BASELINE MONITORING AND CHARACTERIZATION OF ROCKY INTERTIDAL FISH COMMUNITIES IN NORTHERN CALIFORNIA

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ABSTRACT

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A network of new Marine Protected Areas (MPAs) has been established in northern California, covering 137 square miles of coastline, with the goal of maintaining commercially and recreationally important species and to preserve biodiversity. This is the first study in California to create a biodiversity and phylogenetic baseline of rocky intertidal fish communities within MPAs and nearby reference sites. Diversity, abundance, and size structure of intertidal fishes were compared among seven sites from Fort Bragg to Crescent City, CA during the summers and winters of 2014 and 2015. A total of 34 species were collected throughout sampling, just three less than the estimated 37 species based on rarefaction analyses, with the highest diversity and abundance observed at unprotected sites. Many young-of-year recruits of recreationally and commercially important species were collected, indicating the rocky intertidal zone may be an important nursery area for some species. In contrast to previous studies in this region, very few rockfish (*Sebastes)* recruits were found in intertidal areas. Pools had a very high probability of containing fish, but, with the exception of the sculpin *Oligocottus snyderi*, even the most abundant species were detected in less than half of the pools surveyed. While 28 surveys were conducted, an estimated 34 more would have been necessary to detect all species present in the intertidal zone of this region. A phylogenetic analysis was conducted on DNA Barcode data using the mitochondrial cytochrome c oxidase I gene (COI) of intertidal fish including multiple individuals of the same species from different regions along the Eastern Pacific coast. All species were resolved as monophyletic, indicating great potential for use of the COI gene in species identification.

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INTRODUCTION

Rocky shores are the most common littoral habitat on open wave-exposed coasts, occurring at the margins of the oceans throughout the world. Being exposed to tides, waves, and many other conditions from both the aquatic and terrestrial environments, these are perhaps the most dynamic regions of the marine habitat. Organisms living in this transition zone must be able to withstand rapid and sometimes drastic fluctuations in temperature, salinity, pH, wave turbulence, and desiccation, making it a challenging place to live. In addition to these high frequency cycles, the intertidal zone is directly impacted by large-scale, long-term disturbances such as climate change and anthropogenic effects that organisms are not adapted to survive (Sagarin et al. 1999, Brander 2007, Rijnsdorp et al. 2009).

The interface between land and sea where rocky intertidal habitats occur are particularly prone to influence from humans. The most common and destructive anthropogenic effects include pollution (oil spills, eutrophication), over-collection of living resources, introduction of alien species, modification of coastal processes (coastal defenses, siltation), and global change (climate, sea level, ocean acidification). These events may decline in frequency, but will likely continue to increase in magnitude over the next 25 years which will amplify the impact they have on coastal environments (Thompson et al. 2002).

Some of the best-recorded community-level impacts of anthropogenic stress are from oil spills (Clark et al. 1997). These catastrophic spills can be a double-edged sword, where the oil causes significant damage to the environment, but the cleanup through chemical dispersants and physical cleaning can be just as devastating (Hawkins and Southward 1992, Shaw 1992). Along the California coast, the tankering of oil raises the possibility of an oil spill or other impacts to coastal resources.

Recently, the extensive conversion of private to public access of many coastal areas has increased visitation (Davenport and Davenport 2006). An increase in human traffic in intertidal zones tends to detrimentally effect even the most resilient populations (Addessi 1994). There are essentially two main ways that establishing public access can impact rocky intertidal areas visited by people: 1) collecting organisms for food, bait, and aquaria (Addessi 1994, Murray et al. 1999, Clark et al. 2002), and 2) trampling as people explore the area (Van De Werfhourst and Pearse 2007). Recreational gatherers collect a wide variety of organisms from the intertidal to use as fishing bait or for their ornamental value. While daily takes are small, cumulative effects can become substantial (Underwood 1993). Additionally, human trampling and disturbance associated with visiting the intertidal can damage habitats (Newton et al. 1993).

Marine Protected Areas (MPAs) have become a topic of intense focus lately as a tool for fisheries management and conservation. MPAs have been widely recommended for the conservation and management of marine biodiversity, and to increase populations in nearby unprotected areas (Ballantine 1992, Dugan and Davis 1993, Bohnsack 1996, Lauck et al. 1998, Halpern 2003). The Marine Life Protection Act of 1999 (MLPA) laid out plans to design MPAs in California as a network to conserve and restore populations, biodiversity, habitats, and ecosystems. Since 2007, over 850 square miles of coastal waters have been protected in California; the final set of MPAs was established in the North Coast region in December, 2012. The North Coast region protected area extends from Alder Creek near Point Arena (Mendocino County) to the California/Oregon border and includes MPAs that cover approximately 13% of the state's coastal waters in this region.

Fishes inhabiting rocky intertidal environments of the northeast Pacific form an assemblage that is functionally and evolutionarily distinct from those occurring in the subtidal habitats (Yoshiyama et al. 1986). Over 30 species inhabit the rocky intertidal of the northeast Pacific, many of which are confined to intertidal habitats and are rarely (if ever) detected elsewhere (Yoshiyama et al. 1986, Cox 2007). The dominant intertidal fish group in terms of both abundance and diversity is marine sculpins (Cottidae). Marine sculpins and other intertidal fishes have evolved specialized adaptations for the naturally-occurring stressors of intertidal habitats including thermal stress, emersion, and hypoxia (Gibson 1982, Martin and Bridges 1999, Nakano and Iwama 2002, Knope and Scales 2013), but are vulnerable to anthropogenic effects.

A unique life history trait can be observed in northern California where large numbers of young-of-the-year black rockfish (*Sebastes melanops*) typically reside in tidepools during the summer months before moving to the subtidal as juveniles (Studebaker and Mulligan 2008, Lomeli 2009). Measuring intertidal abundance of juvenile rockfish may provide a useful predictor of future adult population dynamics or could be used as a reliable indicator of recruitment for this important fishery species (Bjorkstedt et al. 2002, Wilson et al. 2008).

In addition to baseline monitoring, molecular cataloguing is important for future species identification; especially in juveniles where morphological identification of species is difficult or impossible (Ivanova et al. 2007). For over 40 years DNA has been used to identify species (Manwell and Baker 1963, Woese and Fox 1977) and by the late 1970s and 1980s mitochondrial DNA approaches dominated molecular systematics (Avise 1994). Different techniques and sequences had been used for species identification until Hebert et al. (2003) recently proposed that a single gene, cytochrome oxidase c subunit I, could be used to differentiate the vast majority of animal species. The combination of this gene and work by Tautz et al. (2002, 2003) resulted in a DNA-based taxonomic system called the Barcode of Life. For barcoding sequences to be useful and successful in species identification, DNA sequences need to be more similar within species than between different species. Despite initial criticism of the technique (Lipscomb et al. 2003, Moritz and Cicero 2004), DNA barcoding has potential to become a standard tool for identifying species (Ward et al. 2005, Ivanova et al. 2007; see Waugh 2007 for an overview of benefits and pitfalls). In addition to enabling identification of even very early life stages of fishes where morphological identification may be difficult or impossible, amplification of DNA from the cytochrome *c* oxidase 1 (COI) gene, using a standardized procedure, allows for comparison to reference specimens catalogued in the Fish Barcode of Life Initiative (FISH- BOL; www.fishbol.org; see Ward et al. 2009 for an overview of FISH-BOL).

The objectives of this study were to create a biodiversity baseline for fish living in the rocky intertidal zone, explore questions about the fish communities living there, including recreational and commercial species, and develop a genetic barcode baseline for North Coast rocky intertidal fish. Several questions about the rocky intertidal zone of northern California were addressed from this baseline data: (1) Does biodiversity of rocky intertidal fish differ among sites, between geographic regions (e.g., north and south of the Lost Coast), and with protection level (e.g. MPA or non-MPA)? (2) Does individual size or species composition change with intertidal zone? (3) Do genetic barcodes vary by region or site, and can they be used for identification?

Study Region and Sites

This study was conducted from June 2014 to February 2016 at seven sites along the Northern California Coast: Point St. George and False Klamath Cove (Del Norte County), Palmer's Point (Humboldt County), Ten Mile State Marine Reserve (SMR), MacKerricher State Park State Marine Conservation Area (SMCA), Fort Bragg Cove, and Belinda Point (Mendocino County) (Fig. 1).

The study region is along the boundary of two major biogeographical provinces; cold-temperate Oregonian and warm-temperate Californian. The coastline is exposed to the open ocean with regular wave action comes predominantly from the northwest. Wave height typically ranges from one to two meters, but occasional storms during the winter can produce waves greater than eight meters in height. The average significant wave heights in Del Norte County during 2014 and 2015 were 2.0 meters and 3.1 meters with maximum significant wave heights reaching 7.6 meters and 8.7 meters, respectively. The average seasonal sea surface temperatures (SST) were 10.3°C during the 2014 summer, 12.3°C during the 2014-2015 winter, 10.4°C during the 2015 summer, and 12.2°C during the 2015-2016 winter (data from NOAA marine buoys #46027 and PORO3). The average significant wave heights in Humboldt County during 2014 and 2015 were 2.3 meters and 2.1 meters with maximums of 8.6 meters and 9.9 meters, respectively. Average SST was 12.1°C during the 2014 summer, 13.9°C during the 2014-2015 winter, 12.8°C during the 2015 summer, and 12.5°C during the 2015-2016 winter (data from NOAA marine buoy #46244). The average significant wave heights in Mendocino County during 2014 and 2015 were 2.2 meters and 2.3 meters with maximums of 6.8 meters and 9.0 meters . Average SST was 12.2°C during the 2014 summer, 15.7°C during the 2014-2015 winter, 11.7°C during the 2015 summer, and 13.1°C during the 2015-2016 winter (data from NOAA marine buoy #46014).

The sites were divided into two categories based on separation by the Cape Mendocino and the Lost Coast region. Point St. George, False Klamath, and Palmers Point were called “north” sites while the remaining four sites were called “south” sites. Cape Mendocino, which lies roughly between Ferndale and Rockport, may provide a biogeographical break that exceeds the typical larval drift distance of less than 120 km for many intertidal fishes (Miller and Shanks 2004). This region is also the site of convergent shelf flow that results in either cyclonic coastal eddies or strong offshore transport that form a barrier for larval transport between the two geographic regions (Hayward and Mantyla 1990, Magnell et al. 1990).

Three different rocky intertidal habitat types were sampled in this study: boulder fields, benches, and a combination of the two. Boulder fields typically cover a large region and consist of many boulders emerging from a sandy shoreline. Pools form in the divots between the boulders and are protected during low tide by emerging rocks along the edges of the zone. These areas typically lie near a sandy beach. Benches are large pieces of bedrock that drop of sharply into the ocean. At high tide the bench is completely covered in water, but as the tide goes out it becomes exposed. Pools are formed in the cracks and crevices of the rocks. Sites that are a combination usually have a sharp drop into the subtidal, similar to a bench, but contain many boulders and divots that form pools more similar to boulder fields.

Point St. George (N 41.784, W 124.255), the northern-most site surveyed, is located at the northwest point of Point St. George and connects to the beach that runs along the northern part of the coastal area. The site is a large boulder field that is approximately 90 meters long (extending straight out from shore) and 75 meters wide. The intertidal zone is very exposed to waves during high tide, but is protected by large boulders when the tide recedes.

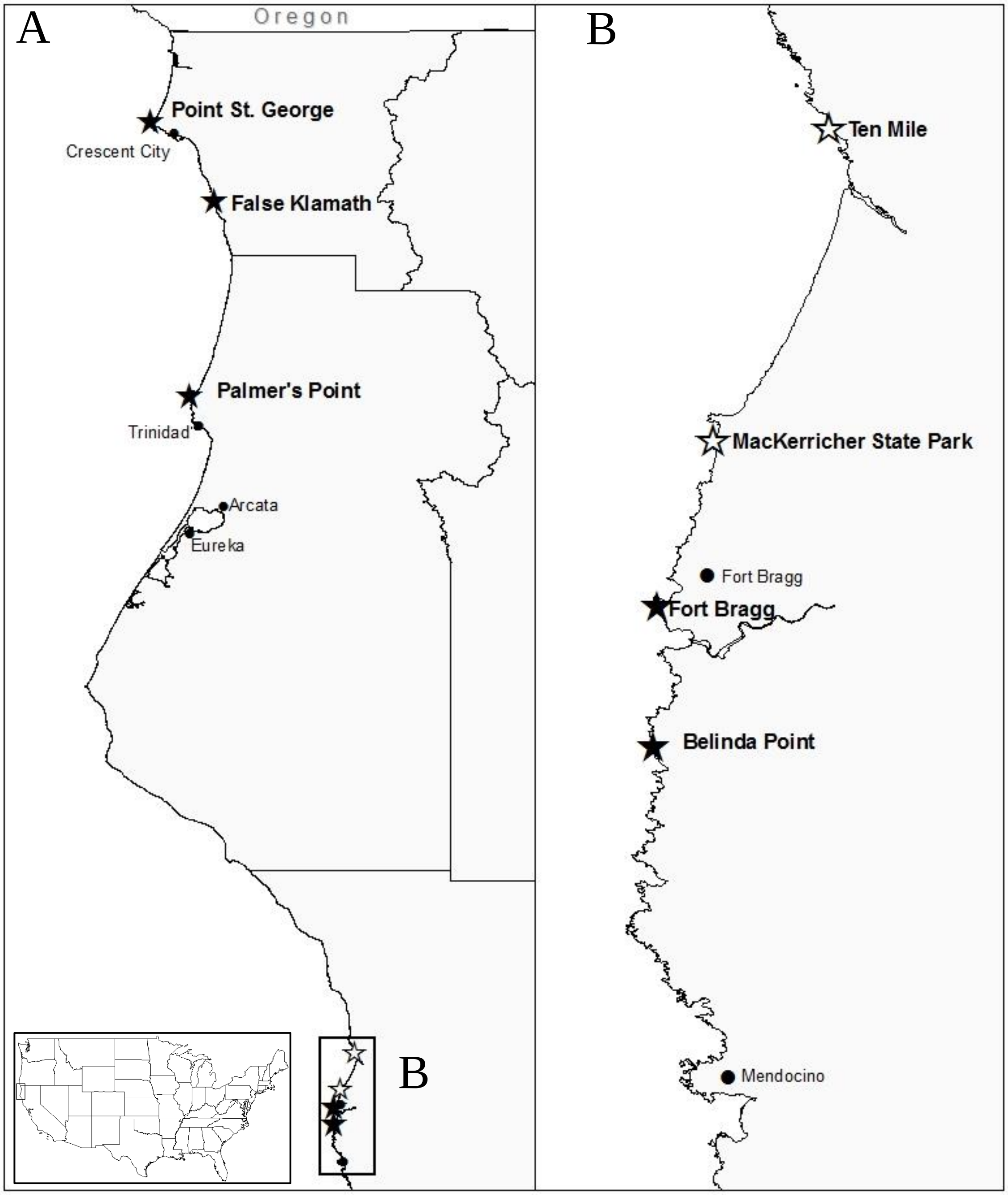

Figure 1. Distribution of rocky intertidal survey locations in Northern California (A) and close-up view of southern sites (B). Protected sites are indicated by white stars which include Ten Mile State Marine Reserve (SMR) and MacKerricher State Park SMCA.

False Klamath Cove (N 41.595, W 124.105) is located at the south end of the beach that surrounds Wilson Creek. This site is also a large boulder field, very similar to Point St. George. At low tide, the intertidal zone is approximately 150 meters long and 110 meters wide.

Palmer’s Point (N 41.131, W 124.163), the only site surveyed in Humboldt County, is located at the western-most point in Patrick’s Point State Park. This site is a wide boulder field that does not extend very far out into the ocean (less than 100 meters). Large rocky outcrops protect the intertidal from waves at low tide, but this site still receives direct wave action during low tide. Palmer’s Point is approximately 40 meters long and 200 meters wide.

Ten Mile State SMR (N 39.568, W 123.772) is one of two MPAs sampled in this study. This site is located about 9 miles north of Fort Bragg, California and is the northern-most site in Mendocino County. Ten Mile SMR is a mix between a boulder field and a bench, which gives it a mixture of characteristics from both. The bench provides protection from most wave action during low tides. A smaller site, Ten Mile SMR is only about 55 meters long and 60 meters wide.

MacKerricher State Park SMCA (N 39.483, W 123.804) is the other MPA sampled in this study and is located just south of Laguna Point along the bluffs of MacKerricher State Park. This site is a true bench habitat that receives almost no impact from waves at low tide, but is completely exposed during high tide. The sampling region is approximately 70 meters long and 35 meters wide.

