FISH-HABITAT ASSOCIATIONS AND THE IMPORTANCE OF DEEP-SEA CORALS IN OLYMPIC COAST NATIONAL MARINE SANCTUARY

By

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To the	Faculty	of W	ashington ⁷	State	Universit	V

The members of the Committee appointed to examine the thesis of KATIE RENEE WRUBEL find it satisfactory and recommend that it be accepted.

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ABSTRACT

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Declining populations and sizes of commercial groundfish have prompted fishery managers to identify important habitats for recovery of fish stocks. Habitats that are important for fish for spawning, breeding, feeding, or growth to maturity, are classified as essential fish habitat (EFH) and designated with some level of protection. However, an EFH Conservation Area was designated with little information on fish distributions and habitat requirements in deep-sea ecosystems off of Washington State. Consequently, in 2006 and 2008 remotely operated vehicle surveys were conducted to identify fish-habitat associations as well as to determine the importance of deep-sea corals as fish habitat. Five major habitat and associated species assemblages were identified from canonical correspondence analysis: (1) boulder habitats dominated by rockfish (Sebastes spp.) as well as globular sponges and small Swifta beringi corals; (2) rock ridge habitats in which structure-forming deep-sea corals and redbanded rockfish (Sebastes babcocki) were associated; (3) coarse soft sediments consisted of Swifta spp. corals and flatfish; (4) mud habitats dominated by flatfish, eelpouts (Zoarcide), and poachers (Agonidae); and (5) habitat generalists occurring over several diverse habitat types. Of the ten deep-sea coral species analyzed, none were found to be selected for by fish as important habitat identified from selectivity indices. Despite low selectivity, corals were still frequently used by

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fish, in which *Primnoa pacifica* and *Lophelia pertusa* had high fish use (~70%) when available. Low-relief habitats with corals had higher fish abundance than similar habitats without corals; however fish abundance was significantly higher on boulder habitats without corals than with corals present. By parsing out the importance of corals as fish habitat over similar physical substrates we found that structure is important in rockfish habitat use. Thus, deep-sea corals are likely important components of EFH over lower complexity or mixed substrates, but may not be as important in high-relief complex boulder habitats. This study provides baseline data for current conditions and future comparisons to determine the efficacy of the EFH Conservation Area in rebuilding fish stocks in deep-sea ecosystems.

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INTRODUCTION

Populations and sizes of commercial groundfish species, such as rockfish (Scorpaenidae: Sebastes), have been declining for decades along the west coast of the United States (Mason 1998; Murray et al. 1999; Starr et al. 2002; Harvey et al. 2006; Levin et al. 2006). Although a few commercial fish stocks have recovered as a result of fishery management [Widow rockfish (Sebastes entomelas), Lingcod (Ophiodon elongatus), and Pacific Whiting (Merluccius productus)], many stocks are still severely depleted (Rosenberg et al. 2006; PFMC 2010; PFMC 2012a). Rockfish are one of the most valuable fisheries on the west coast in that they comprise the majority of the Pacific Coast groundfish fishery, having been commercially targeted since the mid-nineteenth century, and are an important recreational and tribal fishery (Parker et al. 2000; Yoklavich et al. 2000; Love et al. 2002; OCNMS 2011). However, rockfish tend to have low fecundity, are long-lived, and mature late in life making their populations especially susceptible to fishing pressure (Archibald et al. 1981; Love et al. 1990; Parker et al. 2000; Yoklavich et al. 2000; Levin et al. 2006). As a result six Sebastes spp. have been declared overfished (<25% of unfished biomass) by the Pacific Fishery Management Council (PFMC), cowcod, Sebastes levis, yelloweye, S. ruberrimus, canary, S. pinniger, bocaccio, S. paucispinis, darkblotched, S. crameri, and Pacific Ocean perch, S. alutus (Parker et al. 2000; Levin et al. 2006; PFMC 2010). The collapse of these fisheries, due to overfishing, by-catch, habitat degradation, and variable recruitment, has resulted in the need to manage human actions rather than the dynamics of wild populations (Conover et al. 2000).

Fishery stock decline, in association with the reauthorization of the Magnuson-Stevens Fishery Conservation and Management Act (MSFCMA) of 1996 (MSA, 16 U.S.C. § 1801 et seq.) mandated that essential fish habitat (EFH) for all commercially targeted fish species be

defined to aid in ecosystem-based management efforts in rebuilding fish stocks (Rosenberg et al. 2000; Lindholm et al. 2001; NMFS 2010). EFH is defined by Congress as "those waters and substrate necessary to fish for spawning, breeding, feeding or growth to maturity" (MSA, 16 U.S.C. § 1801 et seq.). To define a species' EFH, an understanding of its use of habitat is required for all life history stages. Although some aspects of EFH (i.e. physical and biological) have been described for all federally managed fish species from various studies along the west coast (Stein et al. 1992; Yoklavich et al. 2000; Pacunski & Palsson 2001; Jagielo et al. 2003; Johnson et al. 2003; Tissot et al. 2007; Love & Schroeder 2007; Love et al. 2009), many species' habitat requirements are still unclear at one, multiple, or all life history stages. Analyzing fish behaviors and habitat associations for delineating EFH are frequently accomplished through the use of video platforms such as remotely operated vehicles (ROVs) and human-occupied submersibles (Carlson & Straty 1981; Auster et al. 1995; Pearcy et al. 1989; Stein et al. 1992; Jagielo et al. 2003; Anderson & Yoklavich 2007; Stoner et al. 2007). Studies on fish-habitat associations in Washington State have been limited to shallower habitats (90-150m, Jagielo et al. 2003) and have not been quantified from visual surveys in deep-sea ecosystems (>150m) despite having an area currently designated as an EFH Conservation Area.

Habitat complexity is the physical substrate (rocks, boulders, pinnacles, etc.) as well as the biogenic structure (megafaunal invertebrates) and has been identified as an important aspect of EFH for multiple life history stages of rockfish (Stein et al. 1992; Anderson & Yoklavich 2007; Tissot et al. 2007). For example, juvenile rockfish settle in habitats with high vertical relief (kelp forests, rock ridge, and boulder habitats), indicating complex habitat may act as nursery grounds (Stein et al. 1992; Yoklavich et al. 2000; Anderson & Yoklavich 2007; Tissot et al. 2007; Laidig et al. 2009). Biogenic structure is large complexly shaped, or densely aggregated

invertebrates, that augment the vertical relief of substrates, providing additional shelter, feeding, and spawning locations for fishes (Carlson & Straty 1981; Krieger & Wing 2002; Freese & Wing 2003; Stone 2006; Tissot et al. 2006; Tissot et al. 2007). Furthermore, the MSFCMA reauthorization of 2006 recognizes the importance of deep-sea coral and sponge communities as key habitat for groundfish species and are designated as "habitat areas of particular concern" (HAPC) in some regional Fishery Management Councils (Morgan et al. 2005; NOAA 2010). However, it is still unclear if fish associations with deep-sea corals are a result of the complexity of biogenic structure, the underlying physical habitat, a co-occurrence of the two, or some other driving factor.

Groundfish are hypothesized to be facultative habitat users, using biogenic habitat for shelter, feeding or reproduction, but are not at-risk of becoming extinct in its absence (Auster et al. 1995; Auster 2005). It is important to determine the functional role of biogenic habitats in terms of EFH as these habitats are especially sensitive to anthropogenic disturbances (Auster et al. 1996; Watling & Norse 1998; Dayton et al. 2000; Freese 2001; Brodeur 2001). Mobile, bottom contact fishing gear (i.e. bottom trawls) removes emergent epifauna which reduces habitat complexity and heterogeneity of the seafloor as much as 15-20% in a single pass (Auster et al. 1996; Auster 1998; Moran & Stephenson 2000; Auster 2005). Recovery of deep-sea corals is slow due to slow growth rates, low recruitment, and limited hard substrate in many deep-sea ecosystems (Krieger 2001; Krieger & Wing 2002; Auster 2005; Etnoyer & Morgan 2005). By understanding the distribution of biogenic structure, as well as their utility as fish habitat, fishery managers can better identify areas to limit the impact of bottom contact fishing gear on biogenic habitat, and consequently EFH.

The goal of this research is to determine rockfish-habitat associations within OCNMS using video obtained from an ROV. Our research questions include: 1) What habitats and deep-sea corals are rockfish associated with?; 2) Do rockfish display diel and ontogenetic shifts?; 3) Do rockfish associate with biogenic structures (i.e. deep-sea corals and sponges)?; 4) What are the baseline differences in habitat distribution and species abundance inside and outside a recently protected area?; and 5) What are the anthropogenic impacts (i.e., fishing gear, debris, etc.) on deep-sea corals and sponges in this area?

METHODS

Study Site

Olympic Coast National Marine Sanctuary (OCNMS) was established in 1994 and is located in Washington State at the mouth of the Strait of Juan de Fuca (OCNMS 2011, Figure 1). OCNMS is a highly productive marine ecosystem due to its location in both the Big Eddy (Juan de Fuca Eddy) and the California Current large marine ecosystems, resulting in a large nutrient-rich upwelling zone (Hyland et al. 2005; OCNMS 2011). The Sanctuary covers 8,573 km² of ocean reaching depths of 1,400 meters and supports unique deep-sea habitats in the form of submarine canyon heads (Nitinat, Juan de Fuca, and Quinault), deep-sea corals, and sponges as well as cultural importance through cooperative agreements with four Native American treaty tribes in the region (the Hoh, Makah and Quileute tribes, and the Quinault Indian Nation, OCNMS 2011). OCNMS was glacially carved and is located in the tectonically active Cascadia Subduction Zone, where the North American continental and Juan de Fuca oceanic plates meet (OCNMS 2011). The sanctuary is largely comprised of soft sediment with glacial deposits of cobbles and boulders in limited areas (Intelmann & Cochrane 2006). However, only 25% of the

sanctuary has been mapped, resulting in generalizations of the majority of these seafloor communities (OCNMS 2011). Within OCNMS' boundaries a groundfish EFH Conservation Area was designated, the Olympic 2 Conservation Area (297 km²), protecting 7% of the sanctuary from non-treaty bottom trawling. The Olympic 2 Conservation Area was designated in 2006 following recommendations from the Pacific Fishery Management Council (NMFS 2005; PFMC 2008); however, fish distributions, fish-habitat associations, and fish interactions with deep-sea corals in this area are relatively unknown. Remotely operated vehicle (ROV) surveys were conducted within and outside Olympic 2 to identify areas with deep-sea corals and to quantify fish's interactions with physical and biogenic habitats (Brancato et al. 2007).

