Salinas Lagoon Seining Report

Fall 2023

Permit No.: SCP: S-183400003-20036-001; 4(d) APPS# 26762

Submitted To:
Monterey County
Water Resources Agency

Prepared By:
Jack Eschenroeder
Michael Hellmair
Dana Lee

FISHBIO
1617 S. Yosemite Ave.
Oakdale, CA 95361
209.847.6300

January 2024

Table of Contents

Executive Summary ..... **Error! Bookmark not defined.**
Background 3
Methodology 6
Fish Community Sampling 6
Water Quality Sampling 10
Data Analysis 10
Results 11
Fish Community Sampling 11
Seine Catch 11
Environmental DNA 14
Water Quality Sampling 15
Species Discussion 16
Striped Bass 16
Notable Species Detection – Bluegill Sunfish 16
Conclusions 17
Literature Cited 19
Appendix A – Seasonal CPUE Data 20
Appendix C – Data Management Plan 25
Appendix D – Invasive Species Prevention Plan 27

Executive Summary

In fulfillment of the monitoring recommendations put forth in the 2009 draft Biological Opinion on sand bar management (NMFS 2009), FISHBIO conducted fish community and water quality sampling of the lower Salinas River and Lagoon on October 17 and 18, 2023. This sampling event occurred following a period of lagoon connectivity with the ocean that lasted 261 days from the breaching event on January 3 through reclosure of the lagoon on September 21, 2023. This represents an uncharacteristically long period of connectivity and presented a broad window of opportunity for fish migration to and from the marine environment. A total of 11 of the established sampling stations were subjected to seine sampling.

In total, 11 species of fish were captured via seine sampling. Relatively high catch-per-unit-effort (average = 102.45 fish/net haul) suggests a higher density of fish were present in the lagoon than the densities observed in all fall sampling events since 2010. Notably, a bluegill sunfish (*Lepomis macrochirus*) was observed in the seine sampling during this event, which is the first time this species has been observed across all lagoon sampling events dating back to 2002.

Although the field crew attempted to incorporate hoop nets into the sampling (as was done in May of 2023), the closure of the lagoon and associated increase in water depth precluded the deployment of hoop nets, as the standard seven-foot t-posts were not long enough to allow for placement. Hoop nets will be employed again during spring sampling, provided water levels are sufficiently low.

Notably, no striped bass (*Morone saxatilis*) were captured during this sampling event, which is the first time they have not been detected in a fall sampling event since 2010. In addition, no striped bass eDNA was detected in the four collected samples.

The collection and analysis of four eDNA samples resulted in the detection of two fish species not observed in the seine catch: jacksmelt (*Atherinopsis californiensis*) and western mosquitofish (*Gambusia affinis*). Sequences belonging to hitch (*Lavinia exilicauda*), three-spined stickleback (*Gastrosteus aculeatus*), and yellowfin goby (*Acanthogobius falvimanus*) – all species observed in the seine catch – were also detected. Unassigned sequences belonging to families Cottidae (sculpins) and Cyprinidae (minnows and carps) were detected as well. Notably, the lower number of eDNA samples collected during fall sampling compared to the 12 that were collected in spring sampling may account for the fewer species detected, as a significantly smaller volume of water was filtered.

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 24 times since sampling began in 2002 (seven spring events, four summer events, and 13 fall events: Table 1).

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	-	October

*Sampling conducted by FISHBIO

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 2023a). 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 2023b). Notably, reports on precipitation during third (April through June) and fourth quarter (July through September) of water year 2022-2023 were not yet available on the MCWRA website as of the time of publication.

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	4th (July-Sep 2020)	0.20	10	0.17	18
2020-2021	1st (Oct-Dec 2020)	3.91	24	3.72	30
2020-2021	2nd (Jan-Mar 2021)	7.35	65	7.03	88
2020-2021	3rd (Apr-Jun 2021)	5.75	46	7.33	62
2020-2021	4th (July-Sep 2021)	0.04	20	0.01	6
2021-2022	1st (Oct-Dec 2021)	6.11	154	5.37	150

2021-2022	2nd (Jan-Mar 2022)	0.76	12	1.15	17
2021-2022	3rd (Apr-Jun 2022)	0.44	15	0.38	95
2021-2022	4th (July-Sep 2022)	0.07	47	0.21	172
2022-2023	1st (Oct-Dec 2022)	6.27	158	5.01	140
2022-2023	2nd (Jan-Mar 2023)	7.43	105	13.48	194
2022-2023	3rd (Apr-Jun 2023)				
2022-2023	4th (Jul-Sep 2023)				

