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Morro Bay Estuary Fisheries Monitoring

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Cover photos: Beach seining at Colman Beach (top left), juvenile rockfish (top right), giant kelpfish (bottom left), retrieving otter trawl net (bottom right).

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1 INTRODUCTION

1.1 Background

Estuaries make up a crucial component of marine ecosystems with many fish species completing at least part of their life cycle within these important ecotones. Eelgrass (*Zostera marina*) provides a major source of nursery habitat in estuaries along the West Coast, providing crucial reproductive and rearing habitat for a variety of fish species. The Morro Bay National Estuary Program (MBNEP) has dedicated extensive time and effort to monitor and restore eelgrass in the Morro Bay estuary, California. These monitoring efforts, which have occurred for over twenty years, have documented large fluctuations in eelgrass abundance with a drastic decline of nearly 95 percent observed between 2007 and 2017 when eelgrass area decreased from 344 acres mapped in 2007 to 13 acres mapped in 2017. Beginning in 2019, the MBNEP began to observe a substantial recovery in eelgrass abundance and distribution within the Morro Bay estuary. By 2023, eelgrass covered approximately 750 acres of the Morro Bay estuary, exceeding levels observed prior to the decline (Figure 1) (MBNEP 2024a).

Previous fish monitoring efforts conducted in the Morro Bay estuary compared fish sampling data collected during pre- and post-eelgrass decline conditions (O'Leary et al. 2021; O'Leary2016-2018; Stevens 2006–2007). Results from these studies indicated that following the decline in eelgrass, fish species abundance and biomass remained stable; however, a notable shift in species composition occurred. The shift in species composition was characterized by increases in habitat generalists, such as flatfish (mainly speckled sanddab [*Citharichthys stigmaeus*]) and staghorn sculpin (*Leptocottus armatus*) and decreases in habitat specialists including bay pipefish (*Syngnathus leptorhynchus*) and shiner perch (*Cymatogaster aggregata*).

Bay pipefish

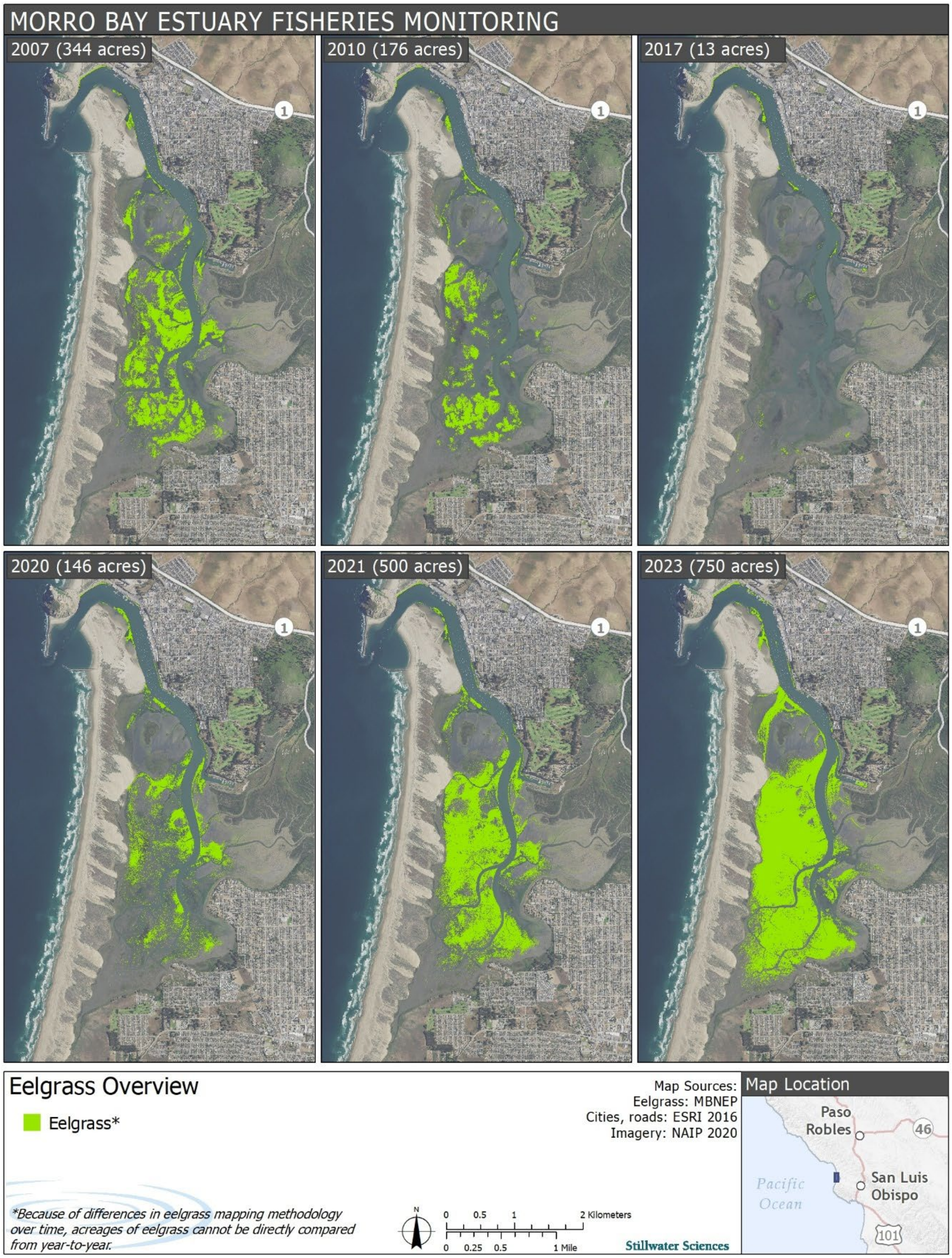


Figure 1. Eelgrass areas in Morro Bay mapped during 2007–2023.

1.2 Goals and Objectives

The goal of this study is to assess how the fish community in the Morro Bay estuary responded to recovery of eelgrass (Figure 1). The objectives of this study were to conduct fisheries monitoring efforts within representative habitats of the Morro Bay estuary to evaluate current fish community conditions based on species composition, richness, and abundance; assess how fish community conditions vary between eelgrass habitat and other habitat types in the bay, and compare current fish community conditions to data collected from previous efforts that were conducted before and after the eelgrass habitat extent declined.

California halibut (*Paralichthys californicus*) (top) and speckled sanddab (bottom)

1.3 Study Area

The Morro Bay estuary is located on California's central coast approximately halfway between San Francisco and Los Angeles. Morro Bay is designated as an Estuary of National Significance and encompasses and area of 2,300 acres. Sampling was conducted at thirteen locations to cover a variety of habitat types present within the Morro Bay estuary including tidal flats, open channel, and shoreline areas (Figure 2). Monitoring locations were located in the vicinity of historic monitoring locations described in O'Leary et al. (2021) with the exception of two beach seine locations (1B-5 and 1B-6) that were added along the sand spit during this study.

Figure 2. Study area and fish monitoring locations.

2 METHODS

Retrieving beam trawl net at monitoring location1A-5

Sampling was conducted at various habitat types during the fall of 2023 and spring of 2024 to account for seasonal variation. Fish capture methods included otter trawls within open channel habitat that is too deep for eelgrass to grow, beam trawls within tidal flat habitat where eelgrass is most abundant, and beach seines along shoreline habitat where eelgrass has a patchy distribution. Results from the 2023 and 2024 monitoring periods which coincided with high eelgrass abundance were compared with historic capture data collected during periods with medium eelgrass abundance (2006–2008) and periods with low eelgrass abundance (2016–2017) as described in O'Leary et al. (2021). In addition, environmental deoxyribonucleic acid (eDNA) sampling was conducted during the fall 2023 and spring 2024 sampling efforts to develop a comprehensive list of fish species occurring in the Morro Bay estuary. Details on each method are described below.

2.1 Capture Methods

To reduce potential sampling bias, various capture methods were used during this study, including otter trawls, beam trawls and beach seines, as described below. Gear specifications and level of effort during the three monitoring periods are summarized in Table 1.

Table 1. Morro Bay fisheries monitoring gear specifications and sampling effort summary.

Gear Type and Dimensions	Sample Seasons (Month and Year)	Site Locations	Sampling Effort (Time/ Distance)	Replicates per Sampling Event
2023–2024				
Otter Trawl Dimensions: 2.5 m x 5.3 m Mesh 38 mm and 6.4 mm[1]	Fall (Sept 2023) Spring (May 2024)	1A-1 Open Water (north bay) 1A-2 Open Water (mid bay) 1A-3 Open water (south bay)	10 minutes (1.0 to 1.7 knots)	3 tows
Beam Trawl Dimensions: 4 m x 2 m Mesh: 3.2 mm	Fall (Sept 2023) Spring (May 2024)	1A-4 Tidal Flat (north bay) 1A-5 Tidal Flat (mid Bay-west side of channel) 1A-6 Tidal Flat (mid Bay-east side of channel) 1A-7 Tidal Flat (south bay)	10 minutes (1.0 to 1.7 knots)	2-3 tows
Beach Seine Dimensions: 15.3 m x 1.8 m Mesh: 6.4 mm	Fall (Sept 2023) Spring (May 2024)	1B-1 Coleman Beach (unvegetated) 1B-2 Coleman Beach (eelgrass) 1B-3 State Park Beach (unvegetated) 1B-4 State Park Beach (eelgrass) 1B-5 Sandspit (unvegetated) 1B-6 Sandspit (eelgrass)	30 m	2-3 hauls
2016–2018				
Otter Trawl Dimensions: 4.6 m x 7.2 m Mesh: 14 mm and 8 mm[1]	Fall (Oct 2016) Summer (Jun 2017)	1A-1 Open Water (north bay) 1A-2 Open Water (mid bay) 1A-3 Open water (south bay)	10 minutes (0.8 to 1.8 knots)	3 tows
Beam Trawl Dimensions: 4 m x 2 m Mesh: 3.2 mm	Fall (Oct 2016) Summer (Jun 2017)	1A-4 Tidal Flat (north bay) 1A-5 Tidal Flat (mid bay-west side of channel) 1A-6 Tidal Flat (mid bay-east side of channel) 1A-7 Tidal Flat (south bay)	10 minutes (0.8 to 1.8 knots)	2-4 tows
Beach Seine Dimensions: 10.2 m x 1.1 m Mesh: 4 mm	Summer (Jul 2016)[2] Spring (Apr 2017 and May 2017) Spring (Apr 2018)	1B-1 Coleman Beach (unvegetated)[2] 1B-2 Coleman Beach (eelgrass)[2] 1B-3 State Park Beach (unvegetated) 1B-4 State Park Beach (eelgrass)	5 to 34 m	2-3 hauls

Gear Type and Dimensions	Sample Seasons (Month and Year)	Site Locations	Sampling Effort (Time/ Distance)	Replicates per Sampling Event
2006–2008				
Otter Trawl Dimensions: 4.6 m x 7.2 m Mesh: 14 mm and 8 mm[1]	Spring (Mar 2007) Summer (Aug 2007) Fall (Nov 2007) Spring (May 2008)	1A-1 Open Water (north bay) 1A-2 Open Water (mid bay) 1A-3 Open water (south bay)	10 minutes (0.8 to 1.8 knots)	3 tows
Beam Trawl Dimensions: 4 m x 2 m Mesh: 3.2 mm	Spring (Apr 2006) Fall (Nov 2006) Spring (Mar 2007) Fall (Nov 2007)	1A-4 Tidal Flat (north bay) 1A-5 Tidal Flat (mid Bay-west side of channel) 1A-7 Tidal Flat (south Bay)	10 minutes (0.8 to 1.8 knots)	3 tows
Beach Seine Dimensions: 15.2 m x 1.2 m Mesh: 4 mm	Spring (Apr 2006) Fall (Nov 2006) Spring (Mar 2007) Fall (Nov 2007) Spring (May 2008)	1B-1 Coleman Beach (unvegetated) 1B-2 Coleman Beach (eelgrass) 1B-3 State Park Beach (unvegetated) 1B-4 State Park Beach (eelgrass)	NA[3]	3 hauls

[1] Otter trawls used variable mesh, the first mesh size value is for the wings and the second value is for the cod end.
[2] Coleman beach not sampled in 2016
[3] Beach seine sampling distance was not reported for 2006–2008

2.1.1 Trawls

Otter trawls and beam trawls were used to sample open water habitat and tidal flats in the Morro Bay estuary during the spring and fall sampling efforts. Otter trawls were used in open water habitat, while beam trawls were used within tidal flats. For both trawl methods, nets were towed for 10-minutes at speeds of 1.0 to 1.7 knots during daylight hours at or near the daily high tide. Two to three tows were conducted at each monitoring location to account for variation between catch composition.

