

Salinas Lagoon Seining Report

Spring 2023



Submitted To:
Monterey County
Water Resources Agency

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June 2023

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This work is funded in part by a Cooperative Endangered Species Conservation Fund Non-Traditional Section 6 Grant (Agreement No. Q2140403) to the Monterey County Water Resources Agency to support the development of the Salinas River Habitat Conservation Plan.

Executive Summary

In fulfillment of the objective of determining the presence and spatial distribution of steelhead in the lower Salinas River to help inform the development of the Salinas River Habitat Conservation Plan (HCP), FISHBIO conducted fish community and water quality sampling of the lower Salinas River and Lagoon on May 9-10, 2023. This sampling event occurred 126 days after a facilitated breaching of the sandbar on January 3, 2023. The sandbar remained breached since that date, subjecting the lagoon to tidal influence and seawater inflow, and providing fish migration opportunities to and from the marine environment. Because of the open river mouth and restrictions related to nesting western snowy plovers (*Charadrius nivosus nivosus*), fewer samples were collected surrounding the lowermost portion of the lagoon than in previous years. In addition, dramatic shifts in sediment resulting from high flows this spring necessitated deviation from previous sample locations in two of the upstream sites. However, with adjustments to sample locations based on availability of suitable habitat, the field crew was able to perform seine hauls at 10 sites, eight of which were identical to those sampled in previous years.

In total, nine species of fish were captured by physical sampling gears, and an additional eight were detected only in environmental DNA (eDNA) samples. Low catch per unit effort during this sampling event suggests a low density of fish in the lagoon. This finding stood apart from those of the preceding two spring sampling events (May 2022 and April 2021), which were marked by very high densities of small, marine-origin fish including Pacific herring (*Clupea pallasii*) and Pacific staghorn sculpin (*Leptocottus armatus*). However, both of these previous sampling events took place during periods of lagoon closure, and the extended lagoon connectivity that occurred in 2023 likely contributed to this shift in relative abundance. Notably, an American shad (*Alosa sapidissima*) was observed in the seine samples, which represents the first time this species has been observed in lagoon sampling.

For the first time, hoop nets were incorporated into the lagoon monitoring this year. The inclusion of this passive gear was intended to capture a more complete representation of the fish community by sampling fish that may be inefficiently captured by seine nets, such as larger bodied and fast-swimming species. Two nets were placed at station 10 (approximately one kilometer upstream of the Highway 1 crossing) and left to soak overnight for a period of approximately 14 hours. These nets captured a total of five species including a white catfish (*Ameiurus catus*), a non-native species that has not been observed in previous lagoon sampling. The hoop nets also captured a single striped bass (*Morone saxatilis*). All striped bass, with exception of the individual captured in the hoop net, were found in sample sites downstream of the Highway 1 crossing. They ranged in size from 160-419 mm in fork length, and each was implanted with a passive integrated transponder (PIT) tag.

The collection and analysis of eDNA samples resulted in the detection of two fish species that were not previously observed in lagoon sampling efforts: white bass (*Morone chrysops*) and green sunfish (*Lepomis cyanellus*). Further, eDNA detected an additional six species of fish that have previously been captured in lagoon sampling efforts but were not observed in the seine or hoop net catch during this event. These included Pacific herring, goldfish (*Carassius auratus*), common carp (*Cyprinus carpio*), Sacramento blackfish (*Orthodon microlepidotus*), tidewater goby (*Eucylcogobius newberryi*), and western mosquitofish (*Gambusia affinis*).

No steelhead (*Oncorhynchus mykiss*) were detected in either the seine or eDNA samples collected in this sampling event. However, water quality data indicate that abiotic factors were not limiting for rearing juvenile steelhead or migrating adult steelhead, as temperatures and dissolved oxygen levels remained within a suitable range for the species.

Background

Monterey County Water Resources Agency (MCWRA) has played a leading role in monitoring and managing the Salinas Lagoon since 1996, when the organization adopted the Salinas River Lagoon Management and Enforcement Plan (MEP) that was developed by the multi-stakeholder Salinas River Task Force. Since that time, the recommended measures included in the MEP have primarily been implemented by MCWRA, its contractors, and the U.S. Fish and Wildlife Service (USFWS). The Salinas River Lagoon project area described in the MEP includes the lower portion of the river from the seasonally present sandbar separating the river from Monterey Bay to approximately two miles upstream.

Beginning in 2002, MCWRA implemented a Lagoon Monitoring Program, and this program was updated in 2010 to incorporate the recommendations of the National Marine Fisheries Service (NMFS) draft Biological Opinion for sandbar management (NMFS 2009). These changes were intended to mitigate potentially negative effects on lagoon-rearing steelhead (*Oncorhynchus mykiss*) belonging to the federally Threatened South-Central California Coast Distinct Population Segment (SCCC DPS; USFWS 1997). One component of this draft Biological Opinion was a requirement for sampling the fish community in the lower river in the spring and summer, in addition to the fall samples that MCWRA was collecting in previous years. In subsequent years, samples were collected by Hagar Environmental Science (HES) in the spring, summer, and fall of 2011, 2012, and 2013, and spring of 2014. Fish community and water quality sampling resumed in the fall of 2020 with surveys conducted by FISHBIO. Sampling was conducted in October of 2020, April of 2021, and May of 2022. In total, Salinas Lagoon seine sampling has been conducted 23 times since sampling began in 2002 (seven spring events, four summer events, and 12 fall events: Table 1).

The fish community composition in the lagoon is largely dependent on freshwater inflow from the Salinas River that affects water quality and habitat conditions. The early winter of 2022/2023 was wetter than average (Table 2), with 158% of normal precipitation (6.27 inches) as measured in Salinas and 140% of normal precipitation (5.01 inches) as measured in King City falling in the first quarter of the 2023 water year (October through December 2022; MCWRA 2023). Conditions remained wetter than average through the spring, with the report for the second quarter (January through March 2023) indicating 105% of average rainfall (7.43 inches) in Salinas and 194% of average rainfall (13.48 inches) in King City (Table 2; MCWRA 2023a).

Table 1. Temporal coverage of Salinas Lagoon sampling events from 2002 to 2023.

Year	Spring	Summer	Fall
2002	-	-	October
2003	-	-	October
2004	-	-	October
2005	-	-	October
2006	-	-	October
2007	-	-	-
2008	-	-	October
2009	-	-	October
2010	-	August	October
2011	May	August	October
2012	April	July	October
2013	April	July	October
2014	April	-	-
2015	-	-	-
2016	-	-	-
2017	-	-	-
2018	-	-	-
2019	-	-	-
2020*	-	-	October
2021*	April	-	-
2022*	May	-	-
2023*	May	-	TBD

*Sampling conducted by FISHBIO

Table 2. Summary of precipitation as a percentage of average by water year quarter. Precipitation accumulation measured at the Salinas Airport and in King City.

Water Year	Quarter	Salinas Precipitation (in)	% of Average Precipitation	King City Precipitation (in)	% of Average Precipitation
2019-2020	4 th (July-Sep 2020)	0.20	10	0.17	18
2020-2021	1 st (Oct-Dec 2020)	3.91	24	3.72	30
2020-2021	2 nd (Jan-Mar 2021)	7.35	65	7.03	88
2020-2021	3 rd (Apr-Jun 2021)	5.75	46	7.33	62
2020-2021	4 th (July-Sep 2021)	0.04	20	0.01	6
2021-2022	1 st (Oct-Dec 2021)	6.11	154	5.37	150
2021-2022	2 nd (Jan-Mar 2022)	0.76	12	1.15	17
2021-2022	3 rd (Apr-Jun 2022)	0.44	15	0.38	95
2021-2022	4 th (July-Sep 2022)	0.07	47	0.21	172
2022-2023	1 st (Oct-Dec 2022)	6.27	158	5.01	140
2022-2023	2 nd (Jan-Mar 2023)	7.43	105	13.48	194

MCWRA can partially regulate the water level in the lagoon via releases to the Old Salinas River through the lagoon outlet slidegate. However, once the lagoon stage exceeds approximately six feet, MCWRA conducts facilitated breaching to prevent flooding of crop fields and residences adjacent to the lower river (USFWS 2007). The sand bar at the Salinas Lagoon remained closed until water over-topped the channel that was excavated by MCWRA crews on January 3, 2023. The lagoon remained open following the facilitated breach, and as of the May 2023 seine sampling, had been open for a period of 126 days. Plotting of lagoon stage prior to and during the sampling period was not feasible this year, as high flows this winter filled the approach channel to the Old Salinas River with sediment, and with the river mouth remaining open, the water level has not been high enough to make surface connection with the slide gate and sensor, resulting in an interruption of logged stage data.