Fort Bragg Cove (N 39.437, W 123.819) is located just south of Soldier Point in the Noyo Headlands area of Fort Bragg, California. Like Ten Mile SMR, this site is a combination of boulders and a bench habitat. It is in a cove, however, which provides some protection from waves even during high tide. Fort Bragg Cove is approximately 60 meters long and 55 meters wide.

Belinda Point (N 39.398, W 123.820) is the southern-most site sampled in this study and is located approximately 3 miles south of the city of Fort Bragg. This site is a bench located in a protected cove, which blocks some of the waves. The bench that was sampled is approximately 125 meters long and 20 meters wide.

MATERIALS AND METHODS

Data Collection

Habitat Description

Distribution and abundance of tidepool fishes may be correlated with various biotic and abiotic factors (Nakamura 1976, Davis 2000), so several basic tidepool characteristics were measured or described. At each tidepool during every sampling period, tidepool water temperature, ocean temperature, air temperature, lowest tide height time, pool dimensions and volume were measured.

The intertidal zone can be delineated by assessing the assemblage of macrophytes and invertebrates present (Menge 2000), but this method is not always useful when dealing with submerged pools. Intertidal zones can be categorized based on the measured height of the pool relative to mean lower low water (MLLW). This can be determined using measuring devices and comparing to the shoreline (Yoshiyama 1981), or it can be estimated based on when the pool becomes fully isolated as the tide recedes, which was sufficient for this study. Pools that are isolated one and a half hours or more before the lowest point of the summer and winter spring tide series were considered "high intertidal" pools, those that became isolated between half an hour and 1.5 hours before the low were considered "mid intertidal" pools, and those that were only isolated in the last half hour before the low were considered "low intertidal" pools.

Sampling Methods

Sites were visited twice per year, once during the summer months (April through August) and once during the winter (November through February). Palmer's Point was sampled monthly during the summer of 2015 and False Klamath was sampled twice during that summer in an effort to locate juvenile rockfish recruits. Tidepools were only sampled on extreme low spring tides when the predicted low tide level was -0.5 feet below MLLW or lower to allow low intertidal pools to become fully isolated. These very low tide cycles occur in the early morning during the summer (typically 0300 to 0900) and at night during the winter (1600-1900). Consequently, much of the sampling occurred at crepuscular periods or in the dark.

At each site a total of three pools distributed across the three intertidal zones (low, mid, and high) were selected and georeferenced. Selected pools were also permanently marked using bolts that were drilled into very large, immovable rocks near the pools and secured into place using Z-SPAR two-part marine epoxy. Bolts were notched to indicate the pool number, and fluorescent zip-ties were attached to the bolts to make locating and identification in future surveys easier. Pools were selected to be of similar surface area, but had to be small enough to be drained and sampled before the tide refilled them and large enough to be permanent pools during surveying (e.g. the water would never evaporate or naturally drain out of them completely). All three pools at a site were sampled on the same low tide to eliminate the possibility of recapturing certain individuals or of fish moving between pools during high tide. Collection and handling of specimens followed an approved protocol from the Institutional Animal Care and Use Committee (#15/16.F.30-A).

A census of the fish assemblage in each pool was attempted by a team of two to three individuals that sampled each empty pool until no more fish were spotted following five minutes of searching. Each pool typically required thirty to sixty minutes of searching, depending on the size and weather conditions. As fish were collected, they were stored in buckets full of seawater, with smaller individuals being separated from larger ones to avoid predation and overcrowding. Once surveying of a pool was finished, the pool was refilled and fish were identified to species, enumerated, and measured (total length in mm). A small subset of fish was vouchered for species verification and to provide tissue for DNA barcoding while the remaining fish were returned to the pool they were found in. Any specimens that were retained were euthanized in a solution of sea water and tricaine methanesulfonate (MS-222), then either preserved in 95% ethanol as a voucher specimen or fixed in a 10% formalin solution and stored in 50% isopropanol for identification. Voucher specimens were deposited into the Humboldt State University Fish Collection, Arcata, California (HSU; Leviton et al. 1985).

Non-destructive methods recommended by Almada and Faria (2004) were used to sample pools as opposed to traditional destructive approaches that killed all fish in the pools using ichthyocides. Once a tidepool was fully isolated it was completely emptied using buckets and a gas-powered water pump. The pools were then thoroughly searched for fish, including regions under boulders, within crevices and among algae and surfgrass. Boulders and rocks that could be moved were picked up to search under, and large gravel was dug through as some fish will bury themselves when frightened. Fish were gathered using handheld dip nets. Headlamps and dive lights were used to search crevices when it was dark. Rockfish were searched for by walking around the mid and low intertidal zone at extreme low tide and searching for rockfish in larger, isolated pools. Since juvenile black rockfish are mostly pelagic, they are easily spotted without having to move boulders or drain pools. If a rockfish was spotted, it was captured with handheld dip-nets. Rockfish found in large channels that remained connected to the ocean were not captured as they were considered subtidal recruits. Pool volume was measured by counting the number of 5 gallon buckets filled with water as the pool was being drained. The remaining amount of water left was estimated to the nearest gallon (typically less than 2 gallons) and added to the total.

Barcoding Procedure

Retained voucher specimens were sequenced at the cytochrome *c* oxidase 1 (COI) gene, the standardized region used for barcoding organisms from the Barcode of Life initiative (Hebert et al. 2003). DNA was extracted using the Chelex method described by Walsh et al. (1991) and all wells in which DNA was being extracted had a volume of 190 µL. A small tissue sample, about the size of a pin head, was taken from the upper caudal fin of voucher specimens and placed into the wells. After centrifuging again, the samples were left to incubate at room temperature overnight (about 12-14 hours) and then boiled at 100 °C for 8 minutes to stop the Proteinase K activity.

For the polymerase chain reaction (PCR), methods followed Ivanova et al. (2007). All PCRs had a total volume of 25.0 µL and included: 10.25 µL ultra pure PCR water, 0.25 µL primer mix, 12.5 µL Master Mix, and 2.0 µL DNA template. The primer mix consisted of 0.025 µL of each primer (4 primers total) and 0.15 µL PCR water. The thermocycler profile for COI consisted of 94 °C for 2 minutes, 35 cycles of 94 °C for 30 seconds, 52 °C for 40 seconds, and 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes. This procedure was replicated to result in two identical 96 well plates, one to be sequenced with the forward primer (M13F) and one for the reverse (M13R). All primer sequences can be found in Table 1.

A primer plate of 96 wells was prepared to be used for sequencing. For this plate, only the forward and reverse M13 primers were used as the sequencing primers. 40 µL of stock primer was added into 360 µL PCR water to create a working primer mix (10 µM). This working primer mix was diluted to 3.2 µM by mixing 320 µL of working primer with 680 µL PCR water. 10 µL of this diluted mix was pipetted into each of the 96 wells on the plate. Two plates were filled with this technique, one containing the forward (M13F) and one containing the reverse sequencing primer (M13R). Primer and PCR product plates were shipped to MCLAB (www.mclab.com, San Francisco, California) for Sanger sequencing using Big Dye chemistry and an Applied Biosystems 3730XL sequencer.

Table 1. Primers used to amplify cytochrome *c* oxidase 1 gene (Ivanova et al. 2007). The M13 tails are highlighted on each primer.

Name	Direction	Primer sequence 5’-3’
VF2_t1	Forward	TGTAAAACGACGGCCAGTCAACCAACCACAAAGACATTGGCAC
FishF2_t1	Forward	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC
FR1d_t1	Reverse	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA
FishR2_t1	Reverse	CAGGAAACAGCTATGACACCTCAGGGTGTCCGAARAAYCARAA
M13F	Forward	TGTAAAACGACGGCCAGT
M13R	Reverse	CAGGAAACAGCTATGAC

Data Analysis

The heterogeneous nature of the intertidal zone can lead to variation of many characteristics among tidepools including pool volume, exposure, tidal height, and exogenous inputs. All of these factors can impact the number of fishes found in the pools. To determine if there was an effect of measurable parameters on the total abundance of fish and number of species (richness) linear regressions were used. Abundance and richness were modelled against temperature, pool surface area, pool volume, and intertidal type (i.e. bench, boulder field, combination). All calculations, unless otherwise specified, were done using program RStudio, version 0.99.491 (RStudio 2012). Pool surface area was roughly estimated by assuming pools to have an ellipsoid shape and using the equation

$$SA = \pi * \frac{L}{2} * \frac{W}{2} \qquad \text{(Equation 1)}$$

where L is the longest straight line that can be drawn from end to end of the pool and W is the widest region perpendicular to L.

Species richness and Simpson’s Index of Diversity, which accounts for richness and relative species abundance, was compared across all sites and intertidal zones with regards to site protection status (protected or unprotected). Seasonal richness and abundance was also examined to determine any effect of temporary occupancy by juveniles of larger, subtidal species. Richness was determined by counting the number of different species caught at each site. Simpson’s Index of Diversity (SID_A) was calculated by using the following equation:

$$SID_A=1-\frac{\sum n(n-1)}{N(N-1)} \qquad \text{(Equation 2)}$$

where n is the number of fish of each species and N is the total number of fish caught. To determine if diversity varied between locations, a 95% confidence interval ($CI_{95\%}$) was calculated for each site using the equation

$$CI_{95\%}=SID_A\pm 2\times\sqrt{var(SID_A)} \qquad \text{(Equation 3)}$$

Variance of the diversity ($var(SID_A)$) was calculated with the equation

$$\mathrm{var}_{SID_A}=\frac{4N(N-1)(N-2)\sum_{i=1}^{S}p_i^3+2N(N-1)\sum_{i=1}^{S}p_i^2-2N(N-1)(2N-3)\left(\sum_{i=1}^{S}p_i^2\right)^2}{[N(N-1)]^2} \qquad \text{(Equation 4)}$$

where N is the total sample size and p_i is the frequency n_i/N (n_i is the *n-th* type). Catch per unit effort (CPUE) was used to determine relative abundance and was calculated for sites as the number of fish caught per pool per sampling effort.

Sample-based rarefaction analyses were conducted on all sites to estimate the number of fish species inhabiting the rocky intertidal in northern California using the program EstimateS, version 9.1.0 (Colwell 2013). Two sets of analyses were done: one on all sites combined and the other as site-specific analyses. Input files were set up as sample-based presence/absence matrices where a 0 indicated the species was absent and a 1 indicated the species was present. For the all sites combined analysis, one input file was created using 28 occasions (4 surveys at each of the seven sites). For the individual sites analyses, 7 input files were created that had 4 sampling occasions. Individual tidepools at each site were combined into one presence/absent data point. When computing rarefaction, 100 randomized runs per test were done. Samples were extrapolated to 84 sampling occasions (all sites combined) or 20 occasions (individual sites) and if an asymptote was reached, that value was assumed to be the maximum number of species.

Detection probabilities were determined for the most common species by comparing the frequency of catch with the total number of sampling events. Detection probability was calculated as the proportion of times that a species was detected in the 82 total pools surveyed for this study (3 pools at 7 sites across 4 survey periods, minus 2 pools that could not be sampled). In addition to being grouped by site, these were also split based on geographic region, habitat type, and season, to determine whether there was a temporal or geographical effect on the probability of finding a species in the intertidal. Only species with an overall detection probability of higher than 12% (individuals were caught on at least 10 separate occasions) were reported.

Phylogenetic trees were constructed using maximum likelihood methods implemented in program MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets (Kumar et al. 2015). Branch support was evaluated using 500 bootstrap replicates. A tree was constructed for family Cottidae, due to its species dominance; an analysis of the remaining species was conducted separately. This allowed for finer resolution of differences in relatedness between Cottids. Additional sequences of each barcoded species were taken from the fish Barcode of Life website to use in the phylogenetic trees (Appendix A). Sequences were selected to span as large a geographical range as possible, with the ideal scenario having one sequence from each state and province on the west coast of North America (ranging from California to Alaska). This enables a range-wide examination of homogeneity in the COI gene within species. Character polarity was provided for the Cottidae family tree by rooting it with an outgroup consisting of one individual from several sister families to Cottidae: Agonidae (*Bathyagonus nigripinnis*), Hemitripteridae (*Blepsias cirrhosus*), Psychrolutidae (*Psychrolutes paradoxus*), and Rhamphocottidae (*Rhamphocottus rhichardsonii*). Mean uncorrected p-distance was calculated for all possible inter- and intraspecies combinations within MEGA7 using all sequences represented in the phylogenetic trees. This allows for the evaluation of the effectiveness of COI for species identification. Heatmaps were constructed to visualize the genetic distances using the function myImagePlot (source: http://www.phaget4.org/R/myImagePlot.R).

RESULTS

Habitat Characteristics

Pool temperatures averaged 13.5 °C and fluctuated between 10 °C and 19.2 °C. Pool temperature tended to be warmer during the winter (averaged 14.37 °C) from being exposed to the warm daytime temperatures and heated by the sun since the extreme low tides are in the evening. Since summer low tides were in the early morning, before sunrise, the pools are cooler (averaged 12.74 °C). Pools averaged 2.78 m^2 (0.97 to 6.6 m^2) in surface area and had an average volume of 434.16 L (54.42 to 1715.3 L). Pools in boulder fields were, on average, larger in surface area but much smaller in volume than those in benches (table 2). This is due to the nature of how the pools are formed; in boulder fields, they fill large, shallow spaces in between rocks and divots in sand, whereas on benches they fill deep cracks and crevices in the solid bedrock.

Linear regressions showed that temperature had no significant impact on the number of fish found in the pools (p=0.25). There was a significant difference between bench and boulder fields in the number of fish per pool (p<0.01) where boulder fields had more fish (17.5 $\text{fish} \bullet \text{pool}^{-1}$) than benches (7.0 $\text{fish} \bullet \text{pool}^{-1}$), but no difference between bench and combination intertidal types (p=0.26). There was also no significant effect of surface area (p=0.71) or volume (p=0.79) on the abundance of fish in tidepools. Volume and surface area did not show any effect on richness (p=0.09 and 0.28, respectively).

Table 2. Site and pool characteristics for each location surveyed.

Location	Site type	Average pool surface area (m^2)	Average pool volume (L)	Latitude	Longitude
Point St. George	Boulder field	2.7	105.2	41.784	-124.255
False Klamath	Boulder field	4.0	208.6	41.595	-124.105
Palmers Point	Boulder field	3.7	449.8	41.131	-124.163
Ten Mile	Combination	2.1	380.9	39.568	-123.772
MacKerricher State Park	Bench	1.5	232.2	39.483	-123.804
Fort Bragg Cove	Combination	2.9	669.4	39.437	-123.819
Belinda Point	Bench	2.6	853.3	39.398	-123.820

Fish diversity and abundance

A total of 1756 fish were caught in the 84 surveyed pools (3 pools at each of 7 sites across 4 survey periods). Collections included 34 species representing eight families, based on taxonomy from the Catalog of Fishes (Eschmeyer et al 2016): Clinidae (kelpfishes), Cottidae (sculpins), Gobiesocidae (clingfishes), Hexagrammidae (greenlings), Liparidae (snailfishes), Pholidae (gunnels), Scorpaenidae (rockfishes), and Stichaeidae (pricklebacks) (Table 3). Cottidae was the most common and had the highest abundance at 1270 individuals (72% of total catch) from 18 different species. The most common species were the fluffy sculpin *Oligocottus snyderi* with 546 individuals and the tidepool sculpin *Oligocottus maculosus* with 390 individuals. All families included multiple species with the exceptions of Gobiesocidae and Liparidae, where the northern clingfish *Gobiesox maeandricus* and the tidepool snailfish *Liparis florae* were the sole representatives, respectively.

Species richness across sites ranged from 13 to 22 species (Fig. 2). At all sites, Cottidae made up most of the catch with Stichaeidae and Gobiesocidae also consistently making up a large proportion of the catches (Fig. 3). Clinidae was caught in fairly high abundance at MacKerricher State Park and Belinda Point, the two bench sites, but was either very rare or nonexistent at other sites. Kelpfishes were only caught at the three southernmost sites. Species richness at protected sites was lower than at the other two southern sites, but was higher than at the two northernmost sites (Fig. 2). The mid

Table 3. Total numbers of each species caught during the entire study period at each site: Point St. George (PSG), False Klamath Cove (FKC), Palmers Point (PP), 10 Mile (10M), MacKerricher State Park (MSP), Fort Bragg (FB), and Belinda Point (BP). The dashed line indicates the division between northern sites (left) and southern sites (right).