Remotely Operated Vehicle Surveys

Remotely operated vehicle (ROV) video surveys were conducted in OCNMS in 2006 and 2008, using the Canadian Scientific Submersible Facility's ROV Remotely Operated Platform for Ocean Sciences (*ROPOS*). *ROPOS* is equipped with high-definition (HD) digital video (forward and vertical facing) and still cameras. Two scaling lasers (spaced 10cm) were used for organism sizing and habitat classification. Navigation data were recorded using the IXSEA GAPS system and pooled to one second intervals. Surveys randomly stratified focus areas with potential deep-sea coral resulting in dives targeting areas of hard substrate identified by side scan sonar (Brancato et al. 2007). Transects (~2m wide) were pre-selected within target sites and spaced 20-80m apart and were conducted 24-hours a day. There were 20 dive sites surveyed in 2006 and 2008 (Figure 2). In 2006, a total of 12 dives took place on 22 May through 4 June, reaching depths ranging between 62-372m. In 2008, 8 dives were conducted July 10-14 at depths ranging between 105-360m. These surveys resulted in 11 sites surveyed outside and 9 inside the

existing Olympic 2 Conservation Area. Additional details on the cruises can be found in OCNMS' dive reports (Bowlby, et al. 2011a; 2011b).

Video analysis

Using the collected video, fish and megafaunal invertebrates were identified and counted and habitat type was recorded for each transect. Habitat patches were identified as areas of uniform habitat for greater than twenty seconds. The bottom half of the video frame (the area below the sizing lasers) was the portion annotated. Substrates were defined using the grain size classification scheme widely used along the west coast and include (in order of increasing grain size); mud (M), sand (S, grains distinguishable), gravel (G, ≥ 4 mm and < 2 cm), pebble (P, ≥ 2 and < 6.5 cm), cobble (C, ≥ 6.5 and < 25.5 cm), boulder (B, ≥ 25.5 cm), flat bedrock (E), and (R) rock ridge (Stein et al. 1992; Yoklavich et al. 2000; Tissot et al. 2007). In this study rock ridge habitats surveyed were comprised of large expanses of high-relief wall habitats (some with a mud veneer) instead of intermittent ridges with exposed rock as found in other west coast studies. Habitat patches were characterized using methods described by Stein et al. (1992): a two-character code was used for each continuous patch of habitat to approximate the percent cover of the two most dominant substrate types. The first character represents the primary habitat (>50% of the patch) and the second character represents the secondary habitat (>20% of the patch). For example, if the habitat patch was >50% mud and >20% cobble it would be classified as mud-cobble (MC), or if the habitat patch was >70% boulder it would be classified as boulder (BB). Instances where the ROV was "off transect," or pausing to take a sample, habitat was recorded as "XX" and no further annotations were made within that portion of video. Habitat

patch area was calculated using transect width (measured by the fixed sizing lasers on the ROV) multiplied by patch length using the navigation data.

Deep-sea corals and sponges were identified to the lowest taxonomic level and sized. Invertebrates were recorded if at least 50% of the individual was within the field of view. Corals and sponges greater than 5cm were recorded and their height (base to tallest branch) and width (broadest point) measured. If the entire coral or sponge colony was not within the field of view a size estimate was recorded and noted as such and the individual was not included in density calculations. Sponges were identified based on growth form, and color was recorded. If corals or sponges were densely aggregated they were grouped based on height categories (5-15 cm, 15-30 cm, and any that were greater than 30 cm were measured individually). Invertebrate conditions were recorded as: not damaged (default condition); dead; overturned (partially or fully in contact with seafloor); partially dead; mat growth; broken or missing branches; detached; gear entangled; or debris entangled. Additionally, any anthropogenic objects [i.e. long line, linear bottom disturbance (e.g., trawl track, cable trenching), crab pot, miscellaneous boat/ship gear (anchor, trawl door, etc.), debris (general, such as bottles, cans, plastic bags), cables, monofilament, nets, rope, rolled boulder, unexploded ordinances (torpedo shaped), or other] were recorded.

Fish were identified to the lowest taxonomic level and sized in 5cm increments using the fixed sizing lasers to measure total fish length. At least 50% of the fish was in the field of view to be recorded. If fish were not able to be identified to species, they were assigned to a taxonomic group or species complex [unidentified juvenile rockfish (juvenile *Sebastes* spp.), unidentified rockfish (*Sebastes* spp.), pygmy/Puget Sound rockfish (*S. wilsoni/S. emphaeus*), darkblotched/bank/sharpchin rockfish (*S. crameria/S. rufus/S. zacentrus*), harlequin/sharpchin rockfish (*S. variegatus/S. zacentrus*) species complex, thornyhead (*Sebastalobus* spp.),

unidentified shark/skate egg case, righteye flounder (Pleuronectidae), or unidentified flatfish].

Additionally, some fish were only identified to family (unless identification to a lower taxonomic group was possible). These include: cusk-eels (Ophidiidae), scuplins (Cottidae), poachers (Agonidae), snailfish (Liparidae), eelpouts (Zoarcide), ronquils (Bathymasteridae), and pricklebacks (Stichaeidae). Schooling fish were noted as such and abundance estimated.

Fish behavior was recorded upon first entrance in the transect area as to exclude any changes in behavior due to the presence of the ROV. Behaviors and associations recorded follow a modified version of Auster et al. (1995) and include: resting, hovering, or swimming. Fish were only associated with deep-sea corals and sponges if they were resting on, were within one body length, or within one meter of the epifauna. Fish greater than one meter in distance from emergent epifauna were assumed to have no association.

In some cases videos had an observed time lag due to drifts in the tape time code from the real recorded time. This time drift was documented and fish observation times were adjusted to the actual time based on the drift after annotation.

Data Analysis

Habitat and species characterization

Observed habitat types were analyzed using a Cluster Analysis (group average linkage method) using Euclidean Distance to group dominant habitat types using the transformed ($x^{0.25}$) density data on 10 abundant deep-sea coral and sponge taxa. Data were transformed to improve data distribution, due to right-skewed, leptokurtic data identified from histograms. Downing (1979) recommends a fourth root transformation for benthic assemblages when the slope of the log-log relationship between variance and mean is near 1.5, slope was 1.6 for our study. Habitat

categories were recorded using habitat patch area, such that primary habitats comprised 70% and secondary habitats 30% of the patch area (multiplying the habitat patch area by 0.7 for primary habitats and 0.3 for secondary habitats). Habitat patches greater than 1m² were included in analyses. Fish and invertebrate density were calculated for each habitat type (#/m²). Size categories established between OCNMS and NOAA's Deep Sea Coral Research and Technology Program (DSCRTP) in NOAA's Deep Sea Coral National Geodatabase standardize deep-sea coral and sponge sizes into three categories: large (>50cm in height), medium (10cm-50cm), small (<10cm) that were included in analyses (DSCRTP 2013).

Differences in habitat area and species abundance inside and outside Olympic 2

Differences in habitat area sampled inside and outside Olympic 2 were calculated to determine differences in sampling effort using a Mann-Whitney U-test. Differences in habitat diversity were compared inside and outside using the Shannon-Weiner diversity index:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

Where p_i is habitat proportion among transects. Diversity scores were compared using a Mann-Whitney U-test to determine if habitats sampled were more diverse inside or outside Olympic 2.

Fish and invertebrate density (#/m²), diversity (Shannon-Weiner), and richness (# of taxa) were compared among transects inside and outside Olympic 2 with a non-parametric Mann-Whitney U-test due non-normally distributed data. Individual fish and invertebrate taxa densities were also analyzed with a Mann-Whitney U-test for difference in taxa density between areas. Small, medium, and large invertebrate densities were compared inside and outside of the protected area. Significance was determined using $\alpha = 0.05$. Univariate statistical analyses were conducted using Minitab statistical package.

Species-habitat associations

The densities of species per habitat patch type were analyzed using canonical correspondence analysis (CCA) to examine how fish communities associate with environmental variables, substrate type and biogenic habitats (McCune & Grace 2002). A CCA has few assumptions, other than unimodality, which works well with this data set considering the high variability. CCA requires two data sets. In this study the main (environmental) matrix was comprised of substrate type using 70% and 30% of habitat patch area for primary and secondary habitats, respectively. Habitat patches greater than 1m² were included in analyses as well as recorded depth for each habitat patch. Deep-sea coral and sponge species that comprised at least 1% total abundance were also included in the main matrix; however, larger, structure-forming species were also included despite low abundance (Paragorgia spp., Lophelia pertusa, and *Primnoa pacifica*; Figure 3). Invertebrate density (#/100 m²) was x^{0.25} transformed to improve data distribution (reduce right-skewness) and meet the unimodality assumption. This resulted in 10 deep-sea coral and sponge taxa, representing >85% of invertebrate observations, being included in the analysis. This data matrix (physical substrate and invertebrate density) was used at the main matrix (environmental matrix) for multivariate analyses.

The second data set (species matrix) was comprised of fish species that comprised greater than 1% total abundance were included in analyses, however, commercially important fish that were <1% abundance were included (lingcod, Pacific Ocean perch, canary, yelloweye, and darkblotched rockfish). Species included comprised >95% of total abundance for fish. This resulted in 25 fish taxa and families included in analyses. The relationship between fish with deep-sea corals, sponges, and habitat variables (substrate type) were analyzed using $x^{0.25}$ transformed fish density (#/100 m²) to improve data distribution (reduce right-skewness) and

meet the unimodality assumption. Habitat patch samples containing zero fish and invertebrate observations were excluded from multivariate analyses resulting in 2,050 habitat patches. Monte Carlo randomization tests were conducted to determine if the distribution pattern could occur by chance (based on 999 permutations) by testing the null hypothesis that there is no relationship between the environmental and species matrices. Each axis was derived from reciprocal weighted averaging of species density within habitat patches constrained by the primary and secondary substrate types of each habitat patch sample as well as the biogenic habitat. Axes scores were standardized by centering and normalizing, the scaling of ordination scores optimized species, and sample scores for graphing were linear combinations of species. Multivariate analyses were conducted using PC-ORD statistical package.

Habitat use and selectivity indexes were calculated on fish densities to determine the use and selectivity of each habitat type by fish species to corroborate our CCA results. Habitat use was calculated using mean fish density (#/m²) over each of the twelve habitat types. Habitat selectivity standardized habitat-use following methods in Anderson and Yoklavich (2007), where the proportional occurrence of each habitat type (i.e. habitat availability) was subtracted from the proportional abundance of each species occurring on that habitat type (i.e. use). Here a positive number indicates more individuals than expected in a given habitat type than random (associated) and a negative number indicates fewer individuals than expected (avoidance). A chisquare goodness of fit test was conducted to determine if species were randomly distributed across habitats.

