Final

SALTON SEA SPECIES CONSERVATION HABITAT

Desert Pupfish Inoculation Plan

Prepared for
California Department of Water Resources

February 2017

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CHAPTER 1
Introduction

1.1 SCH Project Overview

The Salton Sea (Sea) is currently maintained by inflows of agricultural return water but has been shrinking in size as inflows are reduced as a result of water conservation and other water management actions. A rapid and substantial increase in salinity and reduction in the size of the Sea is expected as a result of inflow reductions associated with Imperial Irrigation District's (IID) Water Conservation and Transfer Project (Water Transfer Project), which entails water conservation and transfer transactions pursuant to the Agreement for Transfer of Conserved Water executed by IID and the San Diego County Water Authority in 1998, and supplemented by the 2003 Quantification Settlement Agreement and related agreements (QSA). Declining water inflows will hasten the collapse of the Sea's fish population due to increasing salinity (expected to exceed 60 parts per thousand [ppt] by 2018) and other water quality stresses, such as temperature extremes, eutrophication, and related anoxia and algal productivity. The most serious and immediate threat to the Sea's ecosystem is the loss of fishery resources that support piscivorous birds.

The Salton Sea Species Conservation Habitat (SCH) Project (Project) is located along the southern shore of the Sea in Imperial County. The Project is sponsored by the California Natural Resources Agency (CNRA), and implemented through their departments of Fish and Wildlife (CDFW) and Water Resources (DWR). The SCH Project's purpose is to provide replacement for near-term habitat losses (U.S. Army Corps of Engineers [Corps] and CNRA 2011). The SCH Project's target species are those piscivorous bird species that use the Sea and are dependent on shallow saline habitat for essential habitat requirements within their western geographic range.

The Project objectives include:

1. Provide appropriate foraging habitat for piscivorous bird species.
2. Develop physical structure and microhabitat elements required to support piscivorous bird species.
3. Support a sustainable, productive aquatic community.
4. Provide suitable water quality for fish.
5. Minimize adverse effects on desert pupfish.
6. Minimize risk of selenium toxicity.
7. Minimize risk of disease/toxicity impacts.

The creation, maintenance, and long-term monitoring of 3,770 acres of shallow saline ponds is designed to reduce the scientific uncertainty of identified long-term Sea restoration plans and partially reduce the aforementioned ecological hazards by providing a range of aquatic habitats, over a 75-year period, that will continue to support fish and wildlife species that are dependent on the Sea. The constructed ponds are designed to provide habitat that will support piscivorous bird species and sustainable aquatic communities, provide suitable water quality for fish species, minimize the risk of the bioaccumulation of selenium, and minimize the risk of disease and toxicity to wildlife and plants. Likely fish candidates to be established in the ponds include one or more varieties of tilapia, which are an important forage species for fish-eating birds. California Mozambique hybrid tilapia (*Oreochromis mossambicus x O. urolepis hornorum* hybrid) is the species once abundant in the Salton Sea.

1.2 Purpose of Document

Desert pupfish (*Cyprinodon macularius*) occur in the Project area. This small fish is protected as an endangered species under the federal and state Endangered Species Act. This species may be affected by Project construction, operation and maintenance activities, as disclosed in the Draft Environmental Impact Statement/Environmental Impact Report (DEIS/EIR) (Corps and CNRA 2011) and Biological Opinion (BO) (USFWS 2013). Habitat conditions in the SCH ponds are expected to be suitable for desert pupfish, although the primary purpose of the Project is to provide forage fish for the piscivorous birds, not as a pupfish refuge.

Four conservation measures (USFWS 2013) will be implemented as part of the Project to protect desert pupfish:

1. Monitor the depth of water during maintenance of the Sea pump station. If the water depth is 6 feet or less, the dredging footprint will be surrounded by netting, and desert pupfish will be trapped out of this enclosed space before suction dredging is performed. If salinity levels are beyond the tolerance of desert pupfish (approximately 68 ppt), avoidance and minimization measures would not be required as desert pupfish would not be present.

2. Prepare and implement a desert pupfish inoculation plan if pupfish do not naturally repopulate the ponds 1 year after ponds are filled with water.

3. Prepare and implement an adaptive management and monitoring plan to provide for the monitoring of desert pupfish relative abundance and distribution in the SCH ponds and desert pupfish connectivity from drains around the ponds.

4. Prepare and implement a desert pupfish protection and relocation plan.

The purpose of this document, the SCH Desert Pupfish Inoculation Plan (Conservation Measure 2), is to describe activities for introducing desert pupfish to one or both of the SCH ponds, if SCH managers have determined that desert pupfish have not repopulated one year after ponds are filled with water.

A separate document, the *Desert Pupfish Adaptive Management and Monitoring Plan* (Conservation Measure 3), describes the desired or target condition, suitable monitoring protocol, thresholds, and decision making process by which the SCH managers will determine whether pupfish are present and persisting, and whether management actions such as inoculation should be triggered. Finally, the *SCH Monitoring and Adaptive Management Plan* (MAMP) provides an integrated framework for all Project operations, monitoring, evaluation, and management (CNRA 2015). The DPAMMP will be appended to the MAMP and will be subject to the MAMP's overarching framework.

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CHAPTER 2
SCH Project Description

2.1 Location

The SCH Project is located on the southern shore of the Sea, north of Westmorland in Imperial County, California. The Project site is bounded by Poe Road to the west and Hoskins Road to the east on approximately 4,098 acres of open water, exposed playa, shallow shoreline, and agricultural irrigation ditches and canals, of which 1,750 acres are managed by the U.S. Fish and Wildlife Service (USFWS) as part of the Sonny Bono Salton Sea (SBSS) National Wildlife Refuge (NWR).

2.2 Project Elements

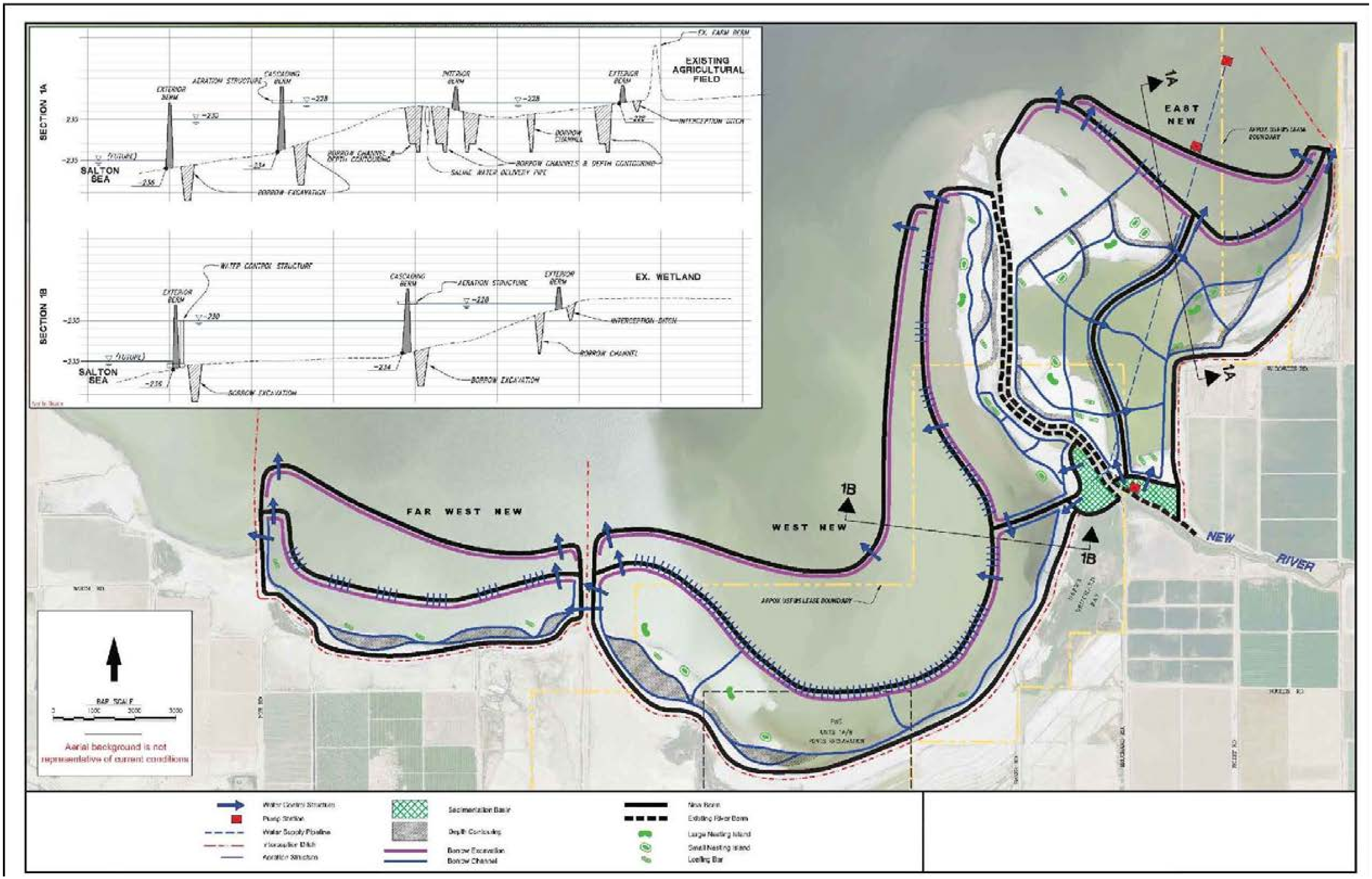
The goal of the SCH Project is to create saline impoundments on the exposed playa to support fish and wildlife dependent on the Sea. The ponds will provide near-term habitat and serve as a proof-of-concept for future phases of the SCH Project.

