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Seasonal fish and invertebrate communities in three northern California estuaries

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Publication Date 2017-12-01

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# SEASONAL FISH AND INVERTEBRATE COMMUNITIES IN THREE NORTHERN CALIFORNIA ESTUARIES

By

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A Thesis Presented to

The Faculty of Humboldt State University

In Partial Fulfillment of the Requirements for the Degree

Master of Science in Natural Resources: Fisheries

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December 2017

### ABSTRACT

# SEASONAL FISH AND INVERTEBRATE COMMUNITIES IN THREE NORTHERN CALIFORNIA ESTUARIES

### Katherine Osborn

The majority of Northern California estuaries are small, flooded, river valleys that are largely unstudied due to their small sizes and remote locations. Yet these estuaries serve as important nursery areas for many marine fish species including rockfish, flatfish, smelt, and herring, and they are vital to anadromous species such as Chinook Salmon (*Oncorhynchus tshawytscha*) and Steelhead (*O. mykiss*). I sampled the summer and winter fish and invertebrate communities of the Big, Mad, and Ten Mile river estuaries. Fish were sampled via beach seine or fyke net and invertebrates were sampled via benthic cores, June 2014-June 2016.

This research is part of a larger suite of studies establishing baseline conditions in Northern California Marine Protected Areas (MPAs). Big and Ten Mile river estuaries in Mendocino County were designated as MPAs in 2012. The Mad River Estuary in Humboldt County was selected as a non-MPA site to investigate its potential as a reference estuary. In the Mad River Estuary, additional sampling was conducted and a diet study was carried out on the feeding habits of two benthic fishes: Pacific Staghorn Sculpin (*Leptocottus armatus*) and English Sole (*Parophrys vetulus*).

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Fish abundance and diversity varied more by season (i.e. summer, winter) than by estuary, while invertebrate diversity varied more by estuary than by season. The Big River Estuary had the strongest ocean connection and the most marine fish and invertebrate species. The Mad River Estuary fish and invertebrate communities were most similar to the Ten Mile River Estuary, which had the least ocean connectivity and species diversity. Additional sampling in the Mad River Estuary showed that fish and invertebrate communities were diverse from spring through fall, and that invertebrate communities within an estuary differed more by upstream distance than by season. Staghorn Sculpin diet in the Mad River Estuary varied by location of capture, but not by season.

#### ACKNOWEDGEMENTS

First, I would like to thank my family and friends for their unwavering support during my graduate work. This thesis would not have been possible without the guidance of my advisor, Tim Mulligan, who provided me with this rewarding research project. Frank Shaughnessy was lead P.I. for the project and a committee member. I would like to thank my other committee members, Darren Ward and Andre Buchheister, for their assistance and guidance. This research depended on volunteer contributions in the field and the lab, especially by Kaitlyn O'Brien, Eric LeBlanc, Chad Martel, Leon Davis III, Georgia Bennett, and Ian Kelmartin. This project was funded by the California Ocean Science Trust and the California Ocean Protection Council (HSU Project #: SO3017), which were both part of the North Coast MPA Management team. The team also included the California Department of Fish and Wildlife and California Sea Grant. Finally, I would like to thank the Fisheries Founding Faculty, Malcolm Oliphant, and the Granite Bay Flycasters for the scholarships they generously provide that allowed me to successfully complete my thesis.

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#### INTRODUCTION

Estuaries occur where rivers meet the tide. These dynamic, highly productive systems attract fish and other wildlife. Many marine fishes move into estuaries to benefit from the high seasonal productivity, or use estuaries as nurseries, due to plentiful prey and low predation pressures (Moyle and Cech 2004). Anadromous adult fishes use estuaries as staging areas before migrating upstream to spawn, and as juveniles before heading out to sea. Estuaries also provide key habitat necessary to life-cycle completion for many fishes. Therefore, the small estuaries that characterize the Northern California Coast have an outsized importance to local fish populations.

People also depend on estuaries, for their roles in ecosystem health and economic prosperity. People capitalize on the abundant resources and convenient locations of estuaries, and have drastically altered them to meet human demands. Alterations include: water diversions upstream, dredging to benefit boating and shipping, and levees that convert tidal marsh into arable land (Gleason et al. 2011). Ecosystem alterations have impaired estuary function, interfering with many services that estuaries provide. Mitigating the effects of such alterations is of great conservation interest, as estuaries provide valuable resources to wildlife and humans. For this reason, several estuaries gained protection when the Marine Life Protection Act (MLPA) was enacted regionally in California from 2000-2012 (Gleason et al. 2013).

In 2012, a final suite of California Marine Protected Areas (MPAs) was unveiled along the Northern California Coast. Roughly half of the new MPAs were designated as State Marine Conservation Areas (SMCAs), which allow limited recreational and tribal take. The MPA Estuary Project was tasked with establishing baseline conditions in newly established estuarine MPAs in Northern California.

I discuss the fish and benthic invertebrate sampling done as part of the MPA Estuary Project in three estuaries: the Big River Estuary SMCA, the Mad River Estuary, and the Ten Mile Estuary SMCA (Figure 1). The Big River Estuary SMCA in Mendocino County prohibits the take of all living marine resources, with the following exceptions: 1) recreational take of surfperch (family: *Embiotocidae*) by hook and line from shore, 2) recreational take of Dungeness Crab (*Metacarcinus magister*) by hoop net or hand, 3) take by select local federally-recognized tribes, and 4) recreational take of waterfowl. The Mad River Estuary in Humboldt County was chosen as a non-MPA site due to physical characteristics that are intermediate to those of the Big and Ten Mile river estuaries. The Ten Mile Estuary SMCA in Mendocino County prohibits take of all living marine resources, except: 1) take by select local federally-recognized tribes, and 2) recreational take of waterfowl.

The Big, Mad, and Ten Mile river estuaries have received relatively little previous study, due to their small sizes and remote locations. Available literature is limited to technical reports, primarily watershed assessments and Total Maximum Daily Load plans; the latter is required under section 303(d) of the Clean Water Act for impaired water sources. All three estuaries are in sparsely populated watersheds where timber harvest is the dominant land-use. The watersheds of all three estuaries are sediment

impaired under the Clean Water Act and are currently managed for the recovery of salmonid populations.



Figure 1. Map of Northern California, with the reference system highlighted in grey and the MPA study systems highlighted in gold. From north to south, the study areas are: Mad River Estuary (light/grey), Ten Mile River Estuary (dark/gold), and Big River Estuary (dark/gold).

We aimed to characterize the seasonal fish and benthic invertebrate communities in the Big, Mad, and Ten Mile river estuaries (Figure 1). To that end, we sampled biannually, summer and winter, June 2014-June 2016. We conducted additional sampling in the Mad River Estuary, quarterly in the first year of the study, June 2014-June 2015, and monthly in the second year, June 2015-June 2016. Finally, I conducted a diet study in the Mad River Estuary on Pacific Staghorn Sculpin (*Leptocottus armatus*) and English Sole (*Parophrys vetulus*), June 2015-June 2016. Staghorn Sculpin are rear-round residents in the Mad River Estuary and were chosen to explore linkages between the benthic invertebrate community and a resident, opportunistic, benthic fish. In contrast, English Sole were selected as a benthic predator that moves into the estuary seasonally to rear.

We expected large seasonal shifts in the fish and invertebrate communities within the Big, Mad, and Ten Mile river estuaries, associated with seasonal changes in estuarine productivity. The dynamism of estuaries results in periods of high productivity and requires resident species to survive daily, seasonal, and annual fluctuations in temperature, salinity, turbidity, and flow. As a result, estuaries typically have a few, highly abundant, resident fish species, but also attract a diversity of transient species during periods of high seasonal production (Moyle 2002). We therefore expected the fish and invertebrate communities of the Big, Mad, and Ten Mile river estuaries to be dominated by hardy estuarine residents in winter and characterized by higher species abundance and diversity in summer. We also expected that the fish and invertebrate communities would vary between upstream and downstream stations. This study had three objectives: 1) provide a baseline of the seasonal fish and benthic invertebrate communities present in the Big, Mad, and Ten Mile river estuaries, 2) examine differences in the fish and invertebrate communities across seasons and locations, and 3) assess the suitability of the Mad River Estuary as a reference site. Big and Ten Mile river estuaries recently gained limited protections and MPA effects were not expected, but this work allows future researchers to test for potential MPA effects using a before-aftercontrol-impact approach, as defined by Eberhardt and Thomas (1991).

Additional sampling in the Mad River Estuary allowed deeper exploration of how community composition changes with upstream distance, and during the transitional seasons of spring and fall. Finally, we chose two benthic fishes for diet analysis to examine whether diet would follow the seasonal patterns expected in fish and invertebrate communities: higher abundance and diversity in summer, and lower abundance and diversity in winter. Additional sampling in the Mad River Estuary enabled: 1) the establishment of baseline fish and invertebrate communities in spring and fall, 2) the determination of whether patterns observed on a biannual basis were supported by increased sampling, and 3) an examination of Pacific Staghorn Sculpin and English Sole diet in the Mad River Estuary.

### **Study Sites**

### **Big River Estuary**

The Big River begins its course in Montgomery Creek in the Mendocino Coast Range, at an elevation of 865 m (NCRWCB 2005b). From there, it runs 67 km out to the Pacific Ocean, draining roughly 470 km<sup>2</sup> (USGS 2011). Annual precipitation averages from 130 cm<sup>3</sup> at the eastern edge of the watershed near Willits, to 100 cm<sup>3</sup> along the coast (NCRWCB 2005b). Near the mouth, the river narrows into a long estuary with limited floodplain habitat and abundant mudflats. The mouth of the river opens onto the northern edge of Mendocino Bay, just south of the town of Mendocino (Figure 2).

Named for the giant redwoods that once lined its banks, the Big River watershed has been actively logged since the 1850's. Historical timber harvest and road building practices increased erosion and blocked upstream migration for salmonids. The river and estuary were greatly altered due to the practice of floating logs downriver to the estuary, which served as a mill pond from 1852-1938 (Warrick and Wilcox 1981). Due to log transport, the channel was widened, large woody debris was removed, and cobble substrates became embedded in fine grain sediments.



Figure 2. Map of Big River Estuary stations and boundaries of the State Marine Conservation Area (SMCA). From east to west: the mouth station and the upstream station.

The estuary is of conservation interest for its scenic beauty and natural resources.

In 1980, the United States Fish and Wildlife Service (USFWS) nominated the estuary for protection under the Unique and Nationally Significant Wildlife Ecosystem Program (Downie et al. 2006). Although the estuary had first priority for protection in California, it did not receive protection until 2002, when the Mendocino Land Trust purchased a 7,334 acre parcel from the Hawthorne Timber Company. The land was donated in 2002 to California State Parks and become part of the Mendocino Headlands California State Park, creating a 74,000 acre wildlife corridor (NCRWCB 2005b). In 2012, the waters of the estuary were designated as an SMCA (Figure 3).



Figure 3. Map of the Big River Estuary State Marine Conservation Area (SMCA). From the Guide to Northern California Marine Protected Areas (CDFW 2013).

These protections should help the Big River Estuary recover from past

disturbances. The Big River has been listed as sediment and temperature impaired since

2003 under section 303(d) of the Clean Water Act. Modern timber harvest practices and

regular road maintenance have led to reduced sedimentation and allowed continued harvest in 55% of the watershed (Downie et al. 2006). The estuary is in the early stages of recovery from habitat simplification and sedimentation. It provides essential wetland habitat and has potential to provide critical salmonid habitat. The establishment of the Big River Estuary as an SMCA also allowed for the first baseline monitoring of the estuary. The Big River Basin Assessment states that, "[the estuary] is unique and should be studied further" (Downie et al. 2006). To this end, we established two polyhaline stations on subtidal muddy beaches at the downstream end of the estuary (Figure 2; Appendix A).

The Big River Estuary is the longest undeveloped estuary in California (Downie et al. 2006) and experienced the strongest tidal influence of our three study systems. Ocean salinities extend roughly 13 km upriver in summer and 5 km in winter during high flows. The tidal signal in daily temperatures was greatest in summer, when coastal upwelling brought cold, nutrient-rich waters into contact with warmer river waters in the estuary. In 2014 and 2015, this signal was 6 °C in summer and 2 °C in fall (Appendix B).

#### Mad River Estuary

The lower Mad River is the historic home of Wiyots, while upriver portions were held by the Whilkut, Nongatl and Lassik tribes (Baumhoff 1958). The river was named for the fiery temper of explorer Dr. Josiah Gregg, whose crew stranded him on the wrong side of the river mouth in 1849 (Gudde and Bright 2004). From its headwaters in the coastal range, the Mad River runs over 180 km, through Trinity and Humboldt counties, draining roughly 1,290 km<sup>2</sup>. Elevation is highest in the headwaters near South Kelsey Ridge, reaching well above the snow line at 1,800 m (Stillwater Sciences et al. 2010). Annual precipitation averages 100 cm<sup>3</sup> along the coast and 200 cm<sup>3</sup> inland (Stillwater Sciences et al. 2010). Before entering the ocean, the river turns abruptly north near the triple junction of the Gorda, North American, and Pacific plates. Our upstream station was located at this bend, which denotes the usual upper limit of the estuary (Figure 4). However, brackish waters can extend as far upstream as the Highway 101 Bridge during king tides. Our second site was located on a sandy beach near the mouth. In June 2015, we permanently added a third site where Norton Creek enters the system. The creek is the last freshwater input before the river mouth and is locally known by the name of one of its tributaries, Widow White Creek.



Figure 4. Map of Mad River Estuary stations. From north to south: the river mouth station, the tributary station, and the upstream station.

The Mad River watershed has been actively logged since the 1850's and is mostly owned by timber companies. The watershed is also used for ranching, farming, gravel mining, and residential development (Stillwater Sciences et al. 2010). Timber harvest, gravel mining, and the associated road construction led to increased erosion and the 1992 listing for the Mad River as sediment impaired under section 303(d) of the Clean Water Act. The river was additionally listed as turbidity and temperature impaired in 2006 (Stillwater Sciences et al. 2010). Addressing these water quality issues is considered critical to the recovery of local salmonid populations.

Coho Salmon were federally listed as threatened in the Mad River in 1997. Chinook Salmon and Steelhead followed in 1999 and 2000, respectively. Similar to the Big River, current management for the Mad River Estuary aims to improve salmonid habitat by decreasing sedimentation and increasing habitat complexity, while maintaining timber harvest and water collections. Water was first diverted from the Mad River in 1933 for residential and industrial use. Today, Humboldt Bay Municipal Water District delivers Mad River water to two thirds of the county.

Water quality and biological data for the Mad River are limited. The Blue Lake Rancheria (BLR) collects water quality data for the Blue Lake Hatchery and Ruth Lake Reservoir. The Mad River Alliance (MRA) collects air and water temperature data from Matthews Dam to the Mad River Boat Ramp, June-October. The California Department of Fish and Wildlife (CDFW) conducted salmonid counts at the Sweasey Dam fish ladder from its construction in 1938 through 1964, when the dam was filled-in. From 1991-2005, CDFW conducted underwater surveys for summer-run Steelhead adults and half pounders. The MRA has since taken over these surveys. From 2009-2011, Humboldt Baykeeper and Alison O'Dowd of Humboldt State University (HSU) carried out toxicity and habitat characterization studies on Widow White and lower Norton creeks.

We chose the Mad River Estuary as a study system due to its proximity to HSU, and to investigate its potential as a reference site to two MPAs, the Big and Ten Mile river estuaries in Mendocino County. Of these three, linear, riverine estuaries, the Mad has a weaker ocean connection than the Big River Estuary, but a stronger ocean connection than the Ten Mile River Estuary, where sandbars can seasonally cut-off marine access (Shaughnessy et al. 2017).

### Ten Mile River Estuary

Named for the distance from its mouth to the mouth of the Noyo River to the south, the Ten Mile River (also known as Ten Mile Creek), is formed at the confluence of the North Fork Ten Mile River and the Middle Fork Ten Mile River. The headwaters of the North Fork Ten Mile River begin at 730 m; the Middle Fork Ten Mile River begins at 750 m in the California Coast Range (NCRWCB 2005a). The Ten Mile River runs 11 km to join to Pacific Ocean, draining approximately 310 km<sup>2</sup> (Durham 1998). River flow varies with precipitation, which averages 100 cm<sup>3</sup> annually along the coast and 180 cm<sup>3</sup> inland (NCRWCB 2005a). The estuary has a narrow, perched mouth, and depends on large storm events to maintain or reestablish ocean connectivity. Sand bars often block or constrict the river mouth in summer, effectively turning the estuary into a freshwater lagoon in some years. Under lagoon conditions, salinities are reduced, ebb and flood tides are delayed, and slack tides can persist for hours within the estuary (Shaughnessy et al. 2017). We established two stations in the Ten Mile River Estuary (Figure 5). The polyhaline downstream station spanned both banks of the river: the southern, sandy shore and the northern, muddy shore. Our second station was a subtidal mudflat approximately one kilometer upstream.



Figure 5. Map of Ten Mile River Estuary stations and boundaries of the State Marine Conservation Area (SMCA). The seine station is to the northwest, fyke station to the southeast.

Timber companies own most of the Ten Mile watershed and have logged the land since the 1870's (Mangelsdorf and Clyde 2000). 1n 1998, the river was listed as sediment impaired under section 303(d) of the Clean Water Act (NCRWCB 2005a). Responsible timber harvest practices, improved road construction standards, and regular road maintenance have since reduced sedimentation while allowing active management for timber in 45% of the watershed (GMA 2000). This success prompted managers to model recommendations for the Big River on the Ten Mile River recovery plan (NCRWCB 2005b).

The Ten Mile River Estuary historically had an important Coho Salmon population, but the last counts estimate that the 1960's Coho population of 6,000 has dropped to fewer than 200 individuals (NCRWCB 2005a). In contrast, Steelhead populations are believed to have surpassed the 9,000 individuals estimated in the 1960's (Mangelsdorf and Clyde 2000). Management for the Ten Mile River aims to recover salmonids by decreasing sedimentation and increasing habitat complexity, similar to the stated management goals for the Big and Mad river watersheds. Salmonids rely on coldwater habitat and low sedimentation, as sedimentation can bury the cobble substrates that salmon rely on for spawning. Although the Ten Mile River is not listed as temperature impaired, the estuary was the warmest of the three study systems, reaching water temperatures above 20 °C in summer and fall (Appendix D). All three systems are also listed as sediment impaired. It is assumed that managing for salmon recovery will also aid other native fishes, as salmonids are especially sensitive to habitat alterations and water quality degradation (NCRWCB 2005a).

The 2012 designation of the Ten Mile Estuary as an SMCA complemented the designation of two other MPAs: the Ten Mile Beach SMCA and the Ten Mile Marine Reserve (Figure 6). Together, these three areas form an MPA region that extends three nautical miles offshore (CDFW 2013). The creation of the Ten Mile Estuary SMCA, like the creation of the Big River Estuary SMCA, should improve conditions for fish by

establishing a baseline of the fish and invertebrates present in the estuary and by

increasing awareness through signage and outreach.