Use of deep-sea corals by fish

The importance of deep-sea corals and sponges as habitat for fish was determined by how often fish use these structures in relation to how often fish use the same physical substrate

lacking deep-sea corals and sponges. These methods are similar to Auster et al. (2005). Mann-Whitney U-tests were conducted on fish density.

Fish associations with biogenic structure were determined using Manly's alpha selectivity index weighting fish distance from coral and sponges: physical contact: 3; resting within 1 body length: 2.5; resting within 1 meter: 2; hovering or swimming within 1 body length: 1.5; and hovering or swimming within 1 meter: 1 weighting among transects. Manly's alpha is calculated:

$$\alpha = \frac{r_{\rm i}}{n_{\rm i}} \sum_{i=1}^{m} \frac{1}{r_{\rm j}/n_{\rm j}}$$

Where r_i is the proportion of coral species i with fish associated, n_i is the total proportion of coral species i observed and m is the total number of coral species included in analyses (Manly 1972; Chesson 1983). Selectivity indices standardized relative use of corals and sponges by fish resulting in an alpha ranging from 0 (avoidance) to 1 (preference) for the ten most abundant corals (used in previous analyses) among transects in which that coral taxa was present. Since alpha is standardized (sums to 1), we can calculated the expected value, where $\alpha_i = 1/m$, indicates there is no preference for coral species i. When α_i is greater than 1/m, then coral species i is preferred by fish. Conversely, when α_i is less than 1/m, then coral species i is avoided. In our study the expected value was 1/m=0.1. To determine if fish were selecting for specific coral taxa we determined overlap with the expected value and calculated confidence intervals (95%) using $\alpha=0.05$:

$$\mu \pm (1.96 \frac{\sigma}{\sqrt{n}})$$

Diel shift

Diel effects were tested to determine if fish abundances differ between day and night. Hart et al. (2010) recommends excluding dusk and dawn periods (8pm-10pm and 4:30am-6:30am) over large- to medium-sized habitats during summer months in the Pacific Northwest to reduce bias due to changing activity patterns of demersal fish. Since these surveys were conducted in the summer field season both years, the dusk and dawn times were eliminated from analyses to eliminate potential activity shifts. Mann-Whitney U-tests were conducted on the area of primary habitat types to determine if there was a significant difference in habitat area sampled between day and night among transects. For primary habitat types that did not differ significantly in area sampled between day and night, fish density was analyzed using a Mann-Whitney U-test to determine differences in fish abundance between day and night surveys.

Ontogenetic shift

Correlations between depth and seafloor type ranked by grain size (smallest to largest) were tested using the Spearman rank correlation. Ontogenetic shifts were calculated using a linear regression to determine if there was a significant pattern between fish size and depth for selected species. For this analysis depth was used as the predictor (x-axis) and fish size was the response (y-axis). Variables (depth and size) were log transformed for some species to meet normality.

Impacts of fishing gear on deep-sea corals

Relative abundance of anthropogenic observations (i.e. fishing gear, trash, etc.) was calculated using the rate of encounter ($\#/100\text{m}^2$). Frequency of deep-sea corals and sponges that

were observed with fishing gear wrapped or entangled were calculated. Deep-sea corals that were damaged or dead and their proximity to fishing gear were recorded using a Nearest Neighbor Analysis. Nearest Neighbor Analysis was conducted in ArcGIS (ESRI) for anthropogenic observations in proximity to all deep-sea corals and sponges observed as well as to damaged and dead individuals to determine the average distance anthropogenic objects are from corals and sponges and how often those corals and sponges were damaged as a potential result of the objects location.

RESULTS

Habitat and species characterization

A total of twenty dive sites (149 transects) were surveyed in 2006 and 2008 sampling an area of 199,450 m² within and outside the Olympic 2 Conservation Area (Figure 4). Average transect width was 2.5m and total transect length was 77km. Fifty-one distinct habitat types were recorded from 2,050 habitat patches. Habitats were pooled and grouped into 12 habitat types based on \geq 65% similarity of invertebrate communities as a result of a Cluster Analysis. The most abundant habitat types were cobble (53,269m²) and sand (40,277m²) substrates. Ridge habitats were the least abundant habitat type available (13,208m²).

Deep-sea coral and sponge observations within OCNMS totaled 47,101 individuals from 33 taxa. Deep-sea corals comprised over half of the observations (54.9%) and sponges 45.1% of observations. The most abundant taxa (84% total invertebrate abundance) included: *Stylaster* spp. (24.5%), globular or ball sponge (22.0%), sponge assemblage (comprised of multiple sponge morphologies, 16.2%), *Swiftia beringi* (14.1%), and *Swiftia pacifica* (7.4%). These abundant taxa tended to be small, non-structure-forming invertebrates. Larger, structure-forming

invertebrates, such as *Paragorgia spp.* (0.7%), *Primnoa pacifica* (0.15%), and *Lophelia pertusa* (0.1%), comprised only 2.05% of total abundance but with a maximum size of 2.10 m. Overall, density of deep-sea corals and sponges was 0.173 individuals m⁻² (SE=0.019). Invertebrates were most abundant on ridge habitats, followed by mud-ridge and boulder-sand habitats (Figure 5Figure 5). However, most of the invertebrates observed were small (<10cm: 0.083m⁻² (SE=0.012), 52.67% total abundance) and medium (10-50cm: 0.089m⁻² (SE=0.013), 47.16% total abundance) sized corals and sponges; large corals occurred at low densities [>50cm: 0.0004m⁻² (SE=0.0001)]. Invertebrate richness was, on average, 6.25 (SE=0.35) taxa per transect.

A total of 57 fish taxa from 16 families were observed from 12,193 individuals. Rockfish species comprised 75% of all observed fish. The most abundant fish (82% total abundance) were unidentified adult *Sebastes* spp. (15.5%), rosethorn rockfish (*S. helvomaculatus*, 12.0%), darkbltoched/bank/sharpchin rockfish species complex (11.0%), *Sebastolobus* spp. (10.8%), pygmy rockfish (6.4%), harlequin/sharpchin rockfish species complex (6.2%), poachers (5.1%), Pleuronectidae (4.6%), Dover sole (*Microstomus pacificus*, 4.2%), redstripe rockfish (*S. proriger*, 4.0%), and hagfish (2.4%). Density of fish, on average, was 0.057 fish m⁻² (SE=0.004). Fish were most abundant on boulder habitats (0.24 fish m⁻²) and density dropped to 0.02-0.06 fish m⁻² in the remaining habitats (Figure 6). Species richness was, on average, 10.3 (SE=0.34) fish taxa per transect.

Differences in habitat area and species abundance inside and outside Olympic 2

There were more transects surveyed inside Olympic 2 (86 transects) than outside (63 transect). However, there was more habitat area sampled outside (107,269 m²) than inside (92,180 m², Z=2.34, P=0.02). Consequently, the area of substrate types surveyed did differ

(Appendix Table 1). Inside Olympic 2 more cobble (Z=2.25, P=0.025) and mud habitats were surveyed (Z=3.25, P<0.001). Outside Olympic 2 there were more boulder (Z=1.96, P=0.047) and gravel habitats (Z=3.45, P<0.001). There was no difference in the area of ridge (Z=1.83, P=0.07), sand (Z=1.06, P=0.29), and pebble (Z=1.91, P=0.05) habitats surveyed inside and outside of Olympic 2 and there was no difference in the diversity of habitats between areas (Z=0.22, Z=0.83).

Overall, deep-sea corals and sponges had a higher pooled density within this EFH Conservation Area relative to outside (Z=2.36, *P*=0.02, Figure 7). However, several individual deep-sea coral and sponge taxa analyzed had no difference in density between areas, *Desmophyllum dianthus* (Z=1.37, *P*=0.13), globular or ball sponges (Z=1.26, *P*=0.21), *Lophelia pertusa* (Z=0.19, *P*=0.84), *Paragorgia* spp. (Z=1.43, *P*=0.15), *Primnoa pacifica* (Z=1.65, *P*=0.09), and *Swiftia pacifica*, (Z=0.27, *P*=0.78, Appendix Table 2). Except for *Plumarella longispina* (Z=4.92, *P*<0.001) and *S. beringi* (Z=2.35, *P*=0.02) which were more abundant inside Olympic 2. There were no *Stylaster* spp., *Anthoptilum grandiflorum*, or *Balanophyllia elegans* observed outside of this EFH Conservation Area. Invertebrate diversity and richness were both higher inside the Olympic 2 Conservation Area than outside (Z=2.15, *P*=0.03 and Z=3.64, *P*<0.001, respectively). Based on size, there was no difference in the density of small invertebrates between areas (Z=1.06, *P*=0.29), but densities of medium (Z=3.18, *P*<0.01) and large (Z=2.15, *P*=0.03) invertebrates were greater within the Olympic 2 Conservation Area.

There was no difference in overall fish density inside and outside of this EFH Conservation Area (Z=0.48, P=0.63). However, several fish taxa displayed a difference in density (Appendix Table 3). There were significantly more Dover sole, hagfish, poachers, thornyheads (Sebastolobus spp.), and righteye flounders observed within Olympic 2 (P<0.01).

Outside of Olympic 2 there were significantly more lingcod, juvenile rockfish, greenstriped (*S. elongatus*), rosethorn, yelloweye, and unidentified rockfish (*P*<0.05). There were several fish taxa that had no difference in density between areas, these include ronquil, darkblotched, redbanded (*S. babcocki*), redstripe, sharpchin rockfish, harlequin/sharpchin and darkblotched/bank/sharpchin species complexes, as well as Pacific Ocean perch, eelpouts, spotted ratfish (*Hydrolagus colliei*), and unidentified flatfish (*P*>0.05). There were no observed canary, pygmy, or yellowtail (*S. flavidus*) rockfish observed within this EFH Conservation Area in these surveys. However, the diversity of fish taxa was higher inside Olympic 2 (H'=1.9) than outside (H'=1.7, Z=2.72, *P*<0.01). Conversely, taxa richness was not significantly different inside and outside (richness=10.3 taxa, Z=0.85, *P*=0.40).