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 remained so for a period of 261 days until the sand berm reformed and the lagoon closed on September 21, 2023. Plotting of lagoon stage prior to and during the sampling period is not feasible for 2023, as high flows during the winter of 2022/2023 filled the approach channel to the Old Salinas River with sediment, and the sediment accumulation combined with the long period during which the river mouth remained open meant that the water level was not high enough to make surface connection with the slide gate and sensor for extended periods of time, resulting in an interruption of logged stage data. However, lagoon stage averaged 4.35 feet during the October sampling event.

Methodology

Fish Community Sampling

Salinas Lagoon sampling is intended to address requirements originally put forth in the draft Biological Opinion for sand bar management (NMFS 2009), which calls for fish community sampling to be conducted in the spring (April–May), summer (June–August), and fall (October–November). 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 ecosystem. Several sample stations were adjusted slightly this fall to accommodate

changes in the configuration of the lagoon and river that occurred following high winter and spring flows. Overall, the mouth of the lagoon was characterized by a marked northward shift, resulting in a configuration that, similar to 2022, combined Stations 1-3 into a single sampling location (Figure 1). Repeating the approach used during the previous sampling in May of this year, an alternative station 8 near the Highway 1 bridge was sampled in addition to the original station 8. In contrast to the spring 2023 sampling event (when the lagoon was under tidal influence), the efficiency of seine hauls was not hampered by tidal currents. In addition, as the water level in the lagoon had increased since the closure of the sandbar, locations that were considered shallow mudflats in spring could be sampled effectively.

FISHBIO field crews conducted seine sampling at 11 stations (1, 4, 5, Alternate 6, 7, 8, Alternate 8, 9, 10, 11, and 12; Figure 1). Seine hauls at all stations were considered effective, typically evidenced by the capture of bottom-oriented fish species that easily evade capture in the event of inefficient seine hauls (such as when the lead line loses contact with the bottom, allowing fish to escape under the net). In previous years, fish community sampling was conducted using a 100-foot beach seine with 1/4-inch mesh. However, beginning with the previous sampling event in May of this year, a 200-foot seine is being used (Figure 2); therefore, the area sampled by each haul is considerably larger than that in previous sampling 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 ft^2 (~1459 m^2), 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. Although crews were prepared to scan all captured striped bass and *O. mykiss* for passive integrated transponder (PIT) tags and implant new tags in any that did not already contain one, no individuals belonging to either species were captured during this sampling event.

Although the field crew had planned for redeployment of the hoop nets used in the May 2023 sampling, excessive water depth resulting from lagoon closure precluded the use of these gears. Hoop nets will be employed again during spring sampling in 2024, provided water depth drops sufficiently by that time.

eDNA samples were collected with single-use aquatic eDNA kits that include a 60-mL syringe, a 5-µm filter cartridge, and a 1-mL syringe of Longmire's solution to stabilize captured DNA for storage and transport (Jonah Ventures, Boulder, Colorado). Whereas eDNA samples collected in

2022 were analyzed using qPCR for the detection of a single species (*O. mykiss*), samples collected since May of 2023 have all been 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 B).

Figure 1. Seine sample stations and eDNA collection sites from October 2023. An interactive map is available at the following link: https://bit.ly/salinaslagoon_Oct2023.

Figure 2. Seine haul in the upper Salinas River Lagoon (Station 11).

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 (µS/cm at 25˚C), conductivity (µS/cm), salinity (parts per thousand; ppt), and dissolved oxygen (mg/L) at the surface.

Data Analysis

Data collected during this sampling effort was 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 was standardized to individuals captured per seine haul. Cumulative and species-specific CPUE was then calculated for the October 2023 sampling event. Data collected during the October 2023 sampling event was also analyzed for species diversity at each sampling location within the lagoon using the Shannon-Weiner Diversity Index.. 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).