2.1.1.1 Beam trawls

Benthic beam trawls were conducted within tidal flat habitat at four monitoring locations throughout the Morro Bay estuary. These monitoring locations included a north bay location (1A-4), one location on each side of the main channel within the mid-bay (1A-5 and 1A-6), and a south bay location (1A-7) (Figure 2). The beam trawl used was approximately 4 meters (m) long with a 2.0 m wide by 0.8 m opening and net mesh size of 0.32-centimeter (cm) mesh, which is the same size as used in previous monitoring periods.

2.1.1.2 Otter trawls

Otter trawls were conducted at three monitoring locations within the main channel of the Morro Bay estuary. These monitoring locations were located near the mouth of the bay (1A-1), one at the north end of the bay (1A-2), and one near the mid-section of the bay (1A-3) (Figure 2). The otter trawl used during the 2023–2024 monitoring period was 2.5 m wide and 5.3 m long held open by two otter doors, the tail, or "cod" end of the net had mesh size of 0.64 cm mesh. This net was smaller than the otter trawl net used during the previous monitoring periods which used an otter trawl that was 4.6 m wide and 7.2 m long.

2.1.2 Beach seining

Beach seining was conducted within shoreline habitat at three areas around the bay. Each area included paired locations with one location having dense eelgrass and the other having little to no eelgrass.[1] Three monitoring locations included shoreline habitat with vegetation (i.e., eelgrass) (1B-2, IB-4, and IB-6) and three locations included unvegetated shoreline habitat (1B-1, 1B-3, and 1B-5) (Figure 2). Beach seining was conducted during daylight hours at or near the daily high tide. During 2023–2024 a beach seine that was 15.3 m long by 1.8 m tall with mesh size of 0.64 cm was used. At each monitoring location, three replicate pulls were conducted over distances of 30 m. Historic monitoring used a beach seine measuring 10.2 m long, 1.1 m high, and had a mesh size of 0.4 cm with pull distances ranging from 5.4 m to 34 m. At the conclusion of each pull, beach seines were retrieved by hand until the seine

Beach seining near the sand spit at monitoring location 1B-6

[1] Beach seining targeted three locations that appeared unvegetated when viewed from a distance; however, small patches of macroalgae and eelgrass were typically observed in very low density at some point along the 30-meter sample distance.

bag reached a high point on the shore. Fish were removed and held in buckets of cold water equipped with oxygen diffusers to create ideal conditions for survival while processing. The volume of water sampled was estimated by the width, length, and depth of each seine pull.

2.2 Fish Handling

All fish specimens were collected, processed, and returned to the water as soon as possible. Fish specimens were removed from the various sampling gear and placed in totes or buckets with cold water equipped with aerators and water was refreshed frequently. All fish were identified to species and fish were measured to fork length. In cases where high numbers of individual species were captured, a representative subset of 25 individuals was measured and the remaining individuals were tallied. Fish were quickly returned to the water once processing was completed. All fish sampling data was collected and recorded in the field.

eDNA sample collection at monitoring location 1A-3

2.3 Environmental Parameters Metadata

Environmental parameters were measured at each monitoring site on each individual sampling day. These parameters included water depth, tide elevation, and water quality conditions. Water depth was measured with a depth finder, or via marks on a depth or stadia rod, depending on depth. Tidal elevation was recorded daily from the Morro Bay harbor Surfline report (https://www.surfline.com/surf-report/morro-bay-harbor/584204214e65fad6a7709cf8?camId=58349cb73421b20545c4b55d). Dissolved oxygen, water temperature, and salinity were measured by MBNEP staff with a handheld YSI Pro 2030 meter (MBNEP 2024b).

2.4 Environmental DNA Sampling

eDNA sampling was conducted during each sampling event. Water samples were collected and filtered in the field. During the September 2023 effort, eDNA samples were collected from sixteen locations within the Morro Bay estuary including each of the thirteen monitoring locations as well as from three tidal channel locations. At most locations a single sample was collected for eDNA analysis; however, at the Coleman Beach monitoring location (1B-2) a total of six samples were collected to compare detection results between replicate samples in order to assess the likelihood of species detection. During May 2024, eDNA samples were collected from nine locations

Water quality measurement at monitoring location 1B-5 covering representative habitats within the Morro Bay estuary and three samples were collected from each location. During each sampling effort, water was collected by submerging a 1-liter sample bottle approximately 0.3 m underwater. Once the water was collected, approximately 100 to 400 milliliters of water was pushed through a syringe filter. Field blanks using distilled water were collected during each day of sampling. Filters were sent to Jonah Ventures Inc. for metabarcoding analysis. Results of the eDNA sampling are summarized by sample location and species detected by season.

2.5 Analysis

Data analysis was conducted in R (version 4.3.2). Survey results were used to test changes in species richness (defined as the total number of species present), community composition (defined as the types of species present and their relative abundance), and abundance (defined as number of fish per haul for trawls and number of fish per m^2 for beach seines) of selected species over time. Species richness for beam and otter trawl samples was calculated as the number of species captured in each replicate haul. Samples were grouped based on eelgrass abundance levels of low (13 acres during 2016–2017), medium (344 acres during 2006–2008), and high (750 acres 2023–2024). Survey results from high eelgrass abundance is based on data collected for this study in 2023 and 2024, and survey results for low and medium eelgrass abundance is based on data analysis provided by Stephens and O'Leary et al. (2021). For each trawl type the relationship between species richness per haul and eelgrass abundance was tested using Generalized Linear Mixed Models (GLMMs) using the 'glmer' function from the R package 'lme4' with "monitoring location" as a random effect to account for repeated measures within sampling events (i.e., 3 replicates per site per sample event). As an additional fixed effect to account for potential seasonal differences in species richness, these models were also tested with "season" (fall = September–November, spring = March–May, summer = June–August). For beach seine samples, seine size and sample distance was used to normalize species richness per seine haul to the area sampled for each haul. The effect of eelgrass abundance on species richness per square meter using a linear mixed effects model (LMM; 'lmer' function within the R package 'lme4') with "monitoring location" as a random effect and "season" an additional fixed effect. Best fit models were selected using Bayesian Information Criterion (BIC). Differences in species richness between beach seine sample locations with eelgrass (i.e., vegetated) and unvegetated sample locations sampled in 2023–2024 were tested using a LMM with "season" as an additional fixed effect and "monitoring location" (Coleman, Sandspit, Windy Cove) as a random effect to account for repeat samples within monitoring locations. "Season" did not improve model fit and was dropped from the final model.

The relative abundance (number of individuals per haul for each period of eelgrass abundance) was calculated for seven species that were detected repeatedly during beam and otter trawl surveys. For each survey method, fish abundance was compared between eelgrass abundance levels for three species that are known habitat specialists in seagrass beds (shiner perch, black surfperch [*Embiotoca jacksoni]*, and bay pipefish) and for three species that are not seagrass specialists (i.e., habitat generalists) which varied between survey method as follows: for beam trawl surveys these included arrow goby [*Clevelandia ios*], speckled sanddab, and staghorn sculpin; for otter trawl surveys these included English sole (*Parophrys vetulus*), speckled sanddab, and staghorn sculpin. Abundance was compared between each period of eelgrass abundance for each species and survey type using non-parametric Kruskal-Wallis tests and for pairwise comparisons of each period of eelgrass abundance using post-hoc Dunn tests.

3 RESULTS

Sampling was conducted during September 14–28, 2023, and during May 6–10, 2024. A total of 8,317 fish representing 24 taxa were captured over the course of the study (Table 1). Beach seine and beam trawls accounted for the bulk of the catch with only three percent of the catch from otter trawls. Although several different fish species were observed during the 2023–2024 monitoring efforts, five different species accounted for the majority of fish captured. Bay pipefish were the most abundant species captured accounting for over 40 percent of the total catch, followed by arroyo goby (20 percent), shiner perch (10 percent), topsmelt silverside (*Atherinops affinis*) (7 percent) and anchovy (5 percent) (Table 2).

Water quality conditions varied between sampling periods and between some sampling locations, except for salinity levels remained similar between efforts at approximately 33 parts per thousand (Table 3). Warmer temperatures and lower dissolved oxygen levels were observed in September 2023 compared to May 2024. During September 2023, water temperatures ranged from 15.1 to 20.4 degrees Celsius (°C). The highest temperatures were measured at beach seine locations and similar temperatures were measured at beam and otter trawl locations. Dissolved oxygen levels recorded in September 2023 ranged from 5.5 to 9.3 milligrams per L (mg/L). The highest dissolved oxygen levels were measured at beach seine monitoring locations and the lowest levels were measured at otter trawl monitoring locations. During May 2024, water temperatures ranged from 10.7 to 17.1°C. The warmest temperature was measured at the beach seine monitoring locations located along the sandspit. Dissolved oxygen levels ranged from 6.5 to 12.7 mg/L. The highest dissolved oxygen level was measured in the south bay beam trawl location and the lowest at the state park beach seine monitoring location (Table 3).

Northern anchovy

Table 2. Number of fish captured by method and habitat type during September 2023 and May 2024 monitoring efforts. *Percent of catch values ≥5% are shaded grey.*

Fish Species (Common Name)	Fish Species (Scientific Name)	Number Captured by Method (Habitat Type)			Total Number Captured	Percent of Catch			Total Percent of Catch
		Beach Seine (Shoreline)	Beam Trawl (Tidal Flats)	Otter Trawl (Open Channel)		Beach Seine (Shoreline)	Beam Trawl (Tidal Flats)	Otter Trawl (Open Channel)	
Arrow goby	*Clevelandia ios*	1,487	202	0	1,689	**39%**	**5%**	0%	**20%**
Bay blenny	*Hypsoblennius gentilis*	0	1	0	1	0%	0%	0%	0%
Bay pipefish	*Syngnathus leptorhynchus*	313	3,155	26	3,494	**8%**	**74%**	**10%**	**42%**
Black surfperch	*Embiotoca jacksoni*	110	157	0	267	3%	4%	0%	3%
California halibut	*Paralichthys californicus*	1	4	0	5	0%	0%	0%	0%
California lizard fish	*Synodus lucioceps*	1	0	63	64	0%	0%	**24%**	1%
Cabezon	*Scorpaenichthys marmoratus*	23	0	1	24	1%	0%	0%	0%
California killifish	*Fundulus parvipinnis*	13	1	0	14	0%	0%	0%	0%
Diamond turbot	*Hypsopsetta guttulata*	7	1	1	9	0%	0%	0%	0%
Dwarf surfperch	*Micrometrus minimus*	101	6	0	107	3%	0%	0%	1%
Giant kelpfish	*Heterostichus rostratus*	12	19	0	31	0%	0%	0%	0%
Northern anchovy	*Engraulis mordax*	420	10	11	441	**11%**	0%	4%	**5%**
Penpoint gunnel	*Apodichthys flavidus*	3	1	0	4	0%	0%	0%	0%
Opaleye	*Girella nigricans*	4	0	0	4	0%	0%	0%	0%
Pile surfperch	*Phanerodon vacca*	5	14	0	19	0%	0%	0%	0%
Rockfish species[1]	*Sebastes Spp*	60	12	0	72	2%	0%	0%	1%
Shiner perch	*Cymatogaster aggregata*	470	399	3	872	**12%**	**9%**	1%	**10%**
Speckled sanddab	*Citharichthys stigmaeus*	53	7	153	213	1%	0%	**58%**	3%
Staghorn sculpin	*Leptocottus armatus*	137	120	6	263	4%	3%	2%	3%
Starry flounder	*Platichthys stellatus*	1	1	0	2	0%	0%	0%	0%
Striped kelpfish	*Gibbonsia metzi*	9	16	0	25	0%	0%	0%	0%
Three-spined stickleback	*Gasterosteus aculeatus*	4	134	0	138	0%	3%	0%	2%
Topsmelt silversides	*Atherinops affinis*	558	0	0	558	**15%**	0%	0%	**7%**
Tubesnout	*Aulorhynchus flavidus*	0	0	1	1	0%	0%	0%	0%
Total		3,792	4,260	265	8,317	46%	51%	3%	100%

[1] Rockfish not keyed out to species.