Species-habitat associations

Substrate type was a significant environmental variable in explaining species communities (Figure 8). An acceptable tolerance level of 0.100000E-12 was reached after 21 and 32 iterations of the first two axes, respectively. The first axis resulted in a significant species-environmental correlation (matrix correlation = 0.731, P=0.001). The strongest gradient (axis 1: eigenvalue = 0.196) explained 10.2% of the variation in species variation in relation to substrate type. Axis one was strongly driven by differences between boulder habitat and all other communities. The other two axes were not significantly different from random, but do show strong species community patterns (axis 2: eigenvalue = 0.091; axis 3: eigenvalue = 0.064) with axis 2 explaining 4.8% of the variance and axis 3 explaining 3.4% of the variance in species communities. Overall, substrate explained 18.3% of species variance. Although this leaves over 80% of the variance unexplained, this is expected due to the high variability and the large

number of zeros in the dataset (Stevenson et al. 1991). Interpreting the variable loadings for the first two axes of the CCA as well as species habitat use and habitat selectivity indexes we grouped species communities in relation to substrate type into five main assemblages.

The first group was boulder habitat and associated species including: S. beringi and globular sponges [specifically green lunar sponge (Latrunculia spp.)] and the majority of rockfish species, pygmy, harelquin/sharpchin, redstripe, yelloweye rockfish, canary, darkblotched/bank/ sharpchin, juvenile, and unidentified rockfish, as well as lingcod (Figure 9). The second grouping was ridge habitats, in which most of the deep-sea corals were positively associated (Balanophyllia elegans, Desmophyllum dianthus, Lophelia pertusa, Paragorgia spp., and *Plumarella longispina*) along with redbanded rockfish. The third habitat group was comprised of coarse soft sediments (sand, gravel, and pebble habitats) in which the only corals associated were Swiftia pacifica and S. beringi and flatfish species (Dover sole and unidentified flatfish). The fourth group was mud habitats in which the coral Anthoptilum grandiflorum, eelpouts, poachers, and flatfish were associated. The last group was made up of habitat generalists, such as rosethorn and sharpchin rockfish (which were positively associated with boulder and cobble habitats respectively, but occurred over multiple habitat types), greenstriped rockfish and *Primnoa pacifica* (primarily occurred on high relief rocky habitats and habitat interfaces), as well as thornyheads which occurred over every habitat type except boulder (Figure 10). All species, except for Dover sole, Pleuronectidae, thornyheads and spotted ratfish, had habitat selectivity indexes that were non-random (χ^2 , P < 0.05, Table 1).

Use of deep-sea corals by fish

Fish density was overall higher with corals present than absent (Z=6.46, P<0.001) but was significant over low-relief and mixed substrates: boulder-sand (Z=5.10, P<0.001), cobblemud (Z=2.72, P<0.01), mud-boulder (Z=3.40, P<0.001), mud-ridge (Z=5.02, P<0.001), pebble (Z=-3.16, P<0.01), and sand-cobble habitats (Z=5.00, P<0.001, Figure 11). However, fish density in boulder habitats was higher without corals present (Z=-2.10, P=0.03) and there was no difference in fish density over remaining habitats [rock ridge (Z=0.87, P=0.38), boulder-cobble (Z=0.22, P=0.83), cobble (Z=1.04, P=0.30), mud (Z=-0.53, P=0.60), and sand habitats (Z=-1.14, P=0.25)].

The majority of fish analyzed (82.5%) were found greater than 1m from a deep-sea coral or sponge and thus had no measured association. However, 2,139 fish (17.5%) did have some level of association with one or multiple invertebrates, with 39 fish (and shark/skate egg cases) (0.3% of fish abundance) having physical contact with an invertebrate. Nearly ~67% of these physical contact associations were with *Paragorgia* spp. The number of corals that were used (i.e. had a fish associated) in relation to their availability resulted in low to moderate use for most corals (10-28%), except for *Lophelia pertusa* and *Primnoa pacifica* corals in which ~70% of those available had fish associated (Figure 12). However, of the ten most abundant deep-sea corals and sponge growth forms, none had mean selectivity indices above the expected value of 0.01. Nearly all coral species were not significantly different from zero (i.e. avoidance), except for *Swiftia beringi* and *Swiftia pacifica* which had selectivity indices significantly greater than zero. However, selectivity indices for these species were less than the expected value, 0.1, indicating that, although fish are not avoiding these corals, fish are not selecting for them either.

Diel shift

Transects sampled during dusk and dawn were eliminated from analyses, removing $37,838\text{m}^2$ (19%) of habitat and 3,180 (27%) of fish sampled from these analyses. Habitat area surveyed did not differ significantly between day and night (Z=0.46, P=0.64), or by substrate type (Appendix Table 4). This data set contained 1403 habitat patches surveyed during the day and 606 at night. A diel shift analysis was conducted for fish density across all habitats. There was no observed diel shift in fish abundance for several fish species analyzed (P>0.5, Table 2). However, diel shifts in species abundance were observed for the darkblotched/bank/ sharpchin and harlequin/sharpchin rockfish complexes, as well as for hagfish, thornhead, redstripe, rosethorn, and unidentified rockfish (P<0.05). All species were more abundant during the day, except harelquin/sharpchin rockfish and hagfish, which were more abundant at night.

Ontogenetic shift

There was a strong negative correlation between depth and seafloor grain size (Spearman rank correlation= -0.764, n= 12, P= 0.004). Smaller grain sizes (i.e. mud habitats) were deeper than cobble and boulder habitats. Several species displayed a significant ontogenetic shift, where smaller individuals occurred at shallower depths than larger individuals of that species (Appendix Table 5). These include poachers, Pacific Ocean perch, darkblotched, pygmy, rosethorn, and unidentified rockfish as well as the darkblotched/bank/sharpchin and harlequin/sharpchin rockfish complexes, thornyheads, and several flatfish species (Dover sole, righteye flounders, unidentified flatfish, P <0.05). Eelpouts ($t_{(112)}$ =-5.31, β =-0.59, P<0.001) and yelloweye rockfish ($t_{(46)}$ =-2.61, β =-0.39, P=0.01) displayed an opposite pattern, where smaller individuals occurred at deeper depths than larger individuals.

Impacts of fishing gear on deep-sea corals

There were few observations of anthropogenic objects (74 observations), with an average density of 0.05 incidents 100m^{-2} (SE=0.007). The occurrence of damaged corals and sponges (n=86) were low in comparison to overall invertebrate abundance, with a density of 0.03 damaged invertebrates 100m^{-2} (SE=0.006). The majority of damaged corals (n=47) were located inside Olympic 2. There were two observations of gear entanglement on a *Paragorgia* spp. and *Stylaster* spp. (0.004% of total invertebrate abundance). *Paragorgia* spp. comprised 71% of the observed damaged corals (n=61) and ~20% of all observed *Paragorgia* spp. were damaged. The average distance of fishing gear (or other anthropogenic observations, i.e. trash) from all observed corals was 445m (SE=7.67), however, distance from damaged corals was much lower at 62.8m (SE=4.57) and <0.1% of corals had an anthropogenic observation within 10m.

DISCUSSION

Our study was the first to investigate the functional role of deep-sea corals as fish habitat along the continental west coast by parsing out the influence of physical substrate in fish associations with corals. We were also successful in determining fish-habitat associations in deep-sea ecosystems off of Washington State. Habitat complexity is an important component in driving species community structure with physical substrate associated with distinct fish species assemblages and deep-sea corals being important for fish in low-relief habitats. By utilizing habitat characterization methodologies widely used along the west coast we were able to compare our results to other studies to identify similarities in substrate driven species assemblages across broad regions. Overall, this study characterizes groundfish communities in Olympic Coast National Marine Sanctuary following the establishment of an EFH Conservation

Area and provides new data on fish distributions, species-habitat associations, and fish use of deep-sea corals in this region to better inform future management decisions.

The Olympic 2 EFH Conservation Area and surrounding sites were comprised of diverse habitat types. Several studies along the west coast have identified substrate as an important indicator of species assemblages (Carlson & Straty 1981; Pearcy et al. 1989; Stein et al. 1992; Pacunski & Palsson 2001; Yoklavich et al. 2000; Jagielo et al. 2003; Johnson et al. 2003; Reynolds 2003; Tissot et al. 2007). In this study seafloor substrates were separated into five groups and species assemblages were similar to other west coast studies; with differences largely found in species composition, lack of small rockfish (pygmy, juvenile and YOY rockfish), and slight differences in rock ridge habitat composition and associated species. These comparisons are summarized in Table 3. In this study, the majority of rockfish species, specifically overfished species (canary and yelloweye rockfish), and other commercially important species (lingcod) were associated with boulder habitats. Boulder habitats had the highest average fish densities (0.24 fish m⁻²). Similar fish densities in boulder habitats were found in other west coast studies (Table 4). However, overall fish density was much lower in Washington (this study; Jagielo et al. 2003), than at Heceta Bank, OR (Stein et al. 1992; Tissot et al. 2007) and several central California studies (Anderson and Yoklavich 2007; Laidig et al. 2009). Jagielo et al. (2003) hypothesized that differences in sampling design (random versus purposive) was the result of difference in fish density; however, our study utilized a purposive sampling design targeting hard substrate and, overall, fish densities were still lower than in the California and Oregon studies. The lower fish abundance observed in Washington likely correlates with the low abundance of observed small (pygmy and juvenile) rockfish or the historical heavy fishing pressure in this region. The second substrate group was rock ridge habitat, in which most of the deep-sea corals

were associated due to high-relief drop-offs and higher currents potentially providing more organic detritus and plankton (Etnoyer & Morgan 2005; Tissot et al. 2006). Other substrate groupings, sand and mud, were dominated by flatfish, eelpouts, and poachers. Commercially important rockfish species and sensitive biogenic structures were associated with high-relief hard substrates (boulder and rock ridge) indicating these habitats may be EFH. Similarities in habitat groupings and corresponding species assemblages among studies indicate that rockfish use comparable habitats along the west coast. These regional similarities allow seafloor composition to be used as an indicator of species assemblages to generate predictions of species distributions along the west coast to better identify areas of EFH.

