Assessment of Native Fishes for Vector Control in Orange County, California

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ABSTRACT: Of the three native freshwater fish species (i.e., threespine stickleback, desert pupfish and arroyo chub) in southern California with a strong potential for consuming immature mosquitoes, the arroyo chub is the best candidate for integrated mosquito management programs in Orange County. At the present time, the California Department of Fish and Wildlife has considered approving two operational approaches for vector control using native fishes: (1) Translocation of native fishes within a watershed to sites below a physical impediment to upstream movement of translocated individuals, and (2) Translocation of fish into adjacent created, isolated aquatic habitat features (e.g., created wetlands). Within Orange County, the distribution of arroyo chub overlaps with source reduction activities carried out by the Orange County Vector Control District in the Arroyo Trabuco (Trabuco Creek and Tijeras Creek) within the San Juan Creek watershed. Numerous isolated water bodies exist within the watershed and in Orange County that could serve as supplemental conservation habitats for enhancing arroyo chub populations and for supplementing arroyo chub numbers for vector control. Discharge data for the Arroyo Trabuco, as well as for several other creeks, in coastal southern California indicate the late winter-early spring high-volume discharge events characteristic of streams in the region have the potential to wash a large proportion of the stocked fish downstream; thus, stocking should be delayed until after this period. It is recommended that between 1,000 and 2,000 arroyo chubs be translocated/stocked between mid-March through late May into Trabuco Creek and lower Tijeras Creek where vector control is currently required. Translocation of fish as early as possible in the spring will enhance the probability that arroyo chub reproduction will produce cohorts of young fish residing in the inundated vegetation of slow-moving sections of the water course where mosquito production is likely to be concentrated. Habitat characteristics favorable to the successful translocation of arroyo chubs and important gaps in our knowledge (e.g., persistence of stocked individuals in natural habitats, outcomes of interactions between native fishes with mosquitofish and introduced piscivores, temporal and spatial variations of important environmental variables in the target sites, estimates of arroyo chub population size, etc.) are discussed.

INTRODUCTION

One component of integrated mosquito management (IMM) programs for lacustrine and riverine wetlands is the use of larvivorous fish. Two species of the mosquitofish (*Gambusia affinis* (Baird and Girard) and *G. holbrooki* Girard) have been used worldwide for nearly a century as biological control agents for mosquitoes (Walton 2007, Walton et al. 2012). Both species are native to the southeastern United States and have native geographic distributions that are broad in comparison to all other North and Central American species in the genus (Moyle 2002). Few fish species can match the wide environmental tolerances and the favorable life history and morphological characteristics of *Gambusia* for mosquito control.

Yet, the hardiness, high reproductive potential, adaptability, aggressiveness and other characteristics that make mosquitofish such a successful predator of mosquitoes in many different aquatic environments worldwide (Swanson et. al. 1996) also make mosquitofish ideal invasive species that have potentially serious negative effects on native fauna and ecosystems (Gratz et al. 1996, Schleier et al. 2008), especially in the southwestern U.S. (Courtenay and Meffe 1989, Mills et al. 2004) and Australia (Arthington and Lloyd 1989). Mosquitofish are purported to prey upon the eggs and immature stages of economically important fish species and competitively eliminate native fish around the world (Myers 1965). Mosquitofish also eat eggs and larvae of native stream-dwelling amphibians in southern California such as the Pacific Chorus Frog, *Pseudacris regilla* (Goodell and Kats 1999) and the California newt, *Taricha torosa* (Gamradt and Kats 1996). The World Health Organization, as well as governmental natural resources agencies and conservation organizations, have urged studies of native larvivorous fishes that can replace the mosquitofish for mosquito control. In many regions of the U.S. outside the native geographic range of the two *Gambusia* species, release of mosquitofish into waters of the United States is no longer permitted. Not surprisingly, there is great interest among agencies responsible for stewardship of wetlands in southern California, such as the U.S. Fish and Wildlife Service (USFWS), California Department of Fish and Wildlife (CDFW) and the Orange County Water District (OCWD), to find a native species to replace *Gambusia* for mosquito control and to extirpate populations of the exotic mosquitofish in sensitive watersheds and habitats. Although *Gambusia* provides effective reductions of mosquitoes in a variety of isolated water features, there is a need to assess the potential use and applicability of native fish populations for vector control in portions of their natural habitat and created aquatic habitats adjacent to their habitat.

The objectives of this study were: (1) To conduct a GIS-based assessment to identify and characterize sites of production of insect vectors within the waters of the United States in Orange County, California where native fish populations could be used to supplement vector control activities, (2) To identify the native fish species amenable for vector control and estimate the number of fish of particular species likely to be stocked per annum, and (3) To review mass-rearing programs for native fishes and to estimate the costs for rearing native fishes. Here, we highlight the findings related to (1) and (2) of the report (Walton et al. 2013) filed with the Orange County Vector Control District (OCVCD).