Table 3. Water quality conditions during fish monitoring efforts conducted in the Morro Bay estuary in September 2023 and May 2024.

Sample Date	Location ID and Description	Sample Method	Average Depth (ft)[1]	Water Temp. (°C)	Salinity (ppt)	Dissolved Oxygen (mg/l)	Dissolved Oxygen (%)
Fall 2023 Sampling Effort							
9/14/23	1A-1 Open Water (north Bay)	Otter Trawl	12.3	15.7	33.8	6.55	80.9
9/14/23	1A-2 Open Water (mid bay)	Otter Trawl	17.9	16.4	33.8	5.48	68.7
9/18/23	1A-3 Open water (south bay)	Otter Trawl	18.9	17.1	33.8	5.04	63.1
9/15/23	1A-4 Tidal Flat (north Bay)	Beam Trawl	5.7	15.5	33.9	8.54	104.9
9/20/23	1A-5 Tidal Flat (mid Bay-west side of channel)	Beam Trawl	5.2	18.2	33.8	7.61	99.0
9/20/23	1A-6 Tidal Flat (mid Bay-east side of channel)	Beam Trawl	4.1	16.3	33.8	6.75	84.5
9/18/23	1A-7 Tidal Flat (south Bay)	Beam Trawl	4.5	--[3]	--[3]	--[3]	--[3]
9/28/23	1B-1 Coleman Beach (unvegetated)[2]	Beach Seine	3.0	15.1	33.6	8.55	104.4
9/28/23	1B-2 Coleman Beach (eelgrass)	Beach Seine	3.0	15.8	32.6	9.20	113.5
9/20/23	1B-3 State Park Beach (unvegetated)[2]	Beach Seine	2.5	19.3	33.1	6.44	85.0
9/20/23	1B-4 State Park Beach (eelgrass)	Beach Seine	2.5	18.3	33.8	6.58	85.5
9/19/23	1B-5 Sandspit (unvegetated)[2]	Beach Seine	3.0	19.8	33.5	6.86	--[3]
9/19/23	1B-6 Sandspit (eelgrass)	Beach Seine	3.5	20.4	33.6	9.25	--[3]
Spring 2024 Sampling Effort							
5/08/24	1A-1 Open Water (north Bay)	Otter Trawl	20.9	10.6	34.4	8.92	99.8
5/07/24	1A-2 Open Water (mid bay)	Otter Trawl	16.5	11.7	--[3]	11.42	105.0
5/06/24	1A-3 Open water (south bay)	Otter Trawl	16.7	14.1	33.3	9.29	109.9
5/09/24	1A-4 Tidal Flat (north Bay)	Beam Trawl	3.7	11.5	34.4	10.64	121.8
5/06/24	1A-5 Tidal Flat (mid Bay-west side of channel)	Beam Trawl	3.7	13.7	32.5	8.59	101.3
5/09/24	1A-6 Tidal Flat (mid Bay-east side of channel)	Beam Trawl	2.7	15.2	33.2	8.39	102.5
5/10/24	1A-7 Tidal Flat (south Bay)	Beam Trawl	3.3	12.6	33.4	12.73	160.5
5/10/24	1B-1 Coleman Beach (unvegetated)[2]	Beach Seine	3.5	14.7	33.5	8.30	100.2
5/10/24	1B-2 Coleman Beach (eelgrass)	Beach Seine	2.2	14.7	33.5	8.30	100.2
5/07/24	1B-3 State Park Beach (unvegetated)[2]	Beach Seine	3.5	10.7	34.1	6.53	72.5
5/07/24	1B-4 State Park Beach (eelgrass)	Beach Seine	4.0	10.7	34.1	6.53	72.5
5/08/24	1B-5 Sandspit (unvegetated)[2]	Beach Seine	2.5	17.1	33.4	10.45	132.5
5/08/24	1B-6 Sandspit (eelgrass)	Beach Seine	2.3	17.1	33.4	10.45	132.5

Notes: ft = feet, °C = degree Celsius, ppt = parts per thousand, mg/L = milligram per liter, % = percent saturation

[1] Average depth refers to water depth at sample location; water quality measurements were all collected at approximately 1-2 feet below the water surface.

[2] Beach seining targeted three locations that appeared unvegetated when viewed from a distance; however, small patches of macroalgae or eelgrass were often observed at some point along the 30-meter sample distance.

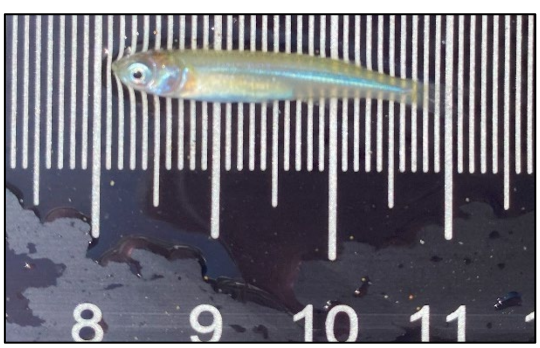
[3.] data not recorded.

3.1 Species Composition

California lizardfish (*Synodus lucioceps*)

Beam trawls were used to sample tidal flat habitat where eelgrass abundance fluctuated between nearly absent during the 2016–2017 monitoring period to abundant during the 2023–2024 monitoring period. Within tidal flat habitat, arrow goby, bay pipefish, shiner perch, and staghorn sculpin were observed during each monitoring period (Figure 3). During the period of low eelgrass abundance, English sole and speckled sanddab made up a large proportion of the total fish captured, while during periods with medium to high eelgrass abundance, English sole and speckled sanddab were either rare or absent from beam trawl catch. Bay pipefish, shiner surfperch, and arrow goby were the most common species captured in tidal flat habitat during periods with medium and high eelgrass abundance; however, staghorn sculpin, arrow goby, and speckled sanddab were the most common species captured during periods with low eelgrass abundance (Figure 3).

Otter trawls were used to sample open channel habitat that is regularly dredged and too deep for eelgrass to grow. Within open channel habitat, speckled sanddab and staghorn sculpin were the only species observed during each monitoring period (Figure 4). During the periods of medium and low eelgrass abundance, speckled sanddab, staghorn sculpin, and English sole were the most common species captured, while during the period of high eelgrass abundance (2023–2024), speckled sanddab, bay pipefish, and California lizardfish (*Synodus lucioceps*) were the most common species captured. No English sole was captured during the 2023–2024 monitoring period and California lizardfish appeared in the catch for the first time during the 2023–2024 monitoring period (Figure 4). A smaller otter trawl net was used during the 2023–2024 monitoring period to reduce potential impacts to eelgrass which likely led to lower overall catch compared to the number of fish caught during previous monitoring periods when a larger otter trawl net was used. While the total otter trawl catch numbers from 2023–2024 may have been lower than what would have been captured with a larger net, a representative portion of the population was likely captured and species composition is not expected to be effected by lower catch numbers.



Topsmelt silversides (*Atherinops affinis*)

Beach seines were used to sample shoreline habitat where eelgrass is patchy. Within shoreline habitat topsmelt silverside, arrow goby, staghorn sculpin, and bay pipefish were typically the most common species captured; however, species composition was highly variable between monitoring periods (Figure 5). During the medium eelgrass abundance monitoring period (2006–2008) no bay pipefish, shiner perch, or speckled sanddab were captured in beach seines but these species were captured during the other monitoring periods. During the high eelgrass abundance monitoring periods (2023–2024) anchovy made up a high proportion of the beach seine catch. Two new beach seine monitoring locations were added along the sandspit during the 2023–2024 monitoring period which likely influenced the species composition. Arrow goby, topsmelt silverside, and shiner perch were the most common species captured along shoreline habitat in 2023–2024 (Figure 5).

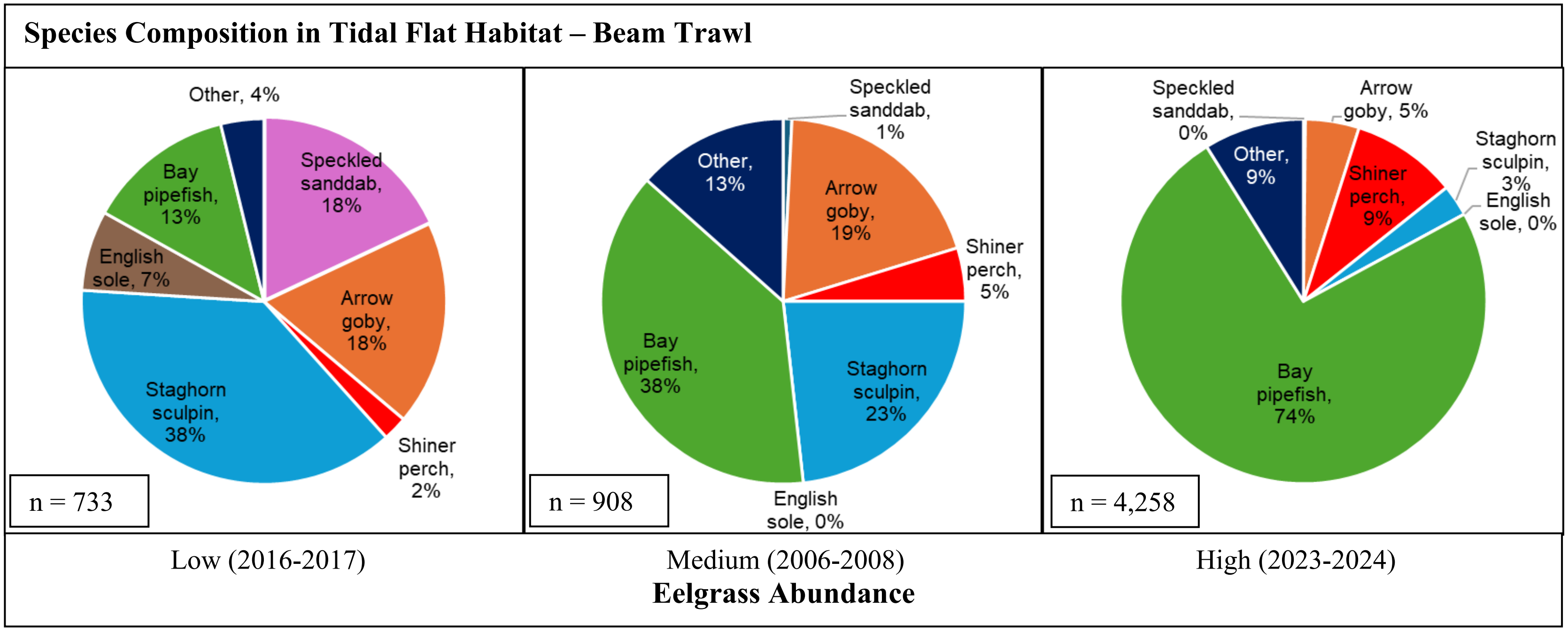


Figure 3. Species composition in tidal flat habitat (beam trawl sampling) during periods of low (2016-2017; left), medium (2006-2008; middle), and high eelgrass abundance (2023-2024; right).