Methodology

Fish Community Sampling

Salinas Lagoon sampling is intended to assist in determining the presence and spatial distribution of steelhead in the lower Salinas River and Lagoon. The purpose of these sampling efforts is to capture any juvenile SCCC DPS steelhead that may be rearing in the lagoon. Objectives include evaluating presence or absence, condition, relative abundance (i.e., catch per unit effort; CPUE), and distribution of juvenile steelhead in the Salinas Lagoon.

The downstream end of the Salinas lagoon is characterized by open, sandy, gradual beaches that are particularly suitable for beach seining. However, the highly mobile nature of the substrate in the lower lagoon, combined with high flows and tidal action, make this portion of the river a very dynamic area. Ongoing lagoon connectivity meant that stations historically sampled along the sandbar separating the river mouth from the ocean (stations 1-3) were not sampled during this event. In addition, several sample stations were adjusted slightly to accommodate changes in the configuration of the lagoon and river that occurred following high winter and spring flows. This included station 5, which was shifted approximately 100 m south of its historic location due to both changes in lagoon configuration and access restrictions related to nesting snowy plovers; and station 6, which was moved to the opposite side of the river due to the formation of a mudflat in the original location. Further, an alternative station 8 near the Highway 1 bridge was sampled in addition to the original station 8. Notably, seine samples collected at stations 7 and 9 were inefficient, as these locations had become shallow mudflats littered with snags. Finally, station 13 was added on the south bank of the lower lagoon to make up for the inability to sample the sandbar stations (1-3).

FISHBIO field crews conducted seine sampling at 11 stations (4, 5, Alternate 6, 7, 8, Alternate 8, 9, 10, 11, 12, and 13; Figure 1). However, as noted above, two of these (7 and 9) were inefficiently sampled due to shallow habitat and the presence of many snags. In previous years, fish community sampling was conducted using a 100-foot beach seine with 1/4-inch mesh. This year, a 200-foot seine was used (Figure 2); therefore, the area sampled by each haul was considerably larger than in previous years. Although a larger net was used, the methodology employed was identical to that in previous years, with the seine being set in a semi-circle a short distance from shore and crew members, pulling the seine onto shore while ensuring the float line stayed above the surface, and keeping the lead line as close to the substrate as possible. At sites where water depths precluded wading, an inflatable raft was used to deploy the seine. These protocols and the dimensions of the net meant that the maximum area sampled by each seine haul was approximately 15,707 ft² (~1459 m²), although the true value was less than this due to the presence of obstacles and variations in wind direction that shifted the net.

Once the seine was pulled onto shore, crews quickly processed captured fish by placing specimens to be measured in aerated recovery buckets and counting extremely abundant species before releasing them. Once all fish were removed from the net, crews applied the standard protocol of recording fork and total length data on at least 30 individuals of each captured species before plus-counting any remaining individuals. For this sampling event, low numbers of captured fish meant that this minimum of 30 individuals of a single species was never exceeded in a single haul; therefore, all captured fish were measured.

Because of a previous NMFS mark-recapture study and tagging of striped bass by FISHBIO staff since 2020 in the Salinas Lagoon, all captured striped bass were first scanned for passive integrated transponder (PIT) tags using an Oregon RFID handheld PIT tag reader before implanting untagged individuals with a 13 mm tag and recording the identification number on the datasheet. Crews then verified the tag number by scanning it once more and sealed the injection site with tissue glue prior to releasing the individual. The same protocol would have been applied to any captured *O. mykiss*, but none were encountered. None of the striped bass captured during this sampling event contained PIT tags, and therefore all were implanted with a new tag.

In addition to beach seining, hoop-style fyke nets were deployed as a means of passively sampling the fish community. The intention of including this additional gear was to obtain a more complete representation of the fish community present in the sampling stations by increasing the odds of capture of fish that may be inefficiently sampled with seine nets, including larger bodied and strong-swimming species. In total, two of these hoop nets were set in the vicinity of station 10 (Figure 3), one facing upstream and one facing downstream. Both nets were placed for a full tidal cycle during flood tide at 6pm on May 9 and recovered during the subsequent flood tide at 8am on May 10.

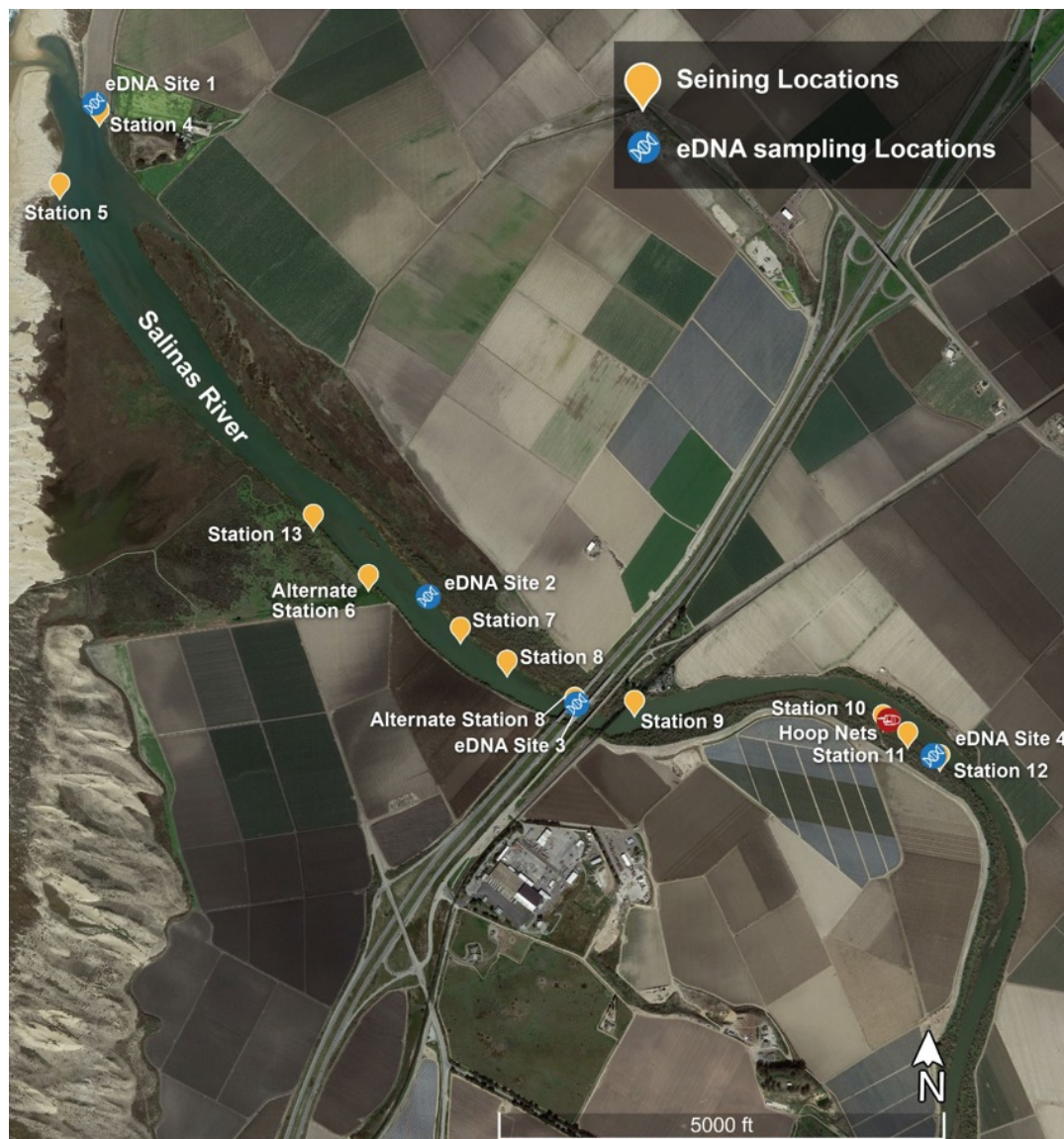


Figure 1. Stations sampled by FISHBIO in May 2023. Interactive map available at https://bit.ly/salinaslagoon_may2023.



