Tidewater Goby Occupancy in the Central Monterey Bay Area: Insights from Environmental DNA Surveys

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Executive Summary

While tidewater goby (*Eucyclogobius newberryi*) have persisted in the lagoon since 2013, detailed information regarding the distribution of the species in the broader geographic area is lacking, including the Watsonville-, Bennet-, Elkhorn-, Moro Cojo- and Tembladero Slough complexes. The purpose of these surveys was to contribute to our understanding of tidewater goby population status, distribution, and ecology in the Salinas area. The presence of nearby populations is ecologically important for tidewater goby in the Salinas River Lagoon as numerous nearby populations are expected to increase the likelihood of recolonization in the event of localized extirpation, thus contributing to the stability, resilience and persistence of the metapopulation. As part of the Low Effect HCP, FISHBIO, in coordination with the Monterey County Water Resources Agency (MCWRA), ICF and the USFWS, developed a study plan for a spatially detailed hybrid survey approach that incorporates environmental DNA (eDNA) and traditional (seining) survey methods to assess tidewater goby distribution and life history characteristics in the Salinas River lagoon and nearby areas, the following goals.

(1) Assess broader tidewater goby distribution in the Monterey Bay area by evaluating occupancy and the presence of nearby potential source/refuge populations using eDNA sample collection.

(2) Provide a more detailed understanding of the size distribution and reproductive patterns of the species in the Salinas River lagoon, and a paired control for eDNA sample collection by seine sampling in the Salinas River Lagoon.

Several of the sampling locations covered in this study have not been previously sampled for tidewater goby, or tidewater goby have never before been detected at these sites. Sampling occurred twice in 2024, in June and October. Tidewater goby eDNA was detected in each geographic cluster (Watsonville, Bennet, Elkhorn, and Moro Cojo sloughs, as well as the Salinas River Lagoon/Old Salinas River) during each event, with exception of sites within the Tembladero Slough area.

These results indicate that tidewater goby are widely distributed in the broader geographic area of the lower Salinas River, including the Watsonville-, Bennet-, Elkhorn-, Moro Cojo- and Tembladero Slough complexes, suggesting that the regional metapopulation is likely relatively stable, and nearby source populations are available for recolonization of suitable habitats in the event of localized extirpation.

This survey also highlights the value of hybrid sampling approaches when feasible, as both methods – eDNA sampling and subsequent qPCR and seining – have imperfect, albeit undetermined, detection probabilities. Whenever possible, a hybrid sampling approach should be favored during future assessments of tidewater goby distribution.

Physical sampling (seining) for tidewater goby was performed under the USFWS' Ventura office subpermit (June sampling event) and under Permit ES98090C (October sampling event).

Introduction

The tidewater goby (*Eucyclogobius newberryi*) is a federally listed endangered species endemic to California (USFWS 1994). Tidewater goby are found in estuarine habitats that are relatively protected from the marine environment, occurring only in brackish environments along the California coast, from San Diego to Crescent City. The number of tidewater goby populations greatly declined during the late 20th century, likely due to heavy coastal development, and the species was listed as Endangered under the Endangered Species Act in 1994.

Tidewater goby habitats are typically separated from the Pacific Ocean by sandbars for most of the year, which effectively isolate populations and prevent fish from moving amongst existing populations or colonizing new habitats. Because migration between populations is rare, substantial genetic differences have developed among tidewater goby populations (e.g. McCraney et al. 2010). The tidewater goby, as a species, is thought to persist as a metapopulation, wherein individual subpopulations in relatively isolated habitats frequently experience extirpation (localized extinctions), to be recolonized during comparatively brief periods of connectivity (Lafferty et al. 1999a, Lafferty et al. 1999b). In the metapopulation model, sub-populations survive and/or remain viable through continual exchange of individuals, or recolonizations after extirpations. Extinction and recolonization rates are higher in the southern portion of the species' range (Lafferty et al. 1999a, Lafferty et al. 1999b), whereas sub-populations are more stable along California's North Coast (Kinziger et al. 2015).

Until their discovery during routine fish monitoring surveys in the Salinas River Lagoon (lagoon) in 2013, tidewater goby were last documented in the lagoon in 1951 (HES 2014). Prior surveys for tidewater goby in 1991, 1992, 2004, and 2010-2012 failed to document the species in the lagoon (USFWS 2013, Hagar Environmental Services [HES] 2012, HES 2013). Presumably, tidewater goby were extirpated from the lagoon due to levee construction and channelization (USFWS 2013). Observations in 2013, and again in 2014, likely represented a natural recolonization event for the species from nearby Bennett Slough or Moro Cojo Slough (HES 2014). Between 2013 and 2014, the tidewater goby population appeared to increase in abundance, and in 2014, tidewater goby were the second most abundant species sampled in the lagoon (only three species were detected; HES 2015). Routine fish monitoring surveys were not conducted by MCWRA (or its contractors) from 2015-2017, but the lagoon and adjacent habitats (Old Salinas River) were sampled by Dr. Brenton Spies in 2014, 2015, and 2017, resulting in the documentation of tidewater gobies during each visit. With the exception of 2019 (no known surveys), periodic (at least annual) sampling in the Salinas River Lagoon since 2018 has confirmed the presence of tidewater goby each year up to and including 2024.

While tidewater goby have persisted in the lagoon since 2013, detailed information regarding the distribution of the species in the broader geographic area is lacking, including the Watsonville Slough, Bennet Slough, Elkhorn Slough, Moro Cojo and Tembladero Slough complexes. Numerous nearby populations are expected to increase the likelihood of recolonization in the event of localized extirpation, thus contributing to the stability, resilience and persistence of the metapopulation.

Tidewater goby habitats are often difficult to sample using traditional techniques such as seining, which requires water sufficiently shallow for biologists to walk, substrate sufficiently firm for the

sampling crew to be mobile, and the habitat to be relatively clear of obstruction (such as rocks, wood, or vegetation) to permit efficient operation of the seine net. Many locations inhabited, or potentially occupied by tidewater goby, therefore present major challenges to the reliable assessment of tidewater goby distribution.

Due to these challenges, eDNA monitoring approaches have been developed as a non-invasive alternative to traditional field methods, primarily because of the difficulty of seining in sloughs and estuaries along California's coast. Also, tidewater goby populations vary considerably in abundance from year to year and eDNA techniques may provide an efficient means of detection even at low abundance (Schmelzle and Kinziger 2016). This sampling approach involves collecting and filtering water samples from an area of interest to search for minute fragments of DNA that animals shed into their environment (Schmelzle and Kinziger 2016). The presence of tidewater goby can then be detected through subsequent laboratory analysis using species-specific genetic primers can then detect target DNA, indicating habitat occupancy by tidewater goby.

To contribute to our understanding of tidewater goby (*Eucyclogobius newberryi*) population status, distribution, and ecology in the Salinas area, FISHBIO, in coordination with the Monterey County Water Resources Agency (MCWRA), ICF and the USFWS, developed a study plan for a spatially detailed hybrid survey approach that incorporated environmental DNA (eDNA) and traditional (seining) survey methods to assess tidewater goby distribution and life history characteristics in the Salinas River lagoon and nearby areas. The study consisted of two distinct, but related, tasks:

(1) Assess broader tidewater goby distribution in the Monterey Bay area by evaluating occupancy and the presence of nearby potential source/refuge populations using a spatially detailed eDNA sample collection at numerous locations with hydrologic connectivity to the lower Salinas basin, and

(2) Provide a more detailed understanding of the distribution and reproductive patterns of the species in the Salinas River lagoon and a paired control for eDNA sample collection by seine sampling in the Salinas River Lagoon to assess distribution, calculate hatch times, and identify peak reproductive periods.

