MCB Camp Pendleton
Arroyo Toad Monitoring Protocol:
1. Summary of results from a workshop on August 27, 2002
2. Monitoring protocol and targeted studies

Prepared for:

Marine Corps Base Camp Pendleton

U.S. DEPARTMENT OF THE INTERIOR
U.S. GEOLOGICAL SURVEY
WESTERN ECOLOGICAL RESEARCH CENTER

MCB Camp Pendleton Arroyo Toad Monitoring Protocol:

1. Summary of results from a workshop on August 27, 2002
2. Monitoring protocol and targeted studies

By Andrea J. Atkinson[1], Brian S. Yang[1], Robert N. Fisher[1], Ed Ervin[1], Ted J. Case[2], Norman Scott[3], and H. Bradley Shaffer[4]

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[1]San Diego Field Station
USGS Western Ecological Research Center
5745 Kearny Villa Road, Suite M
San Diego, CA 92123

[2]Biological Sciences 0116
University of California at San Diego
La Jolla, CA 92093

[3]3655 Lindquist Lane
P.O. Box 307
Creston, California 93432

[4]Section of Evolution and Ecology
University of California
One Shields Ave.
Davis, CA 95616

Sacramento, California
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U.S. DEPARTMENT OF THE INTERIOR
GALE A. NORTON, SECRETARY

U.S. GEOLOGICAL SURVEY
Charles G. Groat, Director

For additional information, contact:

Center Director
Western Ecological Research Center
U.S. Geological Survey
7801 Folsom Blvd., Suite 101
Sacramento, CA 95826

TABLE OF CONTENTS

WORKSHOP ATTENDEES

Scientific Review Panel:

Ted J. Case,	University of California, San Diego
Norman Scott	U.S. Geological Survey (emeritus)
H. Bradley Shaffer	University of California, Davis

Additional Workshop Participants:

Steve Anderson	U.S. Forest Service
Andrea Atkinson	U.S. Geological Survey
Chris Brown	U.S. Geological Survey
Ed Ervin	U.S. Geological Survey
Robert Fisher	U.S. Geological Survey
Stacie Hathaway	U.S. Geological Survey
Tim Hovey	California Department of Fish and Game
Robert Lovich	Marine Corps Base Camp Pendleton
Jamie Uyehara	U.S. Forest Service
Clark Winchell	U.S. Fish and Wildlife Service
Brian Yang	U.S. Geological Survey

1.0 SUMMARY

An all day workshop was held on August 27, 2002 to review and revise the ongoing monitoring protocol for arroyo toads at Marine Corps Base Camp Pendleton. The revised protocol focuses on monitoring the extent of arroyo toad habitat on base and monitoring the proportion area occupied (PAO) of arroyo toad habitat by arroyo toad tadpoles and egg masses. Potential arroyo toad habitat will be divided into approximately 50 blocks and each block will be subdivided into 5-7 sites approximately 200 meters long depending on the total amount of available habitat on the base. The final number of sites and length of each site will be determined after an assessment of the total amount of suitable habitat is completed. Sites will be surveyed for presence of arroyo toad tadpoles and egg masses in a rotating panel design with an estimated 5 or 6 year rotation. One site in each block will be surveyed every year (permanent), while a second site will be rotated within each block in different years (rotating). Most of these sites will be surveyed a 1-2 times annually after breeding has initiated with the visits occurring approximately one month apart. Eight of the 50 blocks will be considered "intensive" blocks and will be surveyed four times.

Table 1. Recommended number of sites per watershed *assuming a 5-year rotation*. Note: These numbers may be adjusted after the assessment of the amount of suitable habitat on base is complete. In this example, a block is made up of 6 sites: 1 permanent plus 5 rotating sites resulting in a 5 year rotation.

			# Regular sites per year		# Intensive sites per year		Total # sites across all years and blocks		
WATERSHED	#regular blocks	# intensive blocks	Perma -nent	Rota-ting	Perma -nent	Rota-ting	Perma-nent	Rota-ting	Total
Santa Margarita	22	3	22	22	3	3	25	125	150
San Mateo	15	3	18	18	3	3	18	90	108
San Onofre	5	2	7	7	2	2	7	35	42
All Watersheds	42	8	42	42	8	8	50	250	300

In addition, the eight intensive blocks should be used for short-term studies to refine key components of the protocol including identification of factors associated with the beginning of arroyo toad breeding, quantifying relationships between number of egg masses, number of tadpoles, and number of adults, examining habitat characteristics associated with tadpoles and egg masses, and examining the relationship between amount of time spent searching, habitat structure, and detection rate of tadpoles. Building upon previous pit-tagging work, toads at the 8 intensive sites will also be scanned for existing pit tags in order to evaluate longevity. Additional research recommendations are also discussed but are not included in the monitoring protocol.

Elements of the monitoring protocol are summarized in Table 2. In addition to the core elements of the monitoring protocol, additional protocol refinement work is also included in the table. A summary of the purpose of each protocol, equipment requirements, etc. is provided in chapter 7.

Table 2. Summary of elements of the Arroyo Toad Monitoring Protocol for Marine Corps Base Camp Pendleton. 2 trained personnel are required for all protocols The final number of sites and site length will be calculated after the total amount of suitable habitat is known.