Results

Fish Community Sampling

Seine Catch

A total of 1,127 fish representing 10 different species were captured via seine in October 2023 (Table 3). Among fall sampling events, this year saw the highest total CPUE since October of 2008 (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 three juvenile bluegill sunfish (*Lepomis macrochirus*; fork length range = 24-36 mm) represents the first time this species has been observed in lagoon seine surveys. However, the detection of eDNA belonging to green sunfish (*Lepomis cyanellus*) during the sampling conducted in May of 2023 had provided some evidence that centrarchids were present in the system. Another notable species observation was starry flounder (*Platichthys stellatus*; Figure 3), which was captured near the Highway 1 Bridge at alternate Station 8 (Figure 1). This species has not been observed in seine sampling since October of 2011, and its presence during the October 2023 sampling likely reflects the recent marine connectivity preceding this event. As in all previous fall sampling events since 2003, no *O. mykiss* were observed.

Figure 3. Starry flounder captured at the alternate Station 8.

Table 3. Cumulative and species-specific catch per unit effort (CPUE) across the 12 fall sampling events ranging from October 2002 to October 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	**Oct 2002**	**Oct 2003**	**Oct 2004**	**Oct 2005**	**Oct 2006**	**Oct 2008**	**Oct 2009**	**Oct 2010**	**Oct 2011**	**Oct 2012**	**Oct 2013**	**Oct 2020**	**Oct 2023***
		Total Seine Hauls	9	17	5	17	9	8	12	12	16	17	12	8	11
Family	**Common Name**	**Scientific Name**													
Clupeidae	Pacific herring	*Clupea pallasii*	0.70	0	6.60	62.90	0.30	194.10	4.40	41.60	56.40	0	0.20	0	2.73
	Threadfin shad	***Dorosoma petenense***	0.20	4.80	27.80	0	0	12.90	0	0	0	0	5.10	29.88	0
Cyprinidae	**Common carp**	***Cyprinus carpio***	0.10	0.30	12.60	3.60	0	0	0.10	0	0	0	0.20	0	0
	Goldfish	***Carassius auratus***	0	0	0	0	0	0	0	0	0	0	0	0.13	0
	Hitch	*Lavinia exilicauda*	30.40	67.60	180	36.70	0.10	20.30	8.50	6.10	0.80	0	0.60	4.13	0.09
	Sacramento blackfish	*Orthodon microlepidotus*	0	3.20	1.40	18.10	0.10	0.60	0	30.30	0	0	0.10	0	0
	Sacramento pikeminnow	*Ptychocheilus grandis*	0	0.10	0	0	0	0	0	0	0	0	0	0	0
Catostomidae	Sacramento sucker	*Catostomus occidentalis*	3.80	13.80	90	18.10	0	0.10	0.10	3.10	0	0	0.10	0.25	0
Osmeridae	Topsmelt	*Atherinops affinis*	0	0	7	0	44.60	10.40	11.20	12.70	21.30	0	0	0	87.55
Salmonidae	Chinook salmon	*Oncorhynchus tshawytscha*	0.10	0	0	0	0	0	0	0	0	0	0	0	0
	Steelhead	*Oncorhynchus mykiss*	0	0	0	0	0	0	0	0	0.10	0	0.10	0	0
Poeciliidae	**Western mosquitofish**	***Gambusia affinis***	0	0.10	640	6.10	0	0	0	0.90	0	0.40	0.10	0	0
Atherinidae	**Inland silverside**	***Menidia beryllina***	0	0	0	0	0	0	0	0	0	0	0	0.38	0.18
Cottidae	Pacific staghorn sculpin	*Leptocottus armatus*	0.60	0.40	1	0.80	0	1.50	0.90	0.50	0.20	0.10	0.30	0.13	0
	Prickly sculpin	*Cottus asper*	0.10	0.40	5.40	0.10	0	0.30	0.20	1.90	0.40	0.40	0.50	1.13	7.73
	Unidentified sculpin	*Cottidae*	0	0	0	0	0	0	0	0	1.70	0	0	0	0