The study was implemented in 2024, consisting of two multi-day sampling events in June and October.

Methods

eDNA Sampling Site Selection

Locations for eDNA sample collection were initially selected based on a desktop review of satellite imagery. Area were identified based on the following criteria: 1) hydrologic connectivity to the Salinas River Lagoon, 2) sheltered from full tidal influence, and 3) (relatively) accessible. Selected locations were reviewed and visited by MCWRA staff to provide information regarding access restrictions and current conditions, ultimately resulting in 39 distinct locations for eDNA sampling. While a small number of the selected locations had been surveyed in the past, most had never been assessed for the presence of tidewater goby. Small discrepancies in repeat sampling locations were attributable to access restrictions (e.g. sensitive wildlife [seals] at Elkhorn Slough East of Moss Landing Harbor) or changed conditions (e.g. Moro Cojo at Castroville Blvd. was dry during the fall sampling event).

eDNA Field Sampling

Upon arrival at a location, geographic coordinates, date, time, water temperature (°C), salinity (parts per thousand; ‰), dissolved oxygen (mg/L), total dissolved solids (mg/l), substrate (gravel, sand, etc.), notes on aquatic vegetation, and any other notable observations were recorded.

Water samples were collected from each location using a Smith-Root ANDe™ system (Thomas et al. 2018; Figure 1) at a target flow rate of 0.5 liters/minute, using a continuous sampling approach (the pump maintains a constant flow rate) to maximize the likelihood of species detection. At all sites, sample collection was conducted from shore using a telescoping pole extension to reduce the risk of contamination between sites (Laramie et al. 2015). Water was filtered until the flow rate slowed to less than 0.1 l/min, resulting in variable volumes filtered at the different sampling locations.

Disturbance of sediment during collection was minimized to the extent possible, as water samples that include sediment can lead to difficulty in filtration (Laramie et al. 2015) and reaction inhibition during qPCR (Eichmiller et al. 2014). Also, degradation rates of DNA in sediment are slower than in the water column and DNA in sediment may be detected months after species absence (Barnes et al. 2014). Additionally, downstream locations were sampled before upstream locations.

Water was filtered from near the surface, as this approach has been shown to improve eDNA detection (Moyer et al. 2014), and even rare benthic species can be more frequently detected from water samples collected near the surface (Balasingham et al. 2018). Furthermore, increased filtration volume compared to benthic samples is possible during transect sampling in the upper water column (decreased likelihood of filter fully clogging).

Single-use, self-desiccating eDNA concentrating filter cartridges (Smith-Root, 47mm diameter, 5µm pore size,) were used for each individual sampling event and location (on rare occasions, high turbidity warranted the sequential use of multiple filters to sample the desired volume of water). Once filtration was complete, the cartridge was carefully removed and immediately inserted into an individual sterile storage pouch for preservation and storage. The pouch was labeled with the

sample number, date, location of sample collection, and volume filtered, and stored in a cool, dark place until genetic analysis.

Preserved filters were sent to Aquatrace Genomics (McKinleyville, CA) for DNA extraction and processing following the completion of each sampling event.

Figure 1. Filtering water for tidewater goby environmental DNA on Elkhorn Slough (near Pick-n-Pull, June 6, 2024).

eDNA Laboratory Analysis

Laboratory processing of samples was performed by Aquatrace Genomics. In the laboratory, procedures for DNA extraction, amplification and analysis followed the methods described in Sutter and Kinziger (2019).

eDNA extraction

In brief, all eDNA extractions were conducted in a dedicated laboratory that is not used for high copy number samples (i.e. analysis of DNA lysates from tissue extractions). Workstations were treated with UV light before each use. Bench spaces, pipettes, centrifuges, and racks were wiped with RNase AWAYTM or 20% bleach before and after each extraction. The eDNA was extracted from filters using a QIAGEN DNeasy Blood and Tissue Kit following the manufacturer's instructions, except that 4µl of RNase was added to the lysate after overnight incubation and QIAGEN's QIAshredder was used for lysate homogenization. Lysis buffer ATL volume was increased to 360µl and proteinase K to 40µl to allow for complete filter submersion (Schmelzle and Kinziger 2016). For the final elution step, only 100µl of Buffer AE was used to increase the final DNA concentration in the elute. All extractions were completed within two weeks of field sample collection.

qPCR

Each qPCR reaction included 5 µl of template DNA, 12.5 µl TaqMan™ Environmental Master Mix 2.0 (Applied Biosystems, 4396838), NC10 probe (200 nM), NC10 forward primer (400 nM), NC10 reverse primer (400 nM); and nuclease-free water sufficient to bring the reaction volume to 25 µl. TaqMan™ Environmental Master Mix 2.0 was used to reduce effects of inhibition in qPCR. Quantitative PCR reactions were performed on an Applied Biosystems QuantStudio™ 6 Real-Time PCR System. Cycling conditions included an initial incubation at 95°C for 10 minutes, followed by 45 cycles of 15 seconds at 95°C and 60 seconds at 60°C. qPCR values are reported in Cycle Thresholds (Ct), or the number of cycles it takes for a reaction to reach the fluorescent threshold and is inverse to the total concentration. A negative control was included (containing all reagents except DNA template was replaced with DNA free water).

Standard Curve, LOD, and LOQ

To quantify the concentration of target DNA in each qPCR reaction, a standard curve was established. The template standard utilized in constructing the standard curve comprised synthetic DNA gBlocks™ Gene Fragments, created by Integrated DNA Technologies, which incorporated sequences from two species loci (steelhead and tidewater goby) concatenated into a single fragment. The process of constructing the standard curve involved an eight-fold serial dilution spanning a range of 0.21 (10^{-12}) to 2,050,491 (10^{-5}) copies per reaction. Within each standard concentration, there were 8 to 16 replicates.

The statistical analysis was conducted using a customized script within the statistical software R.

Physical Sampling

Tidewater goby collection surveys used a two-person crew with a 15 x 4 ft. beach seine (⅛ inch mesh size). Numerous locations were sampled throughout the Salinas lagoon, distributed from near the sandbar/breach location to the Highway 1 Bridge, as well as in the Old Salinas River (OSR) directly behind the slide gate. The locations for sampling have generally remained consistent since FISHBIO began implementing tidewater goby surveys in 2018. Initially, no habitat type was targeted or favored for sampling; rather, approximately equidistant sampling locations were sampled to obtain an adequate overview of the spatial distribution of gobies within

the lagoon. During subsequent sampling events, initially selected locations were revisited. Areas for sampling by beach seine were generally limited by water depth as most sites along the southern shore of the lagoon quickly become too deep for effective seining. Also, areas where substrate is too soft for effective sampling and areas where debris prohibits effective seining could not be sampled

All targeted sampling for tidewater goby was conducted following protocols developed by the United States Fish and Wildlife Service (USFWS 2005, Appendix F). At each sampling location, one to two seine hauls were conducted, depending on the available area that could be sampled efficiently (multiple seine hauls are only possible where sufficient area with adequate depth was available for sampling, the areas sampled by sequential seine hauls did not overlap). After each seine haul, all captured fish are transferred from the seine to an aerated, temporary holding bucket. Once all fish are removed from the seine, they were identified to species level, enumerated and measured.

Following identification and measurement, all sampled fish, including tidewater goby, were released at the site of capture.

Measurements of tidewater gobies were used to evaluate the reproductive period of the species in the Salinas River Lagoon. As growth in fishes is generally continuous and indefinite, differences in size – or range in individual sizes encountered in a population at a particular point in time – can be used as an approximation of the temporal extent of their reproductive period, particularly in small, short-lived species such as the tidewater goby (Hellmair & Kinziger 2014).

