Review and Recommendations of Methods for Monitoring Desert Pupfish (*Cyprinidon macularius*) Presence and Abundance

PREPARED FOR: Imperial Irrigation District

PREPARED BY: Timothy Hamaker/CH2M HILL
Jeff Tupen/CH2M HILL

DATE: November 29, 2006

The purpose of this Technical Memorandum (TM) is to summarize historic and current methods and results of surveys for desert pupfish (*Cyprinidon macularius*) in the vicinity of the Salton Sea. Gathered information is used to inform the development and recommendation of a survey protocol for this species within the Imperial Irrigation District (IID) Service Area. The protocol may be used in existing and future-modified drains, and at pupfish refugia, as anticipated by the USFWS (2002). Field data sheets are appended to this TM as Appendix A.

Introduction/Background

In 2002, IID prepared a draft Habitat Conservation Plan (HCP) for its Water Conservation and Transfer Project (WTP) (CH2M HILL 2002). The HCP includes five habitat-based and three species-specific Conservation Strategies to mitigate adverse environmental effects associated with development and implementation of the WTP. The Desert Pupfish Conservation Strategy (DPCS) is one of the three species-specific conservation strategies.

The overarching biological goal of the DPCS is to maintain viable populations of desert pupfish in the HCP area, which comprises approximately 500,000 acres of agriculture and the water supply and drainage facilities that support this agriculture. Desert pupfish are known or thought to occur in many of the drains that discharge directly to the Salton Sea. The HCP acknowledged that implementation of the WTP could adversely affect pupfish in a number of ways. A decrease in drain water contribution to the Salton Sea could result in a decrease in Sea level, thereby potentially changing or severing existing connectivity among the 29 IID-operated drains that are known to gravity flow to the Sea[1]. Among-drain connectivity is thought to promote and sustain genetic diversity among otherwise isolated groups of pupfish (CH2M HILL 2002, USFWS 2002).

Because the WTP may adversely affect desert pupfish found in drains, IID has committed to develop an approach[2] for determining pupfish presence in drains currently, and in the future[3],

[1] In 2005, the USGS began a 4-year investigation to identify and study selenium in IID-operated drains that gravity flow to the Sea.

[2] Pupfish-4 of the draft HCP and Pupfish Conservation Measure 3 of the In-Valley Biological Opinion (BO) prepared by the United States Fish and Wildlife Service (FWS) direct IID to develop and implement a desert pupfish survey protocol to determine pupfish presence in existing pupfish drains.

maintained by IID. IID has also committed to implement this approach in existing (baseline surveys) and future-modified drains for a period of five to seven consecutive years to determine pupfish presence, and patterns of pupfish drain use. Information collected through this survey effort will be used to inform and adapt management actions for desert pupfish in IID-operated drains.

While pupfish have been successfully collected within IID drains by various researchers, no formalized and/or consistent survey approach (protocol) exists. Similarly, no systematic survey effort to determine pupfish distribution and abundance in the HCP area has been conducted to date. This TM reviews desert pupfish survey work performed to date and suggests a protocol for determining pupfish presence, distribution, and abundance in IID-maintained drains. The protocol may be used to collect baseline information on pupfish presence and patterns of use in drains, and to determine the effectiveness of any adjustments in drain O&M techniques and/or habitat enhancement measures. The protocol should be suitable for use in similar, non-IID-maintained, systems.

Pupfish Listing Background

Observations of desert pupfish in the vicinity of the Salton Sea have occurred since this species was first reported in an Imperial County spring by Girard in 1859 (Black 1980). Following the flooding of the Salton Sink in 1904-1907, desert pupfish colonized what is now known as the Salton Sea (Sutton 1999). In the early 20th century, desert pupfish were reported as abundant at the Salton Sea by several authors (Coleman 1929; Cowles 1934; Barlow 1958, 1961; and Walker 1961, as cited by Black 1980). However, survey efforts by the California Department of Fish and Game (DFG) and others since 1964 indicated that desert pupfish populations had severely declined (Black 1980). The desert pupfish was subsequently listed as endangered by California and the federal government in 1980 and 1986, respectively. Prior to and since its listing, desert pupfish have been surveyed, collected, and monitored in the vicinity of the Salton Sea using a variety of survey techniques.

Desert Pupfish Life History and Habitat Requirements

Extensive reviews of the life history and habitat requirements of desert pupfish have been published over the last century. Miller (1943) reported pupfish ecological requirements and life history. Barlow (1958, 1961) described the social behavior of desert pupfish in both shoreline pools of the Salton Sea and in the laboratory. Black (1980) published the status of desert pupfish in California with a focus on populations and habitats. More recently, Schoenherr (1988) and Marsh and Sada (1993) reviewed extensively the life history of the species.

Pupfish (Cyprinodontidae) prefer shallow, relatively clear water. They are most commonly associated with rooted or floating vegetation and sand-silt substrates (Sutton, 1999) in drains with low flows (Black 1980). Lau and Boehm (1991) found pupfish to be widely distributed in those drains direct (i.e., gravity flow and not pumped) to the Salton Sea. Desert pupfish tend to move together in loose schools of similar age class and, during breeding season, sex. Desert

---

[3] "Future maintained" here refers to pupfish habitat to be constructed by IID in conformance with HCP Conservation Measure Pupfish-3 and BO Pupfish Conservation Measure 1.

pupfish of all ages tolerate extreme salinity, water temperature fluctuations, and low dissolved oxygen concentrations (Black 1980). The critical thermal maximum of 44° C for pupfish is the highest ever recorded for a fish species (Schoenherr 1992, as cited by USFWS 2002). Juvenile desert pupfish survived salinity as great as 98,100 mg/l, or approximately three times the salinity of ocean water (Barlow 1958, as cited by USFWS 2002). Martin and Saiki (2005) reported that desert pupfish abundance was generally highest when habitat conditions and water quality parameters were extreme. This was especially true for pH, salinity, and dissolved oxygen, which appeared to limit the occurrence of non-native fishes.