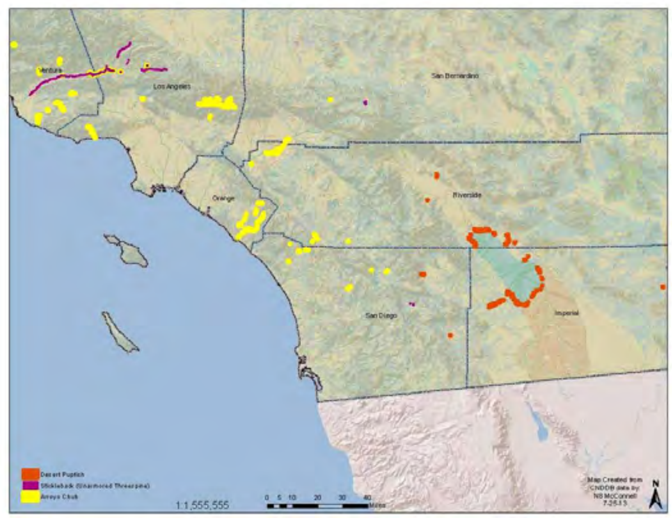
MATERIALS AND METHODS

Three species [i.e., threespine stickleback (*Gasterosteus aculeatus* L.), desert pupfish (*Cyprinodon macularius* Baird and Girard) and the arroyo chub (*Gila orcutti* Eigenmann and Eigenmann)] of native fishes were chosen as potential candidates for IMM programs in Orange County based on biological and ecological characteristics such as life history (e.g., reproductive phenology and distribution of life cycle stages in the water column), known environmental tolerances, diets and potential for mass-rearing. ArcGIS (Esri, Redlands, CA) was used to map the distribution of the native fishes within Orange County and within 8 km of the county borders using distribution data contained within the California Natural Diversity Database (CNDDB; https://www.dfg.ca.gov/biogeodata/cnddb). Inspection and treatment (IT) activities carried out by the OCVCD were extracted from the District's database and mapped. The overlap between the distributions of the three native fish species and sites of problematic vector production were mapped and characterized (i.e., distance of vector control activities, discharge and environmental characteristics if known). In addition to vector control activities associated with waters of the U.S., bodies of standing water that might serve as potential conservation habitat were identified and categorized by surface area.

The interannual and seasonal trends for discharge were characterized at four gage stations in the USGS National Water Information System (http://waterdata.usgs.gov/nwis) in coastal Orange and San Diego counties. The Arroyo Trabuco (= Trabuco Creek plus Tijeras Creek; USGS station number: 11047300) and San Juan Creek (USGS station number: 11046530) stations are located near the terminus of water courses containing the arroyo chub and upstream of their confluence near San Juan Capistrano. The Bonita Canyon (USGS station number: 11048600) and San Mateo Creek (USGS station number: 11046399) stations are on water courses that do not contain the arroyo chub, but may have supported arroyo chub historically. Discharge at these two stations may be indicative of conditions to the north and south of the current arroyo chub habitats in coastal Orange County. The historical distribution of the arroyo chub ranged along the coast from San Luis Obispo County to northern San Diego County (http://ice.ucdavis.edu/aquadiv/fishcovs/ach.gif; also see Fig. 2 in Why 2012). The watershed area of the four streams differs by 20-fold (5.4-109 ha: Walton et al. 2013). Here, the trends for the Arroyo Trabuco are highlighted and discussed relative to the three other sites. The reader is referred to Walton et al. (2013) for a more in-depth presentation of the findings for the three sites.

RESULTS AND DISCUSSION

Candidate Native Fishes for Mosquito Biological Control in Southern California. Three species of native fishes are promising candidates to replace *G. affinis* for biological control of immature mosquitoes in waters of the U.S. in southern California. The threespine stickleback (*Gasterosteus aculeatus* L.), the desert pupfish (*Cyprinodon macularius* Baird and Girard) and the arroyo chub [*Gila orcutti* (Eigenmann and Eigenmann)] are found in watersheds in southern California (Figure 1). These species possess characteristics that are favorable for use as biological control agents for mosquitoes. They are planktivorous for at least a portion of their life cycle (i.e., likely to consume immature mosquitoes), reproduce across an extended period during the year when adult mosquitoes are actively reproducing and are potentially amenable to mass-rearing in tanks and earthen ponds.



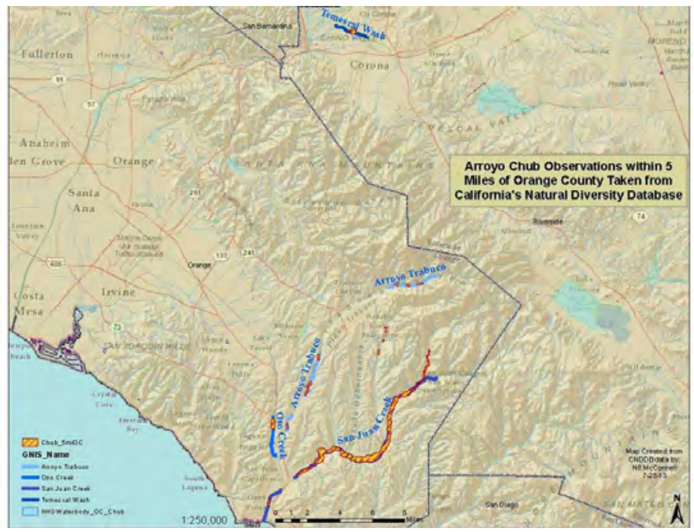
Among these three fish species, the arroyo chub is the best candidate for IMM programs in Orange County. The desert pupfish is not native to Orange County and is listed as an endangered species by the USFWS. The threespine stickleback is broadly distributed across California, includes several morphological variants as well as anadromous populations and populations restricted to inland waters; there is also a federally listed subspecies in southern California. In addition to the inherent difficulties of working with a fish species listed under the Endangered Species Act, the stickleback also has some drawbacks for use in mosquito control such as comparatively narrow environmental tolerances and comparatively low reproductive potential (Offill and Walton 1999, Walton et al. 2007). The arroyo chub is endemic to several southern California watersheds and has been shown to be effective at controlling larval mosquito populations in earthen manmade systems that were devoid of emergent vegetation (Van Dam and Walton 2007) and reducing immature mosquito abundance in experimental wetlands (Henke and Walton 2009).