Species	**PSG**	**FKC**	**PP**	**10M**	**MSP**	**FB**	**BP**
Anoplarchus purpurescens	4	19	2	10	12	20	3
Apodichthys flavidus	13	8	6	3	1	8	3
Apodichthys fucorum	1		1	1	10	2	3
Artedius corallinus		4	1	4	1		
Artedius fenestralis		1					
Artedius harringtoni			1				
Artedius lateralis		7	6	10	6	56	13
Ascelichthys rhodorus	1	2	7	1			1
Cebidichthys violaceus	4	6	26	1	3	5	2
Clinocottus acuticeps	12						
Clinocottus analis				2	1	8	
Clinocottus embryum	1						
Clinocottus globiceps	59	6	41	11		18	5
Clinocottus recalvus	7		2			2	1
Enophrys bison	6		1	4			
Gibbonsia metzi					2		2
Gibbonsia montereyensis					10	2	25
Gobiesox maeandricus	28	48	5	8		25	22
Hemilepidotus hemilepidotus			3				
Hemilepidotus spinosus			5				
Hexagrammos decagrammus				1			1
Hexagrammos lagocephalus						1	
Liparis florae						6	3
Oligocottus maculosus	8	84	211	4	6	76	1
Oligocottus rimensis							5
Oligocottus rubellio					1	1	11
Oligocottus snyderi	129	85	124	41	50	79	38
Phytichthys chirus							1
Scorpaenichthys marmoratus		6	1			1	2
Sebastes carnatus							1
Sebastes melanops				3			
Sebastes miniatus						1	
Xiphister atropurpureus		3	10		1	5	24
Xiphister mucosus		4	8	2	3	10	2
Total	**273**	**283**	**461**	**106**	**107**	**326**	**169**

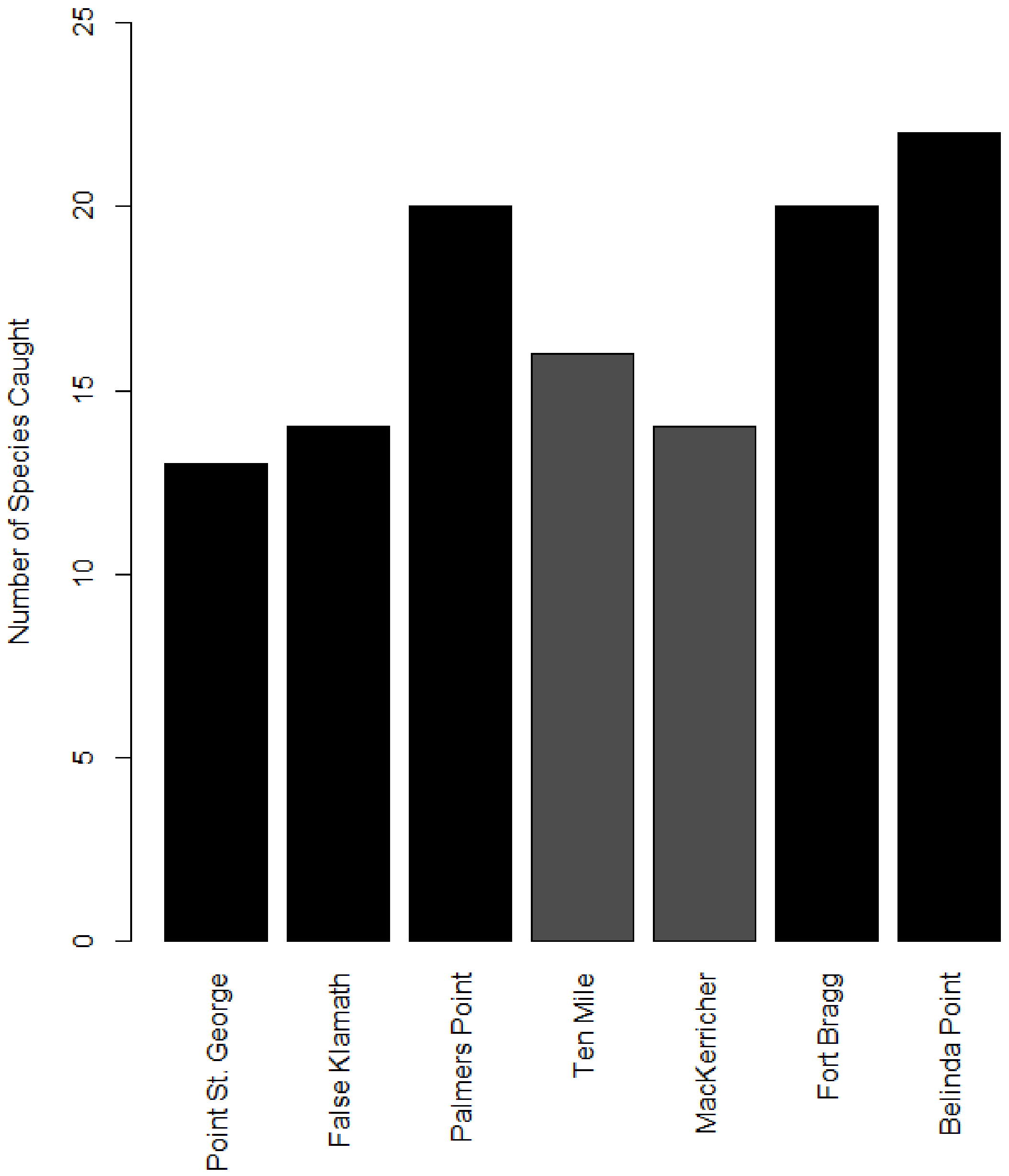

Figure 2. Species richness for each site represented as the total number of species caught throughout all four sampling seasons. Lighter bars indicate sites that are MPAs.

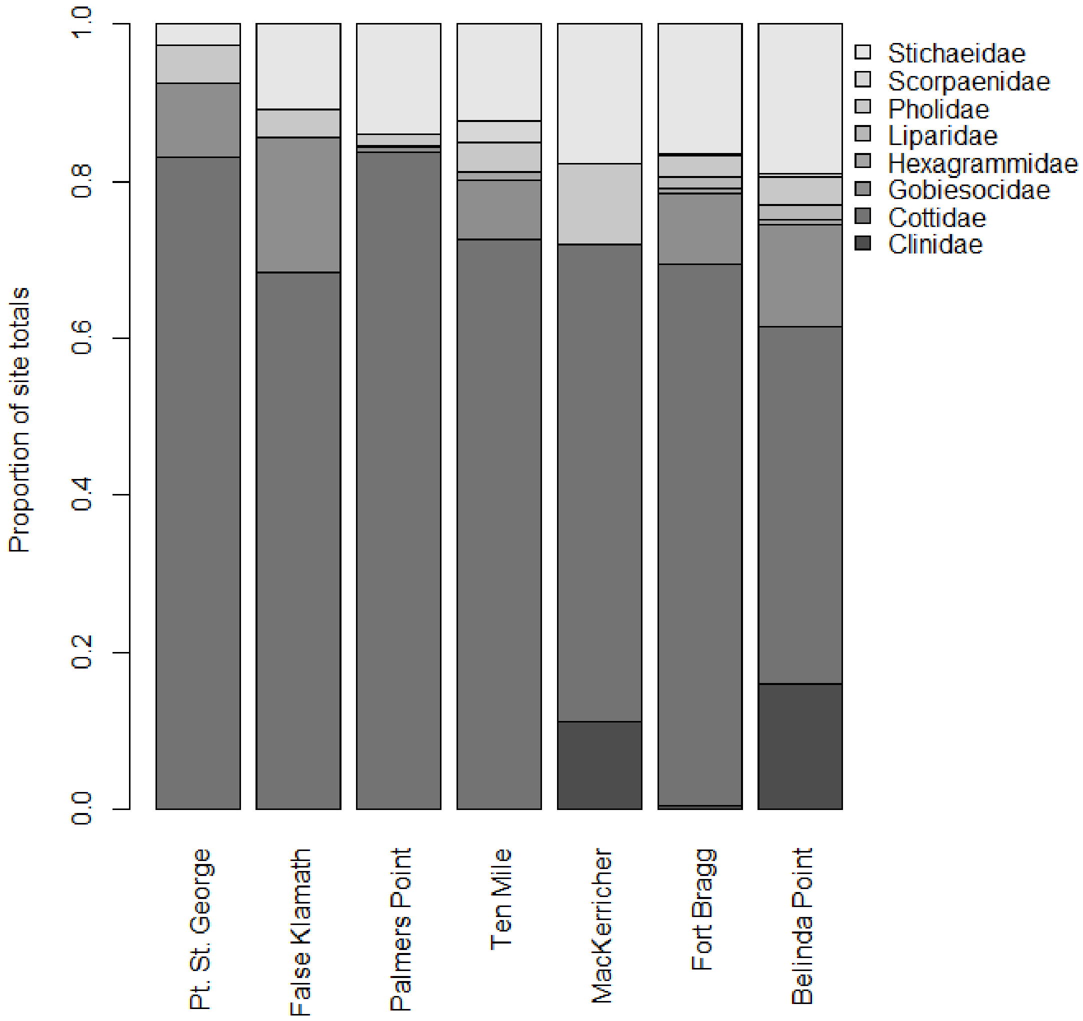

Figure 3. Proportion of fishes caught by family at each site. MacKerricher and Ten Mile are the two protected sites.

and high intertidal zones had greater abundance than the low intertidal zone. Richness was similar in the low and high intertidal zones but greater in the mid zone (Fig. 4).

Diversity, as measured by Simpson's Index of Diversity, did not differ very much across the sites (Fig. 5). There was generally no difference in diversity between sites, except for the northern three sites during the winter where False Klamath was more diverse than Point St. George and Palmers Point. MacKerricher State Park was less diverse than Fort Bragg and Belinda Point during the summer, but did not differ during the winter. False Klamath was the only site that showed a difference in diversity between the seasons, with winter having a higher diversity than summer. The three boulder field sites generally had lower diversities than the other habitat types, but these differences have overlapping confidence intervals. Diversity was lower in the high intertidal zone during both the summer and winter (Fig. 6). Diversity was very similar between seasons for all three intertidal zones.

Abundance varied greatly by site with the most fish being caught at Palmers Point and the fewest caught at Ten Mile (Table 3). Since there was uneven sampling effort at some of the sites due to ocean conditions occasionally making it impossible to drain pools, catch per unit effort was calculated as the average number of fish caught per pool among three pools surveyed during each sampling effort (Fig. 7). Palmers Point had the highest number of fish caught and the highest catch per unit effort, although Point St. George and Fort Bragg had similar numbers of fish per pool.

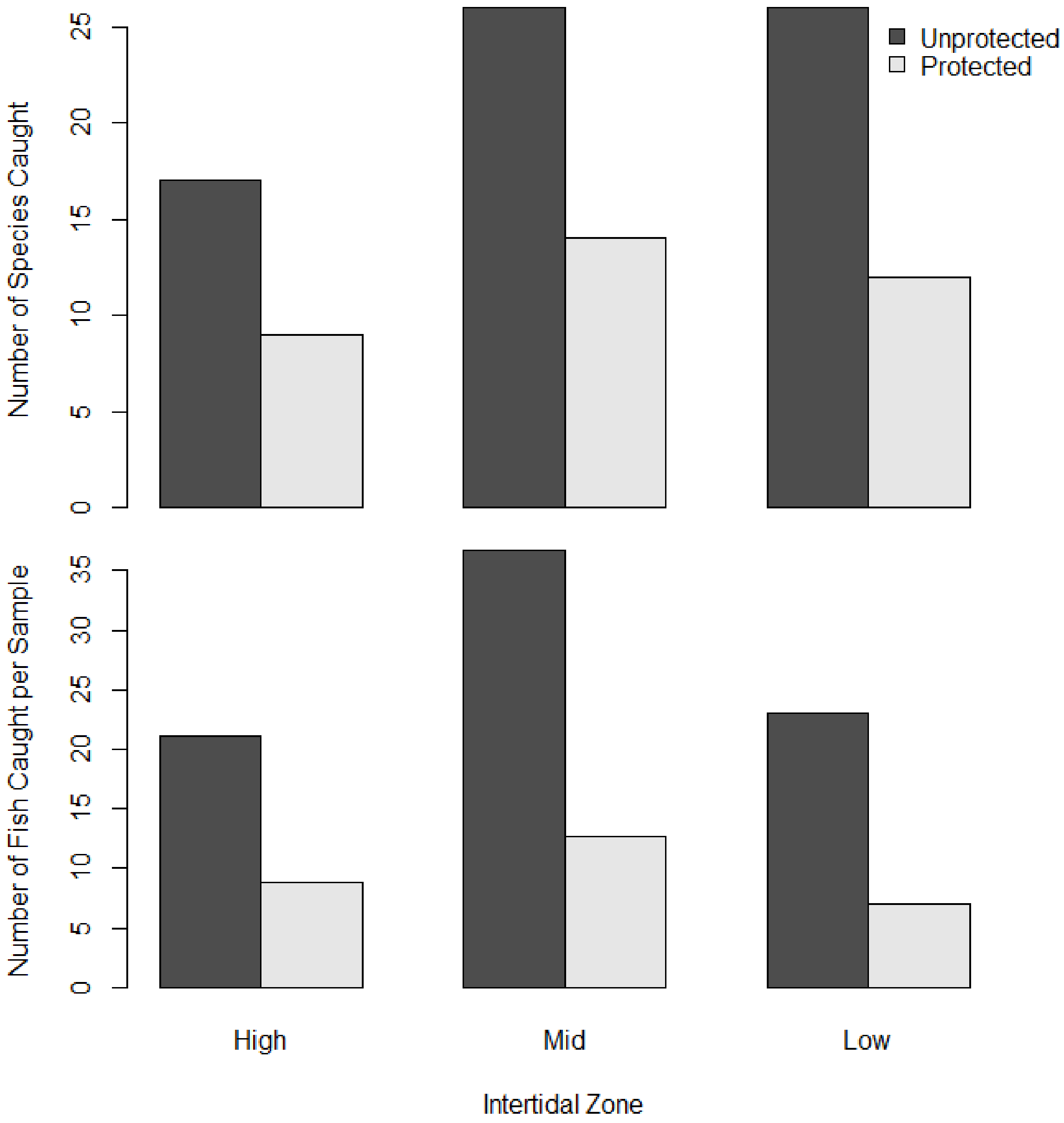

Figure 4. Species richness (top) and CPUE (bottom) based on intertidal zones. Darker bars indicate unprotected sites while lighter bars represent sites located within MPAs. CPUE is measured as the number of fish caught per pool in each zone.

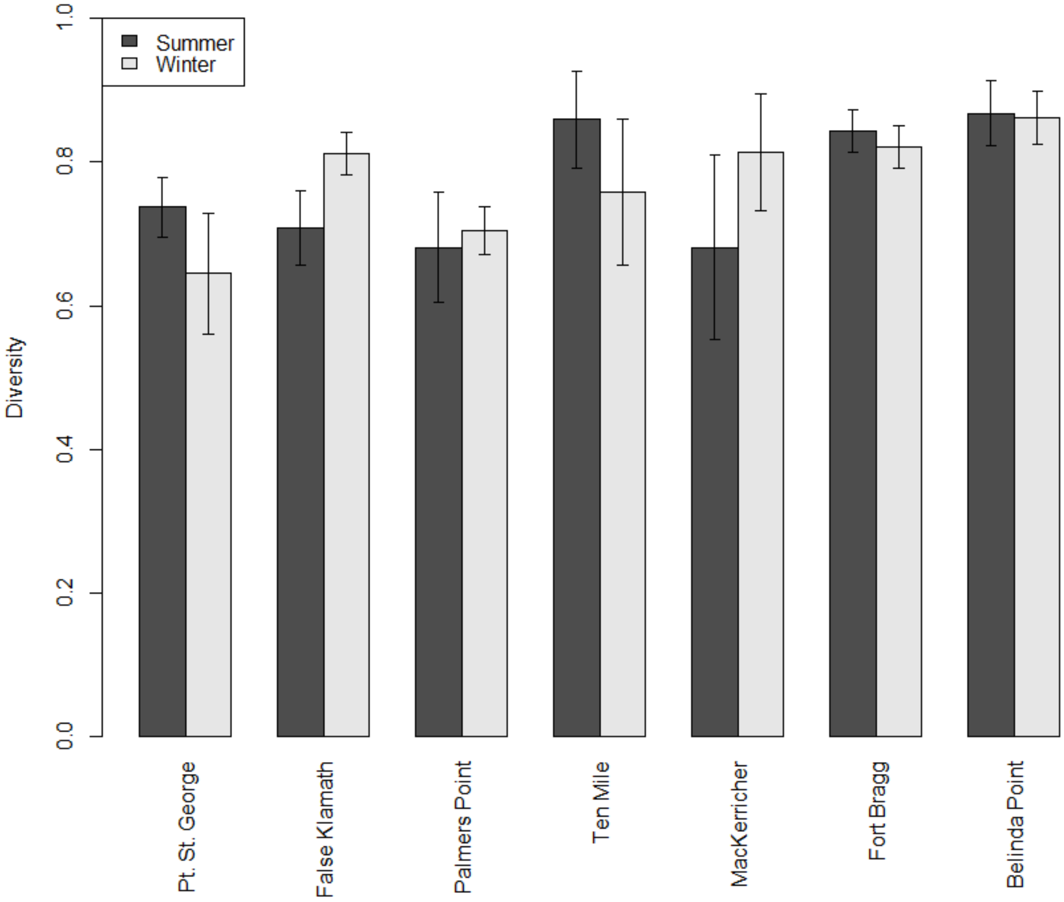

Figure 5. Simpson's Index of Diversity for each season at each site with error bars for 95% confidence intervals.