Protocol	Core Protocol vs Protocol refinement	Purpose	When	Where
1. Habitat suitability classification and survey site selection: Initial classification of habitat as suitable/unsuitable for arroyo toads and survey site selection	Core	Determine extent of potential suitable arroyo toad habitat on base and divide up habitat according to the sampling design	Feb-March; First year & reassess at end of every panel rotation (5-7 yrs)	Portions of the estimated 81 km habitat on base where habitat suitability is unclear
2. Initiation of Breeding: Survey 3-8 of intensive blocks weekly first for calling males and then conduct egg mass surveys.	Core	Determine when arroyo toad egg mass laying initiates. Plan tadpole surveys to start 2 weeks later.	Feb.-Mar. Weekly until breeding initiates	3-8 of the intensive blocks (1-1.4 km long)
3. Proportion sites occupied – regular sites: 1-2 daytime surveys for presence of arroyo toad tadpoles and/or egg masses at “regular” sites. Conduct repeat visit if presence is not detected at first visit. Record habitat variables and presence of invasive species	Core	Monitor through time the proportion of sites occupied by arroyo toad tadpoles. Assess changes in arroyo toad habitat potential and changes in distribution of invasive species.	Mar. – June 1-2 daytime surveys	Regular sites (100-140 sites, 200 m long)
4A. Proportion sites occupied – Intensive sites (option A): 4 repeat surveys for presence of arroyo toad tadpoles and/or egg masses at “intensive” sites.	Core	Similar to #3 above. Additional repeat visits improve estimate of detectability of the protocol which is critical for the analysis	Mar. – July 4 daytime surveys	Intensive sites within Intensive blocks (16 sites, 200 m long, located within the 8 intensive blocks)
4B. Proportion sites occupied – Intensive sites plus protocol refinement (option B) 4 repeat daytime and nighttime surveys of entire “intensive” blocks in which numbers of egg masses, numbers of tadpoles, and numbers of adult arroyo toads are counted	Protocol refinement	In addition to 4A, determine whether changes in the number of egg masses, tadpoles, and adults correlate (or don’t correlate) with each other.	Mar. – July 4 daytime/ nighttime surveys	Intensive blocks (8 blocks 1-1.4 km long)
5. Metamorph surveys – 3 to 3 ½ months after egg masses are found, survey the 8 intensive blocks for presence of metamorphs and count number of abnormal metamorphs out of 50 at each block.	Protocol refinement	Determine whether tadpoles survived to metamorph stage. Determine abnormal development of metamorphs as an indicator of water quality and disease.	June-August 1 daytime survey	Intensive blocks (8 blocks 1-1.4 km long)
6. Optimizing detectability of tadpoles	Protocol refinement	Determine how detectability of tadpoles varies with walking speed, time of year, substrate, and observers	Mar. – June 4 times	2 to 4 of intensive sites (200 m long located within intensive blocks)

2.0 BACKGROUND

Purpose of Review

The purpose and intent of this project is to provide a scientifically valid and cost effective approach for monitoring arroyo toad (*Bufo californicus*) population trends on MCB Camp Pendleton until the species becomes federally delisted, or the Base terminates the program. The protocol will be employed in FY 2003. This monitoring program is an essential component toward meeting the Base's commitment to monitoring. The area to be included in the monitoring design includes the Santa Margarita River, San Mateo Creek, and San Onofre Creek, all of which contain large areas of high quality arroyo toad habitat.

The previous arroyo toad monitoring effort at MCB Camp Pendleton was developed by the environmental contracting firm CPARS (Camp Pendleton Amphibian and Reptile Survey). The final report "Linear Transect Censusing of the Arroyo Toad (*Bufo californicus*) from 1996-2000 on MCB Camp Pendleton" formed the basis for much of the discussion at the workshop and recommendations for a revised arroyo toad monitoring protocol.

Workshop process

Members of San Diego Field Station of the U.S. Geological Survey organized a workshop on August 27, 2002 to scientifically review the existing arroyo toad monitoring protocol results from MCB Camp Pendleton and make recommendations for any changes that might be necessary. Three scientists with a strong background in amphibian monitoring methodologies were invited to serve on a Scientific Peer Review Panel (SPRP):

Ted J. Case, University of California, San Diego
Norman Scott, U. S. Geological Survey (emeritus)
H. Bradley Shaffer, University of California, Davis

The panel was responsible for reviewing all required reading materials beforehand, providing strong review and recommendations regarding the monitoring protocol, and reviewing the resulting workshop summary and recommended protocol.

Additional representatives from USGS, USFWS, USFS, and CDFG were invited to the workshop to provide additional comments and increase coordination and understanding among all involved. These representatives were encouraged to join in the discussion and contributed strongly to the results of the workshop.

Prior to the workshop, USGS representatives assembled background information and reading materials which were mailed out to the Scientific Review Panel (see Appendix A) and put together a draft conceptual model for arroyo toad life history and risk factors to help guide the discussion (see Appendix B).

The MCB Camp Pendleton wildlife biologist, Rob Lovich, provided guidance on Camp Pendleton's history, management, and monitoring needs throughout the process.

During the workshop, the participants discussed and commented on the monitoring plan objectives, the draft conceptual model and how it specifically related to Camp Pendleton, the existing monitoring protocol, and the revised monitoring protocol recommendations and targeted study needs.

USGS representatives assembled the workshop results into this current document for review by the scientific review panel and other workshop participants.

<u>Review of linear transect protocol used from 1996-2000 (Holland *et al*, 2001a)</u>
The protocol used between 1996-2000 to monitor arroyo toads consisted of night-time linear transect surveys for adult and juvenile arroyo toads on 8 1km-long transects in the Santa Margarita, San Onofre and San Mateo watersheds on the base. Between 4-7 surveys were done on each transect per year except in 1996 when only 1-3 were done. Toads were captured, sexed, weighed, and measured. Microsite vegetative cover, air temperature, water temperature, water current, distance from shore, and substrate were also recorded. Based upon Holland *et al* (2001a) and a site tour by Rob Lovich, selection of these 8 sites was assumed to have been predicated upon 1) sampling from a wide geographic distribution of toad habitat on the base, 2) putting transects in known arroyo toad areas of occurrence, and 3) placing transects where they were easily accessible (they appear to start or end at a road or bridge or access point).

Some comments regarding the linear transect protocol by USGS personnel and participants at the workshop include:

- <u>High variability in results between surveys close in time: T</u>he linear transect survey data from Holland *et al* (2001a) show a great deal of variability in the number of toads seen even between surveys in the same year. Although 5 years of baseline data have been accumulated, given the large variability in the numbers, it is difficult to determine how to use these data to determine trends in toad numbers through time. Calculated confidence limits for most sites include zero captures, indicating the low resolving power of these results. However, the linear transect protocol does show presence of adult and juvenile toads in these 8 sites. Calculations of a threshold of the number of surveys with zero captures might be possible.

- <u>Limited ability to extrapolate to the base as a whole: I</u>t is unclear how to extrapolate results from these 8 sites to trends at the rest of the base, particularly since these sites appear to have been selected non-randomly.

