this form of community restructuring have been seen in both oceanic billfish and groundfish populations (Myers and Worm 2003).

Another effect of fishing often observed is changes in fish physiology. Since fisheries tend to select larger, fast-growing individuals from the fish population, they run the risk of altering the genetic information thus changing the evolutionary characteristics of that population (Pauly et al. 2002). Fishing can therefore select against fish with slow maturation because these fish would not have an opportunity to reproduce before being harvested. Research into this phenomenon is limited, but research has shown the prevalence of early maturation in targeted fish species (Kurlansky 1997). Earlier maturation may allow a female fish to spawn sooner in life increasing the spawning potential; larvae from young spawners, however, may experience lower survivability than larvae from older spawning fish, negating any positive affect of increased spawning potential (O'Farrell and Botsford 2006).

By recognizing the negative ecological impacts of overfishing, fisheries managers can take action as needed to stem the problem. As mentioned above, some forms of action have come by means of alternative management practices. Though increasing in popularity, the application of Marine Protected Areas as tools for conservation and fisheries management is still a relatively new strategy (Sobel and Dahlgren 2004).

Marine Protected Areas

Marine reserves, a form of a Marine Protected Area (MPA), were developed based on the idea of eliminating or limiting extraction of fish or any other natural resource within the reserve boundaries (Palumbi 2001). Often referred to as “No-Take” MPAs, Marine Reserves are receiving global attention from fisheries managers, environmental groups, ecologists and various government agencies as a means of conserving marine organisms and restoring depleted fish stocks (Micheli et al. 2004). These reserves operate by protecting local fish stocks as a management tool to augment or stabilize regional fisheries yields (Palumbi 2001). By virtue of their properties, no-take reserves also protect the ecosystem functions of the habitat within their boundaries (Micheli et al. 2004).

Within the MPA category, there are several kinds of Marine Reserves. Each type of reserve utilizes different management strategies to reach different goals. This diversity of strategies and goals can be beneficial for management agencies and biologists in that a reserve can be designed to meet specific management needs. To maximize the benefits of this flexibility, it is necessary to identify the specific conservation needs of a particular ecosystem in order to

effectively achieve protection. Often the predominate conservation goals of a marine reserve stress the desire for protecting biological attributes of marine ecosystems, such as providing critical habitat for a single or several species, maintaining high biomass and species diversity, providing dispersal points for propagules, and establishing refuges from fishing (Allison et al. 1998). Individual reserves are unique in their topography and biodiversity. Where one is effective, another may fail because each situation has unique qualities (Palumbi 2001). To understand the overarching benefits offered by marine reserves this literature review presents a broad comparison across reserves in different ecosystems.

From a fishery perspective marine reserves are a promising management tool. They offer a fundamentally different type of protection not seen in traditional fisheries management practices (Allison et al. 1998). By restricting the access to critically important habitat, such as fish nurseries and spawning grounds, marine reserves specify locations that can and cannot be fished (Hilborn et al. 2004). Protecting a population from fishing pressure often allows fish population structure to be governed by natural mortality instead of fishing mortality (Allison et al. 1998). Marine reserves worldwide have been shown to produce drastic increases in biomass of species that are heavily fished outside reserve boundaries (Palumbi 2004). What often accompanies this increase in biomass is a significant increase in species diversity and population density. Even as benefits may vary by geographic location, the general trend of increased abundance, biomass, and diversity have been documented for a variety of targeted species (Micheli et al. 2004). A recent meta-analysis confirmed the benefits that marine reserves have

on fish populations, and concluded that average abundances of target fish inside reserves were 3.7 times higher than outside reserve populations (Mosqueira et al. 2000).

Similar to fishing, protection within a reserve has the potential to modify the community structure within a marine ecosystem. An increase in biomass of predatory species within a reserve equates to an increase in predation on smaller mid-trophic level species, which are often times not targeted by a fishery. In general, non-target species do not demonstrate the same response (increase in abundance, biomass, and diversity) from the protection of a marine reserve as species targeted by fisheries (Figure 1) (Micheli et al. 2004). This is not to imply that no benefits occur to non-target species. As previously discussed, fishing practices have negative effects on the ecosystem as a whole, thus affecting more than just the targeted fishery stock. Marine reserves offer protection to non-target species by preventing their capture as by-catch and by reducing habitat degradation that occurs during fishing (Allison et al. 1998).

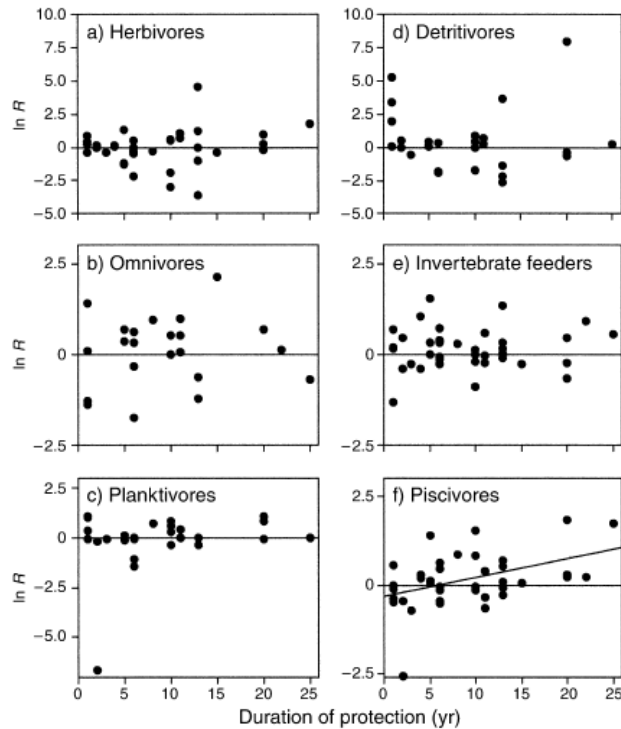


Figure 1. Response ratios ($\ln R$) vs. duration of protection for each of six trophic groups. A statistical significant temporal trend was found only in piscivores. Figure Source: Micheli et al. 2004

A large body of literature provides supportive evidence for the development and implementation of marine reserves; however, individual species response to protection varies depending on family association, trophic level, whether or not the fish were the target of a fishery (level of exploitation), and most importantly body size (Mosqueira et al. 2000). A strong correlation between positive response and species with large body size has been shown (Figure 2)(Mosqueira et al. 2000). Moreover, Micheli et al. (2004) conducted a meta-analysis of 20 studies looking at the effects of reserves on community structure. Their analysis showed that omnivores and other mid-trophic level species responded poorly to reserve protection. This response was most likely explained by the increase in high-trophic level organisms reported in the study (Palumbi

2004). Species benefiting most from protection are the same species most susceptible to the effects of fishing: large, high-trophic level, long-lived, and slow to mature predatory fish (Mosqueira et al. 2000).

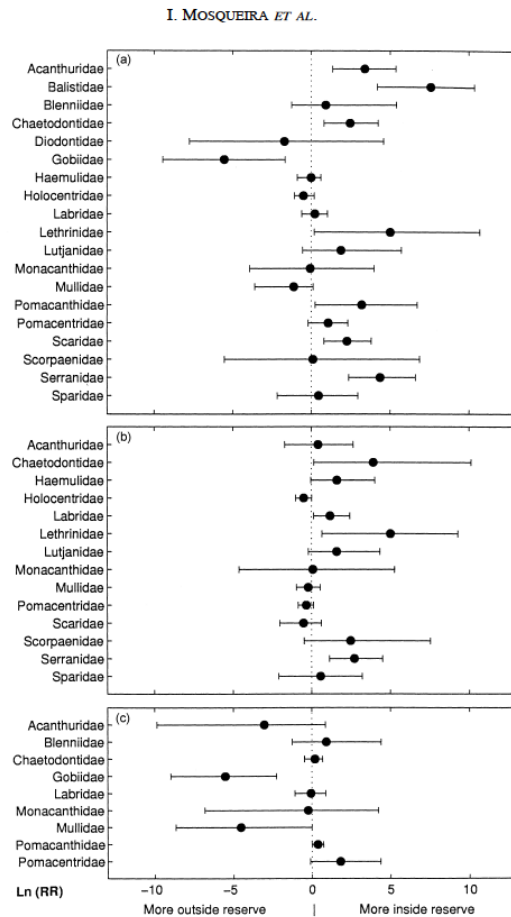


Figure 2. Response Ratios by maximum length groups for (a) all species, (b) Species that are target of fishing and (c) species that are not fished. Figure source: Mosqueira et al. 2000

This has direct implications for marine planners establishing marine reserves to conserve a fishery. Though marine reserves may initially reduce the yield of surrounding fisheries by limiting their access to fish, the long-term ecological benefits may ultimately benefit the fishery (Hilborn et al. 2004, Sumaila et al. 2000). Maintaining a stable protected population of large fish

within a marine reserve may be extremely beneficial to the regional recovery of a previously exploited fishery. Maximum body-size usually correlates to life history parameters such as age at maturity, growth and reproductive output (Mitcheli et al. 2004). As we have examined, these are all parameters selected against when the species is targeted by a fishery. Larger body size also positively correlates with fecundity and LEP (lifetime egg production), which increases reproductive capacity (Plumbi 2004). Simply put, the larger the fish, the more eggs produced, the higher recruitment rate, the greater the growth in abundance of large fish in a region.

Ecology of MPAs

By allowing large fish to congregate, marine reserves ultimately support more eggs, more larvae, and thus more adult fish to supply a neighboring fishery. Three major underlying biological factors that make this possible: Lifetime Egg Production (LEP), larval dispersal, and spillover. The first, Lifetime Egg Production, is the number of eggs produced by a recruit over the course of its lifetime (Botsford et al. 2009). Within a fishery stock LEP is directly correlated with body size. Thus individual small-bodied early maturing fish selected against in a fishery would produce substantially fewer eggs throughout their life (O'Farrell and Botsford 2006). Lifetime egg production becomes very important in quantifying the recruitment rate for a fishery. If the value of LEP drops below the critical replacement value (the number of spawning recruits), the fishery will destabilize and collapse (Botsford et al 2009). The longer female fish live, the more clutches they bare, the higher the recruitment. Though, within a non-fished

population LEP is extremely difficult to estimate (Botsford et al. 2009). As to be expected, an increase in fishing mortality decreases the LEP, hence researchers rely on a ratio (Fractional Lifetime Egg Production or FLEP) between fished and non-fished LEPs to assess the spawning potential ratio (SPR) of a fishery (Botsford et al. 2009). For a fishery to be sustainable FLEP needs be 0, a ratio representing no change in egg production between a fished and non-fished population. This is what a marine reserve provides, an area where the spawning stock enabled to produce the most recruits (see Figure2.). Current management schemes push to use a FLEP of 0.4, allowing a female to only produce 40% of her LEP before being harvested. As figure 3 shows, an F of 40% diminishes the spawner-recruit relationship to levels lower than a non-fished population ($F=0$). Increased LEP becomes an important byproduct of protection through a marine reserve.

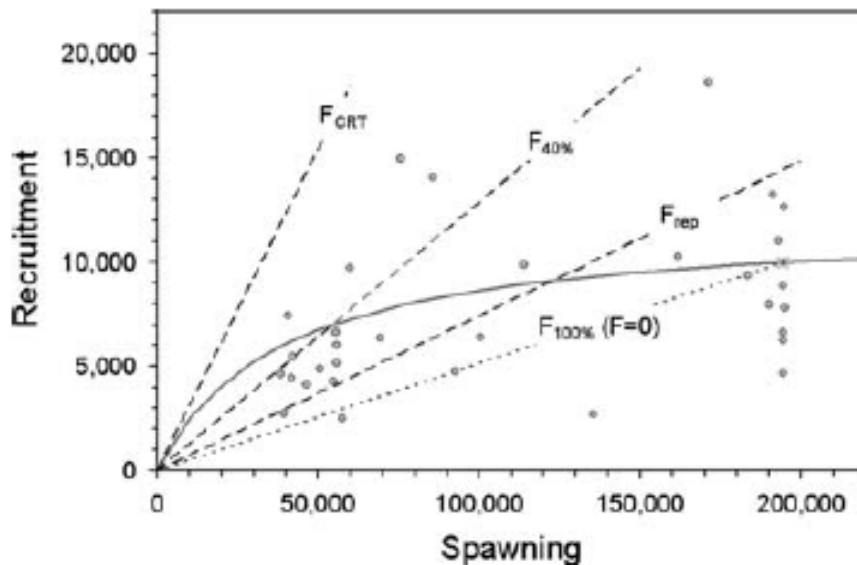


Figure 3. The relationship between recruitment and the spawning stock for a hypothetical fish population. The intersection of replacement line and the spawner-recruit curve show the change in equilibria as the fishing mortality rate (F) is increased and lifetime egg production is diminished. FCRT: Replacement

line associated with critical replacement threshold $F_{40\%}$: Replacement line that represents the fishing mortality rate that reduces LEP to 40% F_{REP} : Replacement line that best fits the data $F_{100\%}$ ($F=0$): Replacement line for a non-fished stock. Figure source: Botsford et al. 2009

The second factor, larval dispersal, can be thought of as propagule diffusion from a central location, or marine reserve. Larval dispersal varies from species to species but can highly determine the potential replenishment of a fishery stock outside reserve boundaries and determine the self-sustainability of species within a reserve (Planes et al., 2008). The greater the distance larvae disperses for a target species propagates outside a reserve could lead to greater distribution of a species. In a marine environment the dispersal distance is positively correlated to pelagic larval duration (PLD), or time spent in the water column as larva (Figure 4) (Bostford et. al. 2009, Shanks. 2009). Though statistical models show that species with longer pelagic larval stages are able to disperse propagules a greater distance than ones whose larval stages are short, the real world outcomes are much more complex. Dispersal results in complex spatial patterns that reflect an interaction of flow patterns that vary in space and time with pattern in survival and behavior (Botford et al. 2009).

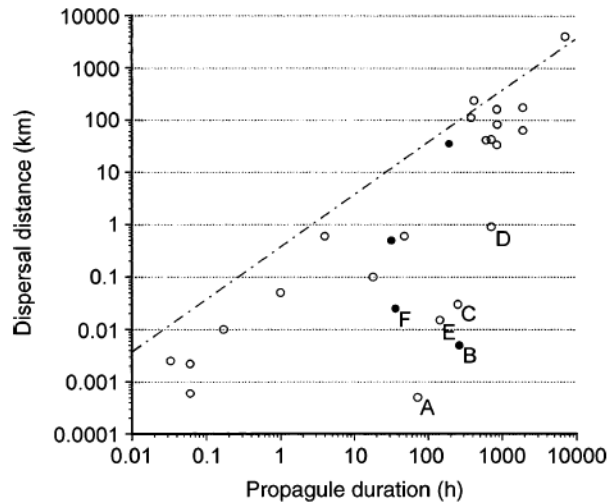


Figure. 4 Estimated dispersal distance plotted as a function of propagule duration. Dashed line is the best fit to the data. Open circle represent animal populations. Closed circles represent plant populations. A significant correlation between propagule duration and dispersal is show for these data. Figure source: Shanks et al. 2003

In a study by Carr and Reed (1993) four conceptual patterns of population replenishment via larval dispersal were distinguished (Allison et al. 1998). These four patterns were then organized along varying axes: distance of propagule transport relative to the scale of reserve and number of population replenishment sources (Fig 5). Their study shows the various ways in which larval dispersal might occur, each four having its own benefit to species that propagate in that method. Populations that exhibit short distance dispersal maintain the ability to be self-replenishing, whereas long distance dispersal populations rely heavily on the influence of a single source population. Whether the source population is a larval stock, single or multiple active breeding populations, or a single isolated breeding population depends on the species of fish. Though each four of the scenarios could be applied to the life history traits of several target fisheries, a common observation of our large bodied, long-lived fish with high PLD is

propagation via long dispersal distance - either from a larval stock that serves several small populations, or a single breeding population whose propagules support other populations. This occurs when migratory or seasonal breeders congregate to form a single large spawning group leaving a larval population that exists absent of the adult population (Fig 5D), or when only one population is healthy enough to promote breeding (Figure 5C). Again, this is highly variable by species, genera and family (Mosqueira et al. 2000.) For example, some members of the rockfish genus *Sebastes spp.*, have low site fidelity, breed in large colonies, and leave propagules to disperse (Figure 5D) while others exhibit high site fidelity, breed in small populations to which the propagules disperse freely between (Figure 5B) (Allison et al. 1998). Knowing which populations support the reproductive success of a species will ultimately affect the placement of marine reserves by asking the question, “What is the spatial structure of the populations?” and “How demographically connected are these populations?”

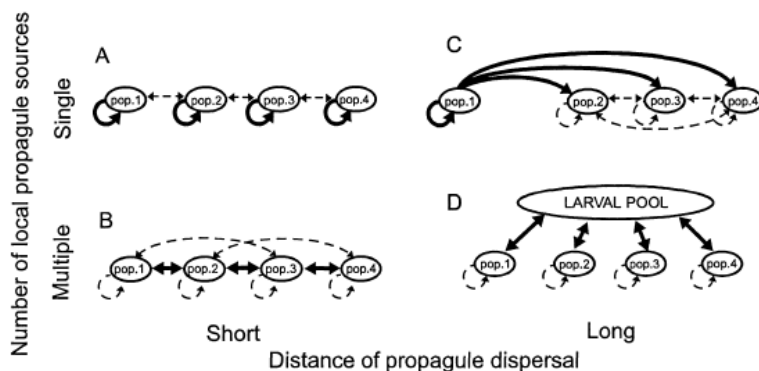


Figure 5. Models of population replenishment: Patterns are distinguished by the distance of propagule dispersal and the number of local propagule sources for a given local population. Ellipses represent isolated adult populations. Bold lines indicate high recruitment rates within or between isolated adult populations. Broken lines indicate low recruitment rates. Figure source: Allison et al. 1998

The third factor is the poorly understood movement of large adult fish to areas outside the reserve. This is known as the “spillover” effect and occurs when there are high quantities of large fish within the boundaries of a reserve (Palumbi 2004). With dense population conditions existing within a marine reserve, competition between cohorts and other species may force fish to venture into unprotected waters. Conservation goals benefit from minimum spillover outside of reserves, while fisheries enhancement may benefit from high spillover (Roberts 2000). As movement rates increase, yield can also increase as fish spend less time in protected reserves (Botsford et al. 2009). A study conducted by McClanahan and Mangi (2000) monitored fish catch per unit effort (CPUE) from the edges of Kenyan marine reserves. Their analysis showed mass, size, and species diversity decreased the farther samples were from the reserve boundary (Palumbi 2004). Another study by Russ and Alcala (1989) analyzed fishing yield in the Philippines before and after the collapse of a marine reserve due to illegal harvesting. This study stated that fishing was 25% more productive when the reserve was in place. When spillover benefits the yield of fishery neighboring a reserve, it also reduces the replacement and buildup of spawning stock inside the reserve. This results in less sustainability and less recruitment (Botsford et al. 2009). The benefit to a fishery from a reserve is dependent on the species being managed and appears to be highly variable across fish families even when mobility is similar (Palumbi 2004).