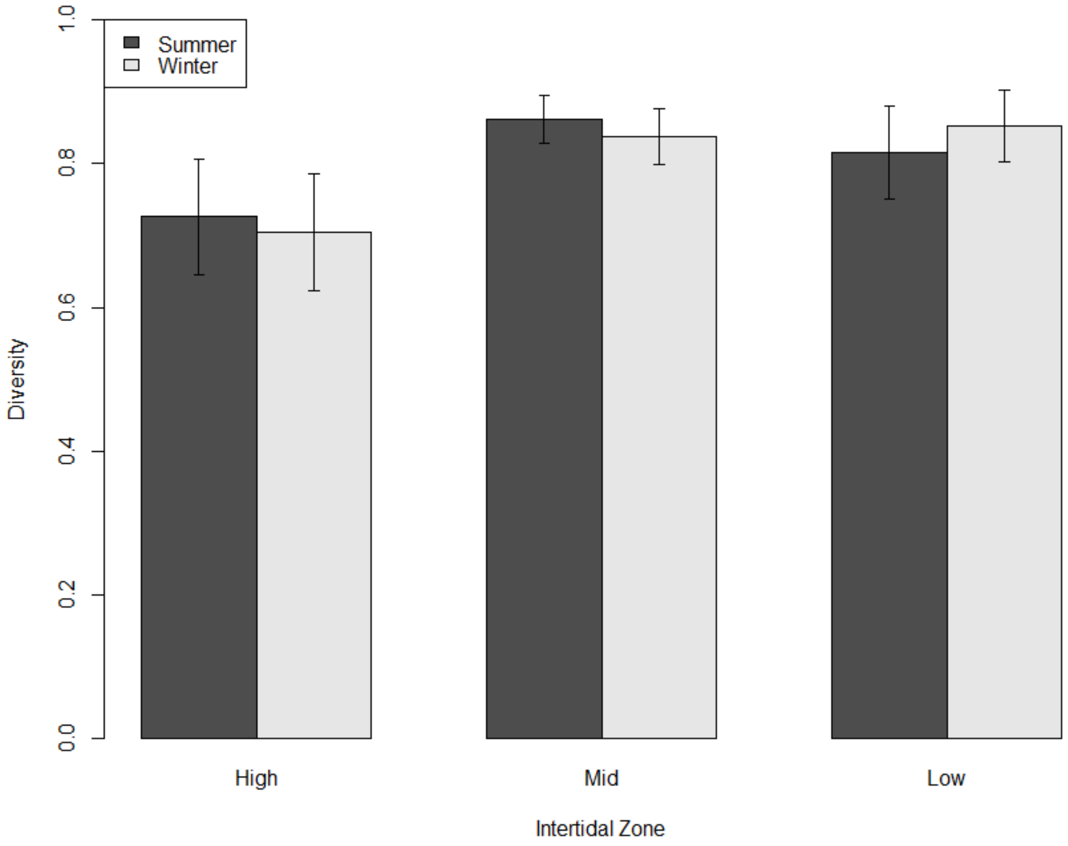

Figure 6. Simpson’s Index of Diversity for each intertidal zone separated by season. Error bars are 95% confidence intervals.

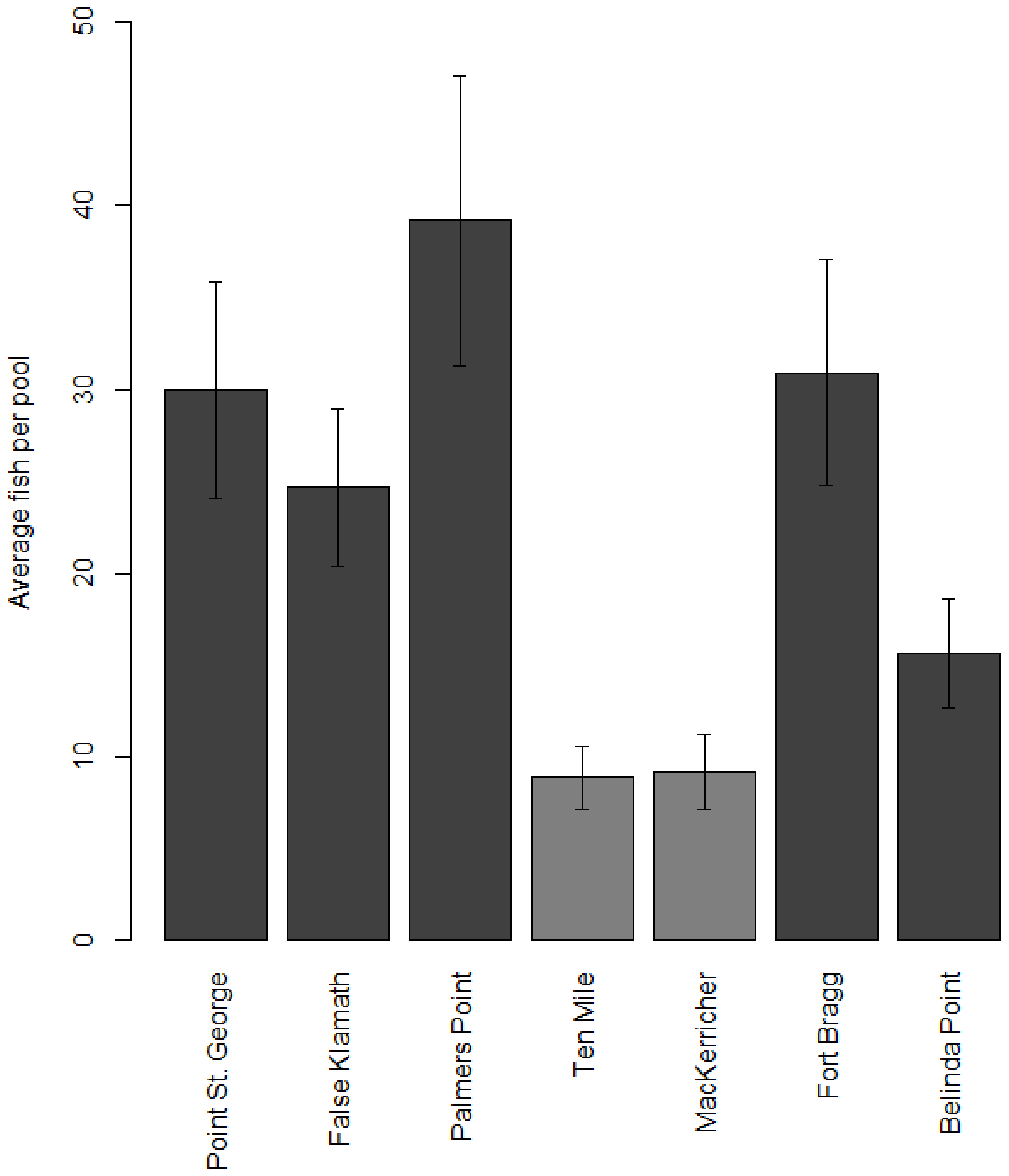

Figure 7. Catch per unit effort measured as the number of fish caught in each pool with standard error for all sites sampled. Light gray indicates sites that are within MPAs.

False Klamath and Point St. George had similar abundances and CPUEs. Apart from Fort Bragg, northern sites had higher abundances and catches per unit effort than southern sites. The two sites located within MPAs had the lowest abundances and the fewest fish per pool, and were both substantially lower than the site with the next fewest fish (Belinda Point).

Rockfish surveys included twenty-eight sampling periods (seven sites sampled across four seasons) and six additional surveys spent solely looking for rockfish at False Klamath (one extra survey) and Palmers Point (five extra surveys). Only five rockfish were caught in southern sites, and none were observed or captured in tidepools in northern sites. Of those five individuals, three were black rockfish *Sebastes melanops* and one was a vermilion rockfish, *Sebastes miniatus*, and one was likely a gopher rockfish *Sebastes carnatus* although due to its very small size it could have been a black-and-yellow rockfish *Sebastes chrysomelas*. At Palmers Point, one juvenile black rockfish was caught in a large channel that was connected to the ocean. At Fort Bragg Cove, four juvenile blue rockfish *Sebastes mystinus* were caught in a very large pool that was connected to the ocean, and small schools of black rockfish and pelagic gopher rockfishes were observed in the shallow subtidal and could be sampled with handheld dipnets. Since these were not isolated in pools, they were not included in the data collection.

Size (total length) frequency distributions were generated for the two most abundant fish species, *Oligocottus snyderi* and *O. maculosus*, collected at all sites during all summer and winter sampling events (Fig. 8). Size ranges were very similar for the two species, ranging from 13-85 mm for *O. maculosus*, and 13-86 mm for *O. snyderi*. Both species had the most individuals in the 25 to 35 mm range during the summer, but during the winter most *O. maculosus* were between 35 and 40 mm while most *O. snyderi* were between 40 and 55 mm. For both species, two peaks can be seen during the summer, centered around 25-35 mm and 50-55 mm for *O. maculosus*, and 25-35 mm and 60-65 mm for *O. snyderi*. These indicate at least two year classes, with a potential very small third age class in *O. maculosus*, centered around 80 mm. Two peaks can be observed in *O. maculosus* during the winter, centered around 35 mm and 70-75 mm. Only one major peak is observed in *O. snyderi* during the winter, centered around 40-55 mm, suggesting that this species likely only survives one winter and very rarely lives through two.

Sample-based rarefaction curves were used to estimate the total number of fish species present in the Northern California intertidal zones as well as to get an idea of how much sampling effort would be required to detect all species (Fig. 9). All twenty-eight samples were combined (three pools at each of seven sites) and extrapolated out by a factor of three to estimate how many species would be encountered from one to 84 pools. There is likely an estimated 37 (31 to 43) species of fish that utilize the intertidal zones, a number that was reached after 61 pools were surveyed. Since this is only three more species than was actually caught, a great amount of effort would be required to detect additional new species. After the 34 species were caught, another six pools would be

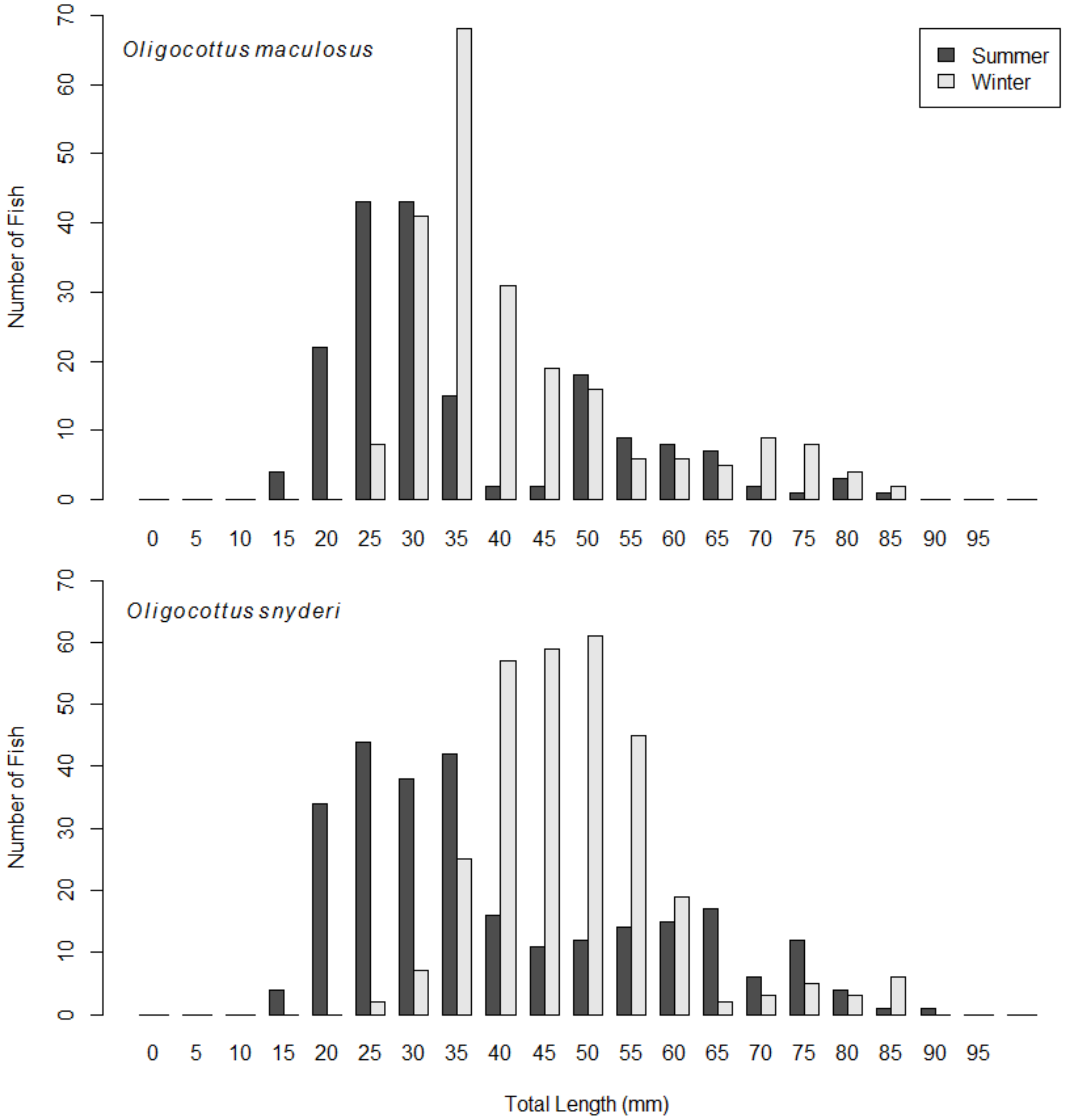

Figure 8. Size (total length) frequency distribution for *Oligocottus maculosus* (top) and *O. snyderi* (bottom) from all sites and years combined but separated by season (winter and summer).