Data Analysis

Von Bertalanffy growth parameters (k and t_0) estimated for a northern California population of tidewater goby (Big Lagoon, Humboldt Co.; Hellmair & Kinziger 2014) were used to derive approximate daily ages for tidewater goby, according to the following formula:

$$L_t = L_\infty * (1 - e^{-k*(t-t_0)}),$$

where L_t is the length at time of capture, L_∞ is 94.18 (the theoretical maximum size for the species), k is 0.67, t_0 is -0.11 and t is the age, in years.

This can be rearranged as

$$t_{days} = \left(\frac{ln\ ln\ \left(1 - \frac{L_t}{L_\infty}\right)}{-k} + t_0 \right) * 365$$

to estimate the daily age of an individual tidewater goby of a known total length (TL) L_t. For example, a fish measuring 25 mm (TL) is estimated to be 128 days old.

Results

eDNA Survey

Spring Summary

During the spring survey, tidewater goby eDNA was detected at ten of 35 locations (Table 1; Figure 2). Environmental conditions varied greatly among sampling locations. Water temperature where samples were collected ranged from 16.9°C to 31.5°C overall, and from 17.1°C to 31.2°C in locations where tidewater goby eDNA was detected (Table 2). Dissolved oxygen ranged from 3.3 mg/l to 27.2 mg/l overall, and from 5.4 mg/l to 23.2 mg/l in locations where tidewater goby eDNA was detected (Table 2). Salinity ranged from 0.01 ppt to 52.66 ppt overall, and from 3.72 ppt to 41.18 ppt in locations where tidewater goby eDNA was detected (Table 2).

It should be noted that the environmental parameters are not necessarily indicative of conditions where tidewater goby reside, as eDNA can be transported by currents (for example, water with suspended eDNA could have flowed into shallow areas where it warmed to over 30°C prior to sampling). Similarly, although eDNA of tidewater goby was found in a sample filtered from water with a salinity of 41.18 ppt, this is not necessarily indicative of direct occupancy of tidewater goby at this exact location at the time of sample collection.

The strongest evidence (positive detection in more than 50% of replicates) of tidewater goby was found in Watsonville Slough at W Beach Road, Bennet Slough E of Hwy 1, Elkhorn Slough at Swiss Canyon, Moro Cojo Slough at Moss Landing, and the Old Salinas River near the slide gate and Portrero Road. Notably, tidewater goby eDNA was not detected at any of the sampled locations in the Salinas River lagoon, despite confirmed physical presence (captured during seining surveys, see below).

Fall Summary

During the fall survey, tidewater goby eDNA was detected at ten of 37 locations (Table 1; Figure 2). Environmental conditions varied greatly among sampling locations. Water temperature where samples were collected ranged from 7.0°C to 22.5°C overall, and from 15.7°C to 22.5°C in locations where tidewater goby eDNA was detected (Table 2). Dissolved oxygen ranged from 0.89 mg/l to 10.56 mg/l overall, and from 0.95 mg/l to 10.56 mg/l in locations where tidewater goby eDNA was detected (Table 2). Salinity ranged from 0.47 ppt to 59.83 ppt overall, and from 0.57 ppt to 56.37 ppt in locations where tidewater goby eDNA was detected (Table 2).

Again, the environmental parameters at the time and location of water filtration are not necessarily indicative of tidewater goby environmental tolerances.

The strongest evidence of tidewater goby was found in Bennet Slough, Elkhorn Slough at Swiss Canyon, Elkhorn Slough East of Kirby Park and Elkhorn Slough at Blohm Road. There were multiple locations where tidewater goby eDNA was detected in both spring and fall, including Watsonville Slough, Bennet Slough, Elkhorn Slough (at Swiss Canyon and East of Elkhorn Road), and Moro Cojo Slough near Moss Landing. Tidewater goby eDNA was also detected in the Salinas River Lagoon, despite no physical capture during seining surveys.

Table 1. Sample locations with their coordinates and the counts of qPCR detections for both Fall and Spring sampling efforts. Detection ratio refers to the fraction of analysis replicates with positive detection of tidewater goby.

General Area	Location	Latitude	Longitude	Spring Detections	Fall Detections
Pajaro Watershed	Watsonville Slough at Shell Rd.	36.8714	-121.8183	0/3	2/6
	Watsonville Slough at W. Beach Rd.	36.8682	-121.8173	2/3	0/6
	Pajaro River at Thurwatcher Road	36.8802	-121.7932	NA	0/6
	Pajaro River Estuary	36.8457	-121.8048	0/3	0/6
Bennet Slough	Bennet Slough N. of HWY 1	36.8263	-121.7760	1/3	3/3
	Bennet Slough E. of HWY 1	36.8246	-121.7772	3/6	3/3
	Bennet Slough W. of HWY 1	36.8204	-121.7861	1/3	0/3
	HWY 1 Pond S. of Bennet Slough	36.8157	121.7856	0/3	0/3
Elkhorn Slough	Elkhorn Slough at Jetty Rd. Crossing	36.8169	-121.7875	0/3	0/6
	Elkhorn Slough East of Moss Landing North Harbor	36.8134	-121.7896	0/3	0/3
	Elkhorn Slough @ Pick-n-Pull	36.8126	-121.7451	0/3	0/6
	Elkhorn Reserve S. of Foundation (on boardwalk)	36.8131	-121.7309	0/6	1/6
	Elkhorn Reserve @ S. Marsh Boardwalk	36.8193	-121.7383	0/6	0/6
	Elkhorn Reserve @ S. Marsh Land Bridge	36.8200	-121.7371	0/3	0/6
	Elkhorn Slough @ Swiss Canyon	36.8300	-121.7329	3/3	6/6
	Elkhorn Slough @ Kirby Park	36.8402	-121.7421	0/3	0/3
	Elkhorn Slough E. of Kirby Park	36.8402	-121.7421	0/3	3/3
	Elkhorn Slough E. of Elkhorn Rd.	36.8563	-121.7548	1/3	1/3
	Elkhorn Slough @ Blohm Rd.	36.8601	-121.7404	0/3	3/3
Moro Cojo	Moro Cojo @ Moss Landing (N. end)	36.7996	-121.7842	0/3	NA
	Moro Cojo @ Moss Landing (S. end)	36.7964	-121.7836	2/3	1/3
	Moro Cojo E. of HWY 1	36.7963	-121.7832	2/6	0/6
	Moro Cojo Slough Downstream of Railroad	36.7892	-121.7535	NA	2/3
	Moro Cojo at Castroville Blvd.	36.7777	-121.7379	0/3	NA (dry)
	South Branch Moro Cojo at Watsonville Road	36.7834	-121.7667	NA	0/3

General Area	Location	Latitude	Longitude	Spring Detections	Fall Detections
Morro Cojo cont'd	Ponds @ Watsonville Rd.	36.7839	-121.7802	0/3	0/6
	Moro Cojo Pond South of Hwy 156	36.7712	-121.7296	NA	0/3
Tembladero Slough	Tembladero Slough @HWY 1	36.7679	-121.7653	0/3	0/6
	Tembladero Slough @ Rec. Trail (Haro St.)	36.7594	-121.7543	0/3	0/3
	Tembladero Slough @ HWY 183	36.7401	-121.7389	0/3	0/3
	East Branch Templadero Slough at Hwy 183	36.7401	-121.7390	NA	0/3
	Tembladero Slough @San Jon Rd.	36.7049	-121.7050	0/3	0/3
Salinas and OSR	Salinas River Lagoon (N. side of beach)	36.7476	-121.8030	0/3	0/6
	Salinas River Lagoon @HWY 1	36.7321	-121.7833	0/6	2/6
	Salinas River Lagoon (near OSR)	36.7498	-121.8015	0/3	0/3
	OSR Near Slide Gate	36.7501	-121.8011	3/3	0/3
	OSR at Monterey Dunes Way	36.7718	-121.7898	0/3	0/6
	OSR at Portrero Rd.	36.7906	-121.7907	2/3	0/6
	Blanco Drain	36.7081	-121.7466	0/3	0/3