- <u>Adult males may not be best indicator of population health: </u>From a logistical point of view, counting numbers of adult toads (especially calling adult males) might be a less sensitive indicator of a problem in arroyo toad breeding than focusing on an earlier life stage such as egg masses or tadpoles. However, the protocol does show presence of juveniles which would allow one to infer that breeding occurred during the previous year.

- Baseline years not easily standardized: The variability in the number and timing of surveys per year and in the number of sampling personnel per survey (1 or 2) might make using this 1996-2000 data as a "baseline" challenging.

- Stressors: The linear transect protocol does not consistently monitor stressors (i.e. bullfrogs, non-indigenous plants, changes in habitat).

The workshop participants pointed out that as much information as possible should be gleaned from the existing data set. This data set contains much valuable information. In addition, it should also be acknowledged that without this basic work performed in Holland *et al* (2001a), it would have been difficult for the workshop participants to develop the revised protocol that is described below. For example, without knowing the high variability within and among years in numbers of adults, monitoring the adult toads might have seemed intuitively the appropriate life stage to monitor.

3.0 OBJECTIVES OF MONITORING PROGRAM DESIGN:

The specific request in the scope of work is
"Monitoring protocol will:

(a) track trends of breeding arroyo toads for the entire base and each of the three occupied drainages
(b) develop a process to determine when trends may approach threshold conditions to warrant management actions or review
(c) not determine the entire arroyo toad population, measure in depth demographics or census upland habitats"

Discussion about the objectives:

The group commented that
1) The monitoring plan needs to be clear about what it can and cannot accomplish.
2) Objective B can be difficult to assess without more knowledge, although we can assess whether the proportion area occupied by tadpoles is stable over time.
3) The group had some problems with Objective C, since it was felt that some knowledge of demographics is necessary in order to establish what the natural range of variability is and what the thresholds for management actions should be. It was recommended that objective C be re-written. The group pointed out that they felt it was their role to propose the most scientifically defensible plan that the base would be wiling to accept.
4) The protocols should allow trends to be distinguished in each of the three watersheds individually as well as the base as a whole.

It was pointed out that the purpose of objective C was to avoid having the base involved in scientific studies with little direct application to meeting regulatory requirements and adaptive management needs on the base itself. It was also explained that a separate effort would be targeting monitoring in upland areas whereas this program is targeting riparian and wetland habitats. Targeted studies that will improve the efficacy of the monitoring program and management and whose direct application to the base is clear would be considered.

4.0 MONITORING PROTOCOL:

Overview

The basic questions that this monitoring program will be used to answer are

- Arroyo toad habitat: What is the extent and distribution of arroyo toad habitat? How is that extent and distribution changing? What is the quality of arroyo toad habitat on base and is it changing?
- Proportion area occupied: What is the proportion of arroyo toad habitat occupied by arroyo toad tadpoles during the breeding season? How does this proportion change with changes in annual precipitation? How does this proportion change in response to pressures such as invasive plants, disturbance by military activities, changes in water management, etc.?

Monitoring of tadpoles was chosen because it was felt that 1) the detectability of tadpoles should be higher and more consistent than the detectability of adult toads, and 2) tadpoles more directly reflect whether successful breeding is or is not occurring. Although some of the workshop participants felt that counting abundance of arroyo toad tadpoles would be difficult because they do not school in large groups, all agreed that detecting presence of the tadpoles should be relatively easy.

The overall monitoring scheme should consist of

1) Assessing changes in the amount and distribution of potential arroyo toad habitat every 5-10 years
2) Potential arroyo toad habitat will be surveyed annually for proportion of area occupied by arroyo toad tadpoles in a rotating panel design using repeated visits to sites to estimate arroyo toad tadpole detectability.
3) "Intensive" sites will be used to gather additional information to refine the protocol and provide information for management to help interpret overall trends. These should preferably be co-located with the previous 8 sites used by Holland *et al* (2001a)

However, it was cautioned that this protocol will need to be tested and refined and the assumptions on which it is based will need to be tested. A key issue is whether the proportion area occupied by tadpoles reflects the status of adult arroyo toad populations.

What is PAO (Proportion Area Occupied)?

The Proportion of Area Occupied (PAO) or more appropriately the "proportion of sampling units occupied" refers to a method of measuring changes in the amount of habitat a species occupies as described in Mackenzie *et al* (2002). The USGS Amphibian Research and Monitoring Initiative (ARMI) is piloting this approach on

Department of the Interior lands throughout the United States.
(http://edc2.usgs.gov/armi/monitoring.asp)
For species such as amphibians which typically show wide annual variation in abundance, measures of habitat occupancy across a wide range of sampling units is hypothesized to be a more useful measure of the status of the population than measuring abundance at a few sites.

However, the ability of the protocol to detect a species on any given visit is frequently less than 100%. That means that even if a species is not detected on a visit, it may in fact still be present. Mackenzie *et al* (2002) presents a method for calculating the true proportion of sampling units occupied by taking the number of sites at which the species was actually detected and revising the number upward based upon the detectability of the protocol. The detectability of the protocol is estimated by doing repeated visits to the sites. This can be done in several ways: 1) by visiting all sites the same number of times (typically 2-6), 2) by visiting "regular" sites a few times (2-3) and a subset of "intensive" sites visited more frequently (4-6), or 3) by revisiting sites only when a species was not detected during the first visit with a maximum of 2-3 visits for regular sites and 4-6 visits for intensive sites.

For example, if 100 sites were visited twice and 45 sites were occupied during the first visit and 56 sites occupied during either the first or second visit and detectability was estimated at 75%, the final estimate of proportion of sites occupied would be 0.60 (60 sites) with a standard error of 0.056.

Mackenzie *et al* (2002) have developed a trial version of computer software that estimates the true proportion of sites occupied called "PRESENCE" which is available on the web site: http://www.proteus.co.nz/
Additional covariates that may affect detectability (i.e. weather) or presence of the species (habitat variables) can also be included.