Figure 6. Left: map of Ten Mile Estuary State Marine Conservation Area (SMCA). Right: map of all three Ten Mile MPAs: Ten Mile State Marine Reserve, Ten Mile Beach SMCA, and Ten Mile Estuary SMCA. From the Guide to Northern California Marine Protected Areas (CDFW 2013).

#### MATERIALS AND METHODS

## Field Collection

Baseline conditions were ascertained for three estuaries: Big River, Mad River, and Ten Mile River. Each estuary was sampled biannually, June and January, summer 2014 through summer 2016. Two stations were selected in each system, to capture some of the variability due to upstream distance (Figures 2, 4, and 5). Two to three beach seines were conducted at each station using a 45.7m by 1.8m seine with 6.4mm mesh (Table 1). Beach seines were conducted when tidal level was 0.3-1.3 m. At one site in Ten Mile River Estuary, beach seining was not possible. Instead, a fyke net (0.7x0.7m wings and lead; two 0.7x1.0m frames with internal fykes; 6.4mm mesh) was set to fish an outgoing overnight tide. All fish were identified to species and total length (TL) was measured to the nearest mm for the first 30 individuals. To sample invertebrates, five benthic cores were taken along the low and mid-tide lines at each station, using a clam gun (diameter = 10 cm) to a depth of no more than 10 cm. The cores were sifted in the field and all visible invertebrates were removed. All work was approved by the Institutional Animal Care and Use Committee (IACUC number 13/14.F.113-A).

	Big River	Mad River		Ten Mile
Field Data Collected	Estuary	Estuary <sup>2</sup>		River Estuary <sup>3</sup>
Beach seine hauls	26		28	17
Fyke net hauls	0		0	5
Benthic cores (low & mid tide)	100		100	100

Table 1. Types of data collected in the Big, Mad, and Ten Mile river estuaries biannually. Sampling was conducted summer and winter, June 2014– June 2016.<sup>1</sup>

To inform biannual sampling across all three estuaries, additional sampling was conducted in the Mad River Estuary (Table 2). This additional sampling was analyzed separately, was more frequent, and included a third station at the mouth of Norton Creek (tributary station, Figure 4). The tributary station was sampled in November 2014 and April 2015 and was added permanently to sampling in June 2015. The first year of the study, beach seines were conducted quarterly following the methods described above. Benthic cores were taken in June 2014 and January 2015 at the mouth and upstream stations, and at the mouth, tributary, and upstream stations in June 2015. The second year of the study, June 2015-June 2016, beach seines were conducted monthly at all three stations when flows allowed. A diet study was also conducted, and up to a total of 36 Pacific Staghorn Sculpin and 36 English Sole were sacrificed monthly for diet analysis. When possible, twelve individuals from each species were taken from each of the three sites for diet analysis. To complement the diet study, benthic cores were added to monthly sampling in September 2015. Benthic samples taken at the third station or as

<sup>&</sup>lt;sup>1</sup>Number of seines per station increased from two to three in June 2015.

<sup>&</sup>lt;sup>2</sup>High flows prevented sampling in the Mad River Estuary in January 2015, sampling was conducted in February instead.

<sup>&</sup>lt;sup>3</sup>Ten Mile River Estuary had a second beach seine station in June 2014 and January 2015, immediately opposite the first station on the far bank of the river. These were collapsed into a single station when a third seine was added to all stations in June 2015. The third seine was performed from the far bank, at the location of the previous second station.

part of monthly sampling were taken along the low-tide line only, and numbered five per

station (Table 2).

River Estuary, June 2014– June 2016.					
		Benthic cores	Staghorn	English	
	Beach seines	(low-tide only)	Sculpin	Sole	
Months	Jun, Nov 2014	Jun 2014			
	Jan, Apr 2015	Jan, Jun 2015			
	Jun-Nov 2015	Sep-Nov 2015	Jun-Nov 2015	Jun-Nov 2015	
	Feb-Jun 2016	Feb-Jun 2016	Feb-Jun 2016	Feb-Jun 2016	
n	127	155	372	72	

Funding guidelines for the MPA Estuary Project explicitly precluded expenditures related to water quality monitoring. Furthermore, the remoteness of our study systems prevented previous collection of water quality data. The MPA Estuary project therefore improved environmental measures throughout the study period. For all sampling events, air temperature, weather, and tidal conditions were recorded. Starting in January 2015, water quality was monitored using a Yellow Springs Instrument (YSI) 6600 Data Logger Sonde. At a single location at each site, measurements were taken for temperature (°C), salinity (ppt), dissolved oxygen (%D.O.), and pH (Appendices E-G).

Environmental data were supplemented by flow data from the United States Geologic Survey (USGS) for the Mad, Navarro, and Noyo rivers; there are no flow gauges on the Big and Ten Mile rivers. The Navarro River lies 13 miles south of the Big River. The Noyo River lies ten miles south of the Ten Mile River. Flow values downloaded from USGS were used to calculate mean flow for the week prior to sampling (henceforth flow). Data were also supplemented by average daily temperature data

<sup>&</sup>lt;sup>1</sup>High flows prevented sampling in the Mad River Estuary in December 2015, January 2016, and March 2016. Sampling was conducted twice in April, 2016 to make up for the lost sampling occasion in March (Appendix F).

downloaded from the National Oceanic and Atmospheric Administration (NOAA), and precipitation data downloaded from the California Data Exchange Center (CDEC). Eric Bjorkstedt of HSU provided Sea Surface Temperature (SST) data for each estuary, which was used to calculate monthly mean SST.

John Largier of the University of California, Davis (UCD) provided additional environmental data for the Big and Ten Mile river estuaries, as part of a complementary study of the physical characteristics of estuarine MPAs. During summer sampling in 2014 and 2015, profiles were collected at stations throughout each estuary (Appendices A and C). In 2014, temperature and salinity profiles were recorded using an YSI CastAway Sonde (Appendices A-D). In 2015, SeaBird19+ recorded profiles for temperature, salinity, dissolved oxygen, and chlorophyll fluorescence. These profiles were used to help determine the salinity regime for sampling stations in the Big and Ten Mile river estuaries (Appendices A and C).

## Sample Processing

In the field, all fish to be sacrificed for diet analysis were bagged by station and seine number, labelled, and placed on ice. Invertebrates were placed in 50 mL tubes by station and core number, and labelled. Upon returning from the field, fish were fixed in 10% formalin. All invertebrate samples were fixed in 5% formalin. After at least a week in formalin, all fish and invertebrate samples were then transferred to 40% isopropyl alcohol. Invertebrate samples were processed by another HSU laboratory (Frank Shaughnessy, HSU Biological Sciences) and identified to the lowest possible taxon. Only presence/absence of each species was recorded for each core. Fish for the diet study were measured to the nearest mm for total and standard length, dissected, and their stomach contents identified to an appropriate taxonomic rank (e.g. order for copepods, family for insect larvae, species for amphipods) used for later analysis. To estimate the number of individuals, only heads were counted.
## Data Analysis

#### Physical and Environmental Data

This study examines seasonal fish and benthic invertebrate communities in the Big, Mad, and Ten Mile river estuaries, as they relate to physical and environmental factors. Physical variables included study system, river kilometers (RKM), and salinity regime. The RKM for each station were determined in ArcMap 10.2.2 and treated as a quantitative variable. Salinity regime was assigned to each station based on salinity profiles from UCD and salinity measurements taken at the time of fish sampling (Appendices A, C, and E-G). Salinity regimes were defined as: oligohaline (0.5-5 ppt), mesohaline (5-18 ppt), or polyhaline (18-30 ppt, FishBase 2017). Environmental data used in analysis included mean daily air temperature (°C), mean monthly sea surface temperature (SST, °C), mean weekly flow (cfs), and mean monthly precipitation (cm). Air temperature, SST, flow, and precipitation values were log transformed prior to analysis.

### Fish Catch Data

Fish catch per unit effort of the beach seine (CPUE<sub>s</sub>) was calculated at each station for species i (Equation 1) as:

$$CPUE_s = \frac{\sum C_i}{N}$$
 [Eqn. 1]

Where  $C_i$  is the total station catch for species *i* for a given sampling date, and *N* is the number of seines conducted at a station (typically three seines). Fish catch per unit effort for the fyke net (CPUE<sub>f</sub>) was calculated separately as (Equation 2):

$$CPUE_f = \frac{c_i}{h_f}$$
 [Eqn. 2]

Where  $C_i$  here is the total catch of species *i* in the fyke net for a given sampling date, and  $h_f$  is the total number of hours the net was fished (typically twelve hours). CPUEs and CPUEf values were used to calculate total species richness for each estuary. Fyke net data was excluded from further analysis. Seine catch values were then used to calculate the Shannon Diversity Index for each station (H'; Equation 3).

$$H' = \sum_{i=1}^{n_i} p_i \log(p_i)$$
 [Eqn. 3]

Where  $p_i$  is the proportion of the total number of individuals of the *i*<sup>th</sup> species caught at a station, and  $n_i$  is the number of species caught at a station. H' values were averaged across stations and plotted by estuary and sampling month. Finally, CPUE<sub>s</sub> values were averaged across stations and used as input for all further analyses.

Three fish species common to all three systems were selected for species analysis: Three-spine Stickleback (*Gasterosteus aculeatus*), Pacific Staghorn Sculpin, and English Sole. Fish length distributions were examined for all three species. A negative binomial

generalized linear model (GLM) was then used to determine predictors of abundance for Three-spine Stickleback, Staghorn Sculpin, and flatfish (i.e. English Sole, Starry Flounder (*Platichthys stellatus*), and Speckled Sanddab (*Citharichthys stigmaeus*)). Predictor variables examined included: season (i.e. summer and winter), system (i.e. Big River Estuary, Mad River Estuary, and Ten Mile River Estuary), RKM, air temperature, SST, and year. For analysis of additional sampling in the Mad River Estuary, predictor variables included: season (i.e. summer, fall, winter, and spring), station (i.e. mouth, tributary, upstream), air temperature, SST, and flow. Multicollinearity was determined using a Variance Inflation Factor (VIF). Predictor variables with a VIF of five or above were excluded from consideration. This led to the exclusion of year for analysis of biannual data and season for analysis of additional sampling in the Mad River Estuary. Model strength was judged by Aikake's Information Criterion corrected for small sample sizes (AICc). Retained models were within four AICc points of the top model and had model weights of at least 10%. Finally, partial residual plots were used to display the relationship between abundance and the predictor variables for the top model for each fish taxa.

Patterns in abundance were examined for the overall fish community using non-Metric Multidimensional Scaling (nMDS) and Permutational Multivariate Analysis of Variance (PERMANOVA), using the vegan (Oksanen et al. 2017) and MASS (Venables et al. 2002) packages in R. An ordination technique frequently used with ecological data, nMDS is favored because it makes few assumptions and stands up well to datasets with large numbers of zeros (Clarke and Warwick 1997). Ordination literally "puts things in order", and consists of a family of multivariate methods that group observations based on their composition (ter Braak 1987). Ecological data are grouped according to the species composition of each sample. Then, researchers can determine whether groupings reflect known ecological gradients, such as temperature, substrate type, or latitude. I grouped samples in nMDS analysis according to system and season for biannual data across all three estuaries. For additional sampling in the Mad River Estuary, samples were grouped according to season and station for fishes, invertebrates, and Staghorn Sculpin diet.

Ordination analysis maximizes rank order correlation among observations without *a priori* information regarding explanatory variables. This makes nMDS a useful exploratory technique for identifying interesting patterns in a dataset. However, as the analysis is not constrained to be a function of explanatory variables, additional techniques must be used to identify significant relationships. PERMANOVA is a common follow-up technique that attributes the similarities between observations to explanatory variables. It thereby determines which explanatory variables significantly drive observed trends.

For this analysis, sample-specific species abundance values from beach seines were square root transformed and standardized using Wisconsin double standardization, which standardizes first by species maxima and then by sample maxima. Transformation and standardization of abundance values allows highly abundant species and less abundant species more equal contributions to the final solution (Legendre and Gallagher 2001). Rare species were removed from analysis to prevent an excessive influence on final results. Rare fishes were defined as any species where total catch over the entire study period was five individuals or fewer. Rare species included Lingcod (*Ophidon*  *elongatus*) for biannual analysis and Sacramento Sucker (*Catostomus occidentalis*) for Mad River Estuary analysis.

Transformed and standardized abundance values were used to generate a Bray-Curtis dissimilarity matrix, which robustly measures ecological distance across variations in species abundance (Faith et al. 1987). This dissimilarity index was used to calculate nMDS solutions in two dimensions. Solutions with stress values of 0.25 or less were retained. Stress is a measure of how closely true patterns in the data match those projected into ordination space. Lower stress values indicate solutions that closely align with ecological distances, while higher stress values indicate less optimal solutions (ter Braak 1987).

PERMANOVA was used to find the extent to which variation in community composition could be attributed to environmental variables. PERMANOVA analyzes the similarities in the Bray-Curtis dissimilarity matrix and identifies which variables are significantly related to how the dataset maps along an ecological gradient (Reum and Essington 2008). It does this by permuting the dissimilarity matrix and calculating a pseudo-p statistic reflecting the likelihood that the null hypothesis is true. For PERMANOVA, the null hypothesis is that the centroids of all groups of data points are equidistant. In other words, a random distribution in the data is likely to result in equidistant groupings, while a non-random distribution will result in non-equidistant groupings. Each pseudo-p-value is compared to the p-value for the original dissimilarity matrix. These comparisons are used to determine the final p-value, which is the likelihood that the original dissimilarity matrix could have been randomly generated. PERMANOVA determines the amount of variation explained by each explanatory variable, after taking all other input variables into account (Clarke and Warwick 1997). The significance of each variable is stated as a p-value and the amount of variation explained by a variable is  $r^2$ . Explanatory variables were retained when they were significant (p < 0.05) and their  $r^2$  value was greater than 0.02. The total variation explained by all explanatory variables combined is  $R^2$ . In ecological datasets,  $R^2$  values of 0.5 or more, or a PERMANOVA that explains 50% of the variation in the data, are considered good solutions (Clarke and Warwick 1997).

Explanatory variables used in the PERMANOVA for the biannual fish communities included: system, season, year, air temperature, SST, flow, and precipitation. The same variables were used in analysis of Mad River data, except station replaced system as a predictor of abundance, and year was removed from consideration due to unequal sampling across years. The explanatory variables used in this analysis were guided by previous analyses of fish communities on the Oregon coast (Desmond et al. 2002).

#### Benthic Invertebrate Data

Prey availability data from the benthos were analyzed in a manner similar to the fish analysis using frequency of occurrence (%FO). Frequency of occurrence was calculated for each station as the number of cores containing the taxa divided by the total number of cores collected multiplied by 100:

$$\% FO = \frac{\# of samples containing taxa}{\# of samples processed} *100$$
 [Eqn. 4]

For biannual sampling, cores were pooled across the low and mid-tide lines (five cores per transect), and %FO was averaged across stations for a given estuary and sampling date. For analyses examining the Mad River Estuary, %FO was only calculated using the low-tide transect lines, because this was the only transect line consistently sampled during additional sampling (i.e. monthly and including the tributary station). %FO was examined at various levels of taxonomic resolution (i.e. all benthic invertebrates, the functional groups shown in table 3, and the highest taxonomic resolution possible). For example, to facilitate comparisons of benthic invertebrate data to fish diet data, %FO was calculated using benthic invertebrate functional groups (Table 3) that reflected the taxonomic resolution that was readily identifiable in stomach contents. Total species richness was used in lieu of a diversity index, where species richness is the total number of species detected in an estuary for a given sampling period.

Patterns in benthic community composition were examined via nMDS and PERMANOVA, using species %FO values. Transect was not significant in preliminary analyses, so transects were pooled across stations for final nMDS analysis. Following the analyses done by Desmond et al. (2002), RKM, air temperature, SST, precipitation, flow, salinity regime, and year were used as input variables for invertebrate data for the biannual analysis. Input variables for analysis of additional sampling in the Mad River Estuary included: season, station, SST, precipitation, and flow.

Functional Group	Scientific Name	BRE	MRE	TMR
Amphiods	Americorophium salmonis	Х	Х	Х
Amphiods	Americorophium spinicorne	Х	Х	Х
Amphipods	Eogammarus confervicolus	Х	Х	Х
Barnacles	Balanus glandula		Х	
Bivalves	Chlamys rubida		Х	
Bivalves	Clinocardium nuttallii			Х
Bivalves	Cryptomya californica	Х		
Bivalves	Macoma balthica	Х		Х
Bivalves	Macoma inquinata	Х		
Bivalves	Macoma nasuta	Х		Х
Bivalves	Mya arenaria	Х		Х
Bivalves	Nutricola tantilla	Х		
Crabs, Shrimp & Mysids	Hemigrapsus oregonensis	Х	Х	
Crabs, Shrimp & Mysids	Neomysis mercedis	Х		Х
Crabs, Shrimp & Mysids	Neotrypaea californiensis	Х		
Insects & Spiders	Chironomidae Larvae		Х	
Insects & Spiders	Non-chironomid Diptera Larvae		Х	Х
Insects & Spiders	Trichocorixa reticulate		Х	
Isopods	Excirolana chiltoni		Х	Х
Isopods	Gnorimosphaeroma noblei		Х	
Isopods	Gnorimosphaeroma oregonensis		Х	
Non-Polychaete Worms	Phoronis pallida			Х
Non-Polychaete Worms	Phoronopsis viridis	Х	Х	Х
Polychaete Worms	Eteone californica	Х		
Polychaete Worms	Hesperonoe complanata	Х		
Polychaete Worms	Mediomastus ambiseta		Х	Х
Polychaete Worms	Neanthes brandti	Х	Х	
Polychaete Worms	Neanthes limnicola	Х	Х	Х
Polychaete Worms	Polydora nuchalis	Х		
Polychaete Worms	Pygospio elegans			Х

Table 3. Common invertebrate species listed alphabetically by functional group for the Big, Mad, and Ten Mile river estuaries, 2014-2016. "X" signifies species presence.