Figure 2. Retrieving a 200-ft beach seine at sampling station 5 on May 10, 2023.



Figure 3. Fyke (hoop) nets set to passively sample fish in the upper Salinas River Lagoon (Station 10).

Water Quality Sampling

After fish processing was completed, crews collected water quality data using a YSI water quality sampling meter in the sampled area. Staff used the YSI to measure temperature (°C), specific conductivity ($\mu\text{S}/\text{cm}$ at 25°C), conductivity ($\mu\text{S}/\text{cm}$), salinity (parts per thousand; ppt), and dissolved oxygen (mg/L) at the surface.

Data Analysis

Data collected during this sampling effort were added to the database of compiled HES and FISHBIO data that was developed in 2021 and has been updated each year since. To ensure comparability with data from HES, all data were standardized to individuals captured per seine haul. Cumulative and species-specific CPUE was then calculated for the May 2023 sampling event. Data collected during the May 2023 sampling event were also analyzed for species diversity at each sampling location within the lagoon using the Shannon-Weiner Diversity Index.

Results

Fish Community Sampling

Seine Catch

A total of 70 fish representing eight different species were captured via seine in May 2023 (Table 3). Total CPUE was only 4.6 percent of that observed in May of 2022, and was the lowest of any spring sampling event to date (Table 3). CPUE from past summer and fall sampling events is also presented in the Appendix for comparison (Tables A1 and A2). The capture of a single American shad (*Alosa sapidissima*; Figure 4) represents the first time the species has been observed in lagoon seine surveys. However, it should be noted that there are historic records of small American shad runs having occurred in the Salinas River (Skinner 1962). As in all previous spring sampling events, no *O. mykiss* were observed.



Figure 4. American shad captured during beach seining on May 10, 2023.

Table 3. Cumulative and species-specific catch per unit effort (CPUE) across the seven spring sampling events from 2011–2023. Note that CPUE is calculated using a single seine haul as the base unit of effort. Non-native species are indicated by bolded common and scientific names.

		Date	May 2011	April 2012	April 2013	April 2014	April 2021	May 2022	May 2023*
		Total Seine Hauls	16	17	14	8	12	10	11
Family	Common Name	Scientific Name							
Petromyzontidae	Pacific lamprey	<i>Petromyzon tridentata</i>	0	0.1	0	0	0	0	0.1
Alosidae	American shad	<i>Alosa sapidissima</i>	0	0	0	0	0	0	0.1
Clupeidae	Pacific herring	<i>Clupea pallasii</i>	0	2.9	89.8	0	104.4	67.8	0
	Threadfin shad	<i>Dorosoma petenense</i>	0	0.1	0	0	0	0	0
Cyprinidae	Common carp	<i>Cyprinus carpio</i>	0	0.2	0	0	0	0	0
	Hitch	<i>Lavinia exilicauda</i>	8.3	11.8	6.5	0	0.1	0.1	2.1
	Sacramento blackfish	<i>Orthodon microlepidotus</i>	0.1	0.9	0	0	0	0	0
	Sacramento pikeminnow	<i>Ptychocheilus grandis</i>	0	0.10	0	0	0	0	0
Catostomidae	Sacramento sucker	<i>Catostomus occidentalis</i>	2.3	1.1	0.2	0	0	0	2.5
Osmeridae	Topsmelt	<i>Atherinops affinis</i>	0	0	0	0	1.1	2.3	0

	Steelhead	<i>Oncorhynchus mykiss</i>	0.1	0.1	0	0	0	0	0
Poeciliidae	Western mosquitofish	<i>Gambusia affinis</i>	0	0	0	0	0.1	0.7	0
Atherinidae	Inland silverside	<i>Menidia beryllina</i>	0	0	0	0	0.6	0	0
Cottidae	Pacific staghorn sculpin	<i>Leptocottus armatus</i>	5.3	0	15.9	0	15.5	18.8	0.5
	Prickly sculpin	<i>Cottus asper</i>	0.2	0.8	0	1.3	0.7	1.5	0.3
Gasterosteidae	Threespine stickleback	<i>Gasterosteus aculeatus</i>	0	6.6	1.9	10.4	0.3	48.5	0.1
Embiotocidae	Shiner surfperch	<i>Cymatogaster aggregata</i>	0.2	0.2	0	0	0	0	0
Moronidae	Striped bass	<i>Morone saxatilis</i>	0.3	2.4	0.6	0	0.1	0.1	0.7
Gobiidae	Tidewater goby	<i>Eucyclogobius newberryi</i>	0	0	0	7.3	0	0	0
	Yellowfin goby	<i>Acanthogobius flavimanus</i>	0	0	0.1	0	0.2	0.1	0
Scianidae	White croaker	<i>Genyonemus lineatus</i>	0	0	0	0	0.1	0	0
Paralichthyidae	Speckled sanddab	<i>Citharichthys stigmaeus</i>	0	0	0	0	0.1	0.1	0
Pleuronectidae	Starry flounder	<i>Platichthys stellatus</i>	0.1	1.1	0	0	0	0	0
Total CPUE			16.9	28.4	115	19	123.1	140	6.4
Native Species			8	11	5	3	8	7	6
Non-native Species			1	3	2	0	4	3	2
Total Number of Species			9	14	7	3	12	10	8

*Whereas in previous years a 100-foot beach seine was used for lagoon sampling, May 2023 sampling used a 200-foot seine. Therefore, CPUE values between 2023 reflect a larger area sampled per net haul, and caution should be exercised in comparing these values to those observed in previous years.

Shannon Wiener Diversity Index (H') values were calculated based on the seine data from each of the sampling stations. This diversity index is a quantitative measurement that takes both species richness and abundance into account and serves as a statistical representation of biodiversity. The index ranges from 0 (no diversity) to 5 (extremely high diversity), but H' values typically range from 1.5 to 3.5 (Gaines 1999). Shannon-Weiner Diversity ranged from 0.64 to 1.04 across the 8 sampling locations where fish were captured in the seine hauls (no catch was observed in stations 9 and alternate station 6), with the highest values occurring near the Highway 1 bridge (Table 4). This increase in diversity at upstream locations is likely driven by the lower salinity in these areas, which allowed for co-occurrence of both the euryhaline species and species with lower salinity tolerances.

Table 4. Shannon-Weiner Diversity (H') values calculated for each sampling station with seine catch data. Sites with no catch (alternate station 6 and station 9) are not included.

Station	Shannon-Weiner Diversity (H')
4	0.68
5	0.69
8	0.63
Alternate 8 (Highway 1 Bridge)	1.04
10	1.01
11	0.88
12	0.65
13	0.64

Hoop Net Catch

The two hoop nets captured a total of five species, each represented by a single individual (one in the net facing downstream and four in the net facing upstream). Notably, the upstream facing net captured a single adult white catfish, which represents the first time this species has been observed in Salinas Lagoon sampling.

Table 5. Total catch of the two hoop nets deployed in station 10. Non-native species are indicated by bolded common and scientific names.

Net	Family	Common Name	Species	Total Length (mm)
Facing downstream	Cyprinidae	Hitch	<i>Lavinia exilicauda</i>	150
Facing upstream	Petromyzontidae	Pacific lamprey	<i>Petromyzon tridentata</i>	450*
Facing upstream	Catostomidae	Sacramento sucker	<i>Catostomus occidentalis</i>	215
Facing upstream	Ictaluridae	White catfish	<i>Ameiurus catus</i>	375
Facing upstream	Moronidae	Striped bass	<i>Morone saxatilis</i>	301

* The total length of captured Pacific lamprey is estimated, as it escaped the net during recovery and was not measured.