MPA Design

Most fish targeted by fisheries go through four distinct life stages: eggs, larvae, juvenile and adult (Pauly et al. 2002). At each life stage different habitats may be required, and because marine reserves provide spatially explicit protection, informed reserve design can protect each life stage. The efficiency of a reserve is greatly enhanced if the design is scientifically sound (Allison et al. 1998). This may seem obvious, but opponents of marine reserve implementation argue that there is scientific uncertainty over optimal reserve locations based on habitat (Roberts 2000). For a reserve to benefit a stressed fishery, it should overlap spatially with essential habitats required by that species. This means protecting enough critical habitat by incorporating all aspects of the habitat utilized by that particular species which in turn requires knowledge of that species life history, including the requirements of each life stage (Allison et al. 1998).

Larval dispersal and adult movements (spillover) are important to the replenishment of a target species and support of a fishery. Depending on the goals set forth by management agencies, reserve designer will incorporate these factors, such as (restate here) into the planning phase. Proper reserve design and establishment would be one that retains sufficient offspring to sustain its own population while also exporting the majority to replenish fishing grounds (Roberts 2000). The effects of successful reserve design may also have ecological benefits by increasing regional diversity. Protecting these “source” areas whereby species recruitment is higher than mortality allows for migration out of the reserve, and increases the potential for sustainable harvesting.

Creating reserves that protect source populations or essential habitat require huge amounts of effort from stakeholders, including scientists and resource managers, policy makers, and the local fishing community. Necessary collaboration may involve cooperative research to work towards the management goal set forth by invested parties. (NRC 2004). A common argument from within this group is centered on reserve size and configuration, asking: “What is better, several small reserves or one large reserve?” The answer to this question depends on the intended goal of the reserve. Reserves implemented for fisheries replenishment may require scaling larger than reserves implemented for the conservation of a particular species. Figure 5 conveys what is known of how several small reserves would work in areas as compared to the effectiveness of one large reserve. Individual breeding populations with short dispersal will best benefit from the local protection of a small reserve. Several small reserves would protect these species because of their high retention of larvae (Roberts 2000). Large reserves would be best suited to protect species with little local retention of larvae or key habitats such as natal/nursery grounds, feeding grounds, migratory routes, etc. (Allison et al. 1998, Roberts 2000). Determining the proper placement and number of areas or reserves will ultimately govern the success of MPAs. Figure 6 illustrates how a reserve design can suit a population of fish with long distance dispersal and little propagule retention outside of the source population. Each reserve site will have to be treated as its own entity, however, there is the possibility for connectivity between reserve sites.

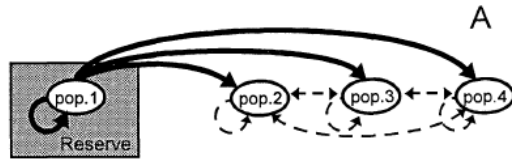


Figure 6. An example of proper reserve placement: Single reserve (shaded box) established on a single-resource population. The reserve protects the reproductive source population. Source: Allison et al 2008.

The analysis of the distribution of biological resources in relation to the physical environment is a challenge that must be met for the successful designation of reserve area (Kracker 1999). The appropriate allocation of space is highly dependent on the understandings of the biological processes within that geographic region, the ecology of the organisms targeted for protection, and the goal of the reserve. Current ecologically based strategies involved in the optimal reserve area selection are often based on specified biodiversity and habitat criteria (Berglund et al. 2012). The relationship of these two criteria can be examined in studies focusing on aspects of species distribution, evenness, and richness as it relates to essential fish habitat (EFH) and the implications that habitat has on population dynamics for targeted species. Research has demonstrated a high correlation between biodiversity and quality of habitat connectivity (Berglund et al. 2012). Specifically, certain species may disperse throughout a region if they are able to translocate from one protected area to another, by way of high quality habitat.

This notion gives rise to what is commonly referred to as “Networked MPAs”. Networked MPAs are a series of marine reserves that work in conjunction with one another to support the movement and migration of fish

within a region. Scenarios where networked reserves are expected to be successful are when target species exhibit home range behavior (Moffitt et al. 2009). For many high-trophic level large fish (such as *Sebastes*), the required habitat of a home range changes with maturity. Different habitat requirements are highly correlated to each life stage, from larvae to mature adult fish (Palsson et al. 2009). These “recruitment pathways” may include a successional use of many types of benthic habitat (Buckley 1997). Changing habitat needs equate to differing home range behavior at each life stage. For several species of the *Sebastes* sp., mature individuals demonstrate high site fidelity, prefer benthic rocky habitats, and rely on pelagic larvae dispersal to propagate throughout a region. Networked reserves have demonstrated their efficiency when specific habitats along a species “recruitment pathways” are targeted (Eisenhardt 2001).

In the past two decades, ecological modeling and marine spatial planning utilizing tools such as GIS have aided in the creation and appropriate placement of reserves (Wrights and Heyman 2008.) Researchers are also applying habitat modeling methods based on remote sensing techniques to better understand the needs of species in certain habitats, what habitats have the greatest abundance, and why species occur where they occur (Valavanis et al. 2008). These efforts directly affect the successful implementation of marine reserves, networked reserves, or any other form of MPA.

The techniques utilized in the ecological modeling and spatial planning of marine reserves can also be adapted for analyzing the effects for established marine reserves. Incorporating the biological needs of target species, the known

ecological benefits of marine reserve protection, and technologies to model and predict species distribution can have wide ranging implications for assessing the regional effects of marine reserves. In order to understand the variation and effectiveness of MPAs, in Chapter 2, I examine the ways in which the ecology of marine reserves affects the surrounding benefit ecosystems region, and fish populations stressed by previous overfishing.

Chapter 2: Manuscript

Introduction

Globally, overfishing is thought to be the primary factor in the ecological and biological deterioration of coastal ecosystems (Jackson et al. 2001, Worm et al 2006). The marine ecosystems of North America's largest Pacific coast estuary, the Puget Sound, are no different. Decades of overfishing, and loss of marine habitat to anthropogenic stressors such as development and pollution contamination have negatively affected most populations and stocks of Puget Sound groundfish (Rice 2007, Tsao et al 2005). The viability of these fisheries has been diminished due to historical exploitation to levels that have now influenced the structure and function of the current Puget Sound marine ecosystem (Williams et al. 2010). An understanding of trends and changes in current groundfish populations are needed to effectively manage and conserve the species within this degraded ecosystem.

Despite historical exploitation, in recent decades there has been a growing interest in reforming fisheries management and recovering threatened stocks (Williams et al. 2010). This interest has focused on examining ways to rebuild depleted stocks of Rockfish (genus *Sebastes*), and lingcod (family *Hexagrammidae*), high-trophic level groundfish that have been adversely affected by recreational and commercial fishing. An early commercial fishery restriction, critical to the recovery of the stocks, was the 1989 prohibition of trawling in the major North and South basins of the Puget Sound (Rice 2007, WAC 220-48-015). Later regulations adopted by the Washington Fish and Wildlife Commission

included the Puget Sound Groundfish Policy to ensure the conservation of habitat and ecosystems used by groundfish to maintain healthy populations of these species (Palsson et al. 1998). Recent management actions specific to Rockfish have targeted the recreational fishery with the implementation of “bag limits” or daily catch quotas, Rockfish Recovery Zones, and the petitioning to list selected species under the Endangered Species Act (Biological Team Review NMFS 2008).

With an increase in the reform of fisheries management regulations that specifically target groundfish, managing agencies (WDFW) implemented the utilization of Marine Protected Areas in 1990 (Van Cleve et al. 2009). As a current form of mitigation, MPAs are geographically established areas that prohibit the harvest of fish. A positive global consensus is building for the use of MPAs for conservation of degraded marine habitats (Allison et al. 1998, Mosquera et al. 2000, Palumbi 2004). However, data and studies have been highly species and region based, demonstrating that target species react positively to the protection offered by MPAs placed in certain habitats (Van Cleve et al. 2009).

This study investigates the regional effect MPAs have on the diversity of groundfish within the San Juan Archipelago, and assesses habitat utilization by target groundfish species. As with any natural resource management tool, continued analysis is often needed to ensure its proper utilization. MPAs are no exception; analyzing the regional effects of established MPAs on marine organisms has led to a greater understanding of their function and implications with respect to their intended goals. Biodiversity surveys and ecological studies

often focus on areas with high concentrations of plant and animal diversity such as intact reserves and protected areas with low levels of human intervention (Chazdon et al. 2009). This is also the case for Puget Sound and San Juan MPAs where studies of biodiversity have focused attention on areas within reserve boundaries (Tuya et al. 2000). But how do these surveys and studies translate to areas outside reserve protection? Within Washington, limited studies have been conducted to understand the effects MPAs have on surrounding fish communities. Additional research is necessary for the continued implementation of marine reserves, such as examining regional biodiversity levels and population status for groundfish at larger spatial scales. By addressing regional biodiversity patterns, scientists can understand how MPAs function as tools for fisheries management and as a means for restoring ecosystem function and reverse the past effects of overfishing (Palsson 2001). The goals of this study are to (1) describe how MPAs affect regional biodiversity levels, and species occurrences through out time and over distance by hypothesizing that samples nearer to MPAs will have significantly different levels in diversity than samples further away, and (2) determine species by species group composition in various habitats to describe species preferences for habitats not targeted by MPA, hypothesizing that species groups will have significant preference for depth and substrate.

By using biodiversity as a metric, this study addresses spatial and temporal variations in measured diversity levels. It is hypothesized that spatial and temporal variables will have a significant effect on levels of diversity throughout the San Juan region, potentially due to the influence of marine reserves.

Observing and quantifying change across these ecological scales will inform managing agencies in the implementation and proper utilization of marine reserves. Analyzing habitat preference by species family group is an effort to validate current MPAs selection and implementation strategies, as knowing that many agencies target specific habitats for protection.

Historical Fisheries Exploitation

The history of harvesting marine fish from the Puget Sound extends to the first peoples to inhabit the region. Similar to the global trend, Puget Sound fisheries have suffered from the distinct effects of increase fishing pressure (Myers and Worm, 2003). Though several species, including groundfish species, are commercially harvested within the Puget Sound, few have received more attention than members of the Salmonid family and Sebastes family. The six species of Pacific salmon are an intricate aspect of the regions ecosystem, economy, and cultural identity. However, these species have also been a source of contention for people in the region. The 1974 federal court case *United States v. Washington*, commonly referred to as the “Boldt Decision”, upheld Washington’s Treaty Tribes rights to 50% of the total allowable catch and responsibility as co-managers of the salmon fishery (Wilkinson, 2000). This was not only a tide turning event for fisheries management in Washington state, but also an event that would have unexpected ecological consequences. In response to the 50/50 allocation of salmon between native and non-native fishers, WDFW urged non-native fishers to target the “abundant” groundfish stocks of the region (Dinnel et al., 2003).

Though commercial groundfish fisheries began in the 1920's, the catch rates for Rockfish species and other groundfish species experienced a rapid decline in catches landed that began in the early 1970's (West 1997). In the early years of the commercial rockfish fisheries, rockfish were often caught as non-targeted by-catch of the Cod (*Gadidae sp.*) fishery. As market demand grew for rockfish and fishing vessels were restricted to the United States waters the Puget Sound in response to the Magnuson Stevens Act of 1976, commercial catch landings for rockfish increased into the 1980's (Williams et al. 2010). This depletion rate of stocks increased rapidly in conjunction with the increased fishing effort that began in the 1970's (WDFW, 2011). Currently several rockfish species and stocks have been categorized as "critical" or "fully utilized", and three species have been ESA (Endangered Species Act) listed by the National Marine Fisheries Service (West 1997, Williams 2010, Biological Review Team NMFS, 2009).

Characteristics of Study Species

Groundfish, a non-taxonomic grouping composed of several different species, have been the focus of decades of research. The examination of groundfish within this analysis groups species into three species groups. Members of this category include Rockfish (genus *Sebastes*), Lingcod (family *Hexagrammidae*), and Pollock, Pacific Cod, Tomcod, and Hake (family *Gadidae*). With the exception of some Gadids, groundfish are long-lived, slow-growing, and late-maturing high-trophic level predatory fish (Pacunski et al. 2013). They function both as predators and prey in the complex trophic-web of

the Puget Sound (WDFW 2011). Species can be found in nearly all habitats and depths, a trait that creates difficulty for management, and the perfect scenario for a commercial and recreational fishery. Several members of the *Sebastes* genus, and the Lingcod *O. elongates*, are targeted within the recreational fishery. Other species, mostly member of *Sebastes* are easily caught as by-catch in the salmon trolling fishery. Management of these species has relied heavily on continuous scientific analyses. Surveys of population dynamics, distributions, species compositions and abundance have contributed to a wealth of knowledge pertinent to Puget Sound groundfish. Unfortunately, the primary cause for these studies has been in response to the continued depletion of groundfish stocks throughout the Puget Sound.

Researching the ecological benefits of Puget Sound and San Juan marine reserves has been accomplished by comparing species populations within reserve protection to populations outside, in similar habitat. Studies have demonstrated the benefits of protection include: increases in fish size, total biomass, and species richness or biodiversity (Palsson 2003 West, 1997). These results have been replicated for several groundfish species over several managed reserves. Conducting this research is a necessary step in establishing and monitoring marine reserves (Palsson 2001); continuous analysis is needed when assessing a dynamic ecosystem such as the marine ecosystems of the Puget Sound. While the measurable and observable benefits of marine reserves are well published, a push towards the creation of networked systems of reserves has proceeded (Moffit et al. 2009, WDFW MPA Work Group 2009). However, thorough analyses

assessing the broader ecological impacts of marine reserves must be conducted. This is necessary to address gaps in management, monitoring, and evaluation, ultimately leading to a lack of current understandings.

Building upon the scientific studies, agency recommendations, and shifting trends towards ecosystem based management practices, the Washington Department of Fish and Wildlife (WDFW) has contributed several resources to the investigation and implication of MPAs within the San Juans Islands. These established MPAs are herein referred to as marine reserves, selected for in this analysis because of the level of protection employed. The goals of these reserves are to protect and conserve target species by prohibiting harvest for groundfish and restricting harvest for other organisms within their boundaries. Of the 22 established reserves managed by WDFW, five occur within the San Juan Archipelago (Van Cleve et al. 2009). As well as WDFW, The Washington Department of Natural Resources (DNR) manages two reserves within the geographic range of this study that employ similar protection levels to support similar goals.

The analysis of the Trawl and ROV datasets provided by Washington Department of Fish and Wildlife comprised a two-part study to determine the regional affects of marine reserves in the San Juan Archipelago. The first, the analysis of eight years of trawl surveys in the San Juan Archipelago, employed a unique methodology of using diversity indices to quantify temporal and spatial variations in levels of diversity of high trophic level ground fish throughout the region. The second, an analysis of ROV survey transects determined species

composition by species group per habitat type and depth. These parameters can be used to assess species expected occurrence rates when habitat data is absent. The utilization of both datasets allowed for a comprehensive examination of the biotic and abiotic factors that affect the implementation of marine reserves in the San Juan region.

Methods

Study Area

All data used for this study was collected within the waters surrounding the San Juan Islands (Appendix A-1). Located off the northwest coast of Washington State, the San Juan Archipelago is comprised of over 450 islands, rocks, and tidally exposed reefs. Depths range from the shallow intertidal to areas over 200m. The regions distinct geomorphology has created an intricate network of straits, channels, and passages between landmasses with complex underwater landscapes. This complexity equates to an abundance of potential habitat for rockfish and other benthic species (Tilden 2004). Compared to other regions of the Puget Sound, the San Juan Archipelago has a significant amount of rocky habitat (Palsson et al. 2009). These waterways represent the San Juan Basin, a distinct Sub-Basin within the North Puget Sound Basin commonly referred to by fisheries manager (Williams et al. 2010). Within the San Juan Archipelago, the Washington Department of Fish and Wildlife manages five San Juan Marine Preserves (Table 1) (Appendix A-2).

Table 1. WDFW Marine Reserves. UML = Uniform Multiple Use, ProRec - Recreational harvest prohibited, ResCom - Commercial harvest restricted, ResAll – All harvest restricted. Source: Van Cleve et al. 2009).

Preserve Name	Acreage	Year Established	Protection Level	Harvest Restrictions
Argyle Lagoon MP	13.00	1990	UML	ProRec/ResCom
False Bay MP	94.70	1990	UML	ResAll
Friday Harbor MP	427.20	1990	UML	ResAll
Shaw Island MP	432.50	1990	UML	ResAll
Yellow and Low Islands MP	187.20	1990	UML	ResAll

Source of Data

The Washington Department of Fish and Wildlife's Marine Fish Division (WDFW) collects and compiles vast amounts of fisheries related data for the Puget Sound and adjoining waters. As per WDFW's mission statement to protect the fish and wildlife resources of the state, the collection of biological and ecological data of native fish species is a primary goal. For fisheries related issues within Washington State data collection on various projects have contributed to a wealth of information. The data collection methods used by WDFW's Marine Fish Division are similar to that of other agencies. Data collection includes the following methods: dive surveys, research trawl surveys, intertidal habitat surveys, and Remotely Operated Vehicle (ROV) surveys. Each method has its benefits for specific analyses. WDFW has used these survey methods to analyze abundance, diversity, species composition, and to gather biological information for several marine organisms.

Washington Department of Fish and Wildlife's Marine Fish Program provided the datasets for the analyses. Two datasets were selected for this study. The first contained trawl survey data collected from 1987 through 2012 and was utilized for the regional biodiversity analysis. The second dataset contained ROV survey data from the 2008 and 2010 survey years and was utilized for the habitat utilization analysis. The trawl and ROV datasets differ by collection methods and spatial scales. These differences allow for separate analyses to be conducted. Prior to acquiring the data, both datasets were sorted by species of interest. Because this analysis focuses on commercially important high-trophic level fish,

all species other than members of *Sebastes*, *Gadidae*, and *Hexagrammidae* were filtered from the dataset. Trawl and ROV surveys were conducted at differing times through out the region. Trawl surveys used in this analysis were conducted during May and June. The ROV survey regime sampled the San Juan Archipelago independent of season, with data collected over 42 days (Pacunski, et al. 2013). Data for mapping was provided by WDFW through personal contact. These data consisted of Washington's MPA inventory geodatabase compiled by Van Cleve and colleagues (2009).

Occurrence Rates and Temporal Variation

Sampling methodology and survey techniques for the Trawl Surveys used are detailed extensively in WDFW's Trawl Survey Field Plan and Manual (Palsson et al. 2002). The general practice for these trawl surveys consisted of station selection, catch processing, and sub-sampling for biological sample collections. An established protocol for station selection ensured that the regionally based and station based systematic surveys were implemented without bias (Palsson et al., 2002). For each survey site, data was collected on location and duration of tow, beginning and end depths, species caught, abundance, and sex/length frequencies.