Desert pupfish are omnivorous, feeding on small invertebrates and detritus picked from the substrate (Sutton 1999), with foraging occurring during daylight hours (Moyle 2002). During warmer months of the year, pupfish move to areas of preferred water temperatures (< 36 °C) within shoreline pools of the Salton Sea and remain there in the shallowest and coolest areas during the evening hours (Barlow 1958, as cited by Sutton 1999). During winter, pupfish move to shallow areas of pools late in the day and remain there through the night (Barlow 1958, as cited by Sutton 1999). Some populations bury themselves in the loose debris on the bottom and become inactive in winter months (Cox 1966, as cited by Sutton 1999).

Desert pupfish can spawn within six weeks of hatching, though most spawn in their second year after reaching approximately 7.5 cm in length (Moyle 2002). Schoenherr (1988) reported that laboratory held pupfish bred from April through October when water temperature was variable, and year round when water temperature was constant. The male desert pupfish generally defends a territory of approximately 1-2 m^2, with territories as small as 45-60 cm^2 (Black 1980). Barlow (1961) described the spawning sequence: a female approaches a prospective male at his territory; the male sidles against the female and she extrudes a single egg; the male then fertilizes the egg and the pair separates. This behavior continues several times with eggs being haphazardly deposited within the male territory. The male defends the territory from other competing males (Black 1980), with no other parental care given thereafter.

Females will produce from 50 to 800 eggs per year. Egg incubation occurs over approximately 10 days at 20 °C (Black 1980). Newly hatched larvae are approximately 4-5 mm in length, and small invertebrates are fed upon within one day of hatching. Larvae double in length within 8 weeks and, depending on water temperature, reach a size of 15-28 mm in 24 weeks (Black 1980). As juvenile fish grow, they become omnivores feeding on a variety of food items, including insect larvae, detritus, algae, and mollusks (Schoenherr 1988). When invertebrates become scarce, algae and detritus are fed upon more frequently, with desert pupfish known to eat their own eggs and larvae (Cox 1972, as cited by Sutton 1999). Life span is variable, but three years is the maximum life expectancy for desert pupfish (Black 1980).

Known Distribution of Desert Pupfish at South Salton Sea Drains

Table 1 summarizes results for pupfish trapping efforts in drains of the south Salton Sea area from 1991 to 2006. Specific survey efforts contributing to the data shown in Table 1 are summarized in the following paragraphs. It should be emphasized that a lack of presence of pupfish at a surveyed drain, as reflected in Table 1, is not evidence of absence, as no surveys were specifically designed to determine absence. Similarly, no surveys summarized in Table 1 explicitly sought to quantitatively determine pupfish abundance and distribution in sampled drains. Further, some efforts (e.g., USGS current study) summarized in Table 1 did not intentionally survey for pupfish. In total, pupfish were collected in all but two[4] of the 36 drains summarized in Table 1. Pupfish populations are known to wink in and out both spatially and temporally (S. Keeney, pers. comm.), and information reported in Table 1 should be considered accordingly as presence/absence snapshots through time and space.

CDFG (1991). In April, May, and June 1991, the CDFG used baited wire minnow traps to survey 30 drains along the south Salton Sea (Lau and Boehm 1991). Traps baited with catfood in perforated plastic bags were set for 20 hours. Traps were set in clear, slow-moving water at depths of 2 to 24 inches. Drains and pools with dense vegetation or stagnant water were not sampled. Rather, traps were set preferentially in or near algal growth or other aquatic vegetation. The number of traps used in a drain varied with drain size and apparent habitat quality. Drains of questionable habitat quality were surveyed. 161 pupfish were captured in 13 of 25 surveyed drains[5]. Vail 5 produced the most pupfish (n = 44).

IID (1993)[6]. From 8 August to 21 September 1993, IID used baited wire minnow traps to survey 31 irrigation drains previously surveyed by the CDFG in 1991 (Remington & Hess 1993). Trapping methods followed that of Lau and Boehm (1991). An average of 6 to 7 traps was used in each drain below the lowest elevation check structure. In addition, two traps were set above the check structure to see if pupfish existed above this presumed pupfish barrier. Drains not producing pupfish after one trapping event were re-trapped. This effort trapped a total of 504 desert pupfish. In most cases, pupfish were not captured in drains with high numbers of tilapia or sailfin mollies. No pupfish were trapped above check structures. The greatest numbers of pupfish were trapped in Trifolium 12 drain (n = 261).

USBR (1999). The USBR surveyed for desert pupfish in five drains of the south Salton Sea from June to September 1999 (Sutton 2002). Baited minnow traps (6 mm mesh size, 38 mm opening) were set for 2-24 hours. Captured pupfish were marked for future identification. Drains were trapped 6 times at 2 week intervals. The purpose of this survey effort was to track pupfish movement within and among sampled drains. Overall, the study could not confirm the use of the Salton Sea as a migration pathway for among-drain movement. It did, however, document small movements of pupfish from drains to nearby shoreline pools. Most (n = 1,441) pupfish were captured in Trifolium 20A.

CDFG (1994, 1996, 1998, 2001, 2003-2006[7]). The CDFG has, through time, surveyed for desert pupfish in various habitat types around the Salton Sea, including both pupfish refugium ponds and drains (E. Konno pers. comm.). Among other survey results, Sutton (1999) published CDFG data for 1994, 1996, and 1998. Pupfish were found within 25 south Salton Sea drains during this 3 year period. Sutton (1999) did not report negative survey results for CDFG sampling in these 3 years. Baited (with catfood) minnow traps are set, with catch results then analyzed for CPUE.