The arroyo chub also possesses characteristics that enhance its survival and proliferation in vector control programs. In addition to broad environmental tolerances (such as tolerance of moderate hypoxia and temperature fluctuations, persistence in backwaters and lentic conditions as well as flowing water), the arroyo chub is a fractional spawner, breeding almost continuously from February to August, although most spawning takes place during peak breeding season in June and July (Moyle 2002). Captive populations held in earthen ponds may continue to reproduce as late as September or early October (W. Walton, personal observation). Adults typically spawn at one year of age (Tres 1992). Laboratory studies have shown the arroyo chub to be omnivorous, feeding on insects, algae and small crustaceans. Greenfield and Deckert (1973) found that 60-80% of the stomach contents consisted of algae. Arroyo chubs are also known to feed on nematodes infesting the roots of a floating water fern (*Azolla*) (Moyle 1976). While invertebrates are an important component of arroyo chub diets in spring, large arroyo chubs feed primarily on benthos (i.e., aquatic insects, snails).

Drawbacks to the widespread use of the arroyo chub for vector control include its listing as a "Species of Special Concern" by the CDFW, concerns of the CDFW that translocation of natural fish populations or stocking of hatchery-reared fish may promote the spread of potential pathogens and parasites, stocking fish may compromise the genetic distinctness of natural populations, and potential hybridization with other minnow species. Due to population declines and loss of habitat, the arroyo chub qualifies as a "Threatened Species" within its native range (Moyle et al. 1995, Veirs and Opler 1998). *Gila orcutti* is endemic to the Los Angeles, San Gabriel, San Luis Rey, Santa Margarita and Santa Ana river systems, as well as Malibu and San Juan creeks (Wells and Diana 1975, Swift et al. 1993; Figure 1). Chub have been introduced and have successfully established populations in the Santa Ynez, Santa Maria, Cuyama and Mojave river systems as well as smaller coastal streams such as Arroyo Grande Creek and Chorro Creek in San Luis Obispo County (Miller 1968, Moyle 1976, Moyle et al. 1995). Recently, arroyo chub were reintroduced into the Arroyo Seco as part of a restoration project carried out near Pasadena (Camm Swift, personal communication). However, as is observed for several minnow species in the region, hybridization occurs readily and poses a concern for conservation efforts of threatened and endangered native fishes. The arroyo chub hybridizes readily with two minnow species endemic to California: the Mohave tui chub (*Siphateles bicolor mohavensis* [Girard]) and the California roach (*Lavinia symmetricus* [Baird and Girard])(Hubbs and Miller 1943, Greenfield and Greenfield 1972, Greenfield and Deckert 1973).

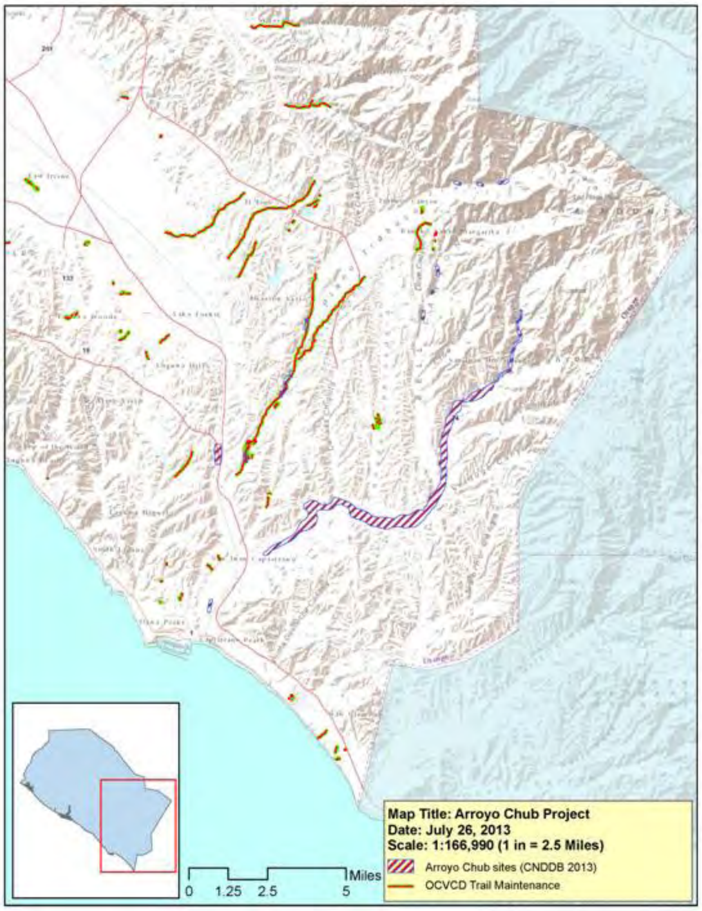
Arroyo chubs have become scarce in their native range because the low-gradient streams, which are their preferred habitat, have largely disappeared due to urbanization (Swift et al. 1993). Arroyo chub populations, as well as those of other native fish, also have declined as a result of the introduction of several sport and non-native fishes to watersheds within southern California (Moyle et al. 1995). Specifically, green sunfish, *Lepomis cyanellus* Rafinesque, and largemouth bass, *Micropterus salmoides* (Lacèpéde), were introduced throughout the state for angling purposes and adults are piscivorous (Baltz and Moyle 1993). The statewide introduction of mosquitofish, *G. affinis*, for mosquito control has also contributed to the declines of native fish populations in California (Moyle et al. 1995). Arroyo chubs tend not to co-occur with red shiners [Lahontan redside, *Richardsonius egregious* (Girard)] that were introduced as forage fish for stocked trout or released by anglers using the species as a baitfish. Piscivory by centrarchids was likely the primary factor that contributed to the failure of stocked arroyo chubs to persist in a wetland within the Prado Basin in western Riverside County, CA (Why 2012).

Within Orange County (Figure 2), the arroyo chub is found in the San Juan Creek drainage in Bell Canyon, Hot Springs Canyon and the upper mainstem of San Juan Creek. Arroyo chub are also found in the Arroyo Trabuco (Trabuco Creek in Trabuco Canyon) and the lower Tijeras Creek. Contrary to the distribution data in the California Natural Diversity Database (CNDDB), the arroyo chub has been extirpated from Oso Creek where red shiners are predominant (John O'Brien, California Dept. Wildlife, personal communication).