The SCH Project area encompasses 3,770 acres of exposed playa at the mouth of the New River, at the southwest end of the Sea (**Figure 1**). Three independent pond units, Far West New, West New, and East New will be constructed and supplied with a combination of water from the New River and the Sea and blended to maintain an optimal salinity range. The SCH ponds will average 2-6 feet deep and have the following approximate maximum areas and volumes:

- Far West New: 514 acres, 2,305 acre-feet
- West New: 1,731 acres, 6,783 acre-feet
- East New: 1,139 acres, 2,870 acre-feet

The initial implementation phase will construct and operate two ponds (320-acres each, designated East Pond and West Pond) to create a total of 640 acres of saline pond habitat in the East New River section of the Project area (**Figure 2**). Each pond will have its own water supply infrastructure that can be operated independently, to allow manipulation of hydrologic parameters such as salinity and residence time as experimental “treatments.” The SCH Project will test different operating regimes (varying salinity and residence time) between the ponds and monitor physical and biological outcomes during the first 10 years.

Figure 1 - Salton Sea Species Conservation Habitat Project Area



Source: Corps and CNRA 2011

Figure 2 - SCH Project - East New River, West Pond and East Pond

Section 2.4.1 and Appendix D of the DEIS/EIR provides a more detailed description of key Project features (Corps and CNRA 2011). Key Project components include: pond berms, pump stations, inflow and outflow structures, water control structures, a sedimentation basin, in-pond habitat features, and an irrigation drain interception ditch. These Project features are shown on Figure 2-8 of the DEIS/EIR and described in Appendix D of the DEIS/EIR. Berms will be constructed to create the necessary pond size, shape, and bottom configuration to provide a range of in-pond aquatic habitats. Interior berms will form smaller impoundments within the ponds and exterior berms will be constructed to separate the ponds from the Sea. Exterior berms will be aligned to not interrupt the flowpath of occasional stormflows from the watersheds that drain into the Sea.

Near the west end of Bowles Road, water from adjacent irrigation drains is currently pumped into an interception ditch, which provides connectivity for pupfish. At completion of construction and pond filling, the agricultural drain water will be pumped directly into the SCH ponds, which will provide extensive pupfish habitat.

2.3 Operations and Maintenance

The new ponds are expected to go through physical and chemical changes following wetting of the playa sediment and initial filling, as described in the MAMP (CNRA 2015). Filling the ponds for the first time after construction is expected to take about three months using the designed pumps, and less time if portable pumps are also used. Both ponds will be filled and maintained at 30 ppt salinity prior to acceptance of construction by the contractor. Initially, the ponds will be allowed to “season” for a period of several weeks to months while undergoing various stages of chemical and biological succession. Water chemistry will likely fluctuate as compounds leach from the newly wetted soils and microbial communities are initiated. Once phytoplankton, zooplankton and other invertebrates are established and salinity is within target range, then tilapia (forage species for piscivorous birds) will be introduced to the ponds.

The ponds will be operated to allow for different combinations of storage, salinity, and residence times (operations scenarios) to investigate how these factors could be adjusted to provide the best conditions for fish and birds. Water will be diverted from the New River and the Sea and blended to achieve the optimal salinity within the ponds. Water from the Sea will be pumped to the ponds via a 24-inch pipe. Water will enter the New River pump station via a 4 feet by2 feet box culvert, where it will then be pumped via a 28-inch pipe to the sedimentation basins and to the ponds via habitat channels. Water from the ponds will return to the Sea via a weir outflow structure.

The SCH Project will be operated during the initial 10-year proof-of-concept period under scenarios designed to test which salinity regime results in the best combination or balance of invertebrate and fish productivity, bird use, seasonal fish survival, and selenium ecorisk minimization (Corps and Resources Agency 2011, 2013). The target salinity range will be 20-40 ppt, occasionally ranging from 0 ppt to 60 ppt. Scenario 1, the first operational scenario to be implemented, will test the lower (20 ppt in West Pond) and upper (40 ppt in East Pond) extremes of the operational range, to increase the likelihood of measuring the effect of salinity on Project performance. Each pond will be operated at the same replacement rate with a different salinity.

Scenario 1 will maintain salinity constant through the year. This scenario will be run for at least two years.

Other operations scenarios could be tested during this 10-year implementation period, depending on management goals and feedback from monitoring. Scenarios 2 and 3 would increase inflow 10 percent or 20 percent, respectively, over the replacement rate. This would produce outflow from the pond and thus reduce the residence time, at the cost of additional pumping. With more information from monitoring, operation scenarios can be adapted to improve Project performance.

Ongoing maintenance will include periodic dredging of the sedimentation basins; maintenance of interior and exterior berms, protective riprap, and pumps; and vegetation, erosion, and vector control. Material excavated from the sedimentation basin will be used to construct habitat features or add to the berms. The pumped diversion from the New River will be maintained to keep the diversion facilities free of sediment. The river bed elevation will also be monitored to detect any downcutting that may occur as the Sea's water level drops. The Sea pumping facilities will be maintained to reduce fouling caused by the hypersaline water flowing through the pumps. Draining the ponds will not be a routine maintenance activity, but may be required if a berm failed or under another type of emergency situation.

Maintenance will typically involve construction equipment such as an excavator at the New River and suction dredging equipment at the pump stations. The sedimentation basins will be constructed and operated in a manner that is unsuitable for desert pupfish. Additionally, desert pupfish do not occur in the New River where the pump intake is proposed.

2.4 Long Term Monitoring and Maintenance

2.4.1 Overview

The SCH Project includes a MAMP to obtain and refine information to inform future restoration efforts and define optimal parameters to support a sustainable, productive aquatic community (CNRA 2015). The main parameters subject to change include salinity, residence time, and depth. The parameters will be controlled by changing the amount and salinity of water delivered to the constructed ponds, the outflow to the Sea, and the total storage in the ponds. The MAMP will help guide post-implementation actions and assess Project performance during the 10-year proof-of-concept period.