### Diet Data

In the Mad River Estuary, a diet study was conducted for two focal species: Pacific Staghorn Sculpin and English Sole. For each fish species, debris and organisms too digested for identification were not considered in analysis. Stomach contents were summarized in terms of %FO (Equation 4), where each stomach was considered as a sample. %FO values were calculated separately by species and by functional group (Table 3). Key prey groups were then determined by plotting prey-specific abundance against %FO (Chipps and Garvey 2007). Prey-specific abundance (A<sub>i</sub>; Equation 5) is defined here as the mean abundance of prey type *i* within stomachs that contain prey *i* (excluding all stomachs that did not contain prey *i*). Following Chipps and Garvey (2007) A<sub>i</sub> is calculated as:

$$A_i = \frac{\sum_{j=1}^{n_i} S_i}{n_i}$$
 [Eqn. 5]

Where  $A_i$  is prey-specific abundance,  $S_i$  is the number of prey *i* within a stomach that contained prey *i*.  $S_i$  is summed across all *j* fish that consumed prey *i*, and  $n_i$  is the total number of fish that consumed prey *i*. As an example, consider six fish captured at a station, three of which have consumed 1, 10, and 7 individuals, respectively, of prey type *i*. The prey specific abundance would be six, as stomachs not containing prey type *i* are excluded from the calculation:

$$A_i = (1+10+7)/(3) = 6$$

Prey groups with a prey-specific abundance of two or less that appeared in fewer than 2% of stomachs were removed from further analysis. When plotting prey-specific abundance

against %FO (Chipps and Garvey 2007), dominant prey groups are defined as those that occur frequently (high %FO) but also have high abundance when consumed (high A<sub>i</sub>).

Diets were also summarized as percent composition by abundance (% $N_i$ ) for each prey group *i* as:

$$\% N_i = \frac{a_i}{\sum_i a_i} *100$$
 [Eqn. 6]

Where  $a_i$  is the abundance of prey group *i* in all stomachs collected in a given sampling period.

Lastly, Staghorn Sculpin diet was analyzed using nMDS and PERMANOVA according to the methods described previously. Staghorn Sculpin diets were modeled using the same factors as used for the benthic invertebrate community analyses to see if diet patterns mimicked those of the prey community.

Results are described in the following order: first, a comparison of fish catch across all three estuaries; second, an examination of monthly fish catch in the Mad River Estuary; third, benthic invertebrate results for biannual sampling across all estuaries and for monthly Mad River sampling; and finally, results from the diet study on Staghorn Sculpin and English Sole in the Mad River Estuary.

#### RESULTS

### **Biannual Fish Sampling**

Forty-two fish species were encountered during summer and winter sampling in the Big, Mad, and Ten Mile river estuaries (Appendix H). Of these fish species, 33 were caught in the Big River Estuary, 34 were caught in the Mad River Estuary, and 18 were caught in the Ten Mile River Estuary. Many of the species unique to the Big River Estuary were coastal sculpins, including: Padded Sculpin (Artedius fenestralis), Bonyhead Sculpin (Artedius notospilotus), and Red Irish Lord (Hemilepidotus hemilepodotus). Additionally, Lingcod, Silverspot Sculpin (Blepsias cirrhosus), Striped Surfperch (Embiotica lateralis), and White Surfperch (Phanerodon furcatus) were only captured in the Big River Estuary. Fishes unique to Mad River Estuary catch included: Jacksmelt (Atherinopsis californiensis), Rough Sculpin (Cottus asperrimus), Northern Anchovy (*Engraulis mordax*), Bay goby (*Lepidogobius lepidus*), Saddleback Gunnel (Pholis ornata), Sacramento Sucker, and Grass Rockfish (Sebastes rastrelliger). No fish species were unique to the Ten Mile River Estuary. Seasonal abundance was highest in summer and lowest in winter for most species, but abundance of three marine species increased slightly in the Big River Estuary in winter: Surf Smelt (Hypomesus pretiosis), Starry Flounder (*Platichthys stellatus*), and Bay Pipefish (*Sygnatus leptorhynchus*; Appendix I). Across seasons, abundance was highest at the downstream stations and lowest at the upstream stations for all three estuaries (Appendix J).

Summer catch was dominated by Topsmelt (*Atherinops affinis*), Pacific Herring (*Clupea pallasi*), Pacific Staghorn Sculpin, and English Sole in the Big River Estuary (Appendix I). In the Mad River Estuary, Staghorn Sculpin and Three-spine Stickleback dominated catch year-round. In the Ten Mile River Estuary, only Staghorn Sculpin consistently dominated the summer catch. However, this excludes fyke net catch at the upstream station in the Ten Mile River Estuary, where in summer we caught thousands of Shiner Surfperch (*Cytomagaster aggregata*) and hundreds of Three-spine Stickleback. Seasonal shifts in overall abundance during biannual sampling were striking in each of the estuaries, ranging from a maximum catch of approximately 450 fish per seine in summer to a maximum of 13 fish per seine in winter (Figure 7).



Figure 7. Mean catch per seine during summer (left) and winter (right) sampling events, June 2014– June 2016, for all three estuaries. From left to right, systems are shown as follows: blue for the Big River Estuary (light), red for the Mad River Estuary (intermediate), and green for the Ten Mile River Estuary (dark). Error bars denote minimum and maximum station catch. Ten Mile catch values are from the mouth station only. Note change in scale of vertical axis between summer and winter.

Increased summer abundances were accompanied by increased species richness

(Figure 8). Species richness tended to follow trends similar to abundance: greatest in the

Big River Estuary, intermediate in the Mad River Estuary and lowest for the Ten Mile River Estuary, where fewer fish species were captured year round, and fewer species were unique to summer catch. However, most fish species contributing to increased species richness were captured rarely, while estuarine residents, such as Pacific Staghorn Sculpin and Three-spine Stickleback, or pelagic schooling species, such as Pacific Herring or Surf Smelt, contributed disproportionately to catch year-round (Appendix I). High summer abundances were largely driven by large catches of a few, highly abundant, species, increasing variability in summer catch across years and systems (Figure 7).



Figure 8. Total fish species richness for biannual sampling. From left to right, systems are shown as follows: blue for the Big River Estuary (light), red for the Mad River Estuary (dark), and green for the Ten Mile River Estuary (intermediate).

Due to low species evenness, shifts in abundance and richness were more pronounced than shifts in diversity, which accounts for relative species abundance (Figures 7-9). Summer and winter Shannon Diversity Index (H') values were often comparable (Figure 9). For example, the Big River Estuary saw a drop in diversity in winter 2015, followed by a corresponding increase in summer 2015. However, the lack of species evenness in summer 2015 and the unusually high diversity in winter 2016 subsequently flattened this trend. Only Mad River Estuary experienced regular drops in diversity during winter sampling events. In the Ten Mile River Estuary, sand bars cut-off ocean access in summer 2014, but the mouth was open in summer 2015, corresponding to relatively low and relatively high species diversity, respectively (Figure 9).



Figure 9. Mean Shannon Diversity (H') and 95% confidence intervals for biannual sampling. Each station was treated as a unique sample. Systems are shown as follows: blue for the Big River Estuary (light), red for the Mad River Estuary (dark), and green for the Ten Mile River Estuary (intermediate). Lines connect seasonal values for clarity. Dotted line represents zero.

Fish size was also examined for three focal species: Three-spine Stickleback, Pacific Staghorn Sculpin, and English Sole. Three-spine Stickleback caught in summer tended to be longer than those caught in winter, except for the last summer of sampling (Figure 10). Size shifts followed a pattern similar to H': Big River Estuary saw size shifts for Three-spine Stickleback in the first year of sampling, but catch of exceptionally large fish in winter 2016 subsequently flattened this trend. Seasonal size shifts were most obvious in the Mad River Estuary, and Three-spine Stickleback length was relatively stable across seasons in the Ten Mile River Estuary (Figure 10).

Pacific Staghorn Sculpin are estuarine residents for the first one to two years of life (Moyle 2002) and only showed modest shifts in length between seasons and systems (Figure 11). In summertime, large numbers of Staghorn Sculpin were caught over a broad range of lengths. Staghorn catch included juveniles as small as 11 mm rearing in the estuary, and mature adults as large as 140 mm.

English Sole move into estuaries in spring and summer as juveniles and return to the ocean by fall. Consequently, we only caught English Sole in the summer. Length of English Sole showed no clear size trends during the study (Figure 12). Size did not appear to differ between estuaries or years. The largest individuals were captured in summer 2015 in the Ten Mile River Estuary (Figure 12).



Figure 10. Length distributions for Three-spine Stickleback (*Gasterosteus aculeatus*) from top to bottom: the Big River Estuary (blue/light), the Mad River Estuary (red/dark), and the Ten Mile River Estuary (green/intermediate).



Figure 11. Length distributions for Staghorn Sculpin (*Leptocottus armatus*). From top to bottom: the Big River Estuary (blue/light), the Mad River Estuary (red/dark), and the Ten Mile River Estuary (green/intermediate).<sup>1</sup>

1: Sample sizes in excess of 180 due to three days of fieldwork in June 2015 where two measuring crews were used.



Figure 12. Length distributions for English Sole (*Parophrys vetulus*) from top to bottom: the Big River Estuary (blue/light), the Mad River Estuary (red/dark), and the Ten Mile River Estuary (green/intermediate).<sup>1</sup>

1: Sample sizes in excess of 180 due to three days of fieldwork in June 2015 where two measuring crews were used.

Abundances of Three-spine Stickleback, Pacific Staghorn Sculpin, and flatfishes were also examined for their relationships to environmental trends. Catch per seine of Three-spine Stickleback during biannual sampling was mostly dependent on RKM (Figure 13). Some of the highest catches for Three-spine Stickleback occurred at the upstream station in the Mad River Estuary, which was 8.2 RKM upstream from the river mouth (Appendix J). In the Big and Ten Mile river estuaries, Three-spine Stickleback abundance was higher at upstream stations than at stations near the mouth. Season and air temperature showed up as secondary predictors of abundance in three of the four top models (Table 4). Secondary predictors reflected increased abundances of Three-spine Stickleback in summer, when air temperatures were higher.



Figure 13. Partial residual plot for the top model predicting Three-spine Stickleback abundance as a function of river kilometers (RKM). All continuous predictor variables were centered and standardized prior to analysis.

Tuble 1. Top four models predicting Timee spine blerkebuer ubundunee.					
Model	Κ	logLik	AICc	delta	weight
Three-spine Stickleback~RKM	3	-51.27	109.67	0.000	0.543
Three-spine Stickleback~RKM+Air Temperature	4	-50.83	111.66	1.981	0.202
Three-spine Stickleback~RKM+Season	4	-51.24	112.49	2.811	0.133
Three-spine Stickleback~RKM+Air					
Temperature+Season	5	-50.24	113.64	3.968	0.075

Table 4. Top four models predicting Three-spine Stickleback abundance.

Abundance of Pacific Staghorn Sculpin was primarily dependent on season (Figure 14), tracking overall seasonal trends in abundance (Figure 7). Air temperature and RKM showed up as additional predictors of abundance in lower-ranked models (Table 5). Staghorn Sculpin are estuarine residents that dominated winter catch in all three estuaries (Appendix I) with catches of five to ten individuals per seine. In summer, Staghorn Sculpin continued to dominate catch in all three estuaries with increased abundances of 50-100 individuals per seine.



Figure 14. Partial residual plot for the top model predicting Pacific Staghorn Sculpin abundance as a function of season.

Table 5. Top three models predicting Pacific Staghorn Sculpin abundance. Model Κ logLik AICc delta weight Staghorn Sculpin~Season 3 -109.2 225.6 0.593 0 -109.1 228.1 Staghorn Sculpin~Season+Air Temperature 4 2.54 0.166 Staghorn Sculpin~Season+RKM 4 -109.2 228.4 2.79 0.147

Due to low catches of English Sole in some years, English Sole abundance could not be analyzed via GLM analysis. Instead catch was pooled for all flatfish species: English Sole, Starry Flounder, and Speckled Sanddab. Flatfishes are seasonal users of estuaries, so unlike Three-spine Stickleback and Staghorn Sculpin, flatfish were not present year-round. Their abundance was best predicted by season and air temperature (Table 6), with a pronounced positive relationship between summer and flatfish abundance (Figure 15). Surprisingly, flatfish abundance was negatively correlated to air temperature. This may be due to the low seasonal variation in coastal air temperatures, or the cool, foggy, weather typical of coastal summers in Northern California. RKM was a weak predictor of flatfish catch, reflecting the larger catches of flatfish at mouth stations as compared to upstream stations. Differences in flatfish abundance between stations were less pronounced than the large seasonal swings in flatfish abundance.



Figure 15. Partial residual plots for the top model predicting flatfish abundance as a function of season and air temperature. All continuous predictor variables were centered and standardized prior to analysis.

Table 6. Top two models predicting flatfish abundance.

Model	K	logLik	AICc	delta	weight
Flatfish~Season+Air Temperature	4	-65.34	140.7	0	0.775
Flatfish~Season+Air Temperature+RKM	5	-65.24	143.6	2.94	0.178

After examining seasonal trends in abundance and diversity, I compared the three estuaries to each other directly using nMDS and PERMANOVA. The resulting ordination had a stress value of 0.179. Stress values close to 0.1 indicate a good solution in ordination that is unlikely to display separations between groups that don't exist in the data. Stress values near or under 0.2 indicate that the ordination portrays a useful picture of true separation of groups within the data (Clarke and Warwick 1997). PERMANOVA of the dissimilarity matrix underlying the ordination resulted in an R<sup>2</sup> value of 0.54, indicating that 54% of the variation in the data was explained by input variables, primarily season ( $r^2 = 0.24$ , p = 0.001) and system ( $r^2 = 0.12$ , p = 0.004). Other input variables included: air temperature, flow, precipitation, and year.

The analysis was able to differentiate between systems, and revealed that the Mad River Estuary overlapped both Big and Ten Mile river estuaries more than they overlapped each other (Figure 16), but had more in common with the Ten Mile River Estuary than with the Big River Estuary. One axis (displayed as nMDS 1 in this ordination) appeared to be associated with system and salinity (Figures 16-19). The other appeared to be associated with season and the associated variables: flow, air temperature, and precipitation.



Figure 16. Two dimensional nMDS by system for biannual sampling. Each point represents sampling at a single station on a single date. Rare species were removed to improve fit. Polygons outline species by the system where they were caught as follows: blue dots for the Big River Estuary, red triangles for the Mad River Estuary, and green squares for the Ten Mile River Estuary.



Figure 17. Species breakdown of a two dimensional nMDS by system for biannual sampling. Rare species were removed to improve fit. Polygons outline sampling instances by system as follows: blue for the Big River Estuary (light), red for the Mad River Estuary (dark), green for the Ten Mile River Estuary (intermediate). Species reflect species loadings for catch in each estuary. Species codes identified alphabetically in the table to the left.



Figure 18. Two dimensional nMDS by season for biannual sampling. Each point represents sampling at a single station on a single date. Rare species were removed to improve fit. Points are filled for summer sampling events and hollow for winter sampling events. Points are colored by system as follows: blue dots for Big River Estuary, red triangles for Mad River Estuary, and green squares for Ten Mile River Estuary. Points for summer samples are filled, winter samples are hollow. Polygons outline summer (rose/dark) and winter (blue/light) sampling events.



Figure 19. Species break down of a two dimensional nMDS by season for biannual sampling. Rare species were removed to improve fit. Polygons outline samples from summer (rose/dark) and winter (blue/light) catch. Species reflect species loadings for seasonal catch. Species codes identified alphabetically in the table to the left.

The Big River Estuary was characterized by a higher diversity of more typicallymarine species, especially Topsmelt (Atherinops affinis), juvenile rockfish (Sebastes spp.), and Kelp Greenling (*Hexogrammos decagrammus*; Figure 17). This was expected, as wide mouth of the Big River Estuary allows brackish waters to extend much farther upstream compared to the other two estuaries. The appearance of Big River Estuary to the right reflects a more marine community, suggesting the ordination may have separated samples according to salinity preference or system. The marine Silverspot Sculpin appeared to the right, and was only found in Big River Estuary (Figure 17). Towards the left of the ordination, Coastrange Sculpin (*Cottus aleuticus*), Prickly Sculpin (Cottus asper), and Coho Salmon (Oncorhynchus kisutch) were captured as juveniles in the low-salinity upper portion of the Mad River Estuary. To test whether community composition was tied to salinity, PERMANOVA was run with two factor variables related to salinity: RKM and salinity regime. Neither factor significantly determined the biannual fish community (p >> 0.05). However, this may have been due to the predominance of polyhaline sampling locations within the biannual beach seine data. In all three estuaries, the downstream stations were polyhaline. In the Big River Estuary, the upstream station was also polyhaline. In the Mad River Estuary, the upstream station was oligohaline. In the Ten Mile River Estuary, the upstream station was excluded from ordination analysis as it was sampled via fyke net. Although RKM and salinity regime were not significant in analysis, salinity preference did appear to be reflected in how species grouped by system.

The Big River Estuary was the most different from the other two systems, driven by a higher diversity of marine fishes and continued catch of marine species in winter 2016 (Figures 9, 17, and 19). Specifically, separation of the Big River Estuary from the other two estuaries was primarily driven by high catches of marine species that gave birth in the estuary, such as Bay Pipefish, Striped Surfperch, and Shiner Surfperch; and by coastal sculpins primarily captured in the Big River Estuary, including Buffalo Sculpin (Enophrys bison) and Bonyhead Sculpin. Separation of the Mad River fish community from the other two estuaries was driven primarily by Coho Salmon and Speckled Sanddab, which were both caught in large numbers during summer sampling (Appendix I). The Mad River Estuary was additionally characterized by the same estuarine species seen in the Ten Mile River Estuary, but stronger ocean influence resulted in a greater diversity of anadromous and marine fishes, and greater overlap with the fish community found in Big River Estuary. Chinook Salmon (Oncorhynchus tshawytscha), English Sole, and Night Smelt (Spirinchus starski) were particularly important in driving overlap between the Big and Mad river estuaries. The Ten Mile River Estuary experienced less ocean influence than the other two systems and had no unique fish species. It was characterized by a hardy estuarine resident fish community of Pacific Staghorn Sculpin, Three-spine Stickleback, and Prickly Sculpin. Steelhead (O. mykiss) and Starry Flounder also typified the Ten Mile River Estuary fish community.

Season explained 24% of the variation in species composition, reflecting the stark difference between the diverse and abundant summer fishes and the few hardy residents of winter (Figure 18). Season is related to temperature, flow, and precipitation.

PERMANOVA found that both the air temperature and flow were significantly related to the distribution of fishes, but the effect was weak; air temperature and flow each explained 6% of the variation in the data (p = 0.02 and 0.017). Precipitation was not significant, but explained a similar amount of variation in the data ( $r^2 = 0.04$ , p = 0.08). Some temporal variation was also attributed to year, which explained 5% of the variation in the data (p = 0.03). Fish species that grouped by season tended to share similar salinity preferences, as most non-resident estuarine users are marine fishes that return to the ocean by winter (Figure 19). Consequently, the summer fish community was primarily driven by the obligate estuarine users Chinook Salmon and English Sole. Meanwhile, winter catch reflected a sculpin-dominated community of estuarine residents, such as Prickly Sculpin, Pacific Staghorn Sculpin, and Three-spine Stickleback. However, some marine fish in the Big River Estuary were more typical of winter catch than summer catch, such as Surfsmelt, which was encountered year-round in the Big River Estuary.