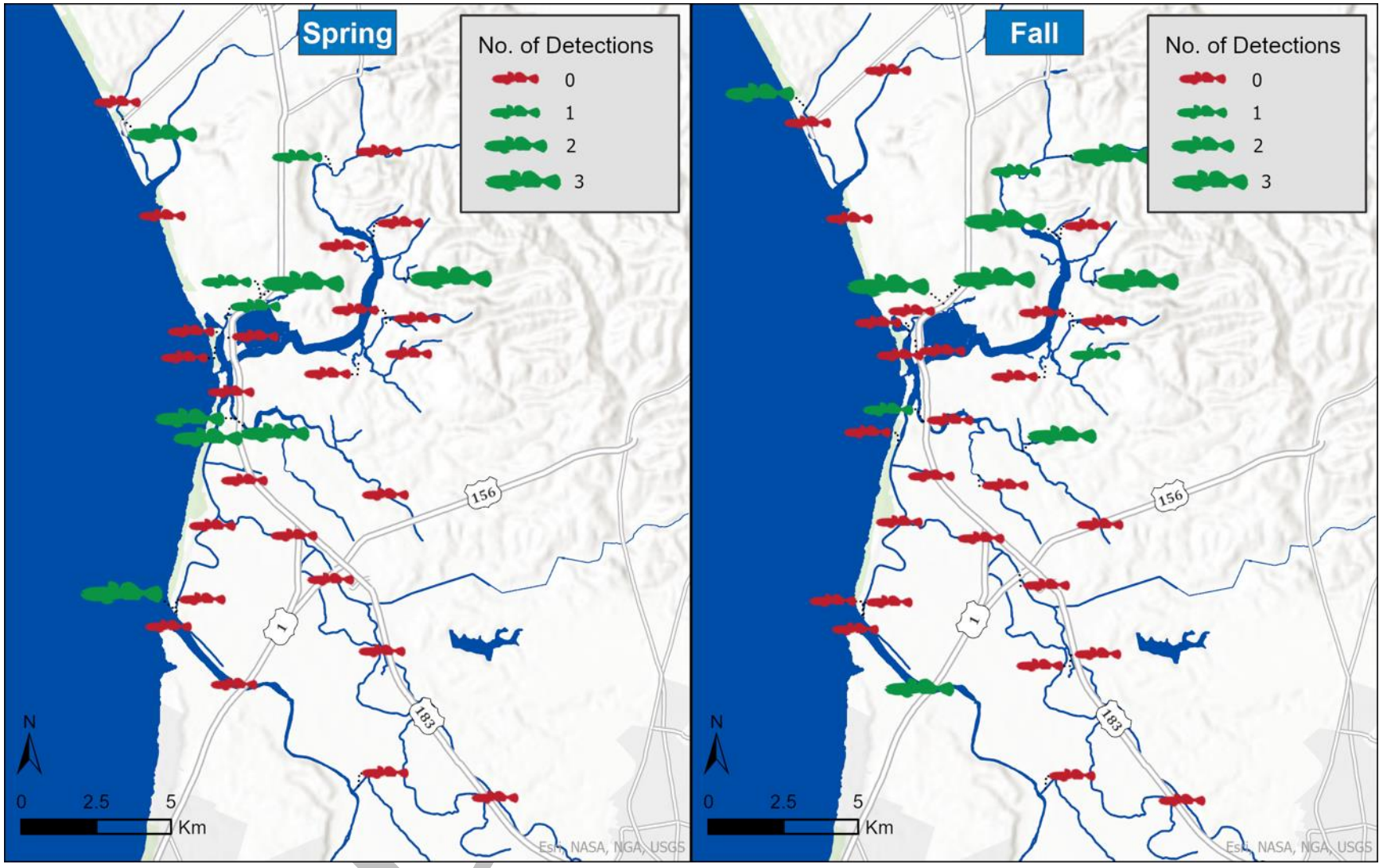

Figure 2. Map of tidewater goby detections resulting from eDNA samples collected in Spring (June) and Fall (October) of 2024. The size of the symbol indicates the fraction of positive samples in analysis replicates (⅓, ⅔, or all replicas with positive detections). Sampling locations and detection frequencies are also summarized in Table 1.

Table 2. Locations where eDNA was sampled to assess tidewater goby distribution, including environmental parameters measured and volume of water filtered. Coordinates for each location are provided in Table 1. Parameter measurements in green indicate events/locations where tidewater goby eDNA was detected.

General Area	Location	Temp. (°C)		D.O. (mg/l)		Salinity (ppt)		Volume filtered (# filters)	
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Pajaro Watershed	Watsonville Slough at Shell Rd.	31.5	17.8	7.1	5.18	8.92	22.42	1.00 (1)	4.80 (2)
	Watsonville Slough at W. Beach Rd.	29.8	17.5	5.9	5.28	6.48	22.08	1.00 (1)	0.35 (2)
	Pajaro River at Thurwatcher Road	-	18.1	-	4.98	-	25.48	-	6.91 (2)
	Pajaro River Estuary	23	17.3	7.8	8.18	0.33	0.47	3.00 (1)	0.93 (2)
Bennet Slough	Bennet Slough N. of HWY 1	31.2	19.0	16.2	8.48	9.5	56.37	1.00 (0)	1.27 (1)
	Bennet Slough E. of HWY 1	23.9	20.1	10.1	7.05	20.46	47.76	2.10 (2)	1.95 (1)
	Bennet Slough W. of HWY 1	23.4	19.6	11.3	1.72	13.45	34.37	1.00 (1)	1.95 (1)
	HWY 1 Pond S. of Bennet Slough	26.9	22.4	7.3	7.61	44.53	59.83	1.00 (0)	1.10 (1)
Elkhorn Slough	Elkhorn Slough at Jetty Rd. Crossing	23	17.6	9.1	9.08	32.44	33.75	0.6 (1)	1.85 (2)
	Elkhorn Slough East of Moss Landing North Harbor	17.9	16.5	7.5	7.42	32.45	32.47	1.00 (1)	1.58 (1)
	Elkhorn Slough @ Pick-n-Pull	27.5	16.5	8.1	6.68	11.7	34.37	1.29 (1)	3.56 (2)
	Elkhorn Reserve S. of Foundation (on boardwalk)	21.2	14.9	12.5	5.87	36	34.42	0.48 (2)	4.26 (2)
	Elkhorn Reserve @ S. Marsh Boardwalk	19.6	14.9	10.0	6.11	34.24	34.27	1.86 (1)	4.75 (2)
	Elkhorn Reserve @ S. Marsh Land Bridge	20	14.9	6.2	5.94	34.14	34.27	1.62 (1)	6.09 (2)
	Elkhorn Slough @ Swiss Canyon	27.7	17.6	5.4	5.53	41.18	46.32	2.00 (1)	6.28 (2)
	Elkhorn Slough @ Kirby Park	23.1	16.6	12.3	5.86	34.67	34.45	2.00 (1)	2.35 (1)
	Elkhorn Slough E. of Kirby Park	26.8	20.4	7.0	0.95	52.66	40.62	0.43 (1)	0.58 (1)
	Elkhorn Slough E. of Elkhorn Rd.	26.1	18.1	23.2	4.81	6.7	35.66	1.02 (1)	3.32 (1)
	Elkhorn Slough @ Blohm Rd.	20.9	15.7	3.3	3.80	1.59	11.79	0.87 (1)	0.61 (1)
Moro Cojo	Moro Cojo @ Moss Landing (N. end)	20.0	-	15.7	-	31.65	-	1.50 (1)	-
	Moro Cojo @ Moss Landing (S. end)	20.8	16.4	15.5	7.18	31.56	32.38	1.00 (1)	3.12 (1)
	Moro Cojo E. of HWY 1	19.8	15.6	8.3	5.63	29.04	32.04	1.08 (2)	6.63 (2)
	Moro Cojo Slough Downstream of Railroad	-	22.5	-	10.56	-	39.68	-	0.89 (1)
	Moro Cojo at Castroville Blvd.	23.1	dry	5.8	dry	5.32	dry	1.25 (1)	-
	South Branch Moro Cojo at Watsonville Road	-	9.6	-	5.66	-	2.00	-	0.11 (1)
	Ponds @ Watsonville Rd.	18.9	7.0	8.4	0.89	1.15	5.15	0.13 (1)	0.08 (1)
	Moro Cojo Pond South of Hwy 156	-	12.4	-	3.68	-	0.57	-	1.24 (1)
Tembladero Slough	Tembladero Slough @HWY 1	18.8	11.9	3.8	7.51	1.35	0.96	0.18 (1)	0.42 (2)
	Tembladero Slough @ Rec. Trail (Haro St.)	18.8	19.4	5.59	9.68	1.31	1.14	0.10 (1)	0.21 (2)