It is difficult to ascertain the actual numbers of arroyo chubs within these watersheds. Quantitative estimates of population size are unavailable. The abundance of arroyo chubs presumably varies annually in relation to flooding and drying. The arroyo chub is adapted to warm, fluctuating streams that were characteristic of the southern California coastal plain (Moyle 2002). Stream discharge varies markedly seasonally, and some streams are intermittent in the lower reaches. Arroyo chubs attain greatest abundance in slow-moving or backwater sections of water courses where inundated vegetation provides important cover for young-of-the-year fish.

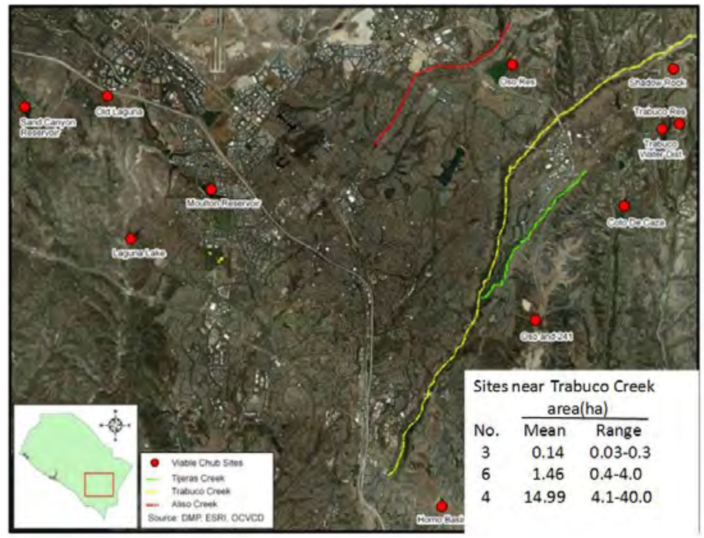
Overlap of Arroyo Chub Populations with Vector Control Activities. The distribution of arroyo chubs overlaps with IT activities carried out by the Orange County Vector Control District in the Arroyo Trabuco (Trabuco Creek and lower Tijeras Creek) within the San Juan Creek watershed (Figure 3). Trail maintenance activities carried out by the OCVCD along Aliso Creek, English Canyon, Dove Canyon, Laguna Canyon, Serrano Creek, within the San Clemente Coastal Streams watershed, at two locations within the upper Santa Ana River watershed, and at several locations within the Newport Bay watershed do not overlap with current arroyo chub distributions in the CNDDB (Figure 3).



At the present time, translocation of arroyo chubs within a watershed to sites below a physical impediment to upstream movement of translocated individuals is the one of the operational approaches for vector control using native fishes that might be considered for approval by the CDFW (Native Fishes Scoping Meeting, OCVCD, 10 July 2012). Trabuco Creek provides the best opportunity for enhancement of native fish populations in areas problematic for vector production. Potential source populations of fish occur upstream of, as well as within, the zone of vector control activities. Moreover, these source reduction activities are the most extensive (in terms of total distance) in southern Orange County. The lower Tijeras Creek is an adjacent site in the same watershed that appears favorable for the enhancement of arroyo chub populations. While impediments to upstream movement of fish are prevalent at the terminus of the water course, it is unknown whether any natural or man-made impediments to upstream movement exist above the trail maintenance activities in the Arroyo Trabuco.

Arroyo chub also are present in San Juan Creek which joins the Trabuco Creek near San Juan Capistrano. An ongoing study of the genetics of the arroyo chub is being carried out by the CDFW. This study should provide information on the relatedness of the *G. orcutti* populations in both water courses. Although trail maintenance activities are not carried out by the OCVCD along San Juan Creek (Figure 3), arroyo chub are found below the confluence of the two stream systems and may form one population within the San Juan Creek watershed.

IT activities also are carried out in numerous isolated habitats that might be amenable for stocking arroyo chub. For example, thirteen habitats near Trabuco Creek range in area from 0.03 to 40 ha (Figure 4). Other prospective sites within Orange County still need to be determined. Sites should maintain water throughout the year and should not contain piscivorous fish such as green sunfish and bass. Coexistence of the arroyo chub with mosquitofish is probably possible, but the outcome(s) of interactions between the two fish species require further study. Ownership of the water bodies needs to be determined, and the owners must be amenable to the stocking of arroyo chubs. The addition of native fish must not jeopardize the existence of endangered or threatened species already present in a site.



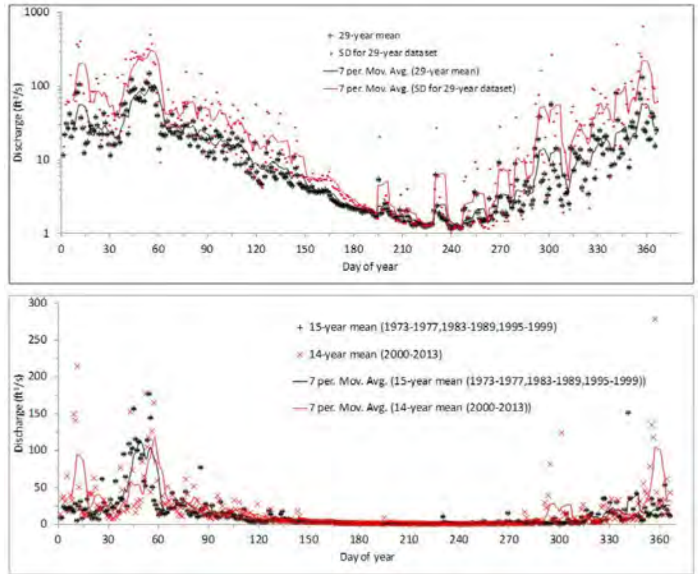
Translocation/stocking of Arroyo Chubs in Trabuco Creek and Tijeras Creek. Several factors should be considered in relation to the translocation/stocking of the arroyo chub within Trabuco and Tijeras Creeks. In addition to the concerns expressed by the CDFW related to the genetics, health and possible differences in the parasite burden of the fish among sites (i.e., stream systems within a watershed and among watersheds), the fish should be relocated as early as possible during the period that adult mosquitoes are reproducing. This strategy should enhance the likelihood that robust numbers of native fish will occur within the sections of the stream system known to produce mosquitoes. The spawning period of the arroyo chub (late February to August: Moyle 2002) begins prior to the onset of host-seeking activity by the majority of mosquito species of concern in the region (in late March-early April) and may extend into October (W. Walton, personal observation) when host-seeking by the mosquitoes tends to decline markedly.