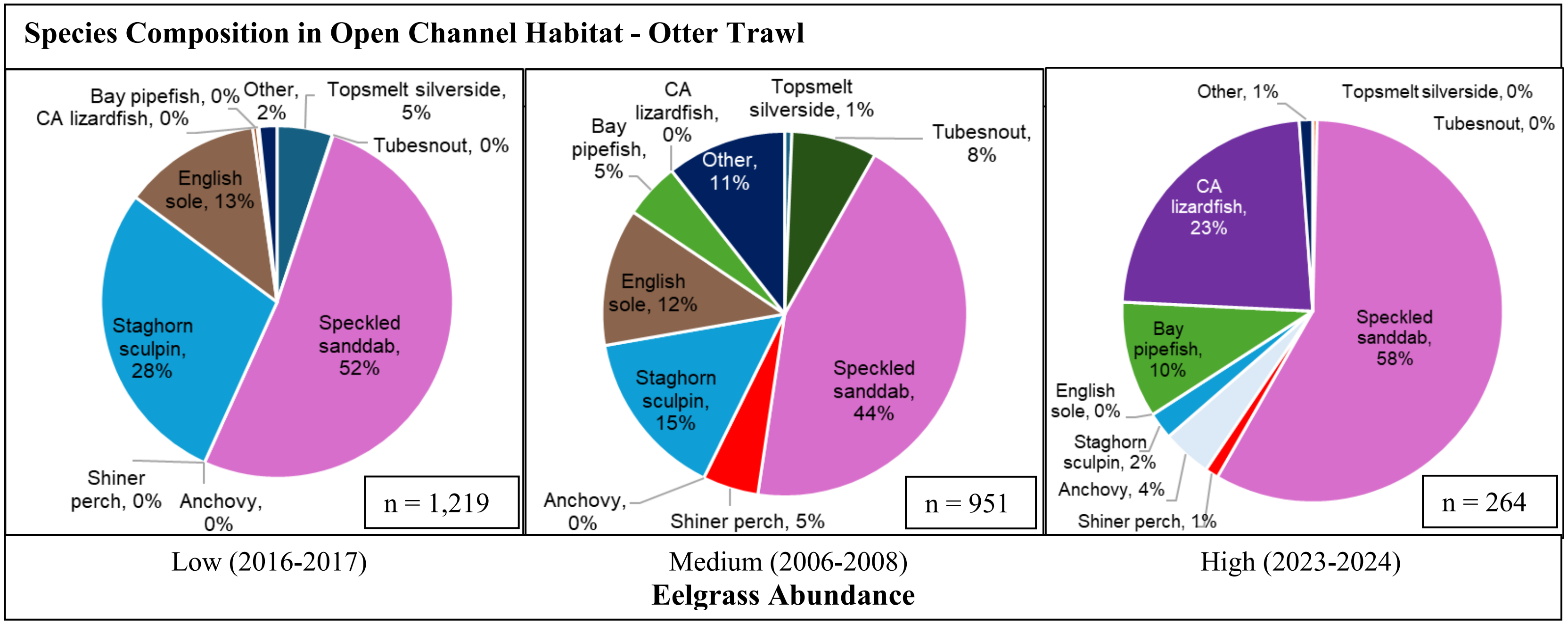


Figure 4. Species composition in open channel habitat (otter trawl sampling) during periods of low (2016-2017; left), medium (2006-2008; middle), and high eelgrass abundance (2023-2024; right).

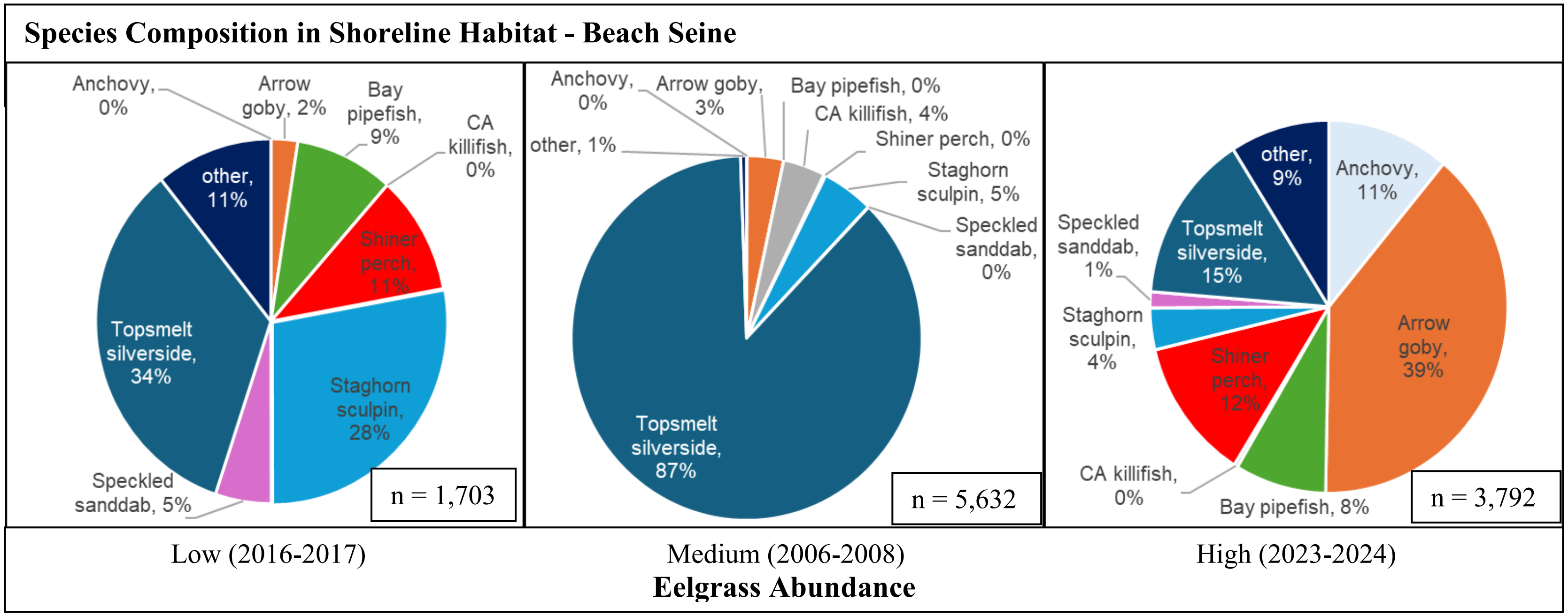


Figure 5. Species composition observed at shoreline habitat (beach seine sampling) during periods of low (2016–2018; left), medium (2006–2008; middle), and high eelgrass abundance (2023-2024; right).

3.2 Species Richness

Species richness varied significantly within tidal flat and shoreline habitat between periods of eelgrass abundance, but samples from within open channel habitat were similar between years. On average, samples from tidal flat habitat collected during the period of high eelgrass abundance contained more species per haul than in the period with low eelgrass abundance ($p = 0.05$), while samples collected from the period with medium eelgrass abundance did not have significantly different species richness compared to other periods (Figure 6). Samples collected within open channel habitat indicated no differences in species richness between periods ($p = 0.83$; Figure 7). Samples collected from shoreline habitat indicate significantly higher species richness when eelgrass was in low abundance compared to both periods with medium and high eelgrass abundance ($p < 0.01$; Figure 8). Season did not have a significant effect on species richness as measured within tidal flat and shoreline habitat; however, there was a significant interaction between season and eelgrass abundance for open channel habitat ($p = 0.04$; Figure 7). On average, more species were captured in the open channel samples in summer compared to fall during the period of low eelgrass abundance and more species were captured in fall compared to spring during the period of high eelgrass abundance. Species richness between tidal flat and open channel habitat is similar with averages ranging from approximately four to five species captured per haul within tidal habitat compared to averages of approximately three species captured per haul in open channel habitat (Figure 6 and Figure 7).

In 2023 and 2024, beach seine samples were collected from eelgrass beds and from unvegetated[2] areas directly adjacent to eelgrass beds. Results showed significantly higher species richness in eelgrass beds compared to adjacent unvegetated areas ($p < 0.01$), although the magnitude of this effect varied by monitoring location (Figure 9).

[2] Beach seining targeted three locations that appeared unvegetated when viewed from a distance; however, small patches of macroalgae and eelgrass were typically observed in very low density at some point along the 30-meter sample distance.

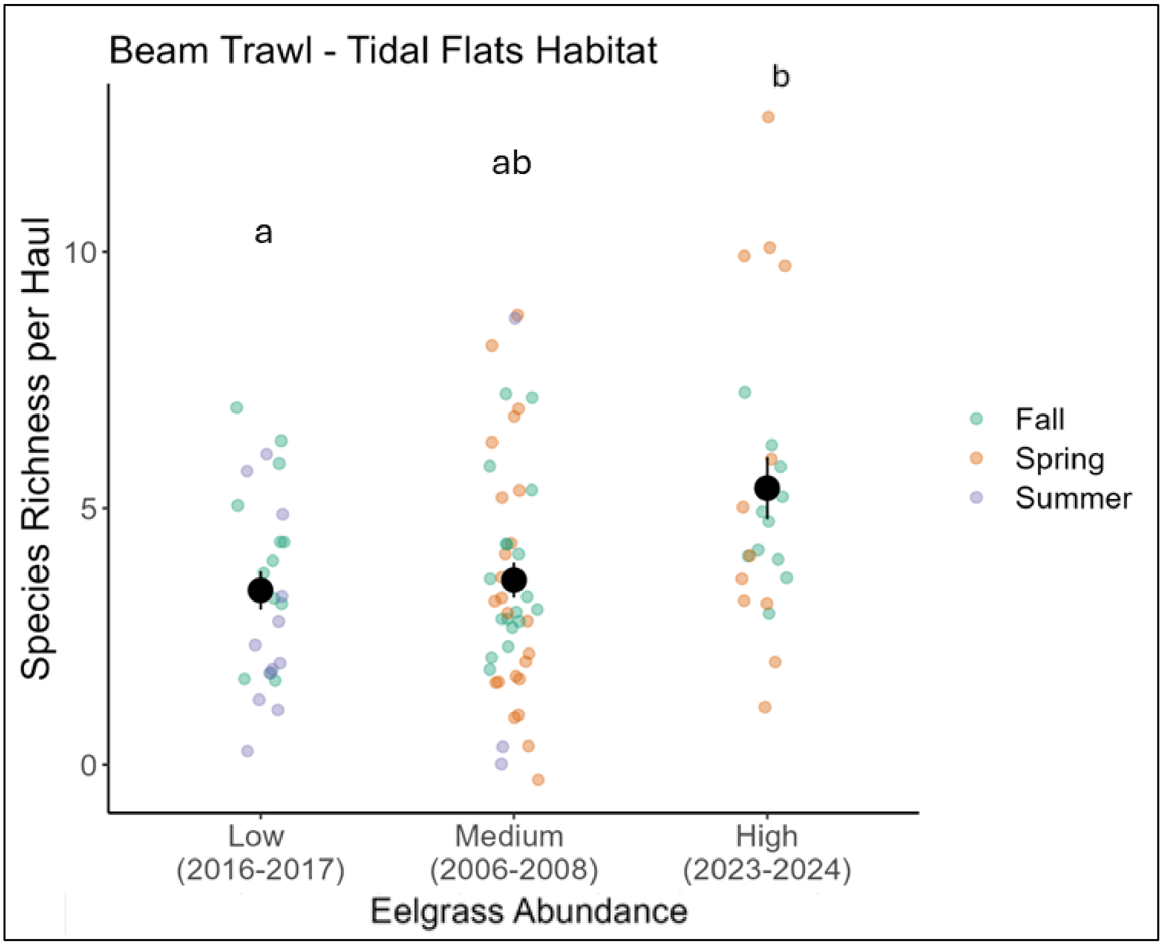


Figure 6. Species richness for tidal flat habitat. Colored points represent species richness from individual trawl replicates. Black points indicate mean ± standard error species richness for each period of eelgrass abundance. Matching lowercase letters indicate statistical similarity (GLMM; p>0.05) between different eelgrass abundance periods.

