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Characterizing Fish Communities using Traditional and Emerging Monitoring Approaches in San Diego County Estuaries

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Characterizing Fish Communities using Traditional and Emerging Monitoring Approaches in San Diego County Estuaries

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by

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ABSTRACT

Fish communities are essential to estuarine health, playing key roles in food webs, nutrient cycling, and ecosystem functioning. However, the persistence of native fish populations is increasingly threatened by the cumulative impacts of coastal development, pollution, and climate change. Rapid, effective monitoring of species distributions is a priority, enabling timely conservation and management. However, the study of any ecosystem is limited by time, resources, and the consideration that research of a system can damage the ecosystem under study.

This research aimed to characterize fish species richness and evaluate the benefits and limitations of different estuary monitoring approaches. Specifically, this study compared traditional methods (block net seining, enclosure traps) with contemporary approaches (community science (iNaturalist) and eDNA metabarcoding) to assess species richness and composition in 2023 across five San Diego estuaries: Tijuana River Estuary, San Diego Bay (Sweetwater Marsh, South Bay Salt Ponds), Mission Bay (Kendall-Frost Marsh), Los Peñasquitos Lagoon, and San Dieguito Lagoon. Additionally, long-term traditional sampling data from 2013 to 2023 at Tijuana River Estuary and San Dieguito Lagoon were compared alongside abiotic variables, including salinity, temperature, and dissolved oxygen.

In 2023, iNaturalist reported the highest species richness at most sites, except Los Peñasquitos and San Dieguito Lagoon, where traditional sampling and eDNA, respectively, yielded more detections. Species richness was lowest at Tijuana River Estuary, consistent with evidence of degraded water quality. Despite strict adherence to sampling protocols, only 10 of 89 eDNA samples yielded sufficient sequence reads, likely due to filter clogging and PCR inhibition caused by high organic content. These findings underscore the value of integrating multiple monitoring approaches, highlight the potential of community science to enhance species inventories, and emphasize the need for improved eDNA protocols tailored to the unique challenges of Southern California estuaries.

Chapter 1: Introduction

This chapter presents an overview of Southern California estuaries and their fish communities, and describes the aims and objectives of this research, which evaluates traditional and emerging approaches for monitoring estuarine biodiversity. It also highlights the ecological significance of these systems and the growing need for robust monitoring tools to guide restoration and management.

1.1 Estuaries in Southern California and the Fish That Inhabit Them

Monitoring fish communities in estuarine and coastal wetland systems is a critical component of ecological assessment. These efforts not only track the outcomes of habitat restoration and compliance with regulatory requirements but also provide insight into long-term ecological responses to climate change and human development (Desmond et al., 2002). In San Diego, traditional fish sampling methods have historically served as the foundation of estuarine monitoring efforts. However, recent advancements have introduced complementary approaches, including environmental DNA (eDNA) metabarcoding, baited remote underwater videos (BRUVs), and community science platforms such as iNaturalist.

Estuaries are among the most biologically productive ecosystems on Earth, supporting diverse fish communities alongside a wide range of other species. In addition to serving as nurseries and critical habitat, they provide essential ecosystem services that benefit human populations (Barbier, 2013; Day, 1981). Such services include cultural services (e.g., tourism and recreation), supporting services (e.g., carbon sequestration and resting areas for migratory birds), regulating services (e.g., nutrient recycling, water purification, and wave and flood attenuation), and provisioning services (e.g., habitat for invertebrates and nurseries for fish) (Barbier, 2013).

Many of these ecosystem services sustain human livelihood and promote economic activity, such as through the filtration of pollutants and support for commercial and recreational fisheries (Booi et al., 2022).

Despite their value, estuaries face significant threats and challenges on a global scale (Booi et al., 2022). Coastal development, channel modification, water diversions, invasive species, and pollution have altered natural hydrologic regimes and habitat structure, resulting in widespread degradation and biodiversity loss within estuaries (Cloern & Jassby, 2012). In severe cases, such as the historical degradation of the Thames Estuary in London, these impacts have caused complete collapses of aquatic communities (Attrill, 1998).

In Southern California, coastal wetland loss has been extensive, with estimates indicating that approximately 90% of these habitats, including bays, estuaries, and salt marshes, have been degraded or destroyed (Zedler et al. 2001). Given their limited size and scarcity in the region, even small estuarine systems hold importance for biodiversity conservation and ecosystem services, including nursery habitat for fish (Allen et al., 2002). At least one species of commercial importance, the California halibut (*Paralichthys californicus*), has been found to depend on California bays and estuaries as nursery habitats (Allen, 1988; Plummer et al., 1983). Additionally, non-commercial fish such as gobies, anchovies, and silversides are highly abundant and play a crucial role as prey for seabirds and commercially important fish (Horn & Allen, 1980).

Monitoring is therefore essential to track ecological change, assess restoration outcomes, and inform adaptive management. In recent years, there has been growing interest in enhancing monitoring techniques to better detect shifts in community structure and ecosystem health. This research contributes to those efforts by evaluating the effectiveness of different methods for

assessing fish assemblages in San Diego County estuaries, with a focus on the strengths and limitations of each approach in monitoring coastal estuarine biodiversity.

1.2 Physical Characteristics of Southern California Estuaries

Southern California is characterized by a Mediterranean climate, marked by low and highly seasonal precipitation, with most rainfall occurring during the winter months. The bays and estuaries in this region are primarily influenced by small, intermittent rivers and streams, resulting in systems that are largely marine-dominated (Allen et al., 2006). These estuaries, often referred to as “negative estuaries”, “intermittent estuaries” or “low-inflow estuaries” (LIEs), are typical in mediterranean climates and along coastal lands which slope steeply to the sea (Largier et al., 1997). Unlike classical estuaries dominated by consistent freshwater inflow and strong salinity gradients, LIEs exhibit seasonal cycles in salinity and temperature, with hypersaline conditions often occurring in late summer. These dynamics result in reduced longitudinal circulation, stagnating flows, and increased vulnerability to pollutant retention (Harvey, 2022; Largier, 2010; Largier et al., 1997).

In addition, estuaries in Southern California vary significantly in size, geomorphology, land use context, and degree of restoration. For example, San Diego Bay has a permanently one tidal inlet, and is highly urbanized, while Los Peñasquitos Lagoon is subject to seasonal inlet closures and active restoration management. Within these systems, diverse sub-habitats, such as tidal channels, mudflats, eelgrass beds, and marsh pools, support distinct fish assemblages at different life stages (Allen, 1982; Valle et al., 1999; Yoklavich et al., 1991). Due to this variability in habitat structure, environmental conditions, and species composition, no single sampling method is considered sufficient. This is partly because these systems vary greatly in extent, making it difficult to establish a single approach that can be applied across all systems to generate long-

term, comparable data. As such, multiple complementary approaches are required, as gear types differ in detection biases, and fish assemblages shift seasonally and across life stages (Yoklavich et al., 1991). This study samples across a range of estuarine conditions to assess the applicability and limitations of different fish monitoring techniques in Southern California systems. In the past, traditional approaches including beach seines and enclosure traps were used to monitor fish communities. However, these approaches are time-consuming and practical only for smaller areas, making them especially challenging to apply in large systems such as San Diego Bay and Mission Bay.

1.3 Fish Communities in Southern California Estuaries

Fish communities in Southern California are primarily composed of estuarine resident estuarine species and marine migrants, with relatively few freshwater or anadromous species due to prevailing saline conditions and limited freshwater inputs (Table 1.1). Estuarine residents, such as gobies and topsmelt, are adapted to tolerate fluctuating salinity and often complete their entire life cycle within the estuary. Marine migrants, by contrast, move between nearshore marine environments and estuaries, using the latter as nursery grounds or foraging habitat during early life stages. Monitoring fish communities provides insight into the ecological condition of estuarine systems, as shifts in species composition, for example, an increase in freshwater taxa, may reflect changes in watershed inputs, such as urban runoff.

1.4 Project Overview

This study aims to evaluate and compare fish monitoring methods in coastal estuaries of Southern California, with a focus on species richness and community composition. Specifically, it

compares traditional techniques (block net seining and enclosure traps) with emerging approaches such as community science (iNaturalist) and environmental DNA (eDNA) metabarcoding.

The research was conducted across five estuarine systems in San Diego County: Tijuana River Estuary, San Diego Bay (Sweetwater Marsh and South Bay Salt Ponds), Mission Bay (Kendall-Frost Marsh), Los Peñasquitos Lagoon, and San Dieguito Lagoon. These systems span different sizes, restoration history, and urbanization, allowing for assessment of monitoring tools across varied ecological contexts.

In addition to sampling data from 2023, long-term traditional sampling data (2013–2023) from Tijuana River Estuary and San Dieguito Lagoon were analyzed alongside abiotic data (salinity, temperature, and dissolved oxygen) to explore potential environmental drivers of species richness.

1.5 Project implications:

This research contributes to a broader effort to develop robust monitoring approaches for estuaries and coastal wetlands in Southern California. Specifically, it focuses on fish monitoring strategies to identify general patterns and the most effective methods for assessing species richness. Beyond species detection, the findings also provide insights into long-term ecological trends and their relationship with key water quality parameters. It also evaluates the feasibility of integrating eDNA into routine monitoring programs, identifying both the challenges and limitations of combining eDNA with traditional approaches within existing management frameworks. By highlighting knowledge gaps and research needs, it aims to inform future applications of eDNA in estuarine monitoring across Southern California.

Tables

Table 1.1. Example of Fish species known to occur within Southern California coastal wetlands and their associated ecological categories (Allen et al., 2006).

Category	Species Name (Scientific Name)
Catadromous species	Striped mullet (*Mugil cephalus*)
Common residents	Pacific staghorn sculpin (*Leptocottus armatus*)
	Bay pipefish (*Syngnathus leptorhynchus*)
	Arrow goby (*Clevelandia ios*)
	California killifish (*Fundulus parvipinnis*)
	Slough anchovy (*Anchoa delicatissima*)
	Deepbody anchovy (*Anchoa compressa*)
Marine Migrants	Topsmelt silverside (*Atherinops affinis*)
	Shiner perch (*Cymatogaster aggregata*)
	Black perch (*Embiotoca jacksoni*)
	Diamond turbot (*Hypsopsetta guttulata*)
	Juvenile California halibut (*Paralichthys californicus*)
	Spotted turbot (*Pleuronichthys ritteri*)
Seasonal Marine Visitors (Spring and Summer)	Californian anchovy (*Engraulis mordax*)
	Gray smoothhound (*Mustelus californicus*)
	Haller's round ray (*Urolophus halleri*)
	Barred sand bass (*Paralabrax nebulifer*)
Alien Species (Established)	Yellowfin goby (*Acanthogobius flavimanus*)
	Rainwater killifish (*Lucania parva*)
	Chameleon goby (*Tridentiger trigonocephalus*)
Alien Species (Potential Threat)	Shimofuri goby (*Tridentiger bifasciatus*)

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was declining before our eyes, but it took regional censuses to recognize the problem, long-term monitoring to identify the causes, and experimental plantings to show why the loss of species matters and which restoration strategies might reestablish species. BioScience **51**:1005-1017.

Chapter 2: Characterizing Fish Species Richness in San Diego Coastal Wetlands Using Traditional and Emerging Monitoring Approaches

2.1 Introduction

This chapter aims to compare traditional monitoring methods with contemporary approaches to assess fish species richness and composition in five estuaries in San Diego County. This research addresses the challenge of selecting appropriate monitoring tools in different estuarine systems, with the goal of informing current monitoring programs and guiding management decisions. More specifically, this chapter has three objectives:

1. Compare species accumulation curves using traditional survey data (block net seining and enclosure traps) collected in 2023.
2. Describe the fish species richness detected by traditional sampling, community science (iNaturalist), and environmental DNA (eDNA) in 2023.
3. Explore the long-term trends in species richness at two estuaries (San Dieguito Lagoon and Tijuana River Estuary) collected using traditional sampling approaches from 2013 to 2023 in the context of salinity, dissolved oxygen and flow.

2.1.1 Threats to Estuarine Fish Communities: Climate Change and Invasive Species

Fish communities play an important role in food chains, nutrient recycling, and maintaining the health of estuaries. In California, many fish species depend on estuaries for at least part of their life cycle (Moyle et al., 2013). However, estuarine species face multiple threats, including habitat loss from coastal development, reduced freshwater inflows due to water diversion, pollution, and

the introduction of non-native species. The persistence of fish communities in estuaries is becoming increasingly uncertain as human development continues and climate change impacts compound (Moyle et al., 2013). Climate change is expected to alter river flows and increase water temperatures, which are anticipated to negatively affect species distributions, reproduction, and overall community stability.

Given these ecological roles and growing threats, understanding how fish communities are structured across California estuaries is essential. In particular, Southern California bays and estuaries differ markedly from those in central and northern regions, both in fish assemblages and habitat use (Allen et al., 2006). These assemblages are adapted to the region's limited freshwater input, which typically occurs only during brief winter rains. As a result, anadromous and catadromous species are largely absent. The diverse habitats found within Southern California estuaries support distinct groups of species, each adapted to specific niches (Allen et al., 2006). For example, gobies inhabit mudflats, striped mullet and anchovy are commonly found in main channels, and marine migrants such as black perch and diamond turbot occupy deeper channels. These diverse habitats make species monitoring challenging, often requiring multiple monitoring approaches required to adequately sample an estuary (Yoklavich et al., 1991).

2.1.2 The Importance of Species Monitoring

The ability to rapidly and accurately track changes in species distributions is widely recognized as a key scientific priority (Pecl et al., 2017), as it enables timely and effective management interventions when needed. However, the study of any ecosystem is limited by time, resources, and the consideration that research of a system can damage the ecosystem under study (Slobodkin et al., 1980). As such, there is no ideal monitoring approach or ecosystem modeling that can provide information about all aspects of a system (Slobodkin et al., 1980). Effective

monitoring of estuaries requires a methodology that can account for various types of habitats, as species and communities have adapted to varying salinity, habitat structure (e.g., vegetation, sediment, and/or hard substrate), velocity of water (affecting dissolved oxygen and turbidity), and temperature.

A cost-effective and useful approach to answer questions about an ecosystem is developing a species list (or distribution data), which consists of the names of species within an area of interest (Slobodkin et al., 1980). The objective of preparing a species list is to record the conditions in a particular place during a particular period, in as much detail as you are capable of (Clench, 1979). A species name can be linked to species-specific knowledge about the environmental conditions and biological requirements of an organism, which in turn provides information about an ecosystem (Slobodkin et al., 1980).

2.1.3 Measuring Fish Species Richness: Challenges and Advancements

Species richness is a key metric in ecological studies and preserving it is a primary goal in conservation efforts (May, 1988). Understanding species composition is crucial for effective natural resource management and sustaining ecosystem function, especially in the face of growing human pressures and climate change. However, accurately estimating species richness remains a challenge. This is because a complete species list is rarely attainable, as species continue to be detected with increasing effort in sampling. With sufficient time and resources, researchers are able to approach a species list that is as complete as possible (Clench, 1979). Despite its importance in biodiversity assessments, species richness is often underestimated due to the inherent difficulty of detecting rare species (Colwell et al., 2012). Additionally, species richness increases non-linearly with effort, making simple standardizations, such as species per unit of sampling effort, unreliable for across site comparisons (Chazdon et al., 1998; Colwell et al., 2012). Furthermore,

comparing species among sites is statistically challenging since the number of observed species is sensitive to the abundance (or number of individuals) in the area sampled (Colwell et al., 2012).

Traditionally, species lists were compiled using capture-and-release surveys, often conducted as part of structured monitoring programs that provide regular and targeted recordings (Isaac et al., 2014). In general, these monitoring schemes are cost-intensive, require dedicated and highly trained personnel, and typically involve standardized protocols (Isaac et al., 2014; Pocock et al., 2015). These processes also tend to be biased toward more developed countries (Moussy et al., 2021). In the southern California region, long-term monitoring programs have been conducted on various estuaries since 2012 by the Tijuana River National Estuarine Research Reserve, the Southern California Coastal Water Research Project (SCCWRP) and the San Onofre Generating Station Restoration Project (SONGS). These surveys have focused on sampling several parameters, including fish in coastal wetlands using seining, enclosure traps and minnow traps and provide insight into fish species richness and abundance. However, these surveys are based on the capture of individuals which can be invasive, time consuming and require a high level of taxonomic expertise, resulting in researchers turning to alternative sampling methods such as community science and genetic tools to monitor ecosystems (Darling et al., 2017). Emerging tools, such as environmental DNA (eDNA) analyses and community science platforms like iNaturalist, can provide more timely and comprehensive assessments of species presence, offering valuable additions to species lists and enhancing our ability to detect invasive species, which are expected to become increasingly prevalent with climate change and associated range shifts (Pecl et al., 2017).

Species accumulation curves (SACs) are a widely used tool for assessing the completeness of a species list by plotting the cumulative number of species against a measure of sampling effort,

such as the number of samples or individuals collected (Băncilă et al., 2014). When SACs reach an asymptote, it indicates that most of the frequently encountered species have been detected and that additional sampling will yield diminishing returns. While complete inventories are rarely attainable, the shape of the curve remains informative for assessing sampling efficiency, community structure, and for comparing diversity across sites or sampling efforts (Băncilă et al., 2014; Colwell et al., 2012). In addition, SACs provide a valuable approach for assessing species richness, as they account for the non-linear relationship between species richness and sampling effort (Colwell et al., 2012). This allows for meaningful data standardization (mitigating sampling-induced biases) and comparison of datasets (Chazdon et al., 1998; Gotelli & Colwell, 2001). Species accumulation curves can be constructed using two primary approaches: sample-based abundance data, which incorporates the number of individuals per species within each sampling unit, and sample-based incidence data, which considers only species presence or absence within sampling units (Gotelli & Colwell, 2001). This study makes use of the latter approach, generating SACs using both randomized (or "smoothed") curves and collector's curves. Randomized SACs average species richness across multiple random permutations of sample order, providing a robust estimate of richness that minimizes bias from sample sequence (Colwell et al., 2012). In contrast, collector's curves retain the original sample order and are useful for visualizing the temporal progression of species detections.

More recently, species richness studies have included community science, a form of research that provides everyone, regardless of their education, an opportunity to contribute towards scientific data. Throughout the past decade, community science has progressed and dramatically enhanced the capacity to cost-effectively assess and manage species populations (Darling et al., 2017). A community science platform that has gained significant traction since its inception in

2008 is iNaturalist. This platform allows individuals to submit records that can be identified through both the software's artificial intelligence and community of scientists and naturalists (Nugent, 2018). iNaturalist's popularity and reach among both the public and the science community is due to the program's user-friendly interface across various devices, automated species identification, and scientist and expert input on the validation of species identifications, which are regularly exported to global biodiversity databases (Encarnação et al., 2021). iNaturalist is considered a useful conservation tool, particularly for countries with limited resources (Darling et al., 2017).

Another emerging method of obtaining distribution data is environmental DNA (eDNA) sampling which has two approaches, an active survey approach which targets a specific species, or a passive survey approach which aims to detect many species. The latter approach, known as high-throughput sequencing (DNA metabarcoding), was employed in this study.

eDNA metabarcoding is a methodology which involves the collection of free-floating DNA from target organisms, which can be extracted from various environmental materials, such as water, soil, or air. As organisms interact with their surroundings, they continuously release DNA through various biological processes, including the shedding of skin cells, excretion of urine and feces, and decomposition of dead individuals. Once released into the environment, the persistence of eDNA varies widely depending on environmental conditions. In temperate aquatic systems, DNA degradation occurs within weeks (Dejean et al., 2011; Thomsen et al., 2012), whereas in cold, arid environments such as permafrost, genetic material can remain preserved for hundreds of thousands of years (Willerslev et al., 2003). After collecting the environmental material of interest, specific gene regions, known as barcodes, are then amplified and sequenced to determine the presence or absence of species.

eDNA has key advantages in species monitoring, which includes the ability to differentiate between morphologically similar species (Thomsen & Willerslev, 2015), and cryptic species (e.g., Hinlo et al. (2017); Shaw et al. (2016); Valdivia-Carrillo et al. (2021)), and has sufficient sensitivity to allow for the detection of rare (e.g., Day et al. (2019); Pukk et al. (2021)) and endangered (e.g., Budd et al. (2023)) species. This is advantageous over traditional surveys which require taxonomic expertise, which is facing a global decline increasing errors from possible misidentifications (Levin, 1984). eDNA is also considered less logistically complicated and more cost-effective (e.g., Akre et al. (2019); Miya et al. (2015)), allowing researchers to collect more samples across a broader spatial area and temporal range (Darling, 2020) in comparison to traditional surveys. Finally, eDNA is considered non-destructive to ecosystems understudy and causes minimal disturbance to communities (Knudsen et al., 2019; Li et al., 2019).

Throughout the past decade, both community science and eDNA sampling have progressed and dramatically enhanced the capacity to cost-effectively monitor the distribution of both native and non-native species (Darling et al., 2017). While no monitoring approach is perfect, they are becoming increasingly valuable as advancements improve their accessibility and usability (Darling et al., 2017).

Various studies have reviewed the applicability of traditional and contemporary sampling techniques to monitor species (Allan et al., 2018; Encarnação et al., 2021; Fediajevaite et al., 2021; Larson et al., 2020). The list of species observed during traditional surveys is considered a small percentage of the actual number of species in an ecosystem, thus an integrated approach, which makes use of contemporary technology to acquire greater volumes and new forms of ecological data, has been recommended (Allan et al., 2018). Although such an approach is favored, little research has been conducted on the feasibility of implementing these methods in regular

monitoring programs. The incorporation of eDNA metabarcoding in regular monitoring plans may allow for the early detection of non-native species, which are introduced by anthropogenic activities or due to "tropicalization", the idea that species are shifting their ranges polewards in response to warming temperatures (Allan et al., 2018; Pecl et al., 2017).

2.1.4 Overview of this Study

This research explored the use of traditional surveys (block net seining and enclosure traps) and two contemporary sampling methods (community science and eDNA metabarcoding) to characterize fish communities in five coastal wetlands in San Diego County. This research also compared long-term species richness data from traditional surveys at two coastal wetlands. The broader aim of this research was to characterize fish species richness and explore the benefits and limitations of using these different approaches in coastal estuary biodiversity monitoring. More specifically, this research had three objectives:

1. Compare species accumulation curves with sampling effort across the five sites using the 2023 traditional sampling data, to understand whether current annual monitoring programs are sufficiently sampling these sites.
2. Describe and compare fish species richness across five coastal wetlands using annual traditional sampling (seining, enclosure traps), community science (iNaturalist), and eDNA metabarcoding collected in 2023. This included a comparison of the number of species detected, differences between native and non-native species detected, and whether sampling approaches are biased towards fish guilds.
3. Explore the long-term trends in species richness at two sites (San Dieguito Lagoon and Tijuana River Estuary) collected using traditional sampling data from 2013 to 2023. Species

richness was explored in the context of three environmental parameters (salinity, dissolved oxygen and temperature).

2.2 Methods

2.2.5 Study Sites

This study was conducted across five coastal wetlands in San Diego County, California, USA, which are referred to as "sites" throughout this research. These sites include San Dieguito Lagoon (SDL), Los Peñasquitos Lagoon (LPL), Kendall-Frost Reserve in Mission Bay (KFR), San Diego Bay Salt Ponds and Sweetwater Marsh National Wildlife Refuge in San Diego Bay (grouped together as SDB), and the Tijuana River Estuary (TJE) (Figure 2.1; Table 2.1). These systems represent the southernmost coastal wetlands along the U.S. West Coast, with the Tijuana River Estuary situated near the U.S.-Mexico border.

Coastal wetlands in San Diego exhibit considerable variation in size and type, ranging from small creeks and fragmented estuaries to extensive bays and harbors (Doughty et al., 2018; Zedler, 1982). SDL, the northernmost site in this study, has a permanently open tidal inlet that is actively managed to remain open, and is identified as the smallest wetland based on estimates by Heady et al. (2014). However, its extent has likely expanded following extensive restoration efforts (Table 2.1; wetland sizes and classifications summarized from Allen et al. (2006); Beheshti et al. (2023); Heady et al. (2014); SCCWRP (2018)). The restoration of the SDL is a requirement of the San Onofre Nuclear Generating Station (SONGS) permit. As a condition of this permit, wetland construction and restoration efforts are required to mitigate the power plant's impact on adult nearshore fish populations across the Southern California Bight, which spans from Point Conception in Santa Barbara to Punta Colonet in Mexico.

LPL has a tidal inlet that regularly closes, primarily due to the constriction of the ocean inlet by a highway and railway (Desmond et al., 2002) and the impact of recent beach renourishment projects north of the estuary (pers. comm.).

SDB and MB are classified as permanently open bays and are among the largest in the region (Table 2.1). The natural marsh habitat within these bays has a long history of anthropogenic disturbance due to dredging, infilling and land use changes (Williams & Zedler, 1999). This study included two sites within SDB: South San Diego Bay Salt Ponds and Sweetwater Marsh National Wildlife Refuge, and one site within MB - Kendall-Frost Reserve (Figure 2.1).

The Sweetwater Marsh National Wildlife Refuge and South San Diego Bay Salt Ponds are situated 18 kilometers and 22 kilometers upstream from the entrance of SDB, respectively. Sweetwater Marsh National Wildlife Refuge, positioned along the bay's eastern side, consists of a network of salt marsh channels and vegetation interspersed with roadways, dikes, and dredged channels. This area has also undergone restoration efforts over the past several decades (Williams & Zedler, 1999). The South San Diego Bay Salt Ponds, located in the bay's southwestern corner, were historically used for salt production. In 2011, approximately 104 hectares of coastal wetlands were restored through sediment redistribution to establish tidal elevations and channels throughout the ponds (SCCWRP, 2018). Native salt marsh vegetation was then planted to support habitat reestablishment.

Kendall-Frost Reserve is located in the northeastern corner of MB and includes approximately 6.5 hectares of remnant salt marsh that previously spanned some 800 hectares in the early 20th century (Patterson, 1965). MB has undergone modifications over the past century due to human activities, including the diversion of the San Diego River, dredging, and infilling to support recreational activities within the bay (Gabrielson, 2002). Kendall-Frost Reserve is

comprised of mudflats with small tidal channels and coastal vegetation (Levin, 1984; Macdonald, 1969).

TJE, characterized by its relatively flat topography, is the third largest system included in this study and has a tidal inlet that rarely closes (Table 2.1). Both TJE and LPL experience frequent and excessive sediment inputs driven by urban development within their respective watersheds (SCCWRP, 2018).

Among the study areas, the TJE experiences the highest levels of contamination from runoff and sewage pollution, a persistent issue within its watershed (Beheshti et al., 2023). Since October 2023, conditions within the estuary have further deteriorated, with approximately 31 billion gallons of untreated sewage, polluted water, and debris flowing into the Tijuana River. This has heightened concerns about public health, coastal water contamination, and the degradation of the TJE (Musegaas, 2024). The ongoing pollution crisis continues to pose serious environmental and health risks, largely driven by rapid urban expansion and insufficient wastewater treatment infrastructure (Musegaas, 2024).

2.2.6 Data Collection: Traditional Survey Data (Seining and Enclosure Traps)

Fish monitoring data collected during the fall (August – October) of 2023 were obtained from two sources: the San Onofre Nuclear Generating Station (SONGS) San Dieguito Wetland Restoration Mitigation Monitoring Program (Schroeter, 2023) (https://marinemitigation.msi.ucsb.edu, accessed on September 24, 2024) and the Southern California Bight Regional Monitoring Program (BIGHT; https://empachecker.sccwrp.org, accessed on August 7, 2024). The latter is an ongoing collaborative effort facilitated by the Southern California Coastal Water Research Project (SCCWRP), involving regulatory agencies,

non-regulatory organizations, and academic institutions. Through this collaboration, multiple organizations contribute to monitoring the ecological health of the Southern California Bight.

The SONGS dataset included block net seining and enclosure trap data from SDL and TJE, while the BIGHT dataset included block net seining data and enclosure trap data for MB (Kendall-Frost), San Diego Bay (Sweetwater Marsh), and LPL, with the latter site only having block net seining data.

For both monitoring programs, field surveys assessed fish species richness along the main channels and tidal creeks of each coastal wetland using a combination of beach seining with blocking nets and enclosure traps (described below, with further details provided in Appendices A and B). Data from blocking nets and enclosure traps were used to compile the species lists used to address Objectives 1 and 2.

2.2.6.1 Beach Seines with Block Nets

Depending on the monitoring program, three to six stations (defined as sampling areas along the coastal wetland) along the main channel were selected (see Figure 2.1 and Table 2.1). At each sampling station along the main channel, two block nets were deployed perpendicular to the shoreline, 7.6 meters apart. Each net extended to a depth of approximately 1.5 meters or a maximum distance of 12.2 meters from shore, whichever was reached first. A beach seine (2 meters high and 7.6 meters wide) was then hauled through the enclosed area, typically covering approximately 100 m^2. Five seine hauls were conducted, followed by a sixth haul, during which the two block nets were retrieved. For LPL, the retrieval of the two block nets was recorded as a single haul, whereas at the other sites they were treated separately as Haul 6 and Haul 7. For consistency across sites, the data from Hauls 6 and 7 were later combined and reported as Haul 6.

Tidal creek sampling followed a similar methodology. Depending on the monitoring program, three to six stations within the tidal creeks were selected. Two block nets were placed 7.6 meters apart across the creek, and a beach seine (2 meters high and 7.6 meters wide) was hauled through the enclosed section for six hauls. To expand the sampling area, an additional block net was placed directly adjacent to the existing net farthest from the tidal inlet. The tidal creek sampling procedure was then repeated within this newly enclosed section.

For both main channels and tidal creeks, all non-goby fish species—except for the yellowfin goby (*Acanthogobius flavimanus*) and longtail goby (*Ctenogobius sagittula*)—were identified, counted, and released.

2.2.6.2 Enclosure Traps

To specifically target gobies, enclosure traps were deployed at each main channel and tidal creek station, except for LPL. Traps were placed at five stations, spaced 10–20 meters apart, in water less than 0.7 meters deep. A Benthic Ichthyofauna Net for Coral/Kelp Environments (BINCKE) was used to conduct multiple hauls through the enclosure trap. Hauls continued until three non-consecutive hauls yielded no fish or invertebrates (as in Anderson and Carr (1998)).

2.2.7 Data Collection: Community Science (iNaturalist)

Data was retrieved from the iNaturalist database (https://www.inaturalist.org/, accessed on 16 April 2024). All observations in 2023 that were considered “research-grade” were included, meaning they had been verified by at least two users, with more than two-thirds of users agreeing on the identification (R. M. Rocha et al., 2024). For each observation, recorded data included the species name, latitude, longitude, and date of observation. The dataset was filtered using the following criteria: (1) observations recorded within San Diego County and (2) all chordates

categorized as “other animals” and “ray-finned fish”. Further refinement was performed to retain only species belonging to *Elasmobranchii* (sharks and rays), *Holocephali* (subclass of cartilaginous fish), and *Actinopterygii* (ray-finned fish), removing all other taxa. To ensure taxonomic accuracy, species names (but not identifications) were cross-checked against the World Register of Marine Species (WoRMS) (https:// www. marinespecies.org/).

The filtered dataset was then imported into ArcGIS Pro, where the *Select by Location* tool was used to identify observations within wetland polygons defined by the San Diego Aquatic Resource Inventory (SDARI)(SFEI, 2024) (accessed on October 22, 2024). SDARI is a high-resolution base map derived from aerial imagery, LiDAR, and ancillary datasets, that is regularly updated by input provided by wetland scientists. The iNaturalist dataset encompassed entire coastal wetland areas (see delineations in Figure 2.1), whereas other datasets, such as environmental DNA (eDNA) and traditional sampling, were limited to stations within each site. For example, iNaturalist observations extended across all of MB, while eDNA and traditional sampling data were restricted to Kendall-Frost Reserve within MB. When referring to these smaller sites, site-specific locations are provided in parentheses (e.g., "MB (Kendall-Frost)").

To reduce redundancy and potential overlap with traditional survey data (given that some monitoring programs upload to iNaturalist), observations of species recorded on the same date as those documented by the SONGS or BIGHT monitoring programs were manually removed. Additionally, duplicate records of the same species at the same location within a one-hour period were excluded.

2.2.8 Data Collection: Environmental DNA (eDNA) Metabarcoding

2.2.8.3 Sample collection, filtering and preservation

Environmental DNA (eDNA) samples were collected at sites throughout San Diego as part of the Southern California BIGHT program in fall 2023 and January 2024 (Figure 2.1, see Appendix C for a detailed description of the sample collection protocol). Each site had 3-6 stations (defined as sampling areas along the coastal wetland). At each station, triplicate 1-liter water samples were collected from a depth of less than 0.5 m using sterile Nalgene bottles. Samples were transported to the laboratory in a cooler on ice. Field negative control samples, consisting of deionized (DI) water, were processed alongside field samples to monitor potential contamination. Field blanks were brought into the field at the time of sample collection and filtered concurrently with the environmental samples to control for potential contamination during transport and handling. All samples were collected by trained personnel following a standardized protocol designed to minimize contamination, including the use of a fresh pair of sterile nitrile gloves at each sampling location. Once collected, samples were filtered away from the site, which generally included a lab setting, pavement parking lot, or backyard, within 24 hours of collection.

Once collected, samples were filtered off-site, typically in a laboratory, paved parking area, or backyard, within 24 hours of collection. Each water sample was transferred to a Kangaroo™ enteral feeding bag and gravity-fed through a coffee filter, followed by a Sterivex™ filter (MilliporeSigma, Burlington, MA, USA). Filtration through the Sterivex™ filter was limited to 40–50 minutes, and the volume of water processed was recorded. Both the coffee and Sterivex™ filters were stored at -20°C. Approximately 1.5 mL of lysis buffer was added to the Sterivex™ filters prior to storage. Field negative controls using deionized water were processed identically and included throughout DNA extraction and sequencing.

2.2.8.4 DNA Extraction

All DNA extractions were conducted in a dedicated molecular laboratory at the SCCWRP in Costa Mesa, California, USA. Two DNA extraction protocols were used for the two filter types.

All DNA extractions from the Sterivex™ cartridges followed a modified DNeasy Blood & Tissue kit protocol (Qiagen Inc., Germantown, MD, USA) optimized to increase DNA yield (Spens et al., 2017). Sterivex™ filters were incubated at 56°C for 20 minutes at 70 rpm with 220 µl of AL buffer, 220 µl sterile PBS and 20 µl of proteinase K. After incubation, 200 µl of molecular grade ethanol was added to the AL buffer/proteinase K mixture and spun through a spin column. AW1 and AW2 were added to wash the columns. The DNA concentration of each sample was measured using a Qubit dsDNA High Sensitivity DNA Quantification Assay (Thermofisher Scientific, Waltham, MA, USA). Finally, the DNA was eluted using 50 µl of AE buffer and stored at -20°C.

DNA extraction from the coffee filters used the DNeasy PowerMax Soil Kit (Qiagen Inc., Germantown, MD, USA). Coffee filters were placed into a falcon tube and vortexed with the PowerBead solution, followed by the addition of C1 and a second round of vortexing. The tubes were then loaded onto a bead-beater (OMNI BeadRupter) set at 4 m^{-s} for two cycles of 30 seconds each. After centrifugation, the supernatant was transferred to a new collection tube, and C2 solution was added before a 10-minute incubation. The samples were then centrifuged again, C3 was added, and a second 10-minute incubation followed. After another round of centrifugation and supernatant transfer, C4 solution was added and poured onto a spin column. The flow-through was discarded, C5 was added to wash the columns, and the DNA concentration was measured with a Qubit dsDNA High Sensitivity DNA Quantification Assay (ThermoFisher Scientific, Waltham, MA, USA). The DNA was eluted using 1 mL of C6 and stored at -20°C.

2.2.8.5 Polymerase Chain Reaction (PCR) and DNA Sequencing

Following DNA extractions from the Sterivex™ and coffee filters, PCR and sequencing were performed at Jonah Ventures (Colorado, USA). Using the MiFishUF and MiFishUR primers (Forward Primer: GTCGGTAA AACTCGTGCCAGC; Reverse Primer: CATAGTGGGGTATCTAATCCCAGTTTG) (Miya et al., 2015), portions of the hypervariable regions of the mitochondrial 12S ribosomal RNA gene were PCR amplified. Following PCR, the amplicons were cleaned, a second round of PCR was performed, and the final indexed amplicons were cleaned and normalized. Next, the final library was sequenced using an Oxford Nanopore Technologies MinION (Oxford, England) with the Ligation Sequencing Kit V14 (cat# SQK-LSK114). A detailed protocol followed by Jonah Ventures is provided in Appendix D.

2.2.8.6 Bioinformatics

Demultiplexing was performed by Jonah Ventures, which involved converting the raw nanopore sequencing data from pod5 to fastq using Dorado v0.7.1 (Oxford Nanopore Technologies, 2024) (Appendix D). Cutadapt v3.4 was used to trim adapters and orient the reads from 5' to 3'. The reads were demultiplexed with pheniqs v2.1.0 (Galanti et al., 2021), after which Cutadapt was used again to extract the target amplicon by removing the primers, discarding any reads where one or both primers were not found or where the resulting amplicon sequences were < 130 bp or > 210 bp. Exact sequence variants (ESVs) were then identified using unoise3 denoising algorithm (Edgar, 2016) in vsearch (Rognes et al., 2016). Final read counts were determined by mapping the raw reads to the identified ESVs using the vsearch function “usearch_global” with a minimum identity of 95%.

Each final ESV was assigned a taxonomy based on a reference database of publicly available sequences on NCBI Genbank (Benson et al., 2005) and a Jonah Ventures voucher sequence records. A detailed protocol followed by Jonah Ventures is provided in Appendix D. Taxonomic assignment was conducted using a consensus taxonomic assignment using either 100% matching reference sequence or all reference sequences within the top 1%, with a threshold for any taxonomic level with >90% agreement with the top hits. The number of fish species detected at each site for 2023 was calculated using R software (version 4.3.3) with RStudio interface (R Core Team, 2022).

A master taxa list was compiled based on species deemed geographically relevant based on available literature (Allen et al., 2002; Allen et al., 2023; Gold et al., 2020). This provided a baseline list of species for initial database matching, enabling the exclusion of species known to be absent from the study area. A gap analysis using R compared the master taxa list to the available 12S sequences available on GenBank, MitoHelper (Lim & Thompson, 2024) and FishCARD (Gold et al., 2020) to identify gaps in fish species sequences records.

2.2.9 Data Analyses

2.2.9.1 Objective 1: Comparing Species Accumulation Curves using Traditional Survey Data

Fish species richness data gathered in 2023 by traditional sampling (seining and enclosure traps) was consolidated across the five sites and used to generate species accumulation curves. For block net seining, a sampling event was defined as six hauls conducted within a single block net. For enclosure trap sampling, a sampling event was defined as one deployed enclosure trap.

Since the data were obtained from two distinct monitoring programs, the number of seining events and enclosure traps varied among sites. Species accumulation curves (SACs) were generated separately for the seining and enclosure trap datasets across all five sites. SACs were used to examine how species richness varied with sampling effort at each site. Curves were produced using 1,000 permutations of the "random" method in the vegan package (Oksanen et al., 2024) in R (version 4.3.3) with the RStudio interface (R Core Team, 2022). The weighted "random" approach averages species richness through linear interpolation of single random permutations (Oksanen et al., 2024). The final point of the species accumulation curve corresponds with the total number of species found at each site in 2023.