<u>Proportion Area Occupied - Pilot Study Design</u>
Potential arroyo toad habitat on the Base will be divided into approximately 50 blocks. Each block will consist of a set number of survey sites that are approximately 200 m long. Sites will be surveyed for presence/absence of arroyo toad tadpoles and egg masses in a rotating panel design. One site within each block will be surveyed every year. The remaining sites in each block will be surveyed once within a rotation of several years. Thus 2 sites within each block will be surveyed each year, one permanent site and one rotating site, for a total of 100 sites surveyed each year (see Table 3). The number of years in the rotation will be determined after the total amount of potential arroyo toad habitat is identified but will likely be five or six years. Each of these sites will be visited a maximum of 2 times annually in the appropriate year. A second visit will only be performed if tadpoles or egg masses are not detected at the first visit. A subset of 8 blocks will be treated as intensive blocks and will be visited more frequently (4 times a year) for presence of tadpoles or egg masses. All 4 visits will be conducted on the intensive sites, even if tadpoles are detected at the first visit, in order to more

Table 3. 5-Year Rotation pattern among groups of sites

Group	# Sites	2003	2004	2005	2006	2007	2008	2009	2010	2011…
Perm (all yrs)	50	X	X	X	X	X	X	X	X	X
A=Year 1	50	X					X			
B=Year 2	50		X					X		
C=Year 3	50			X					X	
D=Year 4	50				X					X
E=Year 5	50					X				

accurately estimate detectability of tadpoles by the protocol and improve accuracy of the calculations. The intensive sites will also be used to conduct the protocol refinement studies.

The number of sites (100 sampled per year) was determined based upon simulations run using the computer program PRESENCE plus the estimate of the amount of arroyo toad habitat on base. For more details see Appendix C. This number of sites should allow detection of *at least* a 50% decrease in site occupancy with an alpha of 0.05 and power of 80% assuming that true tadpole presence is only detected 60% of the time by the protocol. (An alpha of 0.05 means that if there was no true difference in site occupancy, then only 5% of the time would a difference be considered statistically significant by random chance. A power of 80% means that if there is in fact a true difference in site occupancy, that difference will be detected in 80 of 100 times). If detectability of true tadpole presence is higher, e.g. 80%, then the decline detectable by the protocol also improves, e.g. 32% decline in site occupancy instead of 50%. Survey sites would be broken up among the three watersheds approximately as shown in Table 4. Fifty of the sites would be permanent and the remaining 250 or 300 sites would be rotated in a 5 or 6-year rotation (50 sites per year).

Table 4. Recommended number of sites per watershed *assuming a 5-year rotation*. Note: These numbers may be adjusted after the assessment of the amount of suitable habitat on base is complete. In this example, a block is made up of 6 sites: 1 permanent plus 5 rotating sites resulting in a 5 year rotation.

			# Regular sites per year		# Intensive sites per year		Total # sites across all years and blocks		
WATERSHED	#regular blocks	# intensive blocks	Perma -nent	Rota-ting	Perma -nent	Rota-ting	Perma-nent	Rota-ting	Total
Santa Margarita	22	3	22	22	3	3	25	125	150
San Mateo	15	3	18	18	3	3	18	90	108
San Onofre	5	2	7	7	2	2	7	35	42
All Watersheds	42	8	42	42	8	8	50	250	300

Initial classification of habitat as suitable/unsuitable for arroyo toads and site selection
MCB Camp Pendleton has approximately 81 km of potential arroyo toad habitat based upon estimates from the computer program TOPO! Maps and comparison with the map of arroyo toad locations given by Holland and Goodman (1998) (See figure 2). Approximately 10% of the potential arroyo toad habitat on the base is "off-limits" to sampling due to safety reasons and will be excluded.

Habitat on base will first be classified as suitable (potential) or unsuitable habitat for arroyo toads. The upper and lower extents of potential arroyo toad habitat will be identified (i.e. tidal zone to changes in topography away from arroyo toad breeding habitat). Areas suitable and unsuitable to arroyo toads will be mapped using GIS and TOPO! Maps followed by ground-truthing. Areas unsuitable for arroyo toads are expected to be riparian habitat with greater than 3% slope, or lacking sandy substrate, or inundated with brackish water from the estuary. Habitat that has no potential as arroyo toad habitat will be excluded. Otherwise all potential arroyo toad habitat will be included in the sampling design.

Potential arroyo toad habitat will be divided into approximately 50 blocks, depending on the total amount of habitat available. Each block will be divided into 6 or 7 survey sites approximately 200 m long. Each block will contain one permanent site and five to six rotating sites. The final number of sites per block (i.e. length of the rotation) and the final length of each site will be calculated after the actual amount of potential breeding habitat is more accurately determined. Within each block, the years that the sites will be sampled will be randomized (See Figure 1).

The 8 "intensive" blocks will be located within the 8 survey transects used by Holland et al (2001a) because the toads previously pit-tagged by Holland should be in this area.

Figure 1. Stream with two blocks of 6 200m-long sites in which the years they are sampled has been randomized. "Perm" = Permanent sites surveyed in all years. See also Table 3.