The MAMP outlines a monitoring framework to evaluate the effectiveness of the SCH Project and guide adaptive management. The MAMP describes Project objectives, defines expected or desired outcomes, and describes monitoring activities to track progress toward objectives and compliance with regulatory permits during the initial implementation phase. The MAMP also outlines the assessment and decision-making process that will guide adaptive management, including potential management responses that may be triggered by monitoring.

Indicators of SCH Project performance include measures of habitat (e.g., area, depth, physical structure, aquatic plant species/cover, and water quality), target species (e.g., richness, diversity, abundance, habitat use), trophic function (e.g., composition and density of forage species), and stressors (e.g., water quality and selenium concentrations). Key monitoring elements include the following:

- Physical Habitat - flow rate, depth, wetted area, islands, snags, submerged vegetation, and other habitat elements;
- Water Quality - salinity, temperature, pH, dissolved oxygen, nutrients;
- Aquatic Biota - algae, plankton, invertebrates, fish community (species, distribution, abundance);
- Birds - species, abundance and distribution, use of habitat features, breeding and nesting, sick or dead birds; and
- Contaminants - selenium concentrations in water, sediment, bird eggs, and other biota (invertebrates, fish).

For detailed information, see the SCH MAMP.

CHAPTER 3
Desert Pupfish Status and Ecology

3.1 Status and Critical Habitat

The desert pupfish, listed as endangered in 1986, is a small fish, less than 3 inches long, belonging to the Cyprinodontidae family of fishes (Moyle 2002). This species is native to southeastern California, southern Arizona, northern Baja California, and Sonora, Mexico. Naturally occurring populations of desert pupfish are now restricted in the United States to California in the Salton Sea Basin: the Salton Sea, three tributary streams, shoreline pools, and irrigation drains to the Sea (Lau and Boehm 1991, Keeney 2016). This species is found in Mexico at scattered localities along the Colorado River Delta and in the Laguna Salada basin (Hendrickson and Varela-Romero 1989).

Critical habitat was designated in 1986 along portions of the San Felipe Creek, and two of its tributaries, Carrizo Wash and Fish Creek Wash, in Imperial County, California (USFWS 1986). The areas designated as critical habitat include approximately 11 miles of a channel along San Felipe Creek and all of its tributaries and a riparian buffer zone of 100 feet on both sides of the stream channel. A total of approximately 770 acres of critical habitat were designated. The desert pupfish reproduces successfully in San Felipe Creek. These stretches provide adequate food and cover. They are at least partially isolated from predatory and exotic fishes. Recent observations by the California Department of Fish and Wildlife (CDFW) indicate that mosquitofish are the most abundant non-native fish. Tilapia were abundant in San Felipe Creek in 2013-2015 but much reduced in 2016, and sailfin mollies have not been seen since the 1990’s (Keeney 2016).

3.2 Population Status and Genetics

3.2.1 Populations

According to the USFWS 5-Year Review (USFWS 2010), there are collectively 11 extant populations of desert pupfish known in the wild in California (5 populations), Arizona (1), and Mexico (5) (Tier 1 populations in the Recovery Plan). Although many re-introductions have been attempted, approximately 16 transplanted populations of the desert pupfish exist in the wild at present, all in Arizona (Tier 2 populations in the Recovery Plan). There are 46 captive or refuge desert pupfish populations comprised of 27 in Arizona, 14 in California, and 4 in Sonora, Mexico (USFWS 2010, Keeney 2016). The range-wide status of desert pupfish is poor but stable. The fate of the species depends heavily upon future developments in water management of the Salton Sea and Cienega de Santa Clara in Mexico.

Five natural populations persist in California: San Felipe Creek, Salt Creek, Salton Sea, Salton Sea irrigation drains, and Hot Mineral Spa Wash (USFWS 2010). Historically, desert pupfish were abundant along the Sea's shoreline through the 1950s (Barlow 1961). During the 1960s, the numbers declined, and by 1978 they were noted as scarce and sporadic (Black 1980). Currently, desert pupfish numbers in the Sea are relatively low and patchily distributed in Salt Creek and San Felipe Creek, at the mouths of the New, Alamo and Whitewater rivers, within the mouths of irrigation drains that discharge directly to the Sea, and along the southern and northern shoreline of the Sea in shoreline pools (USFWS 2010; Keeney 2013 and 2016). The desert pupfish population in Salt Creek was stable to increasing, and had few non-native species (USFWS 2010; Keeney 2010). However, in the last several years lower Salt Creek has dried completely during summer and water quality has become more problematic (Keeney 2016). San Felipe Creek also has had a stable to increasing population, according to the 5-Year Status Review (USFWS 2010), but in recent years non-native fish have been found here (Keeney 2016).

There are 36 irrigation drains within the Project area that have been monitored by CDFW, USGS and IID over the last 25 years; these desert pupfish populations fluctuate seasonally, but have persisted. While populations in irrigation drains entering the Sea can be abundant (Keeney 2010 in USFWS 2010), fish populations there are still dominated by non-native fish (Martin and Saiki 2005). These drains allow connectivity to shoreline pools, at least seasonally, and may be necessary to prevent desert pupfish from becoming stranded within drain habitats that periodically dry out (Sutton 2002; Keeney 2013). Maintaining these populations and connections in the long-term has been determined to be necessary for the recovery of the species (USFWS 1993). Based on current understanding, this includes maintaining the drain populations and providing for desert pupfish movement between individual drains (USFWS 2010).

In addition to the naturally occurring populations, this species also occurs in several refuge locations (Tier 3 populations) within the Project area. The 14 refuge populations in California occur at Anza Borrego State Park (2), Oasis Springs Ecological Reserve (1), Dos Palmas Reserve (4), Living Desert Museum (4), University of California Riverside (1), Imperial Irrigation District grass carp facility (1), and Borrego Springs High School (1) (Keeney 2016). There was a desert pupfish refuge population at Salton Sea State Recreation Area, but desert pupfish no longer occur there, likely due to bird and raccoon predation as well as lack of soft substrate for fish to burrow into for escape (Keeney 2016). CDFW monitors and maintains these refuge populations.

3.2.2 Genetic Units

As discussed in the 5-Year Status Review (USFWS 2010), much of the research on desert pupfish since the 1993 recovery plan addresses genetics issues and the taxonomy of the *C. macularius* group. Since the isolated nature of desert pupfish populations reduces the flow of genes between sites, inbreeding and genetic drift can be reasonably expected to occur without intervention (Turner 1983, Echelle et al. 2007, Koike et al 2008, Loftis et al. 2009). Based on their work on the natural populations, Loftis (2007) and Echelle et al. (2007) recommended five management units for *C. m. macularius*: Laguna Salada, Cerro Prieto, Cienega de Santa Clara/El Doctor, San Felipe Creek, and the rest of the Salton Sea system (Echelle et al. 2007, Loftis et al. 2009).

Recent genetics studies indicate that the three naturally occurring populations in the Salton Sink: San Felipe Creek, Salt Creek, and Salton Sea (including irrigation drains)–should be treated as one genetic unit (Echelle 1999, Echelle et al. 2007).

3.3 Ecology and Habitat Requirements

This description of ecology and habitat requirements is based on descriptions and reviews in the desert pupfish recovery plan (USFWS 1983), critical habitat designation (USFWS 1986), 5-Year Status Review (USFWS 2010), and the Project's Biological Opinion (USFWS 2013).