Striped Bass Tagging

Scanning indicated that the eight striped bass captured in seine hauls and the single striped bass captured in a hoop net had not previously been implanted with a PIT tag; therefore, a 13-mm tag was injected into each individual prior to release. The striped bass caught during this sampling event is the highest CPUE for the species that has been observed during spring lagoon sampling since 2012, as either fewer or no striped bass were captured in 2013, 2014, 2020, 2021, and 2022 (Table 3). Although the lack of population estimates for striped bass in the system make it infeasible to draw conclusions about shifts in their numbers in the system over the years, their apparent greater relative abundance in May 2023 may have been due to the continued connectivity

of the lagoon with the ocean, allowing striped bass moving in the nearshore environment to enter the river for a longer period of time compared to previous years.

Environmental DNA

A total of 12 eDNA samples were collected as part of this monitoring effort (Figure 1; Table 5). All samples were collected with single-use aquatic eDNA kits (Jonah Ventures, Boulder, Colorado). These self-contained kits include nitrile gloves, a 60-mL syringe, a 5- μ m filter cartridge, and a 1-mL syringe of Longmire's solution to stabilize captured DNA for storage and transport. Three samples were collected at each of four sites ranging from the Old Salinas River slidegate to the upstream-most sample site (Station 12; Figure 1). Samples were collected in triplicate at each site to increase the total volume of water sampled (and thereby increase eDNA detection probability for rare species), and to allow for replicability to improve confidence in the validity of results. Sample volumes ranged from 70 mL to 180 mL (average = 141 mL) due to variation in turbidity across sites that led to faster clogging of the filter in more turbid areas. Whereas eDNA samples collected in 2022 were analyzed using qPCR for the detection of a single species (*O. mykiss*), samples collected in May 2023 were subjected to metabarcoding using MiFish 12S primers, allowing for the detection of multiple species. All samples were submitted to Jonah Ventures for analysis (laboratory methodology available in Appendix).

Of the 12 collected eDNA samples, 11 contained sequences that could be assigned to known species based on available reference libraries. Detected sequences belonged to 14 distinct species (Table 5), including five species that were not detected in either the seine or hoop net samples, two of which were species that had never before been observed in lagoon sampling (green sunfish and white bass; Table 5). Species detections from eDNA generally aligned with observations in the seine catch at each site, but a total of two species detected in the seine samples (American shad and prickly sculpin) and one species detected in the hoop net samples (white catfish) were not observed among the eDNA results. However, it should be noted that there were sequences detected that were assigned to the Clupeidae and Cottidae families, but which could not be resolved to the genus or species level, and these may have belonged to American shad and prickly sculpin. The eDNA samples also detected several sequences belonging to non-fish taxa, including beaver (*Castor canadensis*) and tree swallow (*Tachycineta bicolor*) in site 4, and domestic pig (*Sus scrofa*) in sites 1 and 2. As in previous years, eDNA sampling did not detect *O. mykiss* DNA at any of the sampled locations.

Table 5. Environmental DNA samples and detection results. Each site includes combined results from three replicate eDNA samples. Green cells indicate positive detections, whereas red cells indicate no detection. Non-native species are indicated by bolded common and scientific names. Species not detected in hoop net or seine samples are highlighted in yellow.

Family	Common Name	Site 1	Site 2	Site 3	Site 4
Petromyzontidae	Pacific lamprey*	✓			✓
Clupeidae	Pacific herring	✓	✓	✓	-
Cyprinidae	Goldfish*	✓	✓	✓	✓
	Common carp*	✓	✓	✓	✓
	Hitch	✓	✓	✓	✓
	Sacramento blackfish	-	-	-	✓
Catostomidae	Sacramento sucker*	✓	✓	✓	✓
Centrarchidae	Green sunfish	-	✓	-	✓
Poeciliidae	Western mosquitofish	-	-	-	✓
Cottidae	Pacific staghorn sculpin	✓	✓	-	-
Gasterosteidae	Threespine stickleback	✓	✓	✓	✓
Moronidae	Striped bass	✓	✓	✓	✓
	White bass	-	-	✓	-
Gobiidae	Tidewater goby	✓	-	-	✓
Total Species Detected		9	9	8	10

*The detected *Carassius*, *Catostomus*, and *Cyprinus* sequences were assigned only to the genus level, and the detected *Petromyzontidae* sequences were assigned only to the family level based on percent match to known sequences in available reference libraries, but knowledge of the species in these taxa that are likely to be present to be in the system allows inference of species-level ID.

Water Quality Sampling

Water quality sampling during the seining survey revealed an expected gradient of decreasing salinity with increasing distance from the ocean. Persistent freshwater inflow appears to maintain low-salinity conditions (0.37 parts per thousand [ppt] to 3.84 ppt at the time of sampling; Table 6) throughout the lower lagoon, but periodically higher salinities are expected near the breach location during incoming tides. Dissolved oxygen concentrations were high, exceeding 9 mg/l at all monitored locations, which is likely attributable to a combination of algal photosynthesis and

continuous mixing of the water column due to freshwater inflow and tidal action. Conditions encountered during field sampling were well within the environmental tolerances of the native fish species in the Salinas River basin, including steelhead and tidewater goby.

Table 6. Summary of water quality parameters collected concurrently with beach seining on May 9 and 10, 2023.

Station	Temp (°C)	Dissolved Oxygen (mg/l)	Salinity (ppt)	Conductivity (µS/cm)
4	18.5	11.86	2.21	4,136
5	18.9	12.85	3.84	6,956
Alternate Station 6 (south bank)	17.8	9.97	2.00	3,750
7	16.7	10.80	1.30	2,515
Original Station 8	16.8	10.70	1.47	2,820
Alternate Station 8 (Hwy 1 Bridge)	16.5	9.67	1.20	2,323
9	16.2	9.78	1.10	1,971
10	20.3	9.71	0.37	763
11	20.5	10.65	0.38	767
12	20.2	10.06	0.37	753
13	19.2	11.04	1.62	3,080

Species Discussion

The sampling in May of 2023 is a snapshot of the fish community following a period of 126 days of connectivity with Monterey Bay, which represents a much longer period than has occurred in recent years. This extended connectivity has likely led to differences in the fish community observed during this sampling event, including apparent low relative abundances of most captured species, and higher numbers of striped bass. In the sections below, we provide a discussion of key species observations and implications for lagoon use by rearing juvenile steelhead.

Striped Bass

As noted in previous reports, the results of a collaborative study by NMFS and Trout Unlimited suggest a very large population of striped bass resides in the lower Salinas River, and most of these fish appear to be between two and five years of age (Tommy Williams, personal communication). Between November 2019 and March 2020, the project implanted 237 individual striped bass with PIT tags. Another 527 untagged fish were captured by anglers participating in the study, although they may not all have been unique individuals. In total, only three tagged fish were recaptured by anglers, which precluded accurate estimates of population abundance (the preliminary estimate was 31,053 individuals, with a 95% confidence interval of 15,527–124,210). As large-bodied fish with strong swimming abilities, it is likely that the beach seine used in the Salinas Lagoon surveys is not particularly efficient at capturing striped bass. Despite this gear inefficiency, a total of eight

striped bass were captured via seine during May 2023 sampling. Along with the single striped bass captured in the hoop net, these nine individuals bring the total number of striped bass captured by FISHBIO surveys from October 2020 through May 2023 to 14 individuals. Each of these captured striped bass were untagged, and FISHBIO field crews implanted each fish with a unique PIT tag prior to releasing.