The late winter-early spring high-volume discharge events characteristic of streams in the region have the potential to wash a large proportion of the stocked fish downstream; stocking should be delayed until after this period. Translocation of fish as early as possible in the spring will enhance the probability that arroyo chub reproduction will produce cohorts of young fish residing in the inundated vegetation of slow-moving sections of the water course where mosquito production is likely to be concentrated. Based on the differences in the diets and spatial distribution of fish within native habitats (Moyle 2002) related to age (and size), the young-of-the-year presumably are more important in the consumption of immature mosquitoes than do adult arroyo chubs. Nevertheless, immature mosquito abundance was reduced in the presence of adult arroyo chub as compared to lentic vegetated habitats without fishes (Henke and Walton 2009), but this effect might reflect oviposition deterrence of the mosquitoes (Why 2012) rather than predation of mosquito larvae by the adult arroyo chubs. Moreover, the establishment of fish prior to the peak reproductive period in June and July (Moyle 2002) will ensure that reproductive adults have the greatest chance to acclimate to new surroundings after translocation.

Other factors that should be considered to enhance the production of a new cohort of arroyo chub in environments where fish have been stocked are the characteristics of the habitat related to the survival and reproduction of adults as well as the survival of fry. Arroyo chub prefer slow-moving or backwater sections of warm to cool streams (10 - 24 °C) with muddy or sandy bottoms (Moyle 2002). Whereas arroyo chubs can be found in shallow fast-moving sections of streams with coarse (i.e., rocky, scoured substrate) bottoms, they prefer depositional habitats with depths > 40 cm (Moyle 2002). Habitats with cover (vegetation, root masses, etc.), overhanging banks, deep areas (> 40 cm depth) and/or boulders are favorable for stocking arroyo chub (C. Swift, personal communication). Survival of young fish will be enhanced in habitats containing inundated vegetation. The fish should be stocked into sites where these types of habitats are likely to be present well into the summer.

An additional consideration related to the translocation and stocking of the arroyo chub is the timing and extent of source reduction. Thinning or elimination of inundated vegetation along stream channels reduces mosquito production but also has the potential to reduce the favorability for the survival of young arroyo chub. Presumably the timing of vegetation management is influenced by considerations of the nesting activities of birds. Generally, vegetation management is only permitted after the nesting season; such activities are restricted to autumn and winter when arroyo chub reproduction ceases annually. From the perspective of arroyo chub life history, late-season vegetation management is preferable to late spring-early summer activities.

The interannual pattern of daily discharge is, not surprisingly, similar among the four gage stations for the period between 2001 and 2013 when data are available for all stations. A discontinuous record of daily discharge from autumn 1972 through August 2013 is available for Arroyo Trabuco (Figure 5). A continuous 27-year record of daily discharge is available for San Juan Creek and a continuous 13-year (autumn 2001 through August 2013) dataset is available for Bonita Canyon (Walton et al. 2013). The discharge record for San Mateo Creek is discontinuous, including a period from autumn 1952 through 1968 and a second period beginning in 1994 until August 2013 (Walton et al. 2013). Although the magnitude of the daily discharge is indicative of watershed area and diversions/restrictions of flow within each watershed, the pattern of discharge events reflects the interannual variation in rainfall caused by short-term variation in climate such as El Niño-Southern Oscillation (ENSO) events. Maximum daily discharge events were comparatively greater in 2005 and 2011 than in other years. The ENSO events of 1995 and 1998 are also evident in the daily discharge data at sites.



For each day of the year across years, the mean and the variation of daily discharge is comparatively higher in January through March and from mid-October through December in both Arroyo Trabuco (Figure 5) and San Juan Creek (Walton et al. 2013). Variation in daily discharge declines appreciably around day 150 (May 29 based on a 366-day year to include data for leap years) in Arroyo Trabuco. The monthly mean discharge for April decreases to 14.4 ft^3/s from 25.5 ft^3/s for March and drops to 8 ft^3/s in July (U.S. Geological Survey 2013b). Water flow is unaffected by upstream diversions. In contrast to the Arroyo Trabuco, there is no regulation upstream from the gage station on San Juan Creek, but the Capistrano Water Company diverts water 3.2 km (2.0 mi) upstream of the gage station, and various amounts of diverted water reach the station as irrigation return flow (U.S. Geological Survey 2013a). No discharge was recorded from early May through September 2012. A similar decline in the mean daily discharge through about day 250 (Sept. 6) is observed in both water courses. However, sporadic flooding events are evident in the variation of daily discharge for Arroyo Trabuco (Figure 5). The mean and variation for daily discharge increase in both water courses beginning in early October (~ day 280).