The desert pupfish has a tolerance for high temperatures, high salinities, and low dissolved oxygen concentrations that exceed the levels known for other freshwater fishes (USFWS 1986, 1993). Carveth et al (2006) reported an upper lethal tolerance of 42.4 degrees Celsius (C), plus or minus 0.2 degrees C, when acclimated to a temperature of 30 degrees C. Schoenherr (1990) reported critical thermal maximum (CTMax) as high as 42.6 and 42.7 degrees C, and a critical thermal minimum (CTMin) of 4.4 degrees C. They have been reported to survive oxygen levels as low as 0.1 to 0.4 parts per million (Lowe et al., 1967), and salinities nearly twice that of seawater (Barlow 1958). They are also capable of surviving extreme fluctuations in temperature (Lowe and Heath 1969) and daily salinity changes of as much as 10 to 15 ppt (Kinne 1960). Desert pupfish populations also experience significant temporal fluctuations in distribution and abundance (Varela-Romero et al. 2002).

Although desert pupfish are extremely hardy in many respects, they prefer quiet water with aquatic vegetation (Schoenherr 1992), as long as it is not too dense (Keeney 2016). Habitats include clear, shallow waters with soft substrates associated with cienegas, springs, streams, margins of larger lakes and rivers, shoreline pools, and irrigation drains and ditches. Pupfish typically prefer shallow water, but can occur at greater depths. CDFW captured pupfish in gill nets set at 7-8 feet in the mid-2000s. In San Felipe Creek, pupfish often utilize pools having depths of 3 feet (Keeney 2016). Pupfish seem to prefer a little flow, compared to areas with dense emergent vegetation and/or stagnant areas which often are preferred by sailfin mollies (Keeney 2016). Examples of suitable habitat are shown in **Appendix A**.

Physical habitat characteristics that are generally favorable to desert pupfish (Black 1980, Moyle 1976, Soltz and Naiman 1978, Schoenherr 1992, Keeney 2013 and 2016) include:

- sand-silt substrate, or other soft or unconsolidated substrate such as pea-sized gravel, with some topographic variability
- abundance of filamentous algae and rooted aquatic plants such as widgeon grass (*Ruppia maritima*) and chara, or muskgrass (*Chara* spp.) to provide food, cover and structure for pupfish and their invertebrate prey
- relatively shallow water (1 foot or less in depth), with a range of depths from 0.5 inches gradually sloping to approximately 3 feet
- minimal surface water velocity (< 1 foot per second)
- water temperatures above freezing during the winter

- salinity less than 68 ppt
- channel or pond bottom not completely filled in with emergent vegetation

When desert pupfish were abundant in the shoreline pools of the Sea, Barlow (1958, cited in Sutton 1999) noted that pupfish of all ages moved in and out of shallow areas in shoreline pools during the day, apparently to avoid temperatures higher than 36 degrees C. They spent the nights in the shallowest, coolest areas of the pools. During the winter pupfish did not move into the shallows until mid-afternoon, where they remained into the night (Barlow 1958 cited in Sutton 1999).

Desert pupfish mature rapidly and may produce up to three generations per year. They become sexually mature at 0.6 inches (15 millimeter [mm]) total length (TL), but most do not breed until their second summer when they reach 3 inches (75 mm) TL (Moyle 2002). Spawning occurs throughout the spring and summer months, about late February through late September (Matsui 1981 cited in Lau and Boehm 1991). Spawning males typically defend a small spawning and feeding territory in shallow water. The eggs are usually laid and fertilized on a flocculent substrate and hatch within a few days. After a few hours, the young begin to feed on small plants and animals. Desert pupfish are omnivorous, feeding on small invertebrates and algae, as well as detritus, macrophytes and occasionally their own eggs. Individuals typically survive for about a year (USFWS 1986).

Desert pupfish are vulnerable to competition or predation and thus can be displaced by non-native fishes (Martin and Saiki 2009). Schoenherr (1992) reported behavioral interference by sailfin mollies and Zill's tilapia (*Tilapia zilli*) during mating and spawning. That said, desert pupfish were unusually dominant (greater than 93 percent of the catch) in the U.S. Bureau of Reclamation (Reclamation) ponds, where mosquitofish, sailfin mollies and Mozambique and Zill's tilapia were also present in low numbers (Saiki et al. 2011). U.S. Geological Survey (USGS) sampled the Reclamation ponds with minnow traps in water averaging 7-18 inches deep, with the deepest area 47 inches (Saiki et al. 2011). Pupfish were the dominant species collected from the Reclamation ponds, in contrast to the irrigation ditches where non-native species were most dominant (Saiki et al. 2008).

3.4 Occurrence and Status at Project Area

Surveys for the desert pupfish were not conducted as part of the planning for the SCH Project, but past occurrences of the species are documented in the California Natural Diversity Database (Corps and CNRA 2011). Based on ongoing monitoring by CDFW, desert pupfish are presumed extant in the project area (USFWS 2013). Additionally, because desert pupfish move between the irrigation drains and the Sea (Sutton 2002), desert pupfish could be present anywhere within the edge of the Sea containing suitable habitat and within drains that are tributary to the Sea.

Habitat quality in some irrigation drains has been degraded due to overgrown emergent vegetation that chokes out open habitat and impedes pupfish movement. While IID is responsible for maintaining the drains by clearing sediment and vegetation, it appears that most of these drains have not been maintained regularly, especially in the lower portions nearest to the Sea

(Keeney 2016). There is little, if any, connectivity to the Sea, and what connectivity remains typically occurs during the spring. Many drains have so much dense vegetation in the lower sections that pupfish are no longer able to swim above or below this barrier. Some once-abundant populations in drains and shoreline pools on the south Sea shoreline and northeast of the Project site (Vail 6 Drain, mainly the lower portion which is the shoreline pool located at Lack and Lindsey Roads; and the shoreline pool known as McKendry Pond) have disappeared in recent years due to excessive vegetation and lack of water (Keeney 2016).

Currently, one irrigation drain labeled Salton Sea Sump (SS-8) on the IID drain map book, discharges into the interception ditch at the Phase 1 Project area, east of the New River delta at the west end of Bowles Road. CDFW conducted surveys in 2013 and 2015, but no pupfish were captured or seen (Keeney 2016). On October 11, 2016, a CDFW survey found both the SS-8 drain and the playa where the Phase 1 SCH ponds will be constructed were dry and did not provide suitable habitat for desert pupfish. However, a return survey on November 6 found both water and fish in the SS-8 drain, including desert pupfish, mosquitofish, sailfin mollies and tilapia. Habitat quality was low, given the intermittent flows and lack of established food resources. CDFW staff surmise that a nearby private reserve is the source of some of this drain water in the SS-8 drain.

CDFW staff also surveyed the New River on October 11, 2016. Trapping was conducted (6 minnow traps for 2 hours) in microhabitat that was the most suitable available but still of poor quality for pupfish. Only one western mosquitofish was collected, although others were observed in the river (Keeney 2016). Desert pupfish are not known to occur nor are they expected to occur in the New River because of the high sediment loads, excessive velocities, and presence of predators (Crayon 2013).

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CHAPTER 4
Inoculation (CM-2)

4.1 Overview

The Inoculation Plan includes the following elements:

1. Criteria to evaluate whether ponds will support desert pupfish;
2. Identification of possible source population(s) and quantity of fish to be collected from each source population;
3. Capture and transport methods; and Annual reporting requirements.

The following elements, which are relevant to the Inoculation Plan, are described in the Desert Pupfish Adaptive Management and Monitoring Plan:

1. Desert pupfish population assessment to evaluate population trends in ponds over time; and
2. Contingency plan should the ponds not support viable populations of desert pupfish.

4.2 SCH Pond Criteria

The first step is to evaluate whether conditions in the SCH pond are suitable for desert pupfish. Factors include water quality, food resources, habitat features, and aquatic community. Habitat conditions in the SCH ponds will be monitored following protocols detailed in the SCH MAMP (*Section 5 Monitoring Elements*) (CNRA 2015). This includes water quality, hydrology, aquatic vegetation and structure, and aquatic invertebrates. Habitat attributes for desert pupfish are listed above (*Section 3.3 Ecology and Habitat Requirements*).

Sites for (re)introducing desert pupfish in the target SCH pond(s) will be identified prior to initiation of capture, transport and release, based on availability of suitable habitat and access. Areas that were previously occupied by pupfish will be considered first.