[4] These are Vail 3 and Vail 3A.

[5] Five drains reportedly surveyed by CDFG are not reported in Table 1, as no efforts since that time have been directed at them. They are WP-10 SS-11, Lone Tree Wash, W. of Lone Tree, 3W of Lone Tree, Trifolium 21, and Trifolium 23N.

[6] These surveys were conducted in association with the CDFG (S. Keeney, pers. comm.).

[7] In 2005 and 2006 the CDFG sampled McKendry Pond, U drain, O drain (2006 only), Z Spill drain, Poe drain, Trifolium 14A drain, Trifolium 20A drain, and Lack and Lindsay Pond. Results are added to the USGS 2005 and 2006 effort shown in Table 1 (S. Keeney, pers. comm.).

CDFG pupfish survey data for 2001-2004 are unpublished and were provided to CH2M HILL by Eddy Konno, CDFG, Bermuda Dunes office. Much of the sampling effort in this four year period appears to have focused on the nearshore habitats of the Salton Sea, including marinas, shoreline pools, beaches, and drain mouths. Pupfish were found within 13 of 23 drains surveyed in this 4-year period. Baited minnow traps are set, with catch results then analyzed for CPUE.

USGS (2005, 2006). In August 2005, the USGS began a 4-year study to document selenium in 26 IID operated drains and three shoreline pools directly connected to the Salton Sea. As of the date of this TM, the USGS has completed 6 surveys: two in 2005 (August and October), and four in 2006 (January, April, July, and October). As part of this investigation, the USGS is using baited minnow traps (dimensions 25.4 cm X 25.4 cm X 43.2 cm, with 3.2 mm mesh) to capture mosquitofish and mollies for analysis of tissue selenium. Desert pupfish were captured and released incidental to this fish trapping effort in 16 of 28 sampled locations[8] (Trifolium Storm was not sampled). Ten traps are spaced about 5 m apart and set for approximately 1 hour at each drain/pool sampled. Catfood enclosed in perforated film canisters is used as bait. Between-year variability in pupfish presence at sampled drains is evident in the recent USGS results.

[8] Table 1 identifies 17 locations where pupfish were captured in 2005 and 2006. CDFG found pupfish in O drain in 2006 while the USGS did not (see footnote 7).

TABLE 1.

Summary of desert pupfish distribution in 36 drains at the southern Salton Sea, 1991-2006. Orange cells with a"0" indicate effort with no pupfish captured. Green cells with an "X" indicate effort with 1 or more pupfish captured. Blank cells indicate no capture effort.

	Survey Effort											
	1991 CDFG	1993 IID	1994 CDFG	1996 CDFG	1998 CDFG	1999 USBR	2001 CDFG	2002 CDFG	2003 CDFG	2004 CDFG	2005 USGS	2006 USGS
Drains												
Niland 1	0	X	X					X			X	0
Niland 2	X	0						0			0	0
Niland 3		X						0			0	0
Niland 4	X	0									0	0
O		0	X				0		0	X	X	X
P	0	0	X				0				0	0
Poe	X	X	X	X		X	X		X	X	X	X
Pumice[9]		X									0	X
Q	0	0	X				0				0	0
R	0	X	X	X			X				0	0
S		X	X		X		0	X			0	0
San Felipe Wash	X	X	X	X		X	0				X	0
T	0	0	X				0	0			0	X
Trifolium 1	X	0	X	X			0				0	0
Trifolium 12	0	X	X	X			X				0	0
Trifolium 13	0	X	X		X						X	X
Trifolium 14											0	X
Trifolium 14A		0	X		X		X		0	0	X	0
Trifolium 18	X	0	X								X	X
Trifolium 19	X	0	X	X			0				0	0
Trifolium 20		X	X		X	X	X				0	X
Trifolium 20A[10]				X		X	X		0	X	X	0
Trifolium 22	0	X	X				0				X	X
Trifolium 23	X	X	X	X		X	0				0	X
Trifolium Storm	X	X	X	X	X		X					
U	0	0					X	X	X	X	0	X
Vail 3		0										
Vail 3A		0										
Vail 4A	X	0										
Vail 5[11]	X	X	X						X	X	X	X
Vail 5A	X											
Vail 6[12]	X								X	X	X	X
Vail 7	0	X	X									
Vail Cutoff	0	X	X									
W		0	X		X		X	X			0	0
Z Spill	0	X	X					0	X	X	X	X

[9] USGS is sampling Pumice Pond at the mouth of Pumice Drain.

[10] Synonymous with "former Trifolium 20" drain of others.

[11] USGS is sampling McKendry Pond at the mouth of Vail 5 drain.

[12] USGS is sampling Lack & Lindsey Pond at the mouth of Vail 6 lateral.

Desert Pupfish Survey Methods

Many gear types have been used historically to capture desert pupfish. These include active methods such as seining (Barlow 1958, as cited by Sutton 1999; Schoenherr 1979; Verela-Romero et al. 2003) and throw traps (Winnemiller and Anderson 1997), and passive methods such as umbrella nets (Kordic-Brown 1977) and trapping (Black 1980). Currently, minnow traps, made of either plastic or wire mesh, have been used almost exclusively to collect desert pupfish from shoreline pools, marinas, creeks, laterals, and drains surrounding the Salton Sea (Lau and Boehm 1991; Remington and Hess 1993; Sutton 2002; and Martin and Saiki 2005).