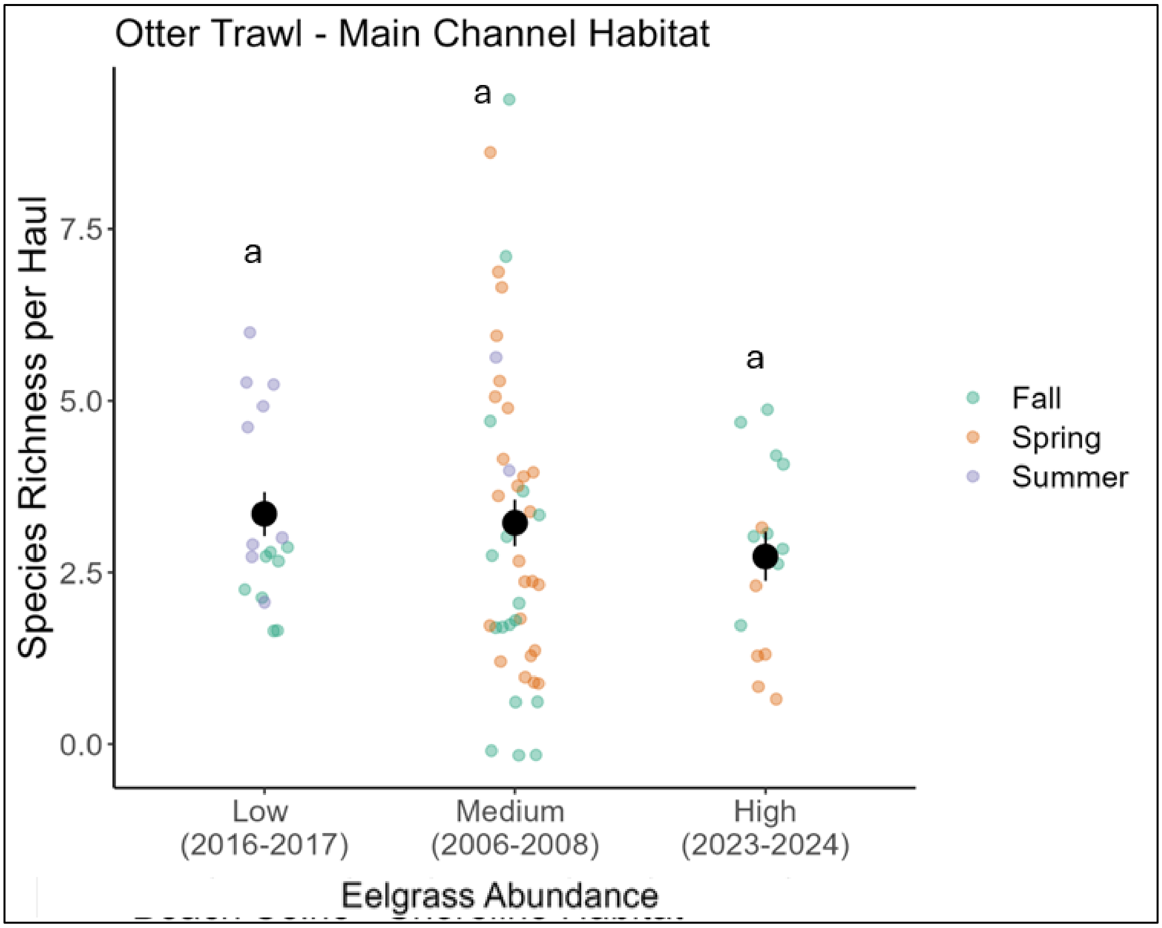


Figure 7. Species richness for open channel habitat. Colored points represent species richness from individual trawl replicates. Black points indicate mean ± standard error species richness for each eelgrass abundance period.

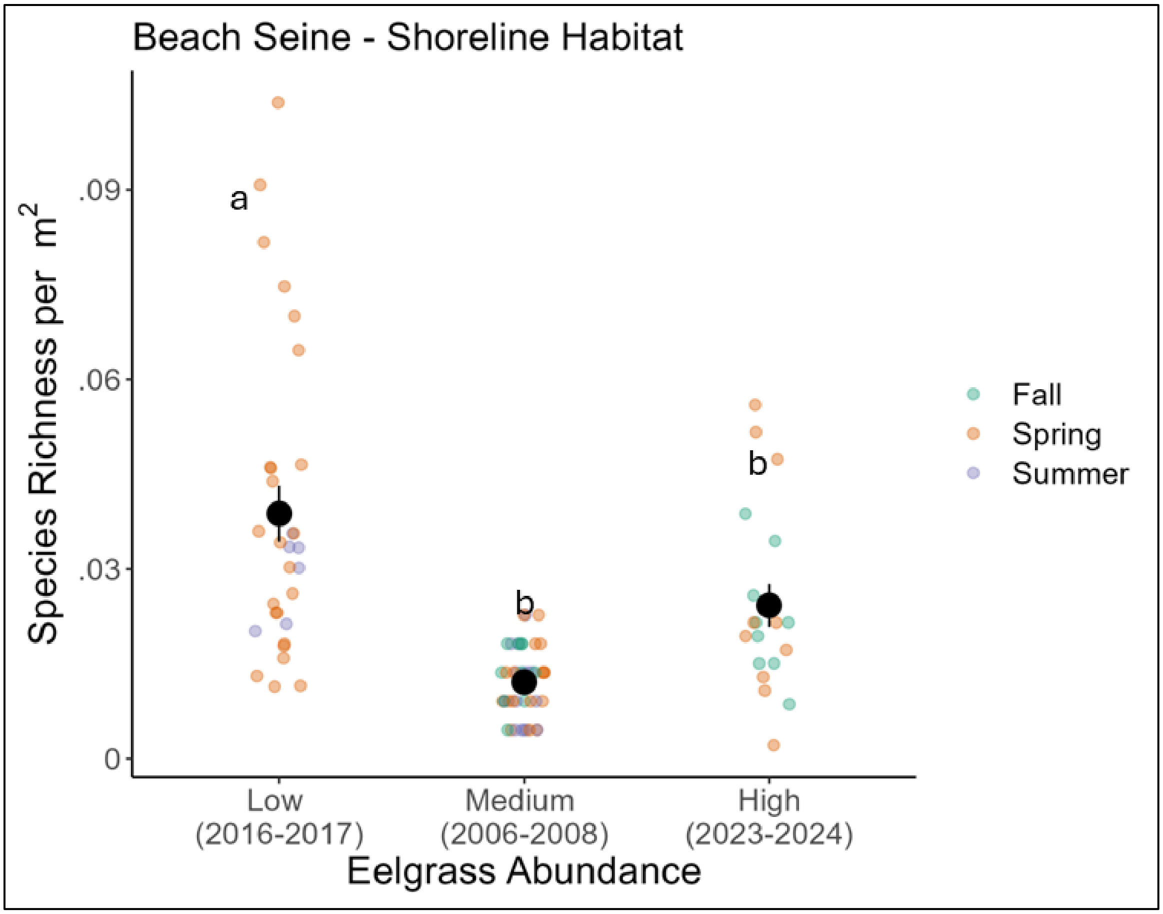


Figure 8. Species richness for shoreline habitat. Colored points represent species richness from individual trawl replicates. Black points indicate mean ± standard error species richness for each period of eelgrass abundance. Matching lowercase letters indicate statistical similarity (GLMM; $p>0.05$) between eelgrass abundance periods.

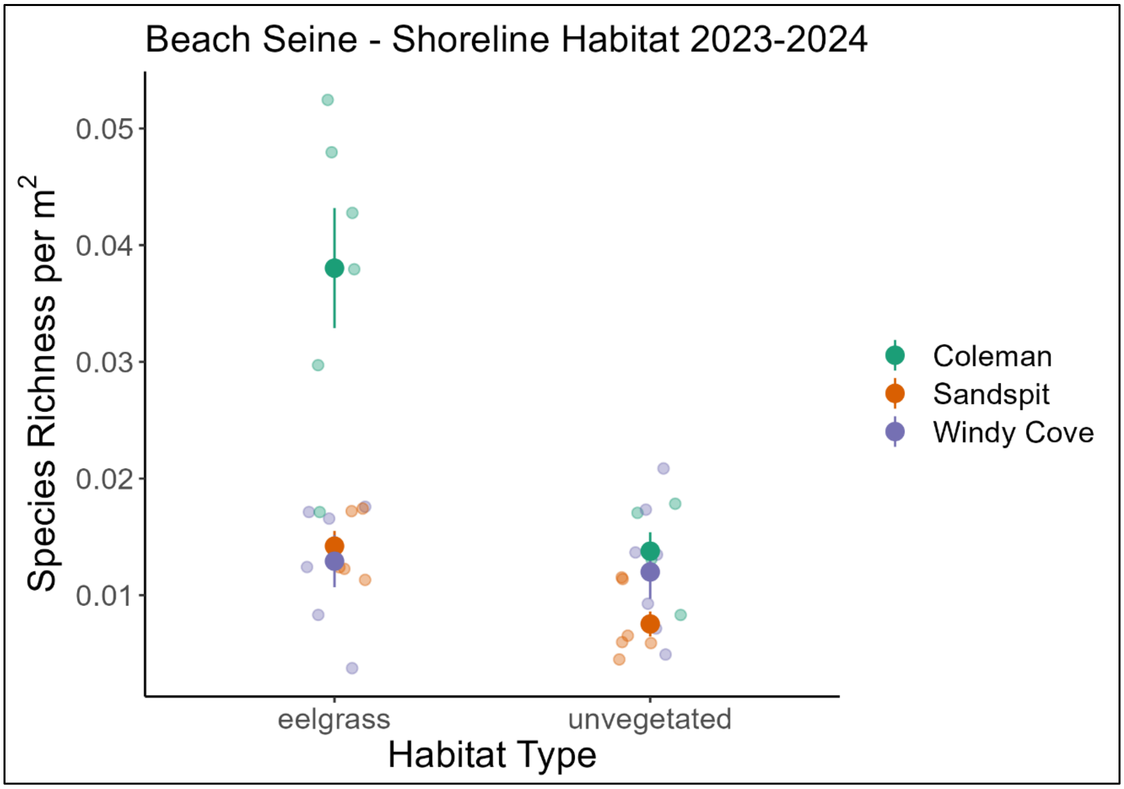


Figure 9. Species richness for shoreline habitat in locations with eelgrass and locations without vegetation sampled in 2023-2024. Solid points indicate mean ± standard error species richness habitat type within each monitoring location. Translucent points indicate species richness from individual sample replicates.

3.3 Abundance

Habitat specialist species (Figure 10) showed a substantial increase in abundance in tidal flat habitat during the period of high eelgrass abundance compared to periods of low and medium eelgrass abundance (shiner perch: $p = 0.003$, black surfperch: $p < 0.001$, bay pipefish: $p = 0.002$; Figure 11). Habitat generalist species (Figure 10) did not follow this pattern. Arrow goby showed similar abundance between all three periods for tidal flat habitat ($p = 0.923$) (Figure 11). Speckled sanddab showed higher abundance during the period of low eelgrass abundance compared to other periods ($p = 0.004$). Staghorn sculpin showed a slight decrease in abundance during the high eelgrass abundance period compared to other periods ($p = 0.045$). In the open channel habitat samples, the highest abundance for speckled sanddab and staghorn sculpin was observed during the low eelgrass abundance period (specked sanddab: $p = 0.010$, staghorn sculpin: $p = 0.031$; Figure 12). Habitat specialists had low abundance in the open channel samples regardless of eelgrass abundance, and abundance did not significantly change between surveys for shiner perch ($p = 0.254$) and bay pipefish ($p = 0.229$). Black surfperch were only found in open channel samples during the medium eelgrass abundance period.