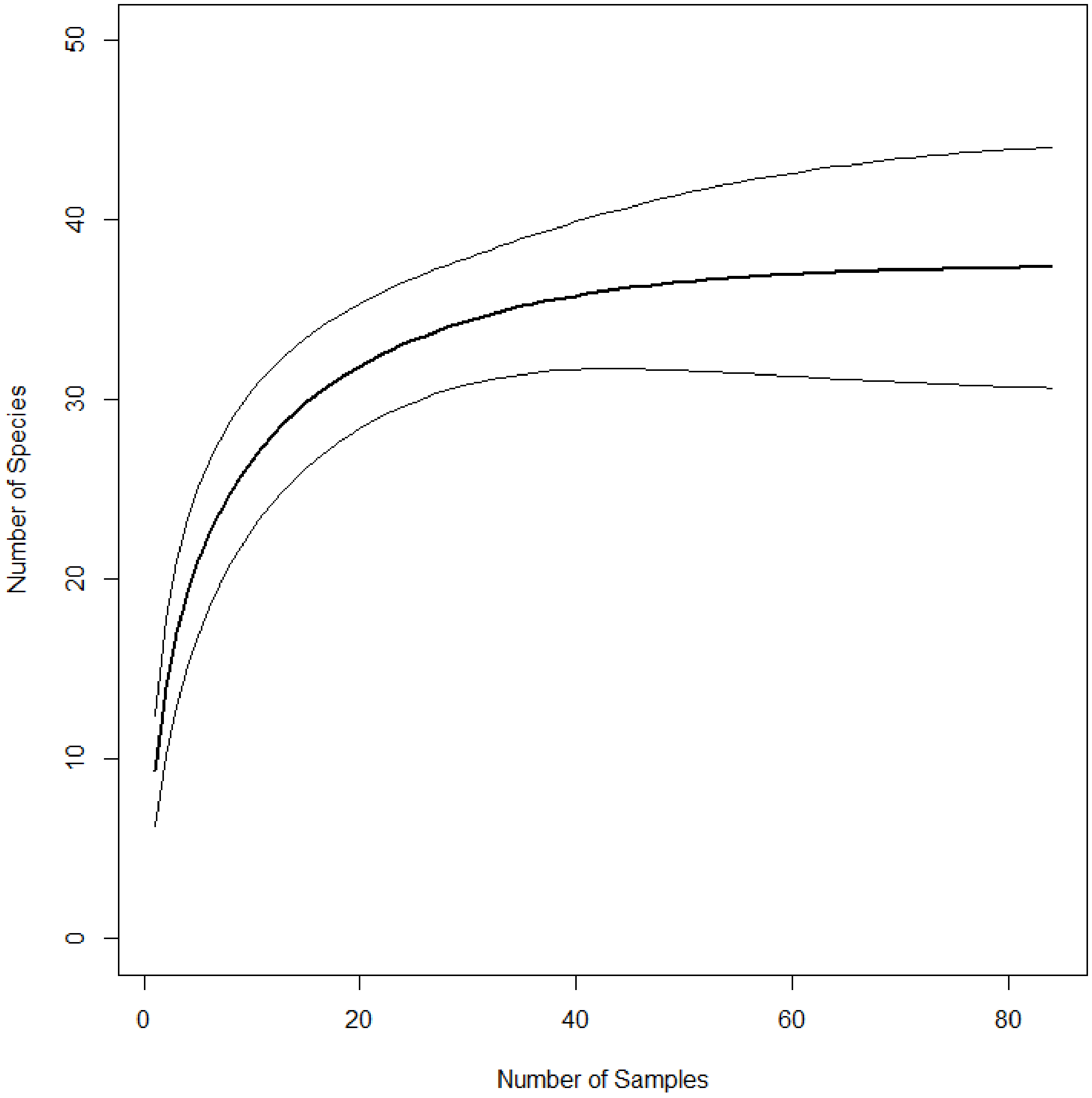

Figure 9. Rarefaction analysis of all 28 sample periods extrapolated to three times the number of samples (84). Thinner lines represent the 95% confidence interval around the estimated number of species (thicker line).

required to find one more species, fifteen additional pools to get two new species, and 34 more pools to get the third and final species.

The same analyses were used for each site individually to determine what sites likely have the most species and to determine if the sampling effort of this study approached a full census of all species at any of the sites (Fig. 10). Sites were extrapolated to 20 samples to estimate how many species would be expected after further sampling (Table 4). None of the extrapolations seemed to asymptote and uncertainty became too large after further extrapolation, so more pools would be required to improve the estimation. While some sites seemed to have most of their species sampled, many require many more samples to detect all of the species present. Since these are extrapolations however, there is a fair amount of uncertainty that accompanies the estimates.

Detection probability

Pools had a very high probability of containing fish, with only two of the 82 pools yielding no fish at all. Since more abundant fish are likely to be spread over a greater distance and in more pools, it is unsurprising that fish caught in greater numbers also had high detection rates. In addition to being the most abundant species, *Oligocottus snyderi* was caught the most frequently by far, occurring in 90% of the pools. The next highest detection rate was from *Oligocottus maculosus*, which was found in 48% of the pools. Detection rates for the rest of the more common species can be found in table 5. Here it is

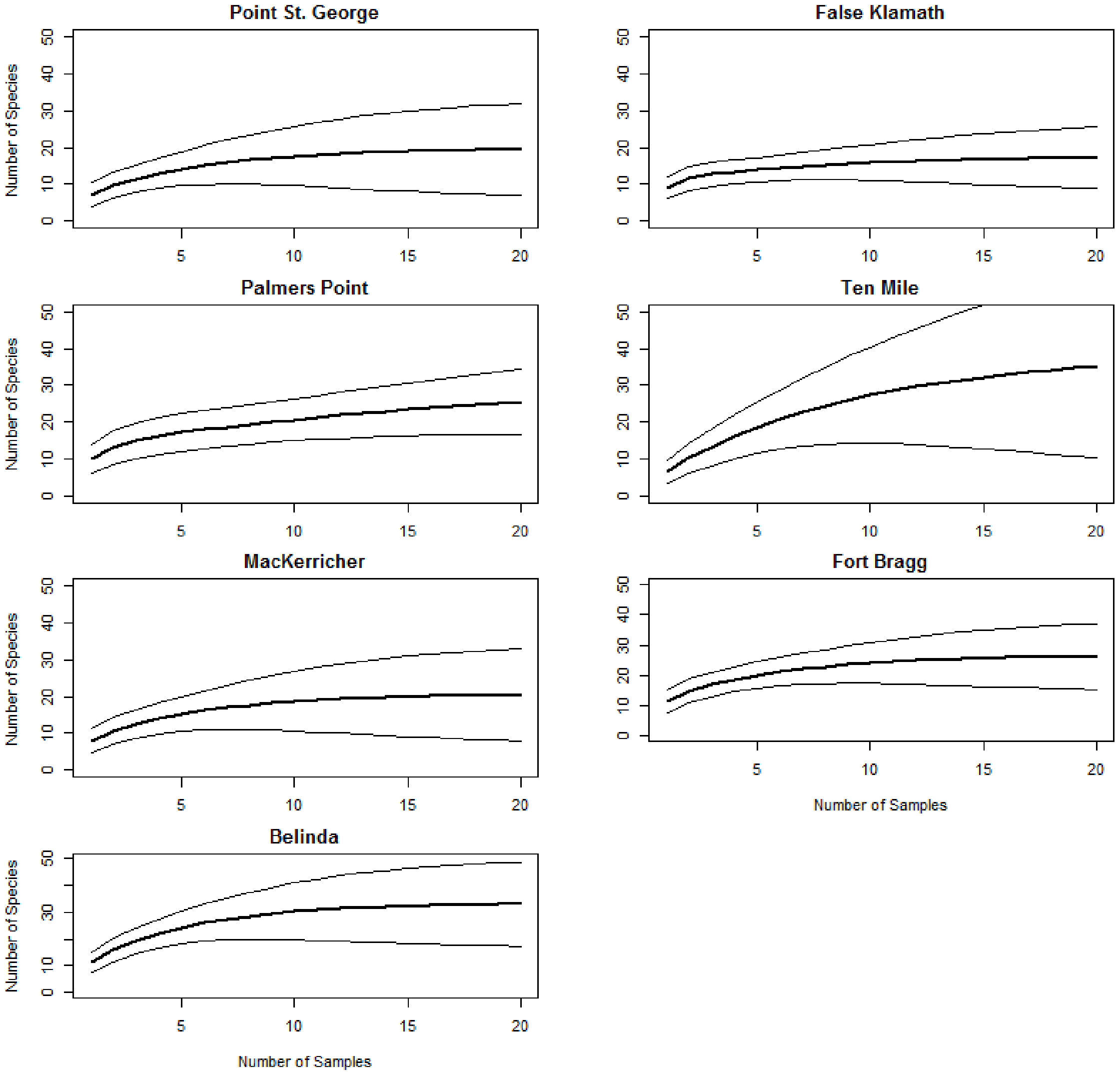

Figure 10. Rarefaction curves for all sites extrapolated out to 20 sampling events. 95% confidence intervals (thinner lines) are plotted around the estimated number of species to be encountered (thicker lines).

Table 4. Estimated number of species that would be detected after 20 sampling events at each site with corresponding 95% confidence intervals. Values were extrapolated out from the species accumulation after 4 sampling events at each site. *False Klamath had one additional sampling event (5 total) and Palmers Point had 5 additional sampling events (9 total).

Site	Estimated species	95% Confidence Interval
Point St. George	19	± 11
False Klamath*	17	± 8
Palmers Point*	26	± 8
Ten Mile	35	± 25
MacKerricher State Park	20	± 11
Fort Bragg	26	± 10
Belinda Point	33	± 16

Table 5. Detection rates for northern California rocky intertidal fishes. The total number of samples (pools drained) was 82, split evenly between the two seasons (summer, winter; 41 each). For habitat type (boulder, bench, mix), 36 samples were in boulder fields (all located in the northern three sites), and 23 were in the bench and mix types each.

Species	Summer	Winter	Boulder	Bench	Mix	Total	Number of pools detected in
Oligocottus snyderi	90.2%	90.2%	91.7%	78.3%	100.0%	90.2%	74
Oligocottus maculosus	51.2%	43.9%	69.4%	26.1%	34.8%	47.6%	39
Clinocottus globiceps	51.2%	34.1%	58.3%	8.7%	52.2%	42.7%	35
Gobiesox maeandricus	34.1%	51.2%	55.6%	30.4%	34.8%	42.7%	35
Artedius lateralis	34.1%	43.9%	22.2%	47.8%	56.5%	39.0%	32
Anoplarchus purpurescens	36.6%	34.1%	27.8%	43.5%	39.1%	35.4%	29
Apodichthys flavidus	22.0%	31.7%	41.7%	8.7%	21.7%	26.8%	22
Cebidichthys violaceus	36.6%	12.2%	33.3%	17.4%	17.4%	24.4%	20
Xiphister atropurpureus	19.5%	17.1%	13.9%	26.1%	17.4%	18.3%	15
Xiphister mucosus	19.5%	17.1%	19.4%	17.4%	17.4%	18.3%	15
Apodichthys fucorum	17.1%	9.8%	5.6%	21.7%	17.4%	13.4%	11
Gibbonsia montereyensis	7.3%	17.1%	0.0%	39.1%	4.3%	12.2%	10
Total	**100.0%**	**95.1%**	**100.0%**	**91.3%**	**100.0%**	**97.6%**	**80**

obvious that seasonal (e.g. *Sebastes*, *Hexagrammos*) and rare (e.g. *Phytichthys chirus*, *Clinocottus embryum*) had the lowest detection probabilities since they were caught on very few occasions.

In general, detection rates were lower in the winter than during the summer, and the only times no fish were caught in a pool were during the winter. There are some exceptions, *O. snyderi* for example, that had the same detection rate in the summer and winter, which is simply because it was so abundant everywhere that even in the winter they were easily found. *Gobiesox maeandricus, Artedius lateralis, Apodichthys flavidus*, and *Gibbonsia montereyensis* were found more frequently in the intertidal during the winter than during the summer.

Cottids, except *Artedius lateralis,* were found much less frequently on bench habitats than boulder fields or a mixed habitat. This could be a result of either habitat preference, or just that these fish were more abundant in the north which is where all the boulder fields were. *Gobiesox maeandricus* seemed to favor boulder fields, likely due to the abundance of small, smooth rocks for them to cling to. The two gunnels showed opposite trends, where *Apodichthys flavidus* was more common in boulder fields but *A. fucorum* was found more frequently in benches or mixed habitats. This is likely due to the latter being rare in the north and more common in the south while *A. flavidus* was more common in the north. The pricklebacks, *Xiphister atropurpureus* and *X. mucosus* did not show much habitat-type preference in terms of detection probability, but when one was caught more frequently, but there appears to be a negative association between these two species, with very few pools containing individuals of both species. *Gibbonsia montereyensis* was found almost exclusively in the bench habitats.

Phylogenetic analysis and DNA barcoding

Genetic barcodes at the cytochrome *c* oxidase 1 (COI) gene were generated for 131 individuals spanning 26 species, which accounts for 76% of the 34 species detected in this study. Of the 26 species, 15 of them were in Cottidae. Three of the species missing included the cabezon *Scorpaenichthys marmoratus*, the calico sculpin *Clinocottus embryum*, and the coralline sculpin *Artedius corallinus*. *Clinocottus embryum* was extremely rare and only one individual was captured throughout the whole study that had to be fixed in formalin for identification, so DNA could not be extracted from the individual. Barcode sequences were accessioned onto the Barcode of Life Database (BOLD) and the specimens used for sequencing have been vouchered into the Humboldt State University Fish Collection (Table 6).

The maximum-likelihood tree for the family Cottidae rocky intertidal sculpins barcoded for this study (plus sequences taken off BOLD for the same species but from different geographic regions) shows that nodes supporting monophyly for each species were always strongly supported (bootstrap values >90) but nodes across geographic samples within species were not well supported (Fig. 11). This supports the idea that between species genetic divergence exceeds within species divergence. A maximum-likelihood phylogenetic tree was also generated for the remaining species not in Cottidae

Table 6. Species, Barcode of Life Identification number (BOLD ID), voucher numbers for specimens accessioned to the Humboldt State University Fish Collection (Museum ID), collection location, and individual identification numbers (Voucher ID) for northern California rocky intertidal fishes.

Species	BOLD ID	Museum ID	Location	Voucher ID
Anoplarchus purpurescens	TPCOT019-16	HSU 5256	Point St. George, CA	Cvio-003
Anoplarchus purpurescens	TPCOT022-16	HSU 5264	MacKerricher State Park SMCA, CA	Apur-004
Apodichthys flavidus	TPCOT028-16	HSU 5332	Ten Mile SMR, CA	Afuc-001
Apodichthys flavidus	TPCOT009-16	HSU 5272	Fort Bragg, CA	Afla-003
Artedius fenestralis	TPCOT001-16	HSU 5333	False Klamath, CA	Afen-001
Artedius harringtoni	TPCOT002-16	HSU 5317	Palmers Point, CA	Ahar-001
Artedius lateralis	TPCOT007-16	HSU 5277	Palmers Point, CA	Alat-006
Ascelichthys rhodorus	TPCOT004-16	HSU 5319	Palmers Point, CA	Arho-002
Cebidichthys violaceus	TPCOT027-16	HSU 5337	False Klamath, CA	Cvio-001
Clinocottus acuticeps	TPCOT005-16	HSU 5259	Point St. George, CA	Cacu-001
Clinocottus analis	TPCOT006-16	HSU 5269	Fort Bragg, CA	Cana-004
Clinocottus globiceps	TPCOT008-16	HSU 5251	Point St. George, CA	Cglo-011
Clinocottus recalvus	TPCOT003-16	HSU 5270	Fort Bragg, CA	Cglo-007
Enophrys bison	TPCOT010-16	HSU 5320	Palmers Point, CA	Ebis-001
Gibbonsia montereyensis	TPCOT026-16	HSU 5343	Belinda Point, CA	Gmon-005
Gobiesox maeandricus	TPCOT020-16	HSU 5328	Point St. George, CA	Gmae-006
Hemilepidotus hemilepidotus	TPCOT011-16	HSU 5239	Palmers Point, CA	Hhem-002
Hemilepidotus spinosus	TPCOT012-16	HSU 5279	Palmers Point, CA	Hspin-001
Liparis florae	TPCOT016-16	HSU 5342	Fort Bragg, CA	Lflo-001
Oligocottus maculosus	TPCOT024-16	HSU 5297	Fort Bragg, CA	Omac-009
Oligocottus rimensis	TPCOT017-16	HSU 5350	Belinda Point, CA	Orim-003
Oligocottus rubellio	TPCOT018-16	HSU 5351	Belinda Point, CA	Orub-005
Oligocottus snyderi	TPCOT023-16	HSU 5255	Point St. George, CA	Osny-012
Sebastes carnatus	TPCOT016-16	HSU 5352	Belinda Point, CA	Scar-001
Sebastes melanops	TPCOT025-16	HSU 5325	Palmers Point, CA	Smel-001
Sebastes mystinus	TPCOT014-16	HSU 5267	Fort Bragg, CA	Smys-001
Xiphister atropurpureus	TPCOT015-16	HSU 5344	Belinda Point, CA	Xatr-002
Xiphister mucosus	TPCOT021-16	HSU 5246	Palmers Point, CA	Xmuc-002