In drain habitats within the Salton Sea Basin, methods other than trapping have produced poor results in capturing desert pupfish (C. Roberts pers. comm., S. Keeney pers. comm.). Some authors indicated that researchers sampling shallower shoreline pools within the Sea have been successful in monitoring pupfish using seines (Barlow 1958). However, the habitat conditions in pools, refuge ponds, agricultural drains, or other watershed features typically inhibit the successful use of seining techniques. Soft substrates, shallow water, heavy vegetation, and steep banks in drains create poor conditions for the effective use of seines.

Other netting methods (i.e. throw nets, umbrella nets) are generally impractical for monitoring pupfish in drains in the Salton Sea due to heterogeneous in-drain and near-drain habitats. For many of the same reasons (i.e., irregular substrates, and heavy vegetative cover) netting is impractical for collecting pupfish in drains. While electrofishing (EF) methods could be effective in environments where electrical conductivity was not excessive (B. Martin, pers. comm.), it is unlikely the USFWS would approve EF for routine monitoring of pupfish in drains of the Salton Sea Basin.

Trapping as Preferred Method

Trapping is the method of choice for collecting pupfish, though trapping results are widely variable, perhaps reflecting the schooling/patchy distribution of pupfish (R. Sutton, pers. comm.). For example, setting a series of traps within the same habitat unit/reach often results in many fish being captured in one trap and few to none in an adjacent trap. Steel minnow traps appear to capture pupfish more consistently than do plastic minnow traps (C. Roberts pers. comm.).

Some or abundant vegetative cover, either as emergent cattails or floating vegetative material, is usually associated with successful catches of pupfish. Shallower and slower moving habitat areas seem to correlate with more successful collections of pupfish. Others have indicated that pupfish can be found in varying depths of water (S. Keeney pers. comm., A Schoenherr pers. comm.).

Several researchers thought that the time of day was important to the success of collecting pupfish in traps, with low light conditions of dawn and dusk contributing to successful collection of pupfish in traps. One researcher preferred long trap set times (overnight), as trap set duration was positively correlated to success in determining presence (A. Schoenherr pers. comm.). However, standard USFWS Section 10(a)(1)(A) recovery permit Terms and Conditions now limit trap set times to 1-2 hours. A primary concern is anoxia events in pre-dawn hours or with overnight sets, so there is some flexibility in when the 1-2 hour trapping sessions can be conducted. Evening hours are an option (C. Roberts pers. comm.). Several sources indicated that pupfish abundance was inversely related to the abundance of competing and predatory fish species. When other fishes are absent or reduced in numbers, pupfish are frequently more abundant.

Proposed Monitoring Approach - General

Bryant (2000) proposed a closed-population removal method using baited minnow traps for surveying salmonids in southeast Alaska. He showed that trap-removal methods were as effective as traditional trap-mark-recapture methods in estimating fish abundance, with the former preferable to the latter due to, among other things, the ability to complete sampling in a single day effort, reducing stress to captured (and then marked and recaptured) fish, and fish only being captured once, which reduces avoidance responses in subsequent trapping events.

Removal methods rely on sequentially sampling (using three or four passes, typically) the target population and analyzing the rate of reduction/depletion in catch among passes. The calculated depletion rate estimates the proportion of the population that has been removed by sampling. Several well-known programs/methods exist to analyze closed-population removal trapping data (e.g., Zippin 1958, White et al. 1982, VanDeventer and Platts 1989, White and Burnham 1999).

Closed-population methods require that populations be isolated from other populations or immigration, and that individuals within a population have equal vulnerability to being caught. Using likelihood-based depletion calculations rather than regression calculations allows some flexibility in establishing the assumption of equal vulnerability (Hayes et al., in prep.).

The trap-removal method shows promise for its application to desert pupfish in irrigation drains within the IID Service Area. The narrow, linear morphology of drains and typical low-flow facilitates relatively easy isolation of drain segments using barrier nets. Pupfish are systematically trapped and removed (and held temporarily in suitable containers or a receiver within the reach, discussed later) from drain segments and using three to four trapping passes within each segment[13].

One of the assumptions of removal sampling is that fish have an equal probability of capture among successive passes. In practice, this is sometimes not a valid assumption, as fish may become increasingly aware of and avoid traps with each pass. Riley and Fausch (1992) reported that trap avoidance sampling bias could be calculated (and accounted for) by using 4 passes (as opposed to 3 passes) and the generalized removal method (calculation) of White et al. (1982). However, Bryant (2000) showed that the probability of capture of salmonids among passes did not appear to be affected substantially using relatively unobtrusive minnow traps (in contrast, for example, to electrofishing avoidance behavior among passes). White et al. (1982) recommended 3 or more passes for removal samplings for two primary reasons: 1) the assumption of constant capture probability can only be tested with three or more passes, and 2) substantial precision is gained over two passes (a worked example showed that four passes produced a threefold improvement in population estimate precision over two passes).

[13] The FWS expressed concern that male, territorial pupfish could be stressed by potentially reintroducing them to foreign territories following completion of multi-pass removal methods. Fishery biologists Peter Moyle and Ron Sutton did not share this concern (Moyle pers. comm., Sutton pers. comm.).

The rate of "depletion" among trapping passes is analyzed to produce a population estimate within each sampled unit (e.g., drain, and/or drain segment). Physical data collected within segments may be used to identify correlations with population results.

It should be noted that implementation of the following protocol should proceed under the direction of a desert pupfish 10(a)(1)(A) permit holder or similarly qualified individual(s), as pupfish will need to be handled. The take associated with this survey effort will be covered by the existing In-Valley Biological Opinion (USFWS 2002), and will be covered in IID's 10(a)(1)(B) Incidental Take Permit when its HCP is finalized. As such, proceeding under the direction of a 10(a)(1)(A) permittee is not a requirement. However, holding such a permit does indicate that the individual(s) in question is/are appropriately qualified to do the work, but there are other ways to demonstrate the required qualifications (C. Roberts pers. comm.).