Data auditing was needed to remove errors from the trawl dataset. Because the trawl database contained data on regions outside the San Juan Archipelago, data were selected from the database after establishing filter parameters. Post-filtering yielded a dataset specific to the San Juan region. After this process was completed, the total number of target species was identified for

each year within the dataset. Within each year, individual samples transects were identified by haul sequence number.

Data for the biodiversity analysis were compiled using surveys from 2001, 2004, 2006, and 2008 - 2012. These survey years represented all years data were collecting in the San Juan region. Prior to 2007, trawl surveys were regionally based, so not all years surveyed had data collected in the San Juan region (WDFW personnel, personal communication). From 1987 to 2007, only three surveys were present for years 2001, 2004, and 2006. In 2008, WDFW changed their data collection strategy to a Puget Sound wide station survey. This implemented sampling by station, and not by region. Under this new collection strategy each station would be sampled twice per year. The two separate sampling strategies affected that amount of samples collected per year (Table 2). The regionally based sampling strategy averaged 39 sites per year. The station based sampling strategy averaged 11.5 sites per year. In 2011, 27 sites were sampled, though still under the station based sampling method, 2011 eleven was analyzed with years of similar sample sizes. A total of 189 sample sites were utilized in this analysis.

Table 2. Years with number (*n*) of Survey Trawls by sampling method. ¹In 2011 technically station based. Analyzed with Regional Sampling due to sample size.

Regional Based Sampling	Station Based Sampling
2001: 40	2008: 12
2004: 35	2009: 12
2006: 41	2010: 12
2011: 27 ¹	2012: 10

Species group demographics were calculated for the trawl dataset. For these analyses, the 14 species observed were compiled into their most similar

taxonomic ‘species’ groups: Sebastes, Gadidae, and Hexagrammidae. These three groups consisted of the total counts for all target species observed over the course of the trawl surveys. (Table 3) For each year, frequencies of occurrence rates (%FO) were compared via ANOVA and Chi-squared contingency methods. Count data for %FO rates were log-natural transformed. To test the effects of depth on species group composition, the average depth per sample was first categorized into two depth strata, Shallow <36.6m, Deep >36.6m. Non-transformed count data for number of species per family present in each sample were utilized in the analysis of species group occurrence rates by depth stratum. This data were incorporated into the spatial analysis.

Table 3. Species observed by species group

Sebastes (genus)	Gadidae (family)	Hexagrammidae (family)
Copper Rockfish, Greenstriped Rockfish, Puget Sound Rockfish, Quillback Rockfish, Redstripe Rockfish, and Redbanded Rockfish	Walleye Pollock, Pacific Cod, Pacific Tomcod, Hake (Pacific Whiting)	Lingcod, Kelp greenling, and WhiteSpotted Greenling,

Biodiversity was calculated for each sample within each year using the Shannon-Weiner and Simpsons D diversity indices (Figure 7) (Magurran, 1998. Keith 2005). These indices measure the species richness and evenness and are commonly used in ecology to describe demographic relationships between organisms within a given sample. A simple abundance calculation (number of species divided by total number of individuals) would demonstrate species richness but does not allude to the distribution of species throughout the sample, or to the commonness relative to other species. Though similar, the Shannon-Weiner and Simpsons D indices utilize different methods for describing the

richness and evenness of a given community of biological sample. First, both indices make no assumption as to the underlying species abundance distribution, and work well with small sample sizes, such as in this study (Magurran 2004). Second, both indices are calculated from the log natural transformation of the abundance data, creating proportional data of the individual per species by sample. The differences in the indices are seen in their utilization and interpretation of data. The Shannon-Weiner assumes that individuals are randomly sampled from an infinitely large community and that all species are represented in the sample (Margurran 2004). Using these assumptions, the equation calculates evenness values to detail species evenness within samples. Though the Shannon-Weiner Index is inclusive of evenness, as separate evenness calculation was also conducted. The Simpson D index incorporates evenness and richness and this index is also considered robust when working with small samples, however, it is weighted towards the most abundant species, assuming that the most abundant species is of greater importance to the ecosystem (Greene 1975).

Shannon-Weiner:

$$H = -\sum p_i \ln p_i$$

$$E_H = H/H_{max} = H/\ln S$$

Simpson D:

$$D = \sum p_i^2$$

$$D = 1/\sum p_i^2$$

(Reciprocal representation for analysis)

$$E_d = D/D_{max}$$

Figure 7. Shannon-Weiner Diversity and Evenness Equation Simpson's D Diversity and Evenness Equation

Evenness, for both indices is represented on a scale between 0 and 1, where 1 equals complete equitability or evenness amongst the sample. Both

indices were utilized in the comparison of means to total catch per year. Unlike the frequency occurrence analysis, the years sampled under the regional sampling scheme were incorporated to other, separate from the years under the station based sampling scheme.

The temporal analyses conducted consisted of three separate tests designed to examine when significant changes in diversity were occurring. These test were to compare diversity and evenness values:

- Between all years sampled.
- Between years by sampling method.
- Within year by depth stratum.

These analyses were conducted using a non-parametric test for comparison, and resampling ANOVAs. Diversity indices values were also examined against abiotic variables of depth (average depth of sample location) and trawl tow length to assess possible correlations.

ROV: Species Composition and Habitat

The ROV surveys conducted in the San Juan Archipelago focused on determining the habitat preference for species of *Sebastes* and other target ground fish. Data for the habitat utilization analysis were compiled from the 2010 ROV dataset. Though both the 2008 and 2010 datasets were present, the 2008 data had recently been reported in Pacunski et al. (2013). The 2008 results would be used to reference the methods of the analysis for the 2010 dataset. Detailed methodology for the deployment and sampling protocol used can be found in Pakunski et al. (2013).

The ROV survey data for 2010 compiled observations made over a total of 139 sample segments. For each segment, the ROV ran approximately 1km, capturing species and habitat structure observations via video camera. The process of video recording as the ROV traveled along the bottom simulated a fish net like “capture event” as if the fish were captured in a trawl net. The video was then later analyzed and all species were recorded with their location and habitat type along each segment.

Data auditing for this data set consisted of truncating the data to include only significant habitat variables, location, sample ID, species groups, and species counts. The significant habitat variables consisted of values for the complexity (structure), and substrate.

The ROV data was utilized to determine the relationship between habitat complexity and species group occurrence. The target species within the ROV data were the same as those for the trawl data. Because these species are often associated with structured and complex habitat, such as rocks, outcroppings, boulders, slopes, and substrate depressions, an analysis of species occurrence by habitat complexity was used to determine any habitat preference observed within the sampled population (Pacunski et al. 2013). Habitat categories for substrate, complexity and relief were compared, jointly and independently, to counts of species abundance. Similar to the analysis for the trawl dataset, the 16 species (including all unidentified but counted species) observed were compiled into their most similar taxonomic ‘family’ groups: Sebastes, Gadidae, and Hexagrammidae.

These three groups consisted of the total counts for all target species observed during the 2010 ROV survey (Table 4).

Table 4. Species by species group

Sebastes (genus)	Gadidae (family)	Hexagrammidae (family)
Canary Rockfish, Copper Rockfish, Greenstriped Rockfish, Puget Sound Rockfish, Quillback Rockfish, Vermillion Rockfish, Yelloweye Rockfish, Yellowtail Rockfish, and Rockfish Unidentified.	Gadidae Unidentified, Pacific Cod	Lingcod, Kelp greenling, Painted Greenling, White-Spotted Greenling, Hexagrammid Unidentified.

Mapping and Spatial Analysis:

ArcGIS 10.0 was used in the spatial analysis of all sampled points. Data from the Trawl dataset were compiled in a concise format, and imported to the geodatabase containing the shapefiles for all of Washington State’s MPAs. Trawl sample locations and latitude/longitude were converted to degree decimal degree prior to georeferencing. All points were projected into NAD_HARN_1983_Washington State Plane_South, to match the projection of the MPA shapefiles. MPAs were selected from the geodatabase by location (the San Juan Archipelago), and managing agency (WDFW and DNR). Both state agencies manage MPAs as functional marine reserves that prohibit the take of groundfish and other organisms.

Diversity Hot Spots were determined using the Hot Spot Analysis “Getis-Ord G_i^* ”. For these analyses data from all eight survey years were examined as one set. The total 188 sample sites were used to generate the Diversity Hot Spot map (one sample had incorrect latitude/ longitude and was discarded). Data selected for this analysis tool were the four values of diversity and evenness. The tool calculates the Getis –Ord G_i^* statistic for each feature in the dataset (these being the sample location points). The distance from each point in the feature

was measured to the 10 closest neighbors as determined by the nearest-k setting. This tool then mapped how clusters of similar diversity values were spatially distributed throughout the surveyed area. Based on the sum of attribute value for a spatially distinct group, the tool calculates the probability of that summation occurring randomly within the total feature dataset. Feature points with high values created hot spots when surrounded by feature points of similar high values. The outputs of this tool are z-scores and p-values that denote significance. Because of the limits of the data collected, the hot spot analysis could not be used to create a model for predicting diversity values at other locations, such as similar methods that utilize Kriging (Bolstad 2008). Presence of Hot and Cool spots were then analyzed by comparing species composition and abundance between the samples comprising the hot spots.

The Spatial Autocorrelation tool (Global Moran's I) was also employed to detect clustering of diversity values. The null hypothesis for this tool states that feature values are randomly distributed across the study area. The outputs of this tool are z-scores, and p-values. Unlike the Getis -Ord Gi* tool the Global Moran's I tool does not locate regions within the data where significant values are clustered (Figure 8).

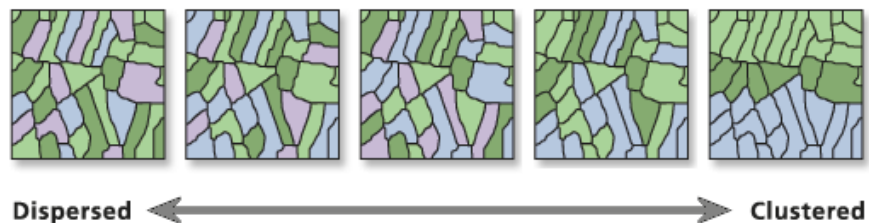


Figure 8 Example of dispersion and clustering (ESRI.com)

Distance from sample location to nearest marine reserve was calculated using the “Near” proximity tool. This gave the Euclidian distance from each sample location to its closest marine reserve. In most instances, the measured distance between reserve and sample point was a relatively straight line that did not cross over land. However, a small number of sample points did. Rather than assume a least cost path of possible fish travel around an obstruction, the determination to utilize Euclidian distance was validated by referenced literature (Tuya et al. 2000). Due to the low number of samples fitting this scenario, the analysis was not likely skewed. The acquired distance from sample point to nearest reserve was then correlated to the values of diversity and evenness.

Presence/Absence data for number of species per species group was also spatially analyzed. The primary tool utilized for this analysis was the Global Moran’s I, to detect any clustering of samples with high counts of species occurrences. Species counts per sample were analyzed using all years for *Sebastes*, *Gadidae*, and *Hexagrammidae*.

Statistical Analysis

All statistical analyses were run and tested using JMP 9.0 (SAS Software). Diversity indices variation by sampling method, and depth stratum were analyzed using the Kolmogorov-Smirnov (KS-test) for non-parametric data. The analysis for index variation over time with regards to sampling method was conducted using similar non-parametric tests. First a resampling ANOVA was utilized to determine if variances were occurring throughout the sampled years. Comparison test of all year pairings utilizing the Wilcoxon method was then run to detect

which year groupings had significant variation in diversity indices values. To determine the effects of two abiotic factors (depth, and trawl tow distance) an analysis of multivariate data employing Pearson's R Pairwise correlations were used. Depth was also categorized and examined for significant effects on diversity indices values using a Chi-Square contingency test.

Habitat variables affecting species presence and composition were analyzed using a series of Chi-Square contingency and ANOVA tests. These tests examined Frequency of Occurrence (FO%) for the three species groups in different depth and habitat categories. For the trawl dataset, FO% was analyzed using a chi-square analysis and an ANOVA. Data were log-natural transformed prior to the ANOVA analysis.

Results

Trawl Frequency of Occurrence

Over the course of the eight surveyed years, a total of 11,749 observations were made of 14 target species. These species were positively identified to the lowest possible taxonomic grouping; for this survey identification to species was possible. The 6 species of rockfish (*Sebastes*) were: Copper rockfish (*S. caurinus*), Greenstriped rockfish (*S. elongates*), Puget Sound rockfish (*S. emphaeus*), Quillback rockfish (*S. maliger*), Redstriped rockfish (*S. proriger*), and Redbanded rockfish (*S. babcocki*). Three species of Hexagrammidae were observed: Lingcod (*Ophiodon elongates*), Kelp greenling (*Hexagrammos decagrammus*), and Whitespotted greenling (*Hexagrammos stelleri*). Four species of Gadidae were observed: Pacific Cods (*Gadus macrocephalus*), Walleye Pollock (*Theragra chalcogramma*), Hake/Pacific Whiting (*Merluccius productus*), and Pacific Tomcod (*Microgadus proximus*). Species frequencies of occurrence rates (%FO) were determined for each year of the trawl surveys. The three species groups differed significantly in their occurrence rates (Likelihood ratio ChiSquare DF =14, 839.33, $p < 0.0001$, Pearsons ChiSquare DF=14, 810.187, $p < 0.0001$). Of the 11,749 observations members of the Gadidae family constituted the vast majority, 92.3% (10,813), of observed fish for all eight years, of which the majority of these observations were Walleye Pollock. The family Hexagrammidae constituted 5.40%, and members of *Sebastes* made up 2.56% of the total observed population. Looking at each year separately, similar significant proportions can be seen (Table 5). Similarly, significance was demonstrated

between the abundance of each species group (ANOVA DF=2, F=17.371, $p < 0.0001$).

Table 5. Frequency of Occurrence and Abundance rates for the three target species groups. Total row represents percentage of total observations and abundance.

Year	Frequency of Occurrence (%FO) and Abundance					
	Gadidae		Hexagrammidae		Sebastes	
2001	81.92	2519	13.59	418	4.49	138
2004	94.55	1595	2.19	37	3.26	55
2006	89.25	1328	5.24	78	5.51	82
2008	98.55	542	0.55	3	0.91	5
2009	96.82	1067	3.18	35	0.00	0
2010	98.73	931	0.95	9	0.32	3
2011	97.38	2231	2.40	55	0.22	5
2012	97.88	600	0.00	0	2.12	13
TOTAL %	92.03	10813	5.40	635	2.56	301

The relationship between depth stratum and species group examined the number of species per group that occurred in each sample. For the genus *Sebastes* and the family Hexagrammidae, the most species caught in a single sample was 3. The most caught for Gadidae was 4. The distribution for number of species present in each sample was shown to be significant by depth stratum only for the families Hexagrammidae and Gadidae (respectively: Likelihood ratio ChiSquare 68.80 and 25.364, $p < 0.0001$). *Sebastes* species were caught in 65 of the 189 samples, of which the number of species per sample was not significantly dispersed between the two depth strata. Species of Hexagrammidae were also caught in 65 of 189 samples, however the number of species present in each sample was shown to increase in the shallow depth stratum. For this family, 68% of single species samples and 100% of the samples containing two or more species occurred in the shallow depth stratum. Gadidae were present in all but 7 of the samples throughout the eight surveyed years. Significance for the deep

depth stratum was shown with 47% of single species samples, 63% of dual species samples, 76% of three species samples, and 90% of four species samples occurring in deep depth stratum.

Diversity and Evenness by Year

Diversity and evenness were compared for each survey year using two diversity index values. The Shannon-Weiner index (H), and the Simpsons D (D) index along with their corresponding evenness calculations were used in determining each samples indices value and mean indices values for each year surveys took place. Prior to the statistical analyses, a comparison of sampling methods utilized to collect diversity data was done. Of the total 189 samples, 143 were under the regional sampling scheme, and 46 were collected under the station sampling method. Diversity and evenness values differed significantly by collection method for all but the Simpsons D index (Table 6). This analysis examined the variability in mean H, D, E_h , and E_d , by sampling methodology employed by WDFW throughout the data collection years.

Table 6. Variation of diversity and evenness values between sampling methods. Kolmogorov-Smirnov test for significance. *Denotes significance.

Diversity Indices	Regional Mean	Station Mean	KS Statistic	D Max deviation	P value
Shannon-Weiner (H)	0.5547	0.4014	0.1084	0.2526	0.0235*
Shannons Evenness (E_h)	0.1884	0.1706	0.0988	0.2303	0.0498*
Simpsons D (D)	1.6137	1.3746	0.0818	0.1906	0.1593
Simpsons Evenness (E_d)	0.1083	0.0457	0.11475	0.2674	0.0138*

Analyses for testing the Shannon (H) and Simpsons (D) diversity and evenness (E_h , and E_d respectively) values for significance for all years sampled were conducted utilizing a resampling analysis of variance (ANOVA) method, and a Wilcoxon Ranked-Sums test. Significance in both Shannon's and Simpsons values ($p < 0.0001$) was found when testing all years together using the resampling ANOVA. Though, this method did not allude to where the significance was occurring, it was possible to examine the decrease in mean diversity and evenness values over all years surveyed. Mean diversity values were shown to fluctuate over 11-year time span of the data collected (Figure 9). The lowest mean diversity values were observed in 2008 for both diversity indices. Mean values were shown to increase from 2008 to 2012, however, they did not surpass the initial diversity values observed in 2001. A non-parametric comparison of pairing using the Wilcoxon method was applied to each diversity and evenness index. Significance for the Shannon and Simpson's diversity indices was found in year pairings between 2001 and all other sampled years except 2006 and 2012. The corresponding evenness indices exhibited similar significance with the exception of Simpson's evenness; this value's significance was found between several year pairings (Table 7).

Table 7. Shannon and Simpsons Diversity Indices: Only observed significant year pairings between all year groupings.