4.3 Source Populations

4.3.1 Population genetics

The Desert Pupfish Recovery Plan (USFWS 1993) provides guidance for re-establishing populations of desert pupfish. A three-tiered hierarchical approach to re-establishment was developed to maintain the integrity of discrete, naturally-occurring stocks, as well as providing exchange of genetic material within re-established populations, and minimizing probability of catastrophic population loss. The extant wild populations of desert pupfish (Tier 1 populations) are the most valuable remaining reservoir of original genetic material. A total of five natural populations (Tier 1) persist in California: San Felipe Creek, Salt Creek, shoreline pools at Salton Sea, Salton Sea irrigation drains, and a wash at Hot Mineral Spa (USFWS 2010).

From these, a second tier of populations can be established in the wild in the best available natural habitats, and among which individuals can be exchanged to maintain genetic variability. Populations designated Tier 2 are replicates of remaining, naturally occurring stocks, composed of re-established populations in the most natural (i.e., historic condition) identifiable habitats within probable historic range. A second suite of re-established populations (Tier 3) will be in the most-natural habitats available after fulfillment of Tier 2 requirements, which may be human-modified to imitate historic conditions and function to optimize balance for genetic diversity and management opportunities (USFWS 1993, 2010).

A Tier 2 population will be considered successfully established if it has survived for 10 years and has required only minor management to persist. Minor management may include minor vegetation removal, fencing, drawing off excess water for wildlife and livestock, population monitoring, management for other native species, and/or pupfish transfers for genetic maintenance. Tier 3 populations may experience major management activities such as new or modified water supply, dredging, major vegetation removal, habitat (re)construction, exotic fish introduction or control, restocking pupfish or supplemental stockings for reasons other than maintaining genetic exchange.

Genetic exchange is to be accommodated between Tier 2 populations derived from a single natural (Tier 1) source, from Tier 2 source populations to their Tier 3 derivatives (but not the reverse), and between Tier 3 populations derived from a single Tier 2 source (but not between Tier 3 populations from different sources).

As noted in the 5-Year Status Review (USFWS 2010), recent genetics studies indicate that the naturally occurring populations (Tier 1) in the Salton sink should be treated as one genetic unit (Echelle 1999, Echelle et al. 2007). As such, all populations in the Salton sink can be considered potential source populations for the SCH Ponds, and use of pupfish from multiple sources in the Salton Sink is encouraged to maintain genetic diversity (Keeney 2016).

4.3.2 Selection Criteria

Selection of source populations will be determined by SCH managers, including CDFW fisheries biologists. CDFW conducts status monitoring of desert pupfish in drains and other locations around the Sea, surveying approximately 20-30 localities annually. Criteria include but are not limited to:

- Large, stable populations (relative abundance and population size based on trapping catch per unit effort [CPUE]) in stable habitats that can sustain collection of pupfish.
- Populations occupying ephemeral habitats with intermittent flows. Rescuing pupfish from drains subject to drying would benefit the species since that population would otherwise be lost.

- Access to property
- Proximity to SCH ponds, to minimize stress in transport

Given recent status and trends from CDFW surveys, the most promising source populations for stocking include:

- San Felipe Creek
- Lower Salt Creek - the lower reach that regularly goes dry
- Hot Mineral Spa – east side, south of Salt Creek and below Bombay Beach
- Varner Harbor – northeast Salton Sea
- Irrigation drains as determined by CDFW based on recent surveys

4.3.3 Number of Fish

The number of fish to be stocked should be based on the numbers known or suspected to actually contribute genes to annual recruitment (the effective population size, N_e), rather than total number of individuals in a population (N) (Minckley 1995). For example, the effective population size of long-lived taxon such as razorback sucker may be a small fraction of the total population, thereby requiring many more individuals (hundreds) for stocking. Far fewer fish are required for short-lived, rapid breeding taxa like mosquitofish (Minkley 1995). Pupfishes are generally small-bodied fish with high reproductive potential that can spawn and propagate with little or no human intervention (Koike et al. 2008).

As reviewed by Koike and others (2008), desert pupfish have extended spawning seasons and can breed at sizes as small as about 0.6 inches (15 mm) standard length (SL) and only two months post-hatching (Kinne 1962, Cox 1966, Constantz 1981). Such attributes allow quick rebound from founder events and other population bottlenecks, and promote large populations in small refuges (Koike et al, 2008). However, the effective population size is undoubtedly lower than the total population. Breeding males are territorial and male reproductive success is highly variable. Pupfish do have alternative male mating strategies, such as sneakers and satellites (Leiser and Itzkowitz 2002), that would moderate the effect of territoriality, but this effect may be especially pronounced in small refuges where a population of several hundred might include only a handful of territorial males (Koike et al., 2008).

For the SCH Project, at least 250 individuals (USFWS 2013) will be used per stocking event per pond. Collections should be supervised by a trained CDFW or USFWS biologist. To minimize the risk of low genetic diversity in the SCH pond population due to low diversity in the source population (Koike et al. 2008), pupfish should be collected from at least two sources. Stocking the new SCH ponds should occur gradually, with a couple stocking events over a period of a few weeks to a few months.

To minimize impacts on the source population, only localities with abundant pupfish populations will be selected. A suggested criterion is no more than 10 percent of adults and/or 10 percent of juveniles should be removed from a locality, but other criteria may be used as determined by trained CDFW or USFWS biologist based on recent survey results. Collections should focus on fish between 0.6-2.2 inches (15-55 mm) TL. CDFW recommends returning to the source population dominant males larger than 2.2 inches(>55 mm TL, blue coloration), gravid females, and fish less than 0.6 inches (15 mm) TL.

However, under the following circumstances, all of the pupfish that can be captured can and should be removed from that specific locality and transferred to the SCH ponds:

- The habitat is in immediate danger of becoming unsuitable due to desiccation, excessive salinity, or other extreme environmental condition,
- CDFW and USFWS determine that a locality has no chance of long-term viability (for example, the Salton Sea when salinity approaches 68-70 ppt).

4.4 Capture and Transport

4.4.1 Timing

In general, inoculations will occur in summer or early fall. Pupfish population numbers are highest during summer, after early spawning has occurred in spring (Keeney 2016). This will maximize the opportunity of collecting sufficient fish for stocking, while minimize the relative impact on source populations due to removal of individuals. Also, this can provide opportunities to rescue pupfish from any locality that is at risk from drying out during summer or fall. Capture and transport will be conducted with care taken to minimize handling and stress as well as exposure to heat, low dissolved oxygen, and crowding.

4.4.2 Capture

Capture will follow CDFW protocols for desert pupfish sampling. The preferred capture method is baited minnow traps, specifically Gee’s minnow trap (9 inch by 17.5 inch, with 1.25 inch-square mesh and double funnel-mouth entrances) of galvanized steel wire. The traps are baited with perforated plastic bags, each filled with 1 ounce of canned, fish-flavored, cat food. The traps are typically deployed in water at least 9 inches deep, sufficient to cover the entire trap.

Traps are set only during the day since pupfish are most active during the day. The preferred duration of trap sets is 1-2 hours, although traps can be checked and emptied, then reset for another 1-2 hours. Overnight sets may result in pupfish mortality due to changes in water quality and sometimes water quantity, presence of too many fishes in traps, vandalism, theft of traps, storm events, and other possible causes. Spacing can be variable due to difference in habitats surveyed, but in the drains, traps should generally be spaced 25-50 feet.

Dip netting can also be used, especially in isolated small areas. Seining is less effective, due to underwater snags and difficulties landing a seine, as well as pupfish escape behavior of diving into soft sediment under the seine’s lead line (Keeney 2016). Seining is also considered more stressful to juveniles than other capture methods (Keeney 2016).