#### Mad River Fish Sampling

Thirty-four species were captured in the Mad River Estuary during biannual sampling and no novel fish species were detected during additional seasonal and monthly sampling (Appendices H and K). More fish species were captured at the mouth and tributary stations, although certain species were unique to upstream catch (Appendix K). For example, Sacramento Sucker juveniles were captured at low salinities (~1 ppt) at the upstream station in fall 2015 and summer 2016. Abundance for most species was higher in summer and fall and lower in winter and spring (Appendix L). Two species had higher abundance in spring than fall: Pacific Staghorn Sculpin and English Sole. Staghorn Sculpin were the most consistently captured species in the Mad River Estuary, and fall abundance was modestly lower than spring (63 vs. 71 CPUE<sub>s</sub>). In contrast, English Sole were captured in relatively large numbers in spring, with an average of 36 fish per seine. By fall, English Sole rarely appeared in catch (CPUE<sub>s</sub> = 2.33). Three-spine Stickleback, like Staghorn Sculpin, dominated summer and fall catch (Appendix L). But it should be noted that summer catch of Three-spine Stickleback was heavily weighted by a single catch of over five thousand individuals in July 2015 at the upstream station. Across seasons, Pacific Herring dominated catch at the mouth and Prickly Sculpin dominated upstream catch (Appendix M). Staghorn Sculpin were captured regularly in large numbers throughout the estuary.

Consistent with biannual sampling, winter had the lowest abundances and summer the highest. Increased sampling revealed that abundance increased slowly through spring, and relatively low abundances sometimes continued through June (Figure 20). In contrast, abundances remained relatively high throughout fall 2015 sampling. From June 2014-June 2016, the largest spike in abundance occurred in July 2015. This was weighted by a single catch of Three-spine Stickleback, previously mentioned (Appendix L). Sampling was not possible in December 2015, January 2016, or March 2016, as high flows made it unsafe to seine the estuary (Figure 20). Conditions allowed for an additional sampling event in early April 2016, which replaced the missed March 2016 sampling dates.



Figure 20. Mean catch per seine across all stations in the Mad River Estuary (red bars). Mean monthly flow is shown on the second vertical axis in cubic feet per second (blue line). Error bars denote minimum and maximum station catch. Flow data courtesy of the USGS. High flows prevented sampling in December 2015 and January 2016.

Although Mad River Estuary catch spiked midsummer, marine species that would contribute to larger catches started moving into the estuary in spring, returning to the ocean in late fall. In April 2015, species richness increased across all stations, most sharply at the mouth of the Mad River, due to an influx of juvenile English Sole, saddleback gunnel, Cabezon (*Scorpaenichthys marmoratus*), and Bay Pipefish (Figure 21). Salmonid smolts such as Coho Salmon, Steelhead, and Chinook Salmon also started entering the estuary from upstream in spring. Diversity values fluctuated but remained high through summer, as osmerids, juvenile rockfish, and juvenile Starry Flounder appeared in catch (Appendix L).



Figure 21. Total fish species richness across all stations in the Mad River Estuary. High flows prevented sampling in December 2015 and January 2016.

Diversity dropped off in November 2015 as juvenile flatfishes, juvenile Copper

Rockfish (Sebastes caurinus), and coastal sculpins, such as Cabezon and Sharpnose

Sculpin (Clinocottus acuticeps) declined in catch (Figure 22). In March 2016, diversity

again increased from low winter values, as Chinook Salmon, Bay Pipefish and Topsmelt appeared in catch. Sampling ceased in June 2016.



Figure 22. Mean Shannon Diversity (H') and 95% confidence intervals for seasonal and monthly sampling in the Mad River Estuary, pooled across stations. High flows prevented sampling in December 2015 and January 2016. Red line represents zero.

Additional sampling in the Mad River Estuary also elucidated size trends, especially for estuarine resident species. Three-spine Stickleback are residents of the estuary, and mate during spring and early summer (Moyle 2002). In 2015 and 2016, Three-spine Stickleback were larger from April through June, due to the appearance of large spawning females in catch (Figure 23). Three-spine Stickleback abundance was best predicted by RKM, flow, and air temperature (Table 7). Monthly SST was also predictive of Three-spine Stickleback abundance in lower-ranked models. Abundance was positively related to upstream distance (Figure 24), reflecting higher catches at the upstream station. Abundance was negatively related to flow, reflecting higher catches in summer, when river flows are lower. Abundance was also negatively related to air temperature, possibly due to the low variability of air temperature along the coast, the cool weather typical of the Northern California Coast, or some combination therein.



Figure 23. Length distributions for Three-spine Stickleback (*Gasterosteus aculeatus*) captured in the Mad River Estuary during seasonal and monthly sampling. High flows prevented sampling in December 2015 and January 2016 (n = 2-210).

 Table 7. Top four models predicting Three-spine Stickleback abundance in the Mad

 River Estuary.

Idver Estaury.					
Model	Κ	logLik	AICc	delta	weight
Three-spine Stickleback~					
Station+Flow+Air Temperature	6	-146.8	308.0	0.00	0.421
Three-spine Stickleback~					
Station+Flow+Air Temperature+SST	7	-145.7	308.7	0.64	0.306
Three-spine Stickleback~					
Station+Flow	5	-149.3	310.1	2.11	0.146
Three-spine Stickleback~					
Station+Flow+SST	6	-149.0	312.3	4.25	0.05



Figure 24. Partial residual plots for the top model predicting Three-spine Stickleback abundance in the Mad River Estuary as a function of station, flow, and air temperature. All continuous variables were centered and standardized prior to analysis.
Another estuarine resident, Pacific Staghorn Sculpin, increased in size from winter through late summer (Figure 25). Larger individuals continued to be caught through fall, even while catch was dominated by smaller fish. The overall size pattern reflects a species that often spends its first year of life in estuaries, reaching sexual maturity before outmigrating to the ocean (Moyle and Cech 2004). Staghorn Sculpin abundance was strongly predicted by station, flow, and SST (Table 8). Air temperature showed up as predictive of Staghorn Sculpin abundance in the second model. Abundance was strongly associated with the tributary station, where Staghorn Sculpin CPUE<sub>s</sub> was twice that of the mouth and tributary stations (Figure 26; Appendix M). Staghorn Sculpin abundance was negatively related to flow and SST, reflecting summer conditions (Figure 26). In summer, river flows are low and upwelling along the coast brings cool, nutrientrich ocean waters to the surface, lowering SST. Staghorn Sculpin increased in abundance in summer catch, similar to all other fish species in the Mad River Estuary.



Figure 25. Length distributions for Pacific Staghorn Sculpin (*Leptocottus armatus*) captured in the Mad River Estuary during seasonal and monthly sampling. High flows prevented sampling December 2015 and January 2016 (n = 20-451).<sup>1</sup>

Table 8. Top two models predicting Pacific Staghorn Sculpin abundance in the Mad River Estuary.

River Estuary.					
Model	K	logLik	AICc	delta	weight
Pacific Staghorn Sculpin~					
Station+Flow+SST	6	-172.3	358.9	0.00	0.782
Pacific Staghorn Sculpin~					
Station+Flow+SST+Air Temperature	7	-172.2	361.6	2.74	0.199

<sup>1</sup>Values in excess of 270 due to one day of field work in June 2015 when two measuring crews were used.



Figure 26. Partial residual plots for the top model predicting Staghorn Sculpin abundance in the Mad River Estuary as a function of station, flow, and sea surface temperature (SST). All continuous variables were centered and standardized prior to analysis.

Size shifts for English Sole were the most pronounced of the three species examined in detail (Figure 27). English Sole increased in size from spring, when they showed up in catch as newly settled juveniles, through summer. The most significant size jump occurred between May and June, 2016. Because English Sole rear in estuaries, it was expected that they get bigger throughout their residency in preparation for outmigration. However, from August through November 2015, fish size actually decreased. This may have been due to new cohorts of smaller fish entering the estuary, or larger cohorts leaving the estuary, so that only the smallest individuals remained.



Figure 27. Length distributions for English Sole (*Parophrys vetulus*) for the months captured in the Mad River Estuary during seasonal and monthly sampling. High flows prevented sampling December 2015 and January 2016 (n = 1-68).

Due to low catches of English Sole during the first season of sampling, all flatfish species were pooled for GLM analysis: English Sole, Starry Flounder, and Speckled Sanddab. Flatfish species were present in the Mad River Estuary seasonally, and their abundance was best predicted by station, flow, and SST (Table 9). Flatfish catch was positively related to the mouth station, where juvenile flatfish were most frequently captured (Appendix M). Similar to Three-spine Stickleback and Pacific Staghorn Sculpin, Flatfish abundance was negatively related to flow and temperature (Figures 24, 26, and 28). In summer, when flow and SST are both low, catches were greatest for all fish species. Catches were lowest in winter, when flows were highest and down-welling increased ocean temperatures off the Pacific Coast (Appendix L).

Model	Κ	logLik	AICc	delta	weight
Flatfish~					
Station+Flow+SST	6	-58.4	131.1	0.00	0.608
Flatfish~					
Station+Flow+SST+Air Temperature	7	-58.1	133.3	2.16	0.206
Flatfish~					
Flow+SST+Air Temperature	4	-62.7	134.4	3.27	0.118

Table 9. Top three models predicting flatfish abundance in the Mad River Estuary.



Figure 28. Partial residual plots for the top model predicting flatfish abundance in the Mad River Estuary as a function of station, flow, and sea surface temperature (SST). All continuous variables were centered and standardized prior to analysis.

Ordination of Mad River Estuary fish catch data was based on a two-axis solution with rare species removed (Stress = 0.219; Figures 29-32). Season was the most important factor determining species composition, explaining almost 20% of the variation in the data ( $r^2 = 0.199$ , p = 0.001). Spring and fall were the most variable sampling periods and the ordination reflected this with a broad spread in spring and fall samples (Figures 29 and 30). The high diversity in these months resulted in a high degree of overlap amongst spring, summer, and fall, while winter samples were closely clumped and separate from other seasons (Figure 29). This reflects the low abundance and diversity of winter catch and suggests that separation of winter samples could be associated directly with season, or could be due to seasonal variation in temperature, flow, or precipitation. Neither air nor water temperature were significant (p > 0.05). Monthly mean SST was significant, but only explained 6% of the variation in the data (p= 0.003). Flow was significant, but only explained 3% variation in the data (p = 0.03). Precipitation was not significant (p > 0.05).



Figure 29. Two dimensional nMDS by season of Mad River fish catch. Rare species removed. Samples collected during the same season are colored and outlined by polygons as follows: light blue for winter, green for spring, rose for summer, and orange for autumn.



Figure 30. Species breakdown of a two dimensional nMDS by season for fish caught in the Mad River Estuary. Rare species removed. Polygons outline sampling instances by season as follows: light blue for winter (dotted), green for spring (dash-dot), rose for summer (solid), and orange for autumn (dashed). Species reflect species loadings for seasonal catch. Species codes identified alphabetically in table on the left.



Figure 31. Two dimensional nMDS by station of Mad River fish catch. Rare species were removed. Polygons outline samples by station as follows: light blue for upstream (light), blue for tributary (intermediate), and navy for the mouth (dark). Samples collected during the same season are color coded as follows: green for spring, rose for summer, orange for autumn, and light blue for winter.



Figure 32. Species breakdown of a two dimensional NDMS by station for fish caught in the Mad River Estuary. Rare species were removed. Polygons outline samples by station as follows: light blue for upstream (light), blue for the tributary (intermediate), and navy for the mouth (dark). Species reflect species loadings for catch at each station. Species codes identified alphabetically in table on the left.

Reflecting the seasonality of species catch, freshwater and estuarine resident species appeared to the left, while marine and estuarine migrant species appeared to the right (Figure 30). Certain species were particularly important in driving seasonal trends. Although winter catch was typified by estuarine residents, the separation of winter samples was mostly driven by Pacific Staghorn Sculpin. Spring sampling was highly variable, driven primarily by large swings in the abundance of Steelhead and Speckled Sanddab. Summer catch was characterized by typically marine species such as Jacksmelt, Penpoint Gunnel (*Apodichthys flavidus*) and Night Smelt. Finally, the fall fish community was characterized primarily by fishes that rear in the estuary as juveniles, including Copper Rockfish, Shiner Surfperch, Bay Pipefish, and English Sole. Some species also masked seasonal trends. For example, Pacific Herring did not appear to be associated with any season, perhaps due to the large but infrequent catches of Pacific Herring in the Mad River Estuary.

Ordination results displayed a visible diagonal gradient from the river mouth to the upstream station (Figure 31), suggesting that station may have been associated with one or both axes. Station location was highly significant in determining species composition, explaining 13% of the variation in the data (p = 0.001), and reflecting the importance of ocean influence in determining the fish community. At the upstream station, the fish community was more stable and was characterized by a predominance of estuarine residents. Separation of the upstream fish community was primarily driven by Steelhead and Coastrange Sculpin (Figure 32). Approximately seven kilometers downstream, the tributary and mouth stations shared similar fish communities, typified by more marine species and greater seasonal variation (Figures 31 and 32). Despite overlap between the downstream stations, some species were primarily detected at the mouth, and drove inter-station differences. These included: Saddleback Gunnel, Cabezon and Pacific Herring. Catch at the tributary station was typified by juvenile fishes, such as Copper Rockfish juveniles and Chinook Salmon smolts (Figure 32).

#### Biannual Benthic Invertebrate Data

Twenty-nine taxa were represented in biannual benthic cores (Table 3). The Big River Estuary had the highest species richness, with 18 invertebrate taxa detected, including seven unique invertebrate species: the Bay Ghost Shrimp (*Neotrypaea californiensis*), three clam species (*Cryptomya californica*, *Macoma inquinata*, and *Nutricola tantilla*), and three polychaete species (*Eteone californica*, *Hesperonoe complanata*, *Polydora nuchalis*). The Mad and the Ten Mile river estuaries each had 14 invertebrate taxa. Six invertebrate taxa were unique to the Mad River Estuary, including *Gnorimosphaeroma* isopods, *Chironomidae* larvae, the barnacle *Balanus glandula*, the clam *Chlamys rubida*, and the water boatman (*Trichocorixa reticulate*). Four invertebrate species were unique to the Ten Mile River Estuary: the clam *Clinocardium nuttallii*, the polychaetes *Mediomastus ambiseta* and *Pygospio elegans*, and the phoronid *Phoronis pallida* (Table 3).

Biannual trends in the benthic invertebrate community were similar to those seen in the fish community, although they were less pronounced. Benthic cores taken in winter were more likely to be empty and contain no invertebrates (Figure 33). There were empty cores taken in summer, but they were rare and occupied cores contained more species (Figure 34). This resulted in a higher %FO (Equation 4) and species richness for benthic invertebrates in summer versus winter (Figures 33 and 34). In analysis, %FO is defined as the proportion of cores at a station containing invertebrates.



Figure 33. Average percent frequency of occurrence of invertebrates in benthic cores. Estuaries are represented from left to right as follows: blue for the Big River Estuary (light), red for the Mad River Estuary (dark), and green for the Ten Mile River Estuary (intermediate).



Figure 34. Total species richness of invertebrates in benthic cores by season and system. Estuaries are represented from left to right as follows: blue for the Big River Estuary (light), red for the Mad River Estuary (dark), and green for the Ten Mile River Estuary (intermediate).

Species richness trends were similar to those seen for fish communities in the three estuaries (Figures 8 and 34). Species richness tended to be highest in the Big River Estuary. The Mad River Estuary was the most seasonal of the three systems, and experienced regular drops in %FO and species richness during both winters of sampling (Figures 33 and 34). Species richness in the Ten Mile River Estuary increased until summer 2015, before steadily decreasing (Figure 34). Both Mendocino estuaries experienced relatively high fish diversity and invertebrate species richness in winter 2016 (Figures 9 and 34). Interestingly, the relatively high species richness of winter 2016 in the Ten Mile River Estuary coincided with the lowest %FO values for the system (50%, Figures 33 and 34).

System was the most important factor determining invertebrate community composition in the Big, Mad, and Ten Mile river estuaries, explaining nearly 20% of the variation in the data (p = 0.001,  $r^2 = 0.197$ ). Another location-related factor, RKM, explained an additional 8% of the variation in the data (p = 0.001). However, RKM may have obscured the impact of system in ordination (Stress = 0.178, Figures 35 and 36). Specifically, samples from the Mad River Estuary had the broadest spread in ordination, overlapping both Mendocino estuaries more than they overlapped each other. This is likely due to RKM: the upstream station in the Mad River Estuary was 8.2 km from the mouth, while the upstream stations in the Big and Ten Mile river estuaries were 2.5 km and 3.8 km from the mouth, respectively. The broader salinity range represented by the Mad River Estuary stations may have swamped important inter-system differences (Figure 35).



Figure 35. Two dimensional nMDS by system of benthic invertebrates captured during biannual sampling. Rare species were removed to improve fit. Polygons outline species by the system where they were caught as follows: blue for Big River Estuary, red for Mad River Estuary, and green for Ten Mile River Estuary.



Figure 36. Species breakdown of a two dimensional nMDS by system of benthic invertebrates captured during biannual sampling. Rare species were removed to improve fit. Polygons outline species by the system where they were caught as follows: blue for Big River Estuary (light), red for Mad River Estuary (dark), and green for Ten Mile River Estuary (intermediate). Species codes listed alphabetically in table on the left.

Across the three estuaries, amphipods and polychaetes were detected in summer and winter cores (Appendix N). Inter-system differences were driven by a more diverse invertebrate fauna in the Big River Estuary year-round that included larger, burrowing, invertebrates such as the Bay Ghost Shrimp and *Macoma* clams (Figure 36). The Mad River Estuary had the most seasonally variable invertebrate community, and was typified by freshwater-loving species unique to Mad River benthic cores: *Gnorimosphaeroma* isopods and *Chironomidae* larvae (Figure 36; Appendix N). In the Ten Mile River Estuary, a diversity of functional groups was represented at low %FO values year-round, from *Macoma* clams more typical of cores taken in the Big River Estuary, to isopods and *Diptera* larvae more typical of cores taken in the Mad River Estuary (Appendix N). Additional locational variability was explained by RKM, and many taxa were unique to certain stations. For example, *Chironomidae* larvae were only found at the upstream station in the Mad River Estuary, while the isopod *Excirolana chiltoni* was predominantly found at the mouth station in the Ten Mile River Estuary (Figure 36).

Seasonal variation in the invertebrate community was not clearly differentiated in ordination (Figures 37 and 38). Season only directly explained 5% of the variation in the data ( $r^2 = 0.047$ , p = 0.03), but flow and precipitation explained 8% and 9% of the variation in the benthic invertebrate community, respectively (p = 0.001). SST explained an additional 4% of the variation in the data, although this was not significant (p = 0.07). Year was not significant either, but inter-annual variation in community composition may have been attributed to flow and precipitation, which vary on a seasonal and inter-annual basis. Seasonal community changes were mainly driven by increased %FO for the



amphipods *Eogammarus confervicolus* and *Americorophium spinicorne* in summer, and by increased abundance and richness in the summer invertebrate community (Figure 38).