Diversity Index		Shannons (H)		Simpsons (D)	
		Z score	p value	Z score	p value
Year pairings	2001-2004	-2.7615	0.0058	-3.0005	0.0027
	2001-2008	-3.0737	0.0021	-2.3031	0.0423
	2001-2009	-2.9538	0.0031	-2.5412	0.0011
	2001-2010	-1.9873	0.0496	-2.0959	0.0361
	2001-2011	-2.2818	0.0225	-2.0324	0.0421

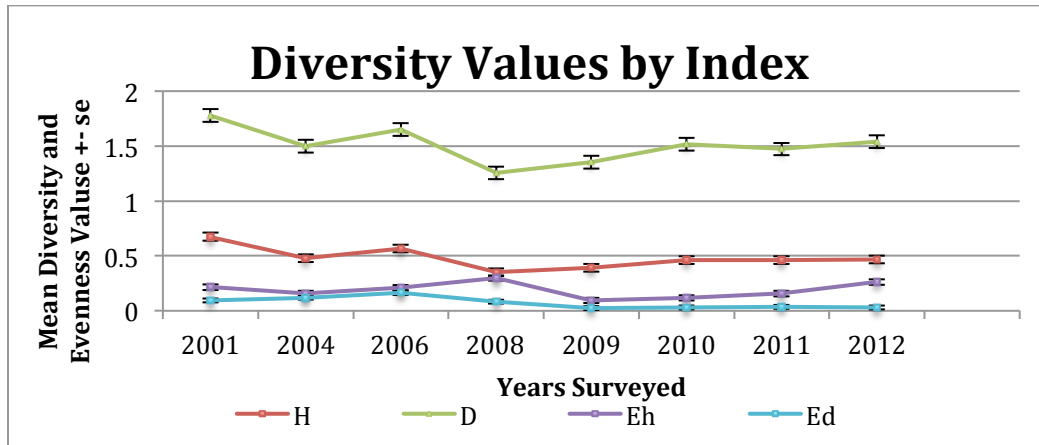


Figure. 9 Mean Values for Shannon-Weiner Index (H, Eh), and Simpsons D (D, Ed) With standard error.

To exclude sampling bias, years were categorized and separated by sampling method and similar sample size. Years 2001, 2004, 2006, and 2011 comprised the regional based sampling method or large sample and were analyzed separately from years 2008, 2009, 2010, and 2012 using a Wilcoxon Ranked-Sums test. The ranked sums analysis one-way chi-square approximation for the years under the regional sampling showed diversity and evenness value varied significantly by sampling year, with the exception of Simpsons evenness (E_H) (H: ChiSquare 8.50409, DF = 3, $p = 0.0361^*$, E_h : ChiSquare 7.7032, DF = 3, $p = 0.0526$, D: ChiSquare 8.8345 DF=3, $p = 0.0316^*$, E_h : ChiSquare 23.4963, DF = 3, $p < 0.0001^*$). Values for these years varied in significance, depending on the pairing of years compared. A Nonparametric comparison for each pair using the Wilcoxon method examined which year pairing demonstrated the most significance. Of the six possible year combinations, the diversity and evenness values for the 2001 – 2011 and 2001 – 2004 were the only years to demonstrate

significant values. (2001 and 2004 respectively: H: $Z = -2.28181$, $p = 0.0225$, $Z = -2.76148$, $p = 0.0058$, E_h : $Z = -2.07641$, $p = 0.0379$, $Z = -2.80587$, $p = 0.0050$, D: $Z = -2.03248$, $p = 0.0421$, $Z = -3.00059$, $p = 0.0027$, E_d : $Z = -3.25852$, $p = 0.0011$, $Z = -4.77580$, $p < 0.0001$). Year pairing 2001-2006 showed no significance for any index value except for E_d ($Z = 2.55546$, $p = 0.0106$).

Years 2008, 2009, 2010, 2012 comprised the station based sampling method. Due to this change in sampling methodology only 12 samples per year were collected. (Table 8.) The ranked sums analysis one-way chi-square approximation showed no significant variance in the values of both indices in diversity and evenness. Further examination using the nonparametric comparison for the 6 possible year pairings also showed no significant variance in the values of both indices in diversity and evenness (all p values greater than 0.05).

Table 8. Shannon's and Simpsons Diversity: Significant year pairings when analyzed by sampling method ($P < 0.05$)

Regional		Station	
2001*	2004*	2008	2009
2001	2006	2008	2010
2001*	2011*	2008	2012
2004	2006	2009	2010
2004	2011	2009	2012
2006	2011	2012	2010

The two abiotic variables addressed were trawl tow distance and depth.

As per the sampling protocol, the target tow distance ranged from 0.2 to 0.4 nautical miles. Tow length was observed to have no linear relation to total catch numbers, and was shown to have no significant correlation to diversity and evenness values (Pearson's R, H: -0.0397, E_h : -0.045, D: -0.0406, E_d : -0.0037).

Depth, when categorized by depth stratum (shallow < 36.6m, and deep >36.6m), showed significant variation by in all was determined for all diversity and evenness values (Wilcoxon, H: $p = 0.0012$, E_h $p < 0.0001$, D: $p = 0.0054$, E_d : $p 0.0005$). A median test for non-parametric data was also performed and showed similar significant values for the four variables. Depth was determined if to exhibit significant negative relationship, when correlated to the diversity indices values by sampling method.

Similarly, when analyzed as a continuous factor, depth was shown to have significant negative correlation to both indices values when analyzed per sampling method. Within the regional sampling scheme, the Simpsons D index for evenness (E_d) was the only value to have a non-significant correlation to depth. Within the station sampling scheme, the Simpsons D diversity index (D) was the only value show non-significance (Table 9).

Table 9. Pairwise correlation: Depth to Diversity Indices by Sampling method* Denotes significance.

Depth Correlation	Region		Station	
	Pairwise Corr. (r)	p value	Pairwise Corr. (r)	p value
H:	-0.3200	<0.0001*	-0.3115	0.0035*
E_h :	-0.3221	<0.0001*	-0.3903	0.0073*
D:	-0.3070	<0.0001*	-0.2100	0.1612
E_d :	-0.0694	=0.4101	-0.3160	0.0324*

ROV: Habitat Preference

During 2010 WDFW’s conducted 139 ROV transects were conducted throughout the San Juan Island Archipelago. Several abiotic variables were determined during the analysis of the 2010 ROV dataset. These variables included: depth, substrate composition, substrate relief, and substrate complexity.

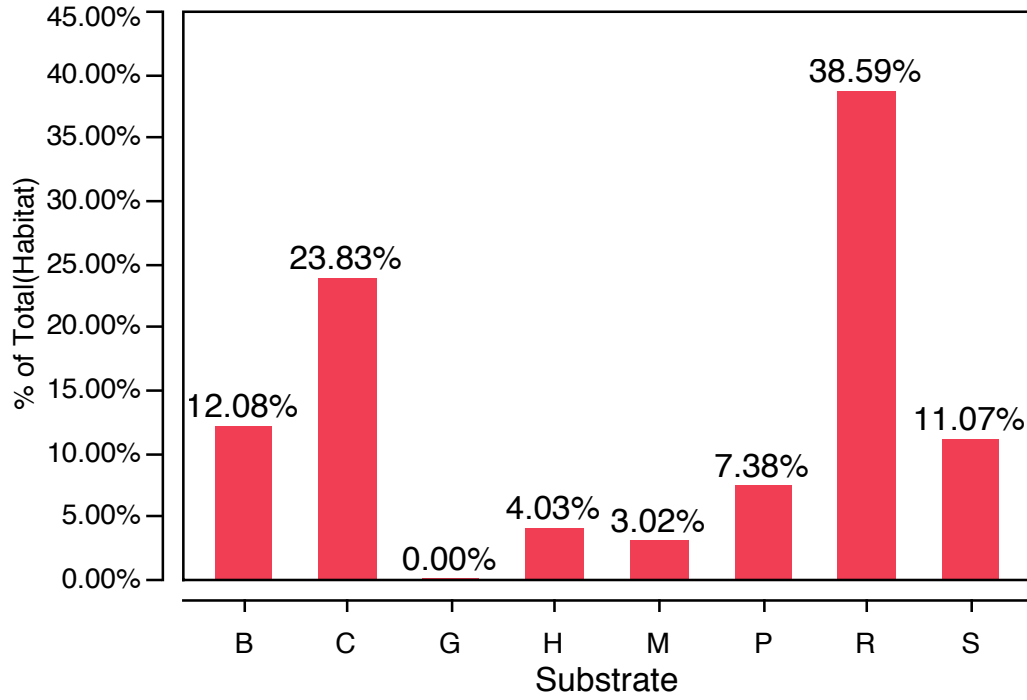
Species compositions were compared to each of these variables with varying degrees of significance. Of the 17 species observed, 13 were positively identified to species. Canary rockfish (*S. pinniger*), Copper rockfish (*S. caurinus*), Greenstriped rockfish (*S. elongates*), Puget Sound rockfish (*S. emphaeus*), Quillback rockfish (*S. maliger*), Vermillion rockfish (*S. miniatus*), Yelloweye rockfish (*S. ruberrimus*) and Yellowtail rockfish (*S. flavidus*) were the positively identified members of the Sebastes genus. The Lingcod (*Ophiodon elongates*), Kelp greenling (*Hexagrammos decagrammus*), Painted greenling (*Oxylebius pictus*), and White-spotted greenling (*Hexagrammos stelleri*) were the positively identified members of the Hexagrammidae family. Unidentified rockfish, unidentified red rockfish and unidentified Hexagrammidae were categorized and counted separately. All observed gadoids were either Pacific Cods (*Gadus macrocephalus*), or were categorized as a family group, Gadidae.

Because of these varying levels in taxonomic ordering, three subgroups: Gadidae, Sebastes, and Hexagrammidae, were used and comprised the summations of all observation for the appropriate species. Members of the Sebastes group comprised 45.98% (1407) of the 3060 species observations. Gadidae observations comprised 45.75% (1400), while Hexagrammidae comprised 8.27% (253) of all observations (Table 10).

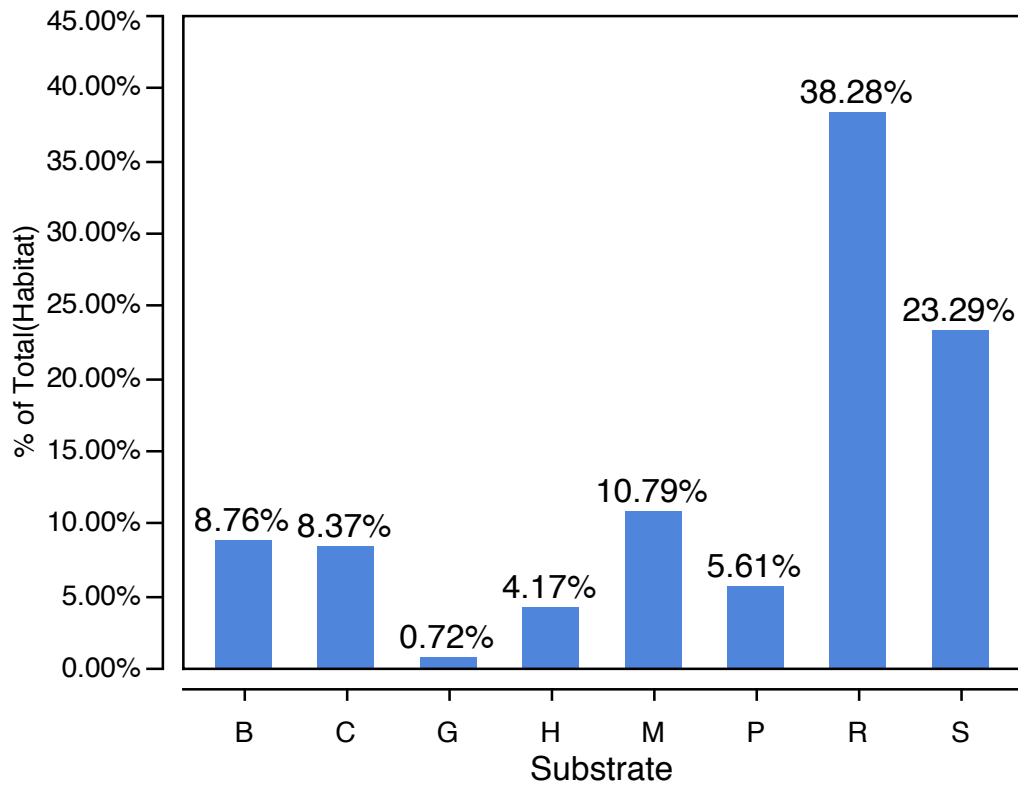
Table 10. Species Group Frequency of Occurrence for all samples.

Species Group	Frequency of Occurrence (%FO)	Total Observation
Sebastes	45.98	1407
Gadidae	45.75	1400
Hexagrammidae	8.27	253
TOTAL	100.00	3060

Relationships between species abundance/occurrence, substrate variables, and depth were evaluated using Chi-Square contingency analyses. Depth stratum was determined using a “count if” function that categorized depth into two classes, Deep (>36.6m) and Shallow (<36.6m) (Pacunski et al, 2013). Eight substrate categories: boulder, cobble, gravel, shell/shell hash, mud, pebble, bedrock, and sand were determined by visual measurements of the substrate when species were encountered (Table 11). The analysis of substrate occurrence and depth showed seven of the eight substrates occurred in both deep and shallow depth strata, with gravel being the exception and not present in any of the shallow observations. Of the eight categories, bedrock and sand contributed to 60.42% of the encountered substrate, with 90.19 % of bedrock and 95.12% of sand observed in the deep stratum (Likelihood ratio ChiSquare 100.665 $p < 0.0001$, Pearson's 106.347, $p < 0.0001$) (Figure 10).



Shallow



Deep

Figure. 10 Habitat Substrate percentages by depth stratum.

All three species groups demonstrated a significant affinity for substrate type (Pearsons ChiSquare, DF =2, 2022.16, $p < 0.0001$). Gadidae observations were made in seven of the eight substrate categories. No members of this species group were observed while the ROV was over a boulder substrate. In most cases, the Gadidae group comprised the highest percentage of total species observation for the seven substrates it was present. The exception to this was the bedrock substrate category, where only 6.7% of the species observed in this substrate were members of the Gadidae species group. Gadidae most frequently occurred in the sand substrate with 40.2% of all Gadidae observations made while the ROV was over sand substrate. The Hexagrammidae group showed the most preference for bedrock substrate with 51.0% of Hexagrammidae observation occurring in this substrate. Members of the Sebastes group were observed most frequently on boulder and bedrock substrate, and comprised 92.8% and 82.3% of the total observations for this boulder and bedrock substrate. Of the total 1407 Sebastes observations, the majority 68.6% occurred on bedrock substrate. The fewest rockfish observations were made in shell hash and mud substrate, collectively comprising 0.7% of the total Sebastes observations (Figure 11).

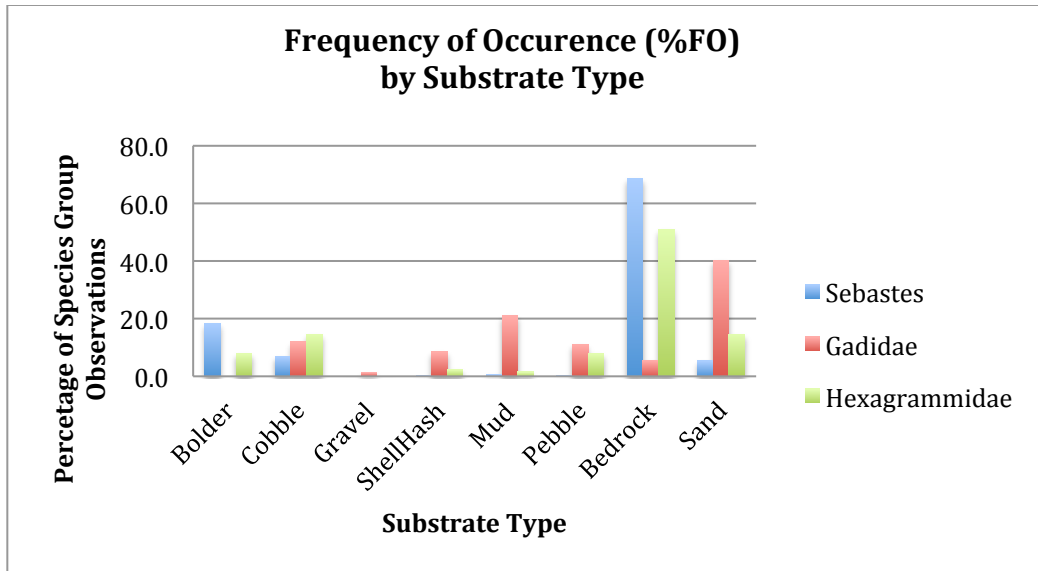


Figure 11. Species group frequency of occurrence per substrate

Table 11. Habitat Substrate Description

Code	Substrate type	Grain Size
B	boulder	0.25-3.0 mm
C	cobble	64-256 mm
G	gravel	2-4 mm
H	shell/shell hash	
M	mud	<0.06 mm
P	pebble	2-64 mm
R	bedrock	continuous
S	sand	0.06-2 mm

Habitat Complexity was also shown to significantly relate to species presence (Pearsons ChiSquare, DF=7, 2315.08 $p < 0.0001$). The Gadidae family was on simple flat habitat through 97.8% of the observations. Hexagrammidae and Sebastes however, were observed to preferred habitat with more structure: 49.2% of Hexagrammid observations and 65.5% of Sebastes observations were made in low but structured habitat (Figure 12). In medium to high complexity habitat, Sebastes accounted for 85%-95% of all fish observations.

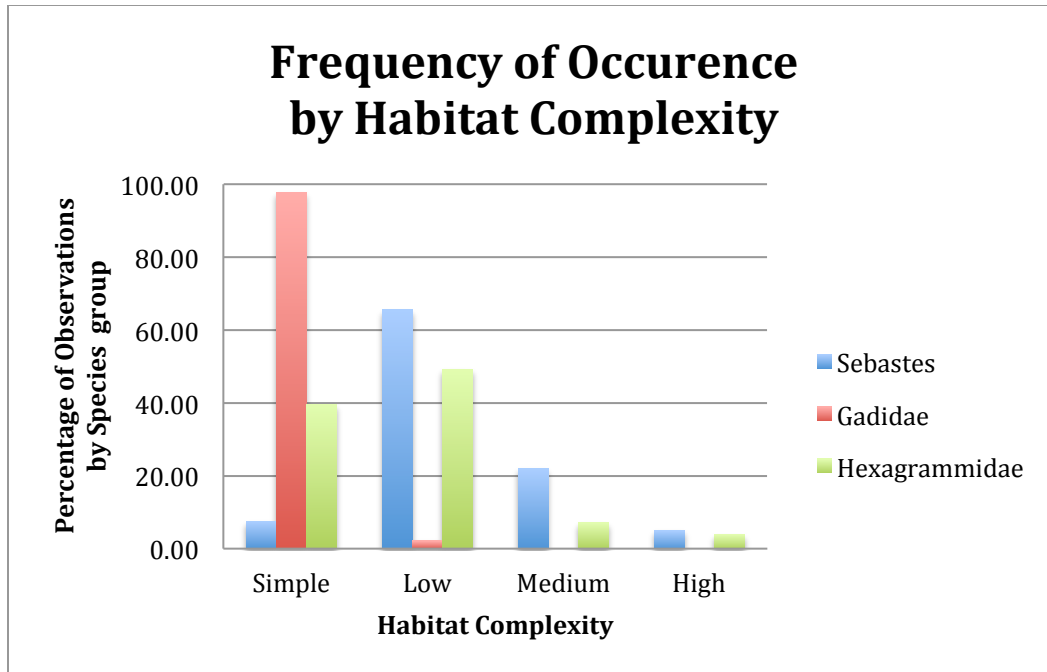


Figure: 12 Species group occurrences per Habitat Complexity

Table 12. Complexity of Benthic Substrate

Complexity	Description
Simple	simple (flat/hummocky w/no crevices)
Low	low (very few crevices, vert. relief 0.5 to 2 meters)
Medium	medium (more than a few crevices, vert. relief >2 meters)
High	high (lots of crevices, Steep slope or wall)

Depth stratum was also shown to be a significant abiotic variable in determining species occurrence rates (Pearsons ChiSquare 589.172, $p < 0.0001$). Over 90% of all observations were made within the deep stratum. Gadidae and Sebastes demonstrated higher occurrence rates, with 96.30% and 91.89% of the observed species in these groups occurring in the deep stratum. Though present in the deep stratum, the Hexagrammids showed a preference for shallower depths. 52.57% of observed Hexagrammids occurring in the shallow depth stratum. Hexagrammidae were the only species group to be higher in abundance in the shallow depth stratum than the deeper, however, this abundance did not allude to a significant preference for the shallow depth stratum (Figure 13).