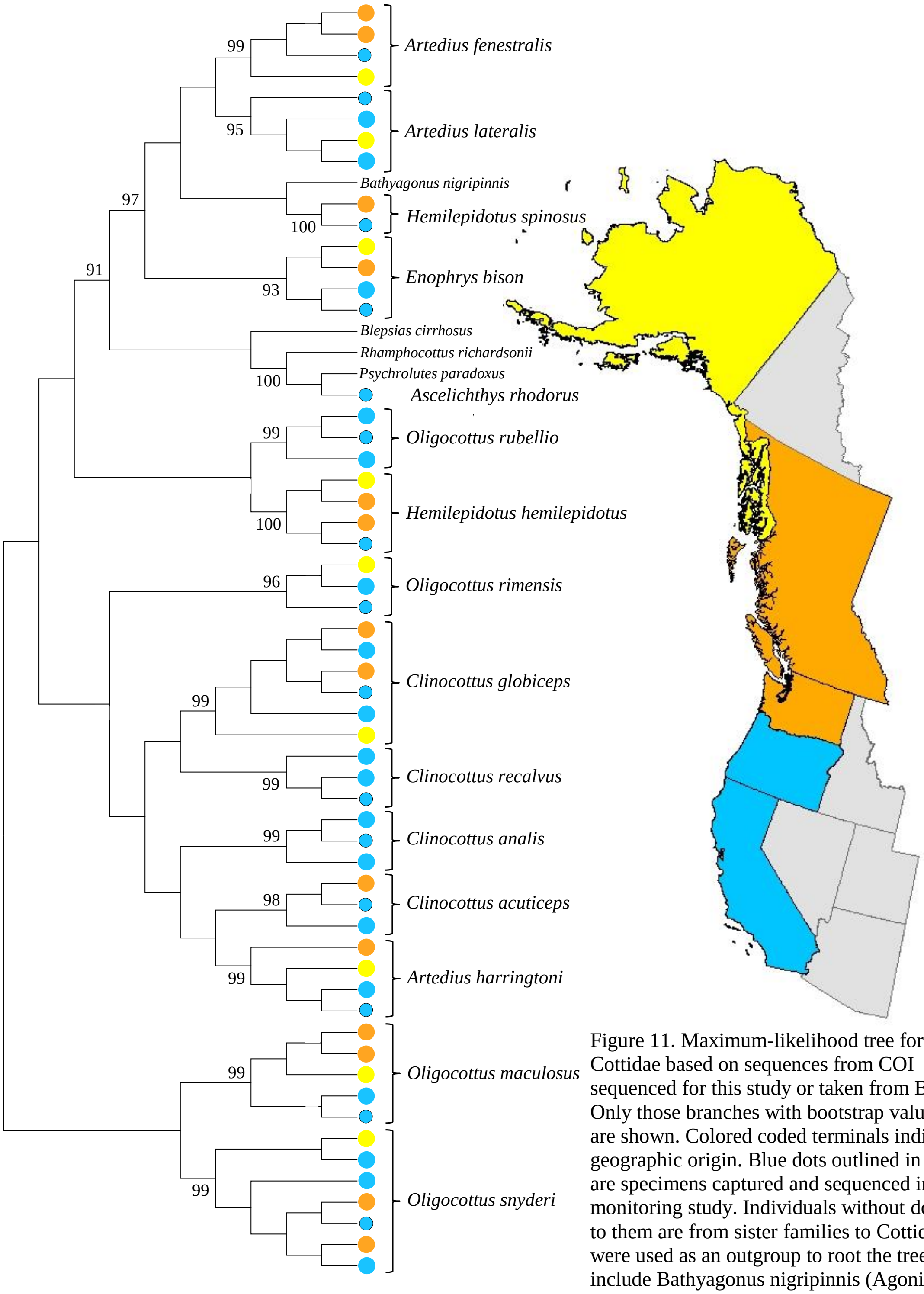

Figure 11. Maximum-likelihood tree for Cottidae based on sequences from COI sequenced for this study or taken from BOLD. Only those branches with bootstrap values >90 are shown. Colored coded terminals indicate geographic origin. Blue dots outlined in black are specimens captured and sequenced in this monitoring study. Individuals without dots next to them are from sister families to Cottidae and were used as an outgroup to root the tree. These include Bathyagonus nigripinnis (Agonidae), Blepsias cirrhosus (Hemitripteridae), Rhamphocottus richardsonii (Ramphocottidae), and Psychrolutes paradoxus (Psychrolutidae).

using sequences from voucher specimens and previously archived material to provide broader geographic representation using sequences from BOLD (Fig. 12). All species were monophyletic with high bootstrap values indicating strong potential for this gene to be used in identification of species. The only exception to the general lack of genetic structure was *Apodichthys flavidus*, where northern sites clustered separate from southern sites, though this is based on only two samples from each of two regions.

Matrices for pairwise genetic distances for species in the family Cottidae indicate there is much more variation between species than within species (Fig. 13). The average number of pairwise nucleotide differences between geographic samples of the same species was 0.16%, but was 16.0% in comparison between species. Based on these results, this sequence can be used to reliably identify species since there is much more variation between species than within species. Within genera, the percentage of sequence difference remained relatively high with only a few pairs being fairly close to identical. These pairs were *Artedius harringtoni* and *A. fenestralis* (11.5% different), *Clinocottus globiceps* and *C. recalvus* (9.6% different), *Hemilepidotus hemilepidotus* and *H. spinosus* (7.8% different), and *Oligocottus maculosus* and *O. snyderi* (10% different). Even with these low percentages between species of the same genus, they remain much higher than the intraspecies sequences.

For all species not in the family Cottidae, interspecies DNA barcode sequences vary much more than intraspecies sequences (Fig. 14). The average variation within a species was only 0.23%, which is substantially lower than the average between species

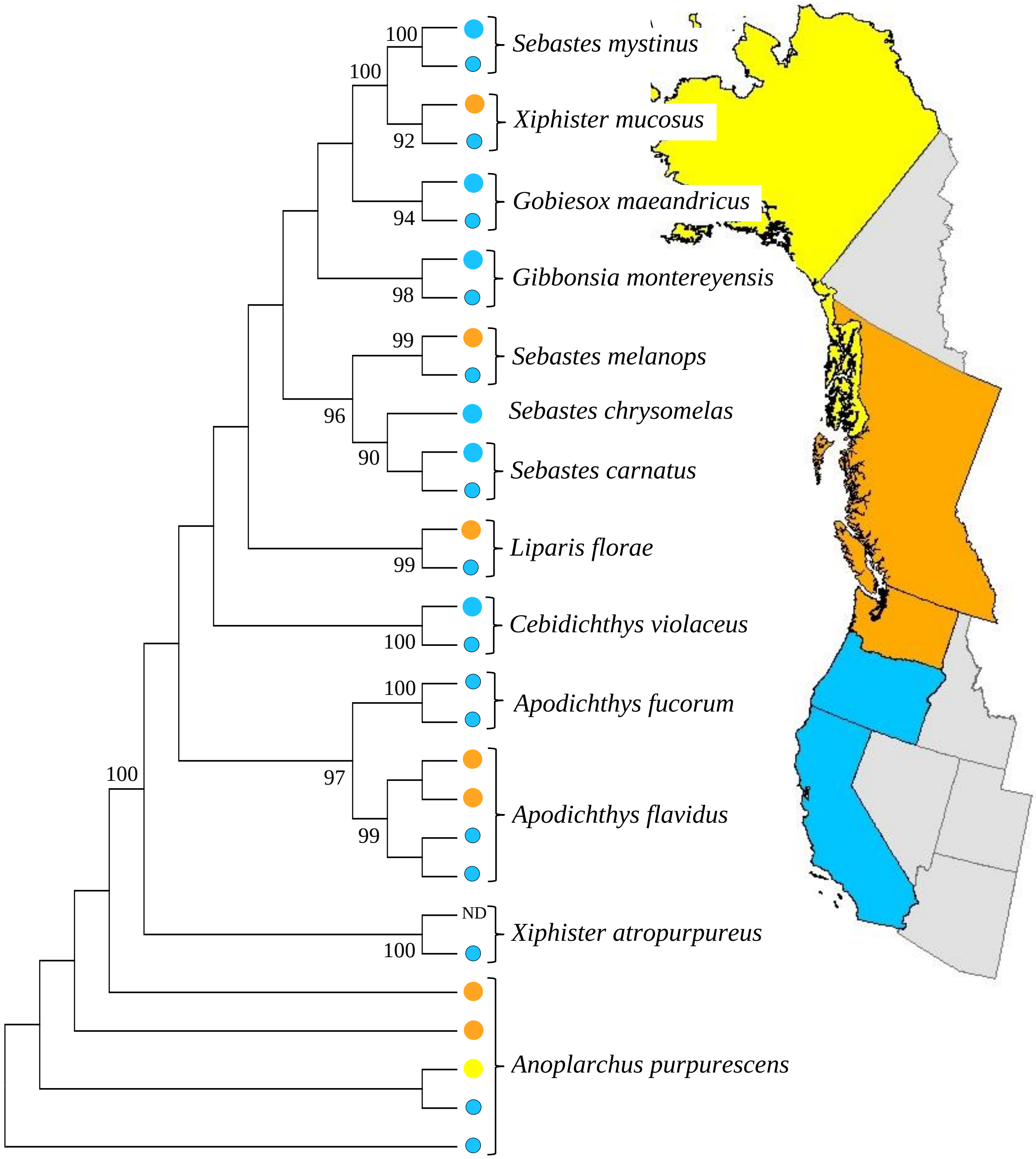

Figure 12. Maximum-likelihood tree for all species that are not in Cottidae based on sequences from COI sequenced for this study or taken from BOLD. Only those branches with bootstrap values >90 are shown. Colored coded terminals indicate geographic origin. Blue dots outlined in black are specimens captured and sequenced in this monitoring study. “ND” indicates no geographic data was available for the sequenced specimen.

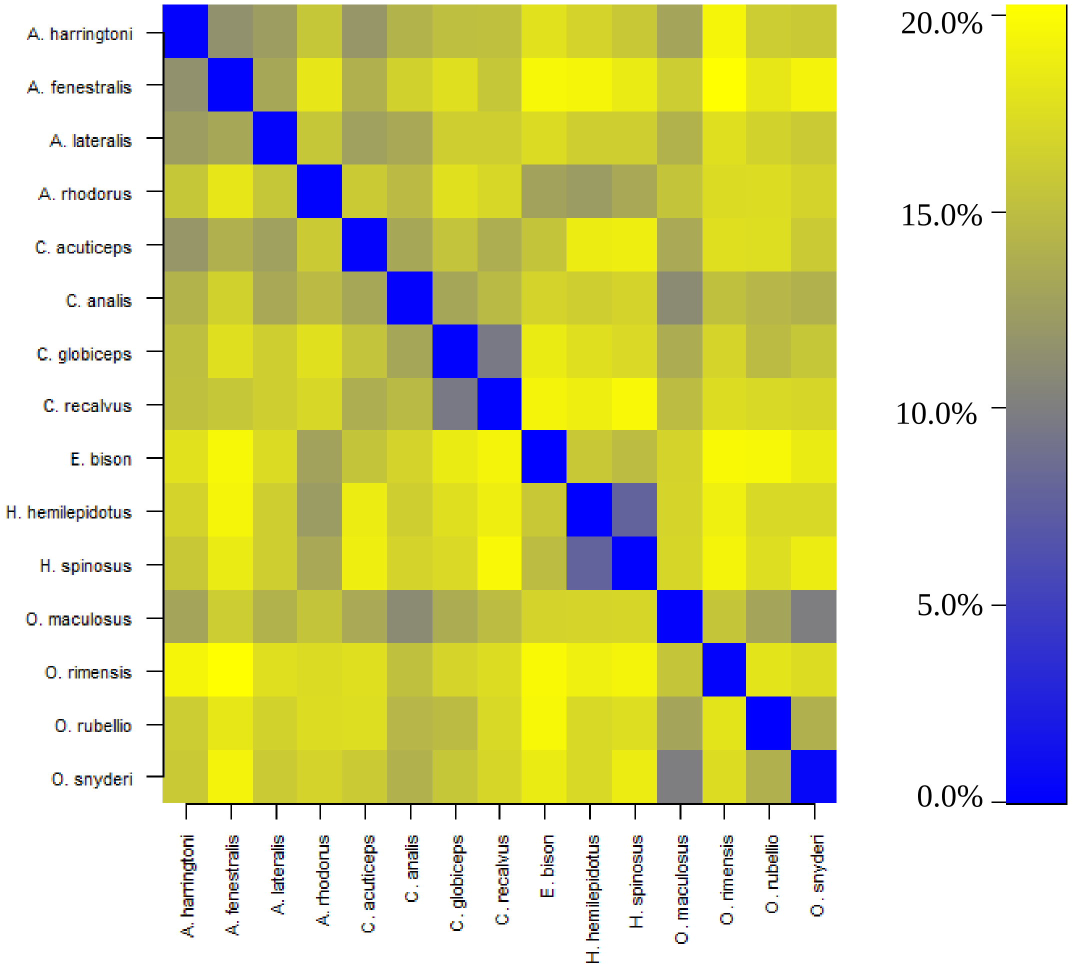

Figure 13. Heatmap of pairwise genetic distances between all species in the family Cottidae sequenced in this study combined with DNA barcodes taken off BOLD. Values along the diagonal represent within-species comparisons whereas values above and below the diagonal, which are symmetrical, represent between species comparisons. Pairwise distances are uncorrected p-distances indicating the proportion of nucleotides that are different between individual sequences.

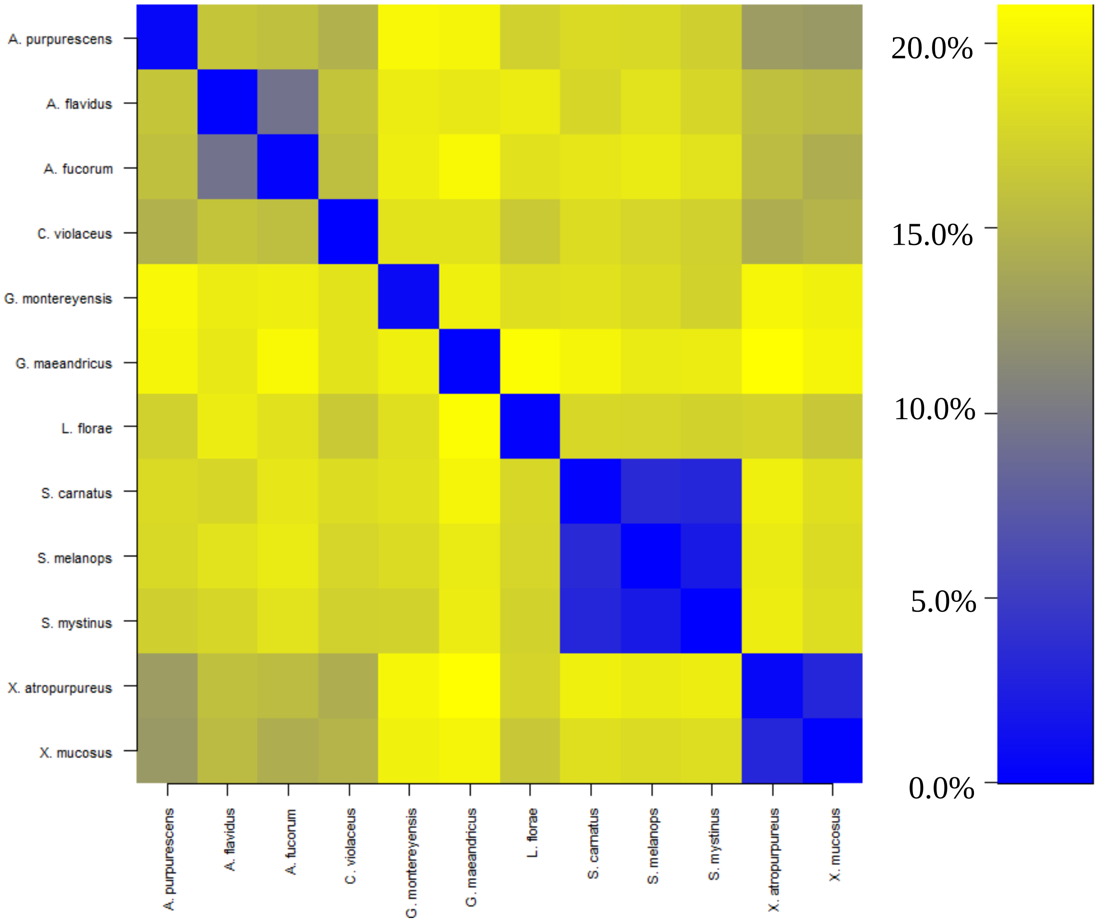

Figure 14. Pairwise genetic distances of all remaining species not in the family Cottidae sequenced in this study combined with DNA barcodes taken off BOLD that were used in the phylogenetic analysis. Pairwise distances are reported as p-distances which indicate the proportion of nucleotides that are different between individual sequences.