2.2.9.2 Objective 2: Comparison of Methods for Fish Species Detection

2.2.9.2.1 Species Richness

Fish species richness data gathered in 2023 by traditional sampling (seining and enclosure traps), iNaturalist, and eDNA across the five study estuaries in San Diego were consolidated into one dataset used to answer objective 2. This dataset was used to provide a comparison of fish species detected by different sampling approaches and to calculate the proportion of species detected by each approach at each site. It is important to note that the sampling methods collected data over three different temporal scales - traditional sampling was conducted annually, iNaturalist data were collected continuously (but irregularly) throughout the year, and eDNA sampling was limited to a single period. The research questions addressed, and the statistical analyses performed, considered these temporal differences and the dynamic nature of community compositions.

Heatmaps were produced using R (R Core Team, 2022) to provide a visualization of which species were detected using each approach. To generate the heatmap, the data were organized into

a matrix with species observations as rows, sites as columns, and binary species presence-absence data as values. The ggplot2 and reshape2 packages (Wickham, 2007) were used to transform and plot the data. From this, the proportion of species detected by different approaches was calculated.

2.2.9.2.2 Community Composition

To characterize the species collected through these approaches, fish communities were assessed by species richness and guilds. Fish were categorized into the 24 guilds defined by Bond et al. (1999), which are based on species' habitat use, feeding methods and behavior. Where fish did not have an assigned guild in literature, guilds were assigned according to their life history traits. The assigned guilds, as well as a description of each guild, allocated to species found in this project are shown in Table 2.2. This data was visualized using the ggplot2 package (version 3.5.1) (Wickham, 2016).

2.2.9.3 Data analysis for Objective 3: Trends in Species Richness Over Time at TJE and SDL

Long-term fish monitoring data were obtained from the San Onofre Nuclear Generating Station (SONGS) San Dieguito Wetland Restoration Mitigation Monitoring Program (Schroeter, 2023) (https://marinemitigation.msi.ucsb.edu, accessed on September 24, 2024). This dataset included fish species richness, recorded using block net seining and enclosure traps (see method in Section 2.2.6.1 and 2.2.2.2), and water quality measurements (temperature, dissolved oxygen, and salinity) collected from 2013-2023. The fish species richness data were collected during the fall (August–October), while the water quality data were collected throughout each year in 15-minute intervals using continuously recording environmental data loggers (e.g., HOBO Dissolved Oxygen Datalogger U26-001, HOBO U24-002-C Conductivity Logger) (see Appendix A for

further data collection details). These long-term datasets were used to assess and compare species richness trends over time across these two systems.

Initial analyses included the generation of collector's curves for both TJE and SDL using vegan on R to show the cumulative number of species observed with increasing sampling effort over time. Data visualizations were created using the ggplot2 package (version 3.5.1) (Wickham, 2016).

Water quality data were processed in R to derive and plot the 7-day moving average of temperature, salinity, and dissolved oxygen at both sites. Additionally, the number of days of hypoxia was calculated based on dissolved oxygen concentrations, with hypoxia defined as <3 mg/L, following SONGS monitoring standards (Appendix A). This was plotted against the number of days of flow recorded for this site. These water quality parameters were then compared to annual species richness trends at the TJE and SDL from 2013 to 2023.

2.3 Results

2.3.1 Objective 1: Comparing Species Accumulation Curves using Traditional Survey Data

For both the block net seining and enclosure trap data, TJE and SDL had the highest number of sampling events, while LPL had the lowest number of sampling events (Figure 2.2). The results from the 2023 block net seining data indicate that fish species richness at each coastal wetland plateaued with varying degrees of sampling effort (Figure 2.2). Fish species richness at TJE plateaued at ~ 10 samples, while SDL, SWM, and KFR plateaued at ~ 20 samples. LPL had insufficient samples (n=4) to reach a plateau indicating that this site is under sampled, however this curve followed a similar trajectory to SDB and SDL. When looking at an equivalent number of samples (n=20), block net seining detected the greatest number of species at SDL, then SDB,

followed by KFR, LPL (despite this site only having 4 sampling events) and finally TJE. Interestingly, SDL had approximately 4.5 times the number of fish species detected compared to the TJE in 2023 when sampling effort exceeded 20 (Figure 2.2.A).

For the 2023 enclosure trap data, San Diego Bay (Sweetwater Marsh) and MB reached a plateau after ~12 samples, while SDL reached a plateau after ~3 samples (Figure 2.2.B). No species were detected using enclosure traps at TJE despite a high sampling effort (60 sample events). Similar to block net seining, at an equivalent sampling effort (20 samples), the highest number of species was detected at SDL, then SWM, followed by KFR and finally TJE, with almost double the number of species detected at SDL than SWM and KFR. Enclosure traps detected four species of Gobiidae: the arrow goby (*Clevelandia ios),* cheekspot goby (*Ilypnus gilberti),* shadow goby *(Quietula y-cauda)*, and an unknown species of Gobiidae (likely a juvenile that could not be identified). Gobies captured in block nets were not counted as part of this study; instead, enclosure traps were specifically used to sample and assess Gobiidae richness. These detections were included in our study to provide a more complete assessment of species richness.

2.3.2 Objective 2: Methods Comparison for Species Detection

2.3.2.1 Traditional Sampling

Overall, a total of 30 species of fish were detected by traditional sampling (a combination of block net seining and enclosure traps). The highest number of species were detected at SDL (n=21) and San Diego Bay (Sweetwater Marsh) (n=16) (Table 2.3). TJE had the lowest number of species detected (n=4), despite having a high sampling effort (Figure 2.3, Table 2.3). Block net seining detected 26 fish species from 20 families across 12 orders (Figure 2.3). The four most widespread species (not referring to abundance) detected across the systems were California killifish

(*Fundulus parvipinnis*) and topsmelt silverside (*Atherinops affinis*) (both found in all systems), as well as arrow goby (*Clevelandia ios*) and shadow goby (*Quietula y-cauda*) (both detected in 4 of the 5 systems) (Figure 2.3).

2.3.2.2 Community Science (iNaturalist)

Of the 1834 iNaturalist observations, a total of 73 species of fish were detected using iNaturalist across the full extent of each coastal wetland in 2023. The highest number of species was detected in MB (n=59), then SDB (n=52), SDL (n=16), TJE (n=11) and LPL (n=7) (Figure 2.3, Table 2.3). The four most widespread species (not referring to abundance) were detected in 4 of the 5 systems and included California killifish (*Fundulus parvipinnis*), sailfin molly (*Poecilia latipinna*, Haller's round ray (*Urolophus halleri*), and bat ray (*Myliobatis californica*).

2.3.2.3 Environmental DNA

2.3.2.3.1 Sequencing Results

Of the 89 samples (42 coffee filters, 47 Sterivex filters) collected at 13 stations across the 5 sites, the eDNA sequencing run produced 413,388 reads. A total of 37 species were detected which included fish and off-target species such as *Homo sapiens* (humans), *Batrachoseps nigriventris* (black-bellied slender salamander), *Sus scrofa* (wild boar), and *Aythya americana* (redhead duck). Sequences that could not be identified to the species level are listed as *Genus* sp.

The proportion of sequence reads assigned to each taxon, including off-target detections grouped as “Other,” is summarized in Supplementary Table S1. Off-target taxa represented only a small fraction of total sequence reads across all sites. Detailed read counts for each site and replicate are also provided in Supplementary Table S1.

After removing off-target species, potential contamination and geographically unlikely taxa, the total eDNA fish data set consisted of 173,121 reads for 26 fish species and 6 taxa identified to the genus level (*Menidia* spp., *Sardinops* spp., *Scomber* spp., *Syngnathus* spp., *Thunnus* spp., *Gymnogobius* spp.). Despite following a consistent protocol across the samples, only 10 (7 coffee filters, 3 Sterivex filters) of the 89 samples had sequence reads above 8,000 base pairs and were considered of sufficient quality to use in further analyses. Such a low success rate was a surprising result, and this is further discussed in Chapter 3. Given the limited eDNA success, these results should be considered preliminary.

2.3.2.3.2 Gap Analysis

A gap analysis revealed that 92% of the species included in our master taxa list, which lists species that are likely to occur in the San Diego region, have reference sequences available in at least one of three databases: GenBank, FishCARD, or MitoHelper (Supplementary Table S2). The remaining species were without reference sequences and therefore require future targeted sampling to improve reference database coverage (listed in Table 2.4).

2.3.2.4 Species Richness Detected by eDNA

A total of 31 species of fish were detected using eDNA. The highest number of species was detected in SDL (n=27), then MB (Kendall Frost) (n=8), TJE (n=7), San Diego Bay (Sweetwater Marsh and South Salt Ponds) and LPL (both with n=6) (Figure 2.3, Table 2.3). The five most widespread species detected across the systems were flathead grey mullet (*Mugil cephalus*) (all systems), Longjaw mudsucker (*Gillichthys mirabilis;* 4 of the 5 systems), topsmelt silverside (*Atherinops affinis*) (4 of the 5 systems), spotted sand bass (*Paralabrax*

maculatofasciatus) (3 of the 5 systems), and California killifish (*Fundulus parvipinnis*) (3 of the 5 systems).

2.3.2.5 Species Richness Comparison Across Methods

Across all the sites, the traditional sampling, iNaturalist and eDNA detected 10 of the same species. iNaturalist detected 44 unique species, while traditional sampling detected 6 unique species and eDNA 1 species (Figure 2.9). The site-specific results showed that iNaturalist recorded the highest proportion of species at MB (77%), San Diego Bay (73%), and the TJE (50%) (Figure 2.10). In contrast, SDL had the greatest proportion of species detected by eDNA (25%), while LPL exhibited the highest contribution from a combination of traditional sampling and iNaturalist (33%).

2.3.2.6 Detection of non-native fish species

Across all monitoring approaches, five invasive species were detected across the study sites in 2023, including the Sailfin molly (*Poecilia latipinna*) at four of the sites, yellowfin goby (*Acanthogobius flavimanus*) at three of the sites, western mosquitofish (*Gambusia affinis*) at two of the sites, and inland silverside (*Menidia beryllina*) and Largemouth bass (*Micropterus salmoides*), each recorded at one site. Only one non-native species was recorded at LPL, while SDL had the highest number of detections, likely a result of the lowest sampling effort at the former and the highest at the latter. Of all wetlands where non-native species were observed, traditional sampling accounted for 25% of detections, a combination of traditional sampling and iNaturalist for 50%, a combination of eDNA and iNaturalist for 14%, and iNaturalist alone for 8%.

2.3.2.7 Community Composition

A comparison of species guilds and detection methods across sites revealed that iNaturalist recorded the highest number of species in guild 19 (substrate-associated hiders in holes and crevices), guild 16 (substrate-associated benthic foragers and mesocarnivores), and guild 22 (substrate-associated diggers and extractors) (Figure 2.11). Traditional sampling primarily detected species from guild 19 and guild 2 (water-column associated, diurnal, selective feeders), while eDNA was most effective at detecting species in guild 19.

2.3.3 Objective 3: Trends in Species Richness Over Time at TJE and SDL

Long-term traditional sampling data (block net seining and enclosure traps) from the TJE and SDL indicate that SDL has consistently supported a higher cumulative fish species richness compared to the TJE (Figure 2.12). From 2013 to 2021, both wetlands exhibited an overall increasing trend in cumulative species richness. However, between 2021 and 2023, cumulative richness at the TJE plateaued, while SDL continued to show an increase in species detections. Annual species richness reveals more variability: TJE experienced a general decline from 2017 to 2023, whereas SDL showed a temporary decrease from 2016 to 2020, followed by a recovery and increase through 2023.

Environmental data from SDL (Figure 2.13) suggest relatively stable conditions over time, with infrequent periods of hypoxia (DO < 3 mg/L) and supersaturation (DO > 10 mg/L) occurring primarily in 2014, 2020, and 2023. In contrast, TJE (Figure 2.14) has experienced frequent and prolonged episodes of both hypoxia and supersaturation since 2013, with particularly pronounced hypoxic events beginning in 2019. This period of increased oxygen stress coincides with the observed decline in annual species richness at TJE (Figure 2.15). These conditions are likely driven

by episodic freshwater inflows and catchment runoff, as indicated by the alignment of elevated flow periods with extended durations of hypoxia (Figure 2.15).

In 2023 only four fish species were detected at the TJE: topsmelt silverside (*Atherinops affinis*), California killifish (*Fundulus parvipinnis*), flathead grey mullet (*Mugil cephalus*), and western mosquitofish (*Gambusia affinis*), the latter being a non-native species in San Diego (Table 2.5). Notably, 2023 also marked the first detection of the non-native fish, inland silverside (*Menidia beryllina*) at SDL (Table 2.6).

2.4 Discussion

Monitoring fish communities in estuarine systems and within associated coastal wetlands is essential for evaluating restoration success, ensuring compliance with mitigation requirements, and understanding long-term ecological trends in the face of climate change and human development (Desmond et al., 2002). In San Diego, traditional fish sampling methods have historically served as the foundation of estuarine monitoring efforts. However, recent advancements have introduced complementary approaches, including environmental DNA (eDNA) metabarcoding, baited remote underwater videos (BRUVs), and community science platforms such as iNaturalist.

This study highlights that complementary monitoring methods are necessary to capture a more accurate representation of fish species richness in San Diego's estuaries. In addition, long-term data shows a sharp contrast between declining richness at TJE (linked to ongoing water quality stressors) and increasing richness at SDL (linked to restoration and improved conditions).

2.4.1 Objective 1: Comparing Species Accumulation Curves using Traditional Survey Data

Sampling effort varied across the five study sites, with TJE and SDL having the highest effort, followed by MB (Kendall-Frost), San Diego Bay (Sweetwater Marsh), and LPL. Species accumulation curves (SACs) indicated that approximately 20 sampling events were required to approach asymptotic species richness across most estuaries. This finding is consistent with Allen (1982), who reported that 66% of fish assemblages in Newport Bay, California, were adequately sampled before reaching 20 sampling events. At SDL and TJE, sampling efforts exceeded 20 events and reached a plateau, whereas LPL exhibited a linear increase in species richness, indicating insufficient sampling and likely underestimation of species richness. As such, future sampling at LPL should have an increased sampling effort. When comparing sites at an equal sampling effort of 20 events, SDL exhibited the highest species richness, followed by San Diego Bay (Sweetwater Marsh), MB (Kendall-Frost), LPL (despite only four total sampling events), and finally TJE.

2.4.2 Objective 2: Comparison of Methods for Fish Species Detection

2.4.2.1 Traditional Sampling

The high species richness at SDL (21 species) can likely be attributed to favorable environmental conditions and adequate sampling effort. Conversely, despite substantial sampling effort at TJE, only four species were detected. While TJE is regarded as one of the most intact salt marshes in southern California and has held National Estuarine Research Reserve status since 1981 (Zedler et al., 2001), it has also experienced long-standing water quality challenges and

sedimentation issues that may have influenced species richness. Further discussion of environmental conditions at TJE is provided in Section 2.4.3 (Objective 3).

Previous research by Desmond et al. (2002), which analyzed 11 years of block net seining data (1987–1998) from TJE, San Diego Bay (Sweetwater Marsh), and LPL, recorded a total of 37 fish species. Nearly 98% of individuals belonged to just seven dominant species: arrow goby (*Clevelandia ios*), topsmelt silverside (*Atherinops affinis*), California killifish (*Fundulus parvipinnis*), shadow goby (*Quietula y-cauda*), longjaw mudsucker (*Gillichthys mirabilis*), and Pacific staghorn sculpin (*Leptocottus armatus*). Most of these species were also detected in the present study across the same three sites, with a few exceptions: arrow goby (*Clevelandia ios*) and shadow goby were not detected at TJE; topsmelt silverside (*Atherinops affinis*) was not detected at LPL; longjaw mudsucker (*Gillichthys mirabilis*) was absent from both TJE and LPL; and Pacific staghorn sculpin (*Leptocottus armatus*) was not detected at TJE, SDB, or LPL using block net seining or enclosure traps. The absence of these species from TJE is likely attributable to reduced water quality, which is discussed in Section 2.4.3 (Objective 3).

Despite its strengths in providing abundance and size data, traditional sampling remains labor-intensive, prone to sampling biases (e.g., net avoidance, habitat inaccessibility), and reliant on increasingly scarce taxonomic expertise. Using these approaches solely to get an estimate on fish species richness may result in cryptic, rare, or nocturnal species being overlooked and underestimated (Yoklavich et al., 1991).

2.4.2.2 Community Science (iNaturalist)

Community science data from iNaturalist yielded the highest number of unique fish species detections across all methods (Figures 2.9 and 2.10), particularly in highly accessible and

recreationally active areas such as MB and SDB. These two bays are the largest systems in this study and encompass a wide range of habitats. In fact, SDB is the third largest bay-estuarine system in California, after San Francisco Bay and Humboldt Bay (Allen et al., 2006), which can likely be attributed to this site's high species richness. California needlefish (*Strongylura exilis*), California butterfly ray (*Gymnura marmorata*) and shortfin corvina (*Cynoscion parvipinnis*) are warm water species that were detected by iNaturalist in SDB. These are tropical/subtropical fish that were previously detected during a study in SDB from 1994 to 1999 (Allen et al., 2002). It is thought that these warm-water species find refuge in shallow portions of SDB, particularly during periods of warmer oceans associated with El Niño events and more recently sustained warming associating with global warming (Allen et al., 2006). El Niño conditions were reported in 2023-2024 and may explain the presence of these species. Further analyses of long-term species richness data would need to be performed to determine whether these species have become established in SDB beyond El Niño conditions.

iNaturalist detected lower species richness in less accessible areas like LPL and SDL, likely due to restricted public access and fishing prohibitions. This suggests that these sites would benefit from organized community science initiatives, such as BioBlitz events, to enhance representation of these systems. Approaches could include the deployment of baited remote underwater videos (BRUVs) or shore-based visual surveys in areas where fishing is restricted.

Although fish are generally considered challenging to document through photographic record (Rocha et al., 2024), the iNaturalist dataset proved to be a valuable resource in this study. The high number of unique species contributed by iNaturalist suggests that community science may be an underutilized tool in current monitoring frameworks and should be more formally integrated into annual reporting efforts. As a freely accessible platform, iNaturalist provides a low-

cost, publicly driven data source that may serve as an early warning system for the detection of new non-native species. (Valdivia-Carrillo et al., 2021)

However, the use of iNaturalist is not without limitations, many of which are addressed in Chapter 3. Observations may be subject to misidentification or geolocation inaccuracies (Contreras-Díaz et al., 2023), emphasizing the need for validation through complementary methods. While species verification protocols such as those proposed by Ackland et al. (2024), which assign confidence scores to key criteria, can improve data reliability, these approaches are time-intensive, typically requiring around 1.5 minutes per observation. This approach becomes impractical in studies with over a thousand records, such as this one. As such, this study was limited to "research grade" observations, which rely on community consensus for species identification. However, even these can occasionally include misidentified records (Contreras-Díaz et al., 2023). To enhance the reliability of iNaturalist data, user education should be prioritized. Promoting best practices, such as avoiding speculative identifications, using relevant tags, and including descriptive comments, can improve the quality and utility of observations. Additionally, increasing participation in underrepresented regions, such as LPL, could improve spatial coverage and enhance the overall effectiveness of iNaturalist as a complementary monitoring tool.

2.4.2.3 Community Composition

Ecological guilds provide an important lens for understanding estuarine fish communities because they group species by shared ecological roles rather than taxonomic identity. Examining guild-level patterns can reveal how well different monitoring methods capture the functional diversity of estuarine systems.

Traditional sampling detected 11 of the 19 guilds (Figure 2.11). The guilds most frequently represented were guild 19 (substrate-associated species that hide in holes or crevices) and guild 2 (diurnally active, water-column associated selective feeders) (Table 2.2). The frequent detection of guild 19 suggests that enclosure traps were effective at capturing Gobiidae, a family typically associated with this guild and are likely to be collected by block net seining and enclosure traps which disturb the channel and tidal creek floor, displacing species along the substrate. Guild 2 includes species such as mosquitofish (*Gambusia affinis*) and topsmelt (*Atherinops affinis*), which are common within these estuarine environments. In contrast, several guilds (7, 9, 11, 12, 14, 17, 20, and 21) were not detected. These guilds include non-schooling, benthic species that often inhabit deeper areas of estuaries, which are generally inaccessible to shallow seining techniques. Additionally, larger, more mobile species were infrequently detected, likely due to their preference for deeper waters beyond the effective range of traditional gear. Nonetheless, a few larger-bodied species such as Haller's round ray (*Urolophus halleri*) and California halibut (*Paralichthys californicus*) were captured. These detections likely reflect the presence of juvenile individuals in shallow nursery habitats. The latter species, of commercial significance, is known to consume small substrate-associated prey, including gobies, during early life stages (Allen, 1988).

Community science data from iNaturalist also showed a bias towards substrate-associated guilds. The highest number of species belonged to guild 19 (substrate-associated species that hide in holes or crevices), guild 16 (substrate-associated benthic foragers and mesocarnivores), and guild 22 (substrate-associated diggers and extractors). The bias towards substrate-associated species is due to community science observations typically occurring in shallow waters where individuals visually observed fish from shore, encountered them during dives in benthic habitats, or captured them through shore-based fishing, which tends to target substrate-associated species.

These results highlight both the strengths and limitations of traditional surveys and iNaturalist in capturing functional diversity. While both approaches are effective at detecting small, substrate-associated, and abundant species, they underrepresent guilds associated with deeper or structurally complex habitats, as well as larger, more mobile species.

2.4.2.4 Environmental DNA (eDNA)

eDNA metabarcoding detected 25 fish species across the five estuarine sites, despite a low success rate, with only 10 of the collected samples yielding sufficient quality data. A review of the approach used and recommendations for future eDNA metabarcoding studies are provided in Chapter 3. Several species were detected exclusively by eDNA at one or more sites, such as kelp clingfish (*Rimicola muscarum*), California sheephead (*Bodianus pulcher*), and blacksmith chromis (*Chromis punctipinnis*), and were not observed in either traditional or iNaturalist datasets. However, the known distribution ranges for these species supports the assumption that these are likely true positives. One potential explanation for this discrepancy is the temporal mismatch between eDNA collection and traditional surveys, which were not necessarily conducted on the same day. Additionally, traditional sampling methods tend to be more effective in shallow, muddy, or sandy habitats, whereas many of the species missed, such as blacksmith chromis (both rocky reef-associated), and kelp clingfish and California sheephead (both kelp forest-associated), are typically found in more structurally complex, hard-bottom habitats that are underrepresented in traditional sampling efforts.

However, when interpreting eDNA results, it is important to account for the potential detection of non-resident or offshore species due to the downstream transport of marine DNA and upstream contributions from freshwater sources. For example, the reef finspot (*Paraclinus*

integripinnis), a species typically associated with coastal habitats such as rocky shores and tidal pools, was detected only through eDNA at LPL. Its presence within the estuarine system is uncertain and may instead reflect passive DNA transport from adjacent marine environments rather than true habitat use. The transport and degradation of eDNA is an active area of research (Andruszkiewicz et al., 2019; Nagarajan et al., 2022), with a growing focus on understanding how eDNA can be reliably linked to species abundance (Harrison et al., 2019). While literature on eDNA dynamics in low-inflow estuaries remains limited, studies in marine systems suggest that eDNA can be transported over tens of kilometers within a few days. Processes such as horizontal advection, decay, and settling are considered primary drivers of eDNA movement in coastal waters (Andruszkiewicz et al., 2019). These estimates are often derived from modeling approaches that rely on assumptions, such as constant eDNA decay rates and settling rates analogous to marine snow, due to the lack of direct measurements in comparable environments. In low-flow marine environments, eDNA concentrations tend to decrease sharply with distance from the source, creating steep spatial gradients. Under such conditions, distinct species assemblages may be separated by as little as 60 meters (Eble et al., 2020; Port et al., 2016).

Most species detected through eDNA in this study aligned with existing species richness records. A comparison of eDNA species detected to long-term historical species richness data at TJE and SDL was performed (Figure 2.3 and Tables 2.5 and 2.6). In terms of TJE, several species detected by eDNA were found to overlap with historical species richness records. Species such as the longjaw mudsucker (*Gillichthys mirabilis*), flathead grey mullet (*Mugil cephalus*), and kelp bass (*Paralabrax clathratus*), have all been commonly recorded through traditional approaches as part of the SONGS monitoring plan. Previous research indicated that dominant fish species in TJE included longjaw mudsucker (*Gillichthys mirabilis*) (detected through eDNA), California killifish

(*Fundulus parvipinnis*) (traditional approaches) and arrow goby (*Clevelandia ios*) (not detected in our study) (Desmond et al., 2002). These same species have also been found to occur in SDB: Sweetwater Marsh by Desmond et al. (2002) and were detected through traditional surveys and iNaturalist during our study. Some detections, such as black surfperch (*Embiotoca jacksoni*) and wrasse (*Halichoeres semicinctus*), were novel for TJE, demonstrating the capacity of eDNA to identify cryptic or previously undetected species.

At SDL, eDNA revealed the highest number of species across the sites and included both historically recorded taxa and new detections. Species such as the bay blenny (*Hypsoblennius gentilis*), California killifish (*Fundulus parvipinnis*), yellowfin goby (*Acanthogobius flavimanus*), longjaw mudsucker (*Gillichthys mirabilis*), and flathead grey mullet (*Mugil cephalus*) have all been commonly recorded through traditional approaches as part of the SONGS monitoring plan. Species such as yellowfin croaker (*Umbrina roncador*) and kelp bass (*Paralabrax clathratus*), detected only once in 2022 and 2013, respectively, through traditional surveys, were identified by eDNA in 2023.

Since eDNA detection is limited by the completeness of reference databases, this study included a gap analysis to assess which species may have been overlooked due to the absence of 12S rRNA reference sequences in publicly available databases. The results indicated that 92% of fish species considered geographically likely to occur in the San Diego region have a reference sequence available in at least one of the following databases: GenBank, MitoHelper and/or FishCard (Supplementary Table S2). The species lacking reference sequences are generally obscure and unlikely to inhabit the study systems. However, five exceptions were noted that were either detected by traditional sampling, iNaturalist or previous research (Table 2.4). These included California halfbeak (*Hyporhamphus rosae*) (previously detected in San Diego Bay by Allen et al.

(2002), cheekspot goby (*Ilypnus gilberti*), specklefin midshipman (*Porichthys myriaster*), slender clingfish (*Rimicola eigenmanni),* California salema (*Xenistius californiensis*), and white seabass (*Cynoscion nobilis*) (likely in MB and San Diego Bay according to online fishing reports). As reference databases and methods continue to improve, eDNA sequence data can be reanalyzed to test for the presence of previously missed or unresolved taxa (Gold et al., 2023).

As with previously discussed approaches, eDNA has several limitations. For example, the MiFish Universal primer is not effective at identifying *Sebastes* (rockfish) species, a key component of coastal California fish communities (Gold, 2020). Moreover, during this study, high sediment and organic matter loads in the estuarine systems resulted in the filters clogging and inhibited PCR amplification (Baidouri et al., 2025). These limitations, combined with the restricted temporal and spatial scope of sampling due to time and funding constraints, reduced the overall resolution of the dataset. Nonetheless, eDNA remains a promising complementary tool for early detection of non-native species and enhancing traditional biodiversity assessments. Further discussion about the limitations and advancements in eDNA has been provided in Chapter 3.

2.4.2.5 Comparison of Methods for Species Detection

Despite extreme differences in the method of each approach, traditional sampling (block net seining and enclosure traps), iNaturalist and eDNA surveys captured 10 overlapping fish species across the five estuaries in San Diego (Figure 2.9). Traditional surveys remain essential for providing information on abundance, life stages, and sex ratios, which are critical for resource management. iNaturalist contributes broad spatial coverage and real-time observations, while eDNA shows potential for detecting cryptic or elusive species. However, the low success rate of

eDNA samples in this study highlights the need for further method refinement and pilot studies for estuaries in southern California.

In this study, eDNA detected only one species not recorded by the other approaches, shared 11 species with iNaturalist, and shared 10 species with both methods (Figure 2.9). These findings are not supported by previous research, which has consistently demonstrated the high sensitivity of eDNA for detecting species that may be overlooked by other methods (Gold et al., 2023; Kelly et al., 2014; Port et al., 2016). eDNA has been shown to detect higher species richness than traditional approaches, including scuba-based visual surveys (Waters et al., 2023), seine netting (Maracle et al., 2024), and aerial surveys (Hunter et al., 2018). For example, Maracle et al. (2024) compared eDNA metabarcoding and seine netting along a 350 km stretch of the Upper St. Lawrence River in Canada, and found that eDNA detected 67 species, nearly double the 38 identified through seining. Similarly, a study in southern California at Big Geiger Cove and Two Harbors found that eDNA captured over 75% of the species observed via SCUBA surveys, as well as additional species missed due to camouflage, nocturnal behavior, or egg deposition patterns (Waters et al., 2023). Incorporating multiple detection methods can help capture a broader range of ecological guilds and provide a more comprehensive understanding of estuarine fish communities. However, due to the limited number of successfully sequenced eDNA samples, it is difficult to draw strong conclusions or compare these results directly with previous research.

A key benefit of both eDNA and iNaturalist compared to traditional surveys is that DNA sequences and iNaturalist photographs serve as verifiable, archivable records linked to specific times and locations, and can be re-examined or re-analyzed as reference databases and taxonomic knowledge improve (Deiner et al., 2017). Unlike traditional seine data, which typically consist of field notes or summary tables, these records provide direct evidence (genetic material or images)

that can be independently validated. For example, as eDNA reference databases and methods improve, sequence data can be reanalyzed to test for the presence of previously missed or unresolved taxa, such as the white seabass (*Atractoscion nobilis*), which is currently lacking from reference databases (Gold et al., 2023).

2.4.2.6 Detection of Non-Native Species

Five non-native fish species were detected across three of the five study sites. These species included inland silverside (*Menidia beryllina*) (detected at SDL), largemouth bass (*Micropterus salmoides*) (detected at LPL), western mosquito fish (*Gambusia affinis*) (detected at TJE and San Diego Bay), sailfin molly (*Poecilia latipinna*) (detected at all sites, except SDL), and yellowfin goby (*Acanthogobius flavimanus*) (detected at all sites, except TJE). These species, apart from the yellowfin goby (*Acanthogobius flavimanus*), are freshwater-associated, and their presence may be linked to "urban drool," a phenomenon driven by anthropogenic freshwater inputs from activities such as water importation, outdoor irrigation, and infrastructure leaks or failures, which collectively contribute excess freshwater to local systems (Bierzychudek, 2022).

2.4.3 Objective 3: Trends in Species Richness Over Time at TJE and SDL

Long-term monitoring shows diverging trends in species richness at TJE and SDL, with richness at TJE declining over the past decade. This decline is likely driven by increasing hypoxia, sedimentation, and episodic sewage discharges from the watershed which extends within both Mexico and the United States (Mladenov et al., 2024; Zedler et al., 2001). Flows in the Tijuana River are often dominated by untreated sewage, groundwater, and stormwater runoff (Mladenov et al., 2024). This issue stems from aging and deteriorating infrastructure, the insufficient capacity

of the International Wastewater Treatment Plant, and the lack of formalized services to support Tijuana's growing population. When volumes exceed the International Wastewater Treatment Plant's capacity (1.0 m^3/s), excess flow is diverted, allowing untreated sewage to enter the estuary and coastal waters, ultimately causing elevated fecal indicator bacteria and eutrophication, which results in low-oxygen conditions. Dissolved oxygen levels are influenced by tidal dynamics, with higher levels during spring tides and high tides (due to seawater influx), and lower levels during neap and low tides, when stratification increases (Ross et al., 2001). In addition to these tidal influences, dissolved oxygen cycles daily, with oxygen concentrations rising during the day due to aquatic plant photosynthesis and falling sharply at night due to respiration (Ross et al., 2001). This results in highly variable dissolved oxygen trends, as is evident in our results which show more pronounced fluctuations at TJE (Figures 2.13 and 2.14). These pronounced fluctuations are likely associated with periods of eutrophication associated with sewage spills, which causes increased plant and algal biomass and amplifies dissolved oxygen extremes (Ross et al., 2001).

Short-term exposure (on the scale of days) to low dissolved oxygen does not generally cause significant harm to estuarine fish communities, though it can alter fish behavior, for example by allowing opportunistic feeding on benthic prey with reduced burial depth (Pihl et al., 1992). In contrast, more prolonged and frequent hypoxic events (on the scale of weeks to months) have been shown in other estuarine systems to reduce fish abundance, increase mortality, inhibit growth, and shift species distributions (Breitburg, 2002; Kramer, 1987). Prolonged periods of supersaturation have also been linked to negative effects on fish, including reduced swimming performance and, in severe cases, mortality (Ross et al., 2001). Similar hypoxic and supersaturation conditions have been documented at TJE in recent years, raising concern that these broader impacts on fish communities may also occur locally. Under such degraded water quality, few species are able to

persist, and those that do are typically generalists with broad environmental tolerances, such as the California killifish (*Fundulus parvipinnis*), flathead grey mullet (*Mugil cephalus*), western mosquitofish (*Gambusia affinis*), and topsmelt silverside (*Atherinops affinis*) (Allen et al., 2006). In contrast with TJE, species richness at SDL has increased since 2020, coinciding with improved water quality and long-term restoration efforts that have expanded estuarine habitat over the past decade (Beheshti et al., 2023).

2.5 Conclusion

Fish play an important role in the ecosystem functioning of estuaries, which are increasingly threatened by biodiversity loss and species shifts associated with climate change. In this study, 25 fish species were recorded using traditional sampling methods (block net seining and enclosure traps), 74 species were documented through iNaturalist, and 22 species were detected using environmental DNA (eDNA) metabarcoding. These results highlight the strengths and limitations of each method in assessing fish biodiversity in southern California's coastal wetlands. Traditional sampling provides robust, standardized data that is essential for long-term monitoring. iNaturalist, a community science platform, offers broad spatial and temporal coverage at no financial cost and contributes significantly to species inventories. eDNA metabarcoding presents a promising, non-invasive tool capable of detecting elusive or non-native species that may be missed by other approaches. However, its effectiveness can be compromised in estuarine environments with high sediment and organic matter loads, which can hinder DNA extraction and sequencing success.

Consistent with findings from previous studies (Gold et al., 2023; Kelly et al., 2014; Port et al., 2016), our results suggest that these methods are best used in combination. While eDNA

and iNaturalist expand the breadth of species detection, traditional sampling provides critical information on fish abundance, size classes, and life stages that are not captured by the other methods.

Long-term trends at TJE and SDL reveal diverging patterns in species richness over the past decade. TJE has experienced a concerning decline in richness, likely driven by increasing hypoxia and supersaturation events linked to ongoing sewage inputs in the watershed. In contrast, SDL has shown a continued species increase, reflecting improving habitat conditions supported by recent restoration efforts.

Future research should incorporate seasonal sampling, as fish assemblages in the region also vary seasonally (Allen et al., 2002). Additionally, emerging non-destructive tools such as baited remote underwater video (BRUV) systems should be considered, as they offer a complementary approach to traditional and molecular methods and are increasingly used in marine and estuarine monitoring.

2.6 Tables and Figures

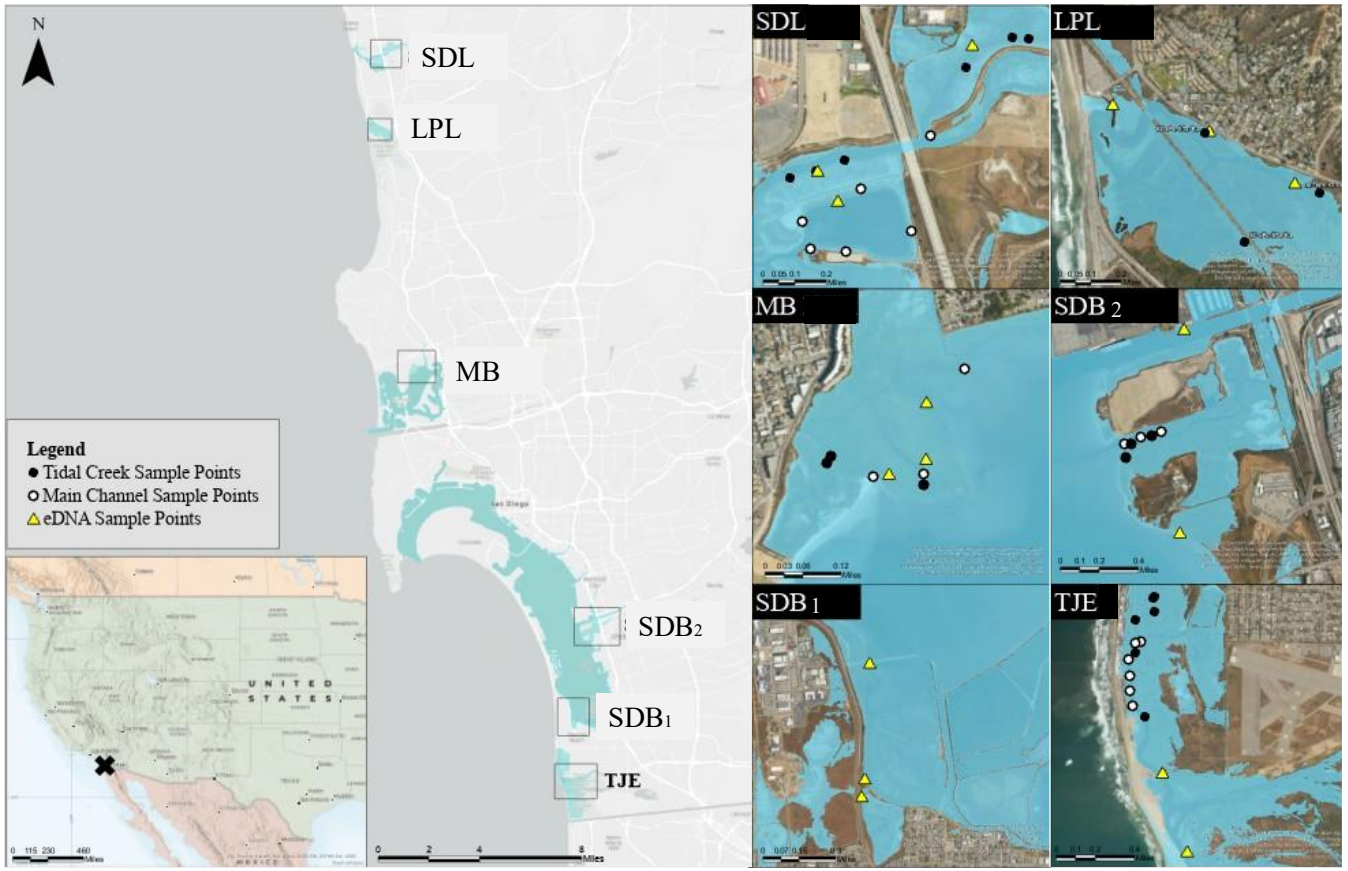

Figure 2.1. Study area comprising five estuaries within San Diego County: San Dieguito Lagoon (SDL), Los Peñasquitos Lagoon (LPL), Kendall-Frost Reserve in Mission Bay (MB), San Diego Bay (grouped here as SDB, with South Bay Salt Ponds shown as SDB_1 and Sweetwater Marsh as SDB_2), and Tijuana River Estuary (TJE).

Table 2.1. Classification and Key Characteristics of the Five Coastal Wetlands Located in San Diego County, California, USA that were Included in this Study, Listed from South to North.