The primary question to be addressed by this survey approach is:

1) What is the population of desert pupfish in a given drain that is directly connected to the Salton Sea (i.e., below the lowest pupfish impasse; typically, elevation control structures)?

A related question(s) that may be addressed using this approach is:

2) Is there variability in pupfish presence and abundance among drains, or among drain segments within a drain? If yes, what factors influence this variability? (e.g., seasonality, drain morphology, water quality, instream or near-stream vegetation, competitors/predators, etc.).

Closed-Population Trapping Protocol (CPTP)

The following describes an application of the CPTP to IID drains that are directly connected[14] to the Salton Sea. Field data and site descriptions are to be recorded on the Desert Pupfish Survey Form, which consists of three sheets. Sheet 1of 3 is the Site Information form. This sheet is used to record physical site information. Only one sheet need be completed for each site surveyed[15]. Sheet 2 of 3 is the Trapping Data form. Trapping effort and catch results are enumerated on this sheet. Each defined segment will require a separate Sheet 2. Sheet 3 of 3 is the Pupfish Data form. Pupfish statistics (size, sex) are recorded on this sheet. Each pass within a segment will require a separate Sheet 3.

1. Select a study area. The USGS in coordination with the IID HCP IT has recently identified 29 irrigation drains that have direct connectivity with the Salton Sea (i.e., they gravity flow, and are not pumped, to the Salton Sea). Pupfish are known to exist in other kinds of waterways/water bodies within the Imperial Valley. The area to be sampled must be clearly defined so that trapping results and estimations may be applied, and inferences/extrapolations made (if necessary), accurately.

---

[14] "Directly connected" implies that pupfish have the opportunity to actively move from drains to the Salton Sea. Drains that are pumped to the Salton Sea are not considered "directly connected". Barriers to movement within drains largely imply IID flashboard check structures for control of instream flow elevation and velocity. At present, only drain segments below the lowest most check structure of a direct-to-Sea drain are assumed to potentially harbor pupfish. However, this protocol may be implanted above check structures if necessary/desirable.

[15] If there are more than 10 segments defined in a survey area, then more than a single sheet 1 will be necessary.

Complete Sheet 1 to summarize the Site Information.

2. Identify, establish, and characterize drain segments. Abundance estimates may be calculated within drains, and within drain segments within a drain. Drain segments should represent somewhat distinctly different "types" of habitats or physical attributes within the sampled drain. Representative photographs should be taken of each segment. Attributes recorded at a segment may/should include average water depth[16] and average segment width, vegetation composition/cover, and/or water clarity/quality (Minckley pers. comm.). In the absence of notable habitat/physical differences within a drain, drain segments need not be established (i.e., a drain may consist of one uniform segment). Describe and quantify (if possible) physical attributes characterizing each established segment. These may be useful in showing relationships between pupfish presence/abundance and drain characteristics. Drain segment origins and termini should be marked with a GPS to determine segment dimensions and facilitate segment-break relocation, if necessary. Complete Sheet 1 to summarize the Segment information at the site. As shown on Sheet 1, water quality parameters may be sampled to further identify potential pupfish presence correlates/covariates. Turbidity may be characterized using qualitative ranks such as clear, slightly turbid, very turbid, or brown/green water.

   Once drain segments have been identified and described, upper and lower block nets of sufficient mesh size (e.g., 3-6 mm) should be erected to isolate one or more drain segments. Segments should be identified and sampled from lowest (nearest to Salton Sea) to highest (nearest up-flow check structure) to minimize turbidity in segments to be sampled thereafter.

3. Sample all drain segments with identical effort among passes. It is critical that sampling effort be equal within a segment among passes, and failure to adhere to this assumption invalidates the protocol. A segment must be fished with the same number of traps among passes, and all traps should be set ("soaked") for the same length of time within a given segment. Four passes are recommended over three passes for reasons described above, and later in this protocol. Note that the USFWS limits trap set times to 1-2 hours, as described previously in this document.

   Ideally, trapping passes among segments would be conducted at the same time to control for potentially confounding time-of-day effects (i.e., Pass 1 would sample all segments at the same time. Pass 2 would then sample all segments at the same time. And so on and so forth). This is more easily accomplished in smaller drains, where staff and equipment are sufficient for the level of effort. In most drains, this will not be possible due to drain size/length. Such time effects may violate the assumption of equal probability of capture among passes. While multi-pass approaches and calculations exist (discussed later) to account for such sources of error, attempts should be made to minimize bias due to time-of-day variability in trapping effort.

---

[16] Surveyors should coordinate with IID to attempt to ensure that drain flows are somewhat invariable for the period of sampling within a given drain, as variable flows due to irrigation runoff may complicate sampling and confound results. If not possible, dynamic drain flows at time of sampling should be recorded for consideration as a covariate factor during analysis of results.

Bryant (2000) suggested that one baited minnow trap (36 cm long, 19 cm wide) could effectively sample an area within a 2m radius of the trap under normal circumstances. Complex habitat areas may require greater trap densities. Trap set times must be standardized (1-2 hour sets should be sufficient) among passes. Randomizing trap locations within a segment is unnecessary. Similarly, trap densities need not be excessive. More important than the recommended trap density stated here (1 trap/2m radius area) is ensuring that the same level of trapping effort (i.e., # of traps and soak time) is exerted within a drain segment among passes[17].

Surveyors must have on hand enough traps to sample the longest drain segment established. Traps are baited with catfood and are deployed every 4 m within each segment. Set times by trap are recorded on a field data sheet (attached). At 1-2 hrs (+/- 10 min), traps are pulled with the pull time recorded, and collected fish are processed quickly. Record trapping effort by segment and pass on Sheet 2.