4.4.3 Handling and Data Collection

Fish handling will be kept to the minimum necessary to remove fish from the trap or net. Fish capture and removal operations will be planned and conducted to minimize the amount and duration of handling. Biologists will maintain captured fish in water to the maximum extent possible during trapping/netting, handling, and transfer for release. Individuals handling fish will ensure that their hands are free of harmful products, including but not limited to sunscreen, lotion, and insect repellent.

Captured fish will be placed in lidded coolers (preferred), buckets or holding tanks that are fitted with aerators. Tanks/buckets should be placed in the shade if possible to prevent increases in water temperature. Desert pupfish will be counted and lengths estimated. If conditions are stressful (i.e., very high temperatures), biologists may discontinue length estimates, especially for the more vulnerable juvenile pupfish.

The directing biologist will document and maintain accurate records of the operations onto data sheets (**Appendix B**). Conditions to be recorded include: specific locations of capture and release, date and time trap was set and pulled, water depth, water temperature, salinity, species and number of fish captured, and observations of substrate type and vegetation. If directed by USFWS or CDFW, species' identification, numbers, and lengths (of the first 30 individuals) for fish species of interest will also be recorded during holding and prior to relocation and release.

Biologists will ensure that water quality conditions are adequate in the buckets, coolers, or holding tanks used to hold and transfer captured fish. Biologists will use aerators to provide well-oxygenated water. Consider using containers that are light-colored, lidded, and fitted with a portable aerator; small coolers meeting this description are preferred over buckets. Containers should be well-rinsed and free of any chemicals or detergent residues. The directing biologist will ensure that conditions in the holding containers are monitored frequently and operations adjusted appropriately to minimize fish stress.

4.4.4 Transport and Release

Holding and transport time will be minimized and conditions will be continuously monitored to minimize stress to captured fish. Diseased fish will not be selected for inoculation (note that visibly diseased fish are not commonly observed, Keeney 2016).

Once the fish arrive at the receiving SCH pond, water quality conditions in the transport container and pond will be measured. If water quality conditions are substantially different (e.g., water temperature differ by more than 3 degrees Celsius, salinity differs by more than 3 ppt), then water from the release site will be gradually mixed into the transport container to acclimatize the fish prior to release. Note that pupfish can tolerate certain water quality extremes well, such as dissolved oxygen above 1.0 milligram per liter (mg/l) and abrupt changes in salinity and temperature (Keeney 2016).

Fish should be introduced into the receiving water by submerging the transport vessel lip and then gently pouring fish into the pond. Any fish that may have died or been injured during transport should be removed, retained and logged for permit purposes. At each inoculation site, it is recommended that fish be released in multiple locations if feasible to distribute them throughout the SCH pond habitat.

4.5 Population Assessment

The desert pupfish population will be monitored and assessed in the course of regular quarterly fish monitoring, as described in the SCH MAMP (*Section 5.5 Fish*) and further detailed in the DPAMPP (a MAMP appendix).

4.6 Performance Criteria and Take

Mortality incidents associated with inoculation activities shall not exceed 5 percent of the individuals collected. If more than 5 percent of the individuals collected are killed, authorized take would be exceeded, and any further inoculation activities would cease, pending notification of the USFWS and approval for subsequent inoculation events.

CHAPTER 5
Reporting and Adaptive Management

5.1 Reporting

A written report will be prepared for each inoculation event and provided to the SCH Project sponsors (CDFW, DWR, Corps) and regulatory agency (USFWS) within 30 days of completion. The summary report will include at a minimum:

- Status and trends of fish and desert pupfish in SCH ponds
- Conditions in SCH ponds – physical conditions, aquatic vegetation, trends in water quality, comparison between ponds
- Rationale and decision criteria for initiating inoculation and selecting release sites in ponds
- Source population – location, status of population, metapopulation genetics, collection site(s) location, habitat conditions, and rationale for selection
- Location of activities
- Methods and equipment
- Conditions during capture, transport, and release (physical habitat, water quality, etc.)
- Desert pupfish captured (numbers, size/age class), transported and released into SCH pond(s)
- Information on fish condition, including mortalities and disposition of specimens

This information will be incorporated as part of the SCH Project's overall Monitoring and Adaptive Management Program. As described in the MAMP, an annual progress report will summarize all monitoring activities and data collected for that calendar year, update prior reports in a cumulative fashion, and include raw data as well as data analysis and comparison with compliance and performance criteria, as applicable. The annual report will describe pond operations scenario, GIS maps of sampling locations, data for each monitoring element (operations, physical, water quality, and biological), habitat conditions and environmental data during monitoring, and any recommendations for improvement of the protocol.

Data, analyses, and publications developed from this Inoculation Plan will be organized, stored, and made publicly accessible through a commonly distributed data management system. Common protocols will be developed and applied when possible. All geospatial data will include full metadata and will be compliant with the Federal Geographic Data Committee (FGDC) standards. CDFW will establish and maintain the data management system. The data collected as part of all components of the broader Sea restoration program will be archived and made accessible in CDFW's Biogeographic Information and Observation System (BIOS) map viewer and all documentation including metadata would be accessible to the public via metadata clearinghouses and CDFW's document library.

CDFW will archive the monitoring reports. Synthesis reports will be prepared at the end of Year 5, and the end of the 10-year proof-of-concept period with final recommendations for long-term SCH management. Data management and quality control will follow guidance in the MAMP and further directions from CDFW as developed.

5.2 Adaptive Management

Procedures for desert pupfish monitoring, capture and relocation will be reviewed following each inoculation event. Adjustments to the Inoculation Plan may be recommended due to changing site conditions, additional phases of Project implementation, status of source population(s), improved gear or methods, or other new information. Minor adjustments are expected to occur over the monitoring period to maintain completeness and feasibility of the monitoring program.

The scientists and managers responsible for the Project will regularly synthesize and analyze the monitoring data and evaluate the status and trends in target resources, and the effectiveness of Project activities. An overall review will be conducted annually to evaluate Project performance. A decision-making framework is established in the MAMP to provide recommendations to SCH managers for maintaining or adjusting operations (CNRA 2015).

All monitoring, evaluation and management elements are integrated into the MAMP. Further details related specifically to desert pupfish management, including population assessment, and triggers to implement the inoculation plan, are described in the DPAMMP, which will be an appendix of the MAMP.

CHAPTER 6
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Appendix A

Suitable Habitat for Desert Pupfish

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These photographs by Sharon Keeney CDFW show examples of excellent habitat conditions for desert pupfish.

Figure A-1	San Felipe Creek, Imperial County
Figures A-2	Dos Palmas Preserve (S-1 Pond), Riverside County
Figures A-3 to A-4	Salt Creek (Upper and Lower), Riverside County
Figures A-5 to A-9	Irrigation drain channel habitat, as illustrated at Avenue 76 Drain at the northern end of the Salton Sea, Riverside County.
Figures A-10 to A-12	Microhabitat features (soft substrate, pondweed) and fishes, including desert pupfish and non-native fish (redbelly tilapia, sailfin molly), in irrigation drain habitat, Avenue 76 Drain, Riverside County.
Figures A-13 to A-14	Desert pupfish and other fishes (mosquitofish, redbelly tilapia) in irrigation drain habitat, Avenue 76 Drain, Riverside County.
Figure A-15	Spawning desert pupfish and male in breeding coloration, at Dos Palmas Preserve, Riverside County.

Figure A-1 – San Felipe Creek, Imperial County, toward south west end of the Salton Sea. This is one of three localities where desert pupfish naturally occur. Photo: Sharon Keeney, CDFW.

Figure A-2 – Dos Palmas Preserve, Riverside County, toward north east side of Salton Sea. The S-1 Pond shown here has excellent pupfish habitat. Photo: Sharon Keeney, CDFW.

Figure A-3 – Upper Salt Creek, Riverside County, one of three creeks where desert pupfish naturally occur. Photo: Sharon Keeney, CDFW.