General Area	Location	Temp. (°C)		D.O. (mg/l)		Salinity (ppt)		Volume filtered (# filters)	
		Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Tembladero Slough cont'd	Tembladero Slough @ HWY 183	25.2	17.4	27.2	8.62	1.29	0.98	0.21 (1)	0.14 (1)
	East Branch Tembladero Slough at Hwy 183	-	18.1	-	6.37	-	0.48	-	0.81 (1)
	Tembladero Slough @San Jon Rd.	22.1	18.1	8.5	5.80	0.01	0.63	0.35 (1)	0.37 (1)
Salinas and OSR	Salinas River Lagoon (N. side of beach)	18.8	15.5	9.0	8.66	3.92	1.23	3.00 (1)	5.44 (2)
	Salinas River Lagoon @HWY 1	20.9	16.8	8.2	6.69	3.61	0.57	3.29 (2)	4.11 (2)
	Salinas River Lagoon (near OSR)	18.1	15.1	9.0	6.18	3.89	1.22	2.00 (1)	2.58 (1)
	OSR Near Slide Gate	17.1	15.2	13.5	6.50	19.37	1.23	0.17 (1)	2.24 (1)
	OSR at Monterey Dunes Way	16.9	14.6	8.3	6.61	10.16	2.32	0.94 (1)	0.86 (2)
	OSR at Portrero Rd.	24.5	16.4	10.7	6.07	3.72	2.45	0.31 (1)	0.69 (2)
	Blanco Drain	21.9	14.9	16.4	7.64	1.41	1.49	0.34 (1)	0.08 (1)

Table 3. Pairwise (aquatic) distances, in kilometers, between eDNA collection locations where tidewater goby eDNA was detected in 2024. Refer to Tables 1 and 2 for a complete list of sampling locations and detection records.

Location	Portrero Rd.	Slide Gate	Salinas Lagoon @HWY 1	Moro Cojo @ Railroad	Moro Cojo @ HWY 1	Moro Cojo @ Moss	Blohm Rd.	Elkhorn Rd.	Kirby Park	Swiss Canyon	Elkhorn Reserve S	Bennet Slough W	Bennet Slough E	Bennet Slough N	W. Beach Rd
Shell Rd	10	16	19	13	9.5	9.5	19.5	18	15.5	15.5	15	9.5	10	11.5	<0.5
W. Beach Rd	10.5	16.5	19	13.5	10	10	20	18.5	16	16	15.5	10	10.5	12	
Bennet Slough N	5.5	11.5	14.5	8.5	5	5	15	13.5	10.5	10.5	10	2	1.5		
Bennet Slough E	4	10	13	7	3.5	3.5	13.5	12	9	9	8.5	0.5			
Bennet Slough W	3.5	9.5	12.5	6.5	3	3	12.5	11	8.5	8.5	8				
Elkhorn Reserve S	9	15	17.5	12	8.5	8.5	10.5	6	6	6					
Swiss Canyon	9.5	16	18.5	12.5	8.5	8.5	6.5	5	1.5						
Kirby Park	9.5	15.5	18	5	8.5	8.5	6.5	5							
Elkhorn Rd.	12	18	21	15	11.5	11.5	1.5								
Blohm Rd.	13.5	19.5	22.5	16.5	13	13									
Moro Cojo @ Moss	2	8	11	3.5	<0.5										
Moro Cojo Hwy 1	2	8.5	11	3.5											
Moro Cojo at Railroad	5.5	11.5	14.5												
Salinas Lagoon @ HWY 1	9	2.5													
Slide Gate	6														

Seining

The intention for this physical sampling effort was to assess the distribution of tidewater goby throughout the lagoon (as monitored twice annually for the past several years), and to capture and measure a relatively large number of tidewater goby (n > 100) to document when juvenile fish appear in the system, their relative abundance compared to larger fish, and permit back-calculation of reproductive timing. This, in turn, would allow an estimate of the distribution of reproductive activity throughout the year and serve to identify the periods when the population is most resilient (or, conversely, most susceptible) to drastic environmental fluctuation (i.e. breaching). However, despite flexibility in choosing sampling locations within the lagoon, sampling during seasons of highest expected abundance, and two sampling events to reach the desired sample number, we were unable to obtain the target sample number despite devoting significant effort.

Spring Summary

Seining was conducted in nine general areas in the Old Salinas River (OSR) and the Salinas River Lagoon on June 6, 2024 (Table 4). Locations for sampling were again dictated by access opportunity, and seining occurred in the same general areas that have been targeted for sampling since 2018. Seine hauls were performed wherever the site characteristics permitted, sometimes with two consecutive hauls in the same general sampling area. Salinity varied substantially across the different areas of the lagoon, ranging from 3.61 parts per thousand (ppt) in the upstream reaches to nearly 19 ppt near the mouth of the lagoon adjacent to the Salinas National Wildlife Refuge. Widgeongrass (*Ruppia* sp.), often associated with tidewater goby, was notably absent throughout the lagoon and was only observed near the slide gate.

Table 4. Summary of sampling locations, effort and catch in the Salinas River Lagoon on June 6, 2024.