Figure 37. Two dimensional nMDS by season of benthic invertebrate species captured during biannual sampling. Rare species were removed to improve fit. Ellipses denote standard deviations for summer (rose/dark) and winter (blue/light) catch. Samples collected in summer are filled, samples from winter months are hollow. Points were color coded by system: blue for Big River Estuary, red for Mad River Estuary, and green for Ten Mile River Estuary.



Figure 38. Species breakdown of a two dimensional nMDS by season of benthic invertebrates captured during biannual sampling. Rare species were removed to improve fit. Polygons outline samples from summer (rose/dark) and winter (blue/light) catch. Species codes listed alphabetically in table on the left.

#### Mad River Benthic Invertebrate Data

Fourteen invertebrate taxa were represented in benthic cores taken biannually in the Mad River Estuary, and no novel taxa were detected during monthly sampling. Monthly sampling of benthic invertebrates commenced in September 2015, when the number of empty cores dropped to zero and total species richness peaked at 14 (Figure 39). Similar to fish abundance, invertebrate occurrence decreased in November 2015 and continued to drop through winter (Figures 20 and 39). Likewise, both fish diversity (Figure 22) and invertebrate species richness (Figure 39) declined in November 2015 and were low through winter sampling. Invertebrate %FO and species richness peaked in summer and fall, when all taxa detected in the Mad River Estuary were found in benthic cores (Table 3; Appendix O). We did not sample for benthic invertebrates in July or August 2015, when the some of the highest fish catches occurred. Throughout the sampling period, benthic cores were dominated by amphipods (A. salmonis, A. spinicorne, and E. confervicolus) and polychaetes (M. ambiseta and Neanthes limnicola) (Appendix O). Gnorimosphaeroma isopods and Diptera larvae also showed up regularly in benthic cores, but were not present in all months. Other benthic invertebrates, including *Hemigrapsus* crabs and *Crangon* shrimp commonly appeared in seine bycatch but rarely appeared in cores (data not shown).



Figure 39. Average percent frequency of occurrence of invertebrates in benthic cores (red bars) and total species richness for each sampling period in the Mad River Estuary (black line). Standard error bars are for frequency of occurrence (dark red lines).

Ordination results for the benthic invertebrate community in the Mad River Estuary differed from fish results. Seasons overlapped to a far greater extent and did not appear in any discernable order (Figures 40 and 41). Rather than appearing separate from the other seasons, winter samples overlapped with other seasons and exhibited the largest spread. Similar to winter, spring samples were characterized by low %FO, low species richness, and a wide spread in ordination. In contrast, summer and fall samples had %FO, high species richness, and samples had the least spread in ordination (Figure 40). Season explained 20% of the variation in species composition for the Mad River Estuary benthic invertebrate community (p = 0.001). Precipitation explained an additional 5% of the variation in the benthic invertebrate community (p = 0.03).



Figure 40. Two dimensional nMDS by season of benthic invertebrates captured in the Mad River Estuary. Rare species were removed to improve fit. Points colored and outlined according to season as follows: green for spring, rose for summer, orange for autumn, and light blue for winter.



Figure 41. Species breakdown of a two dimensional nMDS by season for benthic invertebrates captured in the Mad River Estuary. Rare species were removed to improve fit. Polygons outline seasonal catch: orange for autumn (dashed), rose for summer (solid), light blue for winter (dotted), and green for spring (dash-dot). Species codes identified in table on the left.

Seasonal differences in the benthic invertebrate community were driven by certain taxa. For example, dragonfly larvae and the isopod *E. chiltoni* were key in defining the spring community, while the amphipod *A. salmonis* was more typical of the winter invertebrate community (Figure 41). The summer community was more diverse and abundant, reflected in the appearance of the barnacle *B. glandula* and increased %FO values for the amphipod *E. confervicolus*. The fall community had the highest %FO and species richness, reflected in a cluster of species, including *Gnorimosphaeroma* isopods, the polychaete *N. limnicola*, and the aquatic insect *T. reticulata*. Insects and insect larvae were only found at the upstream station in the Mad River Estuary, indicating the importance of station location in determining the benthic invertebrate community.

Station explained 23% of the variation in the data (p = 0.001). The analysis was able to differentiate between stations (Figures 42 and 43), with a clear upstream to downstream trend starting in the lower right and going diagonally upwards to the middle of the plot, and complete separation between samples taken at the mouth and those taken upstream (Figure 42). Season and station are related to a suite of variables. Season is associated with changes in temperature and flow, while each station was unique in terms of substrate and salinity regime. Therefore, separation by season and station reflect underlying environmental gradients in the data. The stress of the ordination analysis at 0.136 indicates a good ordination solution. Station, season, and precipitation together explained 48% of the variation in species composition.



Figure 42. Two dimensional nMDS by station for benthic invertebrates captured in the Mad River Estuary. Rare species were removed to improve fit. Lines connect samples by station, labelled for clarity as follows: navy for the mouth station (dark), blue for the tributary station (intermediate), and light blue for the upstream station (light). Samples color coded by season: green for spring, rose for summer, orange for autumn, and light blue for winter.



Figure 43. Species breakdown of a two dimensional nMDS by station for benthic invertebrates captured in the Mad River Estuary. Rare species were removed to improve fit. Polygons outline samples by station as follows: pale blue for the upstream site (light), royal blue for the tributary site (intermediate), and navy for the river mouth (dark). Species denote loadings by station. Species codes identified alphabetically in table on the left.

Inter-station differences in the benthic invertebrate community in the Mad River Estuary were driven by certain species. For example, the polychaete *M. ambiseta*, the ribbon worm *Paranemertes peregrina*, the amphipod *A. salmonis*, and *Diptera* pupae were typical of the invertebrate community at the river mouth (Figure 43). The invertebrate community at the tributary station was more similar to that at the mouth and shared less overlap with the upstream station (Figures 42 and 43). The crab *Hemigrapsus oregonensis* was the species most characteristic of benthic cores taken at the tributary station (Figure 43). The invertebrate community at the upstream station was typified by *Chironomidae* larvae and by water boat-man. Upstream cores also contained *Gnorimosphaeroma* isopods and the polychaete *N. limnicola*.

#### Mad River Diet Study

The Mad River Estuary invertebrate community was also studied via the diets of Staghorn Sculpin and English Sole. From June 2015-June 2016, 373 Staghorn Sculpin and 72 English Sole were sacrificed for diet analysis. Twelve Staghorn Sculpin had stomachs that were empty or only contained debris. Six English Sole had empty stomachs. Due to small sample sizes for English Sole in some months, ordination analysis was limited to Staghorn Sculpin diet.

Staghorn Sculpin preyed primarily on invertebrates, although one Staghorn Sculpin had eaten an inviable salmon egg and another exhibited cannibalism. Staghorn Sculpin are opportunistic predators (Moyle 2002) and feed in the mud and in the water column. Their diet in the Mad River reflected both pelagic invertebrates found in the beach seine (e.g. *Crangon* shrimp) and benthic invertebrates found in cores (e.g. *Gnorimosphaeroma* isopods, Table 3). All invertebrate species found in Staghorn Sculpin stomachs were recorded either in benthic cores or beach seine catch. Amphipods, particularly of genus *Americorophium* numerically dominated Staghorn Sculpin diet year-round (Figure 44). Polychaetes were the only other functional group to contribute year-round to Staghorn Sculpin diet, but intact polychaetes were rarely found in stomach contents due to digestion. In some months, *Diptera* larvae, *Gnorimosphaeroma* isopods, and *Crangon franciscorum* also contributed noticeably to Staghorn Sculpin diet (Figure 44).



Figure 44. Percent composition by abundance for prey taxa found in Staghorn Sculpin diet in the Mad River Estuary, for all months collected, June 2015-June 2016.

Dominant prey taxa were identified by plotting prey-specific abundance against frequency of occurrence (Figure 45), where prey-specific abundance is the average number of individuals of a given prey type in a stomach when that prey type is present (Equation 5). This revealed which amphipod species and functional groups were most important to fish diet, by determining how frequently a given prey was consumed and the average number of individuals of that prey found in stomachs containing that prey type. Dominant prey were those prey types that were consumed frequently and in large numbers, such as amphipods (Figure 45). Rare prey were those prey types that were consumed infrequently and in small numbers, such as non-polychaete worms (Round and Ribbon Worms, Figure 45). Because amphipods dominated fish diet, they were analyzed by species. Frequency of occurrence and prey-specific abundance for less common taxa was calculated by functional group.



Figure 45. Prey-specific abundance of key invertebrate taxa plotted against frequency of occurrence in Staghorn Sculpin diet.

All three amphipod species were identified as dominant in Staghorn Sculpin diet, especially those of genus *Americorophium* (Figure 45). When polychaetes were found in diet, only one worm was found on average, but polychaetes were present in 42% of the stomachs dissected, indicating a generalist feeding pattern. Typically, the only sign of polychaetes in stomach contents were the hard acicula, which were presumed to have come from a single individual. However, when intact polychaetes were countable in stomach contents, only one individual, or part of one individual, was usually present. It should also be noted that these data do not account for biomass. For example, a single *C. franciscorum* shrimp could stretch a Staghorn Sculpin stomach to the limit, while many amphipods would be required to achieve the same gut fullness.

Ordination of Staghorn Sculpin diet data gave a stress value of less than 0.2, indicating a helpful ordination solution (Stress = 0.197, Figures 46 and 47). Ordination analysis of Staghorn Sculpin diet failed to differentiate between seasons, and season was not found to significantly determine diet composition (Figure 46, p > 0.05). The upstream station separated out in ordination, and was found to significantly affect diet composition, explaining 19% of the variation in diet (Figure 47, p = 0.007). Consequently, Staghorn Sculpin diet did not change significantly with season, despite seasonal fluctuations in prey communities (Figures 40 and 46). Staghorn Sculpin captured upstream had a diet more dominated by *Chironomidae* larvae and *Gnorimosphaeroma* isopods (Figure 47). Staghorn captured at the tributary and mouth stations were more likely to eat larger organisms, such as *C. franciscorum* shrimp and *Hemigrapsus* crabs.



nMDS 1

Figure 46. Two dimensional nMDS by season for dominant prey groups in Staghorn Sculpin diet. Samples collected during the same season are outlined and colored as follows: green for spring, rose for summer, orange for autumn, and light blue for winter.



Figure 47. Two dimensional nMDS by station for dominant prey groups in of Staghorn Sculpin diet. Samples collected during the same season are colored as follows: green for spring, rose for summer, orange for autumn, and light blue for winter. Shaded polygons outline samples by the location of fish capture: pale blue for the upstream station (light), royal blue for the tributary station (intermediate), and navy for the river mouth station (dark).

Dominant prey items were compared to prey availability in terms of %FO (Figure

48). Staghorn are opportunistic predators, so it is not surprising that they favored prey

types which occurred frequently in the environment: polychaetes and amphipods.

However, Staghorn Sculpin did appear to avoid Diptera larvae and larger prey items,



including *C. franciscorum* shrimp, *Hemigrapsus* crabs, and mysids. This may have been related to fish size, as most fish analyzed were young of the year (Figure 25).

Figure 48. The frequency of occurrence for key prey groups in Staghorn Sculpin diet (left) and benthic cores (right) across all stations and months.

English Sole diet was dominated by much smaller prey that were primarily benthic (Figure 49). Due to their smaller mouths, English Sole ate smaller amphipod species and individuals, with many juvenile-sized amphipods frequently noted in stomach contents. Unlike Staghorn Sculpin, English Sole ate comb jellies, but this was limited to a single individual captured in July 2015. Copepods, especially benthic copepods of the order *Harpactacoida*, made up a significant portion of the diet, and dominated English Sole diet in May 2016, coinciding with the shortest mean total lengths for English Sole over the course of the study (Figures 27 and 49). *Diptera* larvae and small, nonpolychaete worms also contributed to May 2016 diet (Figure 49). In other months, *Harpactacoida* copepods and amphipods, especially *A. salmonis* and *E. confervicolus*, numerically dominated English Sole diets. Larger organisms, such as mysids and *C. franciscorum* shrimp appeared rarely in English Sole diet.


Figure 49. Percent composition by abundance for prey taxa in English Sole diet in the Mad River Estuary, for all months collected, June 2015– June 2016.



Figure 50. Average abundance of key prey groups plotted against frequency of occurrence in English Sole diet.

Similar to Staghorn Sculpin diet, amphipods numerically dominated English Sole stomach contents (Figures 45 and 50). Polychaetes were present in roughly 30% of English Sole stomachs, and usually only one individual was detected (Figure 50). Copepods were found nearly as frequently as the most common amphipod species, and typically appeared in large numbers. Copepod presence in the benthos is not known, as they were too small to be detected in benthic cores. Preference could be determined for other prey groups, and English Sole appeared to favor *E. confervicolus*, the largest amphipod species found in the Mad River Estuary (Figure 51). It should be noted that *E. confervicolus* individuals found in English Sole stomachs tended to be small. English Sole did not consume isopods or crabs completely, and avoided shrimp and mysids, all of which tend to be larger prey items. Ordination analysis was not possible with English Sole diet data, due to small sample sizes in some months.



Figure 51. The frequency of occurrence for key prey groups in English Sole diet (left) and benthic cores (right) across all stations and months.

# DISCUSSION

# **Biannual Sampling**

# **Baseline Seasonal Communities**

Baseline fish and invertebrate communities were determined for the Big, Mad, and Ten Mile river estuaries. Abundance and species richness of fish and invertebrates increased in summer and decreased in winter (Figures 7, 8, 33, and 34). Spikes in summer fish catch were mostly due to large catches of a few, highly abundant species, rather than even increases in diversity. Capturing schools of highly abundant fishes reduced diversity and contributed to high variability in summer catch amongst years and estuaries (Figures 7 and 9). The summer fish community was dominated by periodic users (Figure 19). These were fish that either reared in the estuary as juveniles, such as Starry Flounder and Striped Surfperch, or marine fish that move into estuaries seasonally to feed, including Topsmelt and Night Smelt. The three estuaries shared similar winter fish communities dominated by hardy estuarine residents, primarily Staghorn Sculpin, Three-spine Stickleback, and Prickly Sculpin. Polychaetes and amphipods were present year-round in all systems, often dominating the benthos in winter. In summer, representation of bivalves, crabs, and *Diptera* larvae increased (Figure 38; Appendix N).

The Big River Estuary experienced the greatest tidal influence of the three estuaries and had the greatest diversity of marine fish and invertebrate species (Table 3; Appendix H). Abundance and species richness was relatively high for fish and invertebrates in the Big River Estuary (Figures 7, 8, 33, and 34). The summer fish community was dominated by typically marine species, such as gobies, gunnels, and coastal sculpins. The invertebrate community was characterized by burrowing invertebrates which tend to dwell deeper in the mud: Bay Ghost Shrimp, three clam species that were not detected elsewhere – *C. californica*, *M. inquinata*, and *N. tantilla* – and three unique polychaete species (*E. californica*, *H. complanata*, *P. nuchalis*) (Figure 36; Appendix N). In winter, benthic invertebrate species richness remained relatively high (Figure 34), and catch for three marine fishes increased slightly: Surf Smelt, Starry Flounder, and Bay Pipefish (Appendix I).

The Mad River Estuary was the most seasonally variable of the three estuaries, and experienced regular winter drops both winters in abundance and species richness for fish and invertebrates (Figures 7, 8, 33, and 34). The Mad River Estuary had fewer marine species than the Big River Estuary, but a greater diversity of freshwater fishes and invertebrates, which were captured at the upper end of the estuary (Table 3; Appendix H). Freshwater-dwelling Coastrange Sculpin and Sacramento Sucker were both captured as juveniles at the upstream station (Appendix J). Steelhead and Coho Salmon were also captured upstream as smolts, as they left freshwater. *Chironomidae* larvae were unique to the Mad River Estuary, and were found solely at the upstream station (Figure 36). In summer, the invertebrate community was typified *Gnorimosphaeroma* isopods, which were not found elsewhere (Table 3).

The Ten Mile River Estuary had the least ocean connectivity, becoming a freshwater lagoon in some summers. Abundance and diversity of fishes and invertebrates

were lowest in the Ten Mile River Estuary (Figures 7, 8, 33, and 34). Like the Mad River Estuary, the Ten Mile River Estuary fish community was characterized by salmonids and estuarine sculpins, and the invertebrate community was dominated by amphipods and polychaetes year-round (Figure 17; Appendix N). However, the Ten Mile River Estuary was also home to clams and decapods not found in Mad River Estuary cores (Appendix N). In winter, the invertebrate community was also typified by the presence of non-polychaete worms. In summer, the community was characterized by the clam *C. nuttallii*, the polychaetes *M. ambiseta* and *P. elegans*, and the phoronid *P. pallida*, which were not found elsewhere (Table 3).

# Variation in Fish and Invertebrate Communities

Seasonal changes in the Big, Mad, and Ten Mile river estuaries matched those previously described for Southern California (Horn 1980) and Oregon (De Ben et al. 1990): winter drops in abundance and species richness, and higher species richness in summer and at downstream locations. Fish and invertebrate communities also followed similar patterns within each estuary: the Big River Estuary had the most marine fish and invertebrate communities, the Mad River Estuary experienced the most dramatic drops in winter species richness, and the Ten Mile River Estuary had the lowest species richness (Figures 7, 8, 33, and 34). The fish and invertebrate communities were driven by many of the same factors across estuaries, but the relative importance of these factors differed for the fish and invertebrates. Season drove changes in the fish community ( $r^2 = 0.24$ ), while the invertebrate community was primarily dependent on location ( $r^2 = 0.28$ ).

Seasonality in estuarine fish assemblages was first described in North America for the Atlantic Coast (Cronin and Mansueti 1971), but has since received extensive study on the Pacific Coast (Horn and Allen 1976; Monaco et al. 1992). Allen (1982) described the fish communities in Newport Bay, California, as divided seasonally into periodic and regular users of estuaries. Periodic users depend on temperature and flow signals to determine their movements into and out of estuaries. For example, salmon smolts mature more quickly in warmer waters, which can affect the timing of their outmigration to the ocean (Beckman et al. 1998). As adults, returning salmon will stage at river mouths prior to their upstream journey, and temperature and flow have been shown to change the timing of their spawning migration (Quinn et al. 1997; Richter and Kolmes 2005). Flow also affects recruitment for flatfish species that rear in estuaries. For example, English Sole, Starry Flounder, and Speckled Sanddab spawn offshore and larvae locate estuarine rearing habitat by orienting themselves towards a freshwater plume leaving an estuary (Boehlert and Mundy 1988). Strength of the freshwater plume is determined by flow, and if the plume is weak, larvae may travel further to find suitable estuarine habitat (Demory et al. 1986). Interpretation of the effects of flow as a predictor in analysis of biannual sampling should be treated with caution as flow data came from adjacent watersheds for the Big and Ten Mile river estuaries. Air temperature and flow each explained only 6% of the variation in the overall fish community, but air temperature was identified in GLM analysis as one of the two primary predictors of flatfish abundance. Flow was not included in GLM analysis, but flatfish abundance was lowest in summer 2014, a dry water year, and highest in summer 2016, an above average water year for Northern California (CDEC 2017).