Gasterosteidae	Threespine stickleback	*Gasterosteus aculeatus*	54.3	47	59.8	16.9	0	8.5	6.8	31.7	0.1	3.5	37.5	1	3.27
Embiotocidae	Shiner surfperch	*Cymatogaster aggregata*	0	0	0	0	0	4.50	0.60	0	0.70	0	0	0	0
Moronidae	Striped bass	*Morone saxatilis*	0	0	0	0.40	0	0	0.10	0	0.70	0.20	0.70	0.50	0
Centrarchidae	**Bluegill Sunfish**	***Lepomis macrochirus***	0	0	0	0	0	0	0	0	0	0	0	0	0.27
Gobiidae	**Arrow goby**	***Clevelandia ios***	0	0	0	0	0	0	0	0.10	0	0	0	0	0
	Tidewater goby	*Eucyclogobius newberryi*	0	0	0	0	0	0	0	0	0	0	0.20	0	0.45
	Yellowfin goby	***Acanthogobius flavimanus***	0	0	0	0	0	0	0	0	0	0	0.30	11.38	0.09
Pleuronectidae	Starry flounder	*Platichthys stellatus*	0.10	0	0	0.90	0	0.50	0.70	0.10	0.20	0	0	0	0.09
		Total CPUE	90.40	137.70	212.60	164.60	45.10	253.70	33.70	129	82.60	4.60	46	48.91	102.45
		Native Species	8	7	8	9	4	10	10	9	11	4	10	5	7
		Non-Native Species	2	3	3	2	0	1	2	2	0	1	4	5	3
		Number of Species	10	10	11	11	4	11	12	11	11	5	14	10	10

*Whereas in previous years a 100-foot beach seine was used for lagoon sampling, October 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-Weiner Diversity ranged from 0.29 to 1.08 across the seven sampling locations where multiple species of fish were captured in the seine hauls (no catch was observed in stations 1 and 7, and only single species were captured in stations 6 and 12). Similar to observations during the sampling in May of 2023, two of the highest values occurred near the Highway 1 bridge (stations 8 and 9; Table 4), and one of the further upstream stations (11) exhibited even higher diversity. 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. Stations where no fish were captured are indicated, and stations where only a single species was captured (sites 6 and 12) are assigned an H' value of 0.

Station	Shannon-Weiner Diversity (H')
1	No Catch
4	0.29
5	0.57
6	0
7	No Catch
8	1.04
Alternative 8	0.66
9	1.04
10	0.48
11	1.08
12	0

Environmental DNA

A total of four eDNA samples were collected as part of this monitoring effort (Figure 1; Table 5). Sample volumes ranged from 180 - 480 mL (average = 340 mL) due to variation in turbidity across sites that led to faster clogging of the filter in more turbid areas. Of the four collected eDNA samples, three (all but that collected at Station 2) contained sequences that could be assigned to known species based on available reference libraries. Assigned sequences belonged to six distinct fish species, including two species that were not detected in the seine samples (Table 5). There were sequences detected that were assigned to the Cottidae family (Stations 1 and 3) and Cyprinidae family (Station 3), but which could not be resolved to the genus or species level. 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 directions, whereas red cells indicate no detection. Non-native species are indicated by bolded common and family names. Species not detected in seine samples are highlighted in yellow.

Family	Common Name	Station 1	Station 2	Station 3	Station 4
Atherinopsidae	Jacksmelt	✓	X	X	X
Atherinopsidae	Topsmelt	✓	X	✓	X
Cyprinidae	Hitch	✓	X	X	X
Poeciliidae	**Western Mosquitofish**	X	X	X	✓
Gasterosteidae	Three-spined Stickleback	X	X	✓	X
Gobiidae	**Yellowfin Goby**	X	X	✓	X
	Total Species Detected	3	0	3	1

Water Quality Sampling

Water quality sampling during the seining survey revealed an expected gradient of decreasing salinity with increasing distance from the ocean. Salinities were far higher in the lower Lagoon than those observed during May sampling (2.32-5.30 parts per thousand [ppt] as compared to 0.37-3.84 ppt; Table 6). Conversely, dissolved oxygen (DO) concentrations were far lower than those observed in May and ranged from 4.26-8.50 mg/L compared to values exceeding 9 mg/L at all monitored locations in the spring. This decrease in DO is likely attributable to a decrease in algal photosynthesis, decay of algae and plant biomass, and decreased mixing of the water column due to lower freshwater inflow and loss of tidal action. These low DO conditions would likely be stressful for *O. mykiss*, although lagoon rearing individuals are known to be capable of behavioral modification and movement to areas with suitable environmental conditions (Bond et al. 2022).

Table 6. Summary of water quality parameters collected concurrently with beach seining on October 17 and 18, 2023.