In shoreline habitat samples, abundance of habitat specialists did not show a clear trend with eelgrass abundance (Figure 13). Shiner perch were significantly more abundant during the high eelgrass abundance period compared to other periods. Habitat generalists typically had higher abundance when eelgrass abundance was low except for arrow goby which did not have significantly different abundance levels between eelgrass abundance periods.

Figure 10. Examples of habitat specialists (top) and habitat generalists (bottom) captured in the Morro Bay estuary during 2023–2024 monitoring efforts.

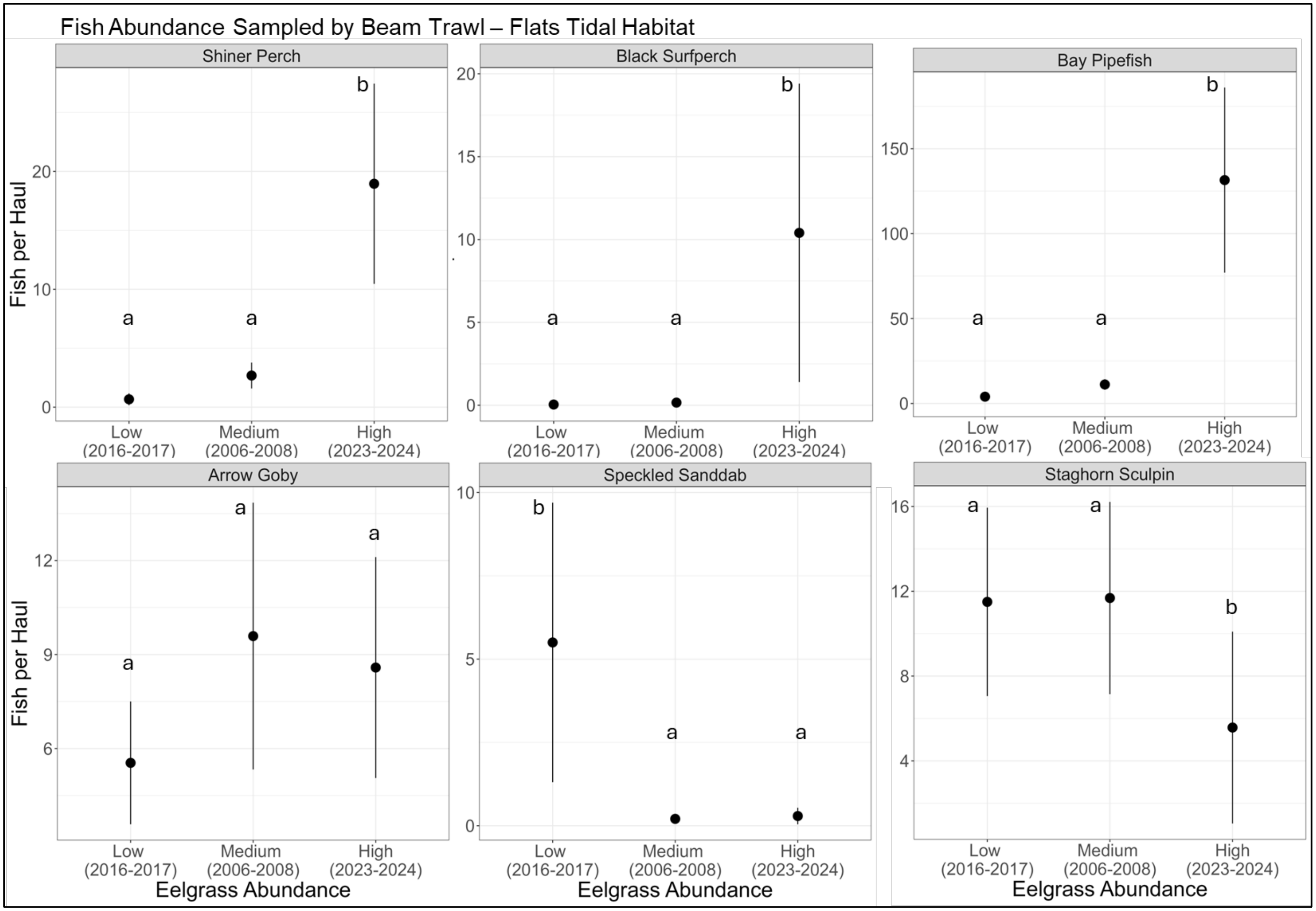


Figure 11. Fish abundance for tidal flat habitat samples during low (2016–17), medium (2006–08), and high (2023–24) eelgrass abundance periods. Matching lowercase letters indicate statistical similarity between timepoints periods (Kruskal-Wallis tests, p > 0.05).

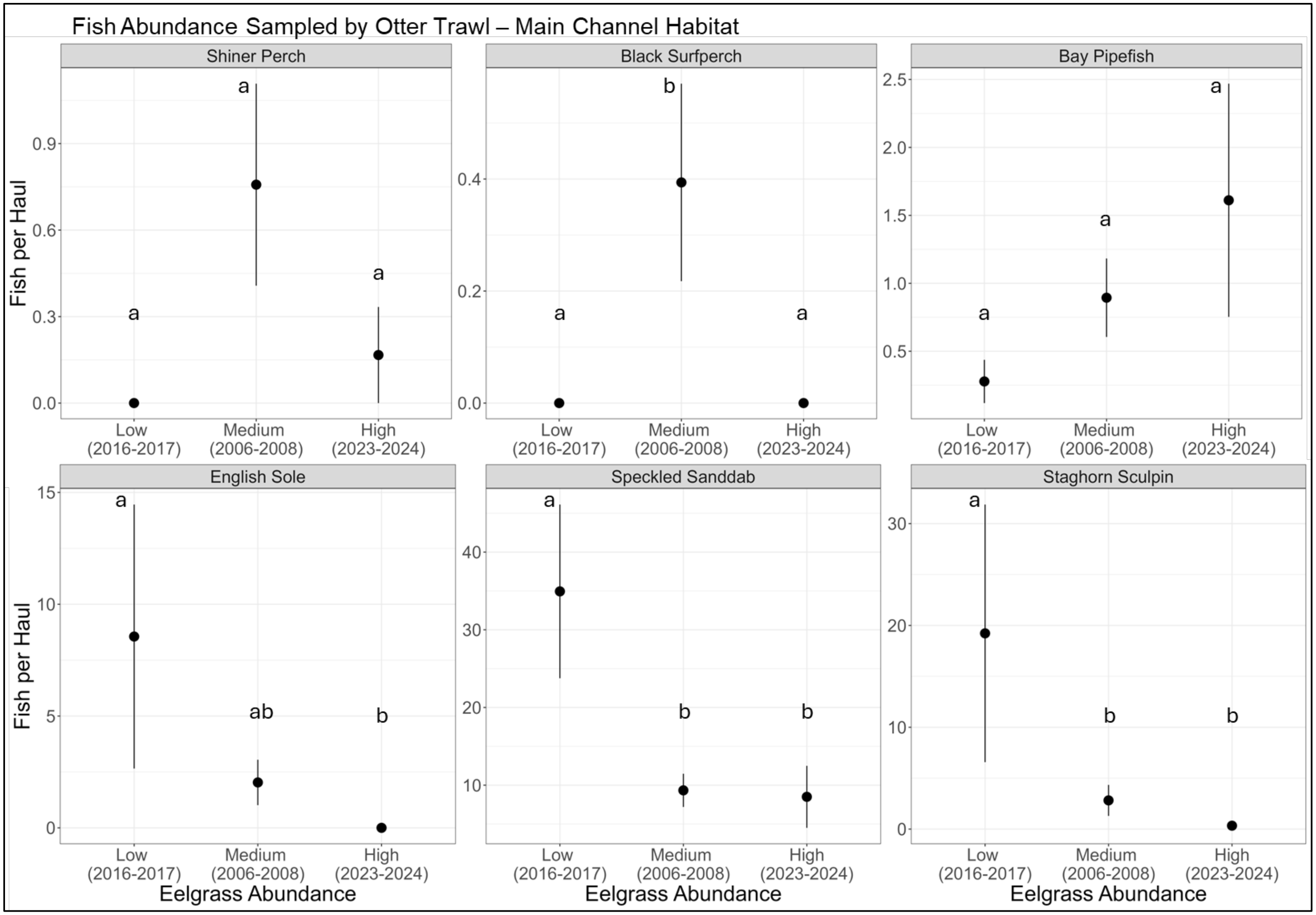


Figure 12. Fish abundance for open channel habitat samples during low (2016–17), medium (2006–08), and high (2023–24) eelgrass abundance periods. Matching lowercase letters indicate statistical similarity between eelgrass abundance periods (Kruskal-Wallis tests, p > 0.05).

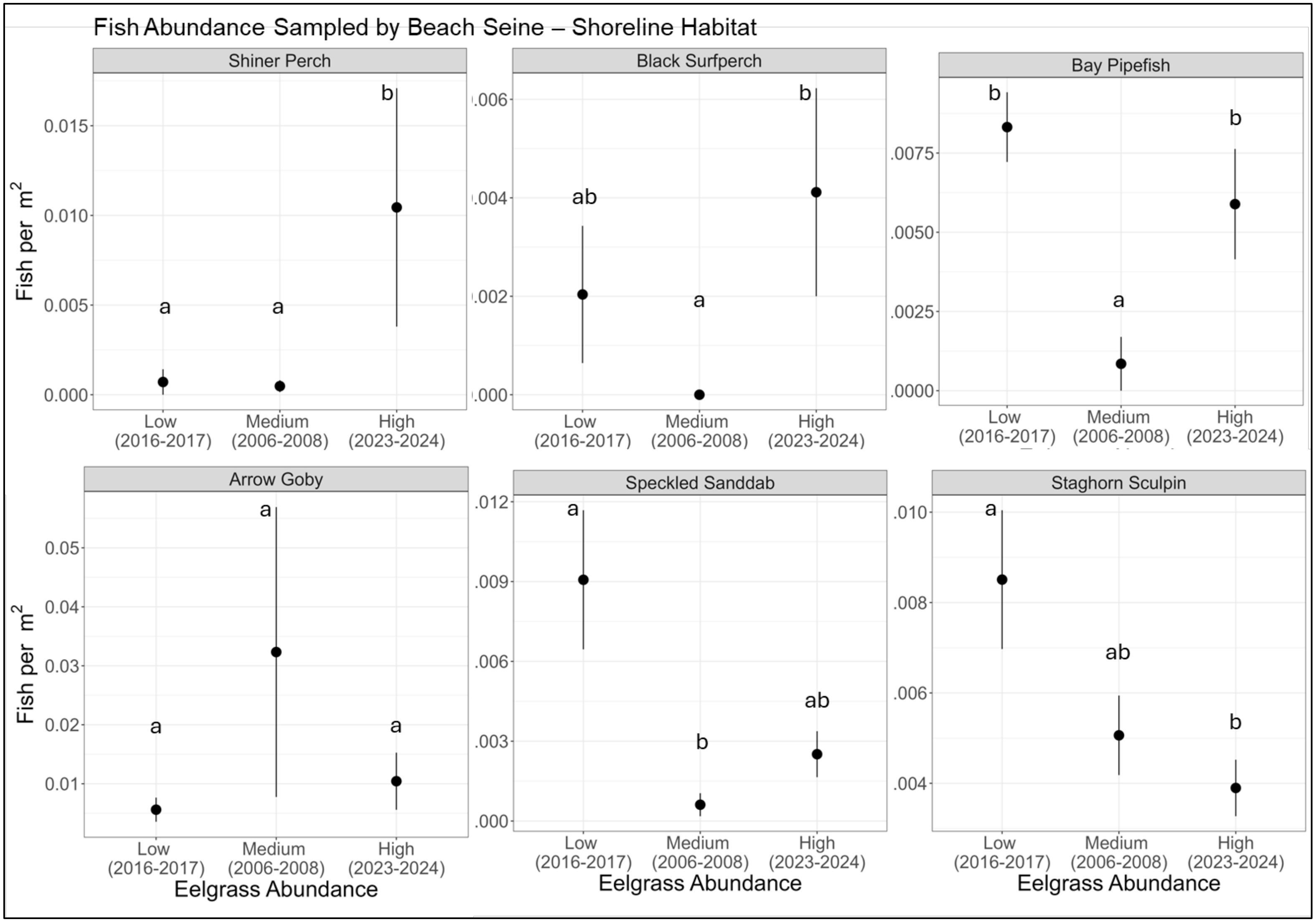


Figure 13. Fish abundance for shoreline habitat seine samples during low (2016–17), medium (2006–08), and high (2023–24) eelgrass abundance surveys. Matching lowercase letters indicate statistical similarity between eelgrass abundance periods (Kruskal-Wallis tests, $p > 0.05$).