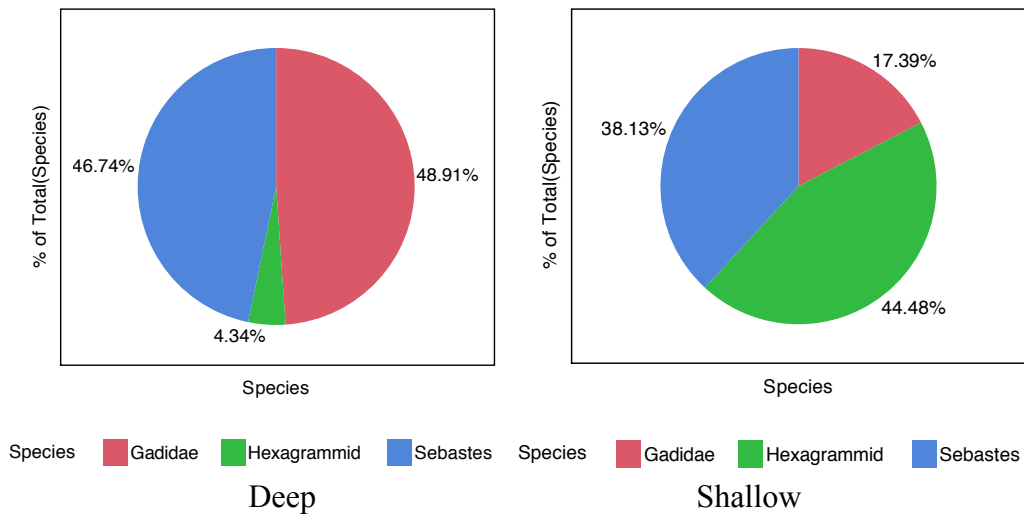


Figure 13 Species group occurrence rates per depth stratum.

Though species group occurrence rates differed significantly between depth strata, an analysis of variance tests for species group composition between depth strata was shown to be nearly significant (ANOVA, DF = 33, F = 2.3585, p = 0.0661). Though non-significant (ANOVA, DF = 1, F = 3.8014, p = 0.0613), the variable for depth stratum was shown to have the most significant influence on species counts. An example, and the only significant interaction for the depth analysis, was made between the species group Hexagrammidae and the deep Depth Stratum (ANOVA, t = -2.12, p = 0.0427).

Spatial Analysis

Spatial analyses for clustering of species and diversity “hot-spots” were run using ArcGIS 10.0 software (ESRI). The Spatial Autocorrelation Global Moran’s I test examined the dispersion of Shannon and Simpson’s diversity and evenness values. All diversity and evenness values, except the Simpsons

evenness, demonstrated significant clustering across the survey area, against the null hypothesis of the test stating no significant clustering (Table 13).

Table 13. Results of the Spatial Autocorrelation Global Moran’s I: All Diversity and Evenness values significantly clustered except for the Simpsons Evenness (E_H). *Denotes significance.

Diversity Index	Moran’s Index	Z-score	p-value
H:	0.3018	5.9452	<0.0001*
E _H :	0.0995	2.0864	0.0369*
D:	0.1766	3.5825	0.0003*
E _D :	0.0830	1.7827	0.0746

The Getis-Ord Gi* Hot Spot analysis spatially assigned significance to sample points with similar diversity levels as their 10 closest neighbors. “Hot Spots” and “Cool Spots” of diversity values are seen throughout the surveyed region. However, the majority of samples were shown to not exhibit significant levels of diversity when compared to their neighbors by this analysis. The analysis of the Shannon’s index values demonstrated significant grouping of diversity value for 68 (36%) of the 188 samples. Within these 68 samples 38 were significantly low values (GiZScores < -2.0, p < 0.05) and 30 were significantly high values (GiZScores > 2.0, p < 0.05) (Appendix B-1). The Simpsons index demonstrated significant groupings of samples 32 (17%) of the 188 samples, with 8 being significantly low values (GiZScores < -2.0, p < 0.05) and 24 being significantly high values (GiZScores > 2.0, p < 0.05) (Appendix B-2).

Both the Shannon’s and Simpson Diversity indices showed a significant hot spot in the NE region of the surveyed area, nearest to Lummi Island (Mean Shannon’s Diversity: 0.7571, mean Simpsons diversity: 1.8764) (Figure 14) (Appendix C-1). The “Lummi” hot spot was comprised of 13 samples. Sample

represented all years surveyed except 2012. Four of the 13 samples were in the deep depth stratum, while 9 samples were in the shallow depth stratum. Ten species were observed in these samples from all three species groups, with total abundance 665 fish. Because presences a single Quilback and Copper rockfish were detected only in only two samples, these species were categorized as *Sebastes sp.* for graphical representation (Table14). Members of the Gadidae family contributed the most to abundance with 545 fish, however samples dominated by Gadidae, were observed to have two or more species present, thus reflecting in diverse samples (Appendix D -1).

Table 14. Species abundance for the13 Samples of the “Lummi” Hot Spot and 12 Samples of the “San Juan” Cool Spot

Species:	Lummi Abundance	San Juan Abundance
Lingcod	3	6
Kelp Greenling	1	0
Pacific Cod	45	78
Pacific Tomcod	236	25
Hake	22	1
Walleye Pollock	309	821
Puget Sound rockfish	2	1
Sebastes spp.	2	6
Whitespotted Greenling	45	0

A noticeable clustering of “Cool” areas was observed in close proximity to the San Juan Island Friday Harbor Marine Reserve (Appendix C-2). This grouping consisted of 12 samples: 7 significant (GiZScores < -2.0, $p < 0.05$) and 5 non-significant samples (4 of which were very close to significant, $p = 0.0502$). Due to the proximity of the samples to a large marine reserve, an examination of the species composition was conducted. The samples represented all years surveyed except 2004. Ten of the 12 samples were in the deep depth stratum,

while 2 samples were in the shallow depth stratum. Similar to the Lummi hot spot, the “San Juan” Cool spot had species from all 3 species groups. Mean value of the Shannon’s index was 0.313. Mean value of the Simpsons index was 1.2132 (Figure 14). The proportions at which species comprised samples were highly dominated by a single species, Walleye Pollock. In total, 8 species were observed with a total abundance of 938 fish caught over the 12 samples. No observations of Whitespotted or Kelp Greenlings were made. Green and Redstripe Rockfish were observed in 3 samples and were categorized as *Sebastes spp.* (Appendix D – 2)

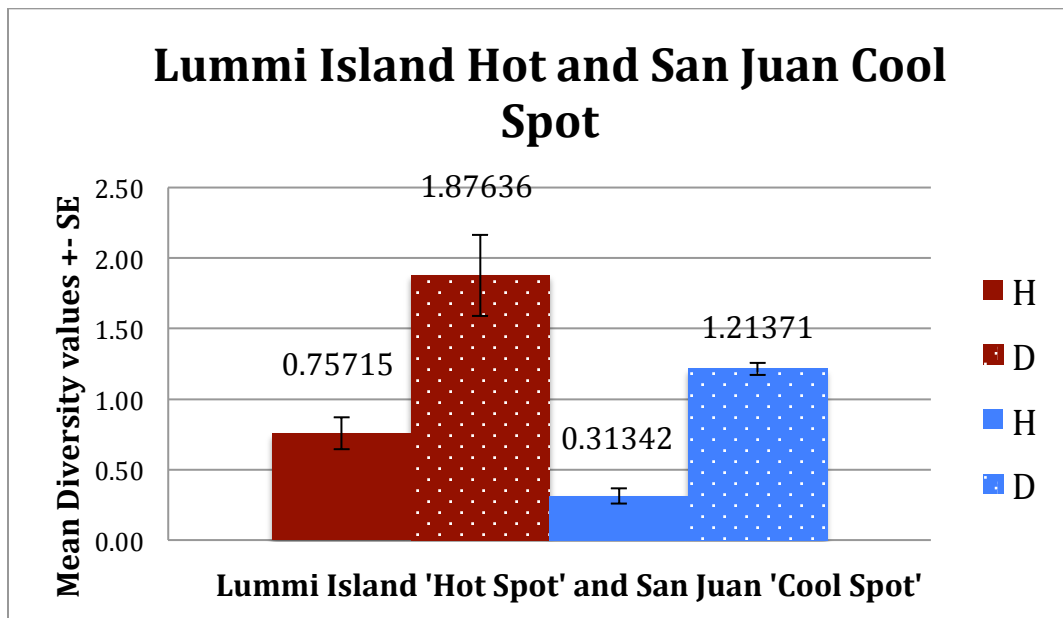


Figure 14. A comparison of the Lummi Island “Hot Spot” and San Juan Island “Cool Spot”. Mean Shannon’s and Simpsons diversity value represented. See Map in Appendix C for reference.

A similar comparison was conducted between two regions surrounding Cypress Island (Appendix C-3). The first region was a “hot spot” area observed off the SW shoreline of Cypress Island (Mean Shannon’s Diversity: 0.85652, mean Simpsons diversity: 2.1829) (Figure 15). Samples represented years 2001,

2004, and 2006. Ten samples were shown to be significant ($\text{GiZScores} > 2.0$, $p < 0.05$), 3 of which were within the Cypress Island Aquatic Reserve. A total of 299 fish were observed between all ten samples. Though members of the Gadidae family were the most abundant group, the majority of samples had more than one species of Gadidae present, similar to the Lummi hot spot (Appendix D-3). All but one of the ten samples was in the shallow depth stratum.

The second region was a grouping of non-significant samples North of Cypress Island. Eleven samples were taken in this region, with a total of 831 fish observed. Six of the samples occurred in the shallow depth stratum, while 5 occurred in the deep depth stratum. These 11 samples were not assigned any significance during the running of the Getis –Ord Gi^* test. Mean diversity values for the 11 samples were less than the Cypress hot spot (Shannon's Diversity: 0.4661, mean Simpsons diversity: 1.55406) (Figure 15). An examination of the species composition showed samples dominated by Walleye Pollock (Appendix D-4).

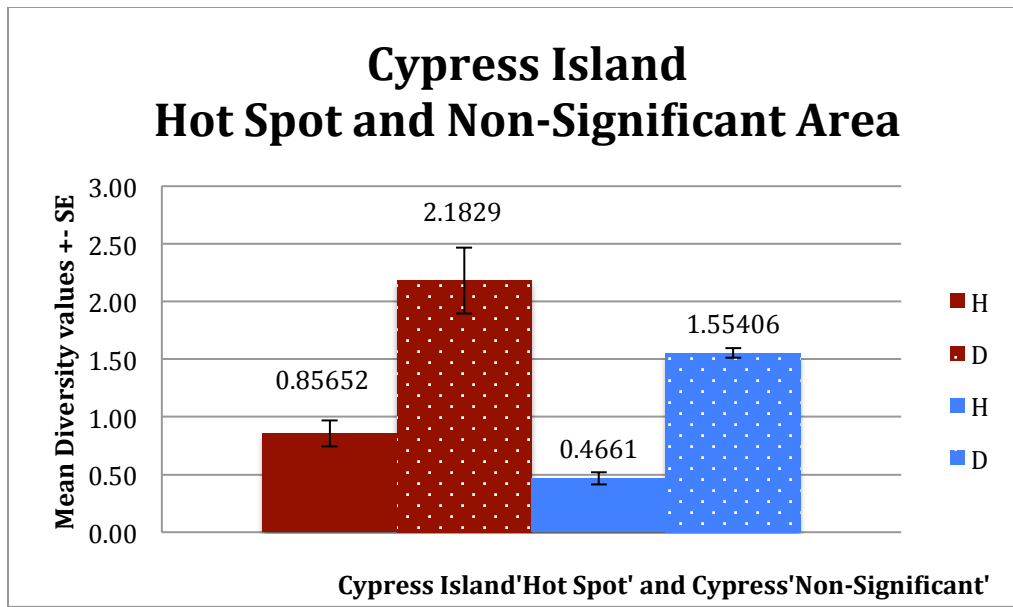


Figure 15. A comparison of the Cypress Island SW “Hot Spot” and Cypress Island N “Non-Significant spot”. Mean Shannon’s and Simpsons diversity value represented. See Map in Appendix C for reference

Using Spatial Autocorrelation Global Moran’s I it was also possible to determine that locations of samples with 0, 1, 2, 3, or 4 species per sample were significantly clustered. This test was conducted for each species group, all of who were shown to exhibit significant clustering by number of species present in each sample (Table 15). This analysis method did not take into account abundance, however, it did illustrate where more species were occurring for each species group (Appendix E-1 to E-3).

Table 15. Results of the Spatial Autocorrelation Global Moran’s I: Significant cluster patterns of samples with 0 to 4 species per species group.

Species Group	Moran’s Index	Z-score	p-value
Sebastes	0.124807	2.5376	0.011158
Gadidae	0.198056	3.9328	<0.0001
Hexagrammidae	0.336231	6.630993	<0.0001

Discussion

Frequency of Occurrence

Frequency of occurrence rates between the ROV data and Trawl data differed drastically. No significant test were done between the two, however, the occurrence frequencies were measured for the same species groups. The vast majority, over 90% of observed species within all but one of the years of the trawl surveys were members of the Gadidae family. In contrast, species of Gadidae only made up 46% of the total observation for the 2010 ROV survey. This variability in frequencies can be attributed to the utilization of two very different survey techniques (number of samples collect and location of samples).

Depending upon depth, the trawl net opening would range between 8 and 14 meters, capturing the fish within its path (Palsson and colleagues 2009). The ROV submersible has a much smaller “capture” area, this being represented by the calculated transect width observed by the mounted camera (Pacunski et al. 2013). ROV transect width varied between 1 and 3 meters (Pacunski et al 2013).

Habitat and depth preferences were demonstrated for all species groups. Within each species group, members of Gadidae, Hexagrammidae, and Sebastes exhibit similar life traits (Love et al. 2002). For example, several species of Sebastes are solitary and prefer nearshore rocky habitat, while other species school, and are observed within a greater depth range ie: the Puget Sound Rockfish (Yates 1988). The variation in life history traits of individual species per family group was not examined for this analysis. By focusing on the general

traits like habitat and depth preference for each species group, it was possible to observe significant variations between the three species groupings.

Several significant observations were made between species groups the environmental variables analyzed in the 2010 ROV survey data. The observations made with these analyses are supported by reviewed literature, and the basic ecology of the three species groups studied (Pacunski et al. 2013, Palsson et al. 2009, Love et al. 2002). Within this study, two species groups, the genus *Sebastes* and family *Hexagrammidae* are composed of species that are associated with complex, structured, rocky habitat. The substrate and complexity categories observed were similarly distributed in both depth strata, though over 90% of the observations were made in the deep depth stratum. Species group composition between the two depth strata was shown to be significantly different, with the only preference for the shallow stratum being by members of the Hexagrammidae family. The observed species within the Hexagrammidae family (Lingcod, Kelp greenling, and Whitespotted greenling) are commonly found at shallower depths (Yates 1998). In both depth strata, observations of any fish were most frequently made while the ROV was over the bedrock substrate. Bedrock substrate offers levels of habitat structure preferred by several rockfish (*Sebastes*) species (Palsson et al. 2009). This was evident by the high frequency of Rockfish observations, and the over all percentage of total organism observations while the ROV was over the bedrock substrate. The family Gadidae represented roughly 46% of the total fish observation of the 2010 ROV survey. The four species observed during the survey exhibit similar life history traits, such as school and

broadcast spawning (West, 1997). The data represented in this study demonstrated that these fish prefer habitat with little complexity and structure as the Gadidae were most frequently observed above a sandy substrate in the deep depth stratum. The results of these occurrence rates for these three in the different environmental variables lend to the interpretation of the diversity data analyzed in conjunction with ROV data.

Measuring Diversity

The utilization of the Shannon's and Simpsons diversity indices represented a different approach to measuring the groundfish communities of the San Juan Archipelago. The data used to compile and calculate these index values are data used to measure the relative abundance, species composition, and biological characteristics of key groundfish species (Palsson and colleagues 2009). As for the use of diversity indices, much care should be taken when selecting which index to use (Yoccoz et al. 2001). The diversity indices chosen for this analysis represent two that are commonly used to in the ecological studies of community structure (Greene 1975, Lande 1996). The Simpsons index was utilized as a measurement the probability that any two individuals within a sample are different species. As a measurement of species diversity for a given number of species, S , within a community (each sample) the index has a maximum value equal to S when all species are equally present (Lande 1996). Similarly, the Shannon's index measure diversity for a given number of species, S , and reaches a maximum value, the log-natural of S , when all species are equally frequent in the community (Lande 1996). The Simpsons and Shannon's index work well with

relatively small samples. The only samples that represented complete evenness, while containing no diversity were samples containing only a single species. These were kept in the analysis because these samples were representative of area that could be dominated by heavy abundance of a single species. This information is useful in understanding the distribution characteristic for some groundfish species.

Measurements of the diversity values for both indices were low when compared to the maximum potential value based on the number of species present in each sample. The community structure between the three species groups is such that the natural abundance of selected species would make for seemingly low diversity. Gadidae have historically occurred at a much higher abundance than Sebastes and Hexagrammidae within the greater Puget Sound region (Palsson et al. 1997, 50 CFR Parts 223 and 224. 2000). Although diversity values may be mathematically low in comparison to the diversity indices maximum potential, the significance of a sample with higher diversity were representative of low counts of Gadidae species, namely Walleye Pollock, and higher abundance of Sebastes and Hexagrammidae species.