Similar annual trends for daily discharge are evident in the two water courses not currently supporting the arroyo chub (Walton et al. 2013). Low summer flows (essentially no measurable flow) are evident in Bonita Canyon. The variation in daily discharge is lower than the long-term daily mean. The decline in the mean daily discharge in San Mateo Creek across spring and summer is similar to that observed in the two water courses containing the arroyo chub (Walton et al. 2013). Interestingly, San Mateo Creek exhibits a marked increase in the mean and variation of daily discharge around day 300 and again during December.

It is possible that recent development within watersheds has altered the patterns of daily discharge. For example, increases in the proportion of area covered by impervious substrate might increase the mean and variation in daily discharge. Installation of structures to capture stormwater runoff might show an opposite effect on daily discharge. The mean daily discharge for the period 2000 - 2013 was greater during December and January than for the discontinuous record from 1972 through 1999 in Arroyo Trabuco (Figure 5). The mean daily discharge for February was equivalently variable for both periods.

In San Juan Creek, mean daily discharge during December increased for the period 2000-2013 relative to the period from 1987 through 1999 (Walton et al. 2013). Surprisingly, the mean daily discharge in January and February during the late 1980s and throughout the 1990s was larger and more variable than during the most recent decade. However, it is difficult to discount differences in interannual variation of precipitation between the two time periods.

Discharge data are the most complete environmental variable dataset available for these sites. Water temperature and water quality data are limited to only a couple of years, if at all, in the USGS datasets. These variables are not recorded in conjunction with source reduction activities. Although we intended to assess the environmental characteristics of habitats producing vectors such as habitat size, water depth, vector abundance, general site conditions, etc., these variables are not routinely recorded along the stream sites where source reduction is being carried out.

The overwinter survival rates of minnows often depend on the interaction between summertime temperatures and food supply. Prevention of starvation during winter is linked to the development of lipid stores during the summer (Meffe and Snelson 1993a, b). These factors are particularly important for fish populations that must survive low water temperatures that accompany freezing conditions during winter. Clearly these conditions are not occurring in Orange County, but poor overwintering survival of larvivorous fishes can affect IMM programs in southern California (Walton et al. 2012). Stocking fish prior to summer would therefore be preferable to stocking fish in late summer and autumn.

An additional consideration when stocking native fish is the abundance of non-native fishes, especially mosquitofish and piscivorous centrarchids in the stream reach to be stocked. The western mosquitofish (*G. affinis*) has negative impacts on the least chub [*Iotichthys phlegethontis* (Cope)] directly through predation and indirectly via exclusion from suitable rearing habitats (Mills et al. 2004, Wagner et al. 2005). In laboratory studies, age-0 least chub sought refuge in the presence of western mosquitofish and spent less time feeding; this resulted in reduced growth and a longer period of time in which least chub were vulnerable to predation (Mills et al. 2004). In natural habitats, western mosquitofish can also reduce the growth of least chub by forcing age-0 least chub to seek refuge from predation by utilizing the cooler pool habitats. Slower growth rate and reduced accumulation of lipid stores which enhance overwinter survival would result from such habitat shifts. Aggressive interactions with western mosquitofish adults (Schoenherr 1981) may cause minnows to reproduce in less favorable habitats (i.e., pools). Young-of-the-year least chub were readily consumed by western mosquitofish, and those not eaten, including adults, experienced reduced growth as a result of competition with western mosquitofish (Mills et al. 2004). Whereas the larger arroyo chub should be less susceptible to the detrimental effects of competition and predation than the least chub, taken together these factors are expected to reduce the size and robustness of chub age classes stocked into habitats where mosquitofish are abundant and predacious centrarchids are prevalent.

How many fish need to be transplanted? Based on previous stocking efforts for the arroyo chub, a minimum of several hundred fish and a maximum of around 2,000 should be transplanted in the region of vector control activities in Trabuco Canyon; however, at present, a more definitive estimate of the number of fish needed cannot be made. The number of arroyo chubs to be transplanted will be a function of suitable habitat for the arroyo chubs, barriers to dispersal upstream, the presence of non-native fishes and other considerations, especially the availability of extant fish to be transplanted. The extent of source reduction activities on Trabuco Creek equals about 10.7 km (6.67 mi) plus an additional 4.0 km (2.5 mi) on Tijeras Creek, a southerly branch (Figure 3). Arroyo chubs are present in both streams (J. O'Brien and C. Swift, personal communication), so suitable habitat is presumably present. Habitats with cover, overhanging banks, deep areas (> 40 cm depth) and/or boulders are favorable for stocking arroyo chub (C. Swift, personal communication). For sites with a high proportion of canopy cover, aquatic vegetation is not very prevalent, but root masses and overhanging banks may provide suitable cover for the fish.

In summer 2008, approximately 300 chubs were translocated from Big Tujunga Wash near the crossing of Oro Vista Avenue (approximately 1 km north of the 210 freeway) and placed in a 4.5 km (2.8 mi) stretch of the Arroyo Seco between the 134 and 210 freeways near Pasadena (C. Swift, personal communication). The introduced fish were placed into two small naturalized areas with cement-lined channels or impassable barriers closely limiting the areas upstream and downstream.