The apparent higher relative abundance of striped bass in the lower Salinas River during this May 2023 sampling event in comparison to previous spring sampling events may be due to continued connectivity of the lagoon with Monterey Bay, which in turn may allow subadult and adult striped bass moving in the nearshore environment to more freely enter the lower Salinas River without having to move through the Old Salinas River and tide gate. This has important implications for the food web, as striped bass are efficient predators that are known to be adept at prey-switching to whichever prey species becomes most abundant in a system (Nobriga and Feyrer 2008). It is possible that the striped bass population shifts their predatory focus as the fish community changes seasonally, but a lack of diet samples precludes testing this hypothesis. Striped bass are generally non-discriminating in the species they prey upon, and they can rapidly take advantage of new prey sources as they become available. These factors combined with the apparently very high abundance of this invasive predator in the Salinas Lagoon has potentially significant implications for juvenile steelhead that may utilize the lagoon habitat for rearing.

New Non-native Predator Detections – Green Sunfish, White Bass, and White Catfish

The inclusion of hoop net sampling and the collection and analysis of eDNA samples resulted in the detection of three additional non-native, predatory fish species. These include the white catfish that was captured in the hoop net, and the green sunfish and white bass whose DNA was detected in collected water samples. Although these species are known to occur in the Salinas watershed (i.e., in the Nacimiento), this is the first time they have been detected in lagoon sampling. The failure to detect any of these species via seine sampling demonstrates the value of incorporating additional methodologies to provide a more complete representation of the fish community present in the lagoon. With the limited data obtained, it is not possible to draw any conclusions about the relative abundance of these species, but each has the capacity to prey upon listed, native species including tidewater goby and juvenile steelhead.

Conclusions

The Salinas Lagoon is a dynamic system that is marked by sudden, dramatic shifts in depth, discharge, and water quality, and associated shifts in the composition of the aquatic community. Historically, this system had an extensive floodplain that was seasonally inundated, and estimates suggest that the area of open water in the lagoon may have been approximately 340 acres in 1910 (NMFS 2007). This expansive wetland likely provided rearing habitat for juvenile steelhead throughout the year. Disconnection of this former wetland habitat, management of the lagoon level to protect agricultural fields and residences, reductions in discharge due to water operations, and the introduction of invasive predators (i.e., striped bass) have reduced the suitability of the Salinas Lagoon for rearing steelhead.

Juvenile steelhead are rarely detected in the lagoon, appearing in only five of the past 23 surveys that occurred between 2002 and 2022 (Table 3; Tables A1, A2). They were last detected during the seining effort in October 2013, and they have not been captured in any of the seining efforts conducted by FISHBIO since 2020. When the species was detected in the lagoon, the CPUE never exceeded 0.1 individuals per seine haul. No steelhead were found during the May 2023 sampling event, although water quality data suggest that abiotic factors such as dissolved oxygen and water temperature have remained within a range suitable for rearing juvenile steelhead. As observed in previous years, it is likely that biotic factors – rather than water quality – are playing a more significant role in reducing juvenile steelhead lagoon use.

Striped bass remain the most abundant anadromous species in the river and likely serve as the most significant remaining connection between the marine and freshwater food webs. Striped bass have been shown to be important predators of juvenile steelhead in other systems in California (Michel et al. 2018) and may occur at very high densities (e.g., 1,227 individuals per river kilometer; Michel et al. 2018). Accurate estimates of the total striped bass population in the Salinas are still lacking, but their relative abundance in the lower river appeared to be significantly higher this year than in the previous five sampling events (as interpreted through higher CPUE). The abundance of this species combined with the species' ability to rapidly adapt to new prey sources as they become available (Nobriga and Feyrer 2008) may be playing a role in limiting use of the lagoon by rearing juvenile steelhead. However, striped bass may focus their predation on seasonally abundant forage fish species (e.g., Pacific herring, threadfin shad) that have been frequently detected in lagoon surveys, and this may limit their impact on any juvenile steelhead utilizing the lagoon as rearing habitat. Further investigation into the ecology and dynamics of the Salinas striped bass population – including studies on their natal origin, movement, dietary composition, and abundance across seasons and years – are warranted in order to investigate their impacts on steelhead and to better comprehend their potential influence on steelhead recovery actions.

Incorporating eDNA sampling into the standard lagoon sampling protocol in 2022 and 2023 provides further evidence that steelhead are either not present or are very rare in the lower river. Further, the use of metabarcoding analysis in 2023 has demonstrated the value of this approach for detecting species that may elude capture in traditional gears such as seines. Taken together, these findings suggest that the continued inclusion of eDNA methodology in future lagoon sampling is warranted. Importantly, failure to detect DNA sequences from a given species in eDNA samples does not provide conclusive evidence of species absence, but failure to detect a species in both eDNA and traditional samples does strengthen confidence that said species is absent or rare. On the other hand, positive eDNA detections definitively demonstrate species presence even if traditional sampling failed to detect them. This tool is not only valuable for increasing the odds of detecting any steelhead that may be present in the lagoon but is also useful for identifying invasive species that may otherwise go undetected, as was observed in the samples collected this spring.

Notably, the use of hoop nets also resulted in the detection of a species that was not previously observed in any lagoon sampling effort. This finding and new detections via eDNA highlight the value of a mixed-gear approach for capturing a more complete representation of the fish community in the Salinas Lagoon. Based on observations in the field over the past three years of sampling by FISHBIO crews, beach seining is of variable efficiency across the sample stations, and changes in river morphology and the presence of debris can make it highly inefficient in certain

locations as site conditions change. This is particularly true in many of the upstream sites, where dense vegetation and abundant debris often impede efficient seining and necessitate annual adjustment of sample stations. Though their use in this sampling event was intended as a proof of concept, the hoop nets were demonstrated to circumvent some of the challenges faced in seine netting and may more efficiently sample certain locations. More strategic and extensive deployment of passive gears like the hoop nets, as well as the inclusion of other gears that are less hindered by the presence of debris (e.g., cast nets), may greatly improve the ability of surveyors to capture a representative sample of the fish community. As such, development of a more comprehensive, multi-gear sampling protocol would be a worthwhile endeavor for ongoing lagoon monitoring.

Literature Cited

- Bollens, SM, and Sanders, AM. 2004. Ecology of larval Pacific herring in the San Francisco Estuary: seasonal and interannual abundance, distribution, diet, and condition." *American Fisheries Society Symposium*. American Fisheries Society.
- [CDFW] California Department of Fish and Wildlife. 2019. California Pacific Herring Fishery Management Plan. <https://wildlife.ca.gov/Fishing/Commercial/Herring/FMP>
- Fleming, K. 1999 Clupeidae. Pages 151-166 in J.J. Orsi, editor. Report on the 1980-1995 fish, shrimp, and crab sampling the San Francisco Estuary, California. California Department of Water Resources, Interagency Ecological Program, Technical Report 63, Sacramento, California.
- Gaines, W. L. 1999. Monitoring biodiversity: quantification and interpretation (Vol. 443). US Department of Agriculture, Forest Service, Pacific Northwest Research Station.
- [HES] Hagar Environmental Science. 2014. Salinas River Lagoon Monitoring Report.
- [MCWRA] Monterey County Water Resources Agency. 2013. Salinas Valley Water Project Annual Fisheries Report. <https://www.co.monterey.ca.us/home/showdocument?id=19052>
- [MCWRA] Monterey County Water Resources Agency. 2023. Report on Salinas Valley Water Conditions for the First Quarter of Water Year 2022-2023. <https://www.co.monterey.ca.us/home/showpublisheddocument/120126/638132829703770000>
- [MCWRA] Monterey County Water Resources Agency. 2023. Report on Salinas Valley Water Conditions for the Second Quarter of Water Year 2022-2023. <https://www.co.monterey.ca.us/home/showpublisheddocument/121635/638176038369970000>
- Michel, CJ, Smith, JM, Demetras, NJ, Huff, DD, and Hayes, SA. 2018. Non-native fish predator density and molecular-based diet estimates suggest differing impacts of predator species on juvenile salmon in the San Joaquin River, California. *San Francisco Estuary and Watershed Science* 16(4).
- Moyle, P. B. 2002. Inland fishes of California: revised and expanded. University of California Press.
- [NMFS] National Marine Fisheries Service. 2007. Biological Opinion: Monterey County Water Resources Agency, Salinas Valley Water Project in Monterey County, California. SWB/2003/2080.