(16.8%). Variation was also high within families, although it became very small within a genus. Within the genus *Sebastes,* variation ranged from 2.3% to 3.6% variation in the sequences. However, this is still much lower than the 0% to 0.2% difference within the same species for those in the genus *Sebastes.* The other genus that shows lower levels of interspecific variation is *Xiphister*. The difference between *X. atropurpureus* and *X. mucosus* was 3.1%, while the difference within the species were 0.5% and 0.1%, respectively.

DISCUSSION

Diversity

This study described the intertidal fish assemblages at seven sites along the Northern California coastline, including two recently established MPAs. Thirty-four species were identified from June 2014 to February 2016 (table 3), exceeding the numbers detected in previous studies for this region (Grossman 1982, Yoshiyama et al. 1986, Cox 2007), Oregon (Chadwick 1976, Yoshiyama et al. 1986), and Central California (Yoshiyama 1981, Boyle and Horn 2006) (table 7). Similar to these previous studies, the family Cottidae had the greatest number of species observed and the highest abundance, most in the genus *Oligocottus*.

A study conducted in Trinidad Bay, California (Moring 1986) reported that *Oligocottus maculosus* was by far the most common species, making up 68% of the total followed by *O. snyderi* at 13%. Cox (2007) reported that *O. snyderi* was the most common species, making up 66% of all the fish and *O. maculosus* only represented 17% of the total abundance. This study found that *O. snyderi* is more abundant (31%) than *O. maculosus* (22%), similar to the results of Cox (2007), but showing less dominance. Since these percentages are both lower and closer together, it suggests that species composition is more even when considered over broader geographic scales as in this study. Refer to Appendix B for more information about other species detected in each study, but note that different sampling techniques were used.

Table 7. Studies of rocky intertidal fish community species richness in Northern California and surrounding regions with the number of species detected in each study. These are compared to this study: MPA baseline characterization.

Study	Region	Number of Species
Michael and Chadwick 1976	Oregon	23
MPA baseline characterization	Northern California	34
Cox 2007	Northern California	24
Grossman 1982	Central California	29
Boyle and Horn 2006	Central California	14
Yoshiyama 1981	Central California	24

Since the southern sites in this study displayed different habitat types and fish assemblages than the northern sites, it is possible these locations were not optimal for *Oligocottus*, or they are less abundant in the slightly warmer southern waters. If this were the case, it would explain the lower dominance in this study when compared to studies in northern waters. When separated, *O. snyderi* made up 33% and 29% of the northern and southern fish abundances, respectively. *Oligocottus maculosus* comprised 30% and 12% of the northern and southern fish abundances, respectively. This indicates that there was no major difference for *O. snyderi*, which ranges along the entire west coast, from Alaska to Baja California. For *O. maculosus*, however, there was a difference which could be due to its natural geographic range not extending as far south (Miller and Lea 1972). Largescale variation in intertidal fish communities along a latitudinal gradient was summarized by Prochazka et al. (1999) which shows that communities switch from being largely sculpin-dominated in the north to having more kelpfishes and gunnels in the south.

Differences in abundance and richness between intertidal zones were observed, but were not consistent with each other. Abundance was similar in the high and low intertidal zones whereas richness was similar in the low and mid intertidal zone, but lower in the high zone (Fig. 4). The cause of this was most likely due to the recruitment of juvenile sculpins to the high intertidal zone. Large numbers of newly settled sculpins (*Oligocottus, Clinocottus*) were typically present in the high intertidal zone whereas very few were ever found in the low intertidal zone. Since so many individuals of a few species were found in these high tidepools, species richness decreased due to lack of space and resources available. These high tidepools were also subject to the most extreme fluctuations in temperature and salinity, making them uninhabitable for more sensitive species. Low intertidal pools were much more complex in structure, containing many caves, crevices, kelp, and surfgrass. This provided more habitat and more food for larger fish that may prey on newly settled juveniles (e.g. *Artedius* spp., *Hemilepidotus* spp.), so more species were observed in smaller numbers. The middle intertidal zone has traits similar to both, where many juvenile fish settle in these somewhat complex, less stressful environments, but there is still some habitat suitable for larger and more species. An example that demonstrates this is Palmer's Point during the summer of 2014. In the high tidepool, there were 61 fish caught (60 of which were Cottids), 4 species, and only 3 sculpin that were greater than 40 mm long, which suggests they were all new recruits. The low intertidal pool contained only 21 fish (14 Cottids) but had 8 species and only four sculpin were less than 40 mm. The middle tidepool had 27 fish (24 Cottids), 6 species, and 10 sculpin that were under 40 mm.

The intertidal is primarily used by permanent residents that are highly specialized for living in the harsh environment, but is also frequently used by the juveniles of subtidal species as a nursery. In this study, juveniles of ten species that are frequently taken in the subtidal or deeper by recreational or commercial fishers were captured (table 8). *Cebidichthys violaceus* is often sought after by poke-pole fishers in the low intertidal to shallow subtidal. *Enophrys bison, Hemilepidotus hemilepidotus,* and *H. spinosus* are

Table 8. Temporary residents caught in northern California rocky intertidal habitats. Total numbers caught were combined from both years and are reported by season, summer (S) and winter (W). Sizes are given in millimeters (mm) of total length. The single number represents the average length (or length of an individual for species where only one or two were caught), and the numbers in parentheses indicate the range of sizes caught during the seasons.

Species	Common Name	Total (S)	Sizes (S)	Total (W)	Sizes (W)
Cebidichthys violaceus	Monkeyface prickleback	24	79 (25-128)	23	98 (48-190)
Enophrys bison	Buffalo sculpin	10	16 (14-18)	1	57
Hemilepidotus hemilepidotus	Red Irish lord	3	113 (112-115)	0	
Hemilepidotus spinosus	Brown Irish lord	2	115	3	101 (95-113)
Hexagrammos decagrammus	Kelp greenling	2	68, 164	0	
Hexagrammos lagocephalus	Rock greenling	1	68	0	
Scorpaenichthys marmoratus	Cabezon	6	68 (46-94)	4	112 (43-169)
Sebastes carnatus	Gopher rockfish	0		1	75
Sebastes melanops	Black rockfish	3	54 (52-56)	0	
Sebastes miniatus	Vermillion rockfish	1	31	0	

larger sculpin that are occasional caught, but typically are not targets in recreational fisheries. *Scorpaenichthys marmoratus* is a large sculpin for which a substantial recreational fishery exists all along the California coast, and is a prized food fish. *Hexagrammos decagrammus* and *H. leptocephalus* are also often taken on rocky reefs and are a relatively valuable fishery. The remaining three, *Sebastes melanops, S. miniatus,* and *S. carnatus* are caught by commercial and recreational fisheries. Except for the monkeyface prickleback, *C. violaceus*, most juveniles of temporary residents were caught during the summer, when juveniles are settling out after their pelagic larvae stage.

For temporary species using the rocky intertidal as a nursery during both seasons (e.g. *C. violaceus, S. marmoratus*), the individuals caught during the winter were overall much larger than those caught during the summer with *H. spinosus* being an exception. The brown Irish lord individuals that were captured were too large to have been newly settled recruits and were likely trapped in the pool when feeding during high tide and since they were detected in very low numbers, it is unlikely they are residents during the juvenile stage. *Cebidichthys violaceus* is frequently a permanent resident of the intertidal, so it is not surprising that the numbers caught were very similar between the summer and winter. All the buffalo sculpin occupying the intertidal during the summer were newly settled recruits. The one individual found in the winter was likely still a young of the year and was just using the intertidal as refuge or was potentially foraging during high tide and got trapped.

Contrary to previous studies in the same region (Studebaker and Mulligan 2008, Lomeli 2009), no black rockfish were found in the rocky intertidal habitats of the northern region of northern California. These results are in stark contrast of previous studies done by Lomeli (2009), and Studebaker and Mulligan (2008), where hundreds to thousands of rockfish were being collected from isolated tidepools in the same areas surveyed in this study (Fig.15). One hypothesis about why there were no rockfish in the northern areas during this study is that the water temperature was warmer so the rockfish may have settled immediately into the subtidal rather than the intertidal. Upwelling patterns and water temperature can have strong influences on recruitment and intertidal fish assemblages (Ritter 2009, Shanks and Pfister 2009). During 2014 and 2015, California experienced a very strong positive El Niño event (Fig. 16), causing warmer water temperatures and less upwelling. This can be seen when looking at changes in sea surface temperature (SST) using 2007 as a baseline and plotting the years this study was conducted (2014-2016) as differences from 2007 (Fig. 17). Sea surface temperature data was not available for the region in 2003-2005 during the Studebaker and Mulligan (2008) study. From the middle of January through March, water temperatures were typically several degrees warmer during this study than in previous years. This is the period when larval rockfish are in their pelagic stage and starting to settle out in central and northern California (Stein and Hassler 1989). Two possibilities arise from this information: either the pelagic larvae grew more quickly as a result of the warmer water and settled out much earlier in the intertidal then moved to the subtidal much earlier as well, or survival of

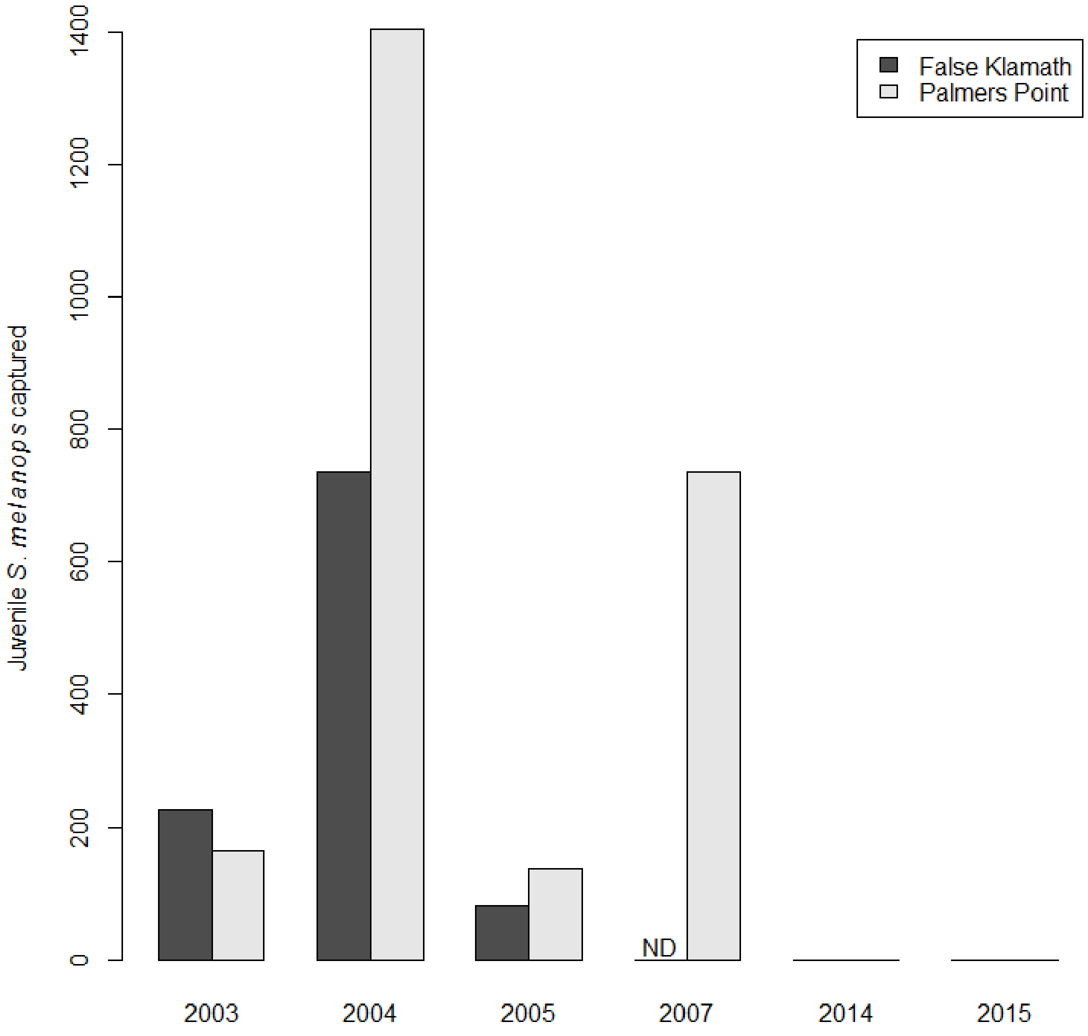

Figure 15. Total numbers of juvenile black rockfish, *Sebastes melanops*, sampled at two sites from 2003 to 2005 (Studebaker and Mulligan 2008), 2007 (Lomeli 2009), and 2014 through 2015 (this study). "ND" indicates no data was collected at False Klamath during 2007.

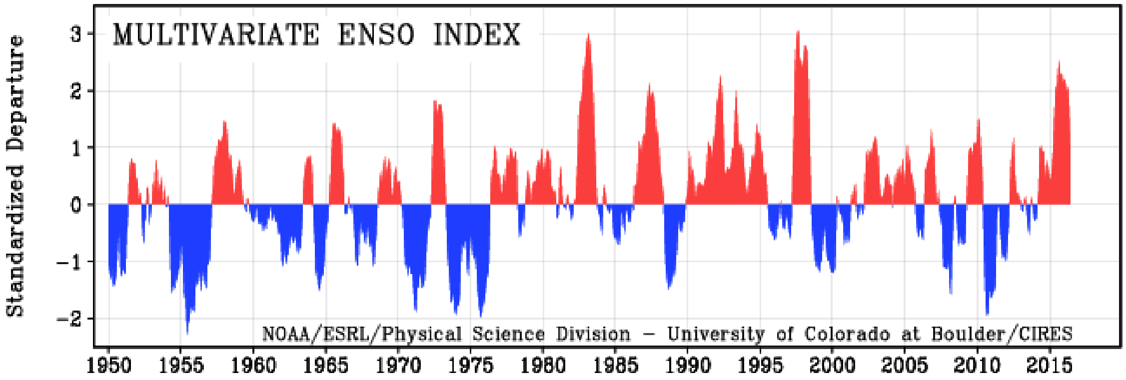

Figure 16. Multivariate El Niño Southern Oscillation (ENSO) index provided and updated by NOAA. Figure taken from http://www.esrl.noaa.gov/psd/enso/mei/.

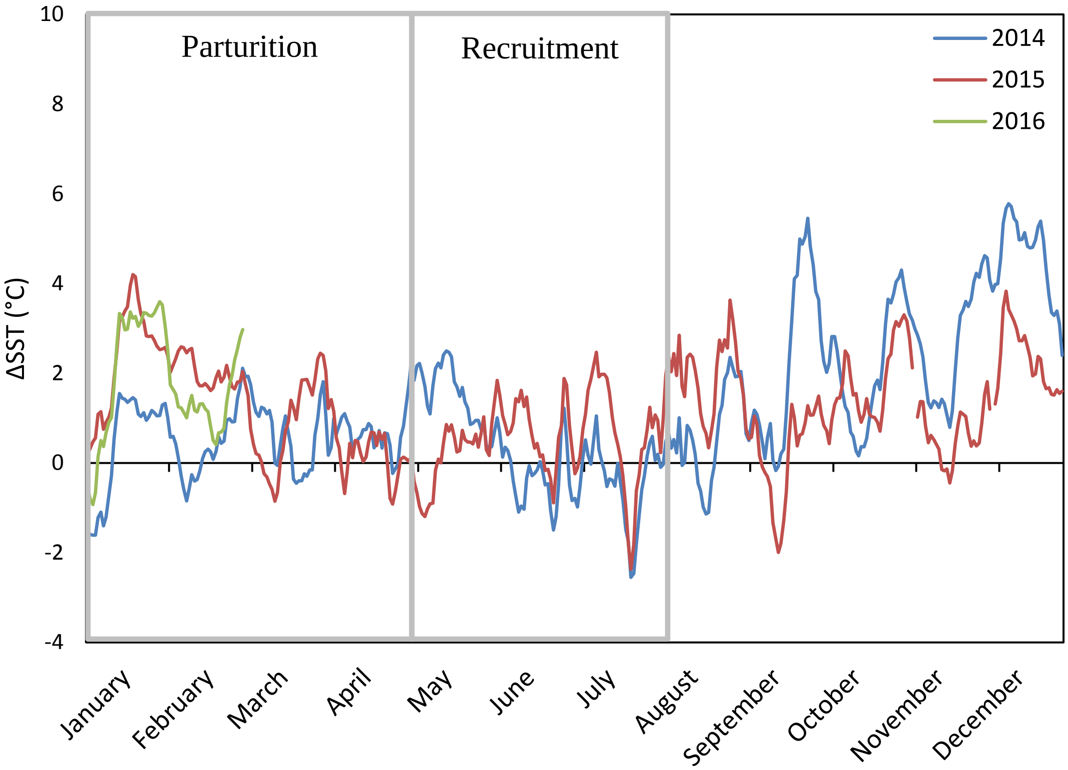

Figure 17. Change in sea surface temperature (ΔSST) from 2007 (during the Lomeli 2009 study) to 2014 through 2016. Temperature data was from the Humboldt Bay North Spit buoy. 2007 was used as a baseline for ocean conditions in which many rockfish were detected in the intertidal. Shaded boxes indicate the time of year when different stages of black rockfish development are occurring.

planktonic larvae was much lower due to inadequate ocean conditions resulting from the El Niño event, so there were very few recruits overall. The former is less likely because the search for rockfish in this study started at the end of March, well before the larvae would start to settle out. The settling (recruitment) stage where black rockfish are typically observed in the intertidal occurs from May to August (Moring 1986, Cox 2007, Studebaker and Mulligan 2008). It is possible that survival was very low, but this will not be realized until this year class reaches harvestable size.