System		Co-ordinates	Extent (Ha) of Estuary [a]	Classification	Inlet Type [c]	Number of Sample Locations	
						eDNA [d]	Seining and Enclosure Traps
1	**TJE**	32°33'59.72"N; 117° 7'45.58"W	353.95	Large river valley estuary[c]	Rarely closed [b]	2	Main channels: 6 Tidal creeks: 6
2	**SDB**	32°35'47.91"N; 117° 7'16.34"W	1058.70[e]	Open Bay/Harbor & intermediate estuary[c]	Perennially Open [b]	6	Main channels: 3 Tidal creeks: 3
3	**MB**	32°47'32.39"N; 117°13'42.67"W	879.52[f]	Open Bay/Harbor, Intermediate estuary[c]	Perennially open	3	Main channels: 3 Tidal creeks:3
4	**LPL**	32°55'53.07"N; 117°15'13.38"W	187.62	Large lagoon [a]	Frequently closed [b]	3	Main channels: 3 Tidal creeks: 0
5	**SDL**	32°58'19.92"N; 117°15'9.31"W	75.2	Large lagoon [a]	Perennially open	3	Main channels: 6 Tidal creeks: 6

[a] Extent (Ha) of Estuary is defined as "estuarine area including adjacent wetlands in hectares", source: Heady et al. (2014)

[b] Source: Desmond et al. (2002)

[c] Source: SCCWRP (2018)

[d] Triplicate 1-litre samples were collected at each eDNA sample and filtered and analyzed.

[e] Extent includes only Sweetwater Marsh and San Diego Bay South Salt Ponds

[f] Extent includes Kendal-Frost Reserve

Table 2.2. Guild Classifications and Definitions used to Categorize Fish in 5 Coastal Wetlands in San Diego, California, USA. Only guilds that were present in this study are listed below. Guild classifications are based on Bond et al. (1999). W.C. indicates water column.

	Guild	Description	Examples
Water Column	Guild 1	Water column foragers, schooling, filter feeding	Threadfin shad (*Dorosoma petenense*), northern anchovy (*Engraulis mordax*), deepbody anchovy (*Anchoa compressa*), slough anchovy (*A. delicatissima*), Pacific sardine (*Sardinops sagax*).
	Guild 2	Selective feeding, diurnal	Mosquitofish (*Gambusia affinis*, specialized neustonic), grunion (*Leuresthes tenuis*), walleye surfperch (*Hyperprosopon argenteum*) <60 mm SL, topsmelt (*Atherinops affinis*) <100 mm SL, white croaker (*Genyonemus lineatus*) <100 mm SL.
	Guild 4	Benthic foragers with schooling and diurnal behavior	Striped Mullet (*Mugil cephalus)*, larger Topsmelt (>100m; *Atherinops affinis*).
	Guild 23	Pelagic mesocarnivores	Pacific bonito (*Sarda chilensis*), Pacific mackerel (*Scomber japonicus*), California barracuda (*Sphyraena argentea*), spiny dogfish (*Squalus acanthias*), California needlefish (*Strongylura exilis*), jack mackerel (*Trachurus symmetricus*).
Substrate-Associated	Guild 5	W.C. foragers, schooling, selective feeding, usually benthic refugers, diurnal	Blacksmith (*Chromis punctipinnis*), señorita (*Oxyjulis californica*) <60 mm SL
	Guild 7	Non-schooling, non-visual	Specklefin midshipman (*Porichthys myriaster*)
	Guild 8	Benthic foragers, schooling, benthic refugers, diurnal, pickers	California killifish (*Fundulus parvipinnis*), señorita (*Oxyjulis californica*) >100 mm SL, kelp surfperch (*Brachyistius frenatus*), shiner surfperch (*Cymatogaster aggregata*)
	Guild 9	Non-schooling, diurnal, engulfers	Kelp bass (*Paralabrax clathratus*), spotted sand bass (*P. maculatofasciatus*), barred sand bass (*P. nebulifer*), giant kelpfish (*Heterostichus rostratus*), sablefish (*Anaplopoma fimbria*), bonefish (*Albula vulpes*).

	Guild 11	Benthic foragers, schooling/nonschooling, diurnal, generalist	Black surfperch (*Embiotoca jacksoni*), dwarf surfperch (*Micrometrus minimus*), white surfperch (*Phanerodon furcatus*), opaleye (*Girella nigricans*) <100 mm SL, garibaldi (*Hypsypops rubicundus*, specialist, territorial, sometimes herbivore).
	Guild 12	Crushers	Rock wrasse (*Halichoeres semicinctus*), California sheephead (*Semicossyphus pulcher*), pile surfperch (*Rhacochilus vacca*).
	Guild 13	Herbivores	Opaleye (*Girella nigricans*) >100 mm SL, halfmoon (*Medialuna californiensis*), zebraperch (*Hermosilla azurea*).
	Guild 14	Nocturnal, generalist	White croaker (*Genyonemus lineatus*) >100 mm SL, black croaker (*Cheilotrema saturnum*), California corbina (*Menticirrhus undulatus*), yellowfin croaker (*Umbrina roncador*)
	Guild 16	Benthic foragers, mesocarnivores	Bigmouth sole (*Hippoglossina stomata*), California halibut (*Paralichthys californicus*), California lizardfish (*Synodus lucioceps*), cabezon (*Scorpaenichthys marmoratus*).
	Guild 17	Substrate sitters, microcarnivores, diurnal	Pacific sanddab (*Citharichthys sordidus*), speckled sanddab (*C. stigmaeus*), longfin sanddab (*C. xanthostigma*)
	Guild 19	Hiders (holes/crevices)	Diurnal Arrow goby (*Clevelandia ios*), cheekspot goby (*Ilypnus gilberti*), shadow goby (*Quietula y-cauda*), bay blenny (*H. gentilis*), reef finspot (*Paraclinus integripinnis*), yellowfin goby (*Acanthogobius flavimanus*)
	Guild 20	Benthic foragers, pickers and scrapers, diurnal	Spotted kelpfish (*Gibbonsia elegans*), stripefin ronquil (*Rathbunella hypoplecta*), rockpool blenny (*Hypsoblennius gilberti*).
	Guild 22	Diggers and extractors	Diamond turbot (*Hypsopsetta guttulata*), c-o turbot (*Pleuronichthys coenosus*), spotted turbot (*P. ritteri*), hornyhead turbot (*P. verticalis*), English sole (*Pleuronectes vetulus*), round stingray (*Urolophus halleri*)

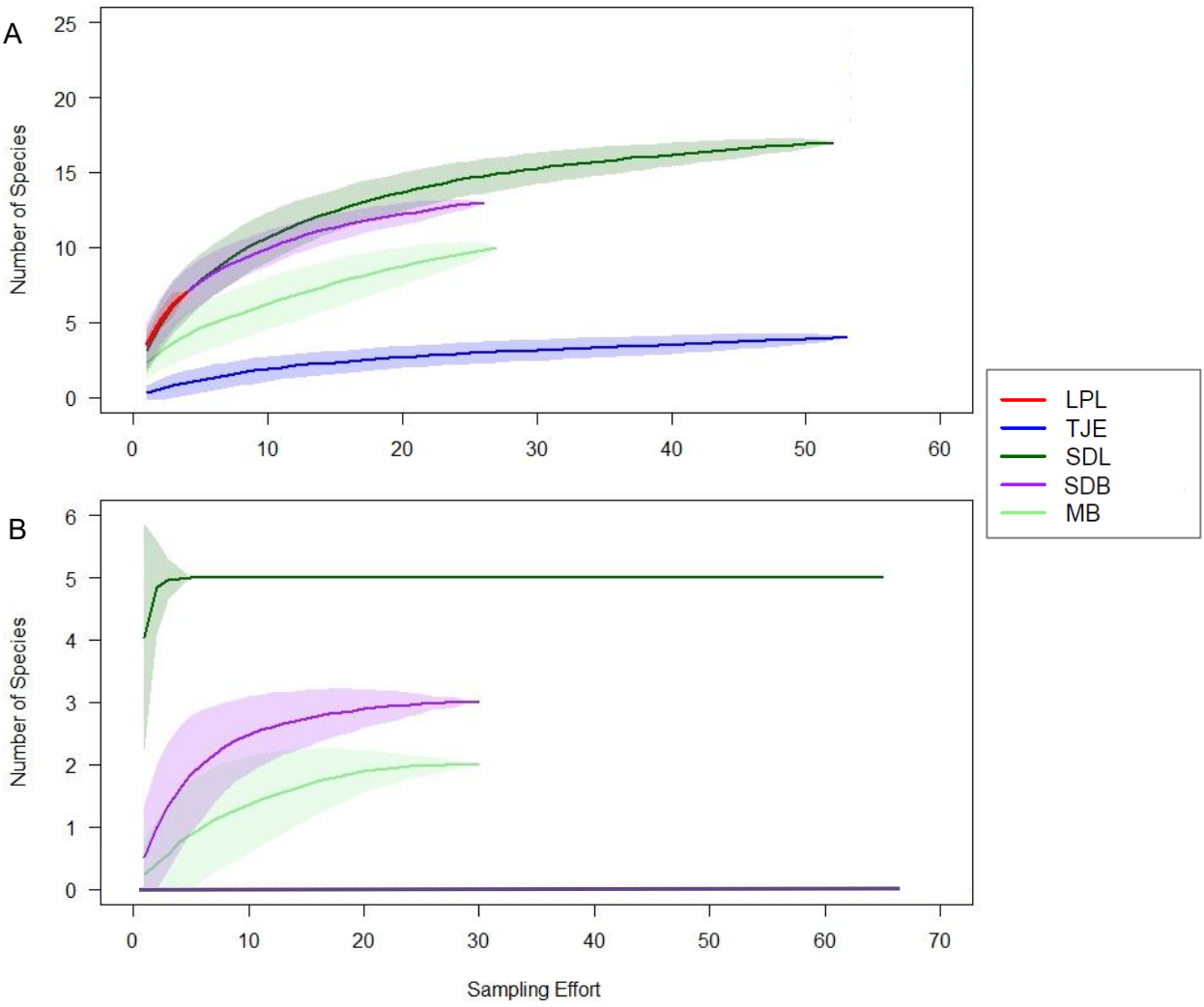

Figure 2.2. Accumulation curves of species richness with a 95% confidence interval (shaded area) across all sites combined, produced using the "random" method with 1,000 permutations using the vegan package in R. **A)** Species accumulation curves for block net seining, with sampling effort being the number of block seines sampled with 6 hauls each; **B)** Species accumulation curves for enclosure trap data, with sampling effort being the number of enclosure traps deployed.

Table 2.3. A summary of the number of species detected using eDNA, traditional sampling, and iNaturalist. "n" values indicate the number of successful samples at each site for eDNA. Sites are listed from south to north.

	Number of Species Detected (2023)			Total Number of Species Across All Approaches
	eDNA	Traditional Sampling	iNaturalist	
TJE	7 (n=1)	4	11	18
SDB	7 (n=2)	16	52	55
MB	8 (n=3)	12	59	62
LPL	6 (n=2)	9	7	12
SDL	27 (n=2)	21	16	39
Total # Species	22	25	74	81

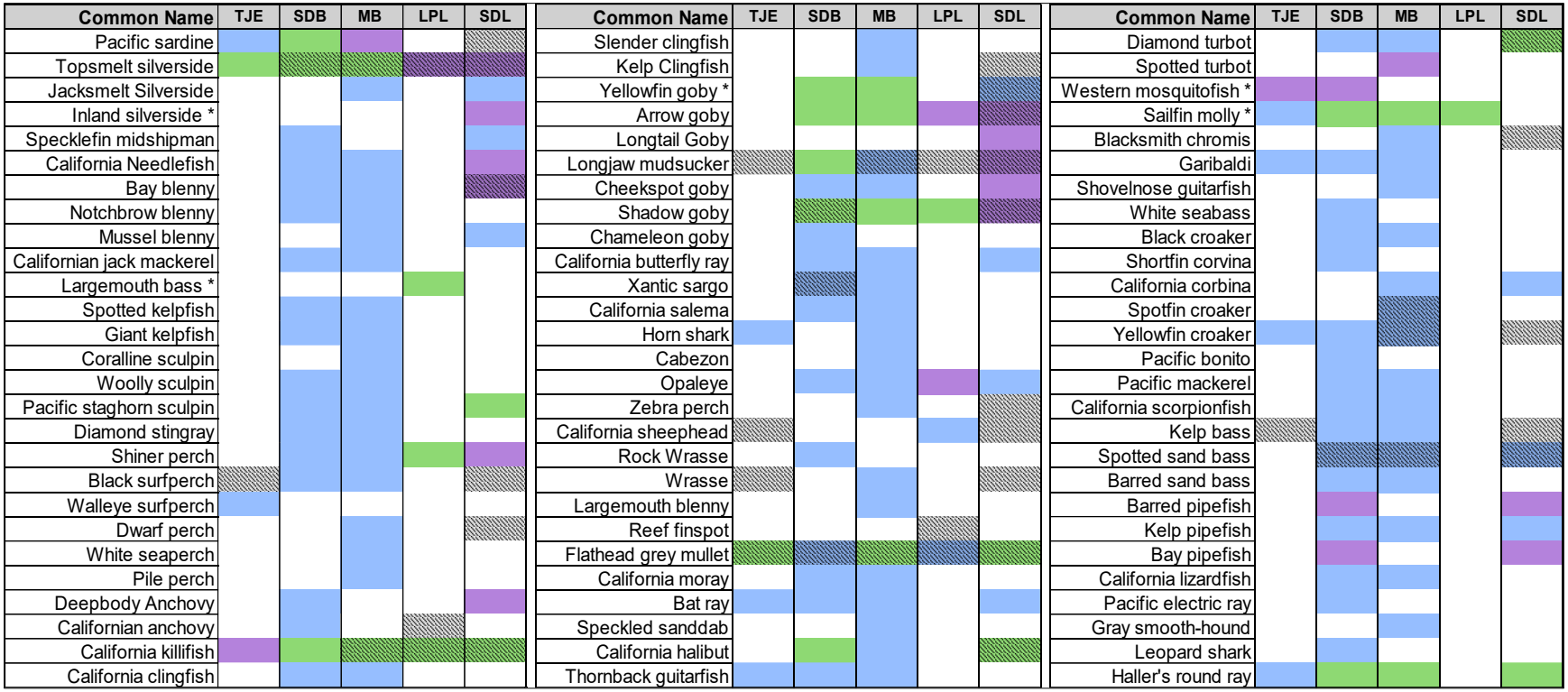

Figure 2.3. A heatmap of species detected in 2023 in coastal wetlands throughout San Diego using traditional approaches (block net seining and enclosure traps) and iNaturalist. Blue indicates the detection of fish species at a site by iNaturalist, purple by traditional approaches, green by both iNaturalist and traditional approaches, and diagonal shading indicates species detected using eDNA. Non-native species are indicated by a *. Sites are listed geographically, with the southernmost site on the left and the northernmost site on the right. Fish species are sorted alphabetically by order of family and then by species name.

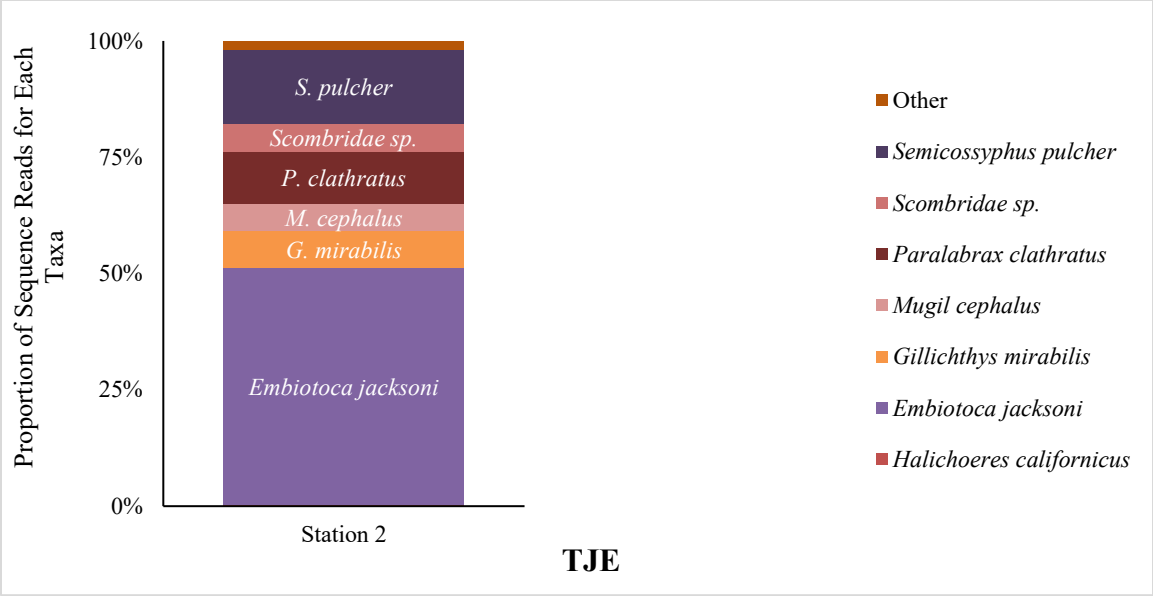

Figure 2.4. Proportion of sequence reads assigned to taxa at the TJE based on eDNA metabarcoding. Only samples with successful sequencing are included. Two stations were sampled with two replicates each, but only one replicate from station 2 yielded sufficient quality for analysis.

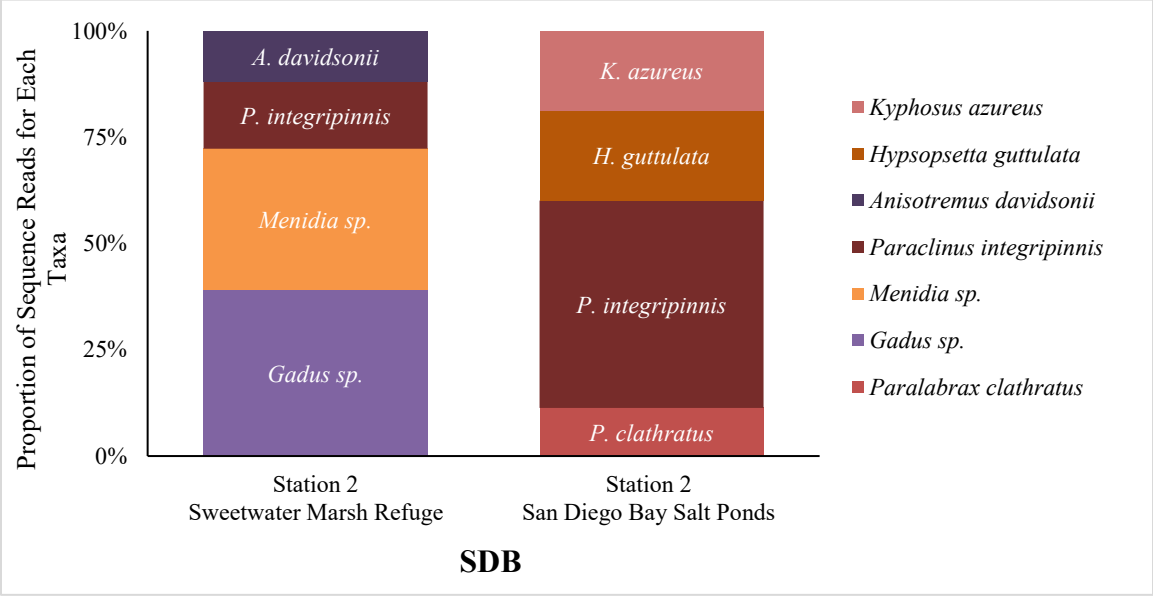

Figure 2.5. Proportion of sequence reads assigned to taxa at SDB based on eDNA metabarcoding. Only samples with successful sequencing are included. Three stations were sampled at the South Bay Salt Ponds and two at Sweetwater Marsh, with two replicates per station. However, only one replicate from station 2 at Sweetwater Marsh and one replicate from station 2 at the South Bay Salt Ponds yielded sufficient quality results.

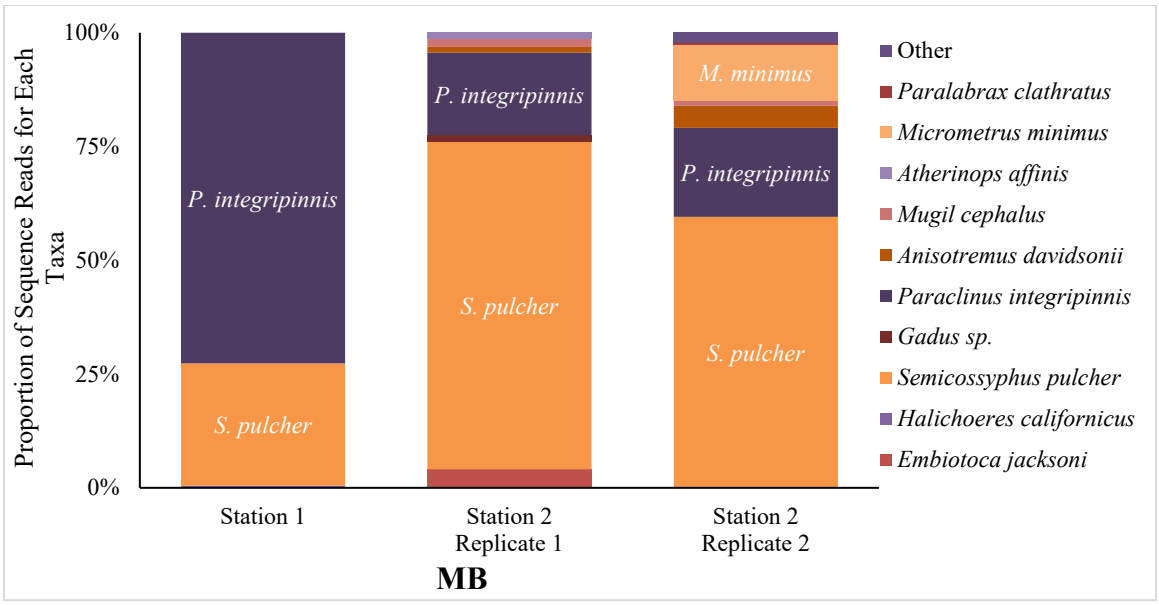

Figure 2.6. The proportion of sequence reads assigned to taxa at MB based on eDNA metabarcoding. Only samples with successful sequencing are included. Three stations with two replicates each were sampled; at station 1, one replicate yielded sufficient quality, while at station 2 both replicates yielded successful results.

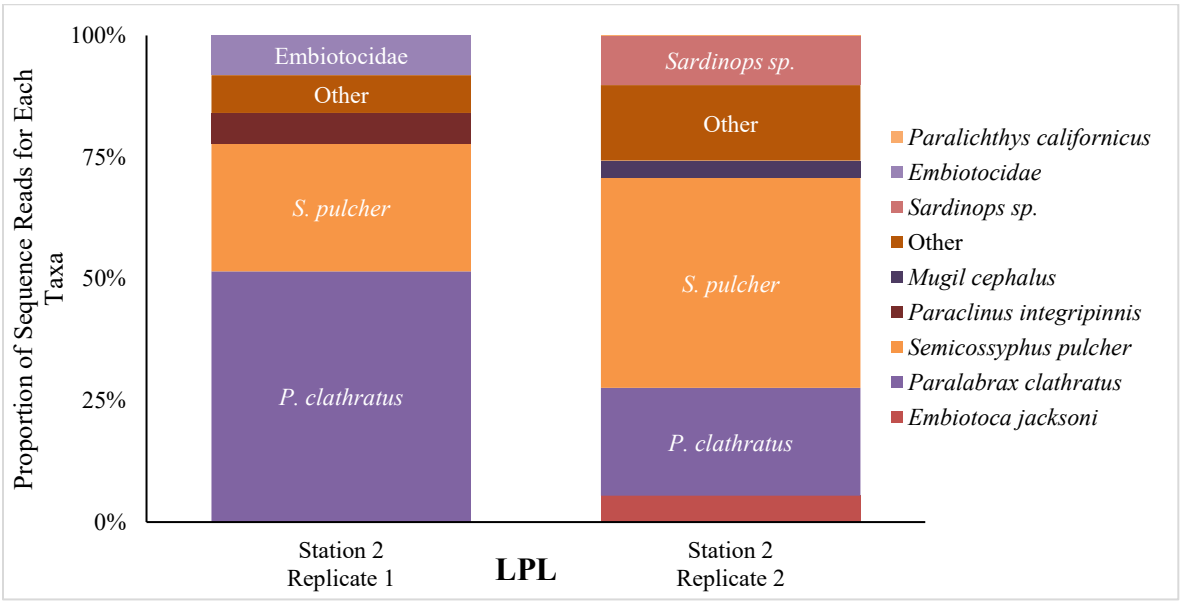

Figure 2.7. The proportion of sequence reads assigned to taxa at LPL based on eDNA metabarcoding. Only samples with successful sequencing are included. Three stations with two replicates each were sampled; at station 2, both replicates yielded sufficient quality results.

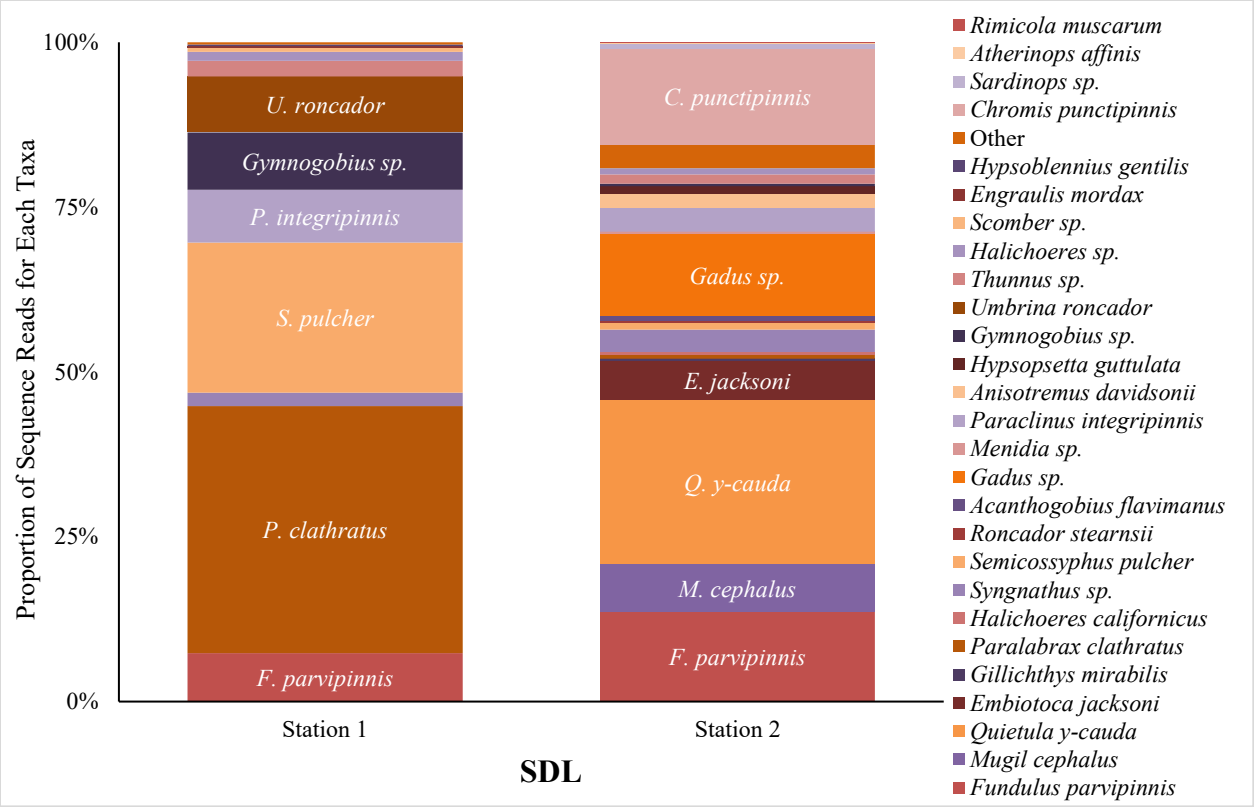

Figure 2.8. The proportion of sequence reads assigned to taxa at San Dieguito Lagoon (SDL) based on eDNA metabarcoding. Only samples with successful sequencing are included. Three stations with two replicates each were sampled; however, only one replicate from station 1 and one replicate from station 2 yielded sufficient quality results.

Table 2.4. List of species identified during the gap analysis that do not have reference sequences in Genbank, MitoHelper or FishCARD. The third column indicates whether each species was detected by iNaturalist or traditional sampling, suggesting potential false negatives in the eDNA dataset.

	Species Name	**Common Name**	**Detected through other approaches?**
1	*Albula gilberti*	Cortez bonefish	No
2	*Cetengraulis mysticetus*	Pacific anchoveta	No
3	*Rhinogobiops nicholsii*	Blackeye goby	No
4	*Cottus asperrimus*	Rough sculpin	No
5	*Cynoscion nobilis*	White seabass	No
6	*Hyporhamphus gilli*	Gill's halfbeak	No
7	*Hyporhamphus rosae*	California halfbeak	Yes, detected by seining (Allen et al., 2002)
8	*Hypsurus caryi*	Rainbow seaperch	No
9	*Ilypnus gilberti*	Cheekspot goby	Yes, by iNaturalist and traditional surveys
10	*Oncorhynchus clarkii clarkii*	Coastal cutthroat trout	No
11	*Porichthys myriaster*	Specklefin midshipman	Yes, by iNaturalist
12	*Ptychocheilus grandis*	Sacramento pikeminnow	No
13	*Rhinobatos productus*	Shovelnose guitarfish	No
14	*Rimicola eigenmanni*	Slender clingfish	Yes, by iNaturalist
15	*Symphurus atricauda*	California tonguefish	No
16	*Torpedo californica*	Pacific electric ray	No
17	*Xenistius californiensis*	California salema	Yes, by traditional surveys

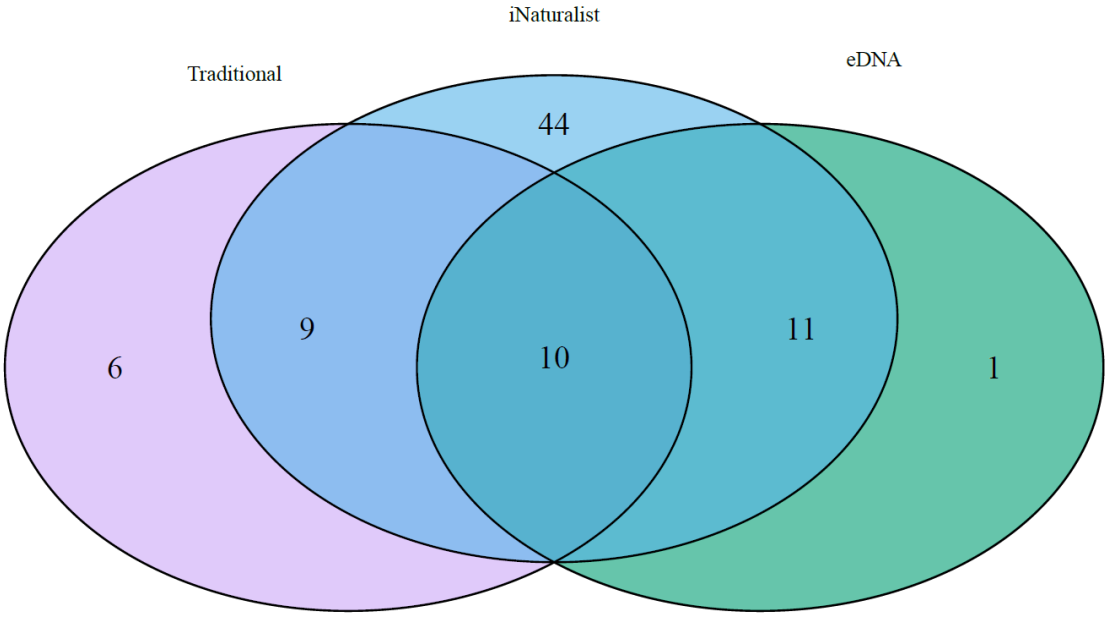

Figure 2.9. Venn diagram of fish species detected across all sites using traditional sampling (block net seining and enclosure traps), iNaturalist and environmental DNA (eDNA).

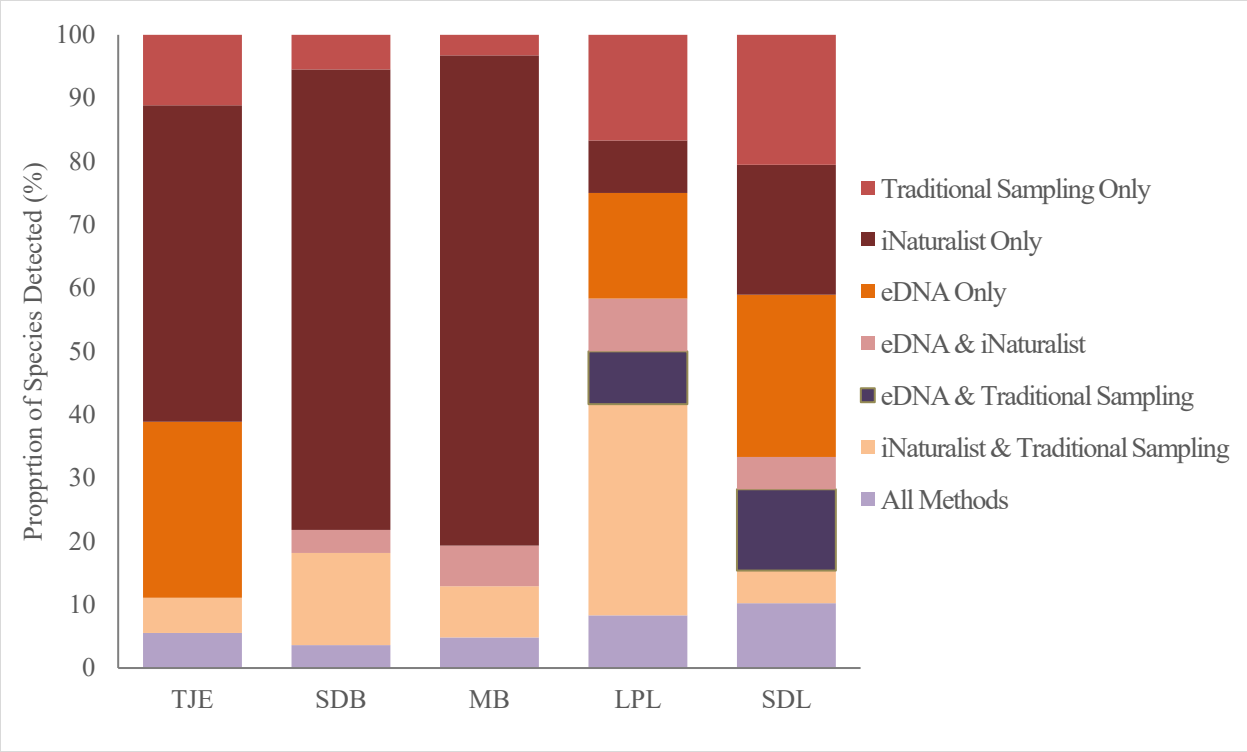

Figure 2.10. Proportion of fish species detected using traditional sampling approaches (seining and enclosure traps) and community science (iNaturalist) at five coastal wetlands in San Diego County, California, USA.

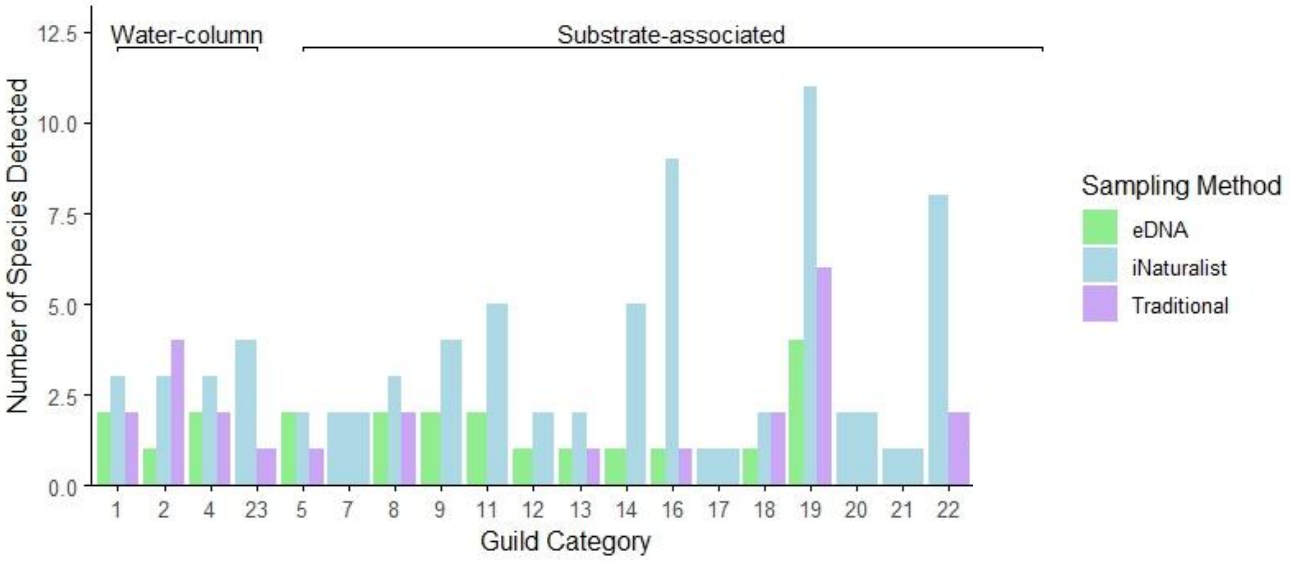

Figure 2.11. Number of fish species grouped by ecological guild and detection method. Descriptions of each guild category can be found in Table 2.2. of the Methods.

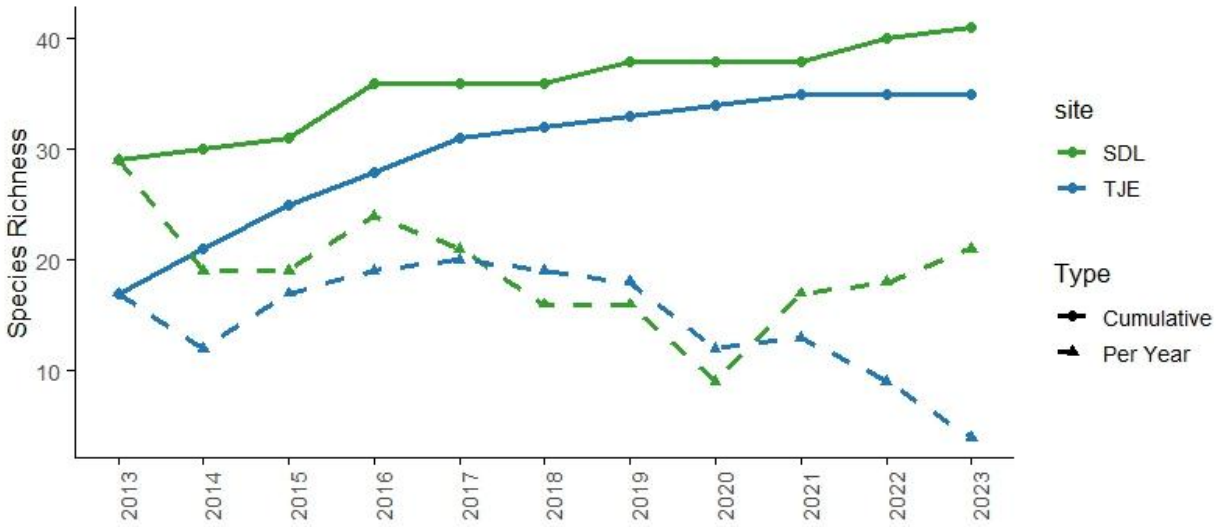

Figure 2.12. Species accumulation curves (solid lines) for fish richness at TJE (blue) and SDL (green) from 2013 to 2023, based on detections from block net seining and enclosure traps conducted during the annual SONGS monitoring program. Dashed lines represent the number of species detected per year at each site.