Deep-sea coral and sponge densities were high compared to other studies along the west coast (Pirtle 2005; Tissot et al. 2006, Table 5). Densities varied based on size, with large (>50cm) corals being rare, as well as over substrate type, with ridge habitats having the highest invertebrate densities. Despite deep-sea corals and sponges having relatively high densities, none were selected as habitat by fish relative to their availability, likely due to the low abundance of large corals observed. This pattern indicates that fish were not selecting for individual coral taxa. However, relatively high usage of corals by fish supports the importance of deep-sea corals as fish habitat, in general, which varied by substrate type. Fish may use corals for multiple reasons: as refuge from predators, co-occurrence in areas of high food availability, corals may attract smaller fish and invertebrates as refuge thus providing food aggregations for larger fish, or fish may select for similar substrate types in which corals settle. Previous studies along the west coast have not parsed out the influence of physical substrate when analyzing fish use of deep-sea corals. For instance, Harter et al. (2009) found that fish density was no different over hard complexity habitats and deep-sea corals in eastern Florida, but did not separate similar physical

substrates with and without these deep-sea corals. Additionally, Stone (2006), using similar methods for defining association [modified from Auster et al. (1995)], found that coral densities observed in the Aleutian Islands (1.23 corals m⁻²) were much higher than in our study, consequently the majority of rockfish observed were associated with corals (83-98%), with 20% having physical contact with these emergent epifauna. However, Stone (2006) was not able determine the importance of physical substrate as a potential driving force for these associations due to lack of similar habitats sampled without corals present. In this study, fish abundance was higher over habitats with corals present, except in boulder habitats. The higher fish density on boulder habitats without corals goes against what we would expect that higher complexity habitats would be selected for by fish. This result may be due to lack of small rockfish in our study that would rely on boulder habitats with corals more as refuge from predators since the majority of corals on boulder habitats were small S. beringi (~10cm height). Small corals do not provide much additional structure to the already complex physical substrate (>2m in vertical relief) of boulders. Additionally, only 2.5% of large deep-sea corals (n=4) occurred on boulder habitats, which may be due to depth related restrictions in colonization since most deep-sea coral species settle at depths greater than 200m (Etnoyer & Morgan 2005) and boulder habitats occurred, on average, <130m. Furthermore, the majority of rockfish observed in our study were adults (>20cm in length), which are more than double in size of S. beringi corals, and instead of using the corals as refuge, were observed utilizing the crevices of the boulder habitats. The presence of small corals may be interfering with fish use of boulder habitats in such a way that fish actually prefer the physical substrate with no corals present in order to better utilize available crevices. Although fish abundance was higher in boulder habitats without corals, in lower relief or mixed habitats fish were more abundant with corals present. This result is likely

due to corals enhancing the low-relief physical substrates, providing additional refuge for fish, or occurring in areas of higher food availability. Auster (2005) found that on the U.S. East Coast, Acadian redfish (*Sebastes fasciatus*) densities were not different over habitats with dense corals from similar physical habitats with dense non-coral epifauna. Results in this study and Auster (2005) indicate that fishes associate with complexity and structure, whether it is biogenic (corals and other emergent epifauna) or physical (boulders). However, additional studies are needed to experimentally test these relationships to determine if there is a facultative relationship.

Deep-sea corals were important aspects of fish habitat in low-relief and mixed substrates. These low-relief habitats are especially at-risk of disturbance from anthropogenic activities. Differences in species abundances inside and outside the Olympic 2 Conservation Area correspond with differences in habitat availability in areas surveyed. Habitats inside Olympic 2 were primarily lower relief, mixed habitats (~51%); consequently more flatfish, poachers, hagfish, and thornyheads were present. This area is protected from non-treaty commercial trawling, however, tribal bottom trawling and other bottom contact activities are still allowed in the boundaries. More boulder habitats were surveyed outside Olympic 2 and as a result higher abundances of rockfish. The lack of protection on important rockfish habitats (i.e. boulder) could result in these habitats being altered and leaves associated fish and coral species vulnerable to fishing pressure including bottom trawling. However, it is important to note that this study only surveyed a small portion of area within Olympic 2 and due to the lack of high resolution bathymetry; we do not know the extent of habitats available within the sites beyond that of the coarser-scale side scan sonar. Future efforts should focus on collecting these high-resolution habitat data to determine associated fish communities within the protected area. These data will aid in current and future efforts in evaluating current groundfish EFH conservation area

boundaries (PFMC 2012b), as species assemblages associated with substrate types in this region are known.

Identifying diel shifts in species activity and abundance is important when determining EFH for groundfish as habitat use may differ depending on the time of the survey. This study took place in relatively deep waters [mean depth = 218m (SE=1.6)] where little light would be likely to penetrate. Hart et al. (2010) found that light penetration reached 50m in Heceta Bank, OR, much shallower than the majority of transects surveyed in this study. However, several species did exhibit diel shifts; the majority of which were more abundant during the daylight hours, except for hagfish and harlequin/sharpchin rockfish. Small fish having a higher abundance during the day and general association with complex boulder habitats is likely in response to prey behavior or to avoid predators (Brodeur 2001; Hart et al. 2010). These findings are similar to Hart et al. (2010) in which rosethorn rockfish were significantly more abundant during the day and hagfish, sharpchin and harelquin rockfish more abundant during the night surveys. However, Hart et al. (2010) took into account species activity, in which species were active if they were not resting on the substrate, in addition to abundance, our study solely analyzed abundance differences. The majority of fish observed in this study, especially in complex boulder habitats, were in contact with the seafloor, so although a species may have been visually more abundant during the day or night it may not have been active during that time. However, our results are consistent with other studies along the west coast, in which small rockfish species (rosethorn rockfish) are diurnal and few large rockfish (sharpchin/harelquin rockfish) and hagfish are nocturnal (Wilkins 1986; Simmonds et al. 1992; Stanley et al. 1999; Hart et al. 2010). Although there are diel shifts in species abundance in this study, these ROV surveys were not designed for diel shift analyses. Future surveys should account for species

activity differences and sample the same transects between day and night to look at overall differences in fish abundance, activity, and habitat associations over the same area.

Young of the year and juvenile rockfish use shallower waters on continental shelves (<100m) and are associated with high relief rocky habitat in studies from central California (Yoklavich et al. 2000; Anderson & Yoklavich 2007), Heceta Bank, OR (Pearcy et al. 1989; Hixon et al. 1991; Stein et al. 1992; Tissot et al. 2007) and the Gulf of Alaska (Carlson & Straty 1981). Most adult rockfish associate with high-relief hard or mixed substrates at depths greater than 100m (Pearcy et al. 1989; Stein et al. 1992; Yoklavich et al. 2000; Laidig et al. 2009). Several species in this study displayed a pattern consistent with an ontogenetic shift, with larger individuals occurring at deeper depths than smaller individuals of that species. This pattern is likely due to complex habitats observed at shallower depths, resulting in more refuge for small fishes. Although most of the juvenile rockfish observed in this study were associated with boulder habitats, these habitats occurred, on average, > 120m. Depths deeper than 100m may be the reason relatively few juvenile rockfish were present (~1% of total fish abundance) compared to other studies along the west coast; ranging from 3.8% of total fish abundance in Davenport, CA (Laidig et al. 2009) to 25.5-39% in Heceta Bank, OR (Tissot et al. 2007; Stein et al. 1992), indicating this area may not be an important nursery ground due to depth restrictions. However, this study surveyed from May-July, in which we observed several gravid rockfish, whereas other studies sampled later in the year (August-October) in which more YOYs were observed (Stein et al. 1992; Yoklavich et al. 2000; Laidig et al. 2009). Additionally, our study did not look at differences in juvenile abundance between years sampled, but interannual variation could account for low juvenile rockfish abundance (Tissot et al. 2007). Future surveys should sample in multiple seasons and across multiple years to determine important habitats for gravid rockfish, nursery grounds for juveniles, and the role of deep-sea corals in these life history stages of rockfish in this region.

Deep-sea corals and sponges are especially susceptible to disturbance and are slow to recover from damage. Anthropogenic impacts (i.e. fishing gear, debris, etc.) had a low frequency of occurrence in this region; despite this low frequency we still had observations of dead and damaged corals. Density of anthropogenic impacts observed in OCNMS (369/km²) was similar to those found by Watters et al. (2010) in southern California (320/km²), but much lower than those in central California (6900/km²), however, distribution of fishing effort along the west coast is the greatest in Washington and Oregon. Differences in gear use by region may account for variation in lost gear, with nearly 75% of trawling occurring in Oregon and Washington (NMFS 2013). Although the initial impact of fishing gear and trash on deep-sea corals is unknown, the average distance of debris observed from corals was over 400m. This distance decreased to 60m in relation to damaged corals; however, it is not clear if these corals were damaged as a direct result of the gear or debris (with the exception of the two corals with entangled fishing gear). Although few negative impacts to organisms from marine debris and lost fishing gear have been observed in the deep-sea (Watters et al. 2010), it is still important to quantify the distribution and types of debris as well as their effect on the seafloor and associated fauna to determine their impacts on remote deep-sea ecosystems.

CONCLUSION

In conclusion, deep-sea corals are likely an important component of essential fish habitat and are at-risk of disturbance from bottom contact anthropogenic activities. Understanding the distributions of and fish associations with deep-sea corals is only recently emerging along the

west coast. Our study is in agreement with other observational studies, in which rockfish associate with available ecologically similar high-relief habitats, both physical and biogenic, when available. During the course of this study, the Pacific Fishery Management Council (PFMC) submitted a request for proposals as part of their 5-year review of groundfish EFH on 31 July 2013. The success of ecosystem-based management approaches depends on our understanding of the functional relationships between federally managed fish species and all aspects of their habitat. This study provides new data on fish-habitat associations in deep-sea ecosystems as well as a baseline characterization of species community structure inside and outside a recently protected area allowing for future studies to determine the efficacy of this EFH Conservation Area in protecting sensitive deep-sea corals and rebuilding depleted fish populations.

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Table 1: Habitat selectivity indexes for 25 species (or species complexes). A positive selectivity index indicates the species was more abundant on (associated with) that habitat type and a negative selectivity index indicates the species was less abundant on (or avoided) that habitat type more often than randomly expected in each of the 12 habitat types. Bolded text represents species having random distributions across habitats (χ^2 , P > 0.05).