Location	Coordinates	Temp. (ºC)	Salinity (ppt)	TWG Catch	Other species (n)	*Ruppia* Presence
Slide Gate	36.749704; -121.801128	18.1	3.89	5	Pac. staghorn sculpin (35), Speckled sanddab (4), Threespine stickleback (2)	Present
OSR	36.750070; -121.801114	17.1	19.37	10	Threespine stickleback (7), Mosquitofish (17)	Absent
Breach Site	36.750506; -121.803841	19.0	4.96	2	Speckled sanddab (7), Pac. staghorn sculpin (9)	Absent
South Berm	36.747189; -121.803235	20.8	14.3	2	Pac. staghorn sculpin (2)	Absent
NWR (downstream 1)	36.746134; -121.802480	20.1	18.56	0	Threespine stickleback (1), Speckled sanddab (4), Pac. staghorn sculpin (6)	Absent
NWR (downstream 2)	36.742671; -121.799886	21.8	13.71	0	Arrow goby (1), Speckled sanddab (3), Pac. staghorn sculpin (2)	Absent
NWR (upstream 1)	36.741381; -121.798735	22.4	7.78	0	Yellowfin goby (1), Inland silverside (5)	Absent
NWR (upstream 2)	36.739192; -121.795568	23.4	6.90	0	Threespine stickleback (3), Pac. staghorn sculpin (2), Prickly sculpin (1)	Absent
HWY 1	36.731993; -121.783053	20.9	3.61	0	Hitch (44), Prickly sculpin (2), Sacramento sucker (13), Pikeminnow (2), Threespine stickleback (11)	Absent

Overall, 19 tidewater goby were captured, all in the lower lagoon and OSR, ranging from 35 mm to 55 mm in total length (TL; Figure 3). Using the above-described age-length relationship, this corresponds to estimated individual ages ranging from 213 to 438 days, suggesting a prolonged reproductive period (225 days). In other words, tidewater goby sampled during this event hatched between March 26 and November 6, 2023.

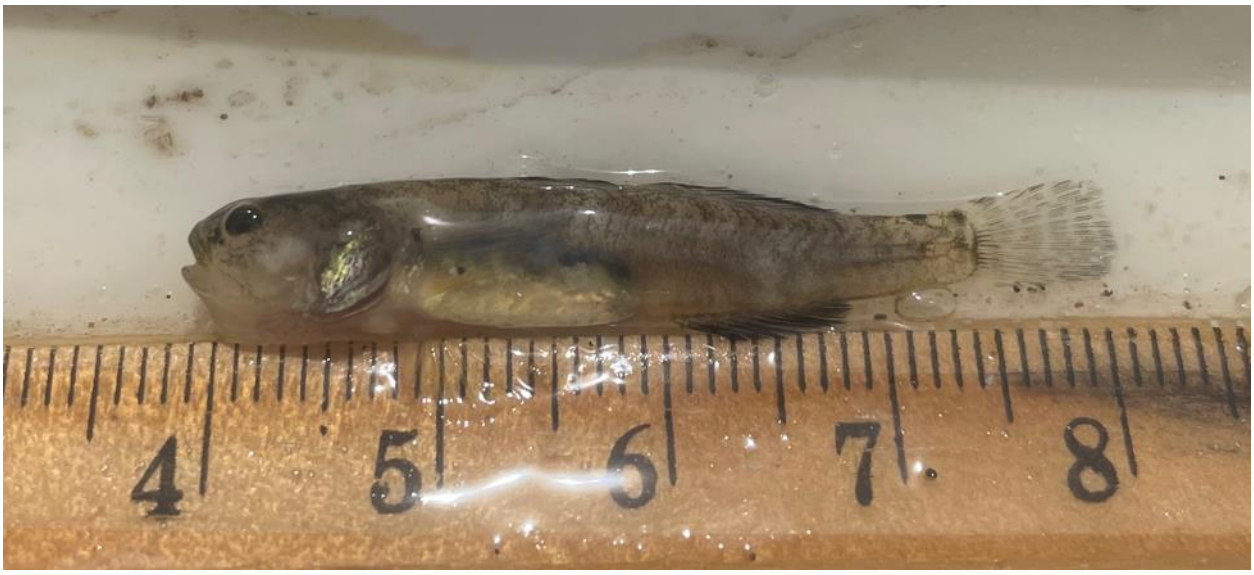

Figure 3. Tidewater goby (42 mm TL) captured near the slide gate in the Salinas River lagoon on June 6, 2024.

Notably, an arrow goby (*Clevelandia ios*; 32 mm TL) was captured in the Salinas River lagoon for the first time since the inception of targeted tidewater goby surveys in 2018. It has been noted that arrow gobies can be found in large numbers in the Salinas River Lagoon, particularly while the sandbar is breached and for some time thereafter (B. Spies, pers. comm.). However, the species rarely co-occurs with tidewater gobies for extended periods of time. Both species have a salinity tolerance of 0-55 ppt (based on laboratory trials), yet tidewater gobies appear to prefer salinities below 15 ppt, and arrow gobies prefer those greater than 15 ppt (Capelli 1997, as cited in Dawson et al. 2002).

Fall Summary

For the first time since FISHBIO implemented tidewater goby occupancy surveys in 2018, no tidewater goby were captured during the fall seining survey in the Salinas River Lagoon or the OSR (Figure 4; Table 5). However, despite a lack of physical capture, detections of tidewater goby environmental DNA suggest population persistence in this habitat, but likely in low abundances at the time of sampling.

Fish diversity was low overall, with inland silverside and threespine stickleback comprising the majority of the catch. Salinity was very low at all sampling locations, decreasing from about 1.2 ppt near the sandbar to 0.7 ppt at the Highway 1 bridge. Ruppia was present only in the OSR.

Table 5. Summary of sampling locations, effort and catch in the Salinas River Lagoon, October 28-29, 2024.

Location	Coordinates	Temp. (ºC)	Salinity (ppt)	TWG Catch	Other species (n)	*Ruppia* Presence
Slidegate	36.749704; -121.801128	15.2	1.22	0	None	Absent
OSR	36.750070; -121.801114	15.2	1.23	0	Fathead minnow (2), Pac. herring (5), Pac. staghorn sculpin (1), inland silverside (4), mosquitofish (TNTC), threespine stickleback (TNTC)	Present
Breach Site	36.750506; -121.803841	17.3	1.16	0	inland silverside (138), threespine stickleback (32), yellowfin goby (1)	Absent
South Berm	36.747189; -121.803235	16.8	1.07	0	Threespine stickleback (3)	Absent
NWR (downstream 1)	36.746134; -121.802480	17.2	1.05	0	Threespine stickleback (4)	Absent
NWR (downstream 2)	36.742671; -121.799886	17.3	1.00	0	Threespine stickleback (6)	Present
NWR (upstream 1)	36.741381; -121.798735	17.5	0.94	0	Inland silverside (TNTC)	Absent
NWR (upstream 2)	36.739192; -121.795568	17.5	0.88	0	Inland silverside (33), threespine stickleback (4), Pacific staghorn sculpin (2),	Present
HWY 1	36.731993; -121.783053	17.7	0.7	0	Prickly sculpin (1), threespine stickleback (TNTC),	Absent