3.4 Environmental DNA Results

A total of 62 unique taxa were detected using eDNA sampling during September and May sampling efforts. In September 2023, eDNA samples were collected from each monitoring location, A total of 33 unique taxa detected between all samples. Detections varied between locations, with some locations returning 0 detections and other locations returning 22 unique taxa detections (Table 4). In several locations, eDNA samples did not detect all the species that were captured at the same location, indicating false negative detections for these locations. At monitoring location 1B-2, where the highest number of taxa were detected, six replicate samples were collected to assess detection probability between replicate samples. Detections between these six replicate samples collected included 2 samples that returned 0 fish detections, 1 sample that returned a single fish detection (Pipefish spp.), 1 sample that detected 18 different fish taxa, and 1 sample that detected 21 different fish taxa. Overall, there appears to be a high likelihood of false detections for the September 2023 samples and many species likely went undetected in the eDNA samples collected. However, eDNA samples detected a higher number of taxa (i.e., 33 taxa) compared to what was observed using traditional sampling methods during both the May and September sampling efforts combined (i.e., 24 taxa).

During May 2024, eDNA sampling methods were modified to collect three replicate samples from a subset of monitoring locations. This allowed us to sample representative habitat of the study area (shoreline, tidal flats, and open channel habitat) without increasing the total number of samples collected. Overall detections in eDNA samples were higher in May 2024 compared to September 2023, with 55 different taxa detected (Table 5). Of the 55 taxa, 13 were wide spread and detected at 8 out of 9 monitoring locations while 18 were rare and only detected at only one location. No fish were detected in any of the three replicate samples collected at monitoring location 1B-5, which is a shoreline habitat location along the sandspit where beach seining resulted in high catch numbers for several species, suggesting false negatives occurred with the eDNA samples analyzed from this location. Overall, eDNA detections from May 2024 detected a higher number of taxa were detected with eDNA sampling compared to the number of taxa captured using traditional sampling methods during both the May and September sampling efforts combined (i.e., 24 taxa).

Table 4. September 2023 eDNA detections (33 taxa).

Species Common Name	Sample Locations																Number of Locations with Positive Detections
	Open Channel			Tidal Flats				Shoreline						Tidal Sloughs			
	1A-1	1A-2	1A-3	1A-4	1A-5	1A-6	1A-7	1B-1	1B-2[a]	1B-3	1B-4	1B-5	1B-6	1C-6	1C-7	1C-9	
Arrow goby				X	X				X					X			4
Black surfperch	X			X	X				X		X			X			6
Blenny spp.					X									X			2
Cabezon				X					X					X			3
California halibut					X												1
California killifish					X								X	X			3
California lizardfish	X			X	X									X			4
California tonguefish									X								1
Crevice kelpfish									X								1
Drum/Croaker spp					X												1
Dwarf surfperch									X					X			2
Cusk-eel family				X													1
Girella species				X													1
Goby spp.				X									X	X			3
Jacksmelt	X			X	X				X								4
Lingcod									X								1
Northern anchovy	X		X	X	X	X	X		X					X	X		9
Penpoint gunnel									X								1
Pile surfperch				X					X								2
Pipefish spp.	X			X	X		X		X					X	X		7
Right-eye flounder spp.					X												1
Rockfish spp.	X			X					X					X			4
Sardine spp.	X			X	X				X								4
Shiner perch	X			X	X				X	X		X		X			7

Species Common Name	**Sample Locations**																**Number of Locations with Positive Detections**
	Open Channel			**Tidal Flats**				**Shoreline**						**Tidal Sloughs**			
	1A-1	**1A-2**	**1A-3**	**1A-4**	**1A-5**	**1A-6**	**1A-7**	**1B-1**	**1B-2**[a]	**1B-3**	**1B-4**	**1B-5**	**1B-6**	**1C-6**	**1C-7**	**1C-9**	
Speckled sanddab	X			X	X				X								4
Staghorn sculpin	X			X	X		X		X					X			6
Striped bass														X			1
Striped seaperch									X								1
Surfperch spp.									X								1
Three-spined stickleback														X			1
Topsmelt silversides	X			X	X				X	X				X	X		7
White croaker									X								1
Woolly sculpin									X								1
Total species detected	**11**	**0**	**1**	**17**	**16**	**1**	**3**	**0**	**22**	**2**	**1**	**1**	**2**	**17**	**3**	**0**	**13**

[a] Six samples were collected from sample location 1B-2 to compare detection results between replicate samples in order to assess the likelihood of species, as such the number of species detected is likely higher than other locations which only included a single sample.

Table 5. May 2024 eDNA detections (55 distinct detections).

Species Common Name	Sample Locations									Number of Locations with Positive Detections
	Open Channel			Tidal Flats			Shoreline			
	1A-1	1A-2	1A-3	1A-5	1A-6	1A-7	1B-2	1B-3	1B-5	
Arrow goby	X	X	X	X	X	X	X	X		8
Bay goby		X	X	X	X	X	X	X		6
Black surfperch	X	X	X	X	X	X	X	X		8
Bay blenny		X		X						2
Blue rockfish			X				X	X		3
Bocaccio rockfish			X	X		X	X	X		5
Cabezon	X	X					X	X		4
California halibut		X	X	X		X	X			5
California killifish		X	X	X	X	X	X	X		7
California sheephead		X								1
California tonguefish		X	X				X			3
Crevice kelpfish							X			1
Diamond turbot						X				1
Dover sole							X			1
Dwarf surfperch				X		X	X			3
Jack mackerel		X	X		X	X				4
Jacksmelt	X	X	X	X	X	X	X	X		8
Kelp greenling							X			1
Lanternfish spp							X			1
Lingcod	X	X	X	X			X	X		6
Longjaw mudsucker				X	X	X	X			4
Monkeyface prickleback	X	X		X			X	X		5
Northern anchovy	X	X	X	X	X	X	X	X		8
Northern lampfish	X									1
Pacific herring	X									1
Pacific sanddab	X									1
Pacific sardine	X	X	X	X	X	X	X	X		8
Painted greenling		X	X					X		3
Penpoint gunnel							X			1
Petrale sole						X				1
Pile surfperch		X	X	X	X	X	X			6
Pipefish spp	X	X	X	X	X	X	X	X		8
Queenfish			X							1
Right-eyed flounder family	X	X	X	X			X	X		6
Rockfish spp	X	X	X	X	X	X	X	X		8
Rubberlip seaperch			X	X				X		3
Sardine spp	X	X	X	X	X	X	X	X		8
Scalyhead sculpin				X						1

Species Common Name	**Sample Locations**									**Number of Locations with Positive Detections**
	Open Channel			**Tidal Flats**			**Shoreline**			
	1A-1	**1A-2**	**1A-3**	**1A-5**	**1A-6**	**1A-7**	**1B-2**	**1B-3**	**1B-5**	
Sculpin spp		X	X			X				3
Shadow goby	X	X	X	X	X	X	X			7
Shiner perch	X	X	X	X	X	X	X	X		8
Smelt spp	X									1
Speckled sanddab	X	X	X	X	X	X	X	X		8
Spotted cusk-eel				X			X			2
Staghorn sculpin	X	X	X	X	X	X	X	X		8
Striped bass		-	X			X				2
Striped mullet	X									1
Striped surfperch	X			X			X	X		4
Surfperch spp	X	X	X	X		X	X	X		7
Three-spined stickleback		X	X		X	X	X			5
Topsmelt silversides	X	X	X	X	X	X	X	X		8
Tubesnout	X									1
Widow rockfish			X	X				X		3
Wolf eel								X		1
Woolly sculpin								X		1
Total species detected	**25**	**29**	**31**	**30**	**19**	**27**	**35**	**27**	**0**[a]	**8**

[a] No species detected at site 1B-5 is likely a result of the sample contamination or inhibition during the amplification process.

Table 6. Comparison of fish detections using traditional capture methods versus eDNA during sampling efforts conducted in Morro Bay in September 2023 and May 2024.

Family	Genus	Species	Common Name	Beach Seine	Beam Trawl	Otter Trawl	eDNA
Anarhichadidae	*Anarrhichthys*	*ocellatus*	Wolf eel				X
Argentinidae	Smelt family						X
Atherinopsidae	*Atherinops*	*affinis*	Topsmelt silverside	X			X
	Atherinopsis	*californiensis*	Jacksmelt				X
Aulorhynchidae	*Aulorhynchus*	*flavidus*	Tubesnout			X	X
Blenniidae	*Hypsoblennius*	*gentilis*	Bay blenny		X		X
		Blenny species					X
Carangidae	*Trachurus*	*symmetricus*	Jack mackerel				X
Clinidae	*Gibbonsia*	*montereyensis*	Crevice kelpfish				X
	Gibbonsia	*metzi*	Striped kelpfish	X	X		
	Heterostichus	*rostratus*	Giant kelpfish	X	X		
Clupeidae	*Clupea*	*pallasii*	Pacific herring				X
	Sardinops	*sagax*	Pacific sardine				X
		Sardine spp					X
Cottidae	*Artedius*	*harringtoni*	Scalyhead sculpin				X
	Clinocottus	*analis*	Woolly sculpin				X
	Leptocottus	*armatus*	Staghorn sculpin	X	X	X	X
	Scorpaenichthys	*marmoratus*	Cabezon	X		X	X
	Sculpin family						X
Cyclopsettidae	*Citharichthys*	*sordidus*	Pacific sanddab				X
		stigmaeus	Speckled sanddab	X	X	X	X
Cynoglossidae	*Symphurus*	*atricaudus*	California tonguefish				X

Family	Genus	Species	Common Name	Beach Seine	Beam Trawl	Otter Trawl	eDNA
Embiotocidae	*Cymatogaster*	*aggregata*	Shiner perch	X	X	X	X
	Embiotoca	*jacksoni*	Black surfperch	X	X		X
	Embiotoca	*lateralis*	Striped surfperch				X
	Micrometrus	*minimus*	Dwarf surfperch	X	X		X
	Phanerodon	*vacca*	Pile surfperch	X	X		X
	Rhacochilus	*toxotes*	Rubberlip seaperch				X
	Surfperch family						X
Engraulidae	*Engraulis*	*mordax*	Northern anchovy	X	X	X	X
Fundulidae	*Fundulus*	*parvipinnis*	California killifish	X	X		X
Gasterosteidae	*Gasterosteus*	*aculeatus*	Three-spined stickleback	X	X		X
Girellidae	*Girella*	*nigricans*	Opaleye	X			
		Girella species					X
Gobiidae	*Clevelandia*	*Ios*	Arrow goby	X	X		X
	Gillichthys	*mirabilis*	Longjaw mudsucker				X
	Lepidogobius	*lepidus*	Bay goby				X
	Luciogobius	Goby species					X
	Quietula	*y-cauda*	Shadow goby				X
Hexagrammidae	*Hexagrammos*	*decagrammus*	Kelp greenling				X
	Ophiodon	*elongatus*	Lingcod				X
	Oxylebius	*pictus*	Painted greenling				X
Labridae	*Semicossyphus*	*pulcher*	California sheephead				X
Moronidae	*Morone*	*saxatilis*	Striped bass				X
Mugilidae	*Mugil*	*cephalus*	Striped mullet				X
Myctophidae	*Stenobrachius*	*leucopsarus*	Northern lampfish				X
		Lanternfish species					X