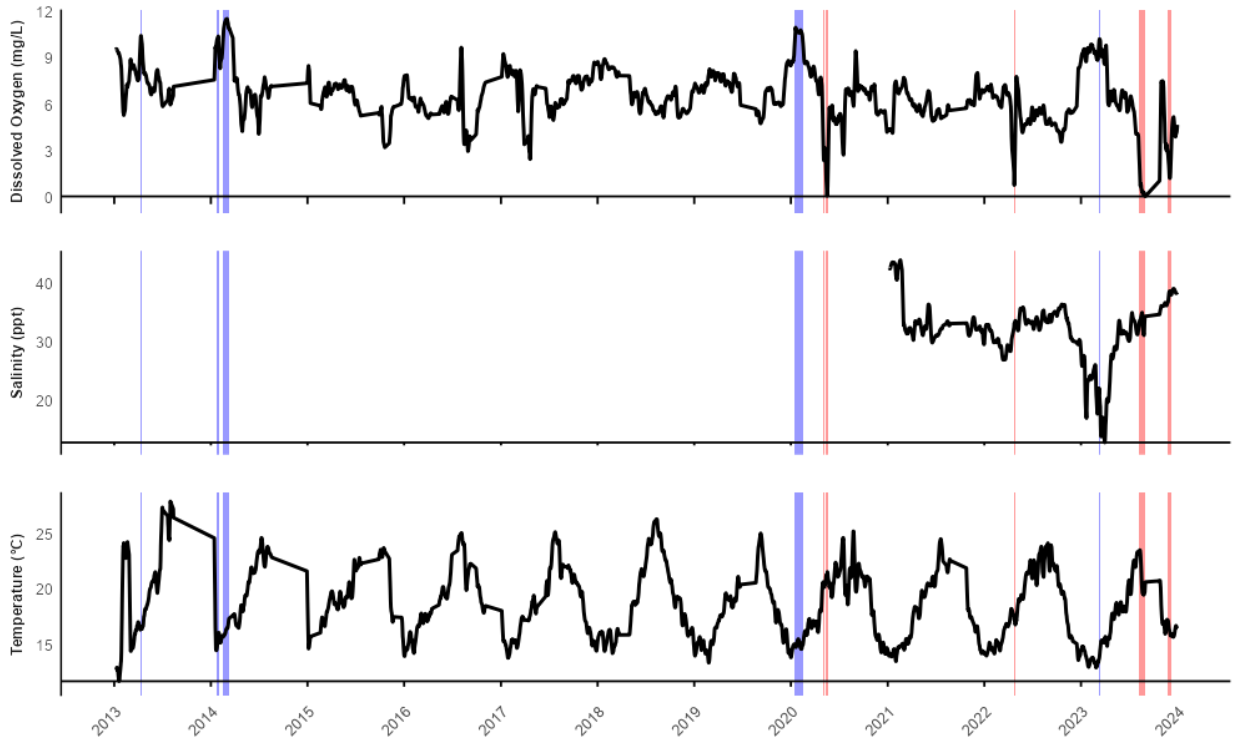

Figure 2.13. Seven-day running averages of dissolved oxygen (mg/L), salinity (ppt), and temperature (°C) recorded at 15-minute intervals at SDL. Red bars indicate periods of hypoxia (dissolved oxygen < 3 mg/L), while blue bars indicate periods of supersaturation (dissolved oxygen > 10 mg/L).

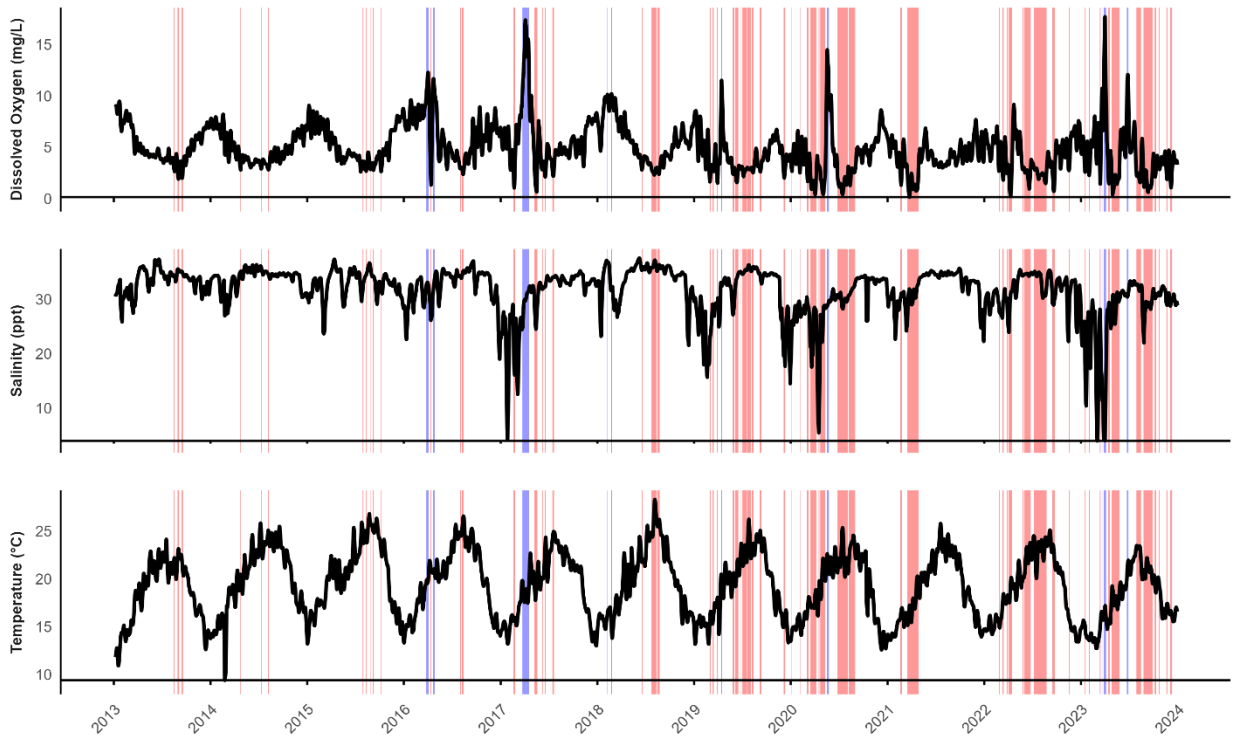

Figure 2.14. Seven-day running averages of dissolved oxygen (mg/L), salinity (ppt), and temperature (°C) recorded at 15-minute intervals at TJE. Red bars indicate periods of hypoxia (dissolved oxygen < 3 mg/L), while blue bars indicate periods of supersaturation (dissolved oxygen > 10 mg/L).

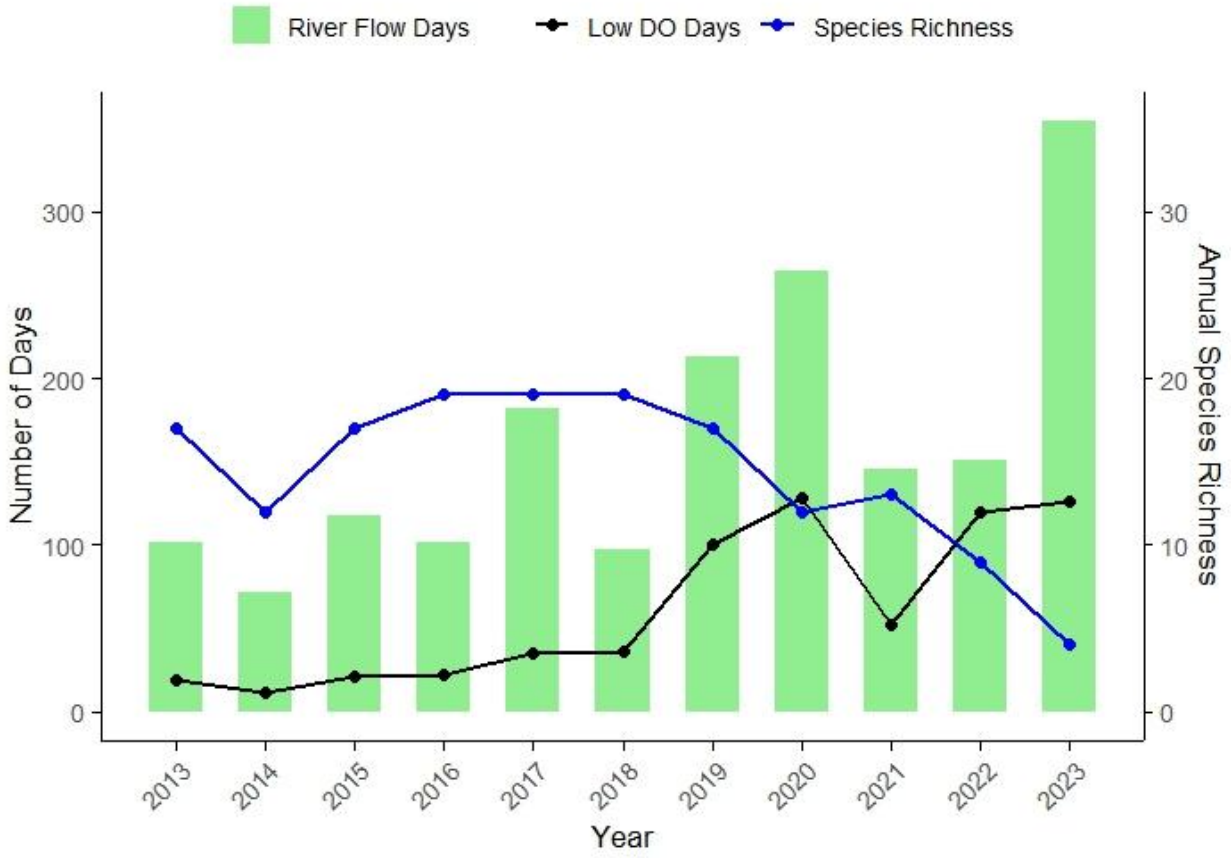

Figure 2.15. Annual number of days with river flow, low dissolved oxygen (DO), and species richness at the TJE. Green bars indicate the number of days per year with measurable river flow (flow > 0 million gallons per day), while the black line and points show the number of days where the 7-day rolling average DO concentration was below 3 mg/L, indicative of hypoxic conditions. The blue line represents annual fish species richness recorded at TJE. Data spans the full calendar year for each variable.

Table 2.5. Fish species detected during annual monitoring of TJE using block seine netting and enclosure traps. "n" refers to the total number of species detected at each site. Non-native species are indicated by *.

Species Name	Common Name	2013 n=17	2014 n=12	2015 n=17	2016 n=19	2017 n=20	2018 n=19	2019 n=18	2020 n=12	2021 n=13	2022 n=9	2023 n=4
Acanthogobius flavimanus	Yellowfin goby *	■		■	■	■	■	■				
Anchoa compressa	Deepbody Anchovy		■	■	■	■	■	■			■	
Anisotremus davidsonii	Xantic sargo											
Atherinops affinis	Topsmelt silverside	■	■	■	■	■	■	■	■	■	■	■
Atherinopsis californiensis	Jacksmelt Silverside											
Clevelandia ios	Arrow goby	■	■	■	■	■	■	■	■	■	■	
Ctenogobius sagittula	Longtail Goby				■		■					
Cymatogaster aggregata	Shiner perch											
Fundulus parvipinnis	California killifish	■	■	■	■	■	■	■		■	■	■
Gambusia affinis	Western mosquitofish *			■	■			■			■	■
Gillichthys mirabilis	Longjaw mudsucker	■	■	■	■	■	■	■		■		
Girella nigricans	Opaleye		■			■	■		■			
Girella spp.		■										
Gymnura marmorata	California butterfly ray	■			■	■		■				
Hypsoblennius gentilis	Bay blenny				■	■	■	■	■	■		
Hypsoblennius spp.												
Hypsopsetta guttulata	Diamond turbot		■				■			■		
Ilypnus gilberti	Cheekspot goby	■										
Kyphosus azureus	Zebra perch											
Leptocottus armatus	Pacific staghorn sculpin											
Menidia beryllina	Inland silverside *											
Mugil cephalus	Flathead grey mullet	■		■	■	■	■			■	■	■
Mustelus californicus	Gray smooth-hound					■	■					
Myliobatis californicus	Bat ray	■										
Ophichthus zophochir	Yellow snake eel							■				
Paralabrax clathratus	Kelp bass								■	■		
Paralabrax maculatofasciatus	Spotted sand bass		■	■		■	■		■			
Paralabrax nebulifer	Barred sand bass					■			■			
Paralichthys californicus	California halibut	■	■	■	■	■	■	■	■	■	■	
Platyrhinoidis triseriata	Thornback guitarfish									■		
Porichthys myriaster	Specklefin midshipman											
Quietula y-cauda	Shadow goby	■		■	■		■	■				
Sardinops sagax	Pacific sardine			■	■		■	■				
Seriphus politus	Queenfish	■			■	■			■			
Sphoeroides annulatus	Bullseye puffer			■								
Strongylura exilis	California Needlefish											
Syngnathus auliscus	Barred pipefish											
Syngnathus leptorhynchus	Bay pipefish											
Umbrina roncador	Yellowfin croaker											
Unknown Species of Atherinopsidae		■	■	■	■	■	■	■		■	■	
Unknown Species of Cottidae		■										
Unknown Species of Gobiidae		■	■	■	■	■	■	■	■	■	■	
Unknown Species of Osteichthyes		■	■	■	■	■		■	■			
Unknown Species of Pleuronectiformes												
Unknown Species of Sciaenidae												
Unknown Species of Syngnathidae												
Urolophus halleri	Haller's round ray				■	■		■	■	■		
Xenistius californiensis	California Salema						■					

Table 2.6. Fish species detected during annual monitoring of SDL using block net seining and enclosure traps. "n" refers to the total number of species detected at each site. Non-native species are indicated by *.

Species Name	Common Name	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
		n=29	n=19	n=19	n=24	n=21	n=16	n=16	n=9	n=17	n=18	n=21
Acanthogobius flavimanus	Yellowfin goby *	■	■	■	■	■	■	■		■		
Anchoa compressa	Deepbody Anchovy	■		■	■	■			■	■	■	■
Anisotremus davidsonii	Xantic sargo											
Atherinops affinis	Topsmelt silverside	■	■	■	■	■	■	■	■	■	■	■
Atherinopsis californiensis	Jacksmelt Silverside				■							
Clevelandia ios	Arrow goby	■	■	■	■	■	■	■		■	■	■
Ctenogobius sagittula	Longtail goby				■	■			■	■		■
Cymatogaster aggregata	Shiner perch	■										■
Fundulus parvipinnis	California killifish	■	■	■	■	■	■	■	■	■	■	■
Gambusia affinis	Western mosquitofish *							■				
Gillichthys mirabilis	Longjaw mudsucker	■	■	■	■	■	■	■	■	■	■	■
Girella nigricans	Opaleye											
Girella spp.												
Gymnura marmorata	California butterfly ray		■									
Hypsoblennius gentilis	Bay blenny	■	■	■	■	■	■			■	■	■
Hypsoblennius spp.		■										
Hypsopsetta guttulata	Diamond turbot	■	■	■	■	■	■	■		■	■	■
Ilypnus gilberti	Cheekspot goby	■										■
Kyphosus azureus	Zebra perch				■							
Leptocottus armatus	Pacific staghorn sculpin	■	■							■		■
Menidia beryllina	Inland silverside *											■
Mugil cephalus	Flathead grey mullet	■		■	■	■	■	■		■	■	■
Mustelus californicus	Gray smooth-hound											
Myliobatis californicus	Bat ray	■		■		■						
Ophichthus zophochir	Yellow snake eel											
Paralabrax clathratus	Kelp bass	■										
Paralabrax maculatofasciatus	Spotted sand bass	■	■	■	■	■	■				■	
Paralabrax nebulifer	Barred sand bass	■	■									
Paralichthys californicus	California halibut	■	■	■	■	■	■	■	■	■	■	■
Platyrhinoidis triseriata	Thornback guitarfish											
Porichthys myriaster	Specklefin midshipman				■							
Quietula y-cauda	Shadow goby	■	■	■	■	■	■	■		■	■	■
Sardinops sagax	Pacific sardine			■		■	■	■				
Seriphus politus	Queenfish				■							
Sphoeroides annulatus	Bullseye puffer										■	
Strongylura exilis	California Needlefish	■		■	■	■	■					■
Syngnathus auliscus	Barred pipefish	■	■	■	■	■	■	■	■		■	■
Syngnathus leptorhynchus	Bay pipefish	■	■	■	■	■				■	■	■
Umbrina roncador	Yellowfin croaker											
Unknown Species of Atherinopsidae		■	■		■	■		■		■	■	■
Unknown Species of Cottidae												
Unknown Species of Gobiidae		■	■	■	■	■	■	■	■	■	■	■
Unknown Species of Osteichthyes		■	■	■	■	■		■		■		
Unknown Species of Pleuronectiformes		■										
Unknown Species of Sciaenidae		■										
Unknown Species of Syngnathidae		■										
Urolophus halleri	Haller's round ray	■	■		■		■	■	■		■	■
Xenistius californiensis	California Salema											

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Chapter 3: Review of Fish Species Monitoring Approaches and Future Directions

This chapter documents the challenges, benefits, and limitations of various fish species monitoring approaches discussed throughout this research, both through firsthand experience and an extensive literature review. The findings aim to inform future research, particularly within Southern California, and contribute to the development and improvement of current and future monitoring programs in the region.

3.1 Review of Traditional Surveys

In this study, traditional survey methods included block net seining and enclosure traps, both of which require the physical capture of fish for identification. These approaches have been used for decades throughout San Diego, providing long-term data on species richness, abundance, and size. Currently, no contemporary method (such as eDNA and iNaturalist) can match the species-specific information provided by these traditional approaches. However, these methods are resource-intensive, requiring frequent sampling by trained experts, which demands significant time and funding. Additionally, traditional surveys are subject to sampling biases, such as net avoidance by fish and macroinvertebrates through burrowing, swimming away, or jumping. Sampling is also restricted to accessible habitats during daylight hours, leading to the underrepresentation of nocturnal species or those inhabiting deeper channels. Furthermore, taxonomic expertise is essential, particularly when distinguishing between morphologically similar species (Levin, 1984; Thomsen & Willerslev, 2015). Detecting rare or cryptic species that occur in low abundance necessitates extensive sampling effort, further increasing the logistical

challenges associated with traditional survey methods (Budd et al., 2023; Day et al., 2019; Pukk et al., 2021).

3.2 Review of Community Science (iNaturalist)

The value of iNaturalist data is contingent on understanding and accounting for any spatial and taxonomic biases (Geurts et al., 2023). These biases also result in some species being disproportionately represented, with observations favoring conspicuous taxa, which limits the platform's ability to generate accurate abundance estimates (Dibattista et al., 2021). Additionally, data quality can be constrained by uncertainties in species identification, observation quality, and georeferencing (Ackland et al., 2024). Uncertainties in species identification may arise from poor taxonomic knowledge, low-quality photographs, or species-specific traits that are difficult to distinguish from photographs, with some species requiring molecular analysis for accurate identification (Barbato et al., 2021).

Georeferencing inaccuracies are most notable for threatened or endangered species, as iNaturalist obscures location data to prevent the misuse of species occurrence records, such as the overexploitation of sought after fisheries species (Contreras-Díaz et al., 2023). When a record for a threatened species is uploaded, the exact longitude and latitude are moved to private fields that are inaccessible to the public. This obscured data is often used in research papers and shared with global repositories such as the Global Biodiversity Information Facility (GBIF), affecting spatial accuracy of species' distribution ranges (Contreras-Díaz et al., 2023). Improved data-sharing frameworks that provide researchers with controlled access to precise coordinates could help mitigate these limitations while still protecting vulnerable species.

To address uncertainties in species identification, verification methods have been developed to assign confidence to iNaturalist observations, such as the approach proposed by Ackland et al. (2024). This approach sums the scores generated in three key criteria: species identification, media quality, and georeferencing accuracy. Assigning confidence scores on average takes 1.5 minutes per observation. However, in cases where observations extend into the thousands (and sometimes millions, as is the case in a study by Geurts et al. (2023) which had 1.7 million iNaturalist observations), this process would require thousands of additional hours, making manual verification unfeasible at scale. This underscores the need to strengthen verification processes to enable iNaturalist's use in larger-scale studies.

The spatial and temporal biases associated with iNaturalist observations have been extensively studied in terrestrial ecosystems; however, few studies have specifically examined these biases in estuarine environments. As such, much of the current understanding of iNaturalist biases is derived from terrestrial studies. For instance, Geurts et al. (2023) analyzed 1.7 million iNaturalist observations from terrestrial habitats in British Columbia, Canada, and found that observations favored areas closer to roads, with as much as 94% of observations within 1km of roads. Additional factors influencing observation patterns included human population density and habitat zones or land cover types (Geurts et al., 2023). Little research has been conducted on spatial biases of iNaturalist observations of fish specifically in estuaries, however, a study by Rocha et al. (2024) explored taxonomic biases associated with marine taxa on iNaturalist. Results suggested that conspicuous groups with high species richness, such as Arthropoda, Mollusca, and Chordata, were well represented, whereas many marine invertebrate groups, including Annelida, Bryozoa, Nematoda, Nemertea, Platyhelminthes, Porifera, Chlorophyta, and Rhodophyta, were underrepresented, likely because they are more cryptic, often sessile, and less visible to non-

specialist observers (Rocha et al., 2024). The predominance of Mollusca and Arthropoda was attributed to these taxa being relatively slow moving and easy to photograph, making them favorable for observations. In addition, these species are more recognizable to people (for example, in comparison to a more cryptic and less familiar bryozoan). Notably, fish were the most frequently recorded vertebrate group, though observations were primarily contributed by divers with specialized, often expensive, underwater photography equipment, potentially limiting accessibility for individuals with lower income (Rocha et al., 2024).

Based on these considerations and preliminary analyses of iNaturalist observation biases along San Diego's estuarine coasts (Figure 1), observations were most frequent in areas closest to roads (commonly used as a proxy for accessibility), near universities and research institutions (likely due to organized field trips and site visits), and within marine protected areas (MPAs). However, upon further examination, the use of road proximity as a metric for accessibility may be less applicable to San Diego, given its dense road network, which results in much of the coastline and estuarine areas being near roads.

Notably, Los Peñasquitos Lagoon exhibited the lowest number of iNaturalist observations (see Results of Chapter 2). This is likely due to restricted public access, with Los Peñasquitos Lagoon having limited trails or entry points. The extent of these biases is expected to vary across different locations, as some regions may experience seasonal variation in public activity, with fewer visitors during colder months. However, in San Diego, seasonality is likely an insignificant factor due to the region's moderate year-round climate, which supports consistent community science participation.

Studies examining biases in iNaturalist observations are crucial for optimizing the use of community science data, as they help identify underrepresented areas along the San Diego

coastline. The findings underscore the importance of targeted marine biodiversity initiatives that encourage public engagement beyond MPAs and areas with easy access. Notably, these preliminary analyses indicated that iNaturalist observations in San Diego County are concentrated in the southern region, with fewer records from northern areas beyond Oceanside (Figure 1). Addressing these gaps through strategic outreach and community engagement efforts could enhance spatial representation in iNaturalist datasets.

Several complexities associated with using iNaturalist became evident throughout this study. When filtering data prior to download, users can select from broad taxonomic groups, including birds, amphibians, reptiles, mammals, ray-finned fishes, mollusks, arachnids, insects, plants, fungi (including lichens), protozoans, and unknown taxa. Since this research focused on fish, selecting "ray-finned fishes" appeared to be the most intuitive approach. However, upon reviewing the downloaded dataset, it became clear that certain fish groups, such as sharks, skates, and rays, were excluded because they were classified under "Animalia" since they are not "ray-finned fishes." To address this, it may be best to add a category for “cartilaginous fishes” to capture this grouping and avoid having to download a large dataset on non-target taxa. This highlights the importance of refining taxonomic filtering post-download to ensure comprehensive representation of the target group.

Additionally, as previously mentioned, iNaturalist is not well-suited for studying threatened or endangered species because it obscures geolocation data to protect sensitive species from potential exploitation. This may be attributed to the data not indicating the presence of the giant sea bass (*Stereolepis gigas*) which has previously been recorded in Mission Bay and San Diego Bay. While researchers can request access to precise location data, obtaining approval can be challenging, especially when working with large datasets.

3.3 Review of Environmental DNA Metabarcoding

Environmental DNA (eDNA) has become a prominent focus of research over the past decade, with numerous studies emphasizing its potential benefits, while others have drawn attention to its limitations. This has led to a degree of skepticism within the scientific community. Many of the advantages of eDNA are outlined in Chapter 2, which include the ability to detect rare species (Day et al., 2019; Pukk et al., 2021), endangered species (Budd et al., 2023), and morphologically similar or cryptic species (Hinlo et al., 2017; Shaw et al., 2016; Valdivia-Carrillo et al., 2021) that may be overlooked or misidentified by traditional methods. Additionally, eDNA approaches often detect a greater number of species compared to conventional sampling techniques and enable broader spatial and temporal coverage due to the relative ease of sample collection. This tool is particularly useful in systems that have hazardous conditions (e.g. high levels of pollution, high gradient streams or adverse weather), which may not be safe to enter to perform more traditional sampling methods. In this study, these advantages were not fully realized due to low amplification and sequencing success rates.

There are four main limitations of eDNA as identified by Nagarajan et al. (2022) and these limitations are discussed in the context of exisiting literature and insight from this research:

Limitation 1: "*eDNA does not give direct information about organism size or absolute abundance*"

The research presented in Chapter 2 was limited to presence/absence data due to the influence of various biotic and abiotic factors on DNA degradation and preservation, affecting the reliability of eDNA for estimating species abundance. The persistence of eDNA is affected by factors such as the condition of the DNA at the time of shedding, the duration since it was released, and environmental conditions like salinity, water temperature, and turbidity (Holmes et al., 2024).

Additionally, the physical characteristics of the DNA molecule, including its length and structure, play a role in its degradation (Nagarajan et al., 2022).

Limitation 2: ***"Detections have some spatial and temporal ambiguity"***

A well-documented limitation of eDNA metabarcoding is the potential for false negatives (failure to detect a species that is present) and false positives (detection of a species that is absent) (Larson et al., 2020). These errors can be minimized through rigorous laboratory protocols, the inclusion of appropriate controls, and measures to reduce contamination. However, in estuarine systems, false positives can also occur through DNA being transported via tributaries, fishing activities, tidal movement, commercial transport, and wastewater discharge. Accounting for such sources is particularly challenging in dynamic marine systems, where eDNA can be transported over long distances, up to 800 meters in some cases (Waters et al., 2023). However, the distance that DNA is transported is dependent on the environment and various abiotic factors, for example tidal movements may transport eDNA from adjacent habitats, resulting in the detection of species outside the targeted survey area (Waters et al., 2023).

Limitation 3: ***"Demographic data such as age and life stage cannot be determined at this time"***

eDNA does not provide information on ecosystem function that is typically collected through traditional sampling methods, such as size frequency distributions and individual abundance (Waters et al., 2023). These data are critical for conservation efforts, particularly as climate change drives ecological shifts. For example, rising temperatures can lead to skewed sex ratios, resulting in genotype–phenotype mismatches with potential long-term impacts on population dynamics (Geffroy & Wedekind, 2020).

Limitation 4: ***"Assays developed in one natural system may not work in others because of differences in biodiversity, chemical inhibitors, and hydrogeochemical differences, and thus require pilot testing."***

While eDNA is widely recognized as a valuable tool for biodiversity monitoring, its application in dynamic and turbid environments such as estuaries presents several challenges (Holmes et al., 2024). The value of eDNA metabarcoding data is contingent on eDNA concentrations and the methods used to collect and analyze the DNA. The former is impacted by species-specific shedding rates, DNA degradation and environmental transport processes (e.g., currents, salinity gradients). The latter is impacted by sampling protocol including filter pore size, sample volume, DNA extraction efficiency, inhibition, primer selection, and PCR amplification biases (Barnes & Turner, 2016; Goldberg et al., 2016).

Our study provided an opportunity to evaluate the effectiveness of current eDNA protocols in Southern California estuaries, with particular attention to PCR inhibition, primer efficacy, and filtration constraints. These challenges were especially pronounced due to the turbid conditions present in estuaries, where high sediment loads and elevated organic matter can interfere with sample processing (Holmes et al., 2024). Turbidity has been documented to negatively impact DNA detection by clogging filters, resulting in reduced volume samples and long filtration times, and in our case, prevented the filtration of the full target volume (1 liter per sample in this study). To compensate, we processed two filters per sample, which increased both workload and costs. The suspended particulate matter in turbid water can include plankton, detritus, and other organic debris, which not only clog filters but also introduce PCR inhibitors such as humic and fulvic acids (Holmes et al., 2024; Matheson et al., 2010). These substances can interfere with enzymatic reactions during DNA amplification, further reducing detection probability.

As part of our protocol, samples were stored at −80°C following filtration and prior to extraction. Although this is a standard approach, freeze-thaw cycles may degrade DNA, particularly in low-concentration samples. DNA quantification using a Qubit fluorometer revealed low yields (<1 ng/μL), consistent with previous findings in high-productivity systems where target fish DNA comprises only a small fraction of total recovered DNA.

Our study made use of the MiFish-U primer, which is widely used to study fish DNA, but is known to also amplify substantial quantities of marine prokaryotes (Gold, 2020). This co-amplification can reduce detection sensitivity for target taxa, lead to inaccurate species identification, and result in inefficient use of sequencing resources. Recent work by Baidouri et al. (2025) identified additional steps to increase primer specificity. This included the use of two different bead-based extraction methods, these being Kingfisher protocol and a Qiagen-based extraction. Following extraction, an additional step was also applied for inhibition removal. This involved using a ZYMO OneStep-96 PCR Inhibitor Removal Kit (CAT: D6035) following the manufacturer's instructions. Following this, 2% E-Gel electrophoresis was performed to verify whether successful amplifications had occurred during PCR. Despite performing these additional steps, this study found that for many sites the target bands were still hidden by amplification of bacteria. Therefore, to further increase target amplification Platinum SuperFi II polymerase was used. For samples which still exhibited inhibition, an additional inhibition removal step was followed to improve amplification. Incorporating these additional steps may improve future eDNA protocols for addressing the challenges of Southern California estuaries.

Baidouri et al. (2025) also recommended using a combination of MiFish-U and MiFish-E4 primers targeting bony fish and elasmobranchs (sharks and rays), respectively, to provide a greater taxonomic coverage. The addition of another primer also reduces the concern that general primers

such as MiFish-U have a greater affinity to sequence some species over others, providing biased results (Thomsen & Willerslev, 2015). Additionally, for some taxa, such as rockfishes, the MiFish 12S barcode lacks the resolution needed to distinguish between closely related species, therefore incorporating additional primers could improve taxonomic resolution (Gold, 2020).

Another consideration that may be evident during PCR, is the formation of primer-dimers, which are artifacts formed by primers binding to each other rather than to the target DNA (Baidouri et al., 2025). Their formation can be minimized by implementing steps to increase specificity, such as using high-fidelity enzymes like Platinum SuperFi II polymerase (Baidouri et al., 2025), and by purifying the amplified libraries (e.g., with BluePippin) to remove short fragments and remaining primer-dimers.

Finally, the accuracy of species identification from sequence reads depends on the quality and completeness of the reference database (Darling et al., 2017). These databases are built by sequencing tissue from individual specimens, a process that relies on taxonomic expertise. Chapter 2 presents a gap analysis conducted to identify species potentially missing from our reference database. Within the California Current Large Marine Ecosystem, extensive efforts have been made to sequence most marine fish species (Gold et al., 2020). Nonetheless, our analysis revealed several gaps in the existing reference databases that should be addressed in future studies.

3.3 Management Recommendations

This chapter outlines the challenges, benefits, and limitations of traditional surveys, community science, and eDNA, with particular emphasis on eDNA constraints and recommendations Each method has different tradeoffs: traditional surveys provide robust data but

are resource-intensive; iNaturalist offers broad coverage but has spatial and taxonomic biases; and eDNA detects rare taxa but is constrained by incomplete databases and inhibitors.

Each monitoring approach has limitations, underscoring the value of integrating multiple methods. Continued research should focus on refining protocols (e.g., addressing PCR inhibition in eDNA, developing taxonomic filters for iNaturalist, and minimizing biases in traditional gear), while also investing in accurate taxonomic resources and expertise. Although new tools reduce the need for field identification, taxonomists remain essential for building reference databases and ensuring correct species identifications.

More broadly, effective monitoring requires clear decision frameworks that link results from all methods to management actions. Without such pathways, there is a risk of inconsistent interpretation or delayed response in time-sensitive scenarios (Darling, 2020). For example, eDNA can serve as an early-warning tool for invasive species, but traditional sampling may be needed to confirm detections before management actions are implemented. Similarly, community science data can highlight under-monitored areas, guide targeted surveys, and provide valuable insight into species presence, serving as a potential early detection tool. Understanding and clearly defining the benefits and limitations of each monitoring approach is essential for managing stakeholder expectations and ensuring results are interpreted correctly. This clarity also helps determine management actions and increases the likelihood that data (whether from eDNA, iNaturalist, or traditional surveys) are applied effectively to support ecological decision-making.

This study demonstrates that no single sampling method is sufficient on its own. Instead, the most effective strategy is a complementary, context-specific approach that balances sensitivity, cost, and practicality, providing a stronger foundation for monitoring fish communities in Southern California estuaries.

Figures

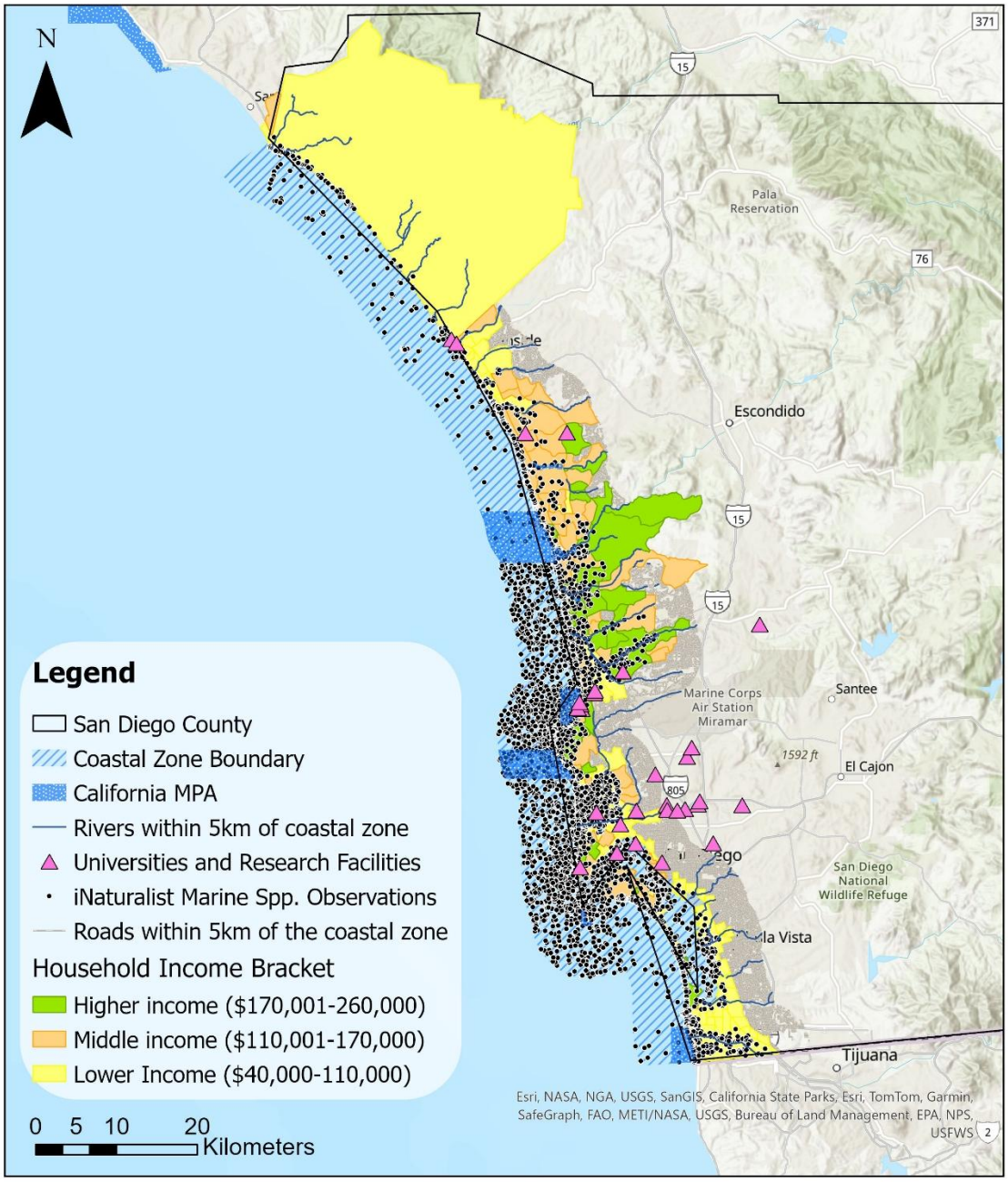

Figure 1. Preliminary analyses showing the distribution of marine species observations on iNaturalist in relation to possible factors that may be causing spatial biases in San Diego County. These factors include proximity to a research facility / university, population density, household income, proximity to roads and whether the area is a marine protected area or not. Note: this iNaturalist data is a different dataset to the iNaturalist data used in Chapter 2.

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Appendix A

MONITORING PLAN FOR THE SONGS' WETLAND MITIGATION PROJECT

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EXECUTIVE SUMMARY

Condition A of the coastal development permit (permit # 6-81-330) issued by the California Coastal Commission (CCC) requires Southern California Edison (SCE) and its partners to create or substantially restore 150 acres of tidal wetlands at an approved location within the Southern California Bight. The purpose of this condition is to serve as out-of-kind mitigation that compensates for past, present and future impacts to fish caused by the operation of San Onofre Nuclear Generating Station (SONGS) Units 2 & 3. A revised plan for wetland restoration at the San Dieguito River Valley, submitted to the CCC by SCE on November 3, 1997, called for the excavation of approximately 115 acres of upland to create tidal wetland and the enhancement of 35 acres of existing tidal wetland through the continuous maintenance of the tidal inlet in perpetuity. Created and restored habitats in this plan included a subtidal basin, channels, and intertidal mudflats and vegetated salt marsh. On October 12, 2005, the Commission approved the Final Restoration Plan and CDP #6-04-88, as conditioned, for the San Dieguito Wetlands Restoration Project. Construction of the wetland restoration project at San Dieguito commenced in August 2006 and was completed on September 29, 2011, with the completion of the inlet opening.