Station	Temp (ºC)	Dissolved Oxygen (mg/l)	Salinity (ppt)	Secchi Depth (ft)
1	19.3	7.27	5.30	5
4	20.0	8.50	4.31	4
5	20.1	8.37	4.70	4
6	18.8	6.30	3.41	4
7	19.1	6.10	3.40	4
8	18.9	6.18	2.88	4

Alternate Station 8	19.4	4.26	2.82	5
9	21.8	6.75	2.32	4
10	19.9	4.89	2.56	4
11	20.4	7.83	2.52	4
12	20.4	4.99	2.48	4

Species Discussion

The sampling in October of 2023 is a snapshot of the fish community following a period of 261days of connectivity with Monterey Bay, which represents a much longer period than has occurred in recent years. This extended connectivity and the reclosure of the lagoon immediately prior to sampling has likely led to differences in the fish community observed during this sampling event, including apparently very high relative abundances. 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. However, the failure to capture any striped bass or detect any striped bass sequences in the collected eDNA samples may suggest that striped bass density was low during the October sampling. It is possible that falling water levels and impending lagoon closure prompted striped bass to emigrate from the lower river in the weeks preceding the sampling event.

Notable Species Detection – Bluegill Sunfish

The capture of a bluegill sunfish (*Lepomis macrochirus*) in Station 9 marks the first time this species has been observed in the lagoon since 2002. It is possible that high flows in the spring flushed bluegill from upstream tributaries and/or reservoirs down to the lagoon. Although the individual captured was a juvenile, larger bluegill have the potential to act as a predator of listed native species like steelhead and tidewater goby. Therefore, future monitoring data should be applied to evaluate the continued presence of this species. However, the dynamic nature of the lagoon may preclude long-term establishment of sunfish populations, as individuals belonging to *Lepomis* species such as bluegill and green sunfish have only been sporadically observed in the lower river.

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 25 surveys that occurred between 2002 and 2023 (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 or October sampling events in 2023, 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.

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 year.

Although hoop nets could not be deployed during October sampling due to high water levels, their capture of species not observed in the seine sampling when they were deployed in the spring highlights the value of including them when conditions allow. Sampling in 2023 has clearly demonstrated 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. 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

Bond, RM, Kiernan, JD, Osterback, AMK, Kern, CH, Hay, AE, Meko, JM, Daniels, ME, and Perez, JM. 2022. Spatiotemporal variability in environmental conditions influences the performance and behavior of juvenile steelhead in a coastal California lagoon. *Estuaries and Coasts*, *45*(6), 1749-1765.

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. 2023a. 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. 2023b. 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

[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.

[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 A – Seasonal CPUE Data

Table A1. 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	*Petromyzon tridentata*	0	0.1	0	0	0	0	0.1
Alosidae	**American shad**	***Alosa sapidissima***	0	0	0	0	0	0	0.1
Clupeidae	Pacific herring	*Clupea pallasii*	0	2.9	89.8	0	104.4	67.8	0
	Threadfin shad	***Dorosoma petenense***	0	0.1	0	0	0	0	0
Cyprinidae	**Common carp**	***Cyprinus carpio***	0	0.2	0	0	0	0	0
	Hitch	*Lavinia exilicauda*	8.3	11.8	6.5	0	0.1	0.1	2.1
	Sacramento blackfish	*Orthodon microlepidotus*	0.1	0.9	0	0	0	0	0
	Sacramento pikeminnow	*Ptychocheilus grandis*	0	0.10	0	0	0	0	0
Catostomidae	Sacramento sucker	*Catostomus occidentalis*	2.3	1.1	0.2	0	0	0	2.5
Osmeridae	Topsmelt	*Atherinops affinis*	0	0	0	0	1.1	2.3	0
	Steelhead	*Oncorhynchus mykiss*	0.1	0.1	0	0	0	0	0
Poeciliidae	**Western mosquitofish**	***Gambusia affinis***	0	0	0	0	0.1	0.7	0
Atherinidae	**Inland silverside**	***Menidia beryllina***	0	0	0	0	0.6	0	0
Cottidae	Pacific staghorn sculpin	*Leptocottus armatus*	5.3	0	15.9	0	15.5	18.8	0.5
	Prickly sculpin	*Cottus asper*	0.2	0.8	0	1.3	0.7	1.5	0.3
Gasterosteidae	Threespine stickleback	*Gasterosteus aculeatus*	0	6.6	1.9	10.4	0.3	48.5	0.1
Embiotocidae	Shiner surfperch	*Cymatogaster aggregata*	0.2	0.2	0	0	0	0	0
Moronidae	**Striped bass**	***Morone saxatilis***	0.3	2.4	0.6	0	0.1	0.1	0.7
Gobiidae	Tidewater goby	*Eucyclogobius newberryi*	0	0	0	7.3	0	0	0
	Yellowfin goby	***Acanthogobius flavimanus***	0	0	0.1	0	0.2	0.1	0
Scianidae	White croaker	*Genyonemus lineatus*	0	0	0	0	0.1	0	0

Paralichthyidae	Speckled sanddab	*Citharichthys stigmaeus*	0	0	0	0	0.1	0.1	0
Pleuronectidae	Starry flounder	*Platichthys stellatus*	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.