Taxa	MM	SS	PP	MR	MB	SC	CM	BS	CC	BC	BB	RR
Canary rockfish	-6.2	-7.6	-8.5	-3.9	-4.2	-8.3	-11.3	27.1	-1.0	-3.0	29.6	-2.7
Darkblotched rockfish	2.4	-5.4	-6.4	1.8	10.1	-11.9	14.4	-5.3	1.8	-1.6	-8.5	8.7
Darkblotched/Bank/ Sharpchin rockfish	-4.1	-8.3	-8.1	1.3	-1.3	-8.9	2.3	-2.2	-1.6	-1.7	30.8	1.7
Dover sole	7.8	1.2	6.9	0.4	-3.3	11.2	-1.4	-6.0	-2.7	-5.5	-8.3	-0.4
Eelpout	42.7	-5.2	-6.2	-3.9	-4.2	-5.8	-9.8	-8.1	-7.7	2.0	9.1	-2.7
Greenstriped rockfish	-5.5	1.0	-2.8	-3.2	-4.2	14.5	-9.2	22.6	3.2	-8.8	-4.9	-2.7
Hagfish	2.2	-4.9	-5.2	4.5	7.9	-9.2	7.8	-7.4	-7.3	17.7	-8.2	2.0
Harelquin/Sharpchin rockfish	-5.7	-8.3	-9.0	-3.9	-3.0	-11.9	-10.8	-8.1	-11.8	-4.5	79.8	-2.7
Juvenile rockfish	0.2	-6.9	8.6	-3.2	-3.5	-9.0	-9.2	6.9	-6.1	-3.8	27.9	-2.0
Lingcod	-5.4	-5.3	-4.0	0.6	-2.0	-2.2	-0.9	12.0	-0.4	-5.0	13.1	-0.5
Pacific ocean perch	-3.1	-8.3	-2.1	3.2	-1.2	-9.9	0.9	-7.1	34.6	-5.1	-2.4	0.3
Poacher	22.3	-6.0	1.6	-0.9	3.8	-4.9	6.9	-2.4	-8.8	-3.6	-8.3	0.3
Pygmy rockfish	-6.2	-8.3	-9.2	-3.9	-4.2	-11.8	-11.3	-7.6	-13.7	-0.9	79.9	-2.7
Redbanded rockfish	-3.5	-8.3	-7.5	53.6	0.2	-8.4	-6.0	-8.1	-9.2	-4.9	-7.6	9.7
Redstripe rockfish	-6.2	-8.3	-9.2	-3.9	-4.2	-9.0	-11.1	14.0	-14.3	-7.5	62.5	-2.7
Righteye flounders	8.7	-5.1	2.0	2.3	-1.7	4.8	4.6	0.2	-4.5	-3.5	-8.1	0.3
Rockfish	-4.5	-8.0	-7.4	-1.9	-3.3	-9.0	-8.4	-3.2	-8.6	-0.2	56.4	-1.8
Ronquil	-5.1	-5.2	-1.9	-2.9	-4.2	2.7	-11.3	9.6	12.8	0.2	8.2	-2.7
Rosethorn rockfish	-3.7	-7.7	-4.1	1.3	1.3	-8.2	-4.2	-0.9	-2.7	6.0	20.0	2.9
Sharpchin rockfish	-2.8	-8.3	-5.8	3.8	6.9	-10.2	16.0	-8.1	4.3	1.8	0.9	1.5
Shortspine/Longspine	6.5	-3.7	-3.8	-0.3	0.5	0.3	6.8	-7.4	6.7	4.1	-8.3	-1.6
Spotted ratfish	-1.4	-7.8	-1.2	-1.7	-2.6	9.1	3.2	-1.7	6.7	2.7	-2.6	-2.7
Unidentified flatfish	3.4	4.1	-0.5	-0.2	-0.1	3.7	-2.6	18.9	-9.9	-9.3	-5.3	-2.3
Yelloweye rockfish	-6.2	-8.3	-7.6	-3.9	-4.2	-8.7	-6.5	6.4	-10.5	-7.0	59.2	-2.7
Yellowtail rockfish	-6.2	-8.3	-3.2	-3.9	-4.2	2.1	-11.3	17.9	-5.4	-6.2	31.5	-2.7

Table 2: Diel shift analysis of fish species were conducted with Mann-Whitney U-tests (*P<0.05, **P<0.01, ***P<0.001) to determine differences in fish abundances between day and night.

Species	% abundance	% abundance	Z -value	P -value
-	during day	during night		
Canary Rockfish	76.3	23.7	0.27	0.78
Darkblotched Rockfish	78.6	21.4	0.02	0.98
Darkblotched/Bank/Sharpchin				<0.001***
Rockfish	84.9	15.1	3.36	
Dover Sole	60.3	39.7	0.92	0.36
Eelpout	48.9	51.1	0.17	0.87
Greenstriped Rockfish	74.2	25.8	0.69	0.49
Juvenile Rockfish	79.8	20.2	0.36	0.72
Harlequin/Sharpchin Rockfish	2.2	97.8	2.33	0.02*
Lingcod	77.5	22.5	0.37	0.71
Hagfish	39.7	60.3	4.12	<0.001***
Pacific Ocean Perch	85.0	15.0	0.16	0.88
Poacher	52.8	47.2	0.33	0.74
Pygmy Rockfish	77.1	22.9	0.59	0.55
Redbanded Rockfish	77.4	22.6	0.35	0.73
Redstripe Rockfish	98.2	1.8	2.14	0.03*
Righteye Flounder	65.1	34.9	0.34	0.73
Rockfish	68.8	31.2	2.80	<0.01**
Ronquil	67.6	32.4	0.01	0.99
Rosethorn Rockfish	75.6	24.4	2.58	<0.01**
Sharpchin Rockfish	80.6	19.4	0.38	0.70
Shortspine/Longspine Thornyhead	60.1	39.9	2.41	0.02*
Spotted Ratfish	80.4	19.6	1.27	0.21
Unidentified Flatfish	58.7	41.3	0.02	0.98
Yelloweye Rockfish	65.9	34.1	0.05	0.96
Yellowtail Rockfish	95.2	4.8	0.58	0.56

Table 3: Habitat groupings and associated species communities comparing similarities and differences in fish assemblages with other west coast studies.

Hobitet erromane	High taxa accominated	Corn & cronna tava accomited	Similar chidise	Omocina ctudiae
Bonlder	Sehastes snn	Globular snonge	Stein et al. 1992; Jagielo et al. 2003; Anderson & Yoklavich 2007: Laidio et al. 2009: Hart et al. 2010	owns Grand o
	Русту	Swiftia berinoi	Stein et al. 1992; Yoklavich et al. 2000; Tissot et al. 2007; I aidio et al. 2009	Anderson & Yoklavich 2007; Hart et
	Redstripe	8,000	Jagielo et al. 2003	
	Yelloweye		Stein et al. 1992; Jagielo et al. 2003; Anderson & Yoklavich 2007; Tissot et al. 2007	Yoklavich et al. 2000
	Canary		Stein et al. 1992; Anderson & Yoklavich; Tissot et al. 2007; Laidig et al. 2009	
	Juvenile rockfish		Stein et al. 1992; Tissot et al. 2007; Hart et al. 2010	
	Harlequin/sharpchin complex			
	Dark blotched/bank/sharpch in complex			
	Lingcod		Yokkavich et al. 2000; Jagielo et al. 2003; Tissot et al. 2007; Laidig et al. 2009	
Rock ridge	Redbanded rockfish	Balanophyllia elegans		Stein et al. 1992; Tissot et al. 2007; Hart et al. 2010
)		Desmophyllum dianthus		
		Lophelia pertusa		
		Paragorgia spp.		
		Plumarella longispina		
,	,			
Sand	Flatfish & Dover sole	Swiftia spp.	Jagielo et al. 2003; Anderson & Yoklavich et al. 2007	
			Stein et al. 1992; Yokkavich et al. 2000; Jagielo et al. 2003; Anderson & Yokkavich et al. 2007; Tissot et al. 2007; Laidig et	
Mud	Flatfish	Anthoptilum grandiflorum	al. 2009	
	Eelpouts		Stein et al. 1992; Jagielo et al. 2003; Tissot et al. 2007	
			Stein et al. 1992; Yoklavich et al. 2000; Jagielo et al. 2003; Anderson & Yoklavich et al. 2007; Tissot et al. 2007; Laidig et	
	Poachers		al. 2009	
-	-		1 0000 F 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
Habitat generalists	Kosethorn	Frimnoa pacifica	Yoklavich et al. 2000; Tissot et al. 2007; Hart et al. 2010	
	Greenstriped		Stem et al. 1992; Yoklavich et al. 2000; Anderson & Yoklavich 2007; Tissot et al. 2007	Laidig et al. 2009
	Sharpchin		Yoklavich et al. 2000; Hart et al. 2010	
	Sebastolobus spp.		Stein et al. 1992; Yoklavich et al. 2000; Tissot et al. 2007	

Table 4: Comparisons of fish density (#/m²) in boulder and mud habitats in similar studies along the west coast of the United States.

Study site	Fish density (#/m²) in boulder	Fish density (#/m²) in mud	Source
	habitats	habitats	
Washington	0.24	0.06	This study
Heceta Bank, OR	0.29	0.20	Tissot et al. 2007; Stein et
			al. 1992
Davenport, CA	0.24	0.20	Laidig et al. 2009
Soquel Canyon, CA	0.20	0.14	Yoklavich et al. 2000
Monterey Bay, CA	1.40	0.10	Anderson and Yoklavich
			2007

Table 5: Invertebrate density in similar studies along the west coast of the United States. Our study only included deep-sea corals and sponges, other studies also included other invertebrates (i.e. anemones, seastars, brachiopods, etc.).

Study site	Invertebrate density (#/m²)	Source
Aleutian Islands	1.23	Stone 2006
Washington	0.17	This study
Heceta Bank, OR	0.06	Tissot et al. 2004
Cordell Bank, CA	0.08	Pirtle 2005
Southern CA	0.15	Tissot et al. 2006

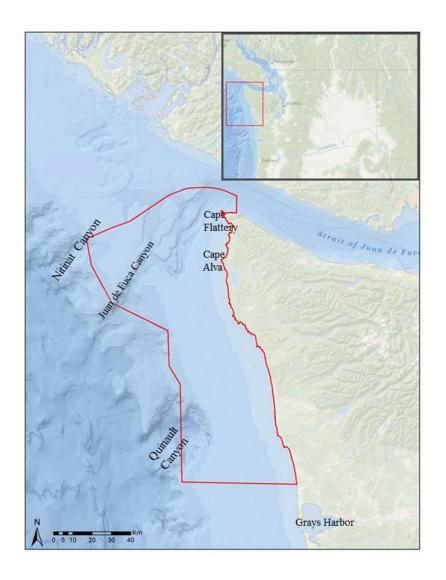


Figure 1: Map of Olympic Coast National Marine Sanctuary (in red), showing bottom topography, located off of the Strait of Juan de Fuca in northern Washington State.