[NMFS] National Marine Fisheries Service. 2009. Biological Opinion for Sandbar Breaching at the Mouth of the Salinas River. Southwest Region, Long Beach, California, December 21, 2009.

Nobriga, ML, and Feyrer, F. (2008). Diet composition in San Francisco Estuary striped bass: does trophic adaptability have its limits? *Environmental Biology of Fishes*, 83(4), 495-503.

Skinner, J. E. 1962. Anadromous fisheries. In: An historical review of the fish and wildlife resources of the San Francisco Bay area (J. E. Skinner, ed.), p. 57–93. California Bay-Delta Authority Science Program and the John Muir Institute of the Environment.

[USFWS] US Fish and Wildlife Service. 1997. Endangered and Threatened Species: Listing of Several Evolutionary Significant Units (ESUs) of West Coast Steelhead. *Federal Register* 62(159): 43937-43954.

[USFWS] US Fish and Wildlife Service. 2007. Biological Opinion on Issuance of Department of the Army Permits to the Monterey County Water Resources Agency for Construction of a Surface Water Diversion Structure in the Salinas River, Near the City of Salinas (Corps File Number 24976S) and for Breaching of the Salinas River Lagoon (Corps File Number 16798S) in Monterey County, California (1-8-06-F .54)

Appendix

Table A1. Cumulative and species-specific catch per unit effort (CPUE) across the four summer sampling events ranging from August 2008 to July 2013. Note that CPUE is calculated using a single seine haul as the base unit of effort. Non-native species are indicated by bolded common and scientific names.

		Date	Aug 2010	Aug 2011	July 2012	July 2013
		Total Seine Hauls	7	9	13	13
Family	Common Name	Scientific Name				
Clupeidae	Pacific herring	<i>Clupea pallasii</i>	35.70	0	0	0.80
	Pacific sardine	<i>Sardinops sagax</i>	0.10	0	0	0
Cyprinidae	Common carp	<i>Cyprinus carpio</i>	0.10	0	0	0.60
	Hitch	<i>Lavinia exilicauda</i>	134.10	4.10	16.20	4.50
	Sacramento blackfish	<i>Orthodon microlepidotus</i>	33.60	0	0.10	0.10
	Unidentified cyprinid	<i>Cyprinidae</i>	0.10	0	0	0
Catostomidae	Sacramento sucker	<i>Catostomus occidentalis</i>	45.90	0	2.60	1.10
Osmeridae	Topsmelt	<i>Atherinops affinis</i>	15.10	0	0	0
Salmonidae	Steelhead	<i>Oncorhynchus mykiss</i>	0	0.10	0	0
Poeciliidae	Western mosquitofish	<i>Gambusia affinis</i>	0	0.40	1.50	0.20
Cottidae	Pacific staghorn sculpin	<i>Leptocottus armatus</i>	33.30	0.60	0.90	0.80
	Prickly sculpin	<i>Cottus asper</i>	5.40	0.40	20	5.10
	Unidentified sculpin	<i>Cottidae</i>	0.30	0	0	0
Gasterosteidae	Threespine stickleback	<i>Gasterosteus aculeatus</i>	347.60	3.40	5.10	21.20
Embiotocidae	Shiner surfperch	<i>Cymatogaster aggregata</i>	13.40	0	0	0
Moronidae	Striped bass	<i>Morone saxatilis</i>	0.40	0	2.40	3.60
Centrarchidae	Largemouth bass	<i>Micropterus salmoides</i>	0	0	0	0.10
Gobiidae	Yellowfin goby	<i>Acanthogobius flavimanus</i>	0	0	0	4.60
	Unidentified goby	<i>Gobiidae</i>	0.50	0	0	0
Sebastidae	Unidentified rockfish	<i>Sebastes spp.</i>	0.20	0	0	0
Pleuronectidae	Starry flounder	<i>Platichthys stellatus</i>	0.90	0.10	0.10	0.10
Total CPUE			666.70	9.10	30.90	42.80
Native Species			15	6	7	8
Non-native Species			2	1	2	5

Total Number of Species	17	7	9	13
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Table A2. Cumulative and species-specific catch per unit effort (CPUE) across the 12 fall sampling events ranging from October 2002 to October 2020. Note that CPUE is calculated using a single seine haul as the base unit of effort. Non-native species are indicated by bolded common and scientific names.

		Date	Oct 2002	Oct 2003	Oct 2004	Oct 2005	Oct 2006	Oct 2008	Oct 2009	Oct 2010	Oct 2011	Oct 2012	Oct 2013	Oct 2020
		Total Seine Hauls	9	17	5	17	9	8	12	12	16	17	12	8
Family	Common Name	Scientific Name												
Clupeidae	Pacific herring	<i>Clupea pallasii</i>	0.70	0	6.60	62.90	0.30	194.10	4.40	41.60	56.40	0	0.20	0
	Threadfin shad	<i>Dorosoma petenense</i>	0.20	4.80	27.80	0	0	12.90	0	0	0	0	5.10	29.88
Cyprinidae	Common carp	<i>Cyprinus carpio</i>	0.10	0.30	12.60	3.60	0	0	0.10	0	0	0	0.20	0
	Goldfish	<i>Carassius auratus</i>	0	0	0	0	0	0	0	0	0	0	0	0.13
	Hitch	<i>Lavinia exilicauda</i>	30.40	67.60	180	36.70	0.10	20.30	8.50	6.10	0.80	0	0.60	4.13
	Sacramento blackfish	<i>Orthodon microlepidotus</i>	0	3.20	1.40	18.10	0.10	0.60	0	30.30	0	0	0.10	0
	Sacramento pikeminnow	<i>Ptychocheilus grandis</i>	0	0.10	0	0	0	0	0	0	0	0	0	0
Catostomidae	Sacramento sucker	<i>Catostomus occidentalis</i>	3.80	13.80	90	18.10	0	0.10	0.10	3.10	0	0	0.10	0.25
Osmeridae	Topsmelt	<i>Atherinops affinis</i>	0	0	7	0	44.60	10.40	11.20	12.70	21.30	0	0	0
Salmonidae	Chinook salmon	<i>Oncorhynchus tshawytscha</i>	0.10	0	0	0	0	0	0	0	0	0	0	0
	Steelhead	<i>Oncorhynchus mykiss</i>	0	0	0	0	0	0	0	0	0.10	0	0.10	0
Poeciliidae	Western mosquitofish	<i>Gambusia affinis</i>	0	0.10	640	6.10	0	0	0	0.90	0	0.40	0.10	0
Atherinidae	Inland silverside	<i>Menidia beryllina</i>	0	0	0	0	0	0	0	0	0	0	0	0.38
Cottidae	Pacific staghorn sculpin	<i>Leptocottus armatus</i>	0.60	0.40	1	0.80	0	1.50	0.90	0.50	0.20	0.10	0.30	0.13
	Prickly sculpin	<i>Cottus asper</i>	0.10	0.40	5.40	0.10	0	0.30	0.20	1.90	0.40	0.40	0.50	1.13
	Unidentified sculpin	<i>Cottidae</i>	0	0	0	0	0	0	0	0	1.70	0	0	0

Gasterosteidae	Threespine stickleback	<i>Gasterosteus aculeatus</i>	54.3	47	59.8	16.9	0	8.5	6.8	31.7	0.1	3.5	37.5	1
Embiotocidae	Shiner surfperch	<i>Cymatogaster aggregata</i>	0	0	0	0	0	4.50	0.60	0	0.70	0	0	0
Moronidae	Striped bass	<i>Morone saxatilis</i>	0	0	0	0.40	0	0	0.10	0	0.70	0.20	0.70	0.50
	Arrow goby	<i>Clevelandia ios</i>	0	0	0	0	0	0	0	0.10	0	0	0	0
Gobiidae	Tidewater goby	<i>Eucyclogobius newberryi</i>	0	0	0	0	0	0	0	0	0	0	0.20	0
	Yellowfin goby	<i>Acanthogobius flavimanus</i>	0	0	0	0	0	0	0	0	0	0	0.30	11.38
Pleuronectidae	Starry flounder	<i>Platichthys stellatus</i>	0.10	0	0	0.90	0	0.50	0.70	0.10	0.20	0	0	0
		Total CPUE	90.40	137.70	212.60	164.60	45.10	253.70	33.70	129	82.60	4.60	46	48.91
		Native Species	8	7	8	9	4	10	10	9	11	4	10	5
		Non-Native Species	2	3	3	2	0	1	2	2	0	1	4	5
		Number of Species	10	10	11	11	4	11	12	11	11	5	14	10

Environmental DNA Metabarcoding Methodology – Courtesy of Jonah Ventures

Sample Process

Sample barcodes were recorded and assigned a well within the 96 well plate or numbered extraction tube. A customized one-ton arbor press along with a removable leather punch was used to open the plastic casing of each filter. Once plastic casing was cut, sample barcodes were recorded and assigned a well within the 96 well plate or numbered extraction tube. The whole filter was removed and transferred to the extraction plate/tube using sterilized tweezers inside a laminar flow hood. The removable leather punch was sterilized between each eDNA filter. Plates or tubes were immediately processed or stored in -20C until the extraction process could be performed.