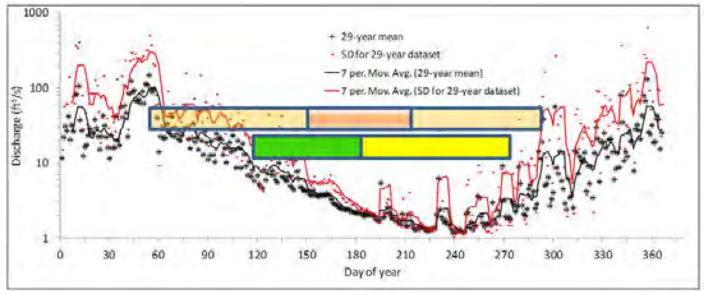
Assuming a transplantation rate of 70 adult fish/km, then 1,050 fish would be needed in Trabuco and Tijeras Creeks to provide a stocking rate comparable to that of the Arroyo Seco reintroduction study. Large numbers of native arroyo chub can be found in San Juan Creek within a 1.6 km or so up and downstream of the mouth of Arroyo Trabuco, and farther up Trabuco Creek near the junction of Oso Creek. Although arroyo chub were present in substantial numbers near O'Neill Park area (C. Swift, personal communication), this population may no longer be present (J. O'Brien, CDFW, personal communication) because of large numbers of red shiners. In 2010, large numbers of arroyo chub were observed in Tijeras Creek upstream of its confluence with Trabuco Creek (C. Swift, personal communication). It seems unlikely that native populations in Trabuco Canyon could sustain the level of harvesting of the aforementioned range of individuals, and hatchery-raised fishes would be needed to supplement the stocked population. There is, however, currently no scientific evidence supporting the aforementioned stocking rate.

Stocking rates of arroyo chub in lentic habitats seem unrealistically high for the lotic habitats of Trabuco Canyon. Van Dam and Walton (2007) used stocking rates of 4.5 kg/ha (mean = 12.5 g/pond as 4 reproductive and 35 larval chubs) and 13.2 kg/ha (mean = 37 g/pond as 31 adult fish) in earthen ponds. Assuming an average stream width of 3 m for 15 km of vector control activities, approximately 17,000 and 50,000 adult arroyo chubs would be needed to provide comparable stocking rates. The number of arroyo chubs present after nearly two years in experimental wetlands was more similar to that of the lower stocking rate than the higher stocking rate (J. A. Henke, unpublished data). Moreover, arroyo chubs and mosquito production are concentrated at the periphery of streams and rivers. Arroyo chubs prefer more marginal, slow or standing flows on the periphery of streams (Feeney and Swift 2008). If mosquito production is problematic, these backwater habitats containing inundated vegetation are likely to be the sites producing mosquitoes. The slow moving regions also can support exotic predators of arroyo chubs, such as sunfish and bass (C. Swift, personal communication), which presumably limit the distribution of arroyo chubs in river systems (i.e., the Santa Ana River: Feeney and Swift 2008) and likely extirpated arroyo chubs from a wetland in the Prado Basin (Why 2012). If about one-tenth of the surface area of Trabuco Creek and Tijeras Creek is conducive for supporting the arroyo chub, then ≤ 1,700 adult arroyo chub would be needed.

Whereas, an estimate of arroyo chub population size and more reliable estimates of the surface area of the two creeks, of suitable chub habitat, and of the regions of problematic mosquito production would be beneficial, as well as other factors (Table 1), this preliminary assessment finds that supplementation of native arroyo chub populations for vector control seems feasible. A characterization of sites that differ in mosquito production, especially the presence and abundance of native fishes in these sites, is suggested. An assessment of the barriers to upstream movement and the amount of suitable habitat for the arroyo chub is needed in the places where source reduction is carried out before the translocation of fish is made. Also, the efficacy of supplementation of native fishes for the reduction in mosquito production and persistence of the stocked fish should be evaluated.

- Habitat characteristics favorable for successful translocation
- Stocking density that facilitates persistence of fish and provides effective mosquito control
- Persistence of stocked individuals in natural habitats
- Temporal and spatial variation of important environmental variables influencing the success of translocations
- Estimates of arroyo chub population size
- The abundance of non-native fishes, especially mosquitofish and piscivorous centrarchids, in the habitats to be stocked.
- Outcome of interactions between arroyo chub and mosquitofish
- A characterization of sites that differ in mosquito production, especially the presence and abundance of native fishes
- An assessment of the barriers to upstream movement and the amount of suitable habitat for the arroyo chub in the places where trail maintenance is carried out.
- Efficacy of supplementation of native fishes for the reduction in mosquito production: direct vs. indirect effects on mosquito abundance
- Hatchery management plans

Based on the aforementioned considerations, it is recommended that the arroyo chub are translocated/stocked between mid-March through late May (Figure 6). Stocking from June through August is possible, but it is expected that annual recruitment for fish stocked in summer will be lower than for fish stocked earlier in the year. The persistence of stocked individuals in natural habitats should be studied.



Mass Rearing of Native Fishes. While native fishes that could potentially serve as alternatives to the mosquitofish as biological control agents for immature mosquitoes, these fishes have been raised by only a few vector control districts in California. Moreover, most of the rearing studies have not been at a scale large enough to be practical for stocking fish into mosquito developmental sites. The greatest success to date has been with the California roach [*Lavinia* (*Hesperoleucus*) *symmetricus* (Baird and Girard); Cyprinidae]. Approximately 4700 and 7300 fish were produced in 2010 and 2011, respectively, but production declined precipitously in 2012 to only 1500 fish (Chris Miller, Contra Costa MVCD, personal communication). The factor(s) contributing to the decline in production is/are unknown; however, brood stock age and food levels are the most likely reasons.

This minnow species was reared in 200-gallon tanks with a recirculating system. Flowing water was required for the fish to initiate spawning, and the primary reproductive period was from April until the end of June. Spawning material for egg deposition was added to tanks containing reproductive adults and then removed so the eggs could be incubated separately from the adult fish. The larvae readily consumed commercial fish food.

California roach has been stocked by the Contra Costa MVCD into abandoned swimming pools at a rate of between 40 and 100 fish/pool. Large adults (~ 2 years old) and 3-month-old young-of-the-year were included in the stocking populations. Approximately 500 individuals also were added to a large wetland; but the success (i.e., persistence of the fish) of that introduction has not been determined.