Addressing temporal variation in the diversity of targeted groundfish species is important for understanding factors that may affect community structure. In this case, several species of groundfish composed a community of fish that have been targeted by conservation efforts in the form of marine reserves (Palsson et al. 2003). While significant temporal variations were observed in diversity and evenness values between the eight sampled years, within-year

variation in diversity and evenness values was only shown significant in one year, suggesting that changes diversity levels occur slowly. This slow change was also demonstrated by the lack of significance in diversity values between the years surveyed under the station based sampling method employed by WDFW. Though these years consisted of smaller samples, they were surveyed consecutively, allowing for a more sensitive means of detection for any regional change in diversity. The observed significant variation in diversity values occurred over time spans from 3 to 10 years from 2001. A significant decrease in mean diversity was demonstrated between 2001, the first year surveyed, and all years except 2006 and 2012. This downward trend in mean diversity values accurately describe changes in community structure over time and space, yet detecting the cause for such change becomes difficult when analyzing the community in this fashion (Greene, 1975).

During the data collection process, WDFW changed sampling methods from regional based sampling to station based (WDFW personal correspondence). This affected the effort and number of samples collected in the San Juan Archipelago each surveyed year and elapsed time between surveyed years. Approximately 76% of the samples analyzed for the study were collected under the regional sampling method. Thus the majority of diversity and evenness values for this analysis were acquired from samples collected under the regional sampling method. Testing the variability in diversity and evenness values by sampling method showed that all diversity values, with the exception of the Simpsons D index, were affected by the sampling strategy employed. However,

since diversity value variations were shown significant in year pairing between the two methods, it is possible to attribute this variation to change in population structure over time more so than sampling method.

Temporal Variation

The temporal variations observed in diversity and evenness values were indicative of the rate at which community structure changes occur within the ecosystem. However, an increase in time did not necessarily correspond to greater significant variation. The most significant variation in diversity values was observed between 2001 and 2008, with 2008 representing the lowest mean diversity values recorded. 2008 also had the lowest number of total fish abundance, and was the first year of the station based sampling method. Changes in abundance are incorporated into the calculations for each diversity index; however, this is assuming that all species have an equal probability of being encountered (Yoccoz, et al 2001). With the survey returning to similar locations each year, species encountered on a previous year had an equal probability of occurring in subsequent years.

Similar to the significance of depth to occurrence frequencies, depth was shown to greatly contribute to change in species composition and was negatively correlated to species diversity. For the diversity values, the shallower samples exhibited higher diversity and evenness levels. Several species of the three groups: Sebastes, Gadidae, and Hexagrammidae inhabit the water column at various depths (Yates, 1988). The most abundant species, Walleye Pollock, are schooling member of the Gadidae family. These fish inhabit various depths,

though are often found in high abundance in deeper regions of the water column (West, 1997). Because of this characteristic, Pollock were often the dominant species in deep stratum samples, resulting in the relatively low diversity values for deep stratum samples.

A counterpart to the temporal variation in diversity values, the spatial distribution of diversity values was also shown to be indicative of sample locations that repeatedly had significantly high or low levels of diversity. The observations made within this analysis are also reflective of areas that experience more intense sampling. With the change from regional to station based sampling in 2008, the survey vessel, *F/V Chasina* trawled in as close to the same location (station) to minimize the variation in sea floor and habitat to provide more powerful inter-annual comparisons (Palsson and colleagues 2009).

Spatial Distribution

The geographic distribution of significantly high and low diversity groupings showed no influence by proximity to a marine reserve. However, within this study it is not possible to conclude that marine reserves have no effect on the diversity values. Descriptive comparisons between significantly “hot” clusters and significantly “cool” clusters were made to describe the species composition within these differing areas. The first two groups to be compared were a “hot” grouping of samples near Lummi Island and a “cool” grouping of sample very close to WDFW’s San Juan Friday Harbor Marine reserve. It was shown that the cool area samples were dominated by a single gadoid species, Walleye Pollock. Though the locations of the samples also were in close

proximity to an established marine reserve, the samples were taken in the deep depth stratum favored by the Gadidae family. The compared “hot” grouping of samples consisted of members of Gadidae as well, however, were species were more evenly distributed throughout the samples. These samples were also predominantly taken in the shallow depth stratum (West 1997).

Of the 188 samples, three fell within the boundaries of the Cypress Island Aquatic Reserve, managed by the Washington Department of Natural Resources. This reserve prohibits the harvest for all species of groundfish, and has been shown to support a vast diversity of intertidal and nearshore fish species (WFC 2011, DNR 2007). The diversity values for the three samples were significantly high, however, the reason for this may be confounded by several factors, including the protection status of the area where these samples were taken. As was shown, diversity values had a negative correlation with an increase in depth. Shallower samples had higher levels of diversity due to the increased presence of multiple species from all three species groups (Coleman et al. 1997). The three samples within the reserve boundaries were statistically similar to seven more samples, just outside the reserve boundary. In total, nine of the ten sample points were in the shallow depth stratum. Though the Gadidae species group demonstrated to prefer the deep depth stratum was present in these samples, their presence was represented by 3 - 4 species being observed in some samples.

The temporal and spatial distribution of samples by species group gave a visual representation for regions of the study area that continually had abundance of more than one species within each species group. Groupings of samples with 1

– 2 species of *Sebastes* were concentrated in a few locations. Notably one location was within close proximity to the San Juan Friday Harbor marine reserve. This reserve, managed by WFDW, restricts the harvest of groundfish and protect habitat suitable for *Sebastes* species (Van Cleve et al. 2009). It is possible that the occurrence of more than 1 species per sample in this area was influenced by the presence of fish using the habitat protected by this marine reserve.

This study demonstrates the significant spatial and temporal variation in diversity values and to shifts in the species composition within the region. Assessing diversity on a regional level for an ecosystem such as the San Juan Archipelago is an important step in monitoring the groundfish community variations. Spatial and temporal trends for the groundfish species represented here are have been poorly understood throughout the Puget Sound region (Williams 2010). These trends are an important means of ecosystem monitoring (Palsson et al. 2003, West 1997). However, the challenge in determining whether the shifts in trends of diversity species composition, and habitat preference are related to the implementation of marine reserves, are representative of natural shifts in community structure, or are being influenced by a combination of factors, makes assessing cause difficult. Further evaluation in the variation of diversity value could be aided by testing potential confounding variables separately. For example, research using similar diversity calculations, multivariate habitat modeling and marine spatial planning has been used to map potential marine reserve locations (Keith 2005, Douvere 2008). The potential for such work in the San Juan Archipelago exist. Though few studies have been done to assess

regional effect of marine reserves, reserve provide an important mitigation strategy for selected groundfish species (Tuya 2000). Combining diversity information, from studies like the one presented here, to future studies of abundance and habitat utilization will add to the understanding of groundfish communities within the San Juan Archipelago.

Chapter 3: Conclusion

Biological Monitoring

Measuring and identifying patterns in biodiversity can be a powerful tool in assessing an ecosystem's capacity to provide for the organisms it harbors (Primack 2010). Monitor diversity in areas where reserves have the potential to augment the biological community structures outside the protected area is especially needed to assess the ecological response to these management strategies. However, accomplishing this task requires monitoring that collects a vast amount of necessary information. Largely the objectives of monitoring biological diversity can be assigned to two categories: scientific and management (Yoccoz et al. 2001). The data utilized for the analyses of this study were collected in efforts to meet both these objectives.

As is common in fisheries management, ecological research is often accomplished by utilizing any suitable data source (NRC, 2004). The Washington Department of Fish and Wildlife have conducted trawl surveys of the Puget Sound since 1987. These surveys have proven to be an invaluable fishery-independent indicator for groundfish population abundance (Palsson and colleagues 2009). Prior to these surveys, population data was derived from catch landing reports (recreational and commercial), and was often not representative of true population status for several species. In 1990, WDFW implemented the use of marine reserves as a means to mitigate declining stocks of groundfish, primarily species of the *Sebastes* genus. Efforts to protect these groundfish species via the utilization of marine reserves are dependent on how the reserves

ecologically function. Tasked with managing five of the marine reserves examined in this study, WDFW relies on biological surveys to detect changes in population and community structure within their marine reserves. In years following the implementation of several marine reserves, WDFW revised their Puget Sound Groundfish Management Plan, adding emphasis to ecosystem-based management and the conservation of biodiversity within the groundfish stocks (Palsson et al. 1998).

Significance of this Study

The selection of the San Juan Archipelago region for this study was important for two main reasons. First, this region has a history of established marine reserves that have been subjected to several studies, thus data on past species abundance was available (Van Cleve and colleagues 2009, Eisenhardt 2001). Second, the San Juan region has been previously subject to intense recreational and commercial fisheries. Thus, marine reserves would potentially have a greater benefit for species in this region over areas that were not subjected to as intense of fishery. Though studies have examined diversity, abundance, and community structure for groundfish populations between reserves and non-protected waters within the San Juan Archipelago (Palsson et al., 2000), none have focused on what large scale regional effects the marine reserves have on the surrounding non-protected areas.

To hypothesize that a marine reserve would have an outward effect on its surrounding environment requires that there be priori knowledge of how that protected area functions in that ecosystem. WDFW surveys of marine reserves

within the San Juan Archipelago have demonstrated benefits such as increased abundance, and size of groundfish within their boundaries (Palsson et al. 2003). In addition to these two factors, larval dispersal can also greatly increase from within marine reserves. The combination of these factors create was had been called the “Reserve Effect” theory, stating that a protected population can be use to supplement a non-protected population through larval dispersal and adult spillover (Allison et al. 1998). The reserve effect may be highly species specific. For example Black rockfish (*Sebastes melanops*), as species of *Sebastes* that congregate over rocky habitat, were shown to exhibit lower levels of larval dispersal than previously assumed by modeling, thus may not contribute progeny to area outside of reserves (Miller and Shanks 2004). Similar larval dispersal studies have observed this characteristic in other *Sebastes* species (Buonaccorsi et al. 2002). The detection of reserve effect is often hampered by a lack of knowledge about biotic and abiotic factors the level of biological and ecological knowledge for the species. Within the San Juan Archipelago, referencing marine reserves as larval distribution points has demonstrated significant variance in dispersal potential base on locations of reserve and influences of surface currents (Engie and Klinger 2007). Other abiotic factors such as El Niño Southern Oscillation, thermocline layers, and ocean acidification need to be clearly investigated as factors affecting the role of marine reserves for groundfish communities (Sato and Wyllie-Echeverria 2004).

The approach of using the Shannon-Weiner and Simpson’s diversity indices represented in this study allowed for trends in diversity and species

evenness to be observed over time, for three distinct families of groundfish. The observed results of this study were only sensitive to the fact that changes in community structure had occurred, but could not determine the cause for the observed changes. An understanding of a “biological timeline” for the species of concern may give clues as to how the community structure of these fish change through time, and how these species will react to protection. With the exception of the Gadidae family, the species examined in this study (Hexagrammidae and Sebastes) exhibit slow growth, late maturity, and occur in relatively few numbers through out the San Juan region (Love et al. 2002, Pacunski et al. 2013). These fish are also high trophic level organisms and once mature are not subjected to much predation. Using the methodology of this study to monitor temporal and spatial variations for these species represents an important way to analyze trends in their community structure.

This study emphasizes the use of quantifying biodiversity as a means of detecting population trends over time and space. There are several other techniques for monitoring community structures; however, biodiversity is often associated with the health of the habitat. In an era where managing agencies such as WDFW are shifting efforts towards ecosystem-based management, surveys that monitor the broad scale changes within an ecosystem will be utilized.

The Continued use of MPAs

Though no observable changes in biodiversity could be attributed the marine reserves of the San Juan Archipelago, I believe the continued utilization of reserves should be a priority of management agencies. As is often the case with

several management strategies, stakeholders and invested peoples expect relatively quick results. Within the context of this study, the five WDFW managed reserves examined have only been established for 23 years (Van Cleve et al. 2009). In some cases since these reserves were since 1992 collecting population data (Palsson et al. 2003). As these reserves were implicitly designed to protect rockfish and other fish associated with rocky habitat, the long-term affect of their protective status may yet to be seen due to these species long life and slow maturation characteristics. This may be evident by the lack in increase in rockfish *Sebastes sp.* densities since surveys in the mid 1990's were conducted, however, Lingcod densities have significantly increased during this time (Palsson et al. 2003). Variations like this demonstrate the need for continued observation within these marine reserves, while also focusing attention to area outside the protected boundaries.

The continued utilization and implementation of marine reserves in Washington State is not without its own hindrances. Currently there are 11 different agencies, managing 127 MPAs within the State (Van Cleve et al. 2009). The differences inherent to the diversity of management practices and goals lend itself to disorganization between agencies in charge of the MPAs. However, preliminary data from a survey sent to 57 stakeholders and invested peoples (including managing agencies) suggest that the majority of correspondents believe that MPAs can: be an effective too to conserve and manage marine resources in the Puget Sound, and believe that a network of marine reserves should be established within the State (Hanlon 2013, unpublished). Positive response to

marine reserve implementation is an encouraging sign that attention is being focused towards the protection and conservation of groundfish species.

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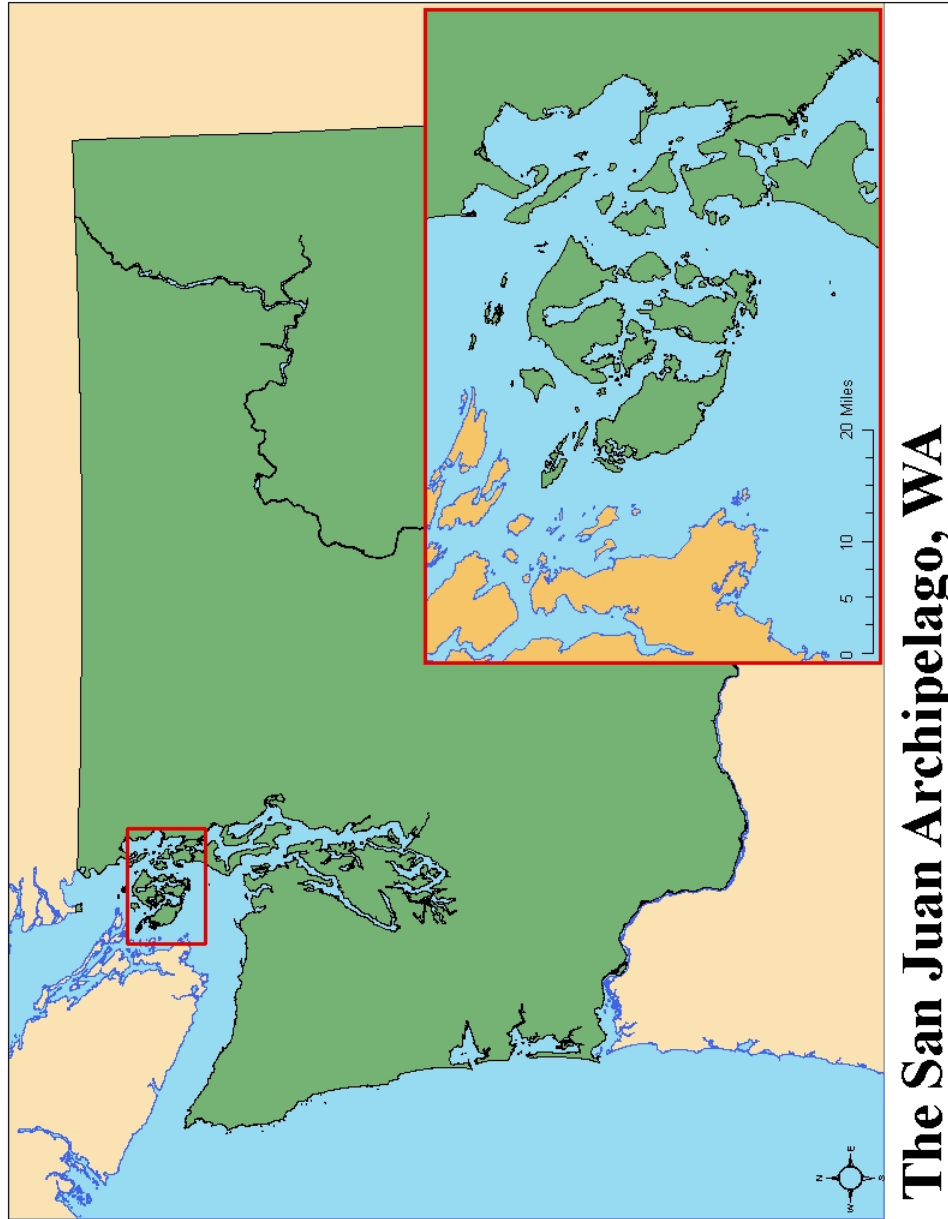
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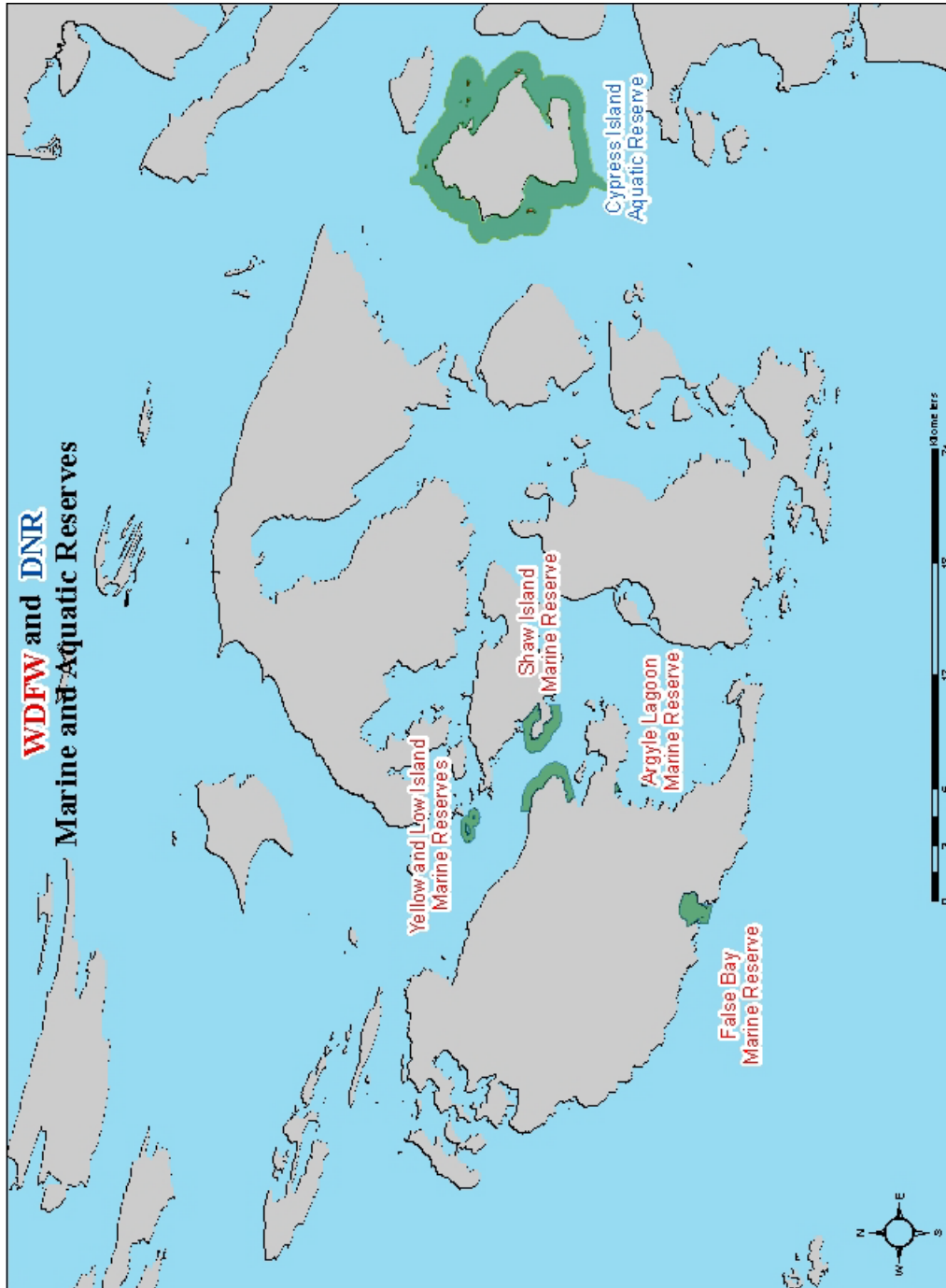
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Appendix A