Extraction

Genomic DNA from samples was extracted using the DNeasy Blood & Tissue Kit (250) (Cat. No. / ID: 69506) according to the manufacturer's protocol. Whole (25mm or 47mm) filters were used for genomic DNA extraction. Genomic DNA was eluted into 200µl and frozen at -20°C.

PCR

Forward Primer: GTCGGTAAACTCGTGCCAGC

Reverse Primer: CATAGTGGGGTATCTAATCCCAGTTTG

Primer notes:

Primer reference: Miya et al 2015

Portions of hyper-variable regions of the mitochondrial 12S ribosomal RNA (rRNA) gene were PCR amplified from each genomic DNA sample using the MiFishUF and MiFishUR primers with spacer regions. Both forward and reverse primers also contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. PCR amplification was performed in replicates of six and all six replicates were not pooled and kept separate. Each 25 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5µl Master Mix, 0.5 µM of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H₂O. DNA was PCR amplified using the following conditions: initial denaturation at 95C for 3 minutes, followed by 45 cycles of 20 seconds at 98C, 30 seconds at 60C, and 30 seconds at 72C, and a final elongation at 72C for 10 minutes. Added 11/2019.

Gel

To determine amplicon size and PCR efficiency, each reaction was visually inspected using a 2% agarose gel with 5µl of each sample as input.

PCR Amplicon Cleanup

Amplicons were then cleaned by incubating amplicons with ExoI/SAP for 30 minutes at 37C following by inactivation at 95C for 5 minutes and stored at -20C.

Barcoding PCR

A second round of PCR was performed to complete the sequencing library construct, appending with the final Illumina sequencing adapters and integrating a sample-specific, 12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95 °C for 30 sec, 55 °C for 30 seconds and 72 °C for 30 seconds.

PCR Normal Pool

Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). 25 µl of PCR amplicon is purified and normalized using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples are then pooled together by adding 5 µl of each normalized sample to the pool.

Sequencing

Sample library pools were sent for sequencing on an Illumina MiSeq (San Diego, CA) at the Texas A&M Agrilife Genomics and Bioinformatics Sequencing Core facility using the v2 500-cycle kit (cat# MS-102-2003). Necessary quality control measures were performed at the sequencing center prior to sequencing.

Bioinformatics

Raw sequence data were demultiplexed using phenix v2.1.0 [1], enforcing strict matching of sample barcode indices (i.e, no errors). Cutadapt v3.4 [2] was then used to remove gene primers from the forward and reverse reads, discarding any read pairs where one or both primers (including a 6 bp, fully degenerate prefix) were not found at the expected location (5') with an error rate < 0.15. Read pairs were then merged using vsearch v2.15.2 [3], discarding resulting sequences with a length of < 130 bp, > 210 bp, or with a maximum expected error rate [4] > 0.5 bp. For each sample, reads were then clustered using the unoise3 denoising algorithm [5] as implemented in vsearch, using an alpha value of 5 and discarding unique raw sequences observed less than 8 times. Counts of the resulting exact sequence variants (ESVs) were then compiled and putative chimeras were removed using the uchime3 algorithm, as implemented in vsearch. For each final ESV, a consensus taxonomy was assigned using a custom best-hits algorithm and a reference database consisting of publicly available sequences (GenBank [6]) as well as Jonah Ventures voucher sequences records. Reference database searching used an exhaustive semi-global pairwise alignment with vsearch, and match quality was quantified using a custom, query-centric approach, where the % match ignores terminal gaps in the target sequence, but not the query sequence. The consensus taxonomy was then generated using either all 100% matching reference sequences or all reference sequences within 1% of the top match, accepting the reference taxonomy for any taxonomic level with > 90% agreement across the top hits.

References:

1. Galanti, Shasha and Gunsalus (2021). Phenix 2.0: accurate, high-performance Bayesian decoding and confidence estimation for combinatorial barcode indexing. BMC Bioinformatics 22.
2. Martin (2011), Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal 17.
3. Torbjørn et al. VSEARCH: a versatile open-source tool for metagenomics. PeerJ 4 e2584 (2016).
4. Edgar and Flyvbjerg (2015), Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 31.
5. Edgar (2016), UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. doi: <https://doi.org/10.1101/081257>.
6. Benson et al. (2005), GenBank. Nucleic Acids Res 33.

Invasive Species Prevention Plan

All field gear used in the Salinas Lagoon was properly disinfected following California Department of Fish and Wildlife Aquatic Invasive Species Disinfection/Decontamination Protocols prior to the start of fieldwork.

A detailed list of the relevant disinfection procedures and preventative measures that were used to prevent the spread of aquatic invasive species in the Salinas Lagoon is listed below.

If equipment is used on the project that was previously working in another stream, river, lake, pond, or wetland within 10 days of initiating work, we implement one of the following procedures to prevent the spread of New Zealand Mud Snails and other aquatic hitchhikers:

- (1) Remove all mud and debris from equipment (waders, nets, watercraft, etc.) and keep the equipment dry for 10 days. OR
- (2) Remove all mud and debris from Equipment (waders, nets, watercraft, etc.) and spray/soak equipment with either a 1:1 solution of Formula 409 Household Cleaner and water, or a solution of Sparquat 256 (5 ounces Sparquat per gallon of water). Treated equipment must be kept moist for at least 10 minutes. OR
- (3) Remove all mud and debris from equipment (waders, nets, watercraft, etc.) and spray/soak equipment with water greater than 120 degrees F for at least 10 minutes. OR (4) Remove all mud and debris from equipment (waders, nets, watercraft, etc.) and freeze equipment below 0 degrees F for at least 48 hours.

Data Management Plan

This data management plan is designed to ensure that project data are collected using peer-approved methods, undergo a quality control and accuracy assessment process, include metadata that meet CDFW's minimum standards.

The following documentation provides evidence of the methods and quality control procedures that were used to meet Grant Agreement requirements.