4. Process Captured Fish. Processing involves enumeration of catch, and size and sex determination if desirable, with these data recorded on the field data sheet. Fish from traps within a segment may be processed together. Recall that segments are identified and established largely based on similar habitat characteristics. As such, fish from intra-segment traps are representative sub-samples of the drain. Record fish data on Sheet 2 and Sheet 3.

Fish that have been processed within a segment on one pass are held until the completion of drain sampling, or until the completion of individual segment sampling[18], whichever comes first. Emptied traps are re-baited and set at the same locations for two or three more passes (three to four passes total per segment). It may be most efficient to stagger trap sets among segments such that while one segment is being trapped, captured fish from another are being processed, so long as trapping effort is held constant among segments and passes. Fish trapped and processed from Segment 1 may be returned to the segment water once all passes within the segment are completed, as long as the block net between Segment 1 and Segment 2 remains (so on and so forth).

5. Materials

A. Steel 3.2 mm mesh minnow traps and trap moorings
B. Catfood (about 60g per trap) in perforated film canisters
D. Data sheets on weather proof paper/pencils
E. GPS
F. Camera
G. PVC holding receiver(s)
J. Time piece (watch, etc.)

---

[17] A FWS reviewer commented that it may be necessary to set traps at densities greater than 1 per 2m radius to avoid violating the assumption of equal vulnerability to trap exposure. Hayes et al. (in prep), cited herein, discuss analytical methods to test this assumption.

[18] Pupfish can be released to a drain segment upon completion of all passes if block nets remain in place until completion of sampling within the entire drain.

M. Measuring board
N. Small aquarium nets
O. Pocket lens
P. Dissolved oxygen meter, thermometer
Q. Portable aerator and power source(s)

Abundance Calculations

The above-noted protocol will produce quantitative estimates of pupfish abundance, both within drains and among drain segments, the latter of which when correlated with drain physical data may indicate factors that control pupfish presence and abundance. This information would be useful in managing drain habitats for pupfish, and modifying drain O&M to benefit both IID processes and pupfish. Population trends may be tracked if the protocol is implemented with sufficient frequency and rigor.

Regression-based methods relating catch to effort were in the past (and still today) used to estimate abundance (e.g., Leslie and Davis 1939, DeLury 1947). Likelihood-based calculation methods (e.g., White et al. 1982) are more commonly used today as they allow testing of removal method assumptions such as equal catch vulnerability among passes. Riley and Fausch (1992) advocated 4 passes over 3 removal passes to account for potential underestimation of abundance using even likelihood-based removal methods.

Van Den Avyle and Hayward (1999) summarized the "Zippin method" (Zippin 1956, 1958) for calculating abundance within a sample area when the "catchability" (i.e., proportion of fish captured to fish present) of fish is high and similar among passes. The equation is:

(1) $$\hat{N} = \frac{C}{1-\hat{p}^{s}}$$

where $\hat{N}$ is the population estimate, C is the total catch over all passes, s is the number of passes, and $\hat{p}$ is the probability of avoiding capture during a pass (= 1-$\hat{q}$, where $\hat{q}$ is capture probability during a pass, or "catchability")

To estimate $\hat{N}$, $\hat{p}$ must be iteratively calculated first:

(2) $$\frac{\hat{p}}{\hat{q}} - \frac{s\hat{p}^{s}}{1-\hat{p}^{s}} = \frac{\sum_{t=1}^{s}(t-1)C_t}{C}$$

where t is the pass number. Charts published by Zippin (1956) are useful in solving Equation (2).

The 95% confidence interval for $\hat{N}$ can be calculated by:

(3) $$\hat{N} \pm 1.96\sqrt{V(\hat{N})},$$

where

(4) $$V(\hat{N})=\frac{\hat{N}(1-\hat{p}^{s})\hat{p}^{s}}{(1-\hat{p}^{s})^{2}-(\hat{q}s)^{2}\hat{p}^{s-1}}$$

When $\hat{q}$ is shown to vary among passes, other methods may be used to estimate $\hat{N}$. Some programs (e.g., CAPTURE of White et al. 1982, MARK of White and Burnham 1999) estimate whether $\hat{q}$ is constant or variable among passes, and calculate $\hat{N}$ accordingly. Most calculations necessary to estimate population abundance, confidence limits about the estimate, and capture probabilities are available as packaged software.

It should be noted here that other trap-removal and analytical methods may be employed using the same multiple pass capture approach advocated here. Capture-mark-recapture options (removal occupancy analyses) may allow better detection of those factors/covariates that control distribution and abundance (J. Groom pers. comm.). As discussed earlier in this document, however, mark-recapture results may be biased by trap aversion. Also, fish handling/marking and multiple captures may harm desert pupfish. Paired sampling designs (removal, versus mark-recapture) may provide a basis for quantifying trap aversion and other sources of variability associated with mark-recapture methods. At present, and at least initially, closed-population removal methods are recommended over closed population mark-recapture methods.

Demonstrating Absence

Demonstrating conclusively that a specific drain lacks pupfish is problematic. Past survey efforts have shown among-year (see Table 1) and within-year variability (USGS, unpublished data) in pupfish presence at a site. This variability may be due, at least in part, to the open nature of pupfish drains direct to the SS, and pupfish potentially moving among these drains. For this reason alone, conclusively demonstrating absence at a specific location would only be useful at a particular point in time. Drains direct to the SS may be recolonized/immigrated-to by pupfish at any time thereafter.