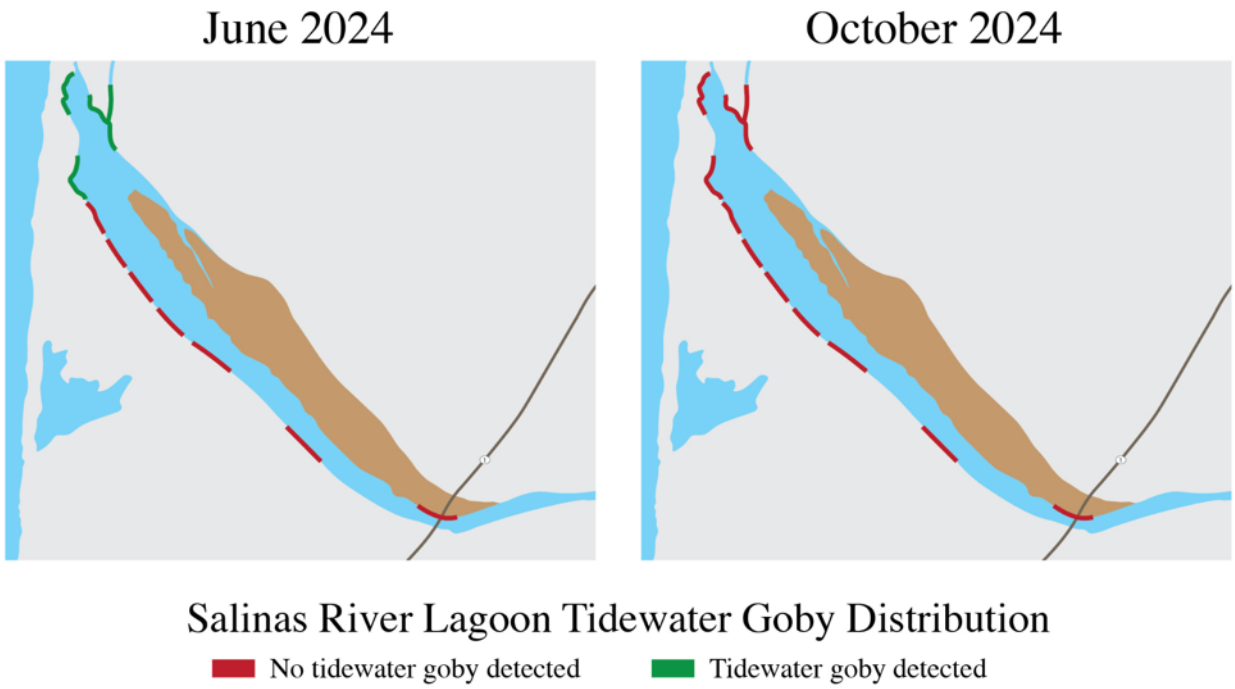

Figure 4. Distribution of tidewater goby in the Salinas River Lagoon in spring and fall of 2024, illustrating presence (green) and non-detection (red), as determined by seining surveys. Note: effort is not standardized.

Discussion

The results of this survey indicate that tidewater goby are widely distributed in the broader geographic area of the lower Salinas River, including the Watsonville Slough, Bennet Slough, Elkhorn Slough, Moro Cojo and Tembladero Slough complexes. Several of the sampling locations covered in this study have not been previously sampled for tidewater goby, or tidewater goby have never before been documented at these sites.

Tidewater goby eDNA was detected during both sampling events at multiple sampling locations, including Bennet Slough North of Highway 1, Bennet Slough East of highway 1, Elkhorn Slough at Swiss Canyon, Elkhorn Slough East of Elkhorn Road, and Moro Cojo Slough at Moss Landing. Elsewhere, although not detected during both sampling events, there is evidence that the general sampling area continued to be occupied, for example in Watsonville Slough (positive detection at W Beach Road in spring, but not in fall; not detected at Shell Road in spring, but positive detection in fall).

Some locations appeared to be at least seasonally occupied, as indicated by clear eDNA signals (positive detection in all replicates) for one of the two sampling events, such as Elkhorn Slough at Kirby Park or Elkhorn Slough at Blohm Road. This finding suggests that the regional metapopulation is likely relatively stable, and nearby source populations are available for recolonization of suitable habitats in the event of localized extirpation. Distances between occupied habitats (approximated by tracing waterways between sampling locations) also seem conducive to dispersal, rarely exceeding 5 km (Table 3). However, as information pertaining to the frequency of migration among suitable occupied habitats is lacking, the possibility remains that the respective populations are relatively isolated, as has been inferred for population clusters that are genetically isolated despite geographic proximity (McCraney et al. 2010). Physical sampling, collecting of genetic samples from individuals, and subsequent analysis of genetic differentiation could inform whether the broader geographic metapopulation is panmictic with frequent exchange of reproducing individuals.

The detection of tidewater goby during one, but not the other, sampling event at a given location could be indicative of seasonal occupancy or imperfect detection. Extensive eDNA sampling in the lagoons, sloughs, and estuaries of the northern California coast suggests that the detection probability for tidewater goby was nearly double that of seining when analyzed with a multimethod occupancy approach (Schmelzle and Kinziger 2016). Despite an imperfect detection probability, eDNA surveys provide a valuable complementary approach to physical sampling, even in environments with high ambient salinities which can have a strong negative effect on DNA availability in the water sample and detection in the qPCR replicate (e.g. Elkhorn Slough at Swiss Canyon, where salinity exceeded 40 ppt at the time of sampling; Foote et al. 2012; Thomsen et al. 2012; Sutter and Kinziger 2019).

Based on the observed ranges in environmental parameters, several locations appeared unsuitable for direct tidewater goby occupancy due to unfavorable environmental conditions (e.g. Bennet Slough N of Highway 1, salinity 56.37 ppt). However, environmental parameters are not necessarily indicative of conditions where tidewater goby reside, as eDNA can be transported by currents (for example, water with suspended eDNA could have flowed into shallow areas where it warmed to about 30°C, e.g. in Watsonville Slough at W. Beach Road or Bennet Slough N. of Hwy 1).

Other habitats appeared suitable based on the environmental parameters measured during sampling; however, the seasonal persistence of these habitats is unknown. For those that are perennial, absence of tidewater goby may be explained by a lack of hydrological connectivity, preventing colonization. Among the regional groups sampled for this investigation, Tembladero Slough was the only area without evidence of tidewater goby occupancy; most likely due to persistent low salinity and poor water quality, owed to its nature as a drainage canal for agricultural runoff.

Despite a lack of physical capture during the seining survey in October 2024 in the Salinas River Lagoon, detections of tidewater goby environmental DNA suggest population persistence in this habitat, but likely in low abundances at the time of sampling. Regrettably, the target number of 100 tidewater goby for a more detailed evaluation of reproductive timing could not be achieved. Physical sampling was scheduled, in part, based on the expectation of highest expected seasonal abundance to coincide with the October sampling event, but inter-annual variation in goby abundance can be drastic (from thousands to millions; Hellmair et al. 2011) without negative implications for long-term population persistence. Regardless, the back-calculation of hatch dates for the 19 individuals sampled in spring suggest a rather prolonged reproductive period of over seven months, indicating resilience to stochastic environmental fluctuation.

The detection record in the Salinas River Lagoon perfectly illustrates the benefit of hybrid surveys, as tidewater goby were not detected by eDNA sampling in spring, despite physical capture during seining, and conversely, tidewater goby were not captured by seining in the fall, but detected using eDNA sampling. Therefore, this survey highlights the value of hybrid sampling approaches when feasible, as both methods – eDNA sampling and subsequent qPCR and seining – have imperfect, albeit undetermined, detection probabilities. Whenever possible, a hybrid sampling approach should be favored during future assessments of tidewater goby distribution.

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Appendix 1 - Site Images for Tidewater Goby eDNA Survey 2024

Figure A1. Watsonville Slough at Shell Road, sampled in Spring (June 5, 2024; top) and Fall (October 22, 2024; bottom).

Figure A2. Watsonville Slough at Beach Road, sampled in Spring (June 5, 2024; no picture) and Fall (October 22, 2024).

Figure A3. Pajaro River Estuary, sampled in Spring (June 5, 2024; no picture) and Fall (October 29, 2024).

Figure A4. Bennet Slough North of Highway 1, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom).

Figure A5. Salinas River Lagoon near OSR, sampled in Spring (June 6, 2024, no picture) and Fall (pictured; October 29, 2024).

Figure A6. OSR near the slide gate, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom).

Figure A7. Salinas River Lagoon on the North side of the beach, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom).

Figure A8. Bennet Slough East of Highway, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom).

Figure A9. Bennet Slough West of Highway 1, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom).