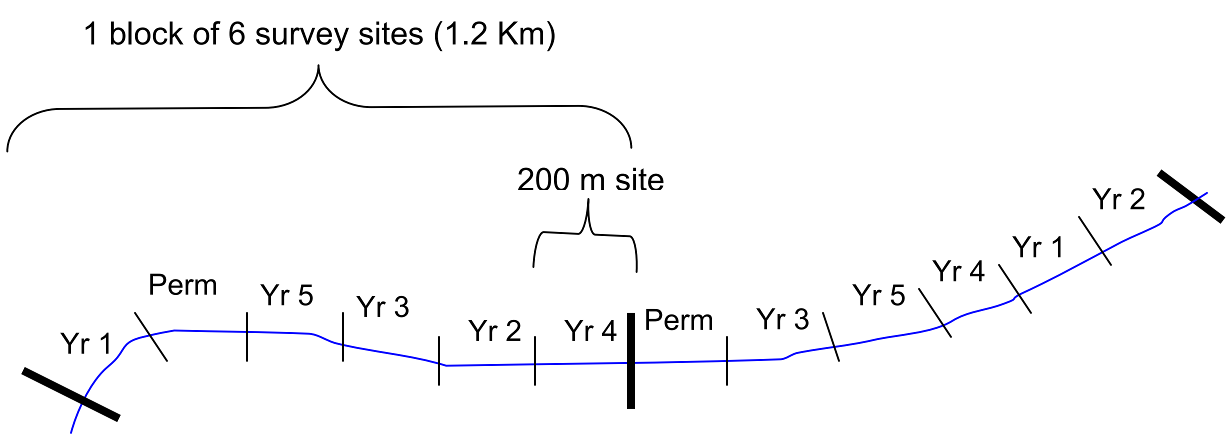
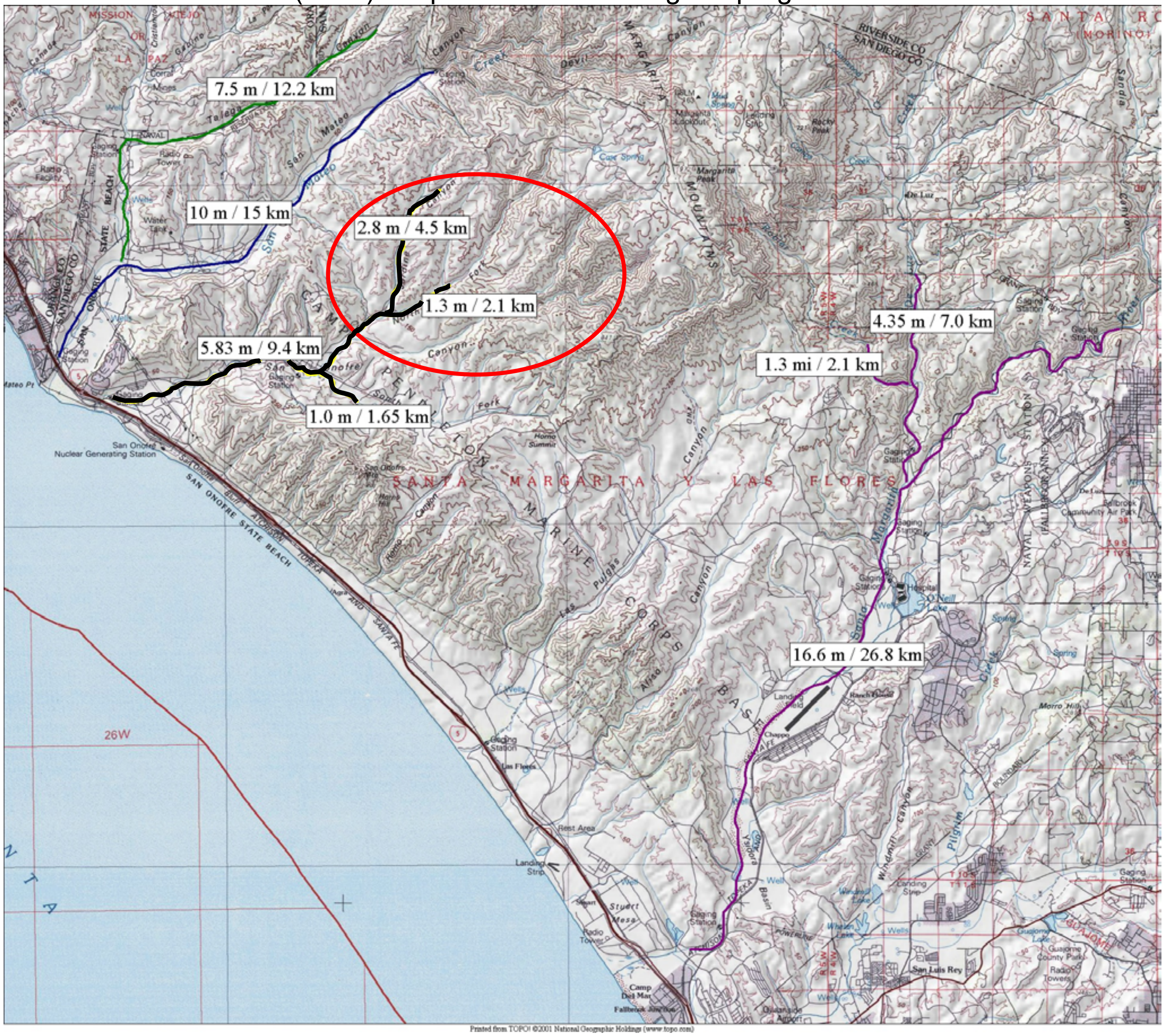


Figure 2. Map of potential arroyo toad habitat and approximate distances derived from arroyo toad locations given by Holland and Goodman (1998). Map was created using the program TOPO!.



Arroyo toad habitat off-limits to sampling due to safety reasons

Initiation and timing of sampling

Once weather temperatures and precipitation and time of year are right to expect calling males, surveys for calling males will be conducted at the 8 intensive sites or a subset thereof. Once arroyo toad males begin calling, weekly surveys will be conducted for egg masses or pairs in amplexus which will signal the actual start of breeding. Variables such as water temperature using Tidbit data loggers, water depth, cloud cover, conditions (clear, overcast, foggy, rainy), and whether moon is visible should be taken. Variables such as moon phase, high and low air temperatures, and precipitation amount and timing can be ascertained from a nearby weather station. All of these factors will be compared with the initiation of toad breeding in order to better refine the model for initiation of breeding presented in the *Arroyo Southwestern Toad Recovery Plan* (USFWS, 1999).

Methodology and variables recorded at each survey site

Approximately two weeks after toads start calling and breeding is confirmed to be occurring at the “intensive” sites, the first of two visits will be made to each survey site. The second visit will be made 1 month later. Since the time to sample all 100 sites will take about 2.5 weeks, the sites will be sampled proportionally each week across the three watersheds to avoid biases caused by sampling all of one watershed 2 weeks before another.

The amount of wet potential arroyo toad habitat within the 200 m site will be estimated. Two observers will walk in the water and on the shore and record the presence of arroyo toad tadpoles within that survey site. Surveyors walking in the water will be especially mindful so as not to disturb sediment that could cover egg masses and negatively affect development of arroyo toads. The presence of arroyo toad egg masses will also be recorded. Surveys will be done during the daytime.