Efforts by the Contra Costa MVCD to mass-rear the Sacramento perch (*Archoplites interruptus* (Girard); Centrarchidae); Sacramento blackfish (*Orthodon microlepidotus* (Ayres); Cyprinidae) and hitch (*Lavinia exilicauda* Baird and Girard; Cyprinidae) have been comparatively less successful than for the California roach (León et al. 2008; C. Miller, CCMVCD, personal communication). Successful rearing of the Sacramento perch through the larval stage requires a diet rich in rotifers. Piscivory by adult fish is potentially problematic for mass rearing. Consumption of young-of-the-year by adults also limited the successful rearing of the Sacramento blackfish (C. Miller, CCMVCD, personal communication).

The threespine stickleback has been used successfully for larval mosquito control in backyard ponds and abandoned swimming pools in the Central Valley (Sacramento-Yolo MVCD 1999; Woody Schoen, personal communication). Twenty to twenty-five adult fish/habitat were stocked into habitats with depths

> 46 cm. However, a poor tolerance for high water temperature and hypoxia, complex mating behavior (e.g., high levels of aggression between males and complex nest construction) and a low reproductive rate (Offill and Walton 1999, Walton et al. 2007) limit the usefulness of this species for mosquito control and in mass rearing programs. The use of this species for vector control is further complicated in southern California because a federally-listed unarmored subspecies, *Gasterosteus aculeatus williamsoni* (Girard) is associated with the Santa Ana River. Although this subspecies has not been collected/observed for nearly fifty years in the Santa Ana River, and has probably been extirpated from the Santa Ana watershed, three relict populations [upper Santa Clara River (Los Angeles County), San Antonio Creek (Santa Barbara County) and Sweetwater River (San Diego County)], and two transplanted populations, exist in southern California (Moyle 2002).

The desert pupfish has been raised successfully in earthen ponds (Walters and Legner 1980; M. Saba, personal observation). Listing of the species under the Endangered Species Act and controversy related to the genetic relatedness of extant populations severely restrict future use of this species for vector control.

The arroyo chub has been raised successfully in an artificial stream at the Riverside-Corona Resource Conservation District (Riverside, CA) and in earthen ponds at U.C. Riverside. The production of arroyo chub in the artificial stream is over 1,000 individuals/annum and cohorts of fish have been used for reintroductions and will be used for a future study in constructed treatment wetlands adjacent to Cucamonga-Mill Creeks (Kerwin Russell, Riverside-Corona Resource Conservation District, Riverside, CA). The wet mass of arroyo chub populations in earthen ponds approximately doubled during 6-week studies spanning the peak reproductive period, and the population growth rate on natural prey assemblages was circa 0.04 individuals/individual/day (Van Dam and Walton 2007).

A very successful program using native fishes for vector control has been implemented in Utah. The Utah Division of Wildlife Resources (UDWR) has developed a partnership with vector control districts in Utah to use the least chub as a substitute for mosquitofish as a biological control agent for mosquitoes. A Memorandum of Agreement (MOA) among the Utah Division of Wildlife Resources, Utah Department of Agriculture and Food and Utah Vector Control Districts established administrative policies and procedures for collecting, holding, distributing, transporting, rearing and releasing mosquitofish (see Appendix A in Walton et al. 2013). Whereas the MOA restricted the use of *Gambusia* by the signatory districts, the use and possession of mosquitofish by districts not signing the MOA is prohibited. The UDWR further developed procedures and policies allowing the vector control districts to rear and distribute the least chub. Under the joint program of the UDWR and the vector control districts, the least chub has been stocked into about 240 backyard ponds near Salt Lake City as part of ongoing research. Last, the UDWR and university researchers conducted a study to understand better the requirements for raising the least chub, the interactions between mosquitofish and least chub and the environmental factors that influence (i.e., potentially limit) the distribution of the invasive mosquitofish. Many of the lessons learned in these research endeavors focused on the least chub are relevant to prospective future efforts for mass rearing the related arroyo chub. Further discussion of the factors to be considered for successful propagation and costs associated with the culture of native fishes can be found in Walton et al. (2013).

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FIGURE LEGENDS

Figure 1. Distribution of three native fish species [desert pupfish: *Cyprinodon macularius* Baird and Girard; arroyo chub: *Gila orcutti* (Eigenmann and Eigenmann); threespine stickleback: *Gasterosteus aculeatus* L.)] which are potential replacements for the mosquitofish [*Gambusia affinis* (Baird and Girard)] as a biological control agent for immature mosquitoes in southern California.

Figure 2. Distribution of the arroyo chub (*Gila orcutti*) in Orange County, California and within 8 km (5 mi) of the county borders. Data taken from California's Natural Diversity Database. Not shown is the presence of *G. orcutti* in the Santa Ana River between the City of Riverside and the Prado Basin.

Figure 3. Overlap of the arroyo chub (*Gila orcutti*) and source reduction activities carried out by the Orange County Vector Control District.

Figure 4. Viable stocking sites for arroyo chub in isolated standing-water habitats near Trabuco Creek. The distribution of sites based on surface area is provided in the lower right.

Figure 5. Mean and variation (SD) for daily discharge (ft^3/s) for 29 years during 1973-2013 (upper panel) and mean daily discharge for periods before or after 2000 (lower panel) at the Arroyo Trabuco gage station in the USGS National Water Information System database. Seven-day running averages depicted.

Figure 6. Mean and variation of daily discharge (ft^3/s) for the Arroyo Trabuco, annual period of reproduction for the arroyo chub (*G. orcutti*) (upper bar: peak period of reproduction is highlighted in the center of the histogram) and recommended timing of translocation of arroyo chub (lower bar). The green histogram in the lower bar represents the better time for translocation based on reproduction of the arroyo chub and annual discharge patterns in the Arroyo Trabuco.

TABLE LEGENDS

Table 1. Some of the additional information needed to evaluate the success and to assist decision-making for the use of the arroyo chub as a component of IMM programs in southern California