Differences in fish communities of the Big, Mad, and Ten Mile river estuaries were secondarily driven by system ( $r^2 = 0.12$ ), which may be related to differences in mouth size and tidal influence amongst estuaries. Bays and estuaries with wider mouths tend to have higher fish abundance and diversity (Horn and Allen 1976). Along the Pacific Coast, estuarine fish communities have been primarily linked to region and estuary type, but diversity within each category is strongly predicted by river mouth depth and tidal extent within the estuary (Monaco et al. 1992). The Big, Mad, and Ten Mile river estuaries fall into the Northern California riverine category described by Monaco et al., and generally follow the trend of greater abundance and diversity in estuaries with a stronger ocean connection. This may partly indicate why the Ten Mile River Estuary, with its narrow mouth and intermittent ocean connection, had no unique fish species.

Total variation explained was similar for the fish and invertebrate communities (54% vs. 50%), but most of this variation was attributed to location-specific factors for the invertebrate community. Most fish species that utilize estuaries return to the ocean prior to winter (Horn and Allen 1978). In contrast, benthic invertebrates must either migrate to different micro-habitats in the winter or die-off with the onset of cooler weather (Munn et al. 2009; Oldenburg 2014). Consequently, the same fish species can range across widely separated estuaries, while benthic invertebrate communities are more likely to be system-specific (Desmond et al. 2002). In our study, each estuary had more unique invertebrate taxa than unique fish species, perhaps reflecting the relatively limited range of some invertebrate taxa.

Distribution of mobility-limited benthic invertebrates is often dependent on location-specific factors, including salinity, substrate type, and toxicity (Chainho et al. 2006; Hemery and Henkel 2015). Teske and Wooldridge (2003) found that distinct benthic communities emerged in muddy and sandy substrates, and that salinity further limited benthic invertebrate distribution. Sediment size and salinity are often correlated within estuaries (Chainho et al. 2006). Each station in our study represented a unique combination of salinity and substrate type, and hosted distinct benthic invertebrate communities: Bay Ghost Shrimp were only found at the downstream station in Big River Estuary, *Chironomidae* larvae were only found at the oligohaline upstream station in the Mad River Estuary, and within the Ten Mile River Estuary, *Diptera* larvae and the amphipod *E. confervicolus* were only found at the upstream station.

Seasonal change in benthic invertebrate communities was mostly attributed to precipitation ( $r^2 = 0.09$ ) and flow ( $r^2 = 0.08$ ), reflecting basin-wide seasonal and interannual variability. Increased rainfall in winter has been associated with decreased abundance and diversity of benthic invertebrates (Silva et al. 2006). Long-term drought has also been linked to changes in estuarine invertebrate fauna, particularly for intermittently-open estuaries, such as the Ten Mile River Estuary (Chuwen et al. 2009). During water year 2014, precipitation along the Northern California Coast was 50-70% of historic averages, and the state was in the third year of a drought (CDEC 2017). In 2015, precipitation along the Northern California Coast increased to roughly 80% of historic averages. Northern California exited the drought in water year 2016 (CDEC 2017). Year was not significant in analysis and season explained little variation in the benthic invertebrate community ( $r^2 = 0.05$ ). However, Ten Mile River Estuary exhibited the highest overall invertebrate species richness in summer 2015, when the mouth remained open, and the lowest species richness in the summer of 2014, when the estuary was cut-off from ocean access. Therefore, fluctuations in invertebrate occurrence and richness in the Ten Mile River Estuary could be reflective of inter-annual variation in precipitation and flow.

# The Mad River Estuary as a Reference Site

The Mad River Estuary was chosen as a non-MPA reference site due to similarities in morphology. Like the majority of west coast estuaries, the Big, Mad and Ten Mile river estuaries are small flooded river valleys that drain relatively small coastal watersheds (Emmett et al. 2000). All three estuaries drain the California Coastal Mountain Range and are too small for significant stratification to occur. Watershed uses and management goals are also similar across the Big, Mad, and Ten Mile river estuaries. All three watersheds are sparsely inhabited, primarily owned by timber companies, and sediment impaired due to erosion from timber harvest and road construction (NCRWCB 2005b, 2005a; Stillwater Sciences et al. 2010). Recent watershed assessments emphasize that the estuarine portions of the Big, Mad, and Ten Mile rivers have the greatest potential for providing high quality salmonid habitat within each watershed, and that estuaries are necessary to life-history completion for salmon and Steelhead (Mangelsdorf and Clyde 2000; Downie et al. 2006; Stillwater Sciences et al. 2010). Current management goals for all three watersheds are continued timber harvest and the recovery of local salmonid populations, through habitat restoration and improved timber practices.

Similarities notwithstanding, there are important morphological and management differences between the Mad River and the two MPAs. First, the Mad River watershed extends above the snow-line (Big River Watershed: 865 m, Mad River Watershed: 1,800 m, Ten Mile River Watershed: 750 m). Second, the river is additionally managed as a municipal water source for Humboldt County (NCRWCB 2005b, 2005a; Stillwater Sciences et al. 2010). In spite of these differences, expected fish species were similar across the three estuaries. Estuarine fish diversity is strongly correlated with ease of ocean access, quantified by the width and depth of the river mouth (Horn and Allen 1978; Monaco et al. 1992). Based on mouth size, the Mad River Estuary would be expected to have diversity intermediate to that of the Big and Ten Mile river estuaries. In fact, there was almost perfect overlap between the fish species present in the Mad and Ten Mile river estuaries. The Big River Estuary fish community had a higher diversity of marine species, but most fishes found in the Big River Estuary were also present in the Mad and Ten Mile marine fish species that dominated seine catch. Estuarine residents must adapt to the daily swings in temperature, salinity, and turbidity inherent in estuaries. Juvenile marine fishes that utilize estuaries as nursery grounds tend to have broad salinity tolerances and leave for the ocean once it becomes too energetically costly to remain in the estuary (Blaber and Blaber 1980). As a result, seasonal effects were more important than inter-estuary differences for the fish communities.

Season was the primary predictor for the overall fish community, as well as Staghorn Sculpin and flatfish abundances (Tables 5 and 6). Only Three-spine Stickleback abundance was best predicted by location, in terms of RKM (Table 4). Despite apparent similarities, there are important caveats. In winter, the fish community of the Big River Estuary was relatively diverse with some marine species increasing in abundance (Figures 8 and 9; Appendix I). In contrast, the Mad River Estuary had the lowest winter fish diversity and the winter community was completely estuarine. The Mad River Estuary was most similar to the Ten Mile River Estuary in winter (Figures 18 and 37), as the Ten Mile River Estuary saw reduced increases in summer diversity compared to the other two systems, perhaps tied to intermittent ocean connectivity (Figures 9 and 34). Finally, although the Mad River Estuary overlapped both the Big and Ten Mile river estuaries more than they overlapped each other (Figures 16 and 35), system remained an important driver of variation in the fish and benthic invertebrate communities ( $r^2 = 0.12$  and 0.20).

Some of the apparent differences between the Big, Mad, and Ten Mile river estuaries are likely due to station choice. In Mendocino, access limitations, MPA boundaries, and sampling requirements limited station choice in the Big and Ten Mile river estuaries. In the Big River Estuary, one station was positioned near the mouth, with a second roughly one kilometer upstream, near the upper MPA boundary. Tidal influence regularly extends beyond this, and salinities at the upstream station regularly exceeded 21 ppt (Appendices A and E). In the Ten Mile River Estuary, the upstream station was also roughly one kilometer from the mouth station, halfway to the upper MPA boundary, where the narrow channel and dense vegetation precluded sampling. Salinity measurements were limited in Ten Mile River Estuary, but did exceed 25 ppt at the upstream station in June 2016 (Appendices C and G). In contrast, the downstream station in the Mad River Estuary was so close to the mouth that it received direct wave action. The upstream station was positioned at the typical upper limit of the estuary, and salinities tended to be low, only exceeding 10 ppt in fall (Appendix F). This led to a strong separation by salinity preference in ordination, strongly driven by the oligohaline upstream station in the Mad River Estuary (Figures 16 and 35). Additional, lower-salinity stations in the Big and Ten Mile river estuaries would allow for a more complete description of estuarine communities and better comparisons between systems. Alternatively, additional downstream stations could be added to Mad River Estuary sampling.

The study period, June 2014-June 2016, marked the strongest El Niño conditions in Northern California since 1997 (Bjorkstedt et al. 2017). The resultant swings in river flow from summer 2014 through summer 2016 changed the position of the mouth of the Mad River and determined whether the mouth of the Ten Mile River was open. El Niño conditions also resulted in elevated SSTs off the Northern California Coast. Flow and SST both affect recruitment success for flatfishes (Boehlert and Mundy 1987). Flow additionally affects temperature, salinity, and turbidity within estuaries. Temperature and salinity determine the distribution of fishes (Cronin and Mansueti 1971), and turbidity affects feeding and recruitment success of pelagic fishes in estuaries (Baxter et al. 1999). The Mad River Estuary, as a non-MPA reference site, accounts for the importance of these large-scale processes in determining fish abundance and distribution, and allows for a before-after-control-impact approach to identify any potential MPA effects.

# Mad River Estuary Sampling

# Baseline in the Mad River Estuary

Results from additional sampling in the Mad River Estuary agreed with and informed results from biannual sampling. Summer communities were abundant and diverse, while winter communities were dominated by hardy estuarine residents, as seen during biannual sampling. Abundance and diversity for fish was highest in summer and lowest in winter, but diversity increased markedly in spring, preceding large increases in catch (Figures 20-22). Abundance and diversity remained high through fall, and only dropped off in winter. Invertebrate %FO and species richness peaked in summer and fall 2015, but benthic cores were not collected in the Mad River Estuary in July and August 2015, when some of the highest fish catches occurred (Figures 20 and 39).

No novel fish or invertebrate species were detected during additional sampling in the Mad River Estuary, but the spring, fall, and tributary station communities were described. In spring, fishes that depend on estuaries for life-cycle completion began appearing in Mad River Estuary catch. These were anadromous fishes such as Steelhead, and flatfish that rear in estuaries as juveniles, including English Sole and Starry Flounder. Invertebrates took longer to repopulate the upper benthos; *Gnorimosphaeroma* isopods and *Chironomidae* larvae made up a small portion of invertebrates detected in springtime benthic cores. In fall, fish abundance and diversity remained high. The fall fish community was similar to the spring community: typified by salmonids, such as Chinook Salmon, and marine species that sometimes rear in estuaries, such as Bay Pipefish and

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Shiner Surfperch. Benthic invertebrate diversity peaked in fall, with the appearance of bivalves, barnacles, and nematodes. The tributary station was mesohaline, characterized by a mix of sand and cobble, and is the last freshwater input before the river mouth. Catch of juvenile rockfish and flatfish was relatively high at the tributary station, while other marine fishes were more frequently captured at the river mouth. The invertebrate community at the tributary station was dominated by amphipods that were also common upstream, such as *A. spinicorne*, as well as typically marine species more common at the mouth, including *H. oregonensis* and *B. glandula*.

# Variation in Fish and Invertebrate Communities

Echoing the biannual analysis, the fish and invertebrate communities in the Mad River Estuary experienced strong seasonal swings in abundance, diversity, and community composition. Correspondingly, season explained 20% of the variation in the fish and invertebrate communities (p = 0.001). However, air temperature, water temperature, and salinity were not significant in analysis, despite the known importance of temperature and salinity in determining the distribution of fishes (Meng et al. 1994) and invertebrates (Hemery and Henkel 2015). Previous studies on the Pacific Coast have failed to link point measurements of water temperature to fish distributions, but did note that SST was significantly tied to fish community composition (Desmond et al. 2002). This may reflect the seasonality of the California Coast, where seasons are determined primarily by shifting ocean currents (García-Reyes and Largier 2012) and where average summer and winter air temperatures only differ by ~5 °C (Emmett et al. 2000).

Fish communities in west coast estuaries are influenced by seasonal upwelling and downwelling, rather than seasonal changes in air temperature (Hickey and Banas 2003). For example, fish that rear in estuaries typically spawn in the ocean and immigrate to estuaries as larvae or juveniles (Boehlert and Mundy 1988). The timing of spawning therefore depends on ocean conditions, including SST, rather than conditions within the estuary. Flow has also been found to be a driving factor for fish communities via timing effects to migration and reproduction cycles (Bottom and Jones 1990). Changes to flow alter the strength of the river plume, which can result in inter-annual differences in which species are detected and when those species are captured. For example, English Sole rely on river plumes to detect estuarine nursery habitat (Demory et al. 1986). The largest diversity and abundance of flatfishes was detected in the Mad River Estuary in June 2016. Thus was preceded by an exceptionally wet spring (Figure 20), increasing the river plume signal and potentially leading to higher abundance and diversity of flatfish in the Mad River Estuary the following summer. SST and flow were both predictive of flatfish abundance and the overall fish community. Although, SST and flow only explained 6% and 3% of the variation in the fish community, respectively.

The importance of environmental variables in driving fish abundance and distribution was more visible for individual fish taxa. Three-spine Stickleback abundance was negatively related to air temperature and flow in the Mad River Estuary (Table 7). Similarly, abundance of Pacific Staghorn and flatfish were each negatively correlated to SST and flow (Tables 8 and 9). Flow and SST are both lower in summer, when our largest catches occurred. Additionally, flow and SST may have affected when and at what size flatfish entered the Mad River Estuary. For example, In April 2015, English Sole were found in large numbers at the tributary station. In 2016, English Sole weren't captured until May. These fish were found at the mouth at significantly smaller sizes than any other sampling month (Figure 27). This indicates that in 2015, English Sole in the Mad River Estuary had fed and migrated upstream by April, reaching a mean size of 27mm. In 2016, English Sole juveniles had only recently entered the estuary in May, had not had a chance to migrate upstream, and had an average length of 22mm.

The low explanatory power of SST and flow for the overall fish community is likely due to two factors. First, the primary drivers of abundance will vary across species,

obscuring the importance of environmental drivers at a community level. For example, abundance of the estuarine resident, Three-spine Stickleback, was primarily driven by RKM. Meanwhile, abundance of flatfish was driven by season and the associated variables SST and flow, reflecting the strong seasonality in flatfish catch. Second, the importance of large-scale processes may be less apparent at the local scale than the regional scale. Fish that utilize estuaries depend on ocean processes, such as SST, and on river factors such as flow, water temperature, and turbidity (Cronin and Mansueti 1971; Monaco et al. 1992). These processes operate on a coastal and basin-wide scale and their influence on local fish communities is difficult to quantify. As a result, changes in the fish community were better explained at a regional scale than at the scale of a single estuary. In fact, measured variables explained 54% of the variation in biannual fish data for all three estuaries, but only 43% of the variation in Mad River Estuary fish data.

Total variation in species was somewhat better explained for invertebrates than fish in the Mad River Estuary ( $R^2 = 0.48$  and 0.43). This was heavily weighted by season, which explained 20% of the variation in the Mad River Estuary invertebrate community. An additional 5% of the seasonal and inter-annual variation was explained by precipitation. For comparison, in biannual analysis only 5% of the variation in the invertebrate community was directly attributed to season, while flow and precipitation cumulatively explained 17% of the variation in the data. This inversion in explanatory power for season versus environmental variables likely reflects the difficulty in attributing local community changes to regional processes. The importance of location within the estuary was emphasized in analysis of Mad River Estuary data. In biannual analysis, RKM was predictive of Three-spine Stickleback abundance, was not significant for the overall fish community, and explained 8% of the variation in the invertebrate community. In Mad River analysis, station predicted Staghorn Sculpin and Three-spine Stickleback abundance, and explained significant variation in the fish and invertebrate communities ( $r^2 = 0.13$  and 0.23). Each station represented a different habitat: from the oligohaline, cobble and mud-dominated upstream station, to the mesohaline cobble-dominated tributary station, to the polyhaline, sandy river mouth station. Consequently, station was an apt proxy for the site-specific variables that are known to most strongly determine benthic invertebrate species composition (Chainho et al. 2006), but the relative importance of these factors could not be determined.

# Staghorn Sculpin and English Sole Diet

Staghorn Sculpin diet did not change seasonally over the course of the diet study June 2015-June 2016, despite strong seasonal trends in the fish and invertebrate communities in the Mad River Estuary. As a generalist consumer, Staghorn Sculpin diet mostly reflected relative prey occurrence, with slight aversion to isopods and polychaetes and a slight preference for the amphipod E. confervicolus (Figure 48). Americorophium amphipods dominated Staghorn Sculpin diet year-round, even in winter when their occurrence declined (Figure 44; Appendix O). Polychaete worms, which occurred regularly compared to other benthic species, appeared in small numbers in 42% of Staghorn Sculpin stomachs. Pacific Staghorn Sculpin feed primarily on amphipods, mysids, and nereid worms (Porter 1964). However, young of the year and adults have also been found to have a varied diet, including small fish, crabs, and other invertebrates (Moyle 2002). Only two individuals in our study exhibited piscivory, one consumed an inviable salmon egg and the other exhibited cannibalism. Previous research has shown that Staghorn Sculpin is the fish species most commonly consumed by older individuals (Mace 1975).

The lack of seasonality in Staghorn Sculpin diet likely reflects the age distribution of the estuarine population. Staghorn Sculpin often spend the first year or two of life in estuaries (Miller 2007) and most Staghorn Sculpin captured in the Mad River Estuary were young of the year, or under 100 mm in length (Figure 25). Analysis of Staghorn Sculpin diet in Alaskan estuaries found weak to moderate monthly shifts in diet composition (Whitney et al. 2017). However, the fish examined were one year of age or older and their diet consisted of juvenile fish, insects, and epibenthic invertebrates. Predator studies of Staghorn Sculpin in Vancouver Island also found monthly variation in diet composition, but monthly shifts in diet were most pronounced for larger size classes, one year of age or older (Mace 1975). The shift from a diet numerically dominated by amphipods to one containing more crabs and fish occurs at one to two years of age (Haertel and Osterberg 1967). Crabs and fish are larger and more mobile than amphipods and polychaetes, and their abundance exhibited strong seasonal trends in the Mad River Estuary (Figure 20; Appendix O). In contrast, seasonal trends were not pronounced for amphipods and polychaetes (Appendix O). Instead, amphipods and polychaetes preferred by Mad River Staghorn Sculpin were available year-round for consumption, explaining the absence of seasonality in Staghorn Sculpin diet (Figure 46).