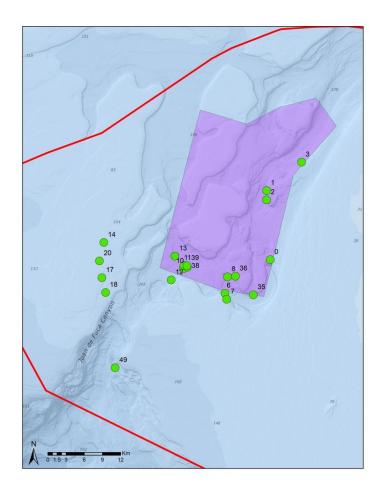


Figure 2: Location of the survey sites (green circles) within study areas in the OCNMS (red boundary), the Olympic 2 Conservation Area (purple polygon).

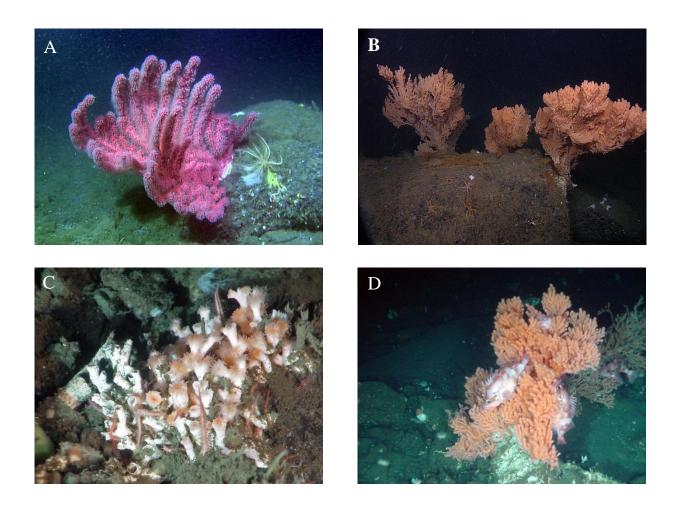


Figure 3: Images of structure-forming corals included in analyses: a) *Paragorgia* spp.; b) *Primnoa pacifica*; c) *Lophelia pertusa*; and d) several sharpchin rockfish having physical contact with a *Primnoa pacifica* coral (Photos courtesy of OCNMS).

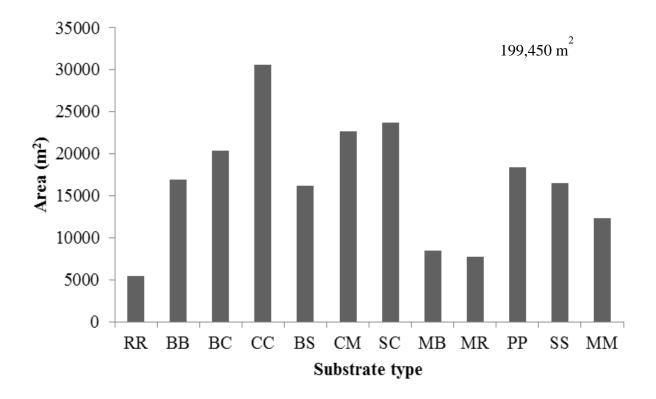


Figure 4: Total habitat area in the twelve habitat categories identified from the results of a cluster analysis. Substratum types recorded were: R = ridge, B = boulder, C = cobble, P = pebble, G = gravel, S = sand and M = mud. The two character code represents the primary (>50%) and secondary (>20%) substrate types, respectively.

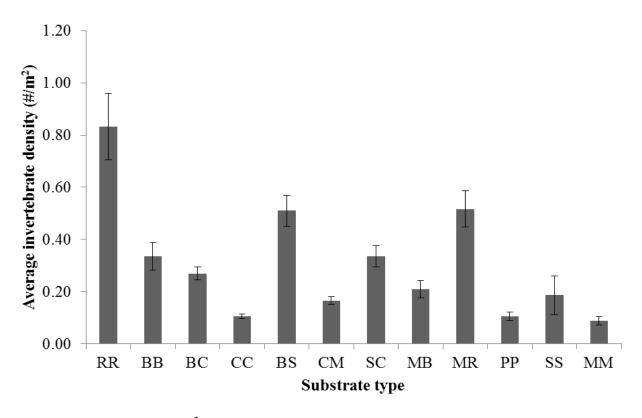


Figure 5: Mean density $(\#/m^2)$ of deep-sea corals and sponges $(\pm 1 \text{ SE})$ across habitat types. See text for description of substrate abbreviations on the x-axis.

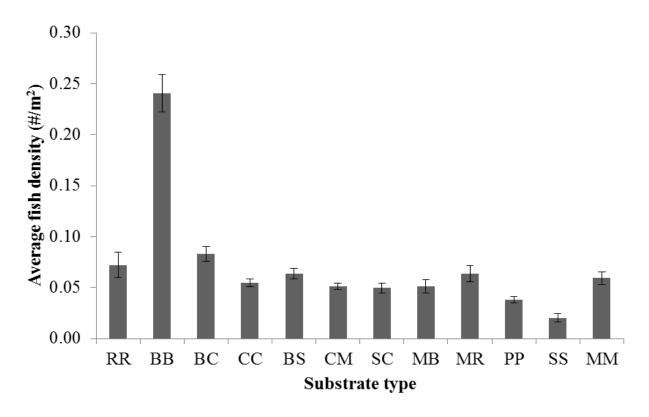


Figure 6: Mean fish density ($\#/m^2$, \pm 1 SE) across habitat types. See text for description of substrate abbreviations on the x-axis.

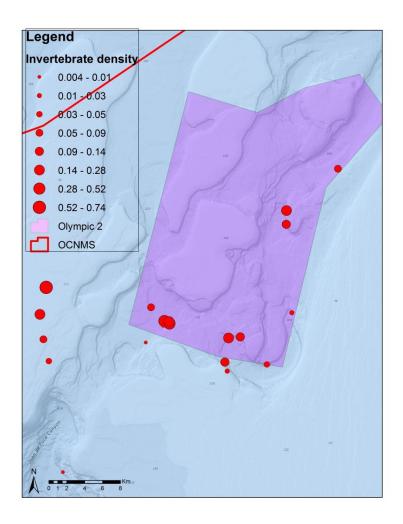
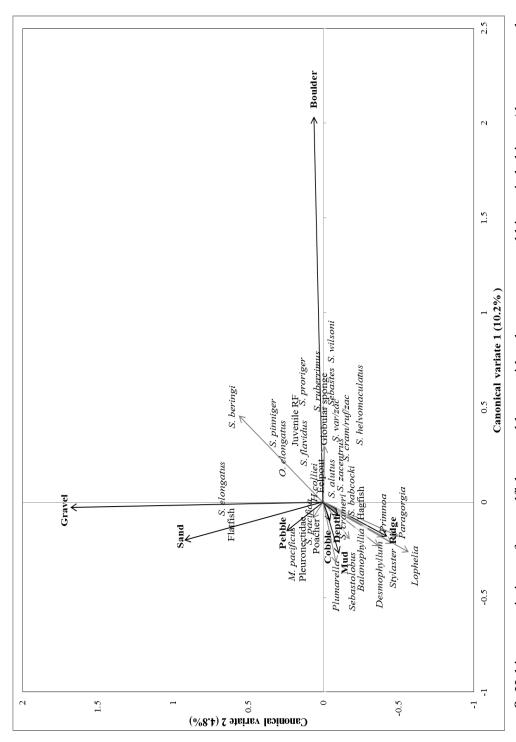


Figure 7: Distribution of coral and sponge density inside and outside Olympic 2.



invertebrate (gray) variables. S = Swiftia (S. beringi and S. pacifica); RF = rockfish (Sebastes); S = Sebastes; O = Ophiodon; Figure 8: Habitat associations of groundfish assemblages with substrate type and biogenic habitats (deep-sea corals and sponges) as determined from a canonical correspondence analysis. Vectors are the eigenvectors of the habitat (black) and

M = Microstomus; H = Hydrolagus.

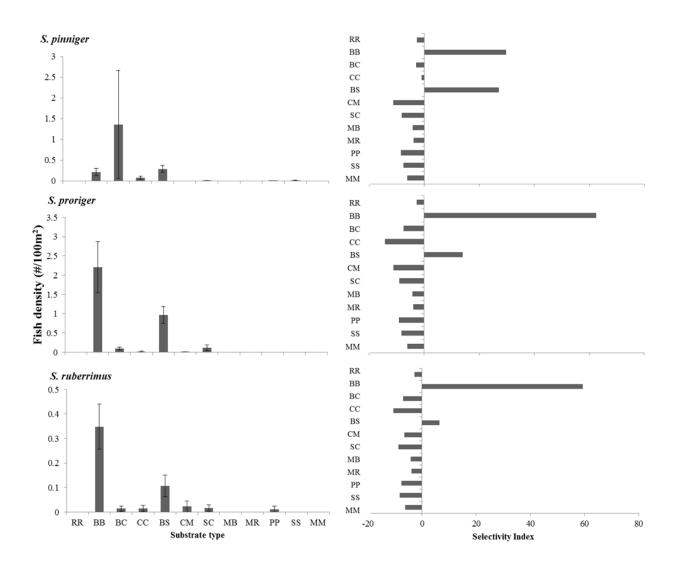


Figure 9: Habitat use and selectivity of boulder associated species: (A) canary rockfish, (B) restripe rockfish, and (C) yelloweye rockfish. Habitat use depicts fish density ($\#/100m^2$, ± 1 SE) in each habitat type. Habitat selectivity depicts the relative use of each habitat type by fish, standardized by habitat availability, where positive values indicate associations and negative values avoidance of habitat types. See text for description of substrate abbreviations on the x-and y-axes.

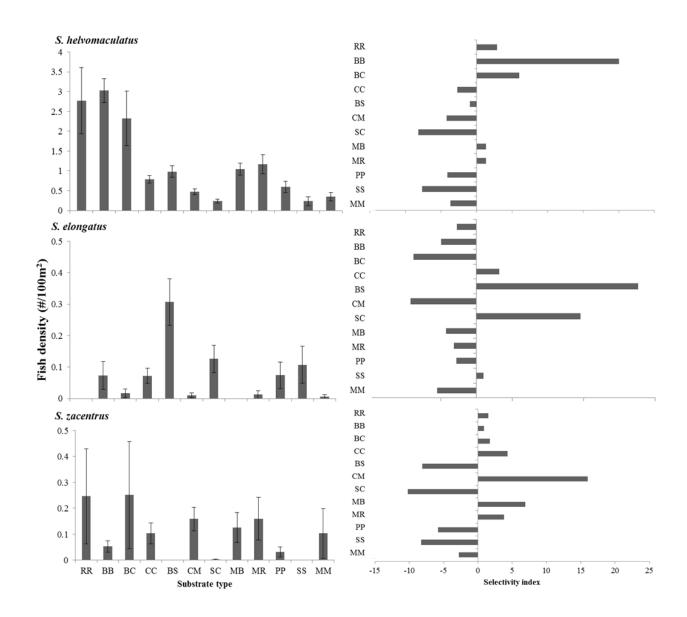


Figure 10: Habitat use and selectivity of: (A) boulder associated rosethorn rockfish, (B) interface/mixed habitat associated greenstriped rockfis), and (C) habitat generalist sharpchin rockfish. See text for description of substrate abbreviations on the x- and y-axes. Interpretation given in Figure 8.