Family	Genus	Species	Common Name	Beach Seine	Beam Trawl	Otter Trawl	eDNA
Ophidiidae	*Chilara*	*taylori*	Spotted cusk-eel				X
		Cusk-eel species					X
Paralichthyidae	*Paralichthys*	*californicus*	California halibut	X	X		X
Pholidae	*Apodichthys*	*flavidus*	Penpoint gunnel	X	X		X
Pleuronectidae	*Eopsetta*	*jordani*	Petrale sole				X
	Hypsopsetta	*guttulata*	Diamond turbot	X	X	X	X
	Microstomus	*pacificus*	Dover sole				X
	Platichthys	*stellatus*	Starry flounder	X	X		--
	Right-eyed flounder family						X
Sciaenidae	*Genyonemus*	*lineatus*	White croaker				X
	Seriphus	*politus*	Queenfish				X
	Drum/Croaker family						X
Sebastidae	*Sebastes*	*mystinus*	Blue rockfish				X
		paucispinis	Bocaccio rockfish				X
		entomelas	Widow rockfish				X
		Rockfish species		X	X		X
Stichaeidae	*Cebidichthys*	*violaceus*	Monkeyface prickleback				X
Syngnathidae	*Syngnathus*	*leptorhynchus*	Bay pipefish	X	X	X	X
Synodontidae	*Synodus*	*lucioceps*	California lizard fish	X		X	X
Total unique detections				**22**	**19**	**9**	**62**

4 DISCUSSION

The Morro Bay estuary is home to a diverse fish community containing numerous fish species (24 unique taxa were captured and 62 unique taxa were detected with eDNA during the 2023– 2024 monitoring efforts), while only a few different species accounted for the majority of fish captured. Results of the recent monitoring efforts show similar levels of species richness between habitat types, while community composition varied by habitat type. In tidal flats where eelgrass is abundant, habitat specialist species are dominant; in open channel habitat where eelgrass is rare, habitat generalist species are dominant; and in shoreline habitat where eelgrass is patchy, habitat specialist and habitat generalist species composition are similar. However, when looking at shoreline habitat with eelgrass, species richness was significantly higher than unvegetated shoreline habitat. Fish abundance was highest in tidal flat habitat compared to other habitat types sampled and bay pipefish accounted for the majority of the fish captured. Overall, eelgrass appeared to highly influence species composition by providing habitat for specialists.

Species richness remained similar between sampling periods, but species composition shifted between periods with varying levels of eelgrass abundance. During periods of medium and high eelgrass abundance, habitat specialists (e.g., bay pipefish) dominated the catch and during periods of low eelgrass abundance habitat generalist species (e.g., speckled sanddab) dominated the catch. Expanded habitat use by habitat specialist species was observed during periods with high eelgrass abundance, and bay pipefish (an eelgrass specialist) was the most abundant fish in both tidal flat and open channel habitat. Expanded habitat use by habitat generalists was observed during periods of low eelgrass abundance, and staghorn sculpin was the most abundant fish captured in tidal flat habitat (Figure 14). However, speckled sanddabs were consistently the most abundant species in open channel habitat during all monitoring periods regardless of eelgrass abundance level.

Species composition observed in the capture data collected in the recent study and in the historic studies indicates a shift between periods of low to high eelgrass abundance; however, most species were observed during each of the different monitoring periods, with a few exceptions (Figure 16). During previous monitoring periods, English sole made up a large portion of the total catch but no English sole were captured or detected in eDNA samples collected during 2023– 2024. Estuaries provide important rearing habitat for English sole, and the presence of other flatfish suggest that conditions are likely suitable for English sole. In addition, a new species, California lizardfish, was captured for the first time during monitoring in 2023–2024. California lizardfish are widely distributed throughout California, but were not previously observed in Morro Bay.

Overall, most species continue to show up in catch data as eelgrass abundance fluctuates between low and high densities but the proportion of individual species to the overall population can change drastically. Abundance data strengthens this conclusion, in particular the abundance of habitat specialist species (e.g., bay pipefish and shiner surfperch) plummeted when there was no eelgrass and then rebounded following the eelgrass recovery. Conversely, habitat generalist species seemed to have varied responses to eelgrass abundance level. For example, speckled sanddab showed significantly higher abundance at all three habitat location types when eelgrass abundance was lowest, while other habitat generalists (i.e., arrow goby and staghorn sculpin) abundance remained similar between varying levels of eelgrass abundance.

Results from the previous monitoring efforts (2006–2008 and 2016–2017) in the Morro Bay estuary found that fish species abundance and biomass remained stable between medium and low eelgrass abundance periods, but a notable shift in species composition occurred (O'Leary et al. 2021). Results from the recent monitoring efforts (2023–2024) found a dramatic shift in species composition toward habitat specialist species (e.g., bay pipefish, shiner surfperch, and black surfperch) during the high eelgrass abundance period. Based on the results of this study, the recovery of eelgrass habitat appears to have a direct influence on fish communities, and apparently these shifts in the fish community can occur within a few years following habitat recovery.

Results from the eDNA monitoring indicate a diverse fish assemblage uses the Morro Bay estuary. The number of unique taxa identified using eDNA (i.e. 62 unique taxa) was nearly three times higher than the number identified using traditional sampling techniques (i.e., 22 unique taxa). No apparent trends were observed between positive eDNA detections and habitat types (i.e., open channel, tidal flats, and shoreline) which is likely due to the large detection range of DNA and the mixing of waters in bay resulting from wind and tidal currents. Detections were higher when multiple samples were collected from each location versus when a single sample was collected at each location.

Although eDNA sampling resulted in a high number of detections, some species were undoubtedly missed. In particular, elasmobranchs (e.g., sharks and rays), which are common in the Morro Bay estuary, were not detected in any of the eDNA samples. Studies have shown mixed results on the ability to detect elasmobranchs using eDNA metabarcoding (Miya et al 2015, Kumar et al 2022) and these effects are potentially compounded in areas with high biodiversity (Kumar et al 2022). Future eDNA sampling efforts in the Morro Bay estuary could use additional primers to increase detection probability for elasmobranchs. While quantitative PCR (qPCR) could be used to maximize detection probabilities for rare species or species of interest such as steelhead (*Oncorhynchus mykiss*) and tidewater goby (*Eucyclogobius newberryi*),

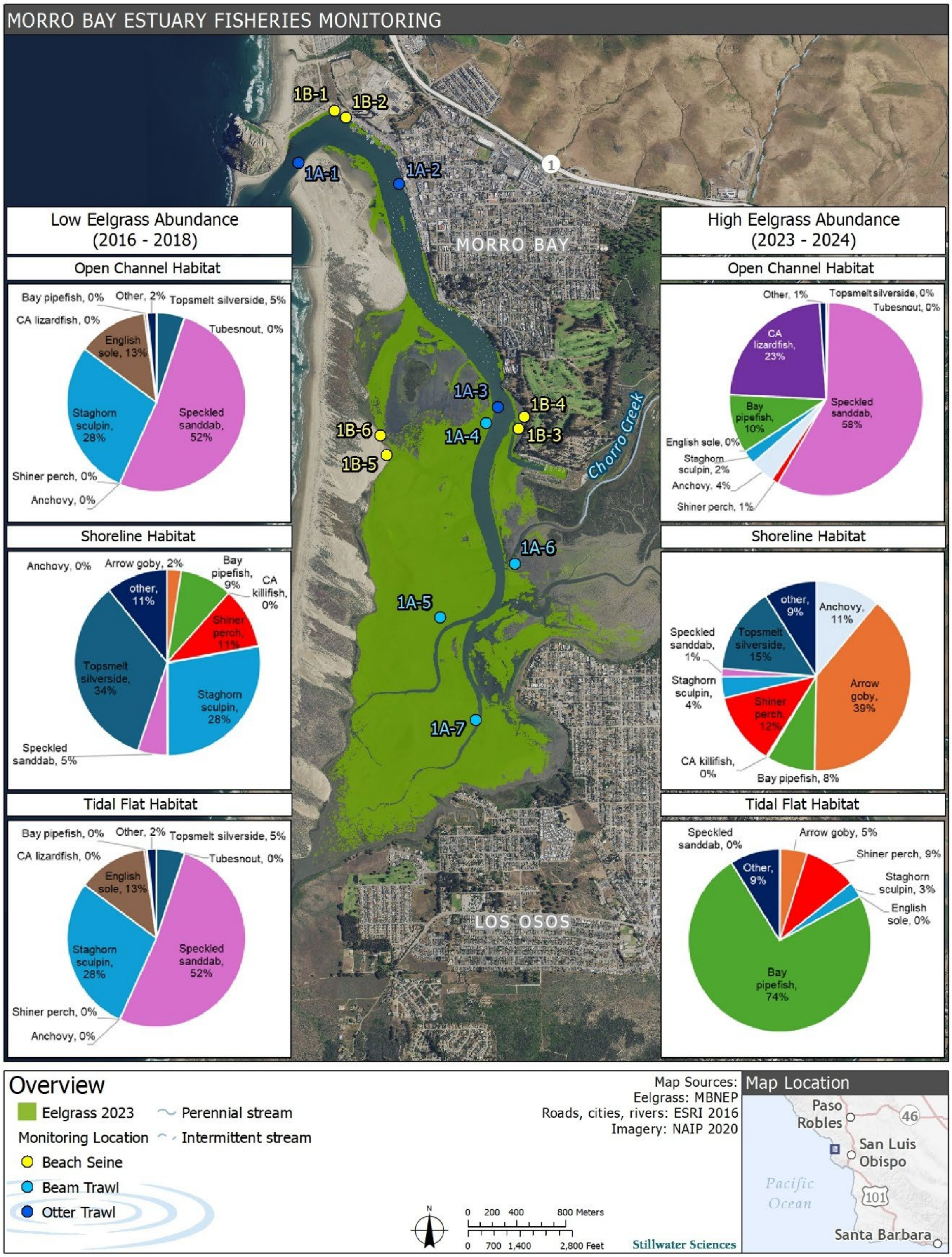


Figure 14. Fish species composition by habitat type during periods with low or high eelgrass abundance.

5 RECOMMENDATIONS

The Morro Bay Estuary experiences large changes in habitat conditions, especially eel grass. It is reasonable to expect that fish communities are responding to these changes, and regular monitoring is recommended to document potential fluctuations and shifts in populations. Future monitoring recommendations include:

- Annual monitoring along shoreline habitats during spring and fall within at least one sample site located at the Coleman Beach, Windy Cove, and Sand Spit sample areas.
- Trawl surveys using both beam and otter trawls conducted every five years
- Annual eDNA sampling during the spring and fall with samples collected from at least four locations in the Morro Bay estuary including
    - in the south bay near Los Osos,
    - near the main tributaries, Los Osos Creek and Chorro Creek,
    - in the mid-section of the bay near Windy Cove, and
    - in the north-section of the bay near Coleman Beach.
- Expand analysis of the eDNA to also target sharks and rays,
- Include qPCR analysis of eDNA samples for any rare species or species of special interest such as steelhead and/or tidewater goby

Based on our results, the highest value monitoring method is beach seines which are easy to deploy and provide representative catch.

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