This study provides a baseline that, along with similar subsequent work will allow evaluation of the effects of MPAs on fish in the intertidal zones of northern California. This study largely reflects the condition of the MPAs only a short time (3-4 years) after they were established, which is not enough time for many of the species that would be directly affected to change substantially. Second, the species found in the intertidal zone are typically not heavily impacted by humans, but rather may be indirectly impacted through a release in predation. In this study, the two MPAs had the lowest abundance of fishes which could mean several things. This lower abundance could simply be due to the habitat being less suitable for fish to survive in, so their abundances would naturally be lower. Another possibility is that the effects of protection from fishing offshore are already being seen. The idea of an MPA is that by closing the area to fishing, more and larger fish will be present offshore. These fish frequently visit the intertidal at high tide to feed on the smaller fish living there. If there are more fish coming in to feed, then they will suppress the numbers of permanent intertidal residents which could be causing the lower abundances found at those sites. While the indirect effects of MPAs and trophic cascades have been well studied for invertebrates and macroalgae, (see Menge 1995 for a review), the effects on intertidal fish have received much less attention.

Detection Probability

Detection of intertidal fish is never perfect, regardless of what method is used. Almada and Faria (2004) provide a review of field collection methods for rocky intertidal fishes including destructive ichthyocides (e.g. Thomson and Lehner 1976, Mahon and Mahon 1994), anesthetics (e.g. Yoshiyama et al. 1986, Griffiths 2000), and non-destructive draining and dip-netting (e.g. Faria and Almada 1999, Griffiths 2003). The overall conclusion from all of these studies is that all methods yield very similar results where the majority of fishes are collected from pools, but the census is rarely, if ever, perfect. It is best to assume that there are always several individuals that remain undetected in the pool. However, these methods are assumed to typically get most individuals in the pool as they have been shown to yield samples that do not vary statistically from those obtained by anaesthetics or killing all the fish in the pool (Faria and Almada 1999).

Since most of the fish caught were *O. snyderi,* it is not surprising that this species had the highest detection probability (Wright 1991, Hanski et al. 1993, He and Gaston 2003, Royle et al. 2005). Many species of sculpin, including *O. maculosus* and *C. globiceps* are typically found in aggregations and seem to settle in pools that are already populated by conspecifics whereas *O. snyderi* tends to avoid forming aggregations and will spread out more evenly throughout the intertidal zone (Morris 1964). This behavior makes it more likely to catch this species in more pools since it forms a less patchy distribution.

Lower overall detection rates in the winter, versus the summer, for rocky intertidal fishes is also likely a function of abundance. This is supported by the observation that there are fewer fish in the intertidal during the winter than during the summer, when conditions are less harsh and many young fish are settling out of their pelagic larval stage. Detection rates were lower for the most part in the winter because there were simply fewer fish due to natural mortality from spring and summer settlement to winter. Also, field conditions were typically less hospitable in the winter than summer, so sampling error may have resulted in more fish being overlooked during bad weather sampling.

Several species were found more frequently in the winter than during the summer (*Gobiesox maeandricus, Artedius lateralis, Apodichthys flavidus, Gibbonsia montereyensis*). *Artedius lateralis* may have been taking advantage of the lack of cover for predation during the winter since the waves tend to kill off most the macroalgae growing in the intertidal. With the tidepools more exposed during the winter, these predators can potentially feed on fishes and shrimp more effectively than in the subtidal. The others were likely easier to detect in the winter when there was less cover in the tidepools and they are very cryptic species.

Oligocottus snyderi was found with the same frequency in the summer and winter, which was probably due to their non-aggregating nature. With fish that cluster together, as their numbers decrease (as they typically do throughout the summer and into winter), areas around the edges of their distribution will lose the species altogether as they die off or move towards the middle of the distribution. With species that don't aggregate, such as *O. snyderi*, as the fish decrease, they won't necessarily leave regions vacant, just less populated so they will still be detected across the entire zone. There is no evidence that *O. snyderi* has a higher survival than other species, so this dispersing behavior is the most probable cause of their consistently high detection rates. In fact, *O. snyderi* has been shown to be less tolerant of high fluctuations in water temperature and salinity and may be stenothermal rather than eurythermal like many of the other Cottids (Nakamura 1976, Nakano and Iwama 2002).

Artedius lateralis is the one exception to Cottids preferring boulder fields over all other types of habitat. This species is a very voracious carnivore, feeding primarily on fishes and shrimp in the intertidal (Yoshiyama 1980) using a lie-and-wait method. In boulder fields, characterized by wide, open pools usually with a sandy or gravel bottom, it may be easier for prey, especially stichaeids, to bury themselves in the sand or gravel to avoid predation. This would make it more difficult for large *A. lateralis* to capture them than when they are in bench habitats and forced to seek shelter in rock crevices.

Gibbonsia montereyensis was only found in southern bench sites (except for one individual) and was almost exclusively captured in very long and narrow crevices filled with water. This is most likely a result of habitat preference, where this fish likes to wedge itself in cracks and crevices or wrap around the base of kelp and surfgrass that grows along the edges of large crevice pools. *Apodichthys fucorum* has similar habitat preferences and was also detected most often in bench sites. Juvenile *A. fucorum*, however, tend to wrap themselves in thick kelp or surfgrass patches, often near open water, which is a common habitat characteristic of mixed bench-boulder field sites.

Phylogenetics

The sculpin family Cottidae has recently been scrutinized due to conflicting results between traditional morphology-based classification and newer genetic-based phylogeny (see Smith and Busby 2014). Recent genetic analyses indicate the family is not monophyletic, and calls for reclassification have been proposed (Smith and Busby 2014). This study on intertidal sculpins supports claims that Cottidae is non-monophyletic, but the analyses here were based only on a single gene and limited taxon sampling. The example that stands out the most from this analysis is the rosylip sculpin, *Ascelichthys rhodorus*, which appears to be very closely related the family Psychrolutidae, or the fathead sculpins, and distantly related to those in Cottidae. Given how morphologically similar the two families are in this region, reclassification of this species into Psychrolutidae would make sense. Also, *Hemilepidotus hemilepidotus* appears to be very closely related to members in the family Agonidae which is less sensible based on morphology. This supports some of the claims made by Smith and Busby (2014) about reclassification of the family Cottidae. Another result that emerged is that, while all the species are monophyletic, the genera are not. This is a common issue because genera are supposed to be very well-defined, but have been shown not to be in this and previous studies (Knope 2013).

One goal that was achieved with this phylogenetic study is the establishment of baseline genetic data that can be used to identify species just by comparing the sequences. This is supported for all the species sequenced in this study, since they were resolved as monophyletic. *Apodichthys flavidus* showed some signs of within species geographic structuring, but the within-species distances were much smaller than between species. Results from this DNA barcoding lay the foundation for application of environmental DNA (eDNA) approaches aimed at community level species inventories and species detection in rocky intertidal habitats.

Baseline characterization of communities is critical for tracking long-term changes, both natural and human-induced. This study was a piece of a largescale project that established biodiversity baselines along the Northern California coast across many different habitat types. The biodiversity baselines established in this project are especially important because they provide a starting point for all MPAs and corresponding reference sites that will allow for critical evaluation of the effectiveness of this protection strategy. An additional benefit of this monitoring program is the ability to understand and predict how disturbances, such as oil spills or climate change, will affect the environment.

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APPENDIX A

Appendix A: List of all sequences and their corresponding Barcode of Life ID (BOLD ID), museum accession number (Museum ID) and the voucher specimen identification code (Voucher ID) used for pairwise distances and phylogenetic analyses accessioned into BOLD that were not from this study.

Species	BOLD ID	Museum ID	Geographic Location	Voucher ID
Anoplarchus purpurescens	DSFAL423-08	UAM 5832-01	Tee Harbor, AK	CWM2008-03
Anoplarchus purpurescens	MFC262-08	SIO 04-103	Puget Sound, WA	MFC263
Anoplarchus purpurescens	TZFPA077-06		Port Hardy, BC	TagR5849
Apodichthys flavidus	MFC263-08	SIO 04-104	Puget Sound, WA	MFC264
Apodichthys flavidus	TZFPB882-08		Port Harvey, BC	0736-A11
Apodichthys fucorum	MFC377-08	SIO 05-172	San Juan Island, WA	MFC378
Artedius fenestralis	FMV453-09	UW118569	Washington	UW118569
Artedius fenestralis	GBGCA12559-15	UAM:Fish:6252:::156671	Kodiak Island, AK	KP827283
Artedius fenestralis	TZFPB879-08	0736-A08	Port Harvey, BC	0736-A08
Artedius harringtoni	GBGCA12563-15	UAM:Fish:4702:::371756	Monterey, CA	KP827280
Artedius harringtoni	GBGCA12564-15	UAM:Fish:6155:::156941	Kasitsna Bay, AK	KP827281
Artedius harringtoni	MFC383-08	SIO 06-4	Whidbey Island, WA	MFC384
Artedius lateralis	GBGCA12566-15	UAM:Fish:2951:::157397	Sitka, AK	KP827287
Artedius lateralis	GBGCA12568-15	UAM:Fish:2976:::371853	Newport, OR	KP827289
Artedius lateralis	RMAYC543-08	UAIC 12989.02	Sonoma, CA	NAFF 2289
Cebidichthys violaceus	MFC386-08	SIO 06-42	San Luis Obispo, CA	MFC387
Clinocottus acuticeps	GBGCA12581-15	UAM:Fish:6179:::157136	Manitoba, BC	KP827295
Clinocottus acuticeps	GBGCA12582-15	UAM:Fish:2973:::371866	Newport, OR	KP827296
Clinocottus analis	BNAFA363-08	UAIC:12989.03	Sonoma, CA	NAFF 2978
Clinocottus analis	MFC034-08	SIO 01-125	San Diego, CA	MFC034

Species	BOLD ID	Museum ID	Geographic Location	Voucher ID
Clinocottus globiceps	GBGCA12594-15	UAM:Fish:6182:::157142	Ucluelet, BC	KP827273
Clinocottus globiceps	GBGCA12596-15	UAM:Fish:2975:::371879	Newport, OR	KP827275
Clinocottus globiceps	GBGCA12597-15	UAM:Fish:2968:::371845	Neah Bay, WA	KP827276
Clinocottus globiceps	GBGCA12599-15	UAM:Fish:2942:::371801	Sitka, AK	KP827278
Clinocottus globiceps	MFC389-08	SIO 06-45	Santa Cruz, CA	MFC390
Clinocottus recalvus	GBGCA12600-15	MLR_05	San Luis Obispo, CA	KP827270
Clinocottus recalvus	MFC390-08	SIO 06-45	Santa Cruz, CA	MFC391
Enophrys bison	BNAFA373-08	UAIC :12989.04	Sonoma, CA	NAFF 2988
Enophrys bison	FMV093-08	UW047706	Puget Sound, WA	UW047706
Enophrys bison	GBGCA12608-15	UAM:Fish:6255:::156665	Kodiak Island, AK	KP827346
Gibbonsia montereyensis	MFC396-08	SIO 06-41	San Luis Obispo, CA	MFC397
Gobiesox maeandricus	MFC397-08	SIO 06-42	San Luis Obispo, CA	MFC398
Hemilepidotus hemilepidotus	DSFAL037-07	CAS 230269	Semidi Islands, AK	SMMOCI007-37
Hemilepidotus hemilepidotus	FMV128-08	UW048795	Puget Sound, WA	UW048795
Hemilepidotus hemilepidotus	GBGCA12612-15	UAM:Fish:6177:::156822	Smith Sound, BC	KP827338
Hemilepidotus spinosus	FMV110-08	UW048010	Puget Sound, WA	UW048010
Liparis florae	MFC245-08	SIO 04-103	Puget Sound, WA	MFC245
Oligocottus maculosus	GBGCA12630-15	UAM:Fish:4698:::371752	Prince William Sound, AK	KP827299
Oligocottus maculosus	GBGCA12633-15	UAM:Fish:6181:::157138	Tofino, BC	KP827302
Oligocottus maculosus	GBGCA12634-15	UAM:Fish:6188:::156838	Bremerton, WA	KP827303
Oligocottus maculosus	MFC405-08	SIO 06-46	Oregon	MFC406
Oligocottus rimensis	GBGCA12637-15	UAM:Fish:2955:::157405	Sitka, AK	KP827319
Oligocottus rimensis	MFC406-08	SIO 06-47	Monterey, CA	MFC407
Oligocottus rubellio	GBGCA12641-15	MLR_01	Big Sur, CA	KP827315
Oligocottus rubellio	MFC407-08	SIO 06-47	Monterey, CA	MFC408
Oligocottus snyderi	BNAFA377-08	UAIC :13496.01	Lincoln, OR	NAFF 2992

Species	BOLD ID	Museum ID	Geographic Location	Voucher ID
Oligocottus snyderi	GBGCA12643-15	UAM:Fish:2972:::371820	Seiku, WA	KP827306
Oligocottus snyderi	GBGCA12645-15	UAM:Fish:4700:::371754	Monterey, CA	KP827308
Oligocottus snyderi	GBGCA12646-15	UAM:Fish:4683:::371913	Ucluelet, BC	KP827309
Oligocottus snyderi	GBGCA12649-15	UAM:Fish:2946:::371790	Sitka, AK	KP827312
Oligocottus snyderi	MFC408-08	SIO 06-42	Cambria, CA	MFC409
Sebases chrysomelas	CMBB020-09		Ventura, CA	RL-4
Sebastes carnatus	SDP103003-13		Santa Barbara, CA	CA-381
Sebastes melanops	TZFPB596-06		British Columbia	TZ-06-RICKER-628
Sebastes mystinus	CCVI005-10		Ventura, CA	RA-2
Xiphister atropurpureus	ANGBF4311-12			JN591554
Xiphister mucosus	TZFPA073-06		Port Hardy, BC	TagR5072

APPENDIX B

Appendix B: List of all species reported from previous intertidal fish surveys of northern California and this study (Hinterman). Note that each study used different sampling techniques that could select for or against certain species.

Species	Moring 1986	Cox 2006	Hinterman
Anoplarchus purpurescens	X	X	X
Apodichthys flavidus	X	X	X
Apodichthys fucorum	X	X	X
Artedius corallinus		X	X
Artedius fenestralis	X	X	X
Artedius harringtoni			X
Artedius lateralis	X	X	X
Ascelichthys rhodorus			X
Atherinops californiensis		X	
Cebidichthys violaceus	X	X	X
Citharichthys stigmaeus	X		
Clinocottus acuticeps	X	X	X
Clinocottus analis			X
Clinocottus embryum			X
Clinocottus globiceps	X	X	X
Clinocottus recalvus			X
Enophrys bison		X	X
Gibbonsia metzi			X
Gibbonsia montereyensis			X
Gobiesox maeandricus	X	X	X
Hemilepidotus hemilepidotus			X
Hemilepidotus spinosus	X	X	X
Hexagrammos decagrammus	X	X	X
Hexagrammos lagocephalus			X
Leptocottus armatus		X	
Liparis florae	X	X	X
Oligocottus maculosus	X	X	X
Oligocottus rimensis			X
Oligocottus rubellio			X
Oligocottus snyderi	X	X	X
Pholis ornata	X	X	
Phytichthys chirus			X
Scorpaenichthys marmoratus	X	X	X
Sebastes carnatus			X
Sebastes melanops	X	X	X

Species	Moring 1986	Cox 2006	Hinterman
Sebastes miniatus			X
Sebastes mystinus	X		
Xiphister atropurpureus	X	X	X
Xiphister mucosus		X	X