If the purpose of determining pupfish absence in a drain is related to a proposed construction project/action, then not capturing pupfish using this protocol may be sufficient to conclude absence without the added effort of detection probability studies. Failure to detect presence in this circumstance is not critical. New construction will still require IID to have a 10(a)(1)(A) permittee or similarly-qualified person(s) present during segment work/dewatering, with an procedure for relocating salvaged pupfish. The closed population approach of this protocol further eliminates/reduces the potential for pupfish re-entering a segment (construction area) after it has been “cleared” of pupfish, thereby reducing the potential for pupfish harm.

As a final point, the USFWS has suggested that information gathered as a result of using this protocol could be useful in determining how much effort might be required to remove a large fraction of fish from a drain or drain segment (J. Groom pers. comm.). Removal effort estimates may then be correlated with drain characteristics. This information may be important when considering pupfish removal necessary with new construction activities (hold, or salvage/relocate), or when conducting management actions such as pupfish predator/competitor removal.

References

Barlow, G. W. 1958. Daily movements of desert pupfish *Cyprinodon macularius* in shore pools of the Salton Sea, California. Ecology 39: 580-587.

Barlow, G. W. 1961. Social behavior of the desert pupfish, *Cyprinodon macularius*, in the field and in the aquarium. The American Midland Naturalist 65: 339-359.

Black, G. F. 1980. Status of the desert pupfish, *Cyprinodon macularius* (Baird and Girard) in California. State of California. The Resources Agency. Department of Fish and Game. 29 pp. +Appendices.

Bryant, M.D. 2000. Estimating fish populations by removal methods with minnow traps in southeast Alaska streams. North American Journal of Fisheries Management 20:923-930.

CH2M HILL. 2002. Draft Habitat Conservation Plan, IID Water Conservation and Transfer Project. June 2002.

Coleman, G. A. 1929. A biological survey of Salton Sea. California Fish and Game 15: 218-227.

Cowles, R. B. 1934. Notes on the ecology and breeding habits of the desert minnow, *Cyprinodon macularius* Baird and Girard. Copia 1:40-42.

Cox, T. J. 1966. A behavioral and ecological study of the desert pupfish, *Cyprinodon macularius,* in Quitobaquito Springs, Organ Pipe Cactus National Monument, Arizona. Ph.D. thesis, Univ. Arizona, Tucson. 102 p.

Groom, J. 2006. USFWS Biologist, Carlsbad, California. Email to Jeff Tupen on 18 July 2006.

Hayes, D.B., J.R. Bence, T.J. Kwak, and B.E. Thompson. In prep. Abundance, biomass, and production. Available at http://www.msu.edu/user/hayesdan/chapter.htm.

Keeney, S. 2006a. CDFG biologist, Bermuda Dunes, California. Phone conversation with Tim Hamaker on 24 February 2006.

Keeney, S. 2006b. CDFG biologist, Bermuda Dunes, California. Emailed comments on revised draft protocol on 31 August 2006.

Konno, E. 2006a. CDFG biologist, Bermuda Dunes, California. Phone conversations with Tim Hamaker on 22 February 2006.

Konno, E. 2006b. CDFG biologist, Bermuda Dunes, California. Phone conversations with Jeff Tupen on 11 July 2006.

Kordic-Brown, A. 1977. Reproductive success and the evolution of breeding territories in pupfish (Cyprinodon). Evolution 31:750-766.

Lau, S. and C. Boehm. 1991. A distribution survey of desert pupfish (*Cyprinodon macularius)* around the Salton Sea, California. Prepared for California Department of Fish and Game, Inland Fisheries Division. Final report for Section 6 Project No. EF90XII-1. November 1991.

Marsh, P. C. and D. W. Sada. 1993. Desert pupfish (*Cyprinodon macularius*) Recovery Plan. Prepared for Region 2 U.S. Fish and Wildlife Service, Albuquerque, NM. September 1993.

Martin, B. 2006a. USGS Biologist, Dixon, California. Phone conversation with Tim Hamaker on 22 February 2006.

Martin, B. 2006b. USGS Biologist, Dixon, California. Meeting with Tim Hamaker on 23 February 2006.

Martin, B. A. and M. K. Saiki. 2005. Relation of desert pupfish abundance to selected environmental variables in natural and manmade habitats in the Salton Sea Basin. Environmental Biology of Fishes 73: 97-107.

Miller. R. R. 1943. The Status of *Cyprinodon macularius* and *Cyprinodon nevadensis*, two desert fishes of western North America. University of Michigan Museum of Zoology. Occasional Paper 473:25 p.

Minckley, C. 2006. USFWS Project Coordinator, Arizona Fisheries Research Office, Parker, Arizona. Email to Tim Hamaker on 4 March 2006.

Moyle, P.B. 2002. Inland Fishes of California. Revised and expanded. University of California Press: Berkeley and Los Angeles, CA. 502 pp.

Moyle, P.B. 2006. Fishery Biologist, UC Davis, California. Phone conversation with Jeff Tupen on 26 October 2006.

Nicol, K. 2006. CDFG biologist, Bermuda Dunes, California. Email to Tim Hamaker on 27 February 2006.

Pister, P. 2006. Desert Fishes Council, Bishop, California. Phone message to Tim Hamaker on 24 February 2006.

Remington, M. D. and P. Hess. 1993. Results of pupfish trapping in Imperial Irrigation District drains (drains discharging directly into the Salton Sea and two shoreline pools); 8 August-21 September, 1993. Environmental Compliance Section, Planning & Technical Service Department, Imperial Irrigation District. September 27, 1993.

Riley, S.C and K.D. Fausch. 1992. Underestimation of a trout population size by maximum-likelihood removal estimates in small streams. North American Journal of Fisheries Management 12:768-776.

Rinne, J. 2006. USFS Biologist, Flagstaff, Arizona. Email to Tim Hamaker on 27 February 2006.