Monitoring by independent contract scientists working for the CCC is being done to: (1) determine whether performance standards established for the wetland mitigation project are met, (2) determine, if necessary, the reasons why any performance standard has not been met, and (3) develop recommendations for appropriate remedial measures. The coastal development permit for SONGS requires that monitoring of the wetland restoration project be done over the full operating life of SONGS Units 2 & 3 (=32 years). In accordance with the SONGS coastal development permit, scientists retained by the Executive Director of the CCC developed a Monitoring Plan to guide the monitoring work and oversee the monitoring studies outlined in the Plan. The SONGS coastal development permit provides a description of the performance standards and the monitoring required for the wetland mitigation project. This Monitoring Plan closely adheres to the monitoring requirements of the permit and includes: (1) a description of the process used to evaluate condition compliance, including a list of performance standards by which the success of the mitigation wetland is judged, (2) descriptions of the specific sampling methods and analyses that are used to evaluate each of the performance standards, (3) an explanation of how project data are managed and archived for future use, and (4) a description of how the results from the monitoring program are disseminated to the CCC, the applicant, and all other interested parties.

This monitoring plan is a living document that will be updated as needed to ensure and maintain rigorous monitoring and evaluation of Condition A in the most cost-effective manner possible. A history of updates to the monitoring plan is provided in Appendix 6 of this document.

1.0 INTRODUCTION

Through its 1991 and 1997 coastal permit actions (permit # 6-81-330-A, formerly 6-83-73) the California Coastal Commission (CCC) adopted permit conditions that require Southern California Edison (SCE) and its partners to create or substantially restore 150 acres of tidal wetlands at an approved location within the Southern California Bight. The purpose of this condition is to provide out-of-kind mitigation that compensates for past, present and future impacts to fish caused by the operation of San Onofre Nuclear Generating Station (SONGS) Units 2 & 3.

On June 11, 1992, the CCC approved SCE's choice of the San Dieguito River Valley as the restoration site that meets the minimum standards identified in the SONGS permit and best meets the objectives of the wetland mitigation requirement. On April 9, 1997, the CCC reaffirmed its prior determination that the San Dieguito River Valley is the restoration site and determined that SCE can propose an additional site for restoration only if achieving all 150 acres of restoration at the San Dieguito River Valley becomes infeasible due to hydrology or other engineering concerns. The CCC also determined that up to 35 acres of enhancement credit could be obtained for inlet maintenance if wetland restoration is done at San Dieguito.

A preliminary plan for wetland restoration at the San Dieguito River Valley (32.969545^0N, 117.26047^0W) was submitted to the CCC by SCE on September 30, 1997. In November 1997, the Commission approved SCE's revised preliminary wetland restoration plan as largely conforming with the minimum standards and objectives stated in the permit. The revised plan called for the excavation of approximately 115 acres of upland to create tidal wetland and the enhancement of 35 acres of existing tidal wetland through the continuous maintenance of the tidal inlet in perpetuity. Created and restored habitats in this plan include a subtidal basin, channels, intertidal mudflats, and vegetated salt marsh. In addition, the plan included the construction of least tern nesting sites, flood control devices such as berms and weirs, and the creation of non-tidal (seasonal) salt marsh, coastal sage scrub and grassland habitats. On October 12, 2005, the Commission approved the Final Restoration Plan and CDP #6-04-88, as conditioned, for the San Dieguito Wetlands Restoration Project. Construction of the wetland restoration project at San Dieguito commenced in August 2006 and was completed on September 29, 2011, with the completion of the inlet opening.

Condition A (Wetland Mitigation) of the SONGS permit requires monitoring, management (including maintenance) and remediation of the wetland restoration be done for a period not less than the full operating life of SONGS plus years monitored without the project attaining compliance with performance standards in the permit. The full operating life of SONGS includes past and future years of operation of SONGS Units 2 and 3 and the decommissioning period to the extent there are continuing discharges (=32 years).

In accordance with Condition D (Administrative Structure) of the SONGS permit, the post-construction monitoring of the wetland restoration will be done independently of SCE and its partners. Scientists retained by the Executive Director of the CCC shall develop the Monitoring Plan, in consultation with SCE and appropriate lead agencies, and will oversee the monitoring studies outlined in the Plan. The present document serves as the Monitoring Plan for the

SONGS' wetland mitigation requirement and provides a general framework to guide the monitoring work. Further details of the monitoring effort will be set forth in biennial work plans. This Monitoring Plan has been reviewed by the Science Advisory Panel (SAP) retained by the Executive Director of the CCC.

It should be noted that the SONGS permit provides a description of the monitoring required for the wetland mitigation project. Specifically, the permit describes the duration of monitoring, the performance standards to be used for judging the success of the restoration, the use of reference sites as a standard of comparison, and the parties responsible for monitoring and evaluating the restoration project. This Monitoring Plan closely adheres to the conditions of the permit and includes a description of the performance standards that will be used to evaluate the success of the restoration and the sampling methods that will be used to make this evaluation. The focus of the CCC Monitoring Plan is on assessing project compliance using the performance standards stated in the permit. Thus, there are a number of issues related to management of the restored wetland that are not included in this document, such as monitoring and maintenance of least tern nesting sites, removal of trash, control and enforcement of public access, mosquito control and development in the watershed. In addition, the CCC or other agencies may add monitoring requirements as part of their regulatory oversight of the wetland restoration project, and this Monitoring Plan does not consider these possible requirements.

2.0 EVALUATION OF CONDITION COMPLIANCE

Condition A identifies the physical and biological performance standards that must be met in order for the wetland restoration project to be considered in compliance with the SONGS permit. The performance standards fall into two categories: (1) absolute standards, which require that the variable of interest be evaluated only within San Dieguito Wetlands, and (2) relative standards, which require that the variable of interest be similar to that measured in natural tidal wetlands within the region. The standards pertaining to topography, tidal prism, habitat areas, plant reproductive success, and exotic species are absolute standards, whereas the standards pertaining to water quality, biological communities, vegetation, *Spartina* canopy architecture, and food chain support, are relative standards that will be evaluated in comparison to reference wetlands.

In this section of the monitoring plan we provide: (1) a list of the performance standards for the wetland mitigation project as provided in the SONGS permit, (2) an explanation of the process used to select the reference wetlands that will be used as a measure of comparison in assessing the relative performance standards, (3) a description of the measure of similarity that will be used to assess compliance of the relative performance standards, and (4) a schedule for the monitoring period.

2.1 Performance standards

The following performance standards will be used to measure the success of the restored wetland and to determine whether remediation is necessary.

Absolute Performance Standards.

Tidal Prism. The designed tidal prism shall be maintained, and tidal flushing shall not be interrupted.

Habitat areas. The area of different habitats shall not vary by more than 10% from the areas indicated in the final restoration plan.

Topography. The wetland shall not undergo major topographic degradation (such as excessive erosion or sedimentation).

Reproductive Success. Certain plant species, as specified in the work program, shall have demonstrated reproduction (i.e. seed set) at least once in three years.

Exotics. The important functions of the wetland shall not be impaired by exotic species.

Relative Performance Standards

Water Quality. Water quality variables [to be specified] shall be similar to reference wetlands.

Fish. Within 4 years of construction, the total densities and number of species of fish shall be similar to the densities and number of species in similar habitats in the reference wetlands.

Macro-invertebrates. Within 4 years of construction, the total densities and number of species of macro-invertebrates shall be similar to the densities and number of species in similar habitats in the reference wetlands.

Birds. Within 4 years of construction, the total densities and number of species of birds shall be similar to the densities and number of species in similar habitats in the reference wetlands.

Vegetation. The proportion of total vegetation cover and open space in the marsh shall be similar to those proportions found in the reference sites.

Algae. The percent cover of algae shall be similar to the percent cover found in the reference wetlands.

***Spartina* Canopy Architecture**. The restored wetland shall have a canopy architecture that is similar in distribution to the reference sites, with an equivalent proportion of stems over 3 feet tall.

Food Chain Support. The food chain support provided to birds shall be similar to that provided by the reference sites, as determined by feeding activity of the birds.

2.2 Reference Wetlands

The SONGS permit specifies that successful achievement of the performance standards will in some cases be measured relative to reference wetlands. The rationale for requiring that the value of a resource in the restored wetland be similar to that in reference wetlands is based on the belief that to be successful the restored wetland must provide the types and amounts of resources that occur in natural wetlands. Resources in natural wetlands, however, vary in

space and time. Differences in physical characteristics of a wetland (e.g., soil, topography, flood regime, tidal hydrology) can cause plant and animal assemblages to differ greatly among tidal wetlands while seasonal and inter-annual differences in weather, nutrient loading, and oceanographic conditions can cause the biological assemblages within tidal wetlands to fluctuate over time.

Ideally, the biological assemblages in a successfully restored wetland should vary in a manner similar to those in the natural wetlands used for reference. Temporal variability, especially of the sort associated with weather (e.g., air temperature, rainfall) or oceanographic conditions (e.g., swell height, sea level anomalies, water temperature) can be accounted for by sampling the restored and natural reference wetlands concurrently. Concurrent monitoring of the restored and natural wetlands will help ensure that regional changes in weather and oceanographic conditions affecting the restored wetland will be reflected in the performance standards, since nearby reference wetlands will be subjected to similar conditions.

The permit requires that the wetlands chosen for reference be relatively undisturbed, natural tidal wetlands within the Southern California Bight (i.e., from Pt Conception to the US/Mexico border). Relatively undisturbed wetlands have minimal human disturbance to habitats (e.g., trampling of vegetation, boating, fishing). Natural wetlands are not constructed or substantially restored. Tidal wetlands are continuously open to the ocean and receive regular tidal inundation. After evaluating more than 40 wetlands within the Southern California Bight three wetlands, Tijuana River Estuary (32.569901^0N, 117.126712^0W, Mugu Lagoon (34.102007^0N, 119.097011^0W), and Carpinteria Salt Marsh (34.401189^0N, 119.537990^0W) were chosen as reference wetlands that best met the criteria of undisturbed, natural tidal wetlands within the Southern California Bight.

2.3 Determination of similarity

A requirement of the SONGS permit is that the response variables used to assess the relative performance standards of the San Dieguito Wetlands Restoration Project (hereafter referred to as “relative performance variables”) be “similar” to those of the reference wetlands. Evaluating whether the performance of the San Dieguito Wetlands is similar to that of the reference wetlands requires that the mean (or in some cases the median) value for a given relative performance variable at San Dieguito Wetlands not be significantly lower than the mean (or median) value at the lower performing of the three reference wetlands. Significance is determined using an approach that utilizes both a formal probability value and an effect size. Generally this is done by means of a t-test except in the case of the performance standards pertaining to vegetation, algae, and food chain support (bird feeding). For the standards pertaining to vegetation and algae, only the mean values are compared because the values are wetland wide estimates made using aerial imagery and thus there are no estimates of variability about a mean value. For the food chain support standard, significance is determined by a resampling procedure in which the effect size is calculated as the proportional difference in the medians of the resampled distributions of the San Dieguito Wetlands and the lower performing reference wetland, and the p-value is the percentile in the distribution of the lower performing reference wetland that is equal to the median value of the San Dieguito Wetlands.

The performance for a particular relative performance variable at San Dieguito Wetlands is considered to be worse than the lower of the three reference wetlands if the p-value for the comparison is ≤ the proportional effect size (i.e., the proportional difference between San Dieguito Wetlands and the worst performing reference wetland). The only exception to this rule is when the p-value and the proportional effect size are both greater than 0.5, in which case assessment for the period is considered inconclusive and additional studies will be done. As an example, if the proportional effect size for a given performance variable was 0.25 (i.e., the mean value at San Dieguito Wetlands was 75% of the mean value at the worst of the three reference wetlands), then a t-test yielding a p-value ≤ 0.25 would indicate the San Dieguito Wetlands Restoration did not meet the performance standard, whereas p-values > 0.25 would indicate that it did meet the performance standard.

The rationale for using the mean value of the worst performing of the reference wetlands is that the reference wetlands are considered to be acceptable measures of comparison for the San Dieguito Wetlands. Hence if the San Dieguito Wetlands Restoration is performing at least as well as one of the reference wetlands, then it should be judged successful. The scaling of the p-value (α) to the effect size recognizes sampling error when estimating mean values and balances the probability of falsely concluding that the San Dieguito Wetlands Restoration is not similar to the reference wetlands when it is (Type I error) with the probability of falsely concluding that the San Dieguito Wetlands Restoration is not similar to the reference wetlands when it is not (Type II error).

To ensure that the San Dieguito Wetlands are not held to a higher standard than the reference wetlands the above procedure is also applied to the three reference wetlands (Tijuana Estuary, Mugu Lagoon, and Carpinteria Salt Marsh) to evaluate whether they would have met the relative performance standards. This is done, for example, by treating Tijuana Estuary as the mitigation wetland and using the other wetlands as the three reference wetlands. The San Dieguito Wetlands are considered similar to the reference wetlands if the number of relative standards met by the San Dieguito Wetlands is equal to or greater than the number of relative standards met by any of the reference wetlands. The above approach ensures that the assessment of similarity is consistent with the SONGS permit requirement that the performance standards be met without the unreasonable requirement that the San Dieguito Wetlands outperform Tijuana Estuary, Mugu Lagoon, and Carpinteria Salt Marsh for every performance standard. Importantly, this approach deals realistically with the inherent variability of nature in a manner that best serves the interests of both the public and SCE.

2.4 The Monitoring Period and Project Compliance

Conditions A and D of the SONGS permit describe the monitoring requirements for the wetland mitigation project, which we summarize below. Additional documentation is provided in Appendix 1.

Fully implemented (Stage 1) monitoring will ensue upon completion of wetland construction and be conducted for a period of not less than 4 years post-construction. All performance standards must be met within 10 years, which is the same amount of time required for the mitigation reef to meet all of the performance standards required of that mitigation project.

Within 4 years of construction, the total densities and number of species of fish, macro-invertebrates and birds must be similar to the densities and number of species in similar habitats in the reference wetlands. The wetland restoration project will be considered *in compliance* when *all of the performance standards have been met for each of three consecutive years*. The duration of the monitoring period will not be less than the full operating life of SONGS plus years monitored without the project attaining compliance with permit standards. SCE will receive mitigation credit for each year that the project is in compliance. The mitigation requirements will be fulfilled when the number of years of compliance equals the total years of operation of SONGS Units 2 & 3, including decommissioning period to the extent that there is continuing operation of cooling water discharge. Remediation may be required if an individual performance standard is not met within ten years or if compliance has not occurred within 12 years. The Executive Director can prolong Stage 1 monitoring or reinstate it if necessary following remediation or degradation of the wetland (resulting in a period of non-compliance).

Condition D of the SONGS permit establishes that upon determination that all of the performance standards have been met for three consecutive years, a scaled back level of monitoring (Stage 2) may ensue. The scaled back monitoring program will be designed and implemented by CCC staff scientists. Reduction in monitoring effort will be based on analyses of data collected during the period in which the project was in compliance. Staff scientists will examine these data to determine the minimum effort that would have been necessary to assess compliance during this period**.** All monitoring, whether it be Stage 1 or Stage 2 must be sufficient for assessing project compliance with the performance standards.

If the restored wetland is in a period of reduced monitoring and if it falls out of compliance for a period of two consecutive years, then to determine if non-compliance is an artifact resulting from a reduction in monitoring effort, full monitoring (Stage 1) may be re-established for those standards that are not being met. If resumption of full monitoring leads to the conclusion that the reduction in monitoring was responsible for non-compliance, then monitoring will remain at the full levels for the duration of the study or until the Executive Director concludes that reduced monitoring could be reinstituted

SCE and its partners are fully responsible for any failure to meet these goals and standards for the full operating life of SONGS Units 2 and 3. If the restored wetland is not in compliance within 12 years post-construction or has not met the biological community standard for fish, macro-invertebrates, and birds within 4 years, then the permittee shall be required to fund an independent study to collect the information necessary to determine what remediation is needed. The permittee shall also be required to implement any remedial measures determined necessary by the Executive Director in consultation with state and federal resource agencies and will provide funds for independent monitoring that evaluates the success of the required remediation. Remediation monitoring may be different from the compliance monitoring required by the permit.

3.0 SAMPLING METHODS AND DATA COLLECTION

3.1 Methods for assessing the relative performance standards

The level of certainty in assessing compliance of the SONGS wetland mitigation project with the performance standards is directly related to sampling effort. Data collected during pre-restoration monitoring at the reference wetlands for invertebrates, fish, and birds together with the advice of experts were used to determine the level of sampling that would likely be needed to detect a 20% deviation from the relative performance standards (i.e., the effect size which is calculated as the proportional difference between the mean values for the San Dieguito Lagoon restoration project and the lowest performing reference wetland) with an 80% probability (i.e., the statistical power calculated as 1- Type II error) using a Type I error (α) = 0.2 (see Appendix 1). However, in practice, only two of the three parameters (effect size, statistical power and α), can be controlled in a sampling design with a fixed sampling effort. The basis of the analysis of monitoring data is to balance risk associated with under-performance of the restored wetland with the risk associated with making errors in assessment (Type I (α) and Type II (β)). Given that statistical power is directly related to both Type I error (α) and effect size, the decision as to whether the restored wetland is in compliance with the relative performance standards will be based on a partially linked relationship between effect size and α, which is equal to the calculated p - value from an analysis.

The following rules will be used when assessing compliance of the relative performance standards:

1) If $\alpha \leq$ effect size for any α ranging from 0.000 to 0.500, then the restored wetland will be considered out of compliance for the period of assessment (α and effect size rounded to three significant figures).
2) If $\alpha >$ effect size for any effect size ranging from 0.000 to 0.500, then the restored wetland will be considered in compliance for the period of assessment (α and effect size rounded to three significant figures).
3) If the effect size is > 0.500 and α is > 0.500 then assessment for the period will be considered inconclusive (α and effect size rounded to three significant figures) and the following steps will be taken:
    a. The sampling design may be revised to increase the statistical power to an expected value of at least 80%. Whether this effort is necessary will be based on the history of the performance of the restored wetland with respect to the performance variable. For example, if the analyses were conclusive in previous periods, then a single inconclusive analysis would not be sufficient to invoke a revision of the sampling design.
    b. If needed, the revised sampling design will be implemented the following year.
    c. If in the following year the standard is met, the standard will be considered to have been met the previous year as well. If in the following year the standard is not met, the standard will be considered to not have been met the previous year as well.

   d. This process will continue until the standard can be assessed, unless the Commission changes the standard set forth in SONGS permit condition A.
4) Monitoring data will be evaluated annually to determine if changes need to be made to the sampling program to bring it closer to the design objective of detecting a 20% deviation from the performance standards (i.e., the effect size) with an 80% probability (i.e., the statistical power) using a type I error (α) = 0.2.

3.2 Data collection

Listed below are the approaches used to evaluate the physical and biological performance standards in Condition A of the SONGS permit. Included is a discussion of the sampling methods used in collecting the information needed to evaluate that standard. In accordance with Condition D of the SONGS permit, final determination of the sampling design (e.g., number and distribution of sampling stations, samples per station) and specific details of the monitoring methods may be adjusted in biennial work programs.

Absolute Performance Standards

1. **Tidal prism**

THE DESIGNED TIDAL PRISM SHALL BE MAINTAINED, AND TIDAL FLUSHING SHALL NOT BE INTERRUPTED.

Approach: The tidal prism is the amount of water that flows into and out of an estuary with the flood and ebb of the tide, excluding any contribution from freshwater inflows (Hume 2005). A reduction in the tidal prism of the restored wetland can have detrimental effects on water quality and alter the area of inundated habitat. Numerical modeling suggested that after restoration, the tidal prism in the lagoon would increase. However, predictions of tidal prism from this modeling are likely to differ from actual values for the as-built wetland since they do not include the effects of friction, which could contribute to a smaller than predicted tidal prism and are not based on the actual as-built topography. Therefore, the tidal prism of the restored wetland will be calculated from empirical measurements on completion of construction and used as the standard of comparison to detect changes in this performance variable during subsequent monitoring.

Since tidal prism can influence the area of planned wetland habitat hit by the tides, the tidal prism standard is evaluated, in part, using criteria set forth in the habitat areas standard, which provides that the areas of the different habitats (subtidal, intertidal mudflat, vegetated salt marsh) shall not differ by more than 10% from planned values (see 3.2.2). The planned tidal volume-elevation relationship indicated that a decrease in tidal prism of greater than 12% could result in a reduction in the area of planned salt marsh habitat (1.3 to 4.5 ft NGVD) of greater than 10%. Since the area of salt marsh habitat may not differ by more than 10% from the planned area, the tidal prism can not be less than 88% of the as-built prism to ensure no more than 10% of planned salt marsh habitat remains exposed during a 4.5 ft tide. Moreover, since a larger than planned tidal prism could increase erosion within the restored wetland, the prism shall also not be larger than 112% of the as-built prism.

Tidal prism is calculated by integrating measurements of tidal flow taken at the Jimmy Durante Bridge (0.9 km from the inlet) using a portable acoustic Doppler profiler/discharge measurement system (SonTek RiverSurveyor or equivalent) towed back and forth across the width of the channel every 15 minutes during an incoming tide. The system is mounted aboard a raft (hydroboard) and features bottom tracking for displaying bathymetry, real-time display of current profiles, and computes discharge. To capture reductions in tidal prism that could require inlet maintenance, or increases in prism that could lead to erosion, tidal prism is measured monthly during spring and neap tides. The performance standard is met if the regression line fit through the prism measurements taken during the monitoring year fall within 12% of the as-built tidal prism values, which were based on measurements of a range of high tides taken in July 2012.

To adaptively manage for deleterious changes in tidal prism, the measurements at Jimmy Durante Bridge may be supplemented with surveys of flow further within the wetland at channels leading to the large basin (W1) and the large intertidal area of W4 and W16. These surveys will be used to proactively identify impeded tidal flow into or out of these areas and inform maintenance action.

SCE's restoration plan calls for the one-time restorative dredging of the inlet channel and lagoon channels followed by routine maintenance dredging approximately every eight months, or as needed, to maintain the inlet channel at the designed configuration. The plan calls for an inlet channel depth of -2.0 to -4.0 ft NGVD and a channel width of 150 ft at MHHW east of Highway 101. The inlet channel west of Highway 101 will be narrower with a depth of -2.0 ft NVGD and a width of approximately 130 ft. Tidal flushing will be uninterrupted if these channel depths and widths are maintained (SCE 2005).

2. **Habitat areas**
THE AREAS OF DIFFERENT HABITATS SHALL NOT VARY BY MORE THAN 10% FROM THE AREAS INDICATED IN THE FINAL RESTORATION PLAN.

Approach: This performance standard is designed to preserve the mix of habitats provided in the Final Restoration Plan (SCE 2005) and to guard against large-scale conversions of one habitat type to another, for example, of vegetated marsh to mudflat. The Final Restoration Plan indicates that subtidal habitat will occur at elevations of < -0.9 ft NGVD, intertidal mudflat will occur from -0.9 to 1.3 ft NGVD, and intertidal salt marsh will extend from 1.3 to 4.5 ft NGVD and specifies the acreages of the different habitats (Appendix 9). While this is useful for planning the acreages of the proposed subtidal, mudflat, and salt marsh habitats, these habitats may not be constrained by planned elevation boundaries. As a result, areas of the three habitats are assessed using criteria based on inundation, elevation, and cover of vegetation.

Areas are assessed as subtidal habitat if they remain continuously submerged. Areas are assessed as mudflat habitat if they are 1) intertidal, 2) located below an elevation of 3.5 ft NGVD and thus subject to regular tidal flooding, and 3) sparsely vegetated, possessing < 5% cover of vegetation as mudflats are by definition intertidal and unvegetated. The upper elevation limit for mudflat for the habitat areas assessment is based on the observation of

surface salt deposits above 3.5 ft NGVD in some areas of the restoration project indicating infrequent tidal inundation. Areas are assessed as salt marsh habitat if they are 1) intertidal, 2) at or below 4.5 ft NGVD, which is the upper limit of tidally influenced habitat for this project, and 3) have a cover of native salt marsh vegetation of ≥ 30% to provide perches and foraging habitat for Belding's Sparrow and other species. Elevation contours are determined using a Real Time Kinematic (RTK) global positioning system (GPS) with a vertical and horizontal accuracy of a few centimeters (typically 3 cm).

Cover of vegetation, water, and bare space are determined using multispectral aerial imagery collected annually in the red green blue (RGB) and near-infrared (NIR) spectrum within 10 x 10 m squares on a grid superimposed over the entire restored tidal wetland. Aerial images are taken in the spring-early summer, which is the period of maximum growth of marsh plants. Because the ability to classify ground cover type based on spectral data varies with weather conditions, the images are calibrated for each aerial survey. The full classification of the aerial image is also inspected at a subset of georeferenced locations to confirm the classification. Nominal pixel size evaluated as vegetated, bare, or water using aerial imagery within each 10 x 10 m plot is 20 x 20 cm. Each 10 x 10 m grid square will be assessed as subtidal, mudflat, or salt marsh habitat using the criteria above. The grid square areas will be summed by habitat with the aid of GIS software and compared to the planned acreages in the Final Plan (SCE 2005) to determine whether they are within 10% of planned values. Grid squares that are not assessed as one of the planned habitats are assigned to an "other" category.

3**. Topography**
THE WETLAND SHALL NOT UNDERGO MAJOR TOPOGRAPHIC DEGRADATION (SUCH AS EXCESSIVE EROSION OR SEDIMENTATION).

Approach: Topographic changes resulting from excessive erosion or sedimentation could impede tidal flow within the wetland altering tidal prism and the areas of planned wetland habitat. Erosion or sedimentation within the restored wetland may result from high volumes of storm run-off, littoral movement of sand that block the inlet channel, or other causes.

This standard is evaluated using visual surveys throughout the restored wetland to identify any sign of substantial erosion or sediment deposition that could impede tidal flow. Surveys are done monthly. Additional surveys will be done following storm events when bank erosion and channel scour and sediment deposition is likely to occur. Constructed berms and associated structures (e.g. culverts and weirs) are a special topographical feature of the restored wetland. These features are also visually inspected during the surveys. Since the success of the restoration depends critically on tidal exchange and the proper functioning of the berms and associated structures, excessive erosion or sediment deposition that impedes tidal flow or a structural weakening of berms will trigger maintenance operations.

The CCC has defined 4.5 ft NGVD as the upper limit of tidally influenced habitat for the calculation of acreage credit for this restoration project. Sediment from the erosion of berms, disposal sites, or other sources may become deposited below this limit, raising

marsh elevation. Because of this, the location of the 4.5 ft contour is of special interest and may be surveyed annually. The area bounded by the 4.5 ft NGVD contour will be calculated to evaluate compliance with the acreage requirement.

4. **Reproductive success**
CERTAIN PLANT SPECIES, AS SPECIFIED IN THE WORK PROGRAM, SHALL HAVE DEMONSTRATED REPRODUCTION (I.E. SEED SET) AT LEAST ONCE IN THREE YEARS.

Approach: The reproductive success of salt marsh plants is evaluated by measuring whether mature seed is produced for seven species that occur in the intertidal habitat of the restored wetland. Species for evaluating plant reproductive success include Parish's Glasswort (*Arthrocnemum subterminale*), Salt Grass (*Distichlis spicata*), Pickleweed (*Salicornia pacifica*, formerly *Salicornia virginica*), Alkali Heath (*Frankenia salina*), Spiny Rush (*Juncus acutus*), Marsh Jaumea (*Jaumea carnosa*), and California Sea Lavender (*Limonium californicum*). These are the most common species found within the restoration site.

Sampling for the setting of mature seed is done in the summer-fall when seed set is expected to be greatest. Ten flower stalks, flower heads, or fruits are collected haphazardly across the wetland from each species for a total of 100 flower stalks, flower heads, or fruits for each species. Seed maturity is determined using reference guides. This standard is successfully met if all of the targeted species set any mature seed in at least one out of every three years.

5. **Exotics**
THE IMPORTANT FUNCTIONS OF THE WETLAND SHALL NOT BE IMPAIRED BY EXOTIC SPECIES.

Approach: Exotic species can cause compositional and functional changes in estuarine ecosystems. Such changes can occur, for example, through the alteration of food webs or the physical structure of habitats (e.g., burrowing activities that affect the stability of tidal channel banks). Monitoring data collected for fish (Section 3.2.7), invertebrates (Section 3.2.8), birds (Sections 3.2.9 and 3.2.13), and vegetation (Sections 3.2.10) are used to assess the prevalence of exotic species.

In addition, to adaptively manage for exotic species, a special survey that covers as much of the wetland as possible that looks for exotic species is conducted once a year. This special survey focuses on plants and visible invertebrates and incorporates a diving survey of the subtidal portion of the main basin (W1). Should areas of infestation be found, appropriate resource agencies will be consulted and targeted studies will be done to assess how the invader is affecting the functioning of the restored wetland. For example, the Australasian isopod *Sphaeroma quoyanum* is known to modify channel bank and marsh edge through its burrowing activities. Should this isopod be found, measurements of erosion rates in infested areas will be compared with those in uninfested areas to ascertain whether the isopod is unduly influencing channel geometry, which could influence tidal flow

and feeding habitat for some shorebirds and waders. *Musculista senhousia* is an invasive mussel from Asia that can occur in extremely high densities (e.g., several thousand per square meter). Should high densities of this mussel be found, studies may be done to determine if this mussel is negatively affecting the densities and species richness of native suspension-feeding species. *Caulerpa taxifolia* is a highly invasive alga that forms a dense mat on any surface, reducing plant and animal density and richness. This alga is of particular concern to State and Federal resource agencies. Should *Caulerpa* be found, appropriate resource agencies will be notified and management actions implemented.

Relative Performance Standards

6. **Water Quality**
WATER QUALITY VARIABLES [TO BE SPECIFIED] SHALL BE SIMILAR TO REFERENCE WETLANDS.

Approach: Because of its documented importance to wetland health, the concentration of dissolved oxygen is used to evaluate water quality within the restored wetland. Dissolved oxygen concentration can change rapidly with inlet closure resulting in adverse effects on estuarine biota. However, dissolved oxygen also varies with location, the tidal cycle and time of day (it is generally higher during the day due to oxygen provided by photosynthesis, and lower during the night due to respiration). Measurements of dissolved oxygen, water temperature, and salinity are made in San Dieguito Wetlands and the three reference wetlands (Tijuana Estuary, Mugu Lagoon, and Carpinteria Salt Marsh) using continuously recording environmental data loggers (e.g., HOBO Dissolved Oxygen Datalogger U26-001, HOBO U24-002-C Conductivity Logger). Instruments are calibrated in accordance with the manufacturer's specified protocol prior to deployment in the field. Data from these instruments are processed every two weeks. There is either one (primary) or two (primary and backup) sampling stations at each wetland location. The coordinates and number of stations at each wetland sampling location are as follows:.

Wetland	Stations	Latitude	Longitude
San Dieguito Wetland	2	32.97072	-117.26255
Carpinteria Salt Marsh	2	34.40063	-119.53953
Point Mugu Lagoon	2	34.11013	-119.09307
Tijuana Estuary	1	32.56833	-117.13128

An oxygen concentration below 3 mg/l is considered hypoxic and sustained concentrations below this value may be detrimental to estuarine biota (ESA, https://www.esa.org/esa/wp-content/uploads/2012/12/hypoxia.pdf). Therefore, one approach is to compare the average length of time dissolved oxygen is continuously below this concentration among wetlands. The water quality standard is evaluated annually by comparing the mean length in hours of continuous hypoxia between San Dieguito Wetlands and the reference wetlands. If the mean number of consecutive hours of DO concentration < 3.0 mg/l is significantly higher in the San Dieguito Wetlands than in the reference wetland with the highest value, then San Dieguito Wetlands fails to meet the standard.

To adaptively manage for decreases in dissolved oxygen concentrations during the year that may reflect poor tidal flushing within San Dieguito Lagoon, measurements of sustained dissolved concentrations of < 3.0 mg/l will trigger action to identify factors contributing to these values and inform maintenance action that may be necessary to raise these concentrations.

7**. Fish**

WITHIN 4 YEARS OF CONSTRUCTION, THE TOTAL DENSITIES AND NUMBER OF SPECIES OF FISH SHALL BE SIMILAR TO THE DENSITIES AND NUMBER OF SPECIES IN SIMILAR HABITATS IN THE REFERENCE WETLANDS.

Approach: Data on the density and numbers of species of fish are collected using 0.4 m^2 x 0.9 m circular enclosure traps, and beach seines (generally enclosing an area of approximately of 100 m^2). Enclosure traps are used in shallow water (≤ 0.7 m deep) to sample gobies (family Gobiidae), which are small, numerically abundant fishes that are poorly sampled by other methods (Steele et al. 2006a). Beach seines in combination with blocking nets are used to sample larger more mobile fishes (Steele et al. 2006b). Fish captured by both methods are identified and counted in the field and returned to the water alive. In cases where species identification is uncertain, voucher specimens are retained for later identification in the laboratory.

Habitats sampled during fish monitoring are main channels-basin and tidal creeks. A potential concern for the monitoring design was that basins of the type constructed in the San Dieguito Wetlands Restoration do not occur naturally in southern California wetlands, and thus cannot be compared to natural reference sites. However, data collected by Marine Ecological Consultants (MEC 1993) on fish abundance from different habitats at San Dieguito Lagoon prior to restoration found that fish assemblages were similar in basin and main channel habitats and thus it is biologically reasonable to treat the constructed basin as main channel habitat in post-construction monitoring. Basin habitat in the San Dieguito Lagoon Restoration is sampled using the same methods employed in tidal creek and channel habitats (i.e. a combination of enclosures and beach seines).

Because estuarine fish are patchily distributed, samples are spaced as widely as practical across these habitats to obtain representative estimates of fish density and species richness. For both fish sampling methods, the number of locations sampled within a wetland is maximized within logistical constraints. Based on available habitat in the reference wetlands (Tijuana Estuary, Mugu Lagoon, Carpinteria Salt Marsh), 6 tidal creek and 6 channel-basin locations within each wetland are sampled by each method (Figs. 1, 2, 3). For enclosure traps, 5 stations spaced 10-20 m apart are sampled within each tidal creek or section of main channel-basin. Sampling an enclosure trap consists of multiple hauls using a Benthic Ichthyofauna Net for Coral/Kelp Environments (BINCKE) through the volume of water trapped by the enclosure until three hauls, which can be non-consecutive, have no fish.

For beach seine sampling, main channel sampling locations are blocked off with two blocking nets rolled out 7.6 m apart on shore. They are deployed perpendicular to the shore reaching a depth of ~1.5 m or a distance of 12.2 m from shore, whichever comes first. A dowel is placed marking the corner of the sampling area and the remaining length of the nets were pulled toward each other to close the blocked area. A beach seine (2 m high x 7.6 m wide) is hauled through the blocked area five times and non-goby fishes were identified to species, counted and released. The two blocking nets were then pulled in and sampled for fish. Tidal creek sampling locations are blocked off with two blocking nets deployed perpendicular to the shore 7.6 m apart and extending across the creek. The beach seine is hauled through the blocked area five times and non-goby fishes are identified to species in the field, counted and released. The blocking net closest to the inlet of the creek is pulled in, sampled for fish, and then redeployed 7.6 m from the upland side of the still deployed blocking net. This second section of the tidal creek is sampled with five hauls of the beach seine and then the two blocking nets are pulled in and sampled for fish. An annual survey using beach seines consists of repeating this method at each main channel and tidal creek location on three different dates during early fall. The location of the seine sampling areas on the three dates is offset to minimize effects of physical alteration of habitat and to avoid sampling areas that might have been affected by previous sampling.

To avoid nesting activity of the Ridgeway's Rail (formerly Light Footed Clapper Rail) in the study wetlands, fish are sampled during early fall. Sampling is also restricted to periods of similar tides and as congruent as possible in timing across all wetlands. Given the logistical difficulties of sampling at night and the tight correlation between daytime and nighttime samples (Merkel & Associates, Inc. 2002), fish are sampled only during daytime.

Since tidal creeks differ from main channel-basin locations in size and hydrology, and thus potentially in fish density and species richness, these two habitats are evaluated separately. The total number of fish are standardized to 1 m^2 for each enclosure or beach seine sample. The averages for enclosures and beach seines are averaged to produce a combined estimate of total density (average number per 1 m^2) for each tidal creek or main channel-basin replicate. Species richness is determined as the number of unique species for each tidal creek or main channel replicate. These replicate values for density and species richness are used to calculate the means and standard errors used to evaluate similarity in total density and species richness of fish in tidal creeks and main channel-basin habitats between the restored and reference wetlands in a given year.

8. **Macroinvertebrates**

WITHIN 4 YEARS OF CONSTRUCTION, THE TOTAL DENSITIES AND NUMBER OF SPECIES OF MACROINVERTEBRATES SHALL BE SIMILAR TO THE DENSITIES AND NUMBER OF SPECIES IN SIMILAR HABITATS IN THE REFERENCE WETLANDS.

Approach: Macro-invertebrate sampling is conducted in tidal creek and main channel-basin habitats in conjunction with enclosure sampling for fish in early fall. Five stations within each tidal creek or section of main channel-basin sampled by enclosure traps for fish are sampled for macro-invertebrates.

Three methods are used to sample macro-invertebrates. Here, macro-invertebrates are defined as those specimens retained on a 0.5-mm mesh screen. First, epifauna (e.g., California Horn Snail, *Cerithidea californica*) are sampled by counting individuals within two sets of 3-25 x 25 cm quadrats spaced uniformly (low, mid, high elevation) at each station on the unvegetated banks of tidal creeks and sections of main channel-basin between the lower limit of vegetation (or, if unvegetated, an elevation of ~1.3' NGVG) and the thalweg, the lowest point of the tidal creek or channel. Second, larger infauna living deeper in the sediments (e.g., Jackknife Clam, *Tagelus californianus*, Ghost Shrimp, *Neotrypaea californiensis*) are sampled adjacent to the quadrats using a 10 cm diameter (large) core pushed into the sediment to a maximum depth of 50 cm. The contents of the 10 cm core are sieved through a 3-mm mesh screen in the field. Animals retained by the 3-mm mesh are identified and counted in the field and returned to the habitat. The contents of the three cores are combined to form one sample for that station.

Finally, smaller invertebrate specimens (e.g., most annelids, *Corophium* amphipods) are sampled using a 3.5-cm diameter (small) core pushed into the sediment to a depth of 6 cm adjacent to the large core samples. The small core samples are preserved on site in 10% buffered formalin, and returned to the laboratory for processing through a 0.5-mm mesh. The small core samples from each station are combined. Specimens are identified and counted under the microscope and archived in ethanol. Invertebrates are identified to the lowest practical taxon for smaller specimens (e.g., polychaetes, oligochaetes, amphipods) and to species for larger specimens (e.g., bivalves, decapod crustaceans). Density of macroinvertebrates sampled using each method are standardized to number per 100 cm^2 and then combined to obtain a density value for each station.

These stations values are then averaged for each tidal creek or main channel location, which are the units of replication giving 6 replicate estimates of macroinvertebrate density in each habitat per wetland. Species richness of macroinvertebrates is evaluated by recording the number of unique species per tidal creek or section of main channel-basin obtained using all sampling methods. Species richness is assessed as the mean number of species in the 6 replicate tidal creeks and sections of main channel-basin for each wetland in a year. These replicate values are used to calculate the means and standard errors used to evaluate similarity in total density and species richness of macroinvertebrates in tidal creeks and sections of channel-basin between the restored and reference wetlands in a given year.

9. **Birds**

WITHIN 4 YEARS OF CONSTRUCTION, THE TOTAL DENSITIES AND NUMBER OF SPECIES OF BIRDS SHALL BE SIMILAR TO THE DENSITIES AND NUMBER OF SPECIES IN SIMILAR HABITATS IN THE REFERENCE WETLANDS.