If after walking the entire 200 meters, arroyo toad tadpoles are not observed, the observers will go back to the areas in the site with the presumed highest quality arroyo toad habitat and continue searching for a set amount of time. This amount of time will initially be 20 minutes, but will be revised after a short pilot study is completed to assess the relationship between search time and detection of tadpoles. If after that set amount of time is complete and tadpoles are not found, the site will be recorded as lacking tadpoles during that survey period.

In this pilot phase, three different metrics will be evaluated: a) proportion total sites occupied by tadpoles, b) proportion sites occupied by tadpoles or egg masses, and c) proportion of sites with wet arroyo toad habitat.

Table 5. Monitoring elements and rationale

General Category	Monitoring elements	Purpose
Key variables recorded at all sites	Is water present in survey site? Presence/absence of arroyo toad tadpoles Presence of egg masses Age category of tadpole	Assess - Proportion sites occupied by tadpoles - Proportion sites with wet suitable habitat and - Proportion sites occupied by tadpoles or egg masses.
Additional habitat variables at first egg mass and/or tadpole sighted (if no tadpole or egg mass is observed at site then measure in pool in best habitat at site)	Time of day Category of substrate where tadpole was found, e.g. sandy bottom, detritus, algal mass, cobble. Are egg masses being covered by excessive sand or silt (excessive turbidity in water)? Percent cover Water depth Water velocity category Water Clarity/Turbidity (can bottom of breeding pools be seen)	Assess - What habitat variables are associated with presence of tadpoles and egg masses? This may become a cofactor later in assessing detectability. - Are egg masses being affected by excessive turbidity?
Additional variables recorded at each site	Weather category (clear, partly cloudy, cloudy/overcast, mist/light drizzle, light rain, heavy rain) Average width of survey site Wet length in survey site Does site have poor, marginal, good, and high potential for arroyo toad breeding based upon gradient, sandy substrate, sandy terraces, and channel braiding? (see page 15) Total number of arroyo toad breeding pools <2 feet in depth and with a sandy bottom Total number of pools > 2 feet in depth Water temperature Air temperature Dominant vegetation type Presence of invasive plants within 50 m of stream (arundo, tamarisk, watercress, fennel) and size category (a few plants, scattered small patches, large contiguous stands) Presence of invasive fish Presence of bullfrogs, crayfish, or African clawed frog. Signs of recent vehicle disturbance Comments on other disturbances/threats (excessive trash) General Comments Observers Survey site number. Start and ending GPS coordinates Start and end time of transect	Assess - What weather variables may influence tadpole detectability? - How much arroyo toad habitat is wet this year? - How much arroyo toad habitat is available and how suitable is it? - Is the amount of arroyo toad breeding habitat changing over time? - Are invasive plants expanding into new areas? Are these associated with changes in occupancy? - Are invasive fauna expanding into new areas or obviously increasing in numbers? Are these associated with changes in occupancy? - Are other signs of disturbance or concern visible?

Assessing potential arroyo toad habitat based upon physical characteristics

As each site is surveyed for presence of arroyo toad tadpoles, it will also be classified as having "high", "good", "marginal", or "poor" arroyo toad breeding habitat potential based upon physical characteristics. The criteria used to identify areas as potential arroyo toad breeding habitat within rivers and streams are first having a gradient (degree of slope) of ≤3 % and preferably less than 2.5%, and then having one or more of the three following physical characteristics: 1) channel substrate type being predominately composed of sand; and 2) the presence of flat sandy terraces immediately adjacent to channel, and 3) having a watercourse of braided channels. Arroyo toad habitat on base is found throughout the drainages although it may shift locally due to periodic flood events. If a river has a slope of less than 3%, then the potential of the habitat for arroyo toad breeding is classified as high, good or marginal based on the number of these physical characteristics that are present. (see Figures 3 and 4).

In brief, as the surveyor walks each 200 meter site, they will determine

- Slope (%) (determined initially from TOPO!® Maps, and then more precisely from start and ending GPS Coordinates)
- Is sandy substrate present (yes/no)?
- Are adjacent sandy terraces present (yes/no)?
- Is channel braiding present (yes/no)?

Which will be used to classify the habitat potential.

At the end of an entire rotation (5 or 6 years), all sites on the base will have been mapped as having high, good, or marginal potential arroyo toad habitat, as well as the excluded unsuitable habitat mentioned earlier. Changes in the amount and location of different habitat categories can be tracked through time.

Figure 3. Potential of habitat for arroyo toad breeding based upon the following physical characteristics: slope of 3% or less plus the presence of one or more of the following: 1) sandy substrate, 2) flat sandy terraces, and 3) braided channels.

	Number of additional physical characteristics present			
Gradient (slope)	**3 of 3**	**2 of 3**	**1 of 3**	**0 of 3**
≤ 3 %	High	Good	Marginal	Poor

<u>High:</u> ≤ 3 % gradient with all three physical characteristics (sandy substrate, flat sandy terraces, and braided channels)

<u>Good:</u> ≤ 3 % gradient with two of the three physical characteristics

<u>Marginal:</u> ≤ 3 % gradient with one of the physical characteristics

<u>Poor:</u> none of the three physical characteristics

Figure 4. Example of arroyo toad habitat with high potential for arroyo toad breeding based upon the following physical characteristics: slope of less than 3%, sandy substrate, flat sandy terraces, and braided channels.