Figure A-4 – Lower Salt Creek, Riverside County, one of three creeks where desert pupfish naturally occur. Photo: Sharon Keeney, CDFW.

Figure A-5 – Irrigation drain (Avenue 76), Riverside County, at the northern end of the Salton Sea. The drain has several outlet channels that provide diverse microhabitats. Note the slow-moving and clear water (not stagnant), sand-silt substrate, abundant (but not too dense) vegetation, including widgeon grass interspersed with open areas. A 2015 survey documented thousands of desert pupfish. Photo: Sharon Keeney, CDFW.

Figure A-6 – Irrigation drain (Avenue 76), Riverside County, with habitat for desert pupfish. Photo: Sharon Keeney, CDFW.

Figure A-7 – Irrigation drain (Avenue 76), Riverside County, with habitat for desert pupfish. Note floating and submerged vegetation, mixed with open areas, and clear water. Photo: Sharon Keeney, CDFW.

Figure A-8 – Irrigation drain (Avenue 76), Riverside County, with habitat for desert pupfish. Note floating and submerged vegetation, mixed with open areas, and clear water. Photo: Sharon Keeney, CDFW.

Figure A-9 – Irrigation drain (Avenue 76), Riverside County, with habitat for desert pupfish. Note aquatic vegetation, and clear slow-moving water. Photo: Sharon Keeney, CDFW.

Figure A-10 –Microhabitat features include soft substrate and submerged aquatic vegetation (wigeongrass) in irrigation drain (Avenue 76), Riverside County. Photo: Sharon Keeney, CDFW.

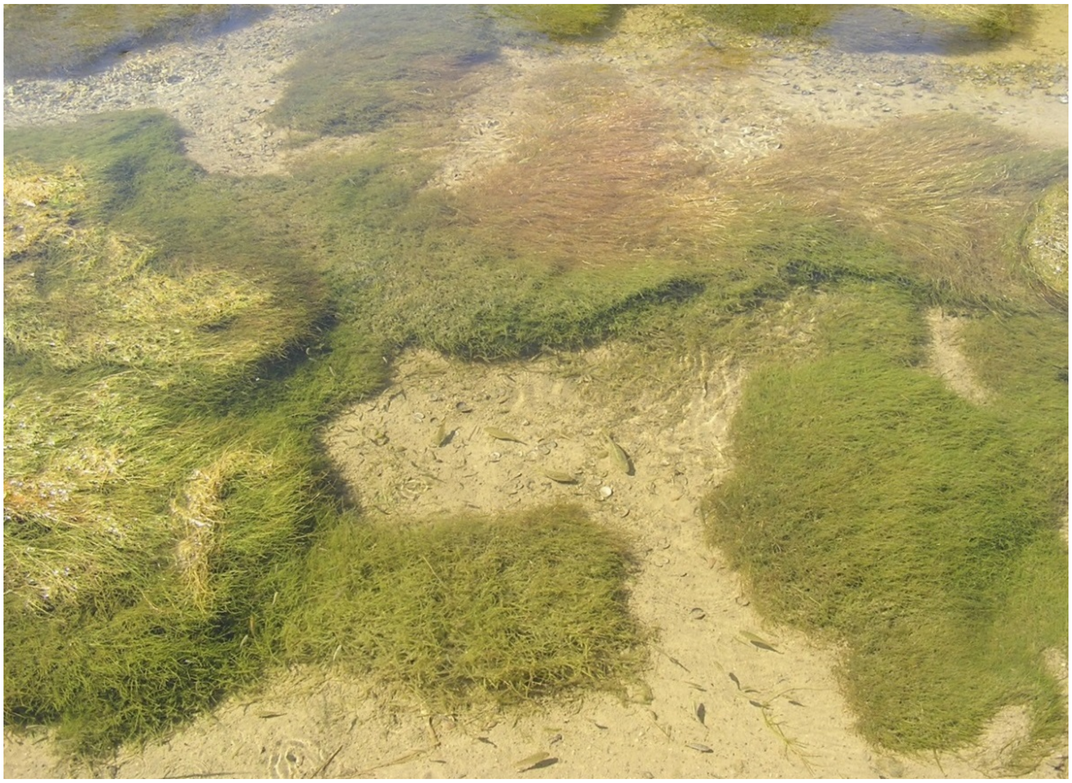

Figure A-11 – Desert pupfish and non-native fishes in drain habitat, with soft substrate and submerged aquatic vegetation (wigeongrass) in irrigation drain habitat, Riverside County. Photo: Sharon Keeney, CDFW.

Figure A-12 – Desert pupfish and non-native fishes (mostly porthole livebearer) in irrigation drain habitat, Riverside County. Photo: Sharon Keeney, CDFW.

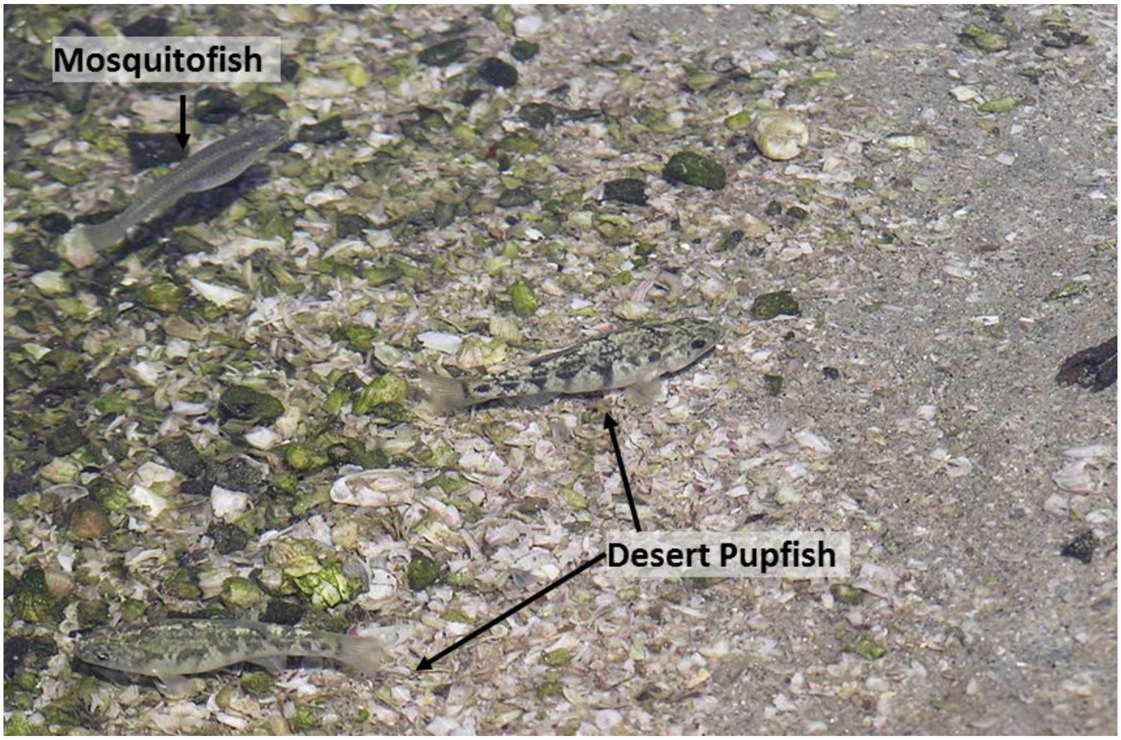

Figure A-13 – Desert pupfish and mosquitofish in an irrigation drain at the north Salton Sea (Arthur 0.5 Drain, Riverside County), showing preferred habitat (soft substrate with structure, and shallow, clear, slow-moving water). Photo: Sharon Keeney, CDFW.