1. **Who collected the data:** Michael Hellmair, Elizabeth Ramsay, Ethan Switzer, Miguel Ibarra
2. **When the data was collected:** May 9-10, 2023
3. **Where the data was collected:** Salinas River Lagoon
4. **How the data was collected (description of methods and protocols):** Sampling was conducted using a 100-foot beach seine with 1/4-inch mesh. At each sampling site, the seine was set in a semi-circle a short distance from shore. Crew members then pulled the seine onto shore, while ensuring the float line stayed above the surface and keeping the lead line as close to the substrate as possible. At sites where water depths precluded wading, an inflatable raft was used to deploy the seine. Once the seine was pulled onto shore, crews quickly processed captured fish by placing specimens to be measured in aerated recovery buckets and counting extremely abundant species before releasing them. Once all fish were removed from the net, crews recorded fork and total length data on at least 30 individuals of each captured species (with the exception of threespine stickleback; *Gasterosteus aculeatus*), before plus-counting any remaining individuals. After fish processing was completed, crews collected water quality data using a YSI water quality sampling meter in the sampled area. Staff used the YSI to measure temperature (°C), specific conductivity ($\mu\text{S}/\text{cm}$ at 25°C), conductivity ($\mu\text{S}/\text{cm}$), salinity (parts per thousand; ppt), and dissolved oxygen (mg/L) at the surface. All data sheets collected in the field were scanned (with electronic copies stored on a server) before the data was entered into a database. Prior to data analyses, the database underwent QA/QC procedures including being checked against field datasheets by two separate individuals. All datasheets were also stored as hard copies at the FISHBIO office.
5. **The purposes for which the data was collected:** Salinas Lagoon sampling is intended to assist in determining the presence and spatial distribution of steelhead in the lower Salinas River and Lagoon. The purpose of these sampling efforts is to capture any juvenile SCCC DPS steelhead that may be rearing in the lagoon. Objectives include evaluating presence or absence, condition, relative abundance (i.e., catch per unit effort; CPUE), and distribution of juvenile steelhead in the Salinas Lagoon.
6. **Definitions of variables, fields, codes, and abbreviations used in the data, including units of measure:** All species field codes are included on the following pages.
7. **The terms of any landowner access agreement(s), if applicable:** Landowner access was granted via personal communication to park a vehicle at the northern end of the Lagoon.
8. **References to any related Department permits or regulatory actions:** Relevant permits are included on the following pages
9. **Peer review or statistical consultation documentation:** All reports were reviewed by multiple parties, including the Grant recipient, and will also be published online and therefore subject to external peer review.
10. **Data licensing and disclaimer language:** All data is the property of Monterey County Water Resources Agency and is subject to their data licensing and disclaimer requirements.

Abbreviation Codes

Common Name	Species Code
American Shad	AMS
Bass Unknown	BAS
Bigscale Logperch	LP
Black Bullhead	BKB
Black Crappie	BKS
Blue Catfish	BLC
Bluegill	BGS
Brook Trout	BKT
Brown Bullhead	BRB
Brown Trout	BT
California Roach	CAR
Catfish Unknown	CAT
Channel Catfish	CHC
Chinook Salmon	CHN
Common Carp	C
Delta Smelt	DSM
Fathead Minnow	FHM
Golden Shiner	GSN
Goldfish	GF
Green Sturgeon	GST
Green Sunfish	GSF
Hardhead	HH
Hitch	HCH
Inland Silverside	MSS
Kern Brook Lamprey	KBL
Kokanee Salmon	KOS
Lamprey Unknown	LAM
Largemouth Bass	LMB
No Catch	NONE
Pacific Lamprey	PL
Pacific Brook Lamprey	BL
Pacific Staghorn Sculpin	PSS
Prickly Sculpin	PRS
Pumpkinseed	PKS

Stanislaus River Station	Station Code
Caswell State Park	ST004X
Caswell State Park – North Trap	ST004N
Caswell State Park – South Trap	ST004S
Oakdale Recreation Area	ST040X
Stanislaus Weir	ST031X
Calaveras River Station	Station Code
Shelton Rd.	CR028X
Merced River Station	Station Code
Gallo Ranch	ME041X
Hatfield Park – North Trap	ME002N
Hatfield Park – South Trap	ME002S

Condition Code	Description
1	Good
2	Fair (partial cell block)
3	Poor (total cell block)
4	No sample taken

Debris Code	Description
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Common Name	Species Code
Rainbow / Steelhead Trout	RBT
Red Shiner	RSN
Redear Sunfish	RES
Redeye Bass	REB
Riffle Sculpin	RFS
River Lamprey	RL
Sacramento Blackfish	SCB
Sacramento Perch	SP
Sacramento Squawfish	SASQ
Sacramento Sucker	SASU
Sculpin Unknown	SCP
Shimofuri Goby	SHM
Smallmouth Bass	SMB
Speckled Dace	SPD
Splittail	SPLT
Spotted Bass	SPTB
Striped Bass	STB
Sturgeon Unknown	STG
Sunfish Unknown	SNF
Threadfin Shad	TFS
Threespine Stickleback	TSS
Tule Perch	TP
Unknown (Unid Juvenile Fish)	UNID
Unknown Centrarchid	CENT
Wakasagi	WAG
Warmouth	W
Western Mosquitofish	MQK
White Catfish	WHC
White Sturgeon	WST
Yellow Bullhead	YEB
Yellowfin Goby	YFG

Tuolumne River Station	Station Code
Grayson	TU005X
Grayson – North Trap	TU005N
Grayson – South Trap	TU005S
Waterford	TU030X
Tuolumne Weir	TU024X

Arroyo Seco River	Station Code
Arroyo Seco River	AS012X

Nacimiento River	Station Code
Nacimiento River	NR001X

Salinas River	Station Code
Upper Salinas	SR109X
Salinas Weir	SR003X

Mark Codes	Description
CFGN	Natural Origin
CFGH	Hatchery Origin
CFG*	Caudal Fin Green
CFR*	Caudal Fin Red
CFO*	Caudal Fin Orange
CFP*	Caudal Fin Pink
CFB*	Caudal Fin Blue
AFG*	Anal Fin Green

LIT	Light
MED	Medium
HVY	Heavy

Weather Code	Description
CLD	Cloudy
RAN	Rainy
CLR	Clear
NIT	Night

AFB*	Anal Fin Blue
TCR**	Top Caudal Fin Red
BCR**	Bottom Caudal Fin Red
DCB**	Double Caudal Fin Red

(* Always indicate stock origin (H or N)
(**) Indicate if mark is specific to location on fish (T or B or D)

Gear Status	Description
0	Set trap
3	Check and raise trap

Relevant Department Permits



State of California – Department of Fish and Wildlife

SCIENTIFIC COLLECTING PERMIT, SPECIFIC USE – Permit

DFW 1379S (NEW 09/01/17)

Specific Use Permit Details:

Specific Use Permit ID: **S-183400003-20036-001**

Reference Title: **Salinas O. mykiss Lifecycle Monitoring Program**

Principal Investigator (PI):

Principal Investigator	PI Status	Approval Status	Conditions
SC-183400006: Andrea N Fuller	Active	Approved	

Geographic Location Details:

Taxonomic Group	Approved County	Approved Locations	Details	Approval Status	Conditions
Fisheries	Monterey	Salinas River	Beach seine surveys will be conducted at several point locations within the Salinas River Lagoon and upstream to the Salinas River Weir location (RM 3). The Salinas Weir will be operated at RM 3 and an RST may be operated (flow dependent) at RM 109.	Approved	

Method Details:

Taxonomic Group	Approved Methods	Details	Approval Status	Conditions
Fisheries	Seine, Beach	Lagoon Seining Surveys will occur one day during seasonal periods (Spring; Apr - May, summer; Jun - Aug, and fall; Oct). The CDFW regional biologist will be notified prior to sampling. Seine hauls will be conducted using a 1/8 inch mesh nylon seine. The seines used are nets suspended between poles with no purse or bunt, featuring a lead line at the bottom and a float line at the top (e.g., traditional beach seine). The seine will be set in a round haul fashion by fixing one end on the beach while the other end is deployed wading upstream and returning to shore in a half circle. Once the lead line approaches the shore, it will be withdrawn more than the cork line until fish are corralled in the bag and the lead line is on the beach. Fish captured in the bag of the seine will be kept submerged in the water until they are transferred to holding containers using dip nets. Each haul is expected to take approximately 5 minutes. Each seine site will have three hauls conducted by a team of at minimum three technicians. Fish from each haul will be separated by size class to avoid risk of predation and placed in aerated 5-gallon buckets prior to processing. Special status species will be given first priority during processing. As recommended by the Tidewater Goby (TWG) Survey Protocol (USFWS 2005) no measurements will be taken from TWG. They will be identified, enumerated, and released at the same point of capture. Any TWG exhibiting signs of physiological stress will be released immediately. Water temperature in the buckets will be kept within 2 degrees of the in-river water temperature. Seining may be conducted in water temperatures up to 70°F (21°C).	Approved	