Staghorn Sculpin diet did depend on their location within the estuary (Figure 47). The cobble substrate and oligohaline waters at the upstream station coincided with a significantly different benthic invertebrate assemblage, and significantly different diet composition for Staghorn Sculpin. Fish captured upstream ate *Chironomidae* larvae and smaller amphipods of the genus *Americorophium*. The tributary and mouth stations, which neighbored each other, hosted similar invertebrate assemblages, and did not drive significant differences in Staghorn Sculpin diet. Gut contents from fish captured downstream were more likely to contain larger prey items, including *C. franciscorum* and *H. oregonensis*. Catch per seine of Staghorn Sculpin also depended on station, so diet reflected Staghorn Sculpin movements through the estuary and prey availability at their point of capture. English Sole diet reflected their use of the Mad River Estuary. English Sole were only present in the estuary seasonally and they grew throughout their rearing period (Figure 27). Copepods were most numerous in the diet of smaller fish (< 40 mm) and amphipods were more numerous in the diet of larger fish (40-60 mm); larger amphipod individuals were noted in the stomach contents of the largest English Sole (> 60 mm). Newly settled English Sole in Humboldt Bay prefer benthic copepods of the order *Harpactacoida*, switching to a polychaete-dominated diet around 50 mm in length (Toole 1980). This likely reflects differences in prey availability between the Mad River Estuary and Humboldt Bay. In Humboldt Bay, polychaetes may dominate, while the Mad River has high occurrences for amphipods from late spring through fall, when English Sole are present (Appendix O).

English Sole were captured almost exclusively at the tributary and mouth stations, where benthic invertebrate communities were largely similar. Consequently, their diet did not vary by location of capture. English Sole diet was also dominated by *A. salmonis* and benthic copepods of the order *Harpactacoida* spring through fall (Figure 49). Other studies of English Sole have noted inter-annual variations in their diet are directly related to river discharge (Kostecki et al. 2010). In this study, flatfish abundance was directly related to river discharge and season (Table 9). It is therefore possible that continued study of English Sole diet would have revealed annual trends related to flow, a driving factor in the flatfish assemblage and the overall fish community in the Mad River Estuary.

# Recommendations

This work examines how fish and benthic invertebrate communities in the Big, Mad, and Ten Mile river estuaries vary by season and location, with a more detailed examination of the fish and invertebrate communities in the Mad River Estuary. All three estuaries have received relatively little attention previously, similar to other small, riverine estuaries along the Pacific Coast. In the hope that future researchers decide to continue this work, or establish biotic baseline data for similar systems, I discuss below three major recommendations to overcome some of the limitations of the present study design: 1) collect continuous temperature, salinity, and turbidity data, 2) narrow the focus to highlight fish and invertebrate prey, complemented by careful station choice, and 3) collect data on pelagic invertebrate prey availability.

1) Funding for this research explicitly precluded expenditures related to water quality monitoring, despite a lack of historical water quality data and previous recommendations that these data be obtained for the Big, Mad, and Ten Mile river estuaries (NCRWCB 2005a; Downie et al. 2006; Stillwater Sciences et al. 2010). This significantly limited our ability to collect water quality data over the course of the study. Estuaries are inherently dynamic, so it is important to capture daily and monthly variation in water temperature, salinity, and turbidity. Such data can be used to generate daily averages and ranges to better understand drivers behind fish and invertebrate distributions. Technologies that reduce the price of water quality monitoring are increasingly available. Year-round deployment of

inexpensive loggers would be the best means to tie trends in the fish and invertebrate communities directly to environmental variables, particularly temperature, salinity, turbidity, and depth.

- 2) The MPA Estuary Project aimed to provide a complete snapshot of the summer and winter plant, invertebrate, and fish communities of Northern California MPAs (Shaughnessy et al. 2017). This broad focus limited the number of stations that could be sampled in each estuary. A narrower focus on the fish and invertebrate prey populations is recommended, so as to allow for additional sampling locations in each estuary. This would benefit fish and invertebrate analysis, as detailed below:
  - a. Stations in the Big and Ten Mile river estuaries did not include the upper reaches of the estuary, limiting comparisons that can be made between the fish communities of the Mad River Estuary and the two MPAs. Additional downstream stations could be added across all three estuaries, and/or oligohaline sampling locations could be added in the Big and Ten Mile river estuaries. Both approaches would more completely describe the fauna of these estuaries and would allow for more direct comparisons to the reference estuary. In the Big River Estuary, the addition of an oligohaline station would also create the opportunity to compare the current study design – with a separate reference system – to a nested study design for the Big River Estuary, with a nested reference site upstream of the MPA boundary. For the Ten Mile River Estuary, upstream sampling

would require compact, lightweight fishing gear that could be deployed in a narrow, heavily vegetated channel.

- b. Each station was characterized by a different substrate, vegetation, and salinity regime. Thus, the relative importance of these habitat variables could not be tested. Increased station coverage could include replicates of habitat variables so that future researchers could separate substrate effects from salinity effects. Benthic invertebrates are important indicators of habitat quality (Van Dolah et al. 1999), and understanding which habitat variables determine community composition is key to informing future management.
- 3) If the diet study is continued, a mysid net should be added to regular sampling in the Mad River Estuary. Prey availability has only been described for the benthos. Mysid sampling would provide data on prey availability in the water column for the larger invertebrates consumed by Staghorn Sculpin and English Sole. Mysid samples have the additional advantage of being relatively cost-effective: laboratory processing time is reduced relative to samples from a zooplankton net, as organisms captured in a mysid net can usually be identified and quantified without the use of a compound microscope.

#### CONCLUSIONS

This work contributes to a standing body of literature documenting the fish and benthic invertebrate communities of estuaries. Like other estuaries along the west coast, the Big, Mad, and Ten Mile river estuaries are characterized by seasonally changing fish assemblages, moderated by location amongst and within estuaries. Winter fish communities were dominated by hardy resident fish species, while summer communities were characterized by increased abundance and diversity, and the appearance of marine fishes in catch. Increased sampling in the Mad River Estuary revealed that spring and fall fish communities are dominated by fish that rely on estuaries for life-cycle completion: salmonids and marine fishes that rear in estuaries as juveniles.

Benthic invertebrate communities in these systems are primarily determined by location, at both the regional and local scales, while occurrence and species richness vary seasonally. In the Mad River Estuary, total variation was better explained for the invertebrate community than the fish community, due to the importance of habitatspecific variables in determining invertebrate communities. The diet of a resident, benthic fish in the Mad River Estuary, Pacific Staghorn Sculpin, varied by location of fish capture, but not by season.

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# APPENDIX A

Appendix A: Station map and top and bottom salinity values for salinity profiles collected by UC Davis in the Big River Estuary. Top: map of the depth profile stations. 2014 stations are in white (light), 2015 stations are in red (dark). Stations that were used in both 2014 and 2015 are pink (intermediate). Bottom: surface and bottom salinity values taken near biannual sampling stations in Big River Estuary.



		Surface Salinity	<b>Bottom Salinity</b>	Salinity Regime
Year	Station	(ppt)	(ppt)	
2014	D	26.2	27.8	polyhaline
2014	E	25.0	30.6	polyhaline
2015	С	31.5	33.5	polyhaline
2015	D	30.5	33.0	polyhaline
2015	E	28.7	33.5	polyhaline

# APPENDIX B





# APPENDIX C

Appendix C: Station map and top and bottom salinity profiles collected by UC Davis in the Ten Mile River Estuary. Top: map of the depth profile stations. 2014 stations are in white (light), 2015 stations are in red (dark). Stations that were used in both 2014 and 2015 are pink (intermediate). Bottom: surface and bottom salinity values taken near biannual sampling stations in Big River Estuary.



		Surface Salinity	<b>Bottom Salinity</b>	Salinity
Year	Station	(ppt)	(ppt)	Regime
2014	D	30.1	30.7	polyhaline
2014	Ι	15.6	31.8	polyhaline
2015	С	32.5	33.0	polyhaline
2015	Е	27.5	32.5	polyhaline
2015	Н	18.7	33.0	polyhaline
2015	J	16.2	30.5	polyhaline

# APPENDIX D




### APPENDIX E

Appendix E: Environmental data for the Big River Estuary. Flow data are from Navarro River, 13 miles south of Big River. Temperatures are in degrees Celsius. Coefficient of variation (C.V.) by station and for the system at bottom of table.

							Daily
		Air	Water		Salinity	Secchi	Flow
Sample Date	Position	Temp	Temp	pН	(ppt)	(m) <sup>1</sup>	(cfs)
Jun 28, 2014	Upstream	23.3	13.6		26.8		4.4
Jun 28, 2014	Mouth	23.3	14.4		28.0		4.4
Jan 5, 2015	Mouth	15.6	9.1	8.9	26.4		179.0
Jan 6, 2015	Upstream	15.6	9.1				164.3
Jun 16, 2015	Upstream	11.5	17.8	8.1	31.0		8.3
Jun 17, 2015	Mouth	12.8	17.0		32.5		7.7
Jan 7, 2016	Upstream	7.8	9.5	7.6	0.3	0.15	3540.9
Jan 7, 2016	Mouth	8.3	9.5	7.7	0.3	0.13	3540.9
Jun 8, 2016	Upstream	17.9	16.6	7.8	21.5	1.06	32.0
Jun 8, 2016	Mouth	19	14.4	7.9	33.3	1.24	32.0
C.V.	Upstream	39.1	29.9	3.2	68.4		
C.V.	Mouth	36.3	26.7	8.0	56.5		
C.V.	Total	35.6	26.8	6.2	58.1		195.9

<sup>&</sup>lt;sup>1</sup> The estuaries were too shallow for a vertical secchi depth, so a horizontal secchi distance was used. The field lead kneeled on shore and a crew member waded away from them while holding the disk underwater. The field lead would then call out when the lines on the disk were no longer visible.

### APPENDIX F

							Daily
		Air	Water		Salinity	Secchi	Flow
Sample Date	Position	Temp	Temp	pН	(ppt)	$(m)^{1}$	(cfs)
Jun 16, 2014	Upstream	16.1	18.6				81.1
Jun 17, 2014	Mouth	15.7	16.0				76.4
Nov 6, 2014	Mouth	17.8	14.7		28.0		195.5
Nov 6, 2014	Tributary	17.8	13.1		8.0		195.5
Nov 7, 2014	Upstream	16.1	15.4		3.0		178.5
Jan 14, 2015	Upstream	15.6					370.9
Jan 14, 2015	Mouth	12.8					370.9
Apr 26, 2015	Mouth	18.3	15.6	8.6	28.0		259.8
Apr 26, 2015	Tributary	18.3	18.9	8.9	4.4		259.8
Apr 27, 2015	Upstream	18.3	17.1	9.0	0.2		239.6
Jun 6, 2015	Upstream	12.8	19.8	7.9	0.5		82.7
Jun 7, 2015	Tributary	12.8	14.8	8.0	35.6		79.9
Jun 7, 2015	Mouth	15.6	16.1	8.3	29.2		79.9
Jul 20, 2015	Mouth	15.6	16.7		33.0	6.90	45.6
Jul 20, 2015	Tributary	16.7	16.7	8.3	31.0	2.40	45.6
Jul 21, 2015	Upstream	17.8	21.7	8.7	1.0	7.15	43.9
Aug 12, 2015	Mouth	17.6	15.5		28.0	10.30	39.8
Aug 12, 2015	Tributary	16.5	19.1		3.0	7.70	39.8
Aug 13, 2015	Upstream	18	20.4		2.0	16.60	39.8
Sep 11, 2015	Upstream	15.6	16.1	7.8	15.3	15.80	36.8
Sep 12, 2015	Tributary	15.6	15.9	8.1	24.7	10.05	37.0
Sep 12, 2015	Mouth	16.1	13.5	8.2	31.9	17.30	37.0
Oct 8, 2015	Mouth	22.8	13.9	7.8	30.2	9.30	41.6
Oct 8, 2015	Tributary	21.6	15.9	7.7	22.5	10.10	41.6
Oct 8, 2015	Upstream	21.7	17.9	7.9	3.2	13.70	41.0
Nov 20, 2015	Upstream	10.9	12.4		0.0	15.00	200.2
Nov 21, 2015	Mouth	11.3	13.0		0.0	10.90	157.5
Nov 21, 2015	Tributary	13.1	13.3		0.0	9.90	157.5

Appendix F: Mad River Estuary environmental data. Temperatures in degrees Celsius.

<sup>1</sup> The estuaries were too shallow for a vertical secchi depth, so a horizontal secchi distance was used. The field lead kneeled on shore and a crew member waded away from them while holding the disk underwater. The field lead would then call out when the lines on the disk were no longer visible.

Appendix F Continued: the Mad River Estuary environmental data. Temperatures in degrees Celsius. Coefficient of variation (C.V.) by station and for the system at bottom of table.

							Daily
		Air	Water		Salinity	Secchi	Flow
Sample Date	Position	Temp	Temp	pН	(ppt)	$(m)^{1}$	(cfs)
Feb 13, 2016	Upstream	12.5	10.5	7.8	0.08	0.31	1298.2
Feb 14, 2016	Mouth	13.5	11.3	7.8	0.9	0.37	1155.2
Feb 14, 2016	Tributary	14.3	12.6	7.8	0.9	0.45	1155.2
Apr 8, 2016	Upstream	14.6	16.3	7.9	0.1	9.60	757.4
Apr 9, 2016	Mouth	14.7	12.4	8.5	31	11.80	737.6
Apr 9, 2016	Tributary	13.5	14.3	8.0	0.8	9.60	737.6
Apr 29, 2016	Upstream	12.9	12.6	7.6	0.1	9.80	776.5
Apr 29, 2016	Mouth	14.5	13.7	7.7	0.2	19.20	686.0
Apr 30, 2016	Tributary	14.1	13.4	7.8	0.2	14.80	686.0
May 12, 2016	Mouth	16.3	16.5	7.4	3.7	17.70	486.5
May 12, 2016	Tributary	11.2	16.6	7.2	3.4	19.40	486.5
May 13, 2016	Upstream	14.6	16.3	7.5	0.1	13.20	477.9
Jun 23, 2016	Mouth	15.6	17.4	7.9	6.3	13.60	105.6
Jun 23, 2016	Tributary	16.3	19.0	8.1	3.6	19.70	105.6
Jun 24, 2016	Upstream	18.1	21.9	8.2	0.1	23.20	101.5
C.V.	Upstream	18.6	20.5	5.9	210.7		
C.V.	Tributary	17.9	14.8	5.4	121.4		
C.V.	Mouth	16.4	12.3	4.7	73.7		
C.V.	Total	17.2	17.3	5.2	123.7		115.3

<sup>&</sup>lt;sup>1</sup> The estuaries were too shallow for a vertical secchi depth, so a horizontal secchi distance was used. The field lead kneeled on shore and a crew member waded away from them while holding the disk underwater. The field lead would then call out when the lines on the disk were no longer visible.

### APPENDIX G

Appendix G: Environmental data for the Ten Mile River Estuary. Flow data are from Noyo River, ten miles south of Ten Mile River. Temperatures are in degrees Celsius. Coefficient of variation (C.V.) by station and for the system at bottom of table.

							Daily
		Air	Water		Salinity	Secchi	Flow
Sample Date	Position	Temp	Temp	pН	(ppt)	$(m)^{1}$	(cfs)
Jun 26, 2014	Mouth	16.7	13.3		31.7		6.6
Jun 27, 2014	Upstream	16.7	13.6		22.3		6.7
Jan 3, 2015	Mouth	11.4					110.4
Jan 4, 2015	Upstream	12.2					101.1
Jun 18, 2015	Mouth	15.6	13.5		30.0		5.5
Jun 19, 2015	Upstream	17.8	15.1		24.5		5.2
Jan 5, 2016	Mouth	15.4	10.7	7.8	3.82	19.7	380.0
Jan 6, 2016	Upstream	9.5	10.1	7.7	0.40		559.5
Jun 6, 2016	Mouth	13.5	15.0	7.3	10.8	19.8	21.0
Jun 7, 2016	Upstream	16.4	15.9	7.7	25.8	11.8	21.0
C.V.	Upstream	25.0	18.8	0.1	65.7		
C.V.	Mouth	8.7	13.6	3.8	72.9		
C.V.	Total	17.3	15.4	2.5	64.4		173.3

<sup>&</sup>lt;sup>1</sup> The estuaries were too shallow for a vertical secchi depth, so a horizontal secchi distance was used. The field lead kneeled on shore and a crew member waded away from them while holding the disk underwater. The field lead would then call out when the lines on the disk were no longer visible.

# APPENDIX H

Appendix H: Species presence (X) in the Big, Mad, and Ten Mile river estuaries, June 2014-2016.

				Ten
		Big	Mad	Mile
		River	River	River
Scientific Name	Common Name	Estuary	Estuary	Estuary
Atherinops affinis	Topsmelt	Х	Х	Х
Atherinopsis californiensis	Jacksmelt		Х	
Catostomus occidentalis	Sacramento Sucker		Х	
Heterostichus rostratus	Giant Kelpfish	Х	Х	
Clupea pallasi	Pacific Herring	Х	Х	Х
Artedius fenestralis	Padded Sculpin	Х		
Artedius notospilotus	Bonyhead Sculpin	Х		
Clinocottus acuticeps	Sharpnose Sculpin	Х	Х	
Cottus aleuticus	Coastrange Sculpin	Х	Х	
Cottus asper	Prickly Sculpin	Х	Х	Х
Cottus asperrimus	Rough sculpin		Х	
Enophrys bison	Buffalo Sculpin	Х	Х	
Hemilepidotus hemilepidotus	Red Irish Lord	Х		
Leptocottus armatus	Pacific Staghorn Sculpin	Х	Х	Х
Oligocottus maculosus	Tidepool sculpin	Х	Х	Х
Scorpaenichthys marmoratus	Cabezon	Х	Х	
Cymatogaster aggregata	Shiner Surfperch	Х	Х	Х
Embiotica lateralis	Striped Surfperch	Х		
Phanerodon furcatus	White Surfperch	Х		
Engraulis mordax	Northern Anchovy		Х	
Gasterosteus aculeatus	Three Spine Stickleback	Х	Х	Х
Gobiesox naeabdricus	Northern Clingfish		Х	
Clevelandia ios	Arrow Goby	Х		Х
Lepidogobius lepidus	Bay Goby		Х	
Blepsias cirrhosus	Silverspot Sculpin	Х		
Hexogrammos decagrammus	Kelp Greenling	Х	Х	
Ophiodon elongatus	Lingcod	Х		
Hypomesus pretiosis	Surf Smelt	Х	Х	
Spirinchus starski	Night Smelt	Х	Х	X

				Ten
		Big	Mad	Mile
		River	River	River
Scientific Name	Common Name	Estuary	Estuary	Estuary
Citharichthys stigmaeus	Speckled Sanddab	Х	Х	Х
Apodichthys flavidus	Penpoint Gunnel	Х	Х	Х
Pholis ornata	Saddleback Gunnel		Х	
Platichthys stellatus	Starry Flounder	Х	Х	Х
Pleuronectes vetulus	English Sole	Х	Х	Х
Oncorhynchus kisutch	Coho Salmon	Х	Х	Х
Oncorhynchus mykiss	Steelhead		Х	Х
Oncorhynchus tshawytscha	Chinook Salmon	Х	Х	Х
Sebastes caurinus	Copper Rockfish	Х	Х	
Sebastes melanops	Black Rockfish	Х	Х	
Sebastes rastrelliger	Grass Rockfish		Х	
Sebastes spp.	Juvenile Rockfish	Х	Х	Х
Syngnathus leptorhynchus	Bay Pipefish	Х	Х	Х

Appendix H Continued: Species list ordered by family for the Big, Mad, and Ten Mile river estuaries, 2014-2016. Where "X" signifies that the given species was captured in the presence of species in system.