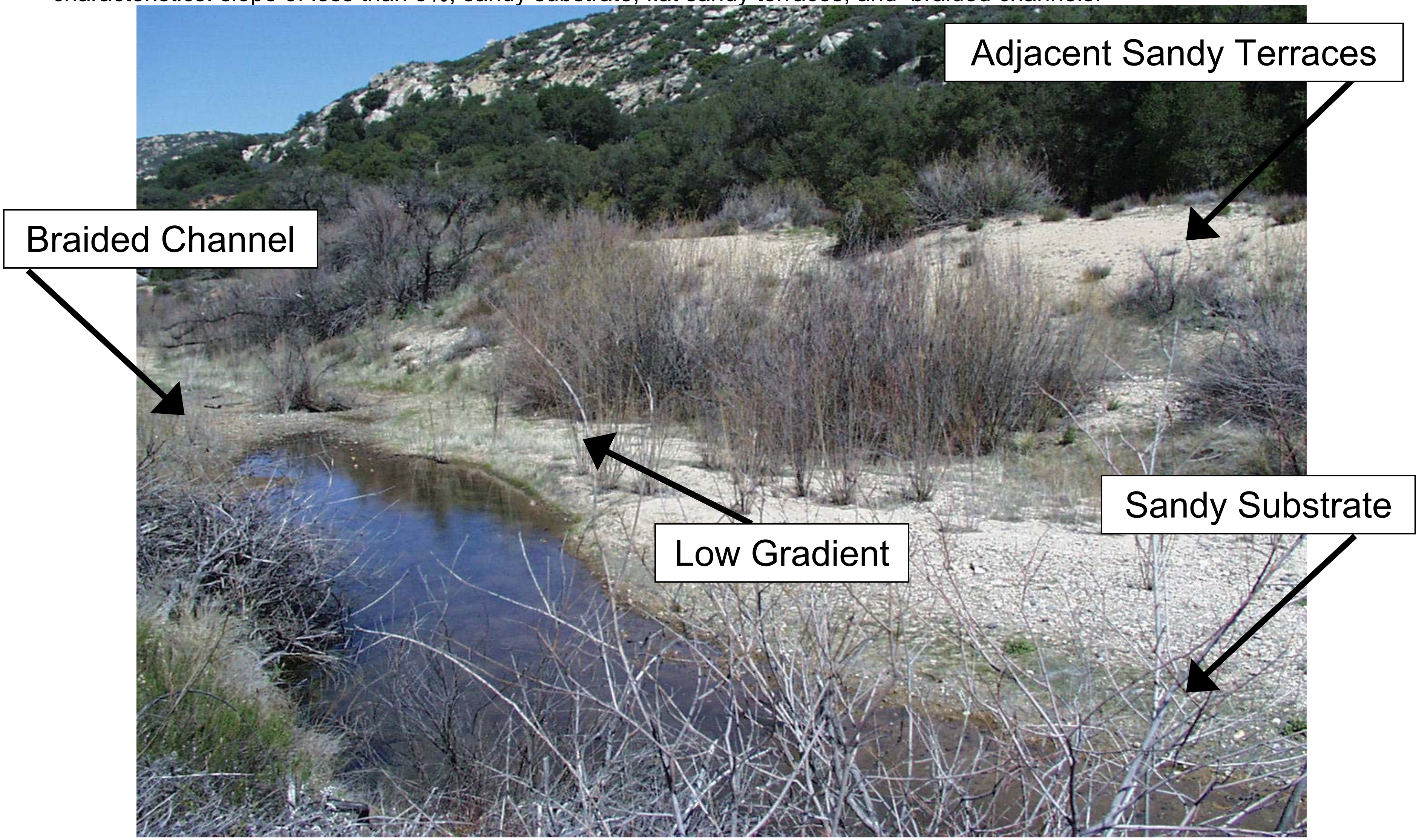
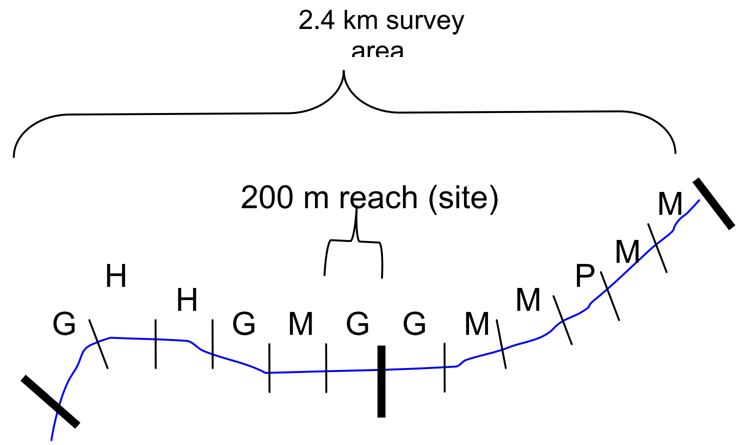


Figure 5. Example of a two, 1.2 km blocks of stream. As each 200 meter is surveyed for arroyo toads, the arroyo toad habitat potential will also be classified according to the number of physical habitat characteristics present. All 2.4 km had at least portions with a gradient of < 3%. The number of physical characteristics present in areas of <3% slope determines the classification of habitat as High (H), Good (G), Marginal (M), or Poor (P) potential arroyo toad habitat.



In this example, 2 sites were classified as high (H) potential arroyo toad habitat, 4 sites were classified as good (G) potential, 5 sites as marginal (M), and 1 site as poor (P).

<u>Methodology and variables recorded at each "intensive" site</u>
Each intensive block will also be used for some protocol refinement studies. Since each block is composed of 6 or 7 200m sites, the entire block will be approximately 1.2-1.4 km in length. These blocks will be placed at the locations of the original transects used by Holland *et al* (2001a).

The intensive sites will generally be used to 1) perform a higher number of presence/absence surveys to increase the accuracy of the proportion area occupied estimate, 2) evaluate when arroyo toad breeding is initiating (as described above), 3) evaluate the relationship between # adults, # egg masses, # tadpoles, 4) refine various aspects of the protocols such as the amount of time observers should spend searching before declaring tadpoles "absent", and 5) potentially evaluate longevity of arroyo toads using toads already pit-tagged by Dan Holland *et al* (2001a)

Once the breeding season has begun, 4 night-time surveys, every 2-3 weeks, will be conducted in each intensive block (all sites within the block) counting the number of adult toads, number of tadpoles, and number of egg masses. Pit tag readers will be scanned across adult toads to see if they contain pit tags and to capture the pit tag numbers. This may require minimal handling of toads. Toads in amplexus will not be disturbed.

A final daytime survey in the late-spring/summer should be conducted for the presence of metamorphs at these 8 sites. Metamorphs may begin appearing from 77 to 105 days after egg laying. Any observed deformities among metamorphs should also be recorded. Deformity rates in a count of 50 metamorphs per site is a typical bioassay method (maximum of 400 across all 8 sites). Increases in deformity rates can indicate a decrease in water quality due to contaminants or can indicate disease or pathogen problems (Blaustein and Johnson, 2003). Changes in water quality can be a concern for both amphibians and humans.