A-1. Study Area

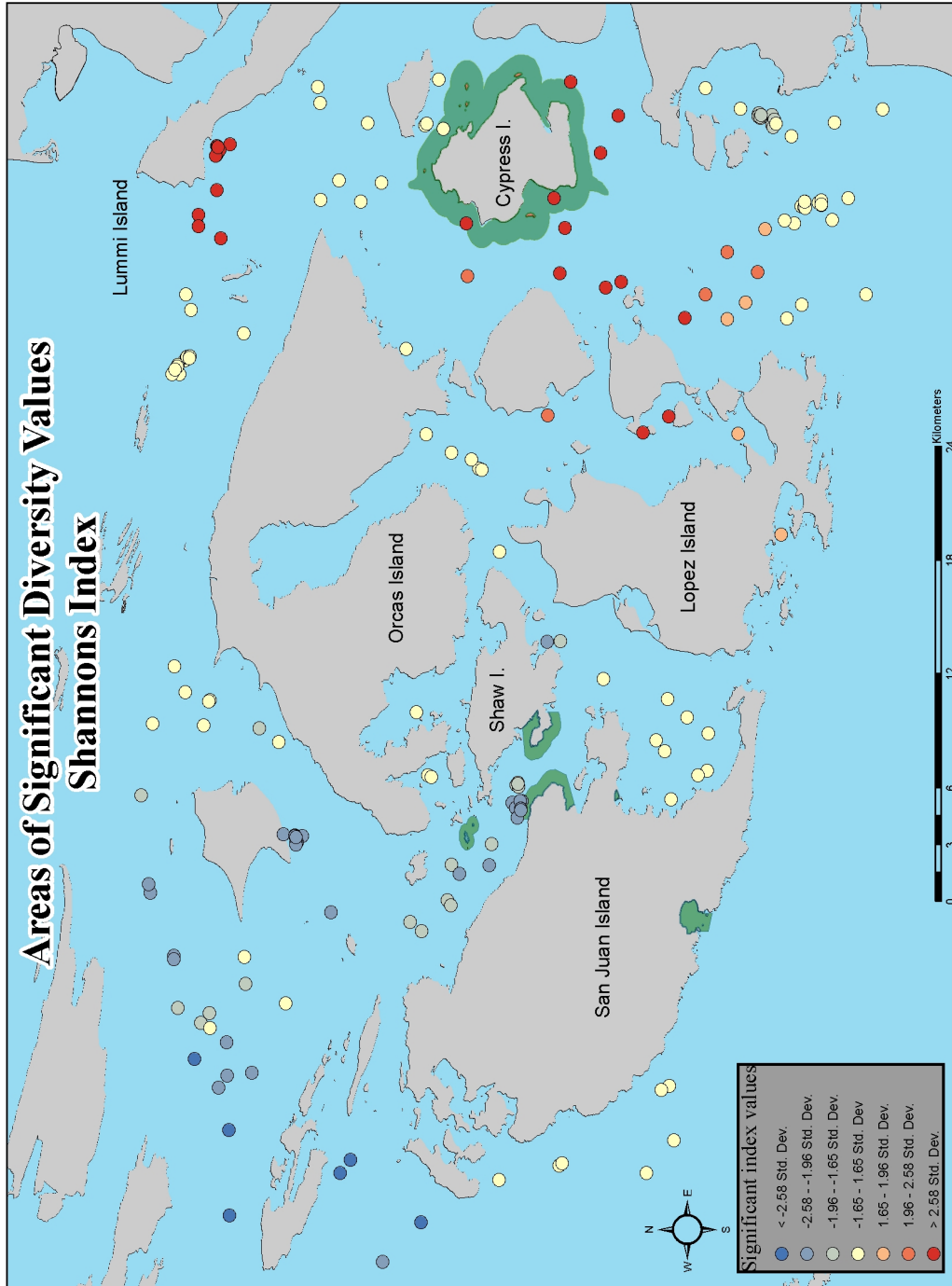


A-2. WDFW and DNR Marine Protected Area Locations

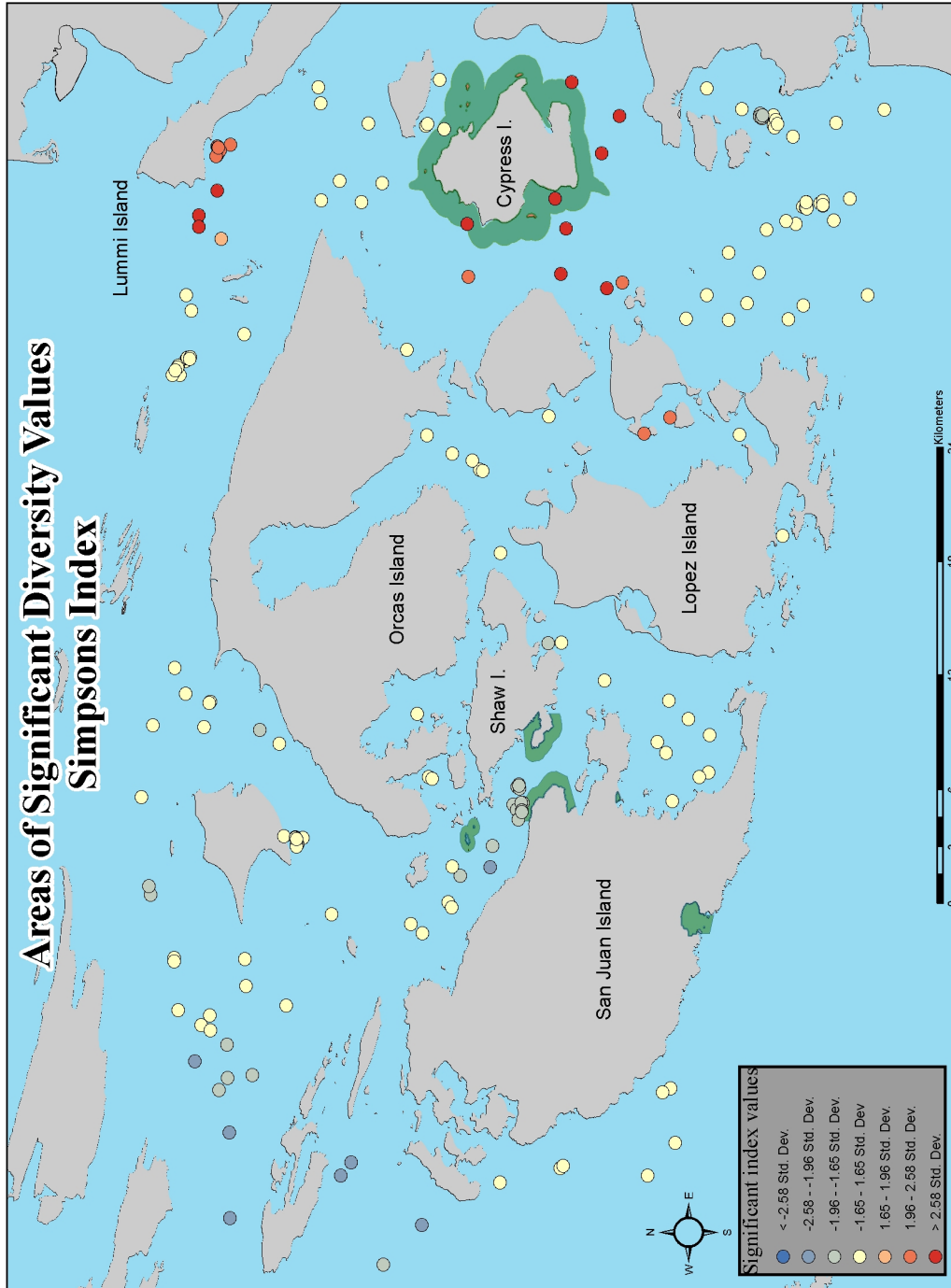


Appendix B

B-1. Shannon's Diversity Map: Significant Grouping

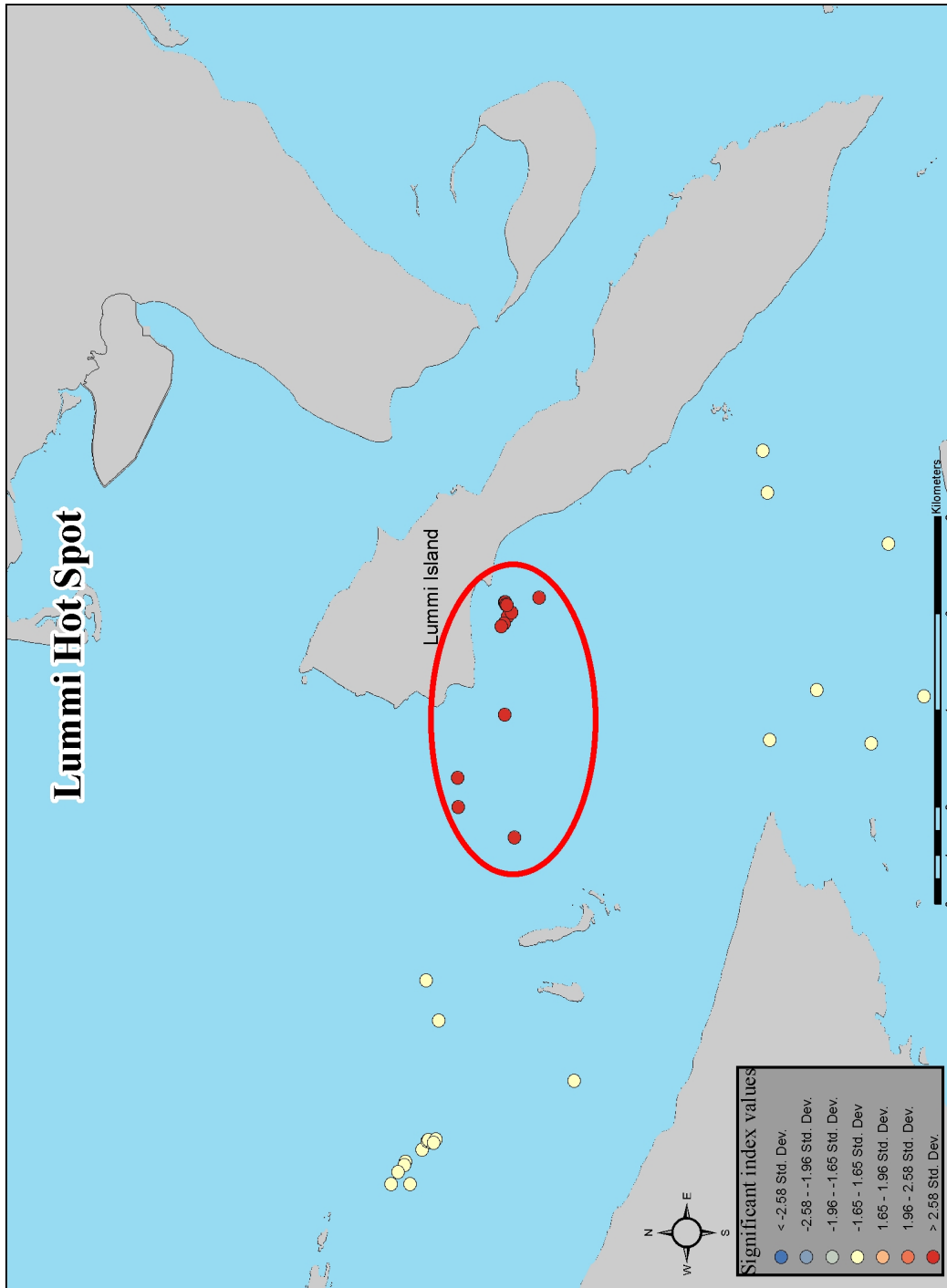


B-2. Simpsons Diversity Map: Significant Grouping

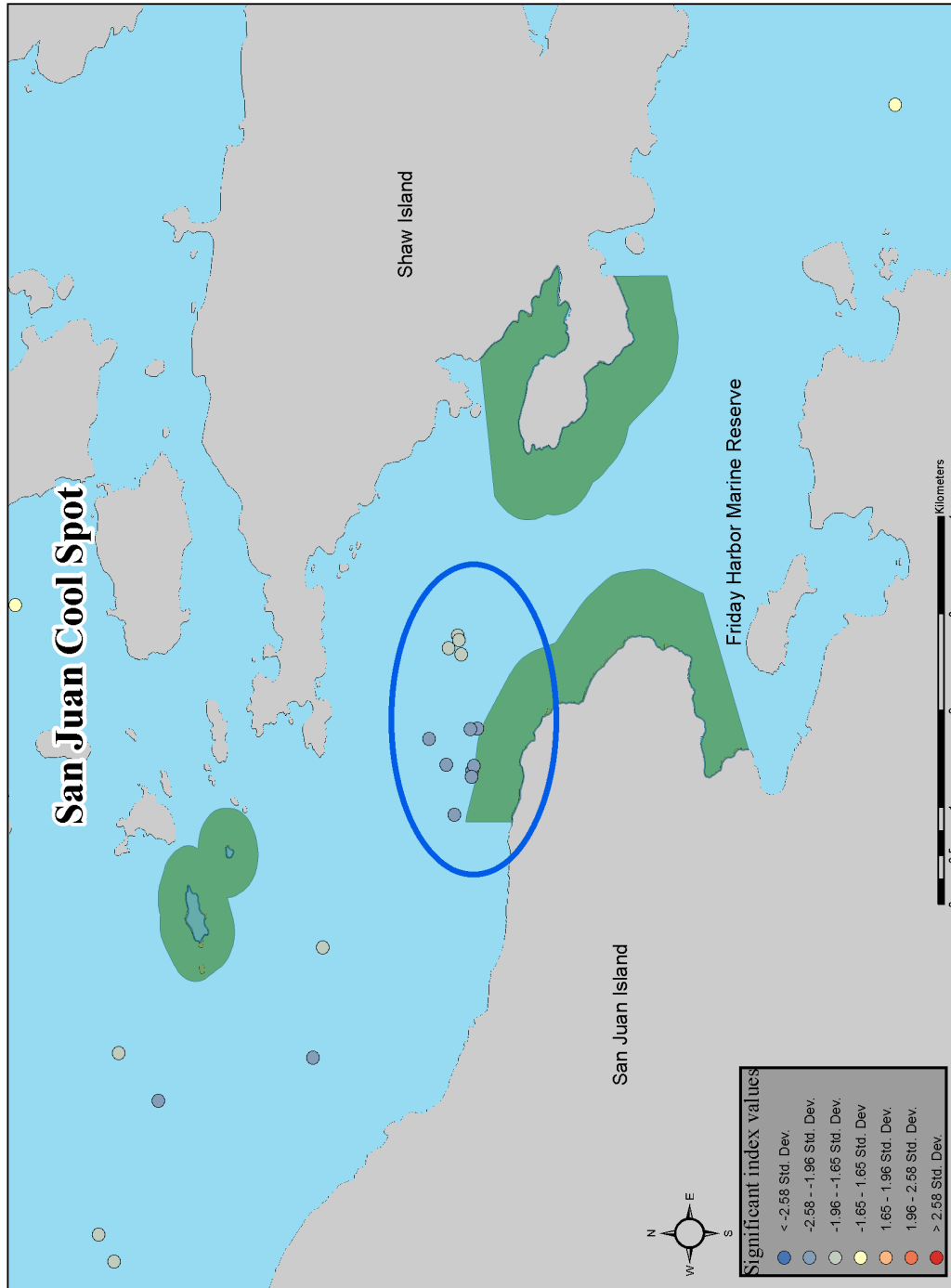


Appendix C

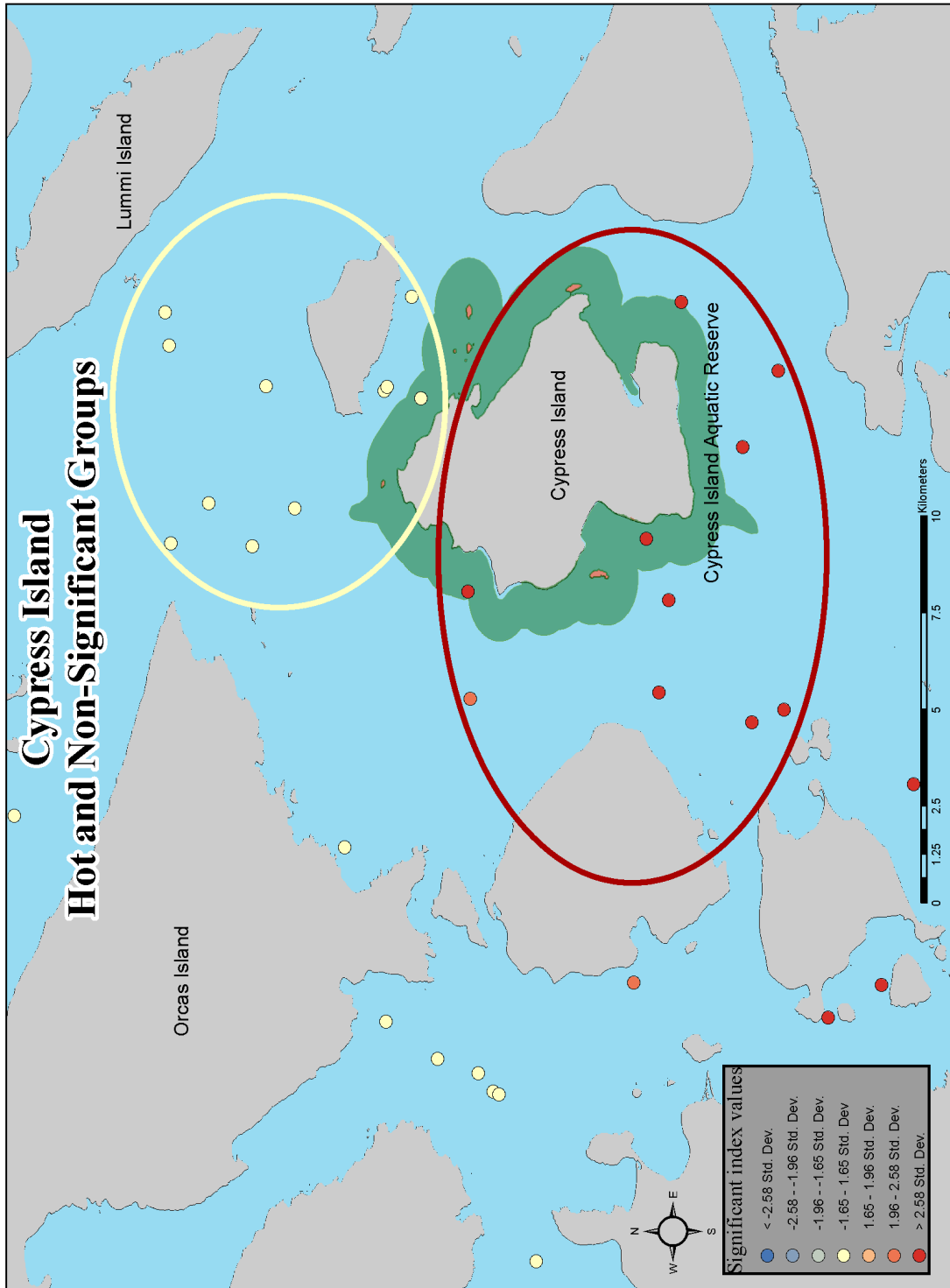
C-1. Location of the Lummi Island Hot Spot: Area of significantly high diversity