Table A2. 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	*Clupea pallasii*	35.70	0	0	0.80
	Pacific sardine	*Sardinops sagax*	0.10	0	0	0
Cyprinidae	**Common carp**	***Cyprinus carpio***	0.10	0	0	0.60
	Hitch	*Lavinia exilicauda*	134.10	4.10	16.20	4.50
	Sacramento blackfish	*Orthodon microlepidotus*	33.60	0	0.10	0.10
	Unidentified cyprinid	*Cyprinidae*	0.10	0	0	0
Catostomidae	Sacramento sucker	*Catostomus occidentalis*	45.90	0	2.60	1.10
Osmeridae	Topsmelt	*Atherinops affinis*	15.10	0	0	0
Salmonidae	Steelhead	*Oncorhynchus mykiss*	0	0.10	0	0
Poeciliidae	**Western mosquitofish**	***Gambusia affinis***	0	0.40	1.50	0.20
Cottidae	Pacific staghorn sculpin	*Leptocottus armatus*	33.30	0.60	0.90	0.80
	Prickly sculpin	*Cottus asper*	5.40	0.40	20	5.10
	Unidentified sculpin	*Cottidae*	0.30	0	0	0
Gasterosteidae	Threespine stickleback	*Gasterosteus aculeatus*	347.60	3.40	5.10	21.20
Embiotocidae	Shiner surfperch	*Cymatogaster aggregata*	13.40	0	0	0
Moronidae	**Striped bass**	***Morone saxatilis***	0.40	0	2.40	3.60
Centrarchidae	**Largemouth bass**	***Micropterus salmoides***	0	0	0	0.10
Gobiidae	**Yellowfin goby**	***Acanthogobius flavimanus***	0	0	0	4.60
	Unidentified goby	*Gobiidae*	0.50	0	0	0
Sebastidae	Unidentified rockfish	*Sebastes spp.*	0.20	0	0	0
Pleuronectidae	Starry flounder	*Platichthys stellatus*	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

Appendix B - Environmental DNA Metabarcoding Methodology

All protocols 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: GTCGGTAAAACTCGTGCCAGC
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 12.5ul 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 Exo1/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). 25µl of PCR amplicon is purified and normalize using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples are then pooled together by adding 5µl 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 pheniqs v2.1.0 [1], enforcing strict matching of sample barcode indices (i.e, no errors). Cutadapt v3.4 [2] was then used 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). Pheniqs 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.

Appendix C – 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, Ethan Switzer, Miguel Ibarra, Marinn Browne
2. **When the data was collected:** October 2023
3. **Where the data was collected:** Salinas River Lagoon
4. **How the data was collected (description of methods and protocols):** Surveys conducted by FISHBIO used a four-person crew with a 200 x 8-foot beach seine (1/4-inch mesh). No particular habitat type was preferentially targeted or favored for sampling; rather, approximately equidistant sampling locations were chosen to obtain an adequate overview of the spatial distribution of the fish community within the lagoon. During subsequent sampling events, initially selected locations were revisited. At each sampling location, one to two seine hauls were conducted. All fish captured during each survey, regardless of method, were identified to species, enumerated, and measured. 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 *O. mykiss* in the lower Salinas River and Lagoon as well as understanding the composition and relative abundance of the overall fish community. Objectives include evaluating presence or absence, condition, relative abundance (i.e., catch per unit effort; CPUE), and distribution of *O. mykiss* and other species 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 below.
7. **The terms of any landowner access agreement(s), if applicable:** Not Applicable
8. **References to any related Department permits or regulatory actions:** Not Applicable
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

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

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**
LIT	Light
MED	Medium
HVY	Heavy

Weather Code	**Description**
CLD	Cloudy
RAN	Rainy
CLR	Clear
NIT	Night

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**
CFG**N**	Natural Origin
CFG**H**	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
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

Appendix D – Invasive Species Prevention Plan

All field gear used in the Salians 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 Watershed 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.