Approach: Birds are sampled by visually identifying and counting (using binoculars or spotting scope) all individuals sighted within 20 -100 x 150 m plots spread across each wetland. The observer walks along the landward edge of the plot or to an acces point that is in close proximity to the plot in order to obtain the best field of view and light conditions.

The time spent identifying and counting birds within each plot is 5 minutes to standardize sampling effort. Birds overflying the plots are counted if within approximately 30 m above the plot. Weather conditions are evaluated at the beginning of each sampling period and sampling is not conducted when weather conditions affect either bird behavior or the visual acuity of the observer. In general, sampling is not conducted under the following conditions: (1) precipitation or heavy fog, (2) winds exceeding 15 mph, or (3) temperatures below 40° F. Nor is sampling conducted when disturbances affect the movement or behavior of birds. Bird sampling is conducted during the same period of the tide cycle (falling and low tide) to reduce the potential effects of this variable on bird abundance. All wetlands are sampled within a few days of one another to reduce the potential effects of factors that might vary among wetlands over time, such as weather, on bird density and species richness.

Bird assemblages in coastal wetlands of southern California exhibit strong seasonal patterns in species richness and density that are driven by the movement of migratory birds. Sampling observations are made during three periods: winter (January, February), spring (April, May), and fall (October, November) that have high bird densities and distinctive species composition. Based on analyses of existing wetland bird data and discussion with bird experts, six sampling surveys are made in each wetland during each seasonal period with 3 surveys taken within each month of each period. Surveys within southern (Tijuana Estuary, San Dieguito Lagoon) and northern (Mugu Lagoon, Carpinteria Salt Marsh) sites are conducted on alternating days (e.g., day 1 = San Dieguito Lagoon, day 2 = Tijuana Estuary, day 3 = San Dieguito Lagoon, day 4 = Tijuana Estuary, etc.).

The number of birds counted within each 1.5 ha plot is averaged across the 18 survey dates (3 periods x 6 surveys per period for each wetland) in a given year to produce an annual mean value for the density of birds in that plot (i.e., number of individuals 1.5 ha^{-1}). The total number of unique bird species observed in a plot is accumulated over all 18 survey dates in a given year to produce an annual mean value for bird species richness of that plot for that year. Yearly mean total densities and mean species richness of birds within each wetland are computed using the 20 plots as replicates for each wetland and these values used for evaluating similarity between the restored and reference wetlands.

10. **Vegetation**

THE PROPORTION OF TOTAL VEGETATION COVER AND OPEN SPACE IN THE MARSH SHALL BE SIMILAR TO THOSE PROPORTIONS FOUND IN THE REFERENCES SITES.

Approach: The percent cover of salt marsh vegetation is evaluated in the restored and reference wetlands in 10 m x 10 m grid squares classified as salt marsh habitat as defined above (Section 3.2.2, Habitat Areas). Estimates of the percent cover of salt marsh vegetation in San Dieguito Wetlands and the reference wetlands are made using aerial imagery taken annually in the red green blue (RGB) and near-infrared (NIR) spectrum at a target resolution of 20 cm x 20 cm pixel size in the late spring or summer. This period also coincides with maximum flowering of some exotic annual species (e.g., mustard) and will maximize the ability to distinguish between native and nonnative vegetation (Section 3.2.5, Exotic species). Because the ability to classify ground cover type based on spectral data

varies with weather conditions, the images are calibrated for each aerial survey. Surveyed benchmarks visible from the air are established throughout each wetland to facilitate geo-referencing of the aerial photographs. The full classification of the aerial images is also inspected at a subset of georeferenced locations to confirm the classification. Mean percent cover of vegetation in the restored and reference wetlands is computed within the 10 m x 10 m grid squares. Since all of the salt marsh habitat is censused in each wetland, comparisons of vegetation cover among wetlands are made using mean values. This performance standard is met if the average percent cover of vegetation within the restored wetland is not lower than that in the reference wetlands.

11. **Algae**
THE PERCENT COVER OF ALGAE SHALL BE SIMILAR TO THE PERCENT COVER FOUND IN THE REFERENCE SITES.

Approach: This performance standard is designed to monitor the development of unusually dense mats of filamentous green macroalgae in the restoration site. Thick mats of algae have the potential to interfere with wetland structure and function by smothering benthic invertebrates and inhibiting bird feeding on mudflats (Everett 1991, Green et al. 2013). Decomposing mats of algae in open water and on salt marsh vegetation can also adversely affect water quality and the survival of marsh vegetation. Estimates of the areas of algal mats are made from the aerial images and 10 m x 10 m grid squares that cover the entire wetland (Section 3.2.10). Since excessive algal growth can be detrimental, the percent cover of macroalgae in the restored wetland must be lower than the value of the reference wetland with the highest cover of macroalgae. All grid areas are censused in each wetland and comparisons of the average percent cover of algae among wetlands are made only using mean values.

To adaptively manage for the excessive growth of macroalgae, qualitative observations for the presence of algal mats will be made during routine water quality monitoring (see Section 3.2.6). Should excessive algal growth be observed at the restoration site relative to the reference sites, measurements of potential factors (e.g., nutrients, tidal flow) that may contribute to mat formation will be made at the restored site to inform a course of action to reduce algal growth.

12. **Spartina canopy architecture**
THE RESTORED WETLAND SHALL HAVE A CANOPY ARCHITECTURE THAT IS SIMILAR IN DISTRIBUTION TO THE REFERENCE SITES, WITH AN EQUIVALENT PROPORTION OF STEMS OVER 3 FEET TALL.

Approach: The canopy of *Spartina foliosa* provides habitat for the state and federally-listed endangered Ridgeway's Rail and other bird species, and canopy heights > 3 feet provide nesting habitat for Ridgeway's Rail (Zedler 1993). The number and height of stems of *S. foliosa* in the restored wetland and in reference wetlands are assessed in 0.1 m^2 circular quadrats placed over the cordgrass every 2 m along a 20 m long transect line extending parallel to the water line and through at least three stands of cordgrass based on the methods developed by Zedler (1993). From the sampling of *S. foliosa*, the mean proportion

of stems >3 feet tall is determined for each cordgrass stand. Maximum heights (excluding flowering stalks) of all stems present in the quadrat are recorded. These values constitute replicates to compare the similarity in the mean proportion of stems >3 feet tall in the restored wetland to the reference wetlands.

13. **Food chain support**
THE FOOD CHAIN SUPPORT PROVIDED TO BIRDS SHALL BE SIMILAR TO THAT PROVIDED BY THE REFERENCE SITES, AS DETERMINED BY FEEDING ACTIVITY OF THE BIRDS.

Approach: The invertebrates and fish inhabiting tidal wetlands provide food chain support for resident and migratory birds. Measurements of food chain support (FCS) provided by the restored wetland to birds is conducted during the same period that birds are sampled to determine bird density and species richness (see Section 3.2.9). This performance standard is evaluated using the density of birds feeding within available mudflat or unvegetated channel within selected plots. A bird will be recorded as feeding if one feeding attempt is made during a 5-minute time interval. Feeding observations are made on shorebirds typically found in all of the study wetlands (e.g., willet, marbled godwit, dowitcher). Observations are conducted during similar tide conditions across wetlands to account for the known influence of tide height on bird feeding activity.

Because bird feeding is evaluated for shorebirds on mudflat, the sample size (number of plots) evaluated for bird feeding varies among wetlands depending on the number of plots that contain mudflat. To ensure that each wetland is weighted equally, the densities of feeding birds are averaged across sample dates for each plot containing mudflat in a given year, then is resampled with replacement 20 times (20 being the targeted sample size). This process is iterated 1000 times, and the mean for each iteration is calculated to produce a dataset of 1000 FCS values for each wetland for a given year.

The four-year running median of the FCS values for each wetland is calculated using a four-year mean of each iteration based on the current year and the previous three years producing 1000 values of the four-year average of the FCS values for each wetland. The four-year median and standard deviation of the FCS values for each wetland is calculated from the resampled distribution of these 1000 values. The four-year running median of the FCS value at San Dieguito Wetland must be similar to that at the reference wetland (as per the methods described in Section 2.3) in order for the San Dieguito Wetland to meet this performance standard for any given year.

3.3 Strategy for dealing with unusual events.

An issue that may occur during the course of monitoring the SONGS wetland mitigation project is the loss of wetland habitat and/or biota at sampling locations within the reference wetlands due to unusual or unforeseen anthropogenic events. Such events would render all or portions of the reference sites inappropriate as comparisons for judging the performance of the restored wetland. An example of such an event might be the loss of sampled habitat due to large-scale dredging operations.

If such an unusual event occurred at Carpinteria Salt Marsh, Mugu Lagoon, or Tijuana Estuary during the monitoring period of the San Dieguito Lagoon restoration project, then the following strategy will be employed:

1. To the extent possible, sampling stations lost or damaged due to human activities will be replaced with stations suitable for use as a reference using the same criteria used for placement as described above (see Section *2.2.*).
2. If the amount of suitable wetland habitat in a reference wetland declines to a point where there are an insufficient number of stations to evaluate a performance standard, then this wetland will be replaced, if possible, with a different reference wetland that contains a similar mix of suitable wetland habitat as the remaining reference wetlands.

4.0 DATA MANAGEMENT

Data management protocols for the wetland mitigation project will follow those developed for the reef mitigation project and are outlined below.

4.1 Daily Field and Data Transfer Procedures

Data management and quality assurance procedures for the wetland mitigation project begin in the field. Upon completion of each field activity, data sheets are checked for completeness and legibility. After these field checks are completed, the data sheets are filed into a field binder for transport back to the laboratory. Upon arrival at the laboratory, data sheets are checked into a survey log that contains entries for the observer, date, and survey location. The log is used to verify that all data assignments for a day have been completed, and all field data have been accounted for.

Data consistency is also verified during the check-in procedure, and any anomalies are brought to the attention of the field supervisor. Senior staff members examine the data sheets for possible misidentification of species, missing data values, and invalid counts. The field supervisor decides how to rectify any errors and implements corrective action to avoid repeating mistakes in the field. Such actions have included retaking data, and providing additional field training for investigators.

4.2 Data Entry and Quality Assurance

All SONGS Mitigation Monitoring data are entered and stored in electronic databases based on Structured Query Language (SQL). The monitoring project's data entry procedures have been designed to facilitate rapid data entry while continuing to ensure the quality and integrity of the data as they are transformed from physical to electronic form.

The vast majority of monitoring data are entered using custom designed web forms. These web forms provide an intuitive, graphical user interface to the project's databases. Each form mimics the exact layout of the data sheets taken into the field, which allows the individual entering the data to electronically transcribe a sheet without replicating key variable entries, or manipulating columns, rows, or formats. Such tasks are processed on the project’s internal web server, which translates the form data into the appropriate format for storage on the project’s data servers. In some cases, these forms can reduce the amount of data a user is

required to enter by over 100 fields for a single data sheet, which translates to significant time savings.

This entry system also allows the implementation of a multi-tiered checking system. Data entered using the web forms are verified in three distinct phases before any information is considered suitable for the final phase databases on which all analyses are done.

1. First, database structure (i.e. foreign key constraints) restricts the values that can be entered into a data table (e.g. the observer entry cell contains only valid entries for observer's names).
2. Second, a JavaScript program is incorporated into each web form used to enter data. These programs include a number of checks (e.g. recognizing invalid data lengths, out of range values, and incorrect formats). Failure of one of these checks prevents the form from being submitted and alerts the user of the error. The system requires errors to be corrected for a form to be successfully submitted.
3. Finally, a third filter occurs on the project's internal web server. After a form is successfully submitted, the web server will check that each data row does not violate any constraint built into the database. If any line of the form fails these tests, the entire form will be rejected until the invalid entry is corrected.

This three phase checking system has greatly reduced the time required for post-entry data checking procedures by eliminating the most common data entry errors. This system has also substantially reduced the number of data checking programs previously required to find these problems, in some cases by as much as 75%.

Three final steps convert the electronically checked databases into the final databases. First, pairs of investigators manually check each data line of the database tables against the field data sheets for correct values. Second, following the manual check, a series of programs are run on the data to check for consistent values between database tables. For example, sampling dates for a given location are checked against the dates recorded into the sampling log. Any inconsistencies are rectified. Once these checks are complete, the data are transferred to a production database that contains all fully checked and verified data. Data from the production database are merged onto a template that populates the data for zero value observations. The templates also contain all pertinent metadata (variable descriptions and sampling methods), which are checked thoroughly prior to posting. At this stage, databases are considered to be in their final form and suitable for analysis.

4.3 Data Storage and Preservation

After the physical data are entered and checked, each data sheet is scanned and converted into a PDF file for electronic storage. The material sheets are then filed in binders by survey type and year, and then added to the monitoring data library located at UCSB's SONGS mitigation office and laboratory in Carlsbad, CA. The PDF data sheets are similarly filed in an electronic library located on the project's data servers.

The project employs a highly redundant, multi-server system to ensure maximum data integrity, preservation, and uptime. The system consists of a central data server, multiple mirror and backup servers located at UCSB's Carlsbad office, the Marine Science Institute on UCSB's main campus in Santa Barbara, CA, and geographically distributed cloud storage.

The central server at UCSB's Carlsbad office acts as the primary management point for all project-related data and files. These files fall into three distinct classes, which are used to determine both the method and format of automated backup and preservation: (1) regular documents (backed up daily in native format), (2) SQL database files (backed up in real time to two mirror servers using native format, and daily to cloud storage in comma delimited text), and (3) statistical and database program files (backed up every hour in native format, and daily to a server on main campus in native format).

Local daily backups are written to a redundant disk array. All valid users for the system can access daily backups of regular documents and statistical or database program files, however, the restoration of SQL database files must be done by a system administrator.

5.0 DISSEMINATION OF RESULTS

The following procedures are followed to ensure efficient and effective communication with SCE, state and federal resource agencies and the general public: (1) CCC contract scientists communicate with SCE and state and federal agencies as needed via phone, email, and face-to face meetings to discuss results and any potential changes in monitoring design, (2) status reports are prepared and submitted to the CCC for public viewing on an annual basis, (3) project related documents are downloadable from the project's public website https://marinemitigation.msi.ucsb.edu/ which also provides information on the history, current status, contact information, and other relevant material pertaining to the monitoring of the SONGS wetland mitigation project, (4)
all monitoring data are deposited annually into the Environmental Data Initiative (EDI) repository (https://portal.edirepository.org) after they have been verified and are freely accessible to the public via the project's website or EDI's data portal (using the Key words UCSB SONGS), and (5) as per Condition D of the SONGS permit, duly noticed annual public workshops are convened to review the overall status of the project, identify problems and make recommendations for solving them, and review activities planned for the following year.

6.0 MANAGEMENT OF THE MITIGATION SITE

The SONGS wetland restoration project (San Dieguito Wetlands Restoration Project) at San Dieguito Lagoon is only part (albeit an important part) of a larger master plan to restore and enhance the San Dieguito River Valley (JPA, 2000). Restoration of non-tidal wetlands and upland habitat, and provisions for public access and viewing are included in the Park Master Plan. Many tasks and programs typically listed in such management plans (e.g., public outreach, watershed management, future land acquisition) are beyond the scope of the SONGS mitigation project, while other tasks (e.g., response to catastrophic events, routine removal of trash and debris, mosquito control) require managed coordinated efforts throughout the entire river park, which is in itself a task that is typically included in the management plans of most ecological reserves. Here, are discussed only those management issues relevant to the SONGS mitigation requirement of creating or substantially restoring 150 acres of tidal wetland that is similar in structure and function to natural undisturbed wetlands in the Southern California Bight.

6.1. Inlet Management

As required by the SONGS permit, SCE has a plan for managing the inlet in perpetuity (SCE 2005). The plan calls for regular monitoring and dredging of the inlet channel, if necessary, to ensure uninterrupted tidal flushing of the restored wetland and provides conditions that would trigger the need for additional maintenance dredging. Data on the depth and width of the inlet channel collected by SCE will be used to determine whether the inlet channel is maintained in an “as designed” condition. If the data indicate substantial sedimentation has occurred in the inlet channel, then maintenance dredging will be implemented to reconfigure the channel to its as designed condition.

6.2. Topography

Degradation of wetland topography and berms may occur over time as a result of erosion, sedimentation and scour. Visual observations, topographic data, and aerial imagery collected as part of the CCC’s post-construction monitoring program will be used to determine the extent to which the topography of the restored wetland or berms have degraded. If these data indicate that major degradation of topography or berms has occurred, then the appropriate corrective action (i.e. dredging, berm repair) will be done to reconfigure the wetland to its as designed condition.

6.3. Control of Weeds and Other Invasive Species

There is a potential for weeds to colonize restored marsh habitats and impede the establishment of desirable marsh species, particularly in areas at high elevations where tidal inundation is less frequent. If in the best professional judgment of CCC staff, invasive exotic species compromise wetland standards and functions, these species will be removed at a frequency that is necessary for marsh plants to become established. The possibility also exists that exotic marine species will invade lower intertidal and subtidal habitats and usurp resources or destroy sensitive marsh habitat typically used by native species. Examples of such species include the green crab (*Carcinus maenas*), Asian mussel (*Musculista senhousia*), isopod (*Sphaeroma quoyanum*), and a green macroalga (*Caulerpa prolifera*), which have invaded several coastal wetlands in California. Unfortunately, controlling the spread of exotic marine species is extremely problematic. The topic of invasive species control in marine environments has received considerable attention in recent years and currently is the subject of several ongoing research programs in California and elsewhere. If exotic marine species are found in the restored wetland, then experts working in this field will be consulted and a program to control the spread of these species will be developed using the most current information.

7.0 CONSTRUCTION IMPACT MONITORING

Conduct surveys to determine if transition habitat may be used to mitigate for impacts to seasonal salt marsh caused by construction.

Areas between elevations of 4.5 ft and 5.0 ft NGVD are defined in the Final Restoration Plan (SCE 2005) as a transitional zone between tidal wetlands and non-tidal or seasonal wetland habitats. Coastal Commission Staff have determined, in accordance with CDP 6-04-088, that transition zone acreage can be used to offset impacts to seasonal salt marsh that occurred during wetland construction provided that the cover of native salt marsh and non-native plants within this zone meets the performance criteria outlined below.

Data on vegetation type and cover will be collected annually in the summer-fall in the constructed transition zone and compared to reference site data to determine if transition zone acreage can be used to offset impacts to seasonal salt marsh during wetland construction. Vegetation type and cover will be determined using point contact sampling at one meter intervals along approximately 100 -10 m long belt transects situated uniformly around the periphery of the restored wetland in the transition zone. Five measurements will be taken at each meter interval perpendicular to the centerline of each transect to achieve 50 sampled points per transect.

Cover of native salt marsh plants in reference seasonal salt marsh habitat adjacent to module W2/3 will serve as the standard of comparison for determining whether transition acreage can be used to offset impacts to seasonal marsh during construction (Fig. 7). The cover of native salt marsh plants in the reference seasonal marsh will be determined using point contact sampling along 10 - 10 m long belt transects situated between 4.5 ft and 5.0 ft NGVD (Fig. 7). Point measurements will be taken every meter as above.

Acreage credit will be assigned for the entire transition (approximately 0.9 acres) contingent on the results of a t-test. To receive acreage credit, the mean cover of native vegetation in the transition must be similar to that in the reference seasonal salt marsh as determined using a one-tailed t-test with α = 0.2 and β = 0.2, thus balancing Type I and Type II errors. If the t-test shows significantly less cover of native vegetation in the transitional habitat of the restored site compared with the reference site then no acreage credit will be given.

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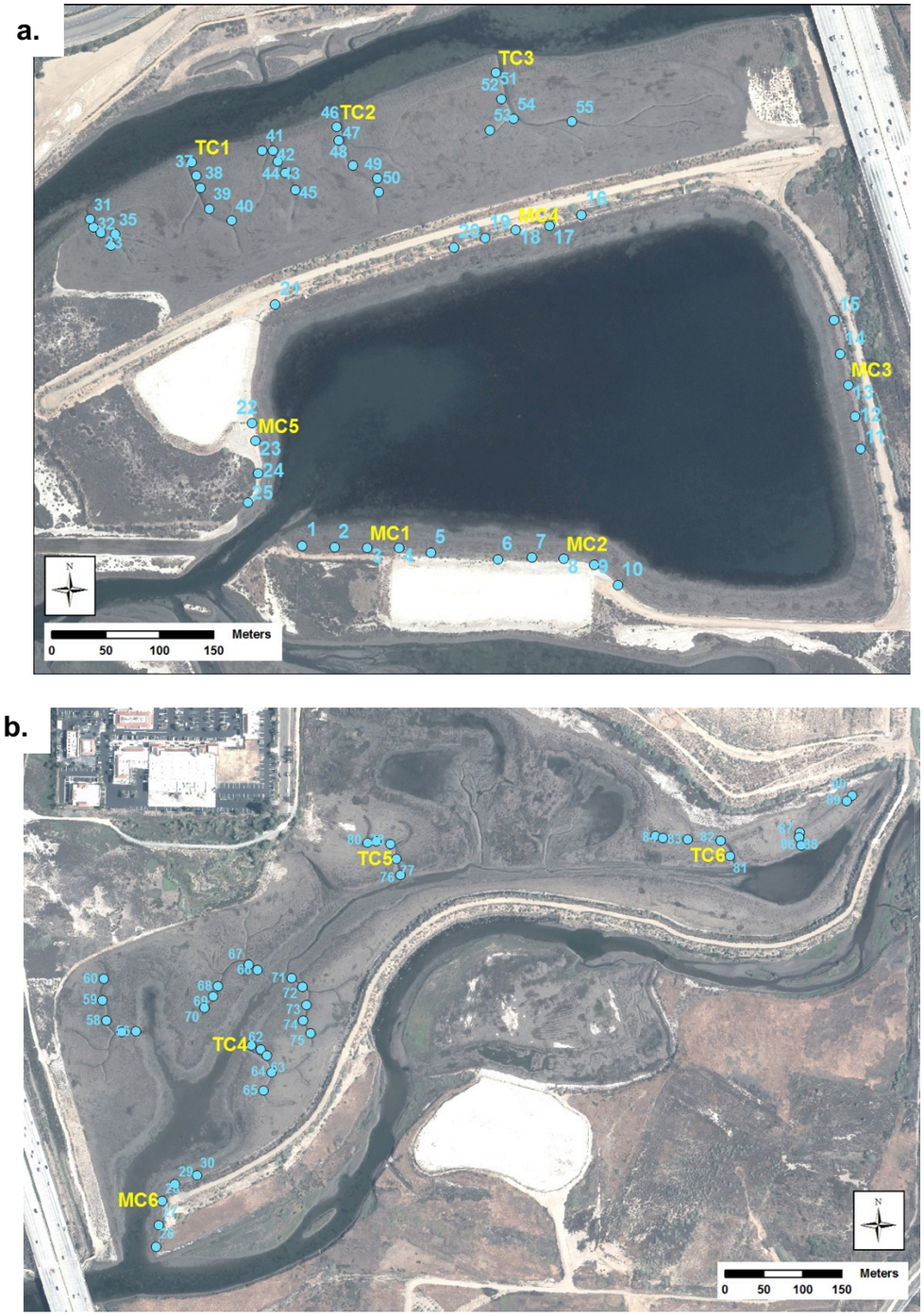


Figure 1. Tidal creeks and sections of main channel sampled for fish and macro-invertebrates in San Dieguito Wetlands a) east side of the Interstate 5 and b) west side of Interstate 5. TC-tidal creek, MC-sections of main channel. See Appendix 3 for updates to sampling locations.

Figure 2. Tidal creeks and sections of main channel sampled for fish and macro-invertebrates in Tijuana Estuary. TC-tidal creek, MC-sections of main channel.

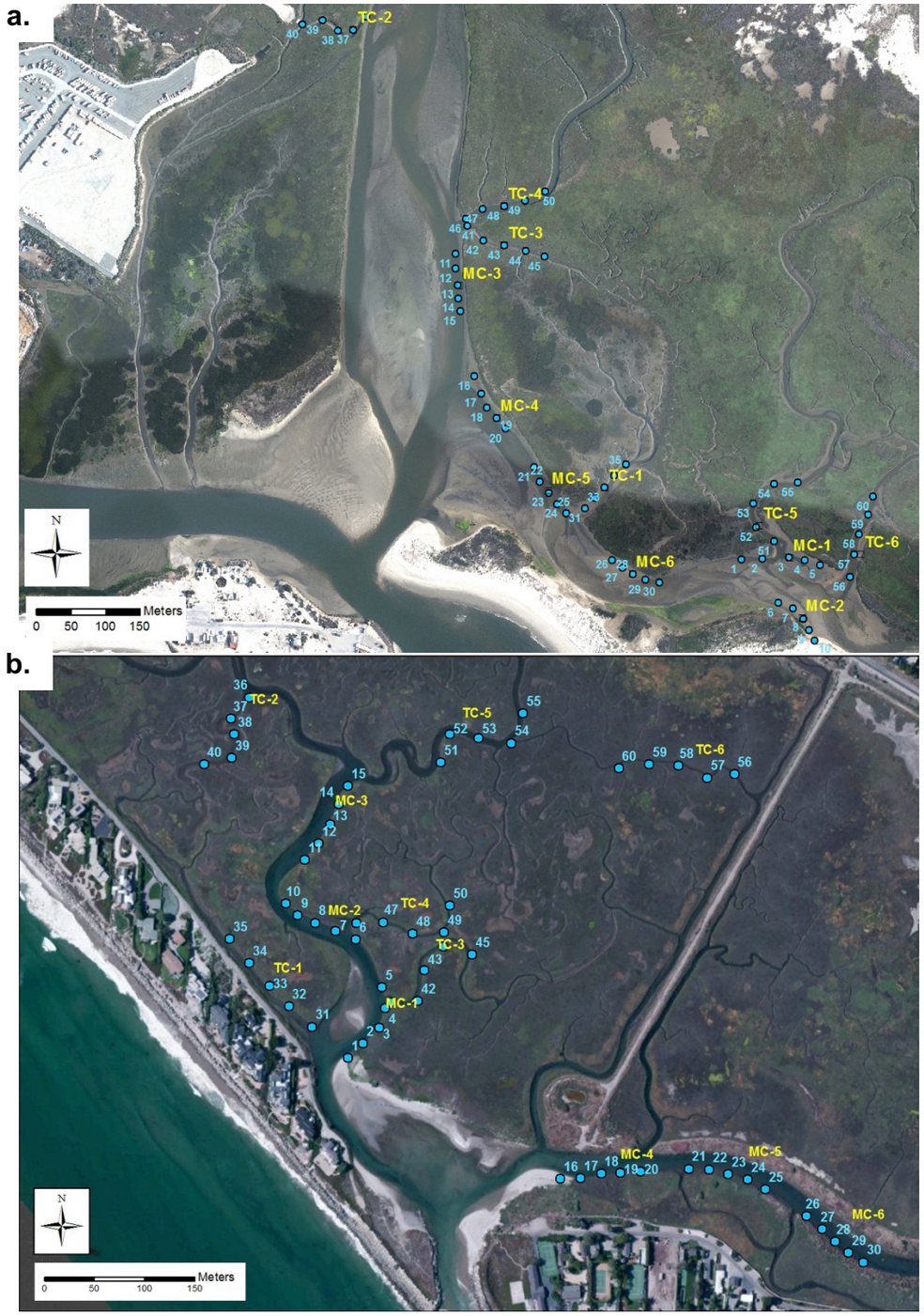


Figure 3. Tidal creeks and sections of main channel sampled for fish and macro-invertebrates in a) Mugu Lagoon and b) Carpinteria Salt Marsh. TC-tidal creek, MC-sections of main channel.

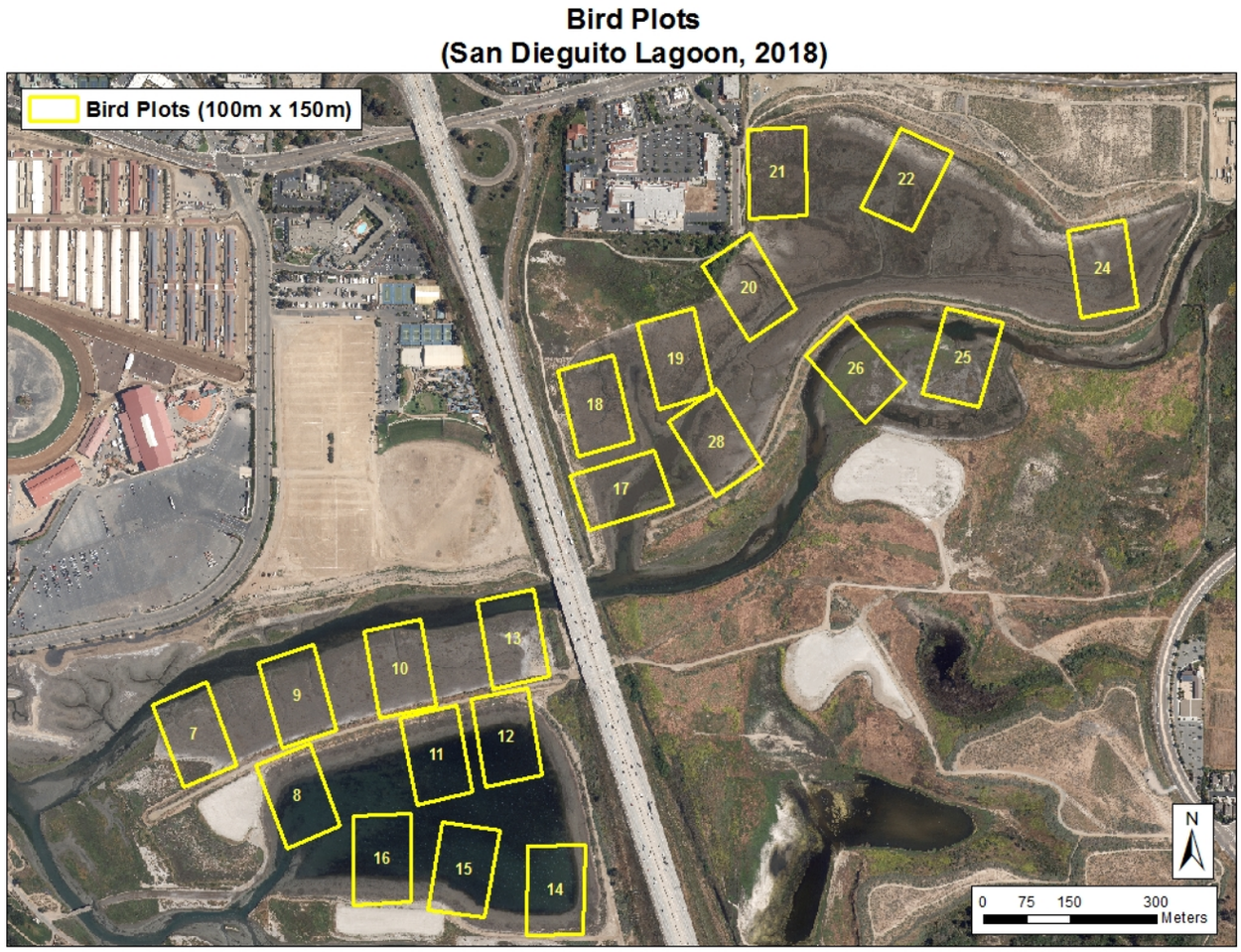
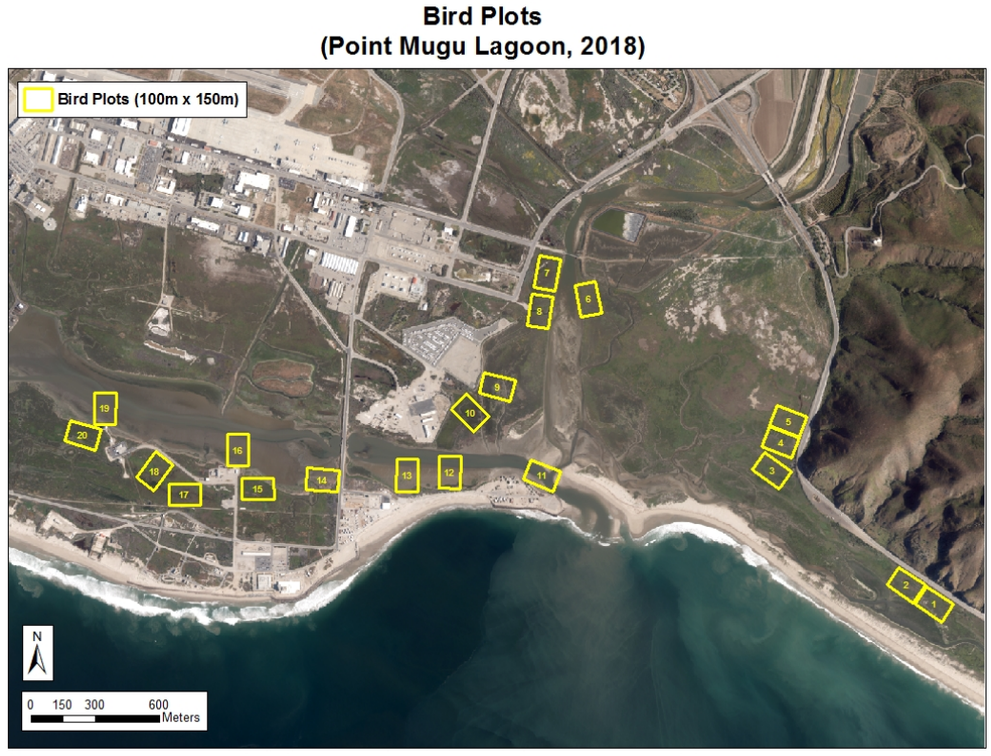


Figure 4. The location of 100 m x 150 m plots sampled for birds in San Dieguito Wetlands.

Figure 5. The location of 100 m x 150 m plots sampled for birds in Tijuana Estuary.

a.



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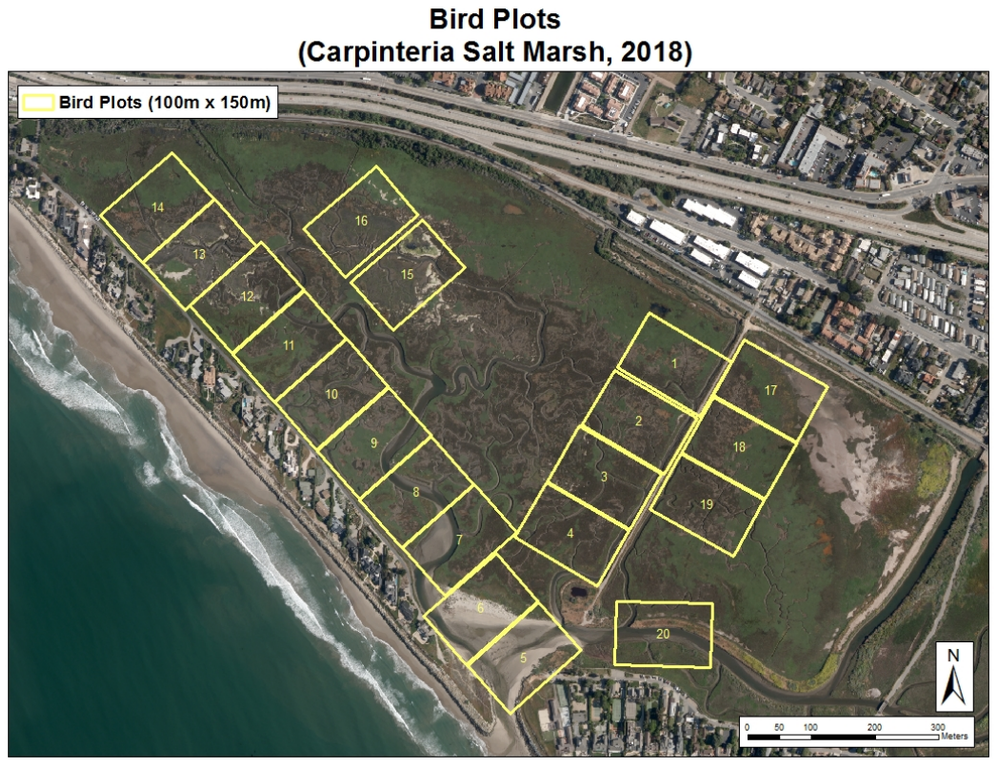

Figure 6. The location of 100 m x 150 m plots sampled for birds in a) Mugu Lagoon and b) Carpinteria Salt Marsh.

Figure 7. The location of 10 m^2 belt transects in a) the transition zone (4.5 – 5.0 ft NGVD) of the restored San Dieguito Wetlands (n = 100) and b) seasonal marsh used as a reference site (n = 10). Transect lengths (a & b) will vary with the width of the transition zone to achieve a 10 m^2 area sampled, but will be approximately 10 m.

Appendix 1

The Definition of Compliance in the Context of the SONGS Mitigation Projects

Prepared for the staff of the California Coastal Commission by:
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EXECUTIVE SUMMARY

The California Coastal Commission (CCC) has required Southern California Edison (SCE) and its partners to construct mitigation projects that provide adequate compensation for the loss of marine resources resulting from the operation of SONGS Units 2 and 3. The CCC is responsible for determining whether these projects are successful. One issue that resides at the core of this determination is the level and duration of performance by the mitigation projects that is needed to achieve compliance with specific conditions of the SONGS coastal development permit. We address this issue below.

The conditions of the SONGS coastal development permit (6-81-330-A) were amended in 1991 to mitigate the adverse impacts of the operation of SONGS Units 2 and 3 on the marine environment. The conditions that were amended to the permit require SCE and its partners to (1) create or substantially restore a minimum of 150 acres of southern California wetlands (Condition A), (2) install fish barrier devices at the power plant (Condition B), and (3) construct an artificial reef large enough to sustain 150 acres of medium to high density kelp bed community (Condition C). A fourth condition (Condition D) requires SCE to fund the Commission's oversight of the mitigation and independent monitoring functions identified in and required by Conditions A, B, and C. Physical and biological standards are identified in conditions A and C that specify how the wetland and reef mitigation projects should perform and the timing and level of monitoring that is needed to evaluate their performance. The specific requirements for attaining compliance of these conditions are discussed in various sections throughout the permit. The purpose of this document is to provide SCE with clear and consistent interpretations of key terms in the SONGS coastal development permit, which provide the basis for assessing compliance of SONGS wetland and reef mitigation projects. We identify the specific sections in the permit that provide support for our interpretations, and provide schedules for the different levels of monitoring that are required to determine whether the wetland and reef mitigation projects are in compliance with Conditions A and C.

INTRODUCTION

The SONGS coastal development permit (6-81-330-A) requires SCE to create or substantially restore a minimum of 150 acres of southern California wetlands (Condition A), and to construct an artificial reef large enough to sustain 150 acres of medium to high density kelp bed community (Condition C). Physical and biological standards are identified in these conditions that specify how the wetland and reef mitigation projects should perform and the timing and level of monitoring that is needed to evaluate their performance is discussed. The purpose of this document is to provide consistent interpretations of key terms in the SONGS coastal development permit (6-81-330-A), which provide the basis for assessing compliance of SONGS wetland and reef mitigation projects. The specific sections in the SONGS permit that provide support for our interpretations are indicated by numerical superscripts in the text and are referenced below (see p. 6 of Appendix 1 Permit language supporting CCC staff's interpretations on SONGS project compliance).