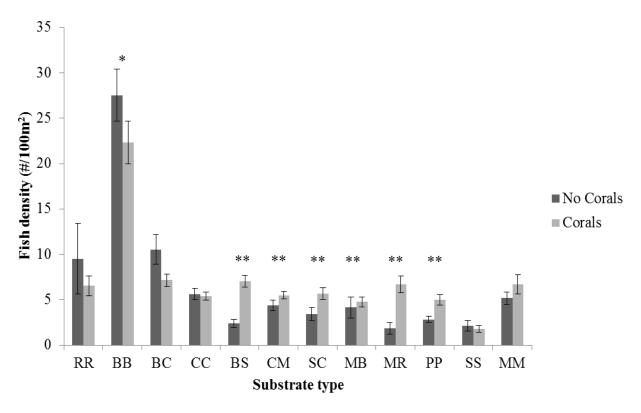


Figure 11: Mean fish density (\pm 1 SE) over habitat types with and without corals. Mann-Whitney U-tests (*P<0.05, ** P<0.01) were conducted on fish density over similar habitat types with and without corals present to determine the relative importance of corals as habitat. See text for description of substrate abbreviations on the x-axis.

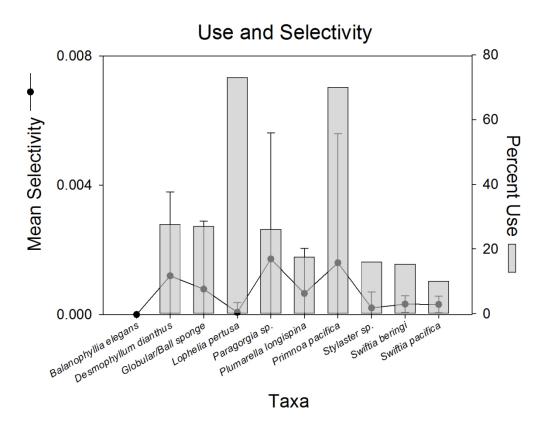


Figure 12: Fish use and selectivity of the ten abundant coral and sponge taxa. Fish selectivity of coral was low, all taxa selectivity were below the expected value was 0.1. Error bars are 95% confidence intervals in which all taxa besides *S. beringi* and *S. pacifica* were not significantly different from zero. Fish use of corals was low to moderate for most coral taxa, but *Lophelia pertusa* and *Primnoa pacifica* corals had high fish use.

APPENDIX

Appendix Table 1: Mann-Whitney U tests (*P<0.05, ** P<0.01, ***P<0.001) were conducted to determine differences in habitat area surveyed inside and outside Olympic 2. Bolded values are those that are significantly greater between areas.

	Inside (m ²)	Outside (m ²)	Z-value	<i>P</i> -value
Total	92,180.27	107,269.97	2.35	0.02*
Ridge	6,212.97	2,433.05	1.83	0.07
Boulder	6,972.47	22,625.58	1.96	0.047*
Cobble	28,088.20	26,177.53	2.25	0.02*
Pebble	6,467.96	12,709.28	1.91	0.05
Gravel	129.72	5,132.61	3.45	<0.001***
Sand	15,557.21	30,556.18	1.06	0.29
Mud	28,751.75	7,635.74	3.25	<0.001***

Appendix Table 2: Mann-Whitney U tests (*P<0.05, ** P<0.01, ***P<0.001) were conducted to determine difference in mean invertebrate density (#/100m2) inside and outside Olympic 2. Standard error in parentheses. Bolded values are those that are significantly greater between areas.

Taxa	Inside	Outside	Z-value	<i>P</i> -value
Anthoptilum grandiflorum	0.02 (0.01)	0.00 (0.00)		
Balanophyllia elegans	0.61 (0.22)	0.00 (0.00)		
Desmophyllum dianthus	1.23 (0.41)	0.52 (0.14)	1.37	0.13
Globular or ball sponge	3.47 (0.47)	4.46 (0.99)	1.26	0.21
Lophelia pertusa	0.08 (0.05)	0.05 (0.05)	0.19	0.84
Paragorgia spp.	0.31 (0.10)	0.05 (0.02)	1.43	0.15
Plumarella longispina	1.66 (0.54)	0.03 (0.02)	4.92	<0.001***
Primnoa pacifica	0.11 (0.04)	0.01 (0.00)	1.65	0.09
Stylaster spp.	0.01 (0.00)	0.00 (0.00)		
Swiftia beringi	16.76 (6.13)	0.14 (0.08)	2.35	0.02*
Swiftia pacifica	0.37 (0.07)	2.51 (0.85)	0.27	0.78

Appendix Table 3: Mann-Whitney U tests (*P<0.05, ** P<0.01, ***P<0.001) were conducted to determine difference in mean fish density (#/100m2) inside and outside Olympic 2. Standard error in parentheses. Bolded values are those that are significantly greater between areas.

Taxa	Inside	Outside	Z-value	<i>P</i> -value
Canary Rockfish	0.00 (0.00)	0.07 (0.03)		
Darkblotched Rockfish	0.02 (0.01)	0.04 (0.02)	0.55	0.59
Darkblotched/Bank/Sharpchin				
Rockfish	0.59 (0.20)	1.02 (0.27)	0.32	0.75
Dover sole	0.51 (0.06)	0.22 (0.04)	4.02	<0.001***
Eelpout	0.06 (0.03)	0.05 (0.02)	0.22	0.83
Greenstriped Rockfish	0.03 (0.01)	0.13 (0.03)	1.98	0.04*
Harlequin/Sharpchin Rockfish	0.01 (0.01)	0.42 (0.28)	0.57	0.57
Juvenile Rockfish	0.01 (0.01)	0.07 (0.02)	2.53	0.01*
Lingcod	0.04 (0.01)	0.15 (0.05)	2.18	0.03*
Hagfish	0.25 (0.06)	0.06 (0.02)	2.27	0.02*
Pacific Ocean Perch	0.08 (0.04)	0.04 (0.02)	0.23	0.82
Poacher	0.50 (0.08)	0.20 (0.05)	5.08	<0.001***
Pymgy Rockfish	0.00 (0.00)	0.50 (0.24)		
Redbanded Rockfish	0.04 (0.01)	0.33 (0.13)	0.70	0.48
Redstriped Rockfish	0.01 (0.00)	0.19 (0.09)	1.83	0.07
Righteye Flounders	0.52 (0.05)	0.29 (0.09)	4.93	<0.001***
Unidentified Rockfish	0.24 (0.05)	1.13 (0.30)	3.69	<0.001***
Ronquil	0.01 (0.00)	0.07 (0.03)	1.43	0.15
Rosethorn Rockfish	0.71 (0.14)	0.84 (0.11)	2.07	0.04*
Sharpchin Rockfish	0.08 (0.02)	0.08 (0.03)	1.16	0.25
Shortspine/Longspine				
Thornyhead	1.10 (0.08)	0.46 (0.09)	6.10	<0.001***
Spotted Ratfish	0.16 (0.03)	0.08 (0.02)	1.18	0.24
Unidentified Flatfish	0.09 (0.02)	0.11 (0.02)	0.75	0.45
Yelloweye Rockfish	0.01 (0.00)	0.03 (0.01)	2.35	0.02*
Yellowtail Rockfish	0.00 (0.00)	0.02 (0.01)		

Appendix Table 4: Differences in area of primary habitats surveyed between day and night conducted with Mann-Whitney U-tests.

Habitat	Area	Area	Z-value	<i>P</i> -value
	surveyed	surveyed		
	during day	during night		
R	4,668.44	2,557.89	1.44	0.15
В	15,301.18	6,818.80	1.27	0.20
С	23,234.67	21,689.16	1.22	0.22
P	12,738.76	2,866.26	1.40	0.17
G	3,679.71	762.47	1.22	0.22
S	32,450.05	8,402.42	1.40	0.16
M	15,838.67	10,601.59	0.53	0.59

Appendix Table 5: Ontogenetic shift analyses for fish were conducted using a linear regression (*P<0.05, ** P<0.01, ***P<0.001) to determine if species sizes increases with depth.

Species	\mathbb{R}^2	t-value	Degrees of	β	<i>P</i> -value
_			freedom		
Canary rockfish	0.5%	-0.74	107	-0.05	0.46
Darkblotched rockfish	26.9%	3.43	32	0.02	<0.01**
Darkblotched/Bank/ Sharpchin rockfish	12.3%	10.96	858	0.05	<0.001***
Dover sole	2.6%	3.49	460	0.02	<0.001***
Eelpout	19.4%	-5.31	112	-0.59	<0.001***
Unidentified flatfish	11.4%	4.47	156	0.04	<0.001***
Greenstriped rockfish	0.1%	-0.36	119	-0.005	0.72
Harelquin/Sharpchin rockfish	4.8%	4.88	472	0.05	<0.001***
Lingcod	2.3%	1.54	99	0.05	0.13
Poacher	12.8%	9.0	551	0.03	<0.001***
Pacific Ocean perch	22.0%	3.32	39	0.06	<0.01**
Pygmy rockfish	0.7%	2.23	717	0.05	0.03*
Redbanded Rockfish	5.2%	-1.93	68	-0.09	0.06
Redstripe rockfish	0.1%	-0.74	373	-0.04	0.46
Unidentified rockfish	3.5%	5.91	963	0.03	<0.001***
Ronquil	0.6%	0.76	90	0.01	0.45
Righteye flounder	15.3%	9.17	467	0.06	<0.001***
Rosethorn rockfish	7.7%	9.03	979	0.021	<0.001***
Sharpchin rockfish	0.1%	-0.34	84	-0.01	0.73
Shortspine/Longspine thornyhead	1.3%	3.73	1088	0.03	<0.001***
Yelloweye rockfish	12.9%	-2.61	46	-0.39	0.01*
Yellowtail rockfish	0.1%	-0.17	38	-0.03	0.87