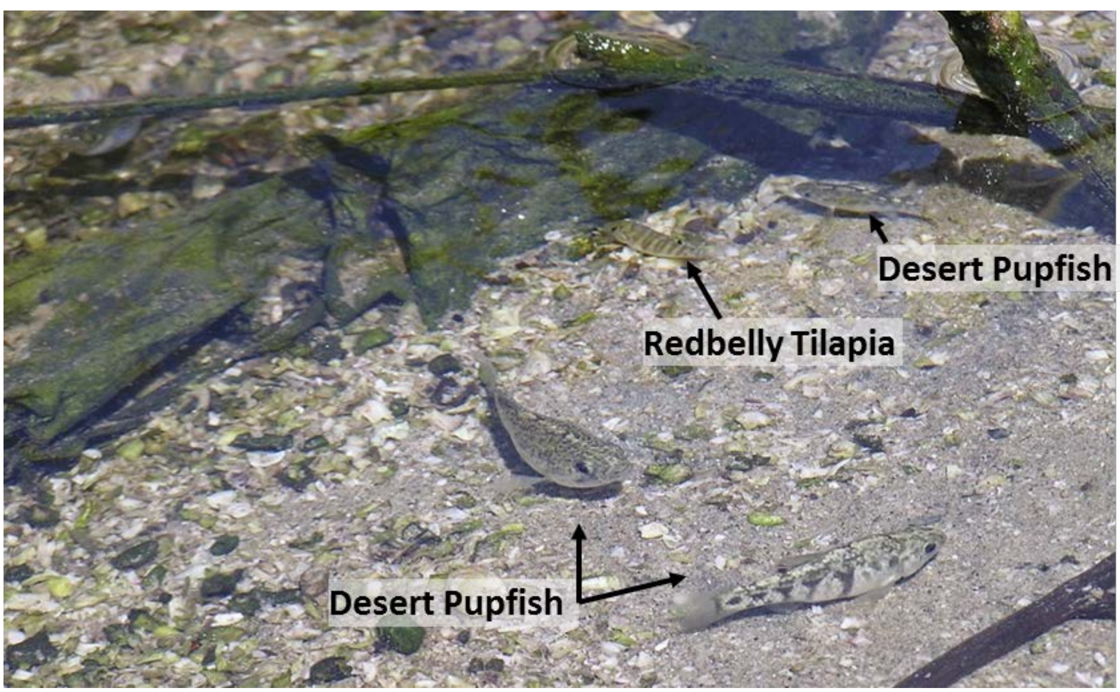

Figure A-14 – Desert pupfish and juvenile redbelly tilapia in an irrigation drain at the north Salton Sea (Arthur 0.5 Drain, Riverside County), showing soft substrate with structure and shallow, clear, slow-moving water. Photo: Sharon Keeney, CDFW.

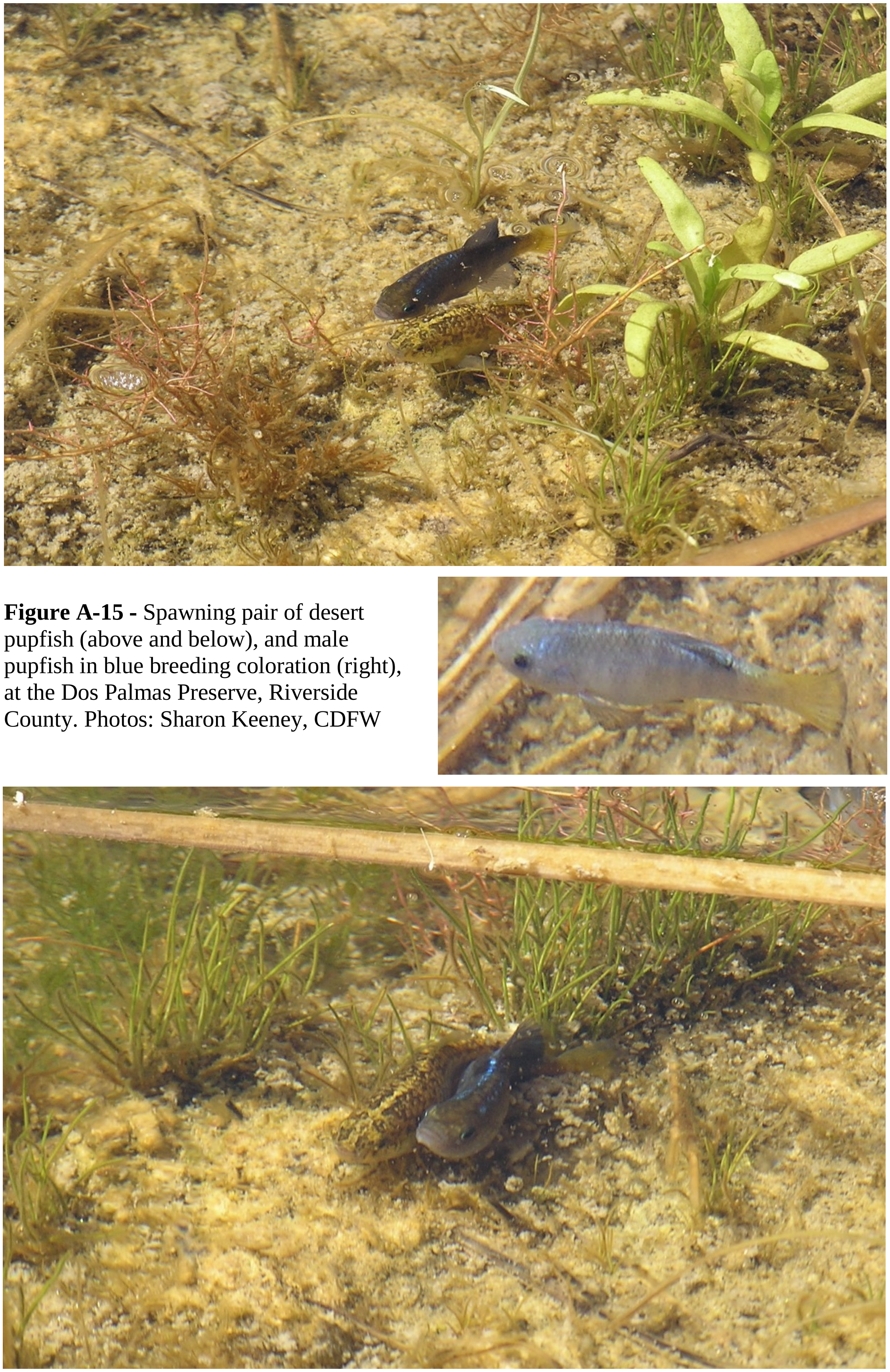

Figure A-15 - Spawning pair of desert pupfish (above and below), and male pupfish in blue breeding coloration (right), at the Dos Palmas Preserve, Riverside County. Photos: Sharon Keeney, CDFW

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Appendix B
Datasheet

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IID Desert Pupfish Trapping Form

Observer: **Date:**

Drain Name: ______________________________

Drain Information:

Length: __________ Width: __________ Depth: __________

Vegetation: ______________________________

Vegetation Density:: ______________________________

Bank Slope*: ______________________________

Salinity: ______________ Conductivity: ______________

Temperature: ______________ TDS: ______________

Turbidity: ______________ Dissolved Oxygen: ______________

pH: ______________ Connectivity: ☐ Yes ☐ No

Water Velocity*: ______________ Substrate: ______________

Traps:

Number of Traps: ______ Spaced: ______ feet apart.

Trap #	Start Time	End Time	Desert Pupfish	Mosquitofish	Sailfin Molly	Tilapia	Red Shiner	
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								

Others (snails, crayfish, etc.): ______________________________

NOTES:

Map of Area

Sketch area of drain trapping. (Note where traps are located by number)

Total Fish Species:

Species	**Total Number**	**Male/Female**	**Length (range)**
Desert Pupfish			
Mosquitofish			
Sailfin Molly			
Tilapia			
Red Shiner			

*Bank Slope: Gentle, Moderate or Steep

Water Velocity: Slow, Medium or High