DEFINITIONS

Monitoring Period: Post-construction monitoring will ensue upon completion of the reef construction and wetland restoration[1, 2]. The duration of such monitoring will last for a period not less than the full operating life of SONGS (defined below) plus years monitored without the project attaining compliance with permit standards[2, 3].

Compliance: The condition in which all performance standards are met.

*Compliance Perio*d: The number of years that a mitigation project is in compliance. The mitigation requirements will be fulfilled when the compliance period equals the total years of operation of SONGS Units 2 & 3, including decommissioning period to the extent that there is continuing entrainment or impingement or discharge of cooling water[3,4].

MONITORING EFFORT

Mitigation Reef (see Figure 1)

1) *Stage 1: Fully implemented monitoring*: Independent monitoring designed and conducted by CCC staff scientists will be done to evaluate the performance of the mitigation reef[5]. The sampling methodology, analytical techniques, and methods for measuring performance of the mitigation reef relative to the performance standards shall be described in the monitoring plan prepared for the mitigation reef[6]. Monitoring will ensue upon completion of the reef construction[2]. All performance standards must be met within 10 years[7,8]. The project will be considered successful when all the performance standards have been met each year for three consecutive years[9]. Hence, fully implemented monitoring will last a minimum of 10 years. All years that the project is in compliance will count towards the compliance period. The level of sampling effort may be reduced during this stage of monitoring if analyses of the data indicate that compliance of the performance standards can be adequately assessed using less sampling effort. Remediation may be required if the performance standards are not met within ten years and if three consecutive years of compliance has not occurred within 12 years [10, 11]. Note that the Executive Director could prolong this stage of monitoring or reinstate it if necessary following degradation of the artificial reef (resulting in a period of non-compliance) or remediation[12].

2) *Stage 2: Annual site inspections:* Monitoring can be reduced to annual site inspections[13,14], which will serve to identify noncompliance with the performance standards, when:
    - **a.** The project has been in compliance with permit standards for at least three consecutive years, and
    - **b.** The project has been evaluated for at least ten years post-construction.

The schedule for monitoring the mitigation reef project is shown in Figure 1.

Restored Wetland (see Figure 2)

1) *Stage 1: Fully implemented monitoring:* Independent monitoring designed and conducted by CCC staff scientists will be done to evaluate the performance of the wetland restoration project[5]. A description of the monitoring can be found in the wetland monitoring plan and details of the monitoring effort will be set forth in a work plan[15]. Monitoring will ensue upon completion of wetland construction[16]. Within 4 years of construction, the total densities and number of species of fish, macro-invertebrates and birds shall be similar to the densities and number of species in similar habitats in the reference wetlands[17]. All performance standards must be met within 10 years, which is the same amount of time required for the mitigation reef to meet all of the performance standards[7,8]. The wetland restoration project will be considered successful when all the performance standards have been met for each of three consecutive years[9]. All years that the project is in compliance will count towards the compliance period. Remediation may be required if all the performance standards are not met within ten years and if three successive years of compliance has not occurred within 12 years[18]. Note that the Executive Director could prolong this stage of monitoring or reinstate it if necessary following remediation or degradation of the wetland (resulting in a period of non-compliance)[12].

2) *Stage 2: Scaled back monitoring:* Upon determination that the project has been in compliance for three consecutive years, a scaled back stage of monitoring will ensue[14]. The scaled back monitoring program will be designed and implemented by CCC staff scientists[5]. Reduction in effort will be based on analyses of data collected during the period in which the project was in compliance. Staff scientists will examine these data to determine the minimum effort that would have been necessary to assess compliance during the period**.** All monitoring, whether it is fully implemented or scaled back, must be sufficient for assessing compliance of the performance standards.

The schedule for monitoring the wetland restoration project is shown in Figure 2.

REMEDIATION

If the mitigation reef or restored wetland is not considered successful within 12 years post-construction or if the restored wetland has not met the biological community standard by year 4, then (at the discretion of the Executive Director):

1) The permittee shall fund an independent study to collect information needed to determine what remediation is required[19].
2) The permittee shall be required to implement any remedial measures determined necessary by the Executive Director in consultation with state and federal resource agencies and will provide funds for independent monitoring that evaluates the success of the required remediation[10,11,19]. Remediation monitoring may be different from the compliance monitoring required by the permit.

If the mitigation reef or restored wetland is in a period of reduced monitoring and if it falls out of compliance for a period of two consecutive years, then to determine if non-compliance is an artifact resulting from a reduction in monitoring effort, full monitoring (Stage 1) may be re-established for those standards that are out of compliance. If resumption of full monitoring leads to the conclusion that the reduction in monitoring was responsible for non-compliance,

then monitoring will remain at the full levels for the duration of the study or until the Executive Director concludes that reduced monitoring could be reinstituted[12]. CCC staff scientists will be responsible for designing and implementing the reduced monitoring program[5].

If resumption of full monitoring leads to the conclusion that non-compliance is due to poor performance of the mitigation project then:

1) The permittee shall be required to fund an independent study to collect the information necessary to determine what remediation is needed [19]
2) The permittee shall be required to implement any remedial measures determined necessary by the Executive Director in consultation with state and federal resource agencies and will provide funds for independent monitoring that evaluates the success of the required remediation[10,11,19]. Remediation monitoring may be different from the compliance monitoring required by the permit.

Permit (No. 6-81-330-A) language supporting CCC staff's interpretations on SONGS project compliance

1. (III.A.3.4). Upon completion of construction of the wetland, monitoring shall be conducted to measure the success of the wetland in achieving stated restoration goals (as specified in restoration plan) and in achieving performance standards, specified below.

2. (III.B. 2.4). Following completion of construction the mitigation reef shall be monitored for a period equivalent to the operating life of SONGS.

3. (III.A.3.0). Monitoring, management (including maintenance), and remediation shall be conducted over the "full operating life" of SONGS Units 2 and 3**.** Full operating life" as defined in this permit includes past and future years of operation of SONGS units 2 and 3 including the decommissioning period to the extent there are continuing discharges. The number of past operating years at the time the wetland is ultimately constructed, shall be added to the number of future operating years and decommission period, to determine the length of the monitoring, management and remediation requirement.

4. (III.B 2.4). The permittee shall insure that the performance standards and goals set forth in this condition will be met for at least the length of time equivalent to the full operating life of SONGS Units 2 and 3…."Full operating life" as defined in this permit includes past and future years of operation of SONGS Units 2 and 3, including the decommissioning period to the extent there are continuing discharges.

5. (III.C.1.0). Personnel with appropriate scientific or technical training and skills will, under the direction of the Executive Director, oversee the mitigation and monitoring functions identified and required by conditions II-A through C. The Executive Director will retain approximately two scientists and one administrative support staff to perform this function.

This technical staff will oversee the preconstruction and post-construction site assessments, mitigation project design and implementation (conducted by permittee), and monitoring activities (including plan preparation); the field work will be done by contractors under the Executive Director's direction. The contractors will be responsible for collecting the data, analyzing and interpreting it, and reporting to the Executive Director.

6. (III.B.2.4. A monitoring plan for the mitigation reef shall be developed by the Commission staff scientists pursuant to Condition D. The monitoring plan shall be completed within six months of approval of a coastal development permit for the mitigation reef proposed in a final plan developed pursuant to this condition. The monitoring plan shall provide an overall framework to guide the monitoring work. The monitoring plan shall describe the sampling methodology, analytical techniques, and methods for measuring performance of the mitigation reef relative to the performance standards identified below.

7. (III.B.2.4). The independent monitoring program for the mitigation reef shall be designed to assess whether the performance standards have been met. If these standards are met after ten years following the completion of construction, then monitoring can be reduced to annual site inspections.

8. (III.B.2.4). If the standards listed above are not met within ten years after reef construction, then the permittee shall undertake those remedial actions the Executive Director deems appropriate and feasible.

9. (III.C.3.0). The mitigation projects will be successful when all performance standards have been met each year for a three-year period. The Executive Director shall report to the Commission upon determining that all of the performance standards have been met for three years and that the project is deemed successful.

10. (III.B.2.4). The permittee shall undertake necessary remedial actions based on the monitoring results and annual site inspections for the full operating life of the SONGS Units 2 and 3.

11. (III.B.2.4). If the standards listed above are not met within ten years after reef construction, then the permittee shall undertake those remedial actions the Executive Director deems appropriate and feasible.

12. (III.C.3.0). If subsequent monitoring shows that a standard is no longer being met, monitoring may be increased to previous levels, as determined necessary by the Executive Director.

13. (III.B.2.4). The independent monitoring program for the mitigation reef shall be designed to assess whether the performance standards have been met. If these standards are met

after ten years following the completion of construction, then monitoring can be reduced to annual site inspections.

14. (III.C.3.0). If the Commission determines that the performance standards have been met and the project is successful, the monitoring program will be scaled down, as recommended by the Executive Director and approved by the Commission. A public review shall thereafter occur every five years, or sooner if called for by the Executive Director.

15. (III.A.3.1). A monitoring and management plan will be developed in consultation with the permittee and appropriate wildlife agencies, concurrently with the preparation of the restoration plan, to provide an overall framework to guide the monitoring work. It will include an overall description of the studies to be conducted over the course of the monitoring program and a description of management tasks that are anticipated, such as trash removal. Details of the monitoring studies and management tasks will be set forth in a work program.

16. (III.A.3.4). Upon completion of construction of the wetland, monitoring shall be conducted to measure the success of the wetland in achieving stated restoration goals (as specified in restoration plan) and in achieving performance standards.

17. (III.A.3.4.b.1). *Biological Communities.* Within 4 years of construction, the total densities and number of species of fish, macroinvertebrates and birds shall be similar to the densities and number of species in similar habitats in the reference wetlands.

18. (III.A.3.4). The permittee shall be fully responsible for any failure to meet these goals and standards during the full operational years of SONGS Units 2 and 3. Upon determining that the goals or standards are not achieved, the Executive Director shall prescribe remedial measures, after consultation with the permittee, which shall be immediately implemented by the permittee with Commission staff direction. If the permittee does not agree that remediation is necessary, the matter may be set for hearing and disposition by the Commission.

19. (III.B.2.4). Executive Director may also use any other information available to determine whether the performance standards are being met. If information from the annual site inspections or other sources suggests the performance standards are not being met, then the permittee shall be required to fund an independent study to collect the information necessary to determine what remediation is needed. The Executive Director shall determine the required remedial actions based on information from the independent study. The permittee shall be required to implement any remedial measures determined necessary by the Executive Director in consultation with state and federal resource agencies, as well as provide funds for independent monitoring that evaluates the success of the required remediation. As described under the funding option (Condition D) of this permit, the cost of remediation shall not be limited if the permittee elects to implement the mitigation reef.

Figure 1. Idealized monitoring schedule for the mitigation reef showing the minimum time periods for the two stages of monitoring: (1) Fully implemented monitoring and (2) annual site inspection. The actual time periods for each stage may be longer, depending on the performance of the project.

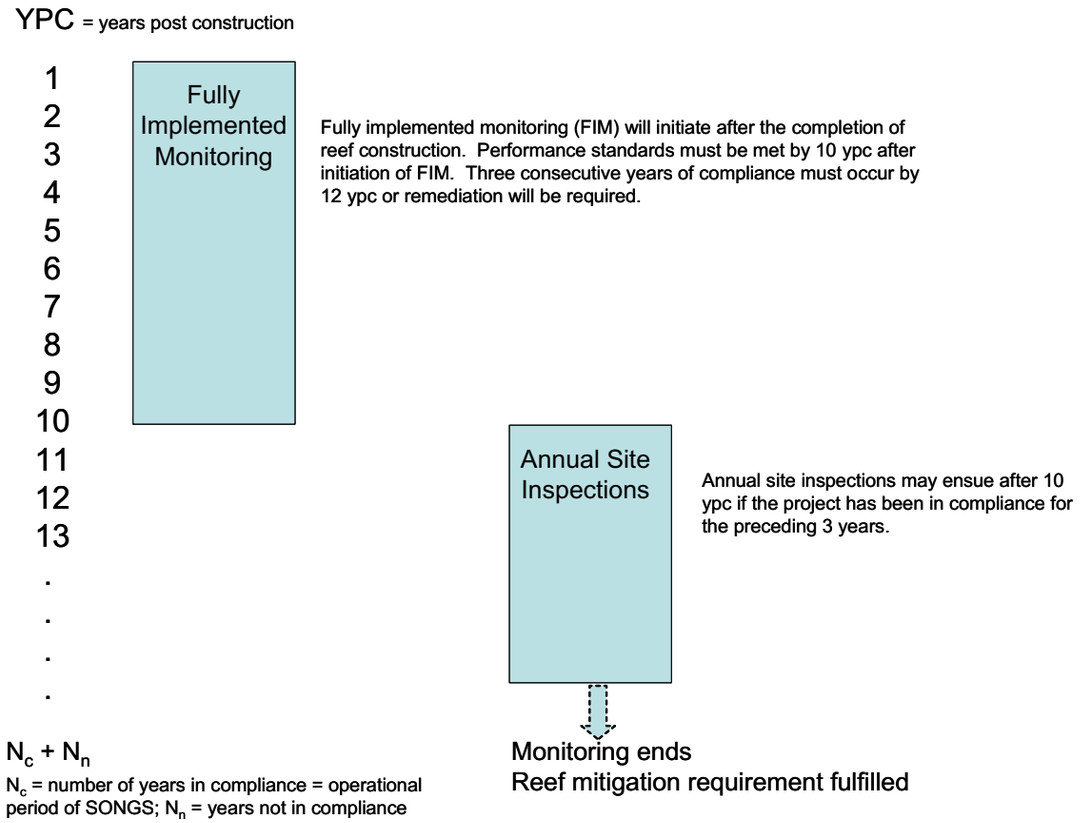
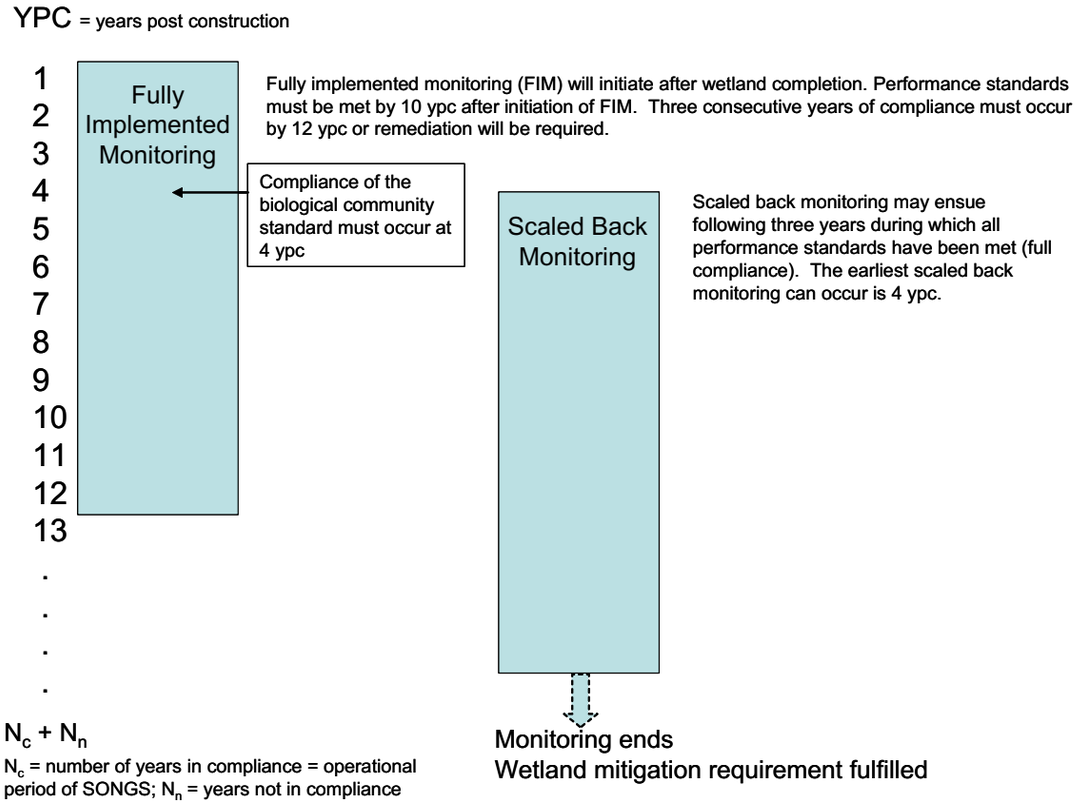


Figure 2. Idealized monitoring schedule for the wetland restoration project showing the minimum time periods for the two stages of monitoring: (1) Fully implemented monitoring and (2) scaled back monitoring. The actual time periods for each stage may be longer, depending on the performance of the project.



Appendix 2

Planned Acres of Subtidal, Mudflat, and Salt Marsh Habitat for the San Dieguito Wetlands Restoration Project as Provided in the Final Restoration Plan (SCE 2005)

Table 1. Table 5.3 from the FRP (SCE 2005) showing planned areas of Subtidal, Mudflat, and Salt Marsh Habitat for the San Dieguito Wetlands Restoration Project. Includes 3.2 acres of mudflat habitat in W16.

Table 5.3 Summary of Net Wetland Habitat Creation - SCE Project Components to Fulfill SONGS Permit Requirements

Habitats	Restored Area (acres)[1] A	Area Required to Compensate for Permanent Impacts (acres)[1,2] B	Area Required to Compensate for Temporary Impacts (acres)[2] C	Net Wetland habitat Creation (acres) A-(B +C)
Tidal Wetland (below +4.5 feet, NGVD)				
Subtidal	32.03	0.00	0.33	31.70
Frequently Flooded Mudflats	11.50	0.00	0.00	11.50
Frequently Exposed Mudflats	10.73	0.00	0.00	10.73
Low Coastal Salt Marsh	17.55	0.08	0.00	17.47
Mid Coastal Salt Marsh	38.37	0.40	2.13	35.84
High Coastal Salt Marsh	21.93	0.56	0.86	20.51
Estuarine Flats Inter Tidal	0.00	0.04	0.00	-0.04
Fresh and Brackish Marsh	0.00	0.08	0.44	-0.52
Riparian Southern Willow	0.00	0.01	0.01	-0.02
Total Tidal Wetland	**132.11**	**1.17**	**3.77**	**127.17**
Nontidal Wetland (above +4.5 feet, NGVD)				
Seasonal Salt marsh	8.65	7.43	14.00	-12.78
Transitional Wetlands	0.82	0.00	0.00	0.82
Estuarine Flats Non Tidal	0.00	0.00	0.21	-0.21
Freshwater Marsh	0.00	0.00	0.00	0.00
Total Nontidal Wetland	**9.47**	**7.43**	**14.21**	**-12.17**
Total Wetland	**141.58**	**8.60**	**17.98**	**115.00**

[1] 4:1 requirement for permanent impacts to B7, B8, NS15, DS32 and Road.
[2] No mitigation is proposed or required for nesting site impacts.

Table 2. Table 4.5 from the FRP (SCE 2005) showing planned areas of Subtidal, Mudflat, and Salt Marsh Habitat for the the Villages Property (W16).

Table 4.5. Summary of Net Wetland Habitat Creation – Villages Project Components[2]

Habitat	*Restored Area (acres)* **A**	*Permanent Wetland Loss (acres)* **B**	*Credits Assigned to Meet SONGS Requirement for SCE Restoration Project (acres)*[1] **C**	*Converted Area (acres)* **D**	*Net Wetland Habitat Creation (acres)* **(A-C)-D**
Tidal Wetland (below +4.5 feet, NGVD)					
Subtidal	0.00	0.00	0.00	0.00	0.00
Frequently Flooded Mudflats	0	0.00	0.00	0.00	0.00
Frequently Exposed Mudflats	5.90	0.00	0.00	0.00	5.90
Low Coastal Salt Marsh	3.60	0.00	0.00	0.00	3.60
Mid Coastal Salt Marsh	4.83	0.00	0.00	0.00	4.83
High Coastal Salt Marsh	6.30	0.00	0.00	0.00	6.30
Fresh and Brackish Water	0.00	0.00	0.00	0.72	-0.72
Total Tidal Wetland	20.63	0.00	0.00	0.72	19.91
Nontidal Wetland (above +4.5 feet, NGVD)					
Seasonal Salt Marsh	0.00	0.00	0.00	5.70	-5.70
Transitional Wetlands	0.14	0.00	0.00	0.00	0.14
Total Nontidal Wetland	0.14	0.00	0.00	5.70	-5.56
Credits Assigned to Meet SONGS Requirement for SCE Restoration Project[1]	0.00	0.00	3.20	0.00	-3.20
Total Wetland	**20.77**	**0.00**	**3.20**	**6.42**	**11.15**

[1] 3.2 acres of the Villages Mitigation Bank will be used to offset permanent wetland impacts from DS32 in order to achieve the 115 acres required for the SCE restoration project.

[2] Based on August 30, 2005 delineation by WRA.

Table 3. Table 5.1 from the FRP (SCE 2005) showing planned areas of Subtidal, Mudflat, and Salt Marsh Habitat including 3.2 acres from W16, but without Villages Property (Table 4.5).

Habitats	WETLAND HABITAT AREA (ACRES) *Module No.*									*Total*
	W1	**W2A**	**W2B**	**W3**	**W4**	**W5**	**W10**	**W16**	**W45**	
Subtidal	31.08	-	-	-	0.95	-	-	-	-	**32.03**
Frequently Flooded Mudflats	5.50	-	-	-	6.00	-	-	-	-	**11.50**
Frequently Exposed Mudflats	1.23	-	-	-	6.30	-	-	3.20	-	**10.73**
Low Marsh	2.92	0.18	-	0.10	10.53	3.82	-	-	-	**17.55**
Mid Marsh	3.13	5.50	-	3.08	25.60	1.06	-	-	-	**38.37**
High Marsh	0.54	1.40	7.50	2.34	2.60	0.45	7.10	-	-	**21.93**
Seasonal Salt Marsh	-	-	-	-	-	-	-	-	8.65	**8.65**
Freshwater Marsh (nontidal)	-	-	-	-	-	-	-	-	-	**0.00**
Transitional Wetlands	0.33	0.00	0.06	0.03	0.24	0.16	-	-	-	**0.82**
Totals	44.73	7.08	7.56	5.55	52.22	5.49	7.10	3.20	8.65	**141.58**

Table 4. Summary of total planned acres of Subtidal, Mudflat, and Salt Marsh Habitat in the San Dieguito Wetlands Final Restoration Plan (SCE 2005), which includes the Villages Property.

Habitat	Acreage from Column A Table 5.3	Acreage from Column A Table 4.5	Total Planned Acres
Subtidal	32.03	0	32.03
Mudflat	19.03*	5.90	24.93
Salt Marsh	77.85	14.73	92.58

*does not include 3.2 acres from W16 shown in Table 5.1

Appendix 3

Changes in the Location of Sampling Sites Due to the Encroachment of *Spartina foliosa* Into Tidal Creeks Sampled in Previous Years

Cordgrass, *Spartina foliosa*, has encroached into six tidal creeks that are sampled for invertebrate and fish densities and species richness. The cordgrass inhibits the use of beach seines and enclosure traps used to sample fish. As a result, sampling of invertebrates and fish was moved from those creeks to the nearest tidal creek that lacked cordgrass in 2019.

Figure 1 shows the location of tidal creeks (TC) and sections of main channel and basin (MC) sampled for fish and macro-invertebrates in San Dieguito Wetlands. This figure has been revised to show the location of sites sampled in 2019 and 2020 (cyan colored dots). Tidal creek stations that were sampled 2012-2018 and not currently sampled are shown as red dots.

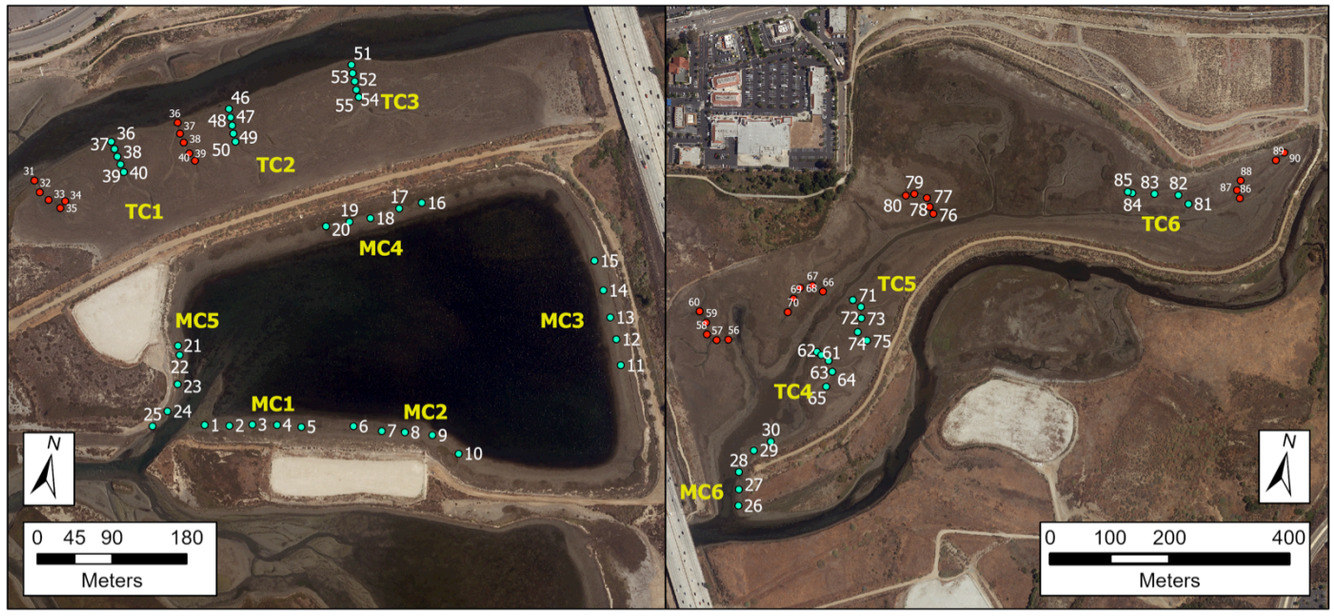


Figure 1. Image showing the locations of tidal creek stations sampled in 2019 and 2020 and moving forward (cyan). Red dots indicate stations sampled prior to 2019.

Appendix 4

Modifications to Performance Monitoring in 2020 due to the COVID-19 Pandemic

To comply with State, Local, and University guidelines regarding the implementation of measures to reduce the spread of COVID-19, elements of performance monitoring of the San Dieguito Wetlands in 2020 were scaled back to reduce the number and contact time of personnel in the field and laboratory. The following section outlines the modifications from the SONGS Wetland Mitigation Monitoring Plan that were made in 2020.

Most performance standards were assessed without modification.

Adjustments to performance monitoring:

Fish Beach Seine sampling
A reduction in the number of beach seines used to sample fish in tidal creek and main channel/basin habitats. Seine sampling is conducted at all wetlands at six main channel and six tidal creek sites. Normally, three seine samples are taken, one each over a three day period. The number of seines taken each site was reduced from three to one.

Bird Surveys
Birds are sampled during the fall, winter, and spring. Spring surveys were missed in 2020 due to a shutdown in research operations. Bird surveys were conducted on schedule in fall and winter as detailed in the Monitoring Plan.

The reduction in sampling effort occurred in all the wetlands and if it did have an effect it appears to be similar across wetlands without imparting bias in evaluating the standards.

Appendix 5

Metadata for the SONGS Wetland Restoration Project

Metadata and data for the SONGS Wetland Restoration Project can be found using the Data tab at the UCSB SONGS Marine Mitigation website (see also Section 5.0, Dissemination of Results):

https://marinemitigation.msi.ucsb.edu/

Appendix 6

Updates to the SONGS Wetland Mitigation Monitoring Plan

Updates in the January 2015 plan.

Addition of Section 7.0, Mitigation of Construction Impacts Monitoring.

The area between elevations of 4.5' to 5.0' NGVD is defined in the Final Restoration Plan (SCE 2005) as a transitional zone between tidal wetlands and non-tidal or seasonal wetland habitats. Coastal Commission Staff have determined, in accordance with CDP 6-04-088, that transition zone acreage can be used to offset impacts to seasonal salt marsh that occurred during wetland construction provided that the cover of native salt marsh and non-native plants within this zone meets the performance criteria outlined in this section.

Updates in the February 2016 plan.

Revision to Section 3.2.9 pertaining to the monitoring of birds.

The approach used to assess the total densities and number of species of birds in San Dieguito Wetlands and the reference wetlands from 2012 to 2015 entailed visually identifying and counting (using binoculars or spotting scope) all individuals sighted within 20 replicate rectangular plots measuring 100 x 150 m spread throughout the wetlands. However, this approach has been revised because the area of different habitat types within the plots (i.e., open water, land) was not standardized to permit a comparison of bird density and number of species in similar habitats between the restored wetland and reference wetlands as required by the permit. Since bird density and numbers of species found in open water may differ from that of land, these two habitats will be evaluated separately. The locations of the plots in San Dieguito Wetlands, Tijuana Estuary, and Mugu Lagoon have been adjusted such that two habitats, open water and land, can be sampled and compared among wetlands.

Updates in the August 2018 plan.

Revision to Section 3.2.9 pertaining to the monitoring of birds.

The approach used to assess the total densities and number of species of birds in San Dieguito Wetlands and the reference wetlands is currently under review by CCC staff. During this review, the approach employed from 2012 to 2015, which entailed visually identifying and counting (using binoculars or spotting scope) all individuals sighted within 20 replicate rectangular plots measuring 100 x 150 m spread throughout the wetlands will be used.

Updates in the June 2021 plan.

Revision to Section 3.2.9 pertaining to the monitoring of birds.

Deleted: "Note: the approach used to evaluate the bird standard provided in the Monitoring Plan updated in 2017 has has been revised in the August 2018 update and is currently under review by CCC staff."

Following review by CCC staff, the approach used to monitor the total densities and number of species of birds in San Dieguito Wetlands will follow the original approach employed since 2012 and as provided in the August 2018 update.

Addition of Appendix 3 showing the adjusted locations of tidal creeks and stations sampled for invertebrates and fish in 2020.

Changes in the location of tidal creek sampling sites was necessary due to the encroachment by *Spartina foliosa* Into tidal creeks sampled in previous years preventing the use of beach seines and enclosure traps used to sample fish.

Addition of Appendix 4 detailing modifications to performance monitoring in 2020 due to the COVID-19 pandemic.

Performance monitoring was adjusted in 2020 to accommodate restrictions to laboratory and field work imposed by the pandemic.

Deletion of several appendices, including three concerning the sampling of fish by Steele et al., which have been published. General reordering of the appendices, including the addition of Appendix 5 containing performance monitoring metadata with the inclusion of some of this information in the section on fish sampling (Section 3.2) and elsewhere in the document.

Updates in the May 2022 plan.

Modification in the method of analysis used to evaluate data pertaining to the relative performance standard for bird species richness.

Bird assemblages in the coastal wetlands of southern California can exhibit strong seasonal patterns in species richness that are driven by the movement of migratory birds. The sampling design used to assess species richness in performance monitoring takes this temporal variability into consideration through sampling birds within three periods during the year, winter, spring, and fall that are expected to have distinctive species composition. During performance monitoring, the number and species of birds are identified and counted within each of the 20 -1.5 ha plots during each of 18 survey dates (3 seasonal periods x 6 surveys per period for each wetland, see Section 3.2.9, Birds).

From 2012 – 2019, the method used to evaluate the species richness standard for birds entailed averaging the number of unique species of birds for each plot across the 18 surveys, then averaging those values across the 20 plots to produce an annual mean value for species richness of birds in each wetland. However, because the number of bird species for each plot is averaged rather than accumulated across the 18 survey dates, this

approach does not take full advantage of the temporal sampling design in capturing the number of unique bird species that frequent a particular plot during overwintering and the spring and fall migration. An alternative approach that accumulates the number of unique bird species within a plot over time better reflects the annual species richness of birds in that plot. Consequently, in 2020, the method of analysis was changed and the total number of unique bird species observed in a plot is accumulated over all 18 survey dates in a given year to produce a value for bird species richness in that plot for the year. Yearly mean species richness of birds within each wetland is then computed using the 20 plots as replicates for each wetland and these values are used for evaluating similarity between the restored and reference wetlands.

Replacement of the wetland metadata text and tables in Appendix 5 with a url to the updated UCSB SONGS Marine Mitigation website that contains links to this information, and the insertion of a reference to Section 5.0, Dissemination of Results.

Appendix B

SOP 9
Fish Seine

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Protocol Reference

TBF. 2021. Fish Beach Seine Standard Operating Procedures. Unpublished protocols. The Bay Foundation, Los Angeles, CA. https://cms.santamonicabay.org/wp-content/uploads/2021/03/L3-Estuarine-Wetland-Manual-V.2_FINAL_web.pdf

Beach Seines. 2020. Tennenbaum Marine Observatories Network, Marine- GEO, Smithsonian Institution. https://marinegeo.github.io/assets/modules/fish-seines/marinegeo_protocol_beach_seines.pdf

Objective

The community assemblage of fish is a key indicator of ecological condition in estuaries. Understanding estuarine fish communities requires measures of fish density and species richness. We recommend three methods for quantifying fish species richness and abundance with the following prioritization:

1) **Fish seines (SOP 9)**
2) Baited Remote Underwater Video (BRUVs) (SOP 8)
3) Baited Traps (SOP 10)

Understanding estuarine fish communities relies on quantification of density and/or species richness of fish. Seines are one of the most widely used gear types for sampling estuarine fishes (e.g., Allen 1982; Allen et al. 1992) because they capture a wide variety of species and are relatively easy to use. However, seines themselves are biased towards smaller, mid-water and sometimes slower fish than other methods such as beam trawls or hook and line fishing. As explored thoroughly in Steele et al. 2006a and 2006b, various factors about seines including mesh size, length, skill of fishers, and block netting can influence the density and species richness estimates. The choice of seine may vary with the project goals and should be carefully considered by the practitioner.

Due to the diversity of fishing methods commonly used across southern California, for the Bight ’23 Estuary regional survey, the main parameter of focus and goal of the survey will be to generate a species list across the Southern California Bight. Due to different seine type, method, and replication, Bight data **cannot** be used to compare species abundance or diversity of fish. Species richness data should be interpreted with caution.

If users are interested in specific fish species (species of protection or priority, e.g., tidewater goby, California halibut, etc.), we recommend targeted surveys. Targeted surveys should consist of more intensive seining, using block nets and enclosure traps to seine until completion. Ongoing efforts can be leveraged to complete targeted surveys, such as efforts conducted by agencies, such as the California Department of

Fish and Wildlife (CDFW), National Oceanic and Atmospheric Administration (NOAA), and Resource Conservation District of the Santa Monica Mountains (RCDSMM) as well as ongoing long-term monitoring programs including the San Onofre Nuclear Generating Station (SONGS) Mitigation Monitoring Program, the Tijuana River Estuary National Estuarine Research Reserve and The Nature Collective's San Elijo Restoration Project.

This SOP provides a standard approach to quantitatively assess the distribution, relative abundances, species richness, and diversity of fish for the EMPA program. If estuary sites do not have a methodology in place for consistent sampling, we recommend beach seining (method detailed below). This provides a fast and efficient way to assess the relative abundance of fish throughout an estuary.

MATERIALS

1. 30 ft l, 6 ft h, 1/8 in mesh beach seine (no bag)
2. 25 m transect tape
3. 3-4, 5 gal buckets or large plastic pool
4. Calipers
5. Rulers or meter sticks

FIELD METHODS – BEACH SEINES

1. Prior to fishing, a discrete water quality measurement should be taken using a YSI or other handheld sensor, following the protocols as outlined in SOP 2.
2. Five seine pulls will be performed at each sampling zone on an ebb tide. The end of the first seine is the start of the second seine, and so on.
    a. Lay out 25 m transect which will mark the seine pull
    b. Stretch out seine, walking along the shoreline
    c. Mark the orientation of how you will pull the seine on the data sheet
        i. Perpendicular to shore – the seine is perpendicular to shore and users are walking along the shore (Figure 1)
            1. This is the preferred method
        ii. Parallel to shore – users start offshore and walk the seine towards the shore moving offshore to inshore (Figure 2, right photo)
    d. Walk the seine 25 m
        i. Close ends at end of transect by forming a C and
        ii. Pull onto land
    e. It is important that the integrity of the seine is not loss during the pull, technicians should ensure that
        i. The lead line remains on the bottom – if the seine gets stuck on a rock or stick, it is possible to carefully lift the lead line over the

obstruction, however if the lead line drifts off the bottom, then the seine should be repeated
        ii. The floats remain on the top of the water
        iii. The lead line rolls due to excess algae or SAV
    f. Transfer fish immediately from the nets into buckets filled with estuary water to be measured and identified to species
3. Measure and count the catch
    a. The first 3 seine pulls will be evaluated for fish abundance and fish standard length
        i. If there are fewer than 10 individuals of a species, all fish standard lengths (most anterior part of the upper or lower jaw to caudal peduncle) should be measured to the nearest millimeter (Figure 3)
        ii. If more than 10 individuals of a given species are collected in a given seine, only the first 10 randomly selected individuals of each species should be measured. The remaining fish of that species (> 10) should be counted and held for release in the buckets
    b. Seine pulls 4 and 5 will be evaluated for species richness. Only the species caught will be recorded, not the number of fish.
    c. Fish that are too small (typically those ≤ 10 mm) to accurately identify in the field should be labeled as juveniles
    d. Record macroinvertebrate or other by-catch data
4. Once a seine has been fully counted and measured, the fish may be released outside of the immediate zone area (to avoid recapture), upstream of the next seine.
5. For each sampling location, the datasheet should be completed, and the following should be filled out -
    a. Project id, site id, and estuary name
    b. Station number
    c. Date
    d. Latitude and longitude of the start and end of each seine pull
    e. Seine elevation (if using an RTK)
    f. Transect location

Figure 1. Perpendicular seine pull - the seine is perpendicular to shore and users are walking along the shore.

FIELD METHODS – BLOCK NETS

NOTE: The SONGS Mitigation Monitoring Program fish seine protocol differs from the above protocol. Below is the methods for block netting, rather than beach seining.