# APPENDIX I

Appendix I: Seasonal abundance in the Big, Mad, and Ten Mile river estuaries, June 2014– June 2016. Abundance calculated as total catch divided by the number of seines.

		Summer	Winter	Summer	Winter	Summer	Winter
		Big	Big	Mad	Mad	Ten Mile	Ten Mile
		River	River	River	River	River	River
Scientific Name	Common Name	Estuary	Estuary	Estuary	Estuary	Estuary	Estuary
Atherinops affinis	Topsmelt	157	0	0	0	7.33	0
Catostomus occidentalis	Sacramento Sucker	0	0	0.33	0	0	0
Heterostichus rostratus	Giant Kelpfish	0.67	0	0	0	0	0
Clupea pallasi	Pacific Herring	102.92	0	187.5	0	0	0
	Sculpins (Unidentified)	0.33	0.33	0.67	0	0	0
Artedius fenestralis	Padded Sculpin	0.67	0	0	0	0	0
Artedius notospilotus	Bonyhead Sculpin	1.33	0	0	0	0	0
Clinocottus acuticeps	Sharpnose Sculpin	0.67	0	1	0	0	0
Cottus aleuticus	Coastrange Sculpin	0	0.33	27.83	0.33	0	0
Cottus asper	Prickly Sculpin	0.75	0.67	25.39	2.67	1	0
Enophrys bison	Buffalo Sculpin	1.42	0	0.5	0	0	0
Hemilepidotus hemilepidotus	Red Irish Lord	0.5	0	0	0	0	0
Leptocottus armatus	Pacific Staghorn Sculpin	92.89	2.39	46.33	20.67	120.67	2.08
Oligocottus maculosus	Tidepool sculpin	0	0.5	0	0	0	0
Scorpaenichthys marmoratus	Cabezon	6.67	0	1.06	0	0	0
Cymatogaster aggregata	Shiner Surfperch	22.67	0	1.5	0	0.5	0
Embiotica lateralis	Striped Surfperch	1.89	0	0	0	0	0

		Summer	Winter	Summer	Winter	Summer	Winter
		Big	Big	Mad	Mad	Ten Mile	Ten Mile
		River	River	River	River	River	River
Scientific Name	Common Name	Estuary	Estuary	Estuary	Estuary	Estuary	Estuary
Phanerodon furcatus	White Surfperch	0.5	0	0	0	0	0
Gasterosteus aculeatus	Three Spine Stickleback	1.63	2.79	33.67	2	0.83	1.33
Clevelandia ios	Arrow Goby	6.97	0.67	0	0	1.92	0
Blepsias cirrhosus	Silverspot Sculpin	2	0	0	0	0	0
Hexogrammos							
decagrammus	Kelp Greenling	1.33	0	0	0	0	0
Ophiodon elongatus	Lingcod	1	0	0	0	0	0
Hypomesus pretiosis	Surf Smelt	0.42	0.83	0	0	0	0
Spirinchus starski	Night Smelt	4	0	434	0.33	18.92	0
Citharichthys stigmaeus	Speckled Sanddab	1	0	0	0	2	0
Apodichthys flavidus	Penpoint Gunnel	1.08	0	0.67	0	0.67	0
Pholis ornata	Saddleback Gunnel	0	0	5.17	0	0	0
Platichthys stellatus	Starry Flounder	0.5	0.67	8.17	0	6	0.78
Pleuronectes vetulus	English Sole	90.03	0	39.17	0	9.08	0
Oncorhynchus kisutch	Coho Salmon	3.33	0	1.67	0	49.5	0
Oncorhynchus mykiss	Steelhead	0	0	0.67	0	0.42	0.33
Oncorhynchus tshawytscha	Chinook Salmon	2.67	0	20.78	0	9.83	0
Sebastes caurinus	Copper Rockfish	3.83	0	1.28	0	0	0
Sebastes melanops	Black Rockfish	0.5	0	1	0	0	0
Sebastes spp.	Juvenile Rockfish	29.67	0	0	0	1.67	0
Syngnathus leptorhynchus	Bay Pipefish	2.17	2.25	0.5	0	1	0

Appendix I Continued: Seasonal abundance in the Big, Mad, and Ten Mile river estuaries, June 2014– June 2016. Abundance calculated as total catch divided by the number of seines.

## APPENDIX J

Appendix J: Station abundance in the Big, Mad, and Ten Mile river estuaries, June 2014– June 2016. Abundance calculated as total catch divided by the number of seines.

		Big River	Big River	Mad River	Mad River	Ten Mile River
Scientific Name	Common Name	Upstream	Mouth	Upstream	Mouth	Mouth
Atherinops affinis	Topsmelt	157	0	0	0	7.33
Catostomus occidentalis	Sacramento Sucker	0	0	0.33	0	0
Heterostichus rostratus	Giant Kelpfish	0	0.67	0	0	0
Clupea pallasi	Pacific Herring	92.83	113	0	187.5	0
	Sculpins (Unidentified)	0	0.33	1	0.33	0
Artedius fenestralis	Padded Sculpin	0	0.67	0	0	0
Artedius notospilotus	Bonyhead Sculpin	0	1.33	0	0	0
Clinocottus acuticeps	Sharpnose Sculpin	0.67	0	0	1	0
Cottus aleuticus	Coastrange Sculpin	0.33	0	18.67	0	0
Cottus asper	Prickly Sculpin	0.67	0.72	36.46	2.96	1
Enophrys bison	Buffalo Sculpin	0	1.42	0	0.5	0
Hemilepidotus hemilepidotus	Red Irish Lord	0	0.5	0	0	0
Leptocottus armatus	Pacific Staghorn Sculpin	63.42	62.17	27.33	36.53	103.77
Oligocottus maculosus	Tidepool sculpin	0.67	0.33	0	0	0
Scorpaenichthys marmoratus	Cabezon	3.67	8.17	0	1.06	0
Cymatogaster aggregata	Shiner Surfperch	21	23.5	0	1.5	0.5
Embiotica lateralis	Striped Surfperch	4	0.83	0	0	0
Phanerodon furcatus	White Surfperch	0.33	0.67	0	0	0
Gasterosteus aculeatus	Three Spine Stickleback	2.43	1.79	42	0.78	1

		Big	Big	Mad	Mad	Ten Mile
		River	River	River	River	River
Scientific Name	Common Name	Upstream	Mouth	Upstream	Mouth	Mouth
Clevelandia ios	Arrow Goby	8.04	1.33	0	0	1.92
Blepsias cirrhosus	Silverspot Sculpin	0.5	3.5	0	0	0
Hexogrammos decagrammus	Kelp Greenling	0.33	2.33	0	0	0
Ophiodon elongatus	Lingcod	0	1	0	0	0
Hypomesus pretiosis	Surf Smelt	0.33	0.72	0	0	0
Spirinchus starski	Night Smelt	4	0	0.33	434	37.83
Citharichthys stigmaeus	Speckled Sanddab	1	0	0	0	2
Apodichthys flavidus	Penpoint Gunnel	0	1.08	0	0.67	0.67
Pholis ornata	Saddleback Gunnel	0	0	0	5.17	0
Platichthys stellatus	Starry Flounder	0.67	0.5	10	2.67	7.32
Pleuronectes vetulus	English Sole	60	110.06	0	39.17	9.08
Oncorhynchus kisutch	Coho Salmon	0.67	6	1.67	0	49.5
Oncorhynchus mykiss	Steelhead	0	0	0.67	0	0.39
Oncorhynchus tshawytscha	Chinook Salmon	2.17	3.17	18.06	23.5	13.33
Sebastes caurinus	Copper Rockfish	2.17	5.5	0	1.28	0
Sebastes melanops	Black Rockfish	0.5	0	0	1	0
Sebastes spp.	Juvenile Rockfish	8.33	51	0	0	1.67
Syngnathus leptorhynchus	Bay Pipefish	1.61	2.78	0	0.5	2

Appendix J Continued: Station abundance in the Big, Mad, and Ten Mile river estuaries, June 2014– June 2016. Abundance calculated as total catch divided by the number of seines.

# APPENDIX K

Scientific Name	Common Name	mouth	trib.	upstream
Atherinops affinis	Topsmelt	Х	Х	Х
Atherinopsis californiensis	Jacksmelt	Х	Х	
Catostomus occidentalis	Sacramento Sucker			Х
Heterostichus rostratus	Giant Kelpfish		Х	
Clupea pallasi	Pacific Herring	Х		
Clinocottus acuticeps	Sharpnose Sculpin	Х	Х	
Cottus aleuticus	Coastrange Sculpin		Х	Х
Cottus asper	Prickly Sculpin	Х	Х	Х
Cottus asperrimus	Rough sculpin			Х
Enophrys bison	Buffalo Sculpin	Х		
Leptocottus armatus	Pacific Staghorn Sculpin	Х	Х	Х
Oligocottus maculosus	Tidepool sculpin	Х		
Scorpaenichthys marmoratus	Cabezon	Х	Х	
Cymatogaster aggregata	Shiner Surfperch	Х	Х	Х
Engraulis mordax	Northern Anchovy		Х	
Gasterosteus aculeatus	Three-spine Stickleback	Х	Х	Х
Gobiesox naeabdricus	Northern Clingfish	Х	Х	
Lepidogobius lepidus	Bay Goby		Х	
Hexogrammos decagrammus	Kelp Greenling		Х	
Hypomesus pretiosis	Surf Smelt	Х		
Spirinchus starski	Night Smelt	Х		Х
Citharichthys stigmaeus	Speckled Sanddab	Х		
Apodichthys flavidus	Penpoint Gunnel	Х	Х	
Pholis ornata	Saddleback Gunnel	Х	Х	
Platichthys stellatus	Starry Flounder	Х	Х	Х
Pleuronectes vetulus	English Sole	Х	Х	Х
Oncorhynchus kisutch	Coho Salmon	Х	Х	Х
Oncorhynchus mykiss	Steelhead	Х	Х	Х
Oncorhynchus tshawytscha	Chinook Salmon	Х	Х	Х
Sebastes caurinus	Copper Rockfish	Х	Х	
Sebastes melanops	Black Rockfish	Х		
Sebastes rastrelliger	Grass Rockfish	Х	Х	
Syngnathus leptorhynchus	Bay Pipefish	Х	Х	Х

Appendix K: Mad River Estuary species presence (X) by station (trib = Tributary).

### APPENDIX L

Appendix L: Seasonal abundance in the Mad River Estuary, June 2014– June 2016. Abundance calculated as total catch divided by the number of seines.

Family	Scientific Name	Common Name	Summer	Fall	Winter	Spring
Atherinopsidae	Atherinops affinis	Topsmelt	8.67	67.44	0	0.33
Atherinopsidae	Atherinopsis californiensis	Jacksmelt	6.67	2.67	0	2
Catostomidae	Catostomus occidentalis	Sacramento Sucker	0.33	0.33	0	0
Clinidae	Heterostichus rostratus	Giant Kelpfish	0	0.33	0	0
Clupeidae	Clupea pallasi	Pacific Herring	187.5	0	0	0
Cottidae		Sculpins (Unidentified)	1.67	0.67	0	0.33
Cottidae	Clinocottus acuticeps	Sharpnose Sculpin	7.83	18.5	0	0
Cottidae	Cottus aleuticus	Coastrange Sculpin	24.17	12.92	0.33	0.67
Cottidae	Cottus asper	Prickly Sculpin	239.52	200.28	2	37.41
Cottidae	Cottus asperrimus	Rough sculpin	0	0.33	0	0
Cottidae	Enophrys bison	Buffalo Sculpin	0.5	0.33	0	0.33
Cottidae	Leptocottus armatus	Pacific Staghorn Sculpin	103.97	63	29.33	70.55
Cottidae	Oligocottus maculosus	Tidepool sculpin	0	0.67	0	0
Cottidae	Scorpaenichthys marmoratus	Cabezon	0.96	1	0	0.67
Embiotocidae	Cymatogaster aggregata	Shiner Surfperch	63.83	10.44	0	3.17
Engraulidae	Engraulis mordax	Northern Anchovy	0	0.33	0	0
Gasterosteidae	Gasterosteus aculeatus	Three Spine Stickleback	1142.11	245.04	2	9.58
Gobiesocidae	Gobiesox naeabdricus	Northern Clingfish	0.33	0.5	0	0
Gobiidae	Lepidogobius lepidus	Bay Goby	0	0.33	0	0
Hexagrammidae	Hexogrammos decagrammus	Kelp Greenling	1	0	0	0

Family	Scientific Name	Common Name	Summer	Fall	Winter	Spring
Osmeridae		Smelt (Unidentified)	0	0.33	0	0
Osmeridae	Hypomesus pretiosis	Surf Smelt	0.33	0.67	0	0
Osmeridae	Spirinchus starski	Night Smelt	434	0	0.33	0
Paralichthyidae	Citharichthys stigmaeus	Speckled Sanddab	0	7	0	0
Pholidae	Apodichthys flavidus	Penpoint Gunnel	1.56	0.83	0	0.33
Pholidae	Pholis ornata	Saddleback Gunnel	5.5	1.33	0	0.67
Pleuronectidae	Platichthys stellatus	Starry Flounder	6.73	0.67	0.33	0
Pleuronectidae	Pleuronectes vetulus	English Sole	27.97	2.33	0	36
Salmonidae	Oncorhynchus kisutch	Coho Salmon	1.42	0	0	0.56
Salmonidae	Oncorhynchus mykiss	Steelhead	2	0.67	0	0.56
Salmonidae	Oncorhynchus tshawytscha	Chinook Salmon	35.44	1.17	0	0.6
Sebastes	Sebastes caurinus	Copper Rockfish	2.29	2	0	0
Sebastes	Sebastes melanops	Black Rockfish	1	0	0	0
Sebastes	Sebastes rastrelliger	Grass Rockfish	0	1	0	0
Sebastes	Sebastes spp.	Juvenile Rockfish	0.33	0.33	0	0
Syngnathidae	Syngnathus leptorhynchus	Bay Pipefish	5.11	12.33	0	0.5

Appendix L Continued: Seasonal abundance in the Mad River Estuary, June 2014– June 2016. Abundance calculated as total catch divided by the number of seines.

## APPENDIX M

Appendix M: Station abundance in the Mad River Estuary, June 2014– June 2016. Abundance calculated as total catch divided by the number of seines.

Family	Scientific Name	Common Name	Mouth	Tributary	Upstream
Atherinopsidae	Atherinops affinis	Topsmelt	14.53	15.2	4.44
Atherinopsidae	Atherinopsis californiensis	Jacksmelt	1.44	3.5	0
Catostomidae	Catostomus occidentalis	Sacramento Sucker	0	0	0.33
Clinidae	Heterostichus rostratus	Giant Kelpfish	0	0.33	0
Clupeidae	Clupea pallasi	Pacific Herring	187.5	0	0
Cottidae		Sculpins (Unidentified)	0.44	0.33	1.22
Cottidae	Clinocottus acuticeps	Sharpnose Sculpin	6.33	4	0
Cottidae	Cottus aleuticus	Coastrange Sculpin	0	1.08	8.1
Cottidae	Cottus asper	Prickly Sculpin	5.99	62.96	53.89
Cottidae	Cottus asperrimus	Rough sculpin	0	0	0.33
Cottidae	Enophrys bison	Buffalo Sculpin	0.39	0	0
Cottidae	Leptocottus armatus	Pacific Staghorn Sculpin	19.29	56.19	25.36
Cottidae	Oligocottus maculosus	Tidepool sculpin	0.67	0	0
Cottidae	Scorpaenichthys marmoratus	Cabezon	1.03	0.5	0
Embiotocidae	Cymatogaster aggregata	Shiner Surfperch	0.79	7.27	51.33
Engraulidae	Engraulis mordax	Northern Anchovy	0	0.33	0
Gasterosteidae	Gasterosteus aculeatus	Three Spine Stickleback	3.7	45.81	256.39
Gobiesocidae	Gobiesox naeabdricus	Northern Clingfish	0.33	0.5	0
Gobiidae	Lepidogobius lepidus	Bay Goby	0	0.33	0
Hexagrammidae	Hexogrammos decagrammus	Kelp Greenling	0	1	0

Family	Scientific Name	Common Name	Mouth	Tributary	Upstream
Osmeridae		Smelt (Unidentified)	0.33	0	0
Osmeridae	Hypomesus pretiosis	Surf Smelt	0.5	0	0
Osmeridae	Spirinchus starski	Night Smelt	434	0	0.33
Paralichthyidae	Citharichthys stigmaeus	Speckled Sanddab	3.5	0	0
Pholidae	Apodichthys flavidus	Penpoint Gunnel	0.5	0.44	0
Pholidae	Pholis ornata	Saddleback Gunnel	3.08	1.33	0
Pleuronectidae	Platichthys stellatus	Starry Flounder	2.67	0.56	7.58
Pleuronectidae	Pleuronectes vetulus	English Sole	17.8	3.33	0.33
Salmonidae	Oncorhynchus kisutch	Coho Salmon	0.33	0.39	1.33
Salmonidae	Oncorhynchus mykiss	Steelhead	0.67	0.33	0.58
Salmonidae	Oncorhynchus tshawytscha	Chinook Salmon	11.88	10.31	9.36
Sebastes	Sebastes caurinus	Copper Rockfish	1.03	0.92	0
Sebastes	Sebastes melanops	Black Rockfish	1	0	0
Sebastes	Sebastes rastrelliger	Grass Rockfish	1.67	0.33	0
Sebastes	Sebastes spp.	Juvenile Rockfish	0.33	0.33	0
Syngnathidae	Syngnathus leptorhynchus	Bay Pipefish	1.53	1.6	6.5

Appendix M Continued: Abundance by station in the Mad River Estuary, June 2014– June 2016. Abundance calculated as total catch divided by the number of seines.

#### APPENDIX N

Appendix N: Mean frequency of occurrence for key prey groups (Table 3). From top to bottom: the Big River Estuary, the Mad River Estuary, and the Ten Mile River Estuary, June 2014-June 2016. Dotted line denotes 100% occurrence.



### APPENDIX O

Appendix O: Mean frequency of occurrence for key prey groups (Table 3) found during seasonal and monthly sampling in the Mad River Estuary. Dotted line denotes 100% occurrence.