Roberts, C. 2006a. USFWS biologist, Carlsbad, California. Phone conversation with Tim Hamaker on 24 February 2006.

Roberts, C. 2006b. USFWS biologist, Carlsbad, California. Email to Jeff Tupen on 18 July 2006.

Saiki, M. 2006. USGS Biologist, Dixon, California. Email to Tim Hamaker on 28 February 2006.

Schoenherr, A. 2006. Professor, Fullerton College, Fullerton, California. Phone conversation with Tim Hamaker on 2 March 2006.

Schoenherr, A.A. 1979. Niche separation within a population of freshwater fishes in an irrigation drain near the Salton Sea. Bulletin of the Southern California Academy of Sciences 78: 46-55.

Schoenherr, A.A. 1988. A review of the life history of the desert pupfish, *Cyprinodon macularius*. Bulletin of the Southern California Academy of Sciences 87: 104-134.

Schoenherr, A.A. 1992. A natural history of California. University of California Press. Los Angeles, California. 772 pp.

Scoppetone, G. 2006. USGS Biologist, Reno, Nevada. Phone conversation with Tim Hamaker on 14 March 2006.

Sutton, R. J. 1999. The desert pupfish of the Salton Sea: a synthesis. Prepared for the Salton Sea Authority, La Quinta, California. August 5, 1999.

Sutton, R.J. 2002. Summer movements of desert pupfish among habitats at the Salton Sea. Hydrobiologia 473: 223-228.

Sutton, R.J. 2006a. USBR Biologist, Denver, Colorado. Phone conversation with Tim Hamaker on 23 February 2006.

Sutton, R.J. 2006b. USBR Biologist, Denver, Colorado. Phone conversation with Jeff Tupen on 30 October 2006.

United States Fish and Wildlife Service. 2002. Draft Biological Opinion on the Bureau of Reclamation's voluntary biological conservation measures and associated conservation agreements with the California Water Agencies and the Imperial Irrigations District's Water Conservation and Transfer to San Diego Count Water Authority. Prepared for Bureau Director Lower Colorado River Region, Boulder City, Nevada. Prepared by Assistant Field Supervisor, Carlsbad Fish and Wildlife Office, Carlsbad, CA. FWS-IMP-2628.10. 64 pp.

Van Den Avyle, M.J. and R.S. Hayward. 1999. Dynamics of exploited fish populations. Chapter 6 in C.C. Kohler and W.A. Hubert (eds.) Inland Fisheries Management in North America. Second edition. American Fisheries Society, Bethesda, MD.

Van Deventer, J.S. and W.S. Platts. 1989. Microcomputer software system for generating population statistics from electrofishing data. Users guide for MicroFish 3.0. General Technical Report USDA Forest Service. Intermountain Research Station, Ogden, Utah.

Verela-Romero, A., G. Ruiz-Campos, L. M. Yépiz-Veláquez, and J. Alaníz-García. 2003. Distribution, habitat and conservation status of desert pupfish (*Cyprinodon macularius)* in the lower Colorado River. Reviews in Fish Biology and Fisheries 12: 157-165.

Voeltz. J. 2006. Biologist, Arizona Game and Fish Department, Phoenix, Arizona. Email to Tim Hamaker on 28 February 2006.

Walker, B. W. 1961. The ecology of the Salton Sea, California, in relation to the sportfishery. California Department of Fish and Game. Fish Bulletin 113:204.

White, G.C. and K.P. Burnham. 1999. Program MARK: Survival estimation from populations of marked animals. Bird Study 46 Supplement, pp. 120-138.

White, G.C., D.R. Anderson, K.E. Burnham, and D.L. Otis. 1982. Capture-recapture and removal methods for sampling closed populations. Los Alamos National Laboratory Report No. LA-8787-NERP. Los Alamos, New Mexico.

Winnemiller, K. O. and A. A. Anderson. 1997. Response of endangered desert fish populations to a constructed refuge. Restoration Ecology 5(3): 204-213.

Zippin, C. 1956. An evaluation of the removal method of estimating animal populations. Biometrics 12:163-169.

Zippin, C. 1958. The removal method of population estimation. Journal of Wildlife Management 22:82-90.

Appendix A: Desert Pupfish Survey Forms

Sheet 1 of 3: Site Information

Sheet 2 of 3: Trapping Data

Sheet 3 of 3: Pupfish Data

Desert Pupfish Survey Sheet 1 – Site Information

Site Name: ______________________________

Site Description: ______________________________

____

Observers: ______________________________

No. of Segments (if applicable): _______

Segment No.	Length (m)	Width (m)	Depth (m)	Description (substrate type, vegetation composition and density, photo ID references, structures in segment, etc.)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Segment Water Quality

Segment No.	Date	Time (24-hr)	D.O.	Temp	Turbidity	Salinity
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						

Desert Pupfish Survey Sheet 2 – Trapping Data

Site Name: ____

Segment No: ___________ **GPS Location:** ______________________________

Observers: ____

Notes (weather, etc.) : ____

	Pass 1	Pass 2	Pass 3	Pass 4
Date				
# Traps				
Set Time				
Pull Time				

↓Species[1]
 # →

DP				
GMB				
SLM				
RSH				
MT				
RT				
BG				

1 DP (desert pupfish), GMB (western mosquitofish), SLM (sailfin molly), RSH (red shiner), MT (Mosambique tilapia), RT (redbelly tilapia), BG (bluegill)

Desert Pupfish Survey Sheet 3 – Pupfish Data

Site Name: ____

Segment No: _______________

Pass No: _____________

Total Pupfish Captured: ____________

Size (TL) by sex:

Males	Females	Juveniles / Indeterminate