Figure A10. Elkhorn Slough East of Moss Landing Harbor, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom). Access to the exact location sampled in Spring was not possible due to area closures.

Figure A11. Elkhorn Slough at Jetty Road Crossing, sampled in Spring (June 6, 2024; top) and Fall. (October 29, 2024; bottom).

Figure A12. Highway 1 Pond East of Elkhorn Slough, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom)

Figure A13. Moro Cojo at Moss Landing on the North End, sampled in Spring (June 6, 2024).

Figure A14. Moro Cojo at Moss Landing on the South end, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom).

Figure A15. OSR at Portero Road, sampled in Spring (June 6, 2024; no picture) and Fall (October 29, 2024).

Figure A16. Elkhorn Slough at Pick-n-Pull, sampled in Spring (June 6, 2024; top) and Fall (October 29, 2024; bottom).

Figure A17. Elkhorn Slough at Blohm Road, sampled in Spring (June 6, 2024; top) and Fall (October 28, 2024; bottom).

Figure A18. Elkhorn Slough East of Elkhorn Road, sampled in Spring (June 6, 2024; top) and Fall (October 28, 2024; bottom).

Figure A19. Elkhorn Slough at Kirby Park, sampled in Spring (June 6, 2024; top) and Fall (October 30, 2024; bottom).

Figure A20. Elkhorn Slough East of Kirby Park, sampled in Spring (June 6, 2024; no picture) and Fall (October 30, 2024).

Figure A21. Elkhorn Slough at Swiss Canyon, sampled in Spring (June 6, 2024; top), and Fall (October 30, 2024; bottom).

Figure A22 Moro Cojo at Castroville Boulevard, sampled in Spring (June 6, 2024; top). The site was visited, but not sampled in Fall (October 22, 2024; bottom), as the location was dry.

Figure A23. Tembladero Slough at Highway 183, sampled in Spring (June 6, 2024; no picture) and Fall (October 22, 2024).

Figure A24. Tembladero Slough at San Jon Road, sampled in Spring (June 6, 2024, no picture) and Fall (October 22, 2024).

Figure A25. Blanco Drain, sampled in Spring (June 6, 2024; top) and Fall (October 22, 2024; bottom).

Figure A26. Tembladero Slough at the recreation trail on Haro Street, sampled in Spring (June 6, 2024; top) and Fall (October 22, 2024; bottom).

Figure A27. Salinas River lagoon at Highway 1, sampled in Spring (June 7, 2024; top) and Fall **(**October 22, 2024; bottom).

Figure A28. Tembladero Slough at Highway 1, sampled in Spring (June 7, 2024; top) and Fall. (October 30, 2024; bottom).

Figure A29. OSR at Monterey Dunes Way, sampled in Spring (June 7, 2024; top) and Fall (October 29, 2024; bottom).

Figure A30. Ponds at Watsonville Road, sampled in Spring (June 7, 2024; top) and Fall **(**October 30, 2024; bottom).

Figure A31. Moro Cojo East of Highway 1, sampled in Spring (June 7, 2024; no picture) and Fall (October 29, 2024).

Figure A32. Elkhorn Reserve South of foundation on the boardwalk, sampled in Spring (June 7, 2024; top) and Fall (October 30, 2024; bottom).

Figure A33. Elkhorn Reserve at the South Marsh Boardwalk, sampled in Spring (June 7, 2024; top) and Fall (October 30, 2024; bottom).

Figure A34. Elkhorn Reserve at the South Marsh Land Bridge, sampled in Spring (June 7, 2024; top) and Fall (October 30, 2024; bottom).

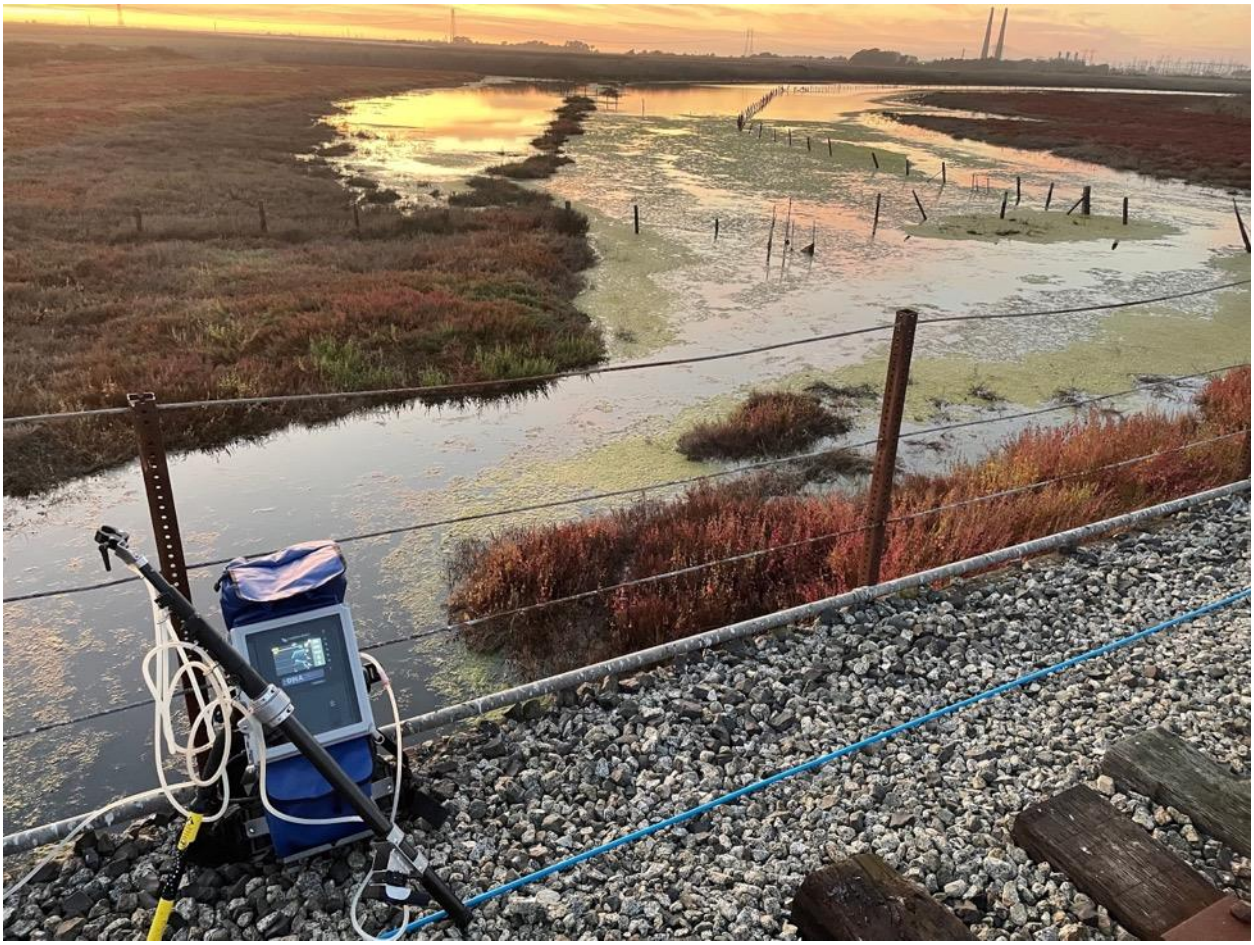

Figure A35. Moro Cojo Slough downstream of the railroad, sampled in Fall (October 29, 2024).

Figure A36. South Branch Moro Cojo at Watsonville Road, sampled in Fall (October 30, 2024).

Figure A37. Moro Cojo Pond South of Highway 156, sampled in Fall (October 30, 2024).