C-2. Location of the San Juan Island Cool Spot: Area of significantly low diversity

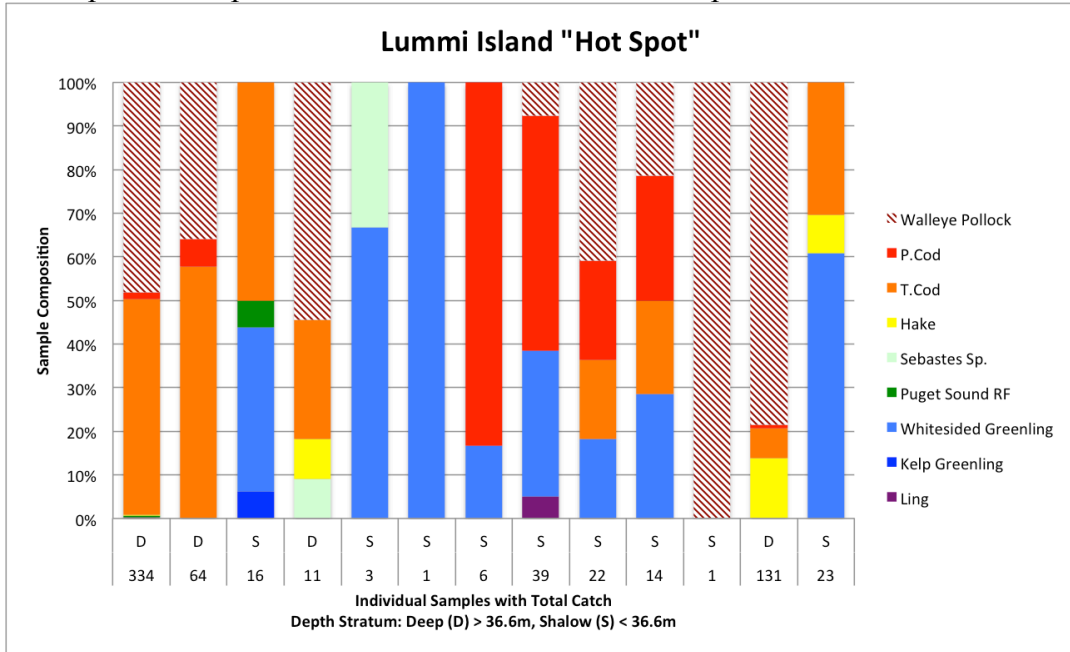


. C-3 Location of the Cypress Island Hot and Non-Significant Spots

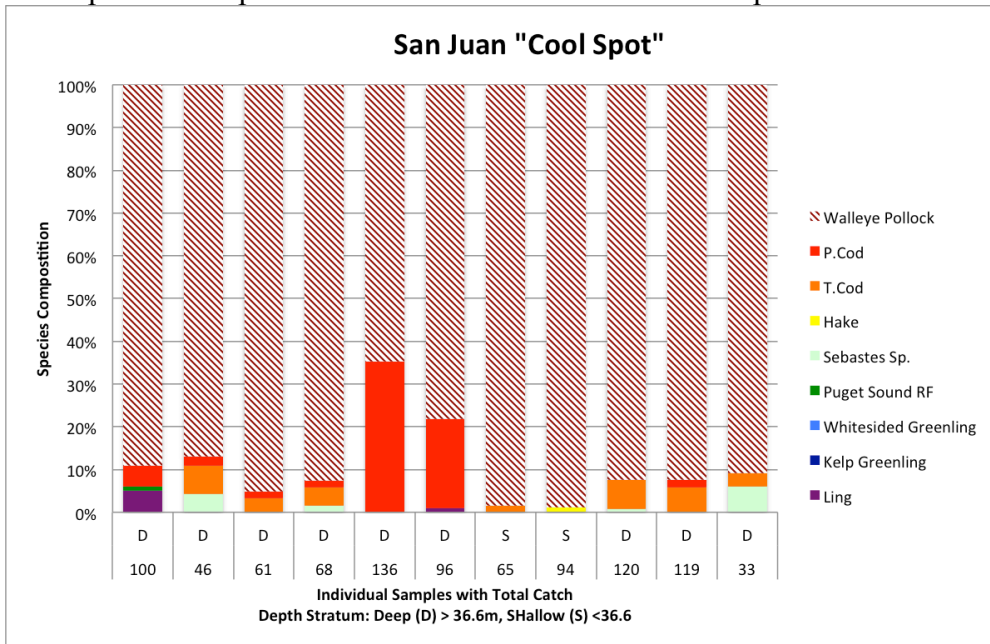


Appendix D

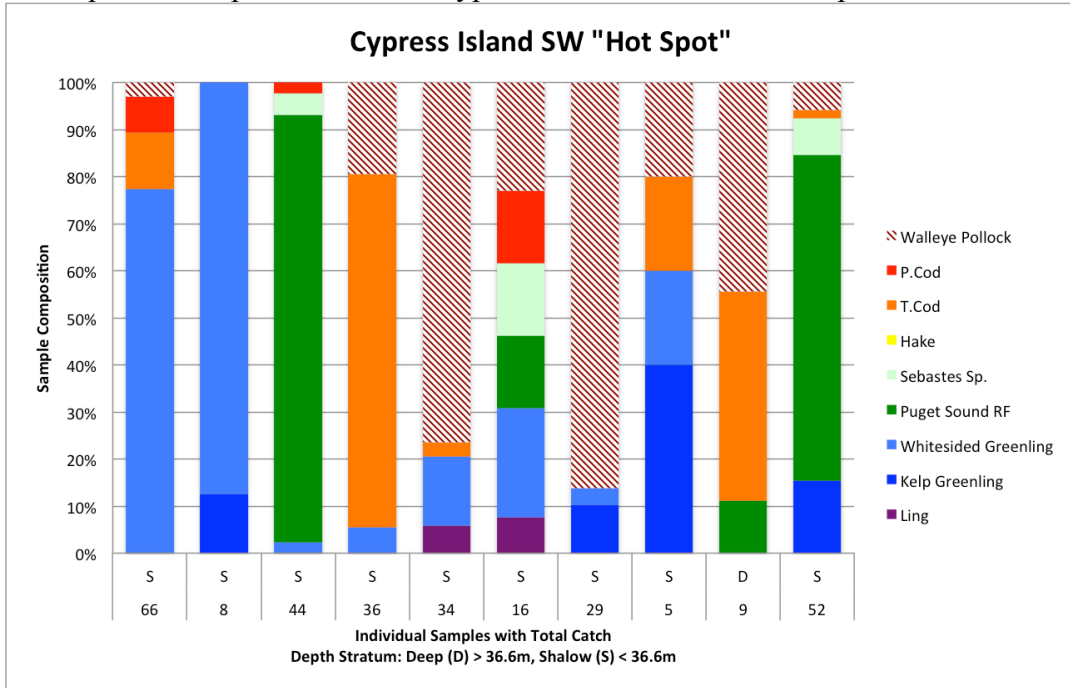
D-1. Species composition for the Lummi Island "Hot Spot"



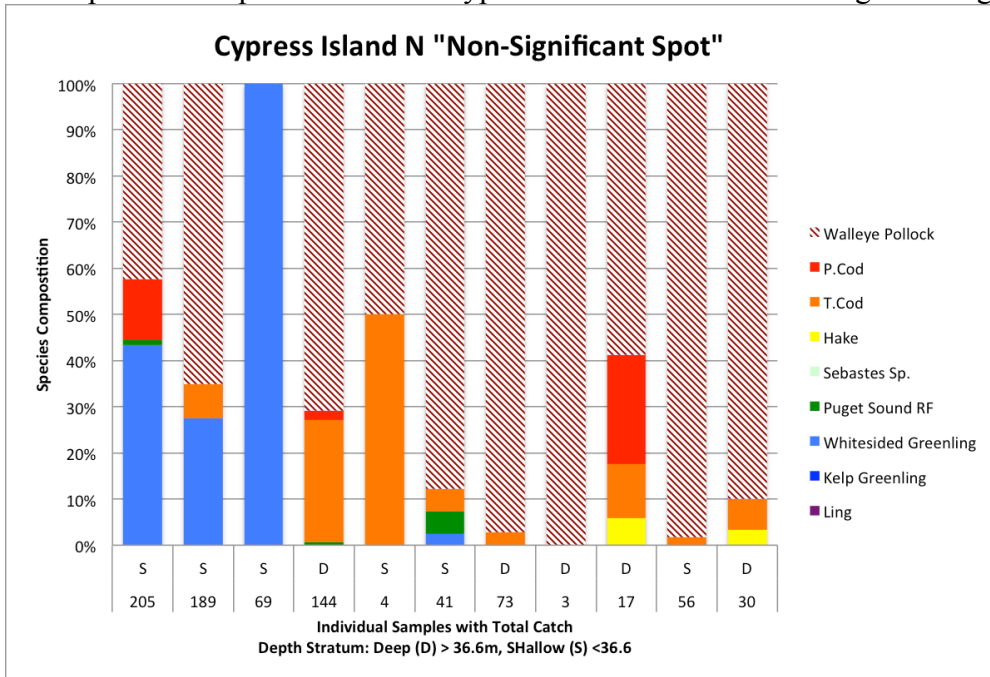
D-2. Species Composition for the San Juan Island "Cool Spot"



D-3. Species composition for the Cypress Island SW area “Hot Spot”

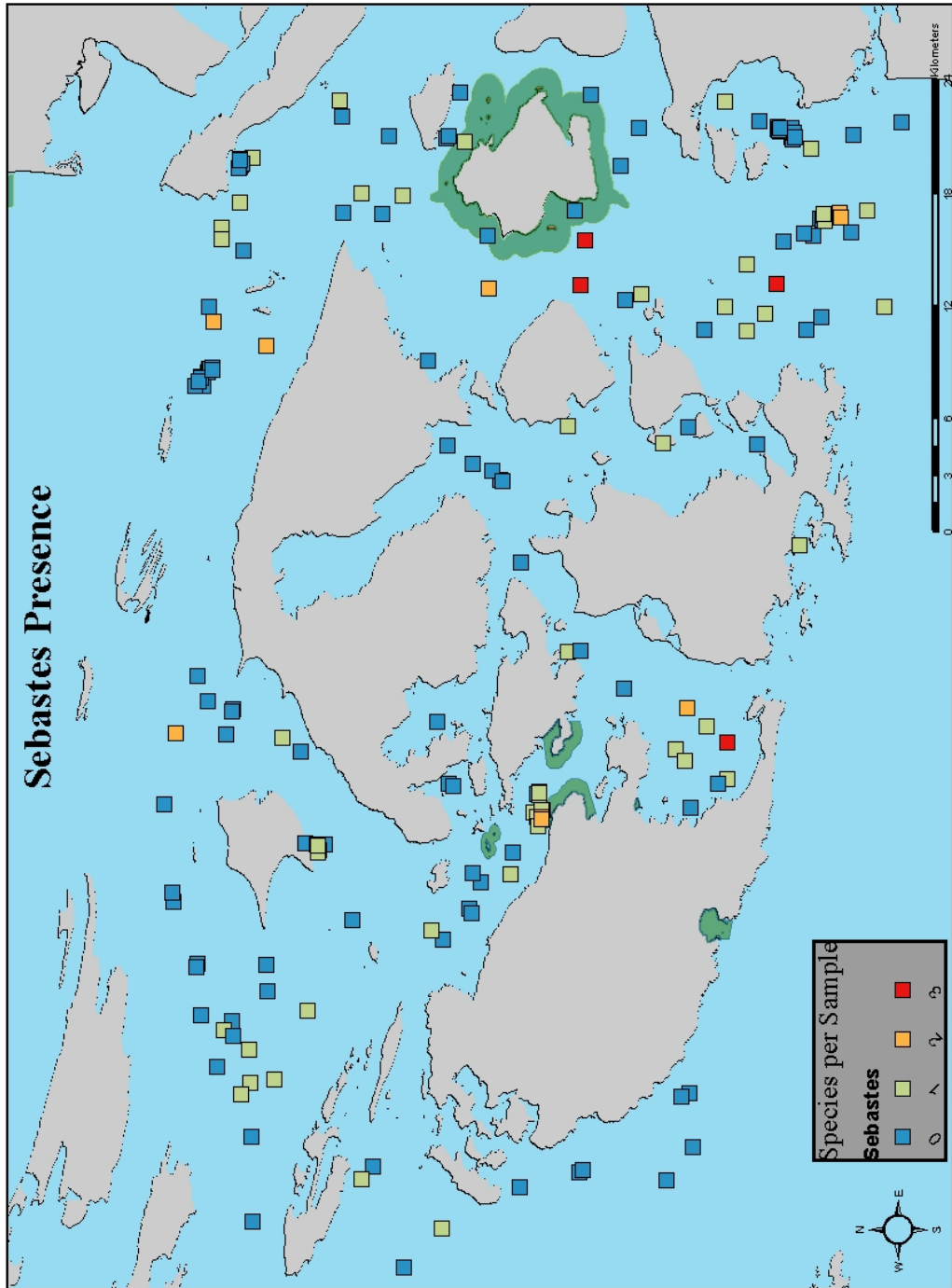


D-4. Species Composition for the Cypress Island Northern Non-Significant group

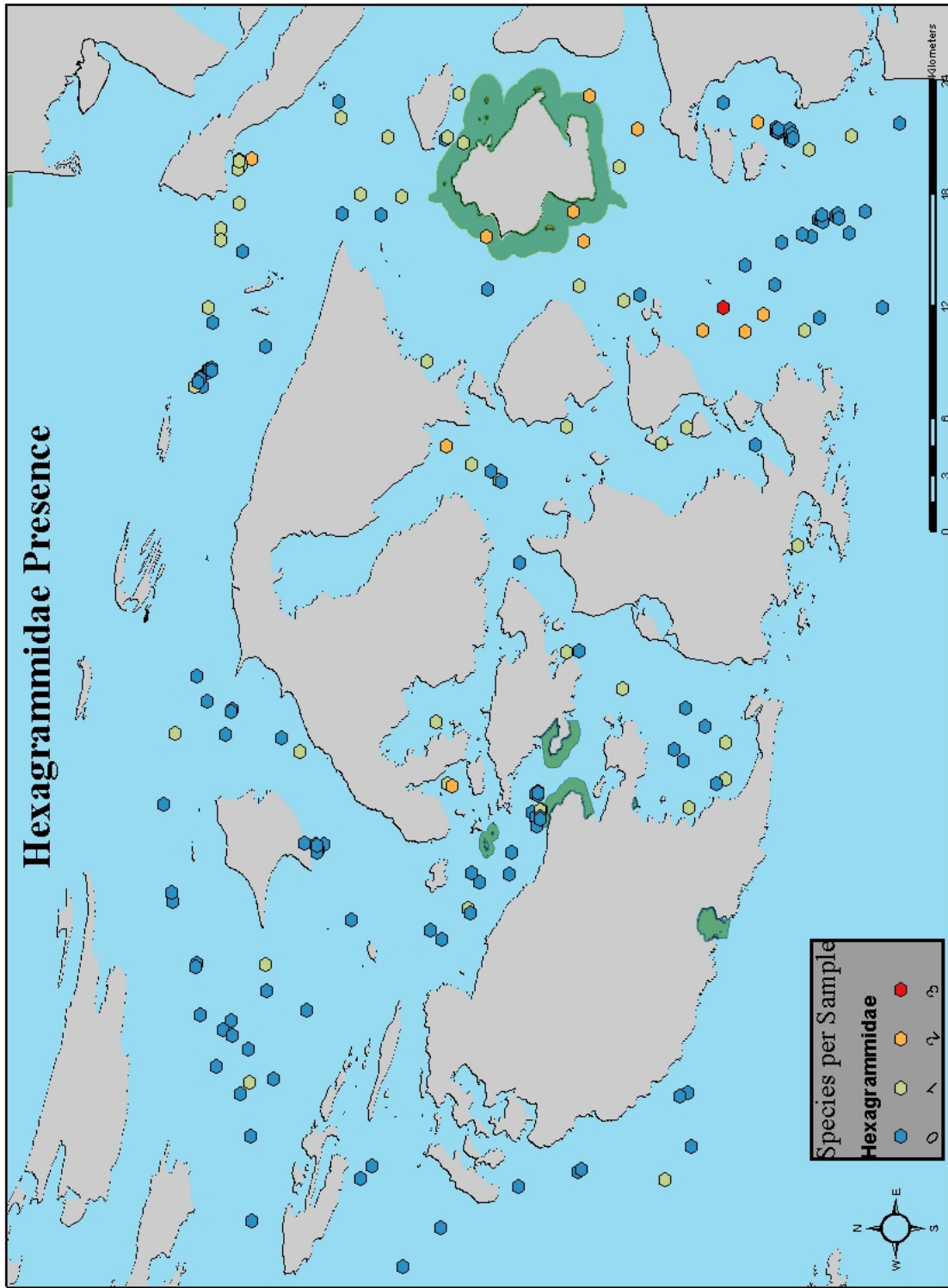


Appendix E

E-1. *Sebastes*: occurrence by number of species present per sample



E-2. *Hexagrammidae*: occurrence by number of species per sample



E-3. *Gadidae*: occurrence by number of species per sample