1. Main channel seining: To outline the survey area, run a 25 ft transect perpendicular to the shoreline and place wooden dowels every 2-3 ft starting on either side of the transect tape until the water level is approximately waist high. When the appropriate depth is reached, continue to install dowels parallel to shore to complete the survey area outline.
    a. If the current is strong, more dowels may be needed
2. Wait 15 minutes after installing dowels
3. Unroll each blocking net (40 ft and 80 ft) 25 ft apart along the shoreline.
4. Deploy both nets while keeping the lead line flush with the bottom, tracing the perimeter of the survey area set by the dowels. The two blocking nets will overlap once the survey area is fully enclosed.
    a. Hang the float line on the dowels to prevent movement from wind or tide.
5. Once the survey area is fully enclosed, stretch the 25 ft beach seine along the far side (deepest side) of the enclosed area, parallel to shore.
6. One person should be on either side of the seine.
7. Drag the seine toward the shore with the lead line taut along the bottom.
8. Once onshore, carefully lay the net out and retrieve all organisms and place in buckets with fresh estuarine water until the seine has been fully checked and all

individuals can be identified, counted, measured (to distinguish recruits vs. adults), and released outside of the blocking nets/survey area.
    a. Record all fish except burrowing gobies (these are captured using a separate fish survey technique).
9. Repeat for five hauls
10. Remove the dowels holding the blocking nets and pull the deepest ends of the two blocking nets into shore simultaneously, maintaining their overlap.
    a. Drag the 40 ft seine in with the 80 ft seine encircling it to prevent fish from escaping.
    b. Including the blocking nets, there should be seven hauls in total.
    c. Record the water depth and algae/vegetation cover at the end of the survey
11. Repeat this process for three days at main channel (n=6) site. Note: The survey area is moved each sampling day ~30 ft (or as much as possible) to avoid habitat disturbed from seining days prior.
12. Tidal creek sampling: To outline the survey area, simultaneously place dowels from one side of the tidal creek to the other, spaced 25 ft apart while unrolling two 40 ft blocking nets secured by the dowels. This will establish two sections for sampling, TC-A and TC-B.
    a. Stretch the 25 ft beach seine along the bank of one side of the tidal creek and pull towards the opposite bank side, keeping the lead line taut.
        i. Carefully pull the seine up the bank face. For steep bank faces, this will require multiple people to ensure the lead line remains in contact with the bottom as the seine is full onto the marsh plain.
    b. Once onshore, carefully lay the net out and retrieve all organisms and place in buckets with fresh estuarine water until the seine has been fully checked and all individuals can be identified, counted, measured (to distinguish recruits vs. adults), and released outside of the blocking nets/survey area.
        i. Record all fish except burrowing gobies (these are captured using a separate fish survey technique).
    c. Repeat for five hauls
    d. Remove only the dowels holding the blocking net for section TC-A, while keeping the dowel along the bank in place. Have one person hold the seine and dowel along the bank in place while the other person pulls the seine in along the remaining blocking net for TC-B.
        i. Once both ends of the seine are along the bank, carefully pull the blocking net up the bank face.
        ii. Once onshore, carefully lay the net out and retrieve all organisms and place in buckets with fresh estuarine water until the seine has been fully checked and all individuals can be identified, counted, measured (to distinguish recruits vs. adults), and released outside of the blocking nets/survey area.
            1. Record all fish except burrowing gobies (these are captured using a separate fish survey technique).
            2. TC-A will have 6 hauls in total

            a. Record the water depth and algae/vegetation cover
e. Use the blocking net previously used for TC-A to set the survey area for TC-B. Simultaneously deploy the dowels and blocking net out, spaced 25 ft from the existing blocking net.
    i. Repeat the above process (25 ft seine, five hauls, record data for each haul)
    ii. Pull both blocking nets in, keeping them overlapped. Carefully pull both blocking nets onto the marsh plain and identify, count, measure, and release all caught individuals.
        1. TC-B will have 7 hauls in total
            a. Record the water depth and algae/vegetation cover

Figure 2. The use of blocking nets in tidal creek (left) and main channel (right) habitats. The right photo demonstrates a parallel seine pull.

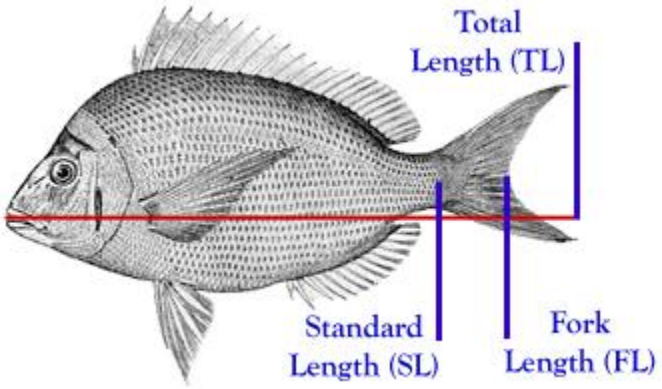

Figure 3. Standard length on an example fish (source: https://fishionary.fisheries.org/tag/standard-length/)

References:

Allen, D. M., S. K. Service, and Ogburn-Matthews, M.V. 1992. Factors influencing the collection efficiency of estuarine fishes. Transactions of the American Fisheries Society 121:234–244.

Allen, L. G. 1982. Seasonal abundance, composition, and productivity of the littoral fish assemblage in upper Newport Bay, California. Fishery Bulletin 80:769–790.

Steele, M.A., Schroeter, S.C, and Page, H.M. 2006a. Sampling Characteristics and Biases of Enclosure Traps for Sampling Fishes in Estuaries. Estuaries and Coasts 29(4): 630-638.

Steele, M.A., Schroeter, S.C, and Page, H.M. 2006b. Experimental Evaluation of Biases Associated with Sampling Estuarine Fishes with Seines. Estuaries and Coasts 29(6B): 1172-1184.

QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC)

SCCWRP will provide training for all field teams prior to the start of the sampling period. This training will include how to deploy and conduct seine surveys. Training will occur in the field and will also serve as an intercalibration exercise to ensure that all field teams are collecting and interpreting data in the same way, as well as to test species identification. Following initial training, each team will explore their specific systems with wildlife specialists appointed by the landowners (Fish and Game, etc.) to identify areas of sensitive habitat that should be avoided during sampling. These trainings will be set up to determine specific routes to sampling areas that can be adhered to during each sampling event. The Lead Scientist from each organization will be responsible for ensuring that their field personnel have been trained properly on all field methods and procedures that will be used during the survey. It will be their responsibility to review the

Field Operations Manual with their field crews, and to make sure that each person understands that these procedures must be followed during the survey. Personnel that cannot perform a required operation will not participate in conducting that operation.

While it is expected that the lead taxonomists of each organization will have a wide range of knowledge of the common seine caught species, it is not expected that all persons making field identifications will know every species. *It is, therefore, very important to avoid guessing when finalizing any identification*. An error made in the identification of an organism may result in an irretrievable error in the database because most of the organisms that are identified in the field are returned to the sea. If there is any question regarding the identity of a specimen, that specimen shall be returned to the laboratory for final identification and a voucher should be collected. Once the final identity of any specimen has been ascertained in the organization's laboratory, that change will be made on the fish species sheets by crossing out the original name (do not erase the original name) and writing the correct name. Conversely, if it has been determined that a species cannot be identified at the organization's laboratory, the specimen will be sent to SCCWRP for identification.

All data collected on the data sheet should be entered clearly. Field crews should double check that all fish tallies are counted and the final numbers calculated for each fish species per seine pull. Team Leaders should also double check that the Data sheets have been completely filled out before leaving a transect site.

Appendix C

SOP 4
Water eDNA

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PROTOCOL REFERENCE

California Molecular Methods Workgroup SOPs for DNA sampling best practices and water eDNA collection: https://github.com/stheroux/MMWG/

OBJECTIVE

Environmental DNA methods provide an opportunity to survey multiple taxa at one time from an environmental sample, including microbes, fish, and mammals. In conjunction with the other biological indicators, water eDNA samples will be collected and analyzed to profile species biodiversity. This SOP describes the collection and preservation of water eDNA samples using gravity filtration.

Instructional video: https://youtu.be/sQ_zVaddKt8

MATERIALS

Field:

1. Kangaroo gravity feeding bags
2. MasterFlex tubing, cut into 2 inch pieces
3. Adapter for connecting Sterivex to tubing
4. 0.22um Sterivex filters
5. Coffee filters and filter holder
6. Sterivex labels with sample site code, date, and replicate number
7. Sterivex blue caps
8. 2ml screw cap tube pre-loaded with preservation solution
9. Sample collection bottle (500ml or 1L) *****
10. 3 mL syringe*
11. DNAway
12. Kimwipes
13. MilliQ water
14. Nitrile gloves
15. Duct tape or binder clip for securing Sterivex filter (ie to bucket)
16. Whirl Pak or Ziploc bags labeled with sample site code, date, and replicate number

* Items should be sterilized before use and between sampling sites to prevent cross-contamination. To sterilize, soak in acid wash (1% solution of hydrochloric or nitric acid) or bleach (final concentration 1-5%), rinse 3x in DI H2O, and autoclave. Alternatively, soak in bleach solution (final concentration 1-5%) and rinse 3x with DI H2O.

Field methods: Water column

Instructional video: https://youtu.be/sQ_zVaddKt8

Sample Collection:

One kangaroo bag will be used ***per sampling station*** – filtering 1 field blank and 3 replicates. The field blank should be filtered first before filtering the replicates. ***Only 1 field blank is needed per estuary.***

1 Kangaroo pouch
⇓
100ml MilliQ water field blank
⇓
3 x 1L replicates

If field crews are short on time, water samples can be collected following the instructions below and then **brought back to the lab for filtering**. Samples can be collected using either 1L Nalgene bottles or 1L whirlpaks. Samples should be kept **cold on ice** until filtering (samples should be filtered within 24 hours).

Preparation:

1. Prior to removing eDNA supplies or collecting the sample, put on **nitrile gloves.**
2. Open eDNA supplies on a flat, dry, **clean** stable surface (lab bench, folding table, small storage container, upside down bucket, etc.).
    a. **Sterilize the surface** before unpacking the eDNA supplies with a Clorox or bleach wipe or DNAway.

Collection of field blank:

3. **Before sample collection**, one field blank should be processed per estuary.
    a. Filter ~100ml of MilliQ water through a clean Kangaroo pouch and Sterivex filter following the instructions below.

Collection of water eDNA sample:

4. Sample water should be collected at high tide using a 1L sample bottle. Surveyors can wade into the water, so that samples are collected in the intertidal zone at high tide. There should be at least 1 m of water at time of collection.
5. Stand in the water until the **sediment settles**, and then begin collecting the samples.
6. Rinse sample bottle 3x with ~10ml of sample (estuarine) water; discard rinse water offshore or away from sampling site. Fill bottle with 1L of sample water. If bottle numbers are limited, large WhirlPaks can be used for water collection.
7. Carry sample water back to shore.

8. After this stage**, samples could be brought back to lab for filtering**. Samples should be kept **cold on ice** until filtering and filtered within 24 hours.

Filtering with coffee filter, Sterivex filter, and kangaroo bag:

9. Ensure that Kangaroo bag tubing switch is in “off” position.
    a. Load a coffee filter onto the filter holder and place in opening of kangaroo pouch; hold filter holder and kangaroo pouch steady with 2 hands (**Figure 1)**.
    b. Pour sample water into coffee filter, allowing volume to filter through before adding more water. This should take ~5-10 minutes.
    c. After all water has passed through the coffee filter, remove filter squeezing out remaining liquid. Avoid touching the inside of the filter. Store coffee filter in labeled whirlpak. Place on ice immediately.
10. Hang Kangaroo bag out of direct sunlight, climate control preferred, about 4-6 feet above the floor. Place container/bucket underneath bags to collect outflow (or let drain on floor if boat or outdoor site filtering allows).
    a. The best and fastest filtering will occur when the bag is high enough so that the filter line is straight and extended.
11. Open the Sterivex filter and label with site name, station number, sample replicate, and date using the provided labels. Attach the Masterflex tubing to the purple end of the Kangaroo bag tubing. Insert the white adapter to the other end of the Masterflex tubing. Attach (twist) the Sterivex filter to the white adapter (**Figure 2**).
12. Use a binder clip or duct tape to secure the Sterivex filter pointing into the bucket. Avoid the Sterivex filter touching the mud or sediment.
13. Open Kangaroo tubing switch to allow water to begin flowing.
14. Stop filtering after **40-50 minutes** even if less than 1L has been filtered.
    a. **Record the volume filtered on the sample sheet.**
15. When done filtering, use a 3 mL syringe to expel the remaining water out of the Sterivex filter. Expel the remaining water 3x until the filter is dry. Cap the larger end of the Sterivex with a blue cap.
16. Proceed to **Sample Preservation** (below). Between stations, rinse filter holder with DI water 3x and wipe down with DNAway.

Figure 1. Coffee filter and kangaroo pouch.

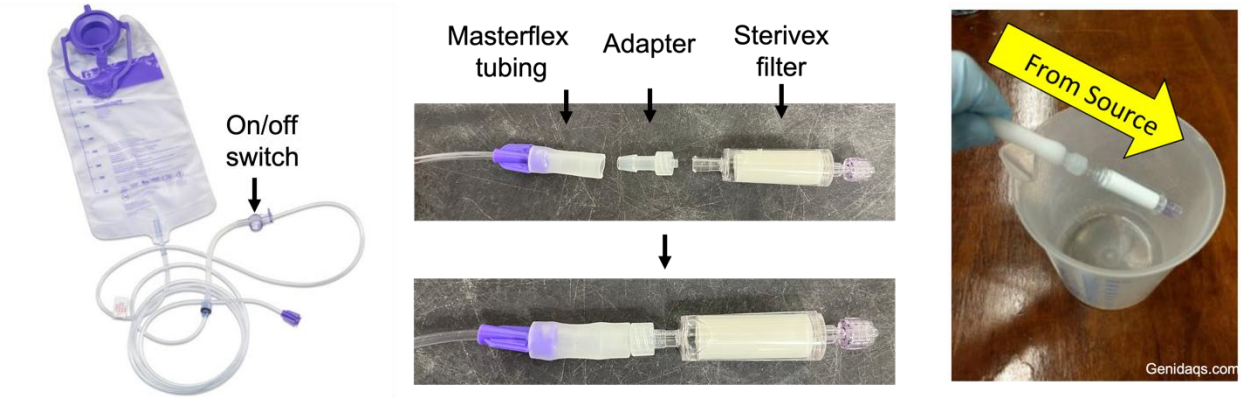

Figure 2. Plan A. Left: Kangaroo feeding bag. Center: Assembling the Masterflex tubing, adapter, and Sterivex filter. Right: Sterivex draining into bucket.

Sample Preservation:

17. Load ~1.5 ml of preservation solution using a disposable pipette. Insert the pipette into the open end of the Sterivex and flood the membrane with the preservation solution. When complete, cap the remaining end of the Sterivex with another blue cap (**Figure 3**).
18. Soak the Sterivex membrane by inverting the Sterivex 3x.
19. Place the labelled Sterivex into a labeled whirlpak
    a. All three replicates can go into the same whirlpak or bag.
20. Keep tubes with filters on ice until able to be transported to -20°C or -80°C freezer. Samples at -20°C are stable for six months.
21. Repeat all steps for as many replicates as requested (default = 3 replicates). A single kangaroo bag can be reused for three replicates within a sampling station. Each replicate requires its own Sterivex filter.

**Filtering before storage will help preserve the integrity of the eDNA. The freeze-thaw cycle is known to degrade DNA, especially extracellular DNA which we are targeting in the water samples. Additionally, thawing large volumes of water will take a long time and could result in more DNA decay.

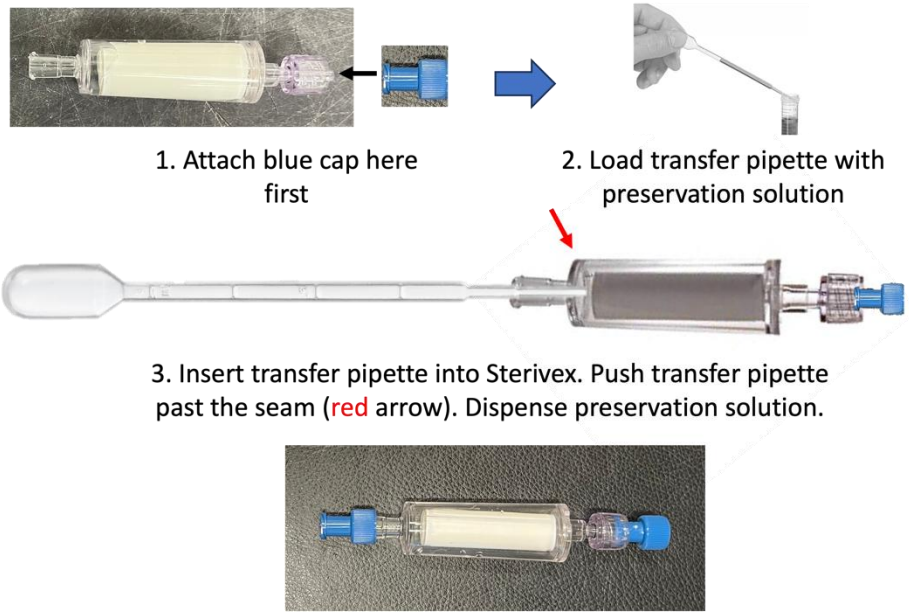

Figure 3. Steps for loading Sterivex filter with preservation solution.

QUALITY ASSURANCE/ QUALITY CONTROL (QA/QC)

Field personnel must be thoroughly trained in the proper use of sample collection gear and must be able to distinguish acceptable versus unacceptable water grab samples in accordance with pre-established criteria.

Field personnel must be thoroughly trained to recognize and avoid potential sources of sample contamination (e.g., non-sterile sampling supplies, poor sterile technique).

All data collected on the eDNA data sheet should be entered clearly. Team Leaders should double check that all eDNA samples have been collected and properly filtered and that the Data sheets have been filled out before leaving a transect site. eDNA Filters relinquished to SCCWRP need to have the following eDNA COC documentation: SCCWRP eDNA CoC template_forBight.xlsx

Appendix D

Jonah Ventures MiFishU Test Methods

Sample Process

1.0

Raw samples were not processed by Jonah Ventures.

Extraction

2.0

DNA extraction was not handled by Jonah Ventures for samples associated with this batch.

PCR

3.18.3

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

4.1.1

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

5.1.1

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

6.2.1

A second round of PCR was performed to complete the sequencing library construct, appending the final Illumina sequencing adapters and integrating sample-specific, dual index sequences (2 x 10bp). 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

8.2.1

Final indexed amplicons from each sample were cleaned and normalized using mag-bind normalization. A 15µl aliquot of PCR amplicon was purified and normalized using Cytiva SpeedBead magnetic carboxylate modified particles (#45152105050250). Samples were then pooled together by adding 5µl of each normalized sample to the pool.

Sequencing

9.9.1

Sample library pools were prepped for sequencing on an Oxford Nanopore Technologies MinION (Oxford, England) using the Ligation Sequencing Kit V14 (cat# SQK-LSK114). Final basecalling was completed using AWS Batch and EC2 g5.xlarge instances running ONT's dorado software (v0.7.1) in super high accuracy mode using the dna_r10.4.1_e8.2_400bps_sup@v5.0.0 basecalling model.

Bioinformatics

10.11.3

Raw Nanopore sequencing output was converted from pod5 to fastq format using dorado v0.7.1 [1] and the super-high accuracy basecalling model. Cutadapt v3.4 [2] was then used to trim outer adapters and reorient reads in a consistent 5' to 3' direction. The resulting reads were sorted into individual samples by demultiplexing with pheniqs v2.1.0 [3], allowing no more that one mismatch in each of the paired 10 bp molecular indices. Cutadapt was then used again to extract the target amplicon by removing the gene primers, discarding any reads where one or both primers were not found or where the resulting amplicon sequences were < 130 bp or > 210 bp. Exact sequence variants (ESVs) were then identified for each sample using the unoise3 denoising algorithm [4] as implemented in vsearch [5]. Only reads that were observed 4 or more times and with a maximum expected error rate [6] > 1 bp were considered as candidate ESVs and putative chimeras were filtered using the uchime3 algorithm [7]. Final read counts were determined for each sample by mapping unfiltered raw reads to the identified ESVs using the vsearch function usearch_global with an minimum identity of 95%. For each final ESV, a consensus taxonomy was assigned using a custom best-hits algorithm and a reference database consisting of publicly available sequences on NCBI GenBank [8], as well as Jonah Ventures voucher sequences records. Reference database searching used vsearch to conduct exhaustive semi-global pairwise alignments, and final 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. A consensus taxonomic assignment 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. Oxford Nanopore Technologies. (2024). Dorado basecalling software (Version 0.7.1). GitHub. https://github.com/nanoporetech/dorado.

2. Martin (2011), Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. Journal 17.

3. Galanti, Shasha and Gunsalus (2021). Pheniqs 2.0: accurate, high-performance Bayesian decoding and confidence estimation for combinatorial barcode indexing. BMC Bioinformatics 22.

4. Edgar (2016), UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. doi: https://doi.org/10.1101/081257.

5. Torbjørn et al. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4 e2584 (2016).

6. Edgar and Flyvbjerg (2015), Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 31.

7. Edgar (2016), UCHIME2: improved chimera prediction for amplicon sequencing. doi: https://doi.org/10.1101/074252.

8. Benson et al. (2005), GenBank. Nucleic Acids Res 33.

Supplemental Table S1. Relative frequency distributions of species reads in of fish species richness across 5 estuaries in San Diego

Order	Family	Genus	Species	% match	# species	Total	TJE Station 2 Coffee Filter	SDB Sweet Water Station 2 Coffee Filter	SDB Salt Ponds Station 2 Coffee Filter	SDL Station 2 Coffee Filter	LPL Station 2 Coffee Filter	KFR Station Coffee filter	MB KFR Station 1 Coffee Filter 2	SDL Station 1 Sterivex 1	LPL Station 2 Sterivex 1	MB KFR Station 2 Sterivex 3
Labriformes	Labridae	Semicossyphus	*Semicossyphus pulcher*	100	1	46695	0	0	0	398	10184	19079	2443	5193	2199	7199
Blenniiformes	Labrisomidae	Paraclinus	*Paraclinus integripinnis*	100	1	24169	0	1651	4132	1322	0	6294	6598	1830	529	1813
Perciformes	Serranidae	Paralabrax	*Paralabrax clathratus*	100	1	20039	592	0	959	258	5221	132	0	8554	4323	0
Cyprinodontiformes	Fundulidae	Fundulus	*Fundulus parvipinnis*	100	1	10591	5117	0	0	5013	0	0	0	461	0	0
Gobiiformes	Gobiidae	Quietula	*Quietula y-cauda*	100	1	10103	1114	0	0	8989	0	0	0	0	0	0
Gadiformes	Gadidae	Gadus		100	4	8811	0	4111	0	4566	0	0	0	0	0	134
unk_order	Pomacentridae	Chromis	*Chromis punctipinnis*	100	1	5365	0	0	0	5365	0	0	0	0	0	0
unk_order	Embiotocidae	Embiotoca	*Embiotoca jacksoni*	100	1	4644	792	0	0	2170	1275	0	0	0	0	407
unk_order	Unknown	Unknown				4287	0	0	0	0	3636	0	0	0	651	0
Mugiliformes	Mugilidae	Mugil	*Mugil cephalus*	100	1	4249	1603	0	0	2646	0	0	0	0	0	0
unk_order	Embiotocidae	Micrometrus	*Micrometrus minimus*	100	1	3945	0	0	0	0	0	3945	0	0	0	0
Lutjaniformes	Haemulidae	Anisotremus	*Anisotremus davidsonii*	100	1	3702	0	1264	0	764	0	1549	0	0	0	125
Atheriniformes	Atherinopsidae	Menidia		100	3	3650	0	3503	0	147	0	0	0	0	0	0
Clupeiformes	Clupeidae	Sardinops		99.4	2	2372	0	0	0	0	2372	0	0	0	0	0
Pleuronectiformes	Pleuronectidae	Hypsopsetta	*Hypsopsetta guttulata*	100	1	2236	0	0	1795	441	0	0	0	0	0	0
Gobiiformes	Gobiidae	Gymnogobius		89.3	3	2128	0	0	0	150	0	0	0	1978	0	0
unk_order	Sciaenidae	Umbrina	*Umbrina roncador*	100	1	1933	0	0	0	0	0	0	0	1933	0	0
Syngnathiformes	Syngnathidae	Syngnathus		100	3	1738	26	0	0	1250	0	0	0	462	0	0
Centrarchiformes	Kyphosidae	Kyphosus	*Kyphosus azureus*	100	1	1585	0	0	1585	0	0	0	0	0	0	0
Cyprinodontiformes	Fundulidae	Fundulus	*Fundulus parvipinnis*	96.4	1	1211	0	0	0	0	0	0	0	1211	0	0
Scombriformes	Scombridae	Thunnus		100	5	978	0	0	0	511	0	0	0	467	0	0
unk_order	Unknown	Unknown				939	0	0	0	939	0	0	0	0	0	0
Mugiliformes	Mugilidae	Mugil	*Mugil cephalus*	99.4	1	782	0	0	0	0	213	389	0	0	0	180
Gobiiformes	Gobiidae	Gillichthys	*Gillichthys mirabilis*	100	1	707	593	0	0	114	0	0	0	0	0	0
unk_order	Unknown	Unknown				702	0	0	0	0	0	702	0	0	0	0
unk_order	Embiotocidae			93	3	684	0	0	0	0	0	0	0	0	684	0
Labriformes	Labridae	Halichoeres		99.4	2	638	0	0	0	323	0	0	0	315	0	0
Mugiliformes	Mugilidae	Mugil	*Mugil cephalus*	99.4	1	602	0	0	0	0	602	0	0	0	0	0
Labriformes	Labridae	Halichoeres	*Halichoeres californicus*	100	1	314	87	0	0	117	0	67	43	0	0	0
Gobiiformes	Gobiidae	Acanthogobius	*Acanthogobius flavimanus*	100	1	274	0	0	0	274	0	0	0	0	0	0
unk_order	Unknown	Unknown				243	0	0	0	243	0	0	0	0	0	0
Clupeiformes	Clupeidae	Sardinops		100	2	219	0	0	0	219	0	0	0	0	0	0
Scombriformes	Scombridae	Scomber		100	3	171	0	0	0	21	0	0	0	150	0	0
Gobiiformes	Gobiidae	Quietula	*Quietula y-cauda*	99.4	1	165	0	0	0	165	0	0	0	0	0	0
Atheriniformes	Atherinopsidae	Atherinops	*Atherinops affinis*	99.4	1	132	0	0	0	0	0	0	0	0	0	132
Atheriniformes	Atherinopsidae	Atherinops	*Atherinops affinis*	99.4	1	96	0	0	0	96	0	0	0	0	0	0
Clupeiformes	Engraulidae	Engraulis	*Engraulis mordax*	100	1	88	88	0	0	0	0	0	0	0	0	0
unk_order	Sciaenidae	Roncador	*Roncador stearnsii*	100	1	80	0	0	0	80	0	0	0	0	0	0
Scombriformes	Scombridae	Thunnus		100	3	75	0	0	0	0	0	0	0	75	0	0
Caudata	Plethodontidae	Batrachoseps	*Batrachoseps nigriventris*	89.7	1	68	0	0	0	0	0	0	0	68	0	0
Blenniiformes	Blenniidae	Hypsoblennius	*Hypsoblennius gentilis*	100	1	66	0	0	0	17	0	0	0	49	0	0
unk_order	Unknown	Unknown				56	0	0	0	56	0	0	0	0	0	0
Clupeiformes	Engraulidae	Engraulis	*Engraulis mordax*	99.4	1	52	0	0	0	0	0	0	0	52	0	0
Primates	Hominidae	Homo	*Homo sapiens*	100	1	49	0	0	0	49	0	0	0	0	0	0
Pleuronectiformes	Paralichthyidae	Paralichthys	*Paralichthys californicus*	100	1	30	0	0	0	0	30	0	0	0	0	0
unk_order	Unknown	Unknown				17	0	0	0	17	0	0	0	0	0	0
Artiodactyla	Bovidae	Ovis		100	2	17	0	0	0	0	17	0	0	0	0	0
Perciformes	Serranidae	Paralabrax	*Paralabrax maculatofasciatus*	100	1	14	14	0	0	0	0	0	0	0	0	0
Gobiiformes	Gobiidae	Clevelandia	*Clevelandia ios*	100	1	14	14	0	0	0	0	0	0	0	0	0
Blenniiformes	Gobiesocidae	Rimicola	*Rimicola muscarum*	94.6	1	14	0	0	0	14	0	0	0	0	0	0
unk_order	Unknown	Unknown				11	0	0	0	0	11	0	0	0	0	0
Primates	Hominidae	Homo	*Homo sapiens*	100	1	10	0	0	0	0	0	0	0	10	0	0
						TOTAL	**10040**	**10529**	**8471**	**36734**	**23561**	**32157**	**9084**	**22808**	**8386**	**9990**

Supplemental Table S2. List of species expected to occur within the study sites and the corresponding reference sequence database.

Reference sequences were collected from existing GenBank records

Supplemental Table 1. List of Geographically Relevant Fish Species (Southern California Region)

Number	Species	GenBank Accession (If Applicable)	FishCARD	GenBank	MitoHelper
1	*Acanthogobius flavimanus*	LC843965.1			
2	*Acipenser medirostris*	X95023.1			
3	*Acipenser transmontanus*	X95024.1			
4	*Albula gilberti*	N/A			
5	*Alloclinus holderi*	MH830492.1			
6	*Alosa sapidissima*	LC091584.1			
7	*Ameiurus catus*	DQ421868.1			
8	*Ameiurus melas*	DQ421856.1			
9	*Ameiurus natalis*	DQ421865.1			
10	*Ameiurus nebulosus*	JX899750.1			
11	*Amphistichus argenteus*	JN125222.1			
12	*Anchoa compressa*	N/A			
13	*Anchoa delicatissima*	DQ912036.1			
14	*Anisotremus davidsonii*	N/A			
15	*Apodichthys flavidus*	LC092023.1			
16	*Artedius corallinus*	LC126226.1			
17	*Artedius fenestralis*	LC091923.1			
18	*Artedius notospilotus*	N/A			
19	*Atherinops affinis*	AY268927.1			
20	*Atherinopsis californiensis*	N/A			
21	*Atractoscion nobilis*	MK902914.1			
22	*Aulorhynchus flavidus*	LC091880.1			
23	*Blepsias cirrhosus*	LC036769.1			
24	*Brachygenys californiensis*	N/A			
25	*Brachyistius frenatus*	XM_069700705.1			
26	*Caranx sexfasciatus*	LC848708.1			
27	*Carassius auratus*	LC795718.1			
28	*Catostomus occidentalis*	N/A			
29	*Caulolatilus princeps*	N/A			
30	*Cephaloscyllium ventriosum*	N/A			
31	*Cetengraulis mysticetus*	N/A			
32	*Chanos chanos*	XM_030792555.1			
33	*Cheilotrema saturnum*	N/A			
34	*Chilara taylori*	LC091830.1			
35	*Chromis punctipinnis*	FJ616322.1			
36	*Citharichthys sordidus*	LC145943.1			
37	*Citharichthys stigmaeus*	LC092080.1			
38	*Citharichthys xanthostigma*	AF488499.1			
39	*Clevelandia ios*	LC092048.1			
40	*Clinocottus acuticeps*	LC091912.1			
41	*Clinocottus analis*	XM_068601332.1			
42	*Clupea pallasii*	LC091582.1			
43	*Rhinogobiops nicholsi*	N/A			
44	*Cosmocampus arctus*	N/A			
45	*Cottus aleuticus*	LC091944.1			
46	*Cottus asper*	LC091946.1			
47	*Cottus asperrimus*	N/A			
48	*Ctenogobius sagittula*	N/A			
49	*Cymatogaster aggregata*	LC091978.1			
50	*Cynoscion nobilis*	N/A			
51	*Cynoscion parvipinnis*	DQ533185.1			
52	*Cyprinella lutrensis*	AF148326.1			
53	*Cyprinus carpio*	LC091587.1			
54	*Dorosoma petenense*	EU552664.1			
55	*Elops affinis*	AF454710.1			
56	*Embiotoca jacksoni*	XM_069174085.1			
57	*Embiotoca lateralis*	LC091976.1			
58	*Engraulis mordax*	LC091576.1			
59	*Enophrys bison*	LC091947.1			
60	*Eucyclogobius newberryi*	XM_072465622.1			
61	*Fundulus heteroclitus*	XM_021312282.2			
62	*Fundulus parvipinnis*	N/A			
63	*Gambusia affinis*	LC091875.1			
64	*Gasterosteus aculeatus*	LC091881.1			
65	*Genyonemus lineatus*	LC036870.1			
66	*Gibbonsia elegans*	DQ533203.1			
67	*Gibbonsia metzi*	U90364.1			
68	*Gibbonsia montereyensis*	U90365.1			
69	*Gila orcuttii*	KM282420.1			
70	*Gillichthys mirabilis*	KF415367.1			
71	*Girella nigricans*	KC136546.1			
72	*Gobiesox maeandricus*	LC092040.1			
73	*Gobiesox rhessodon*	KY656395.1			
74	*Gymnothorax mordax*	N/A			
75	*Gymnura marmorata*	N/A			
76	*Haemulon flaviguttatum*	N/A			
77	*Halichoeres semicinctus*	MG665335.1			
78	*Hemilepidotus hemilepidotus*	LC091927.1			
79	*Hermosilla azurea*	KC136547.1			

80	*Hesperoleucus symmetricus*	N/A			
81	*Heterodontus francisci*	XM_068010770.1			
82	*Heterostichus rostratus*	HQ158801.1			
83	*Hexagrammos decagrammus*	LC091901.1			
84	*Hippocampus ingens*	KY065829.1			
85	*Hippoglossina stomata*	N/A			
86	*Hypanus dipterurus*	N/A			
87	*Hyperprosopon anale*	JN125225.1			
88	*Hyperprosopon argenteum*	JN125226.1			
89	*Hypomesus nipponensis*	LC492327.1			
90	*Hypomesus pretiosus*	LC091618.1			
91	*Hypomesus transpacificus*	XM_047020679.1			
92	*Hyporhamphus gilli*	N/A			
93	*Hyporhamphus naos*	N/A			
94	*Hyporhamphus rosae*	N/A			
95	*Hypsoblennius gentilis*	U90389.1			
96	*Hypsoblennius gilberti*	U90390.1			
97	*Hypsoblennius jenkinsi*	N/A			
98	*Hypsopsetta guttulata*	AF488487.1			
99	*Hypsurus caryi*	N/A			
100	*Hypsypops rubicundus*	FJ616348.1			
101	*Hysterocarpus traskii*	N/A			
102	*Ictalurus furcatus*	XM_053618457.1			
103	*Ictalurus punctatus*	XM_017460688.3			
104	*Ilypnus gilberti*	N/A			
105	*Kyphosus azureus*	KC136547.1			
106	*Labrisomus xanti*	U90373.1			
107	*Lampetra tridentata*	LC091546.1			
108	*Lepidogobius lepidus*	LC092050.1			
109	*Lepomis cyanellus*	KM282423.1			
110	*Lepomis gibbosus*	KM282424.1			
111	*Lepomis macrochirus*	LC277930.1			
112	*Leptocottus armatus*	LC091940.1			
113	*Leuresthes tenuis*	XM_072374929.1			
114	*Lucania parva*	N/A			
115	*Lythrypnus dalli*	KF415411.1			
116	*Lythrypnus zebra*	JX024884.1			
117	*Medialuna californiensis*	KC136408.1			
118	*Menidia beryllina*	KX686069.1			
119	*Menticirrhus undulatus*	N/A			
120	*Merluccius productus*	LC091818.1			
121	*Micrometrus minimus*	N/A			
122	*Micropterus salmoides*	XM_038708334.1			
123	*Microstomus pacificus*	LC145944.1			
124	*Morone saxatilis*	XM_035666330.1			
125	*Mugil cephalus*	LC843960.1			
126	*Mustelus californicus*	N/A			
127	*Mustelus henlei*	N/A			
128	*Mustelus mustelus*	JX978294.1			
129	*Myliobatis californica*	KM364985.1			
130	*Neoclinus blanchardi*	MH830493.1			
131	*Neoclinus uninotatus*	MH830494.1			
132	*Oligocottus maculosus*	LC091941.1			
133	*Oncorhynchus clarkii clarkii*	N/A			
134	*Oncorhynchus keta*	LC091629.1			
135	*Oncorhynchus kisutch*	LC091627.1			
136	*Oncorhynchus mykiss*	LC799145.1			
137	*Oncorhynchus tshawytscha*	LC091628.1			
138	*Ophichthus zophochir*	DQ645656.1			
139	*Ophiodon elongatus*	LC091904.1			
140	*Oxyjulis californica*	MG665337.1			
141	*Oxylebius pictus*	LC104539.1			
142	*Paraclinus integripinnis*	U90367.1			
143	*Paralabrax clathratus*	AY072660.1			
144	*Paralabrax maculatofasciatus*	MK902875.1			
145	*Paralabrax nebulifer*	MK902857.1			
146	*Paralichthys californicus*	N/A			
147	*Percina macrolepida*	N/A			
148	*Phanerodon furcatus*	N/A			
149	*Phanerodon vacca*	LC091972.1			
150	*Pholis laeta*	LC092021.1			
151	*Pholis ornata*	LC092019.1			
152	*Pimephales promelas*	XM_039650154.1			
153	*Platichthys stellatus*	LC552481.1			
154	*Platyrhinoidis triseriata*	N/A			
155	*Pleuronectes vetulus*	LC145937.1			
156	*Pleuronichthys coenosus*	LC145920.1			
157	*Pleuronichthys decurrens*	LC145935.1			
158	*Pleuronichthys guttulatus*	AF488487.1			
159	*Pleuronichthys ritteri*	N/A			
160	*Pleuronichthys verticalis*	AF488489.1			
161	*Poecilia latipinna*	XM_015047642.1			
162	*Pogonichthys macrolepidotus*	AF081860.1			

163	*Porichthys myriaster*	N/A			
164	*Porichthys notatus*	LC091844.1			
165	*Psettichthys melanostictus*	LC145938.1			
166	*Pseudobatos productus*	N/A			
167	*Pseudupeneus grandisquamis*	N/A			
168	*Ptychocheilus grandis*	N/A			
169	*Quietula y-cauda*	N/A			
170	*Raja binoculata*	LC091570.1			
171	*Raja inornata*	LC091569.1			
172	*Rhacochilus toxotes*	N/A			
173	*Rhacochilus vacca*	LC091972.1			
174	*Rhinobatos productus*	N/A			
175	*Rhinogobiops nicholsii*	N/A			
176	*Rimicola eigenmanni*	N/A			
177	*Rimicola muscarum*	KY656398.1			
178	*Roncador stearnsii*	N/A			
179	*Sarda chiliensis*	LC092074.1			
180	*Sardinops sagax*	LC091585.1			
181	*Scomber japonicus*	LC092075.1			
182	*Scorpaena guttata*	N/A			
183	*Scorpaenichthys marmoratus*	LC091934.1			
184	*Sebastes auriculatus*	LC104501.1			
185	*Sebastes caurinus*	LC104532.1			
186	*Sebastes melanops*	LC104516.1			
187	*Sebastes rastrelliger*	DQ678121.1			
188	*Sebastes serranoides*	DQ678163.1			
189	*Semicossyphus pulcher*	XM_069670307.1			
190	*Seriola lalandi*	LC590911.1			
191	*Seriphus politus*	N/A			
192	*Sphoeroides annulatus*	MK902882.1			
193	*Sphyraena argentea*	N/A			
194	*Spirinchus starksi*	LC091611.1			
195	*Spirinchus thaleichthys*	LC091615.1			
196	*Squatina californica*	HM637766.1			
197	*Strongylura exilis*	AF231542.1			
198	*Symphurus atricauda*	N/A			
199	*Syngnathus auliscus*	N/A			
200	*Syngnathus californiensis*	KY065874.1			
201	*Syngnathus leptorhynchus*	LC091882.2			
202	*Synodus lucioceps*	LC021072.1			
203	*Tetronarce californica*	N/A			
204	*Thaleichthys pacificus*	LC091616.1			
205	*Torpedo californica*	N/A			
206	*Trachurus symmetricus*	LC091962.1			
207	*Triakis semifasciata*	LC104355.1			
208	*Tridentiger barbatus*	LC732537.1			
209	*Tridentiger bifasciatus*	LC762650.1			
210	*Tridentiger trigonocephalus*	LC772844.1			
211	*Umbrina roncador*	N/A			
212	*Urolophus halleri*	N/A			
213	*Xenistius californiensis*	N/A			
214	*Xystreurys liolepis*	MK617042.1			
215	*Zalembius rosaceus*	N/A			
216	*Zapteryx exasperata*	N/A			