Additional variables that will be measured are the same as the regular sites described above.

Training

Personnel involved in arroyo toad surveys will need to receive standardized training in species identification at the different lifestages as well as in the specific protocols of habitat assessment.

Egg masses: USFS personnel noted that arroyo toad egg masses can sometimes be confused with *Bufo boreas* egg masses, especially early in the season. Experienced personnel can tell them apart. But inexperienced personnel can mis-identify *Bufo boreas* egg masses as arroyo toad egg masses early in the season. It is recommended that such early identifications be re-checked in subsequent weeks when the egg mass is older or with the presence of arroyo toad tadpoles. USFS personnel felt that the error rate was only 10% at the worst.

Tadpoles: arroyo toad tadpoles can be confused with *Hyla cadaverina* tadpoles, so training of correct recognition of arroyo toad tadpoles will be important as well.

Exotic species: Some additional training will be necessary for recognition of exotics that are considered important to record.

Additional monitoring needs

Since alterations in flows and alterations in water quality are a concern with regards to how they will affect arroyo toads in addition to how they will affect human activities, it is recommended that the base biologist coordinate with existing water flow monitoring and water quality monitoring occurring in the rivers at the base and upstream of the base to acquire this monitoring data. If arroyo toad populations appear to be declining, the data will be available to assess whether there appears to be a correlation between long term changes in water quality and flows and changes in the extent of arroyo toad breeding habitat and in occupancy by arroyo toads. While not proving that one causes the other, a correlation can provide working hypotheses that can be tested, possibly with experiments.

There is increasing concern about presence of estrogens in treated sewage effluent and estrogen mimics (e.g. atrazine) in agricultural water runoff and what those effects may be on wildlife (Hayes *et al*, 2002). Often this is not monitored in general water quality measures. At the minimum it might be wise for Camp Pendleton to take a baseline measure of estrogen and estrogen mimic levels in water in the Santa Margarita and the

San Mateo or save samples for later analysis if the need arises. Then these data would be available for comparison in the future if concerns are raised about whether estrogen levels have increased.

<u>Analysis and Interpretation:</u>
Due to widely fluctuating annual precipitation patterns, the pilot study should be continued for 5-10 years to establish a baseline for how arroyo toad occupancy varies with annual precipitation. Once the baseline range of variation is established and assuming that a decline is not occurring during the first 5-10 years, tentative warning limits can be established for management, for example, if occupancy falls outside the 90% confidence limits of the expected occupancy.

During the first 5-10 years before a baseline is established, if two years are determined to have significant differences in proportion area occupied, then a series of questions can be asked to help determine if management intervention is needed:

Is there an associated change in the amount of annual precipitation and amount of wet habitat? (annual precipitation should be available from a local weather station; amount of wet habitat is assessed on the ground during surveys)

If the amount of precipitation and wet habitat do not appear to be a concern, then is there a decrease in the amount of arroyo toad breeding habitat within the sites (i.e., open sandy areas where breeding can occur?) Are exotic plants reducing this area? Are flushing flows needed to periodically reconfigure the habitat?

Is there a pattern to the decrease in occupied sites that can be linked to a cause? Is the decrease all occurring on one watershed or all watersheds? Are declines associated with increased incidence of arundo or tamarisk? Has a new exotic plant or animal species been observed such as African clawed frogs or fire ants? Are new training activities being conducted in areas where occupancy is decreasing?

Is there a decrease in the number of breeding females? (at the intensive sites this can be assessed by counting the number of egg masses as well as looking at egg mass to adults ratios) What was the proportion area occupancy in the previous year?

Is there a decrease in egg mass and tadpole survivorship? At the intensive sites, are metamorphs observed? At the regular sites, were problems with excessive siltation of egg masses or vehicle damage observed? Were there major storm events that could have washed away egg masses and tadpoles? (this could be assessed from storm records and gaging station data)

It may not be possible to discern the cause of a decline from the above design although some hypotheses may be generated and others excluded. More focused studies may be needed such as investigating whether tadpoles are infested with a disease, comparing current water quality and estrogen levels with a baseline level, investigating the associations between a new exotic and arroyo toads, or determining whether water management is causing flushing out of egg masses and tadpoles or conversely, excessive mortality through desiccation.

Other Comments
Adult night time surveys: although it was felt that the tadpole monitoring protocol should be refined by comparing it with other life stages, the workshop participants couldn't justify having an ongoing adult nighttime survey program beyond this protocol refinement period since the variability in the data was so high. However, leaving out some simultaneous check on adult populations to go with the tadpole monitoring made several participants nervous since trends in one life stage are not always related to trends in another life stage. Counting numbers of egg masses should provide a surrogate for number of breeding females, since females only lay one egg mass in a season. However since USFS found that number of egg masses correlated strongly with the amount of rainfall, this may indicate that not all females breed every year.

Using coverboards to monitor arroyo toad metamorphs was discarded as an idea since this might provide habitat for argentine ants.

5.0 PROTOCOL REFINEMENT STUDIES

The monitoring protocol as described should be considered a pilot study. The workshop participants mentioned several questions which should be considered to better refine the recommended protocol.

P1) Factors that influence tadpole detectability and time spent searching: What is the relationship between the amount of time spent searching and a) detectability, b) # tadpoles and c) life stage (size) of tadpoles. How long do you search before saying tadpoles are not detected at a given 200 meter stretch or a sub-section within that 200 meter stretch?

A pilot study should be conducted to evaluate the amount of search time that should be spent per site before declaring a site "tadpoles not detected". Two surveyors, an observer and recorder, will go up and down a 200 m stretch 2 times at different walking speeds

Minutes	Walking speed	# Tadpoles	% detected from those detected at 30 minutes
10	20 m/min	10	45%
30	7.5 m/min	22	100%

One surveyor will record while the other calls out sightings of arroyo toad tadpoles and egg masses. Locations and approximate number of tadpoles and number of egg masses at each sighting should be recorded. The recorder also silently records additional sightings that they see but the observer does not. After every 100 meters, observer and recorder will switch. Locations should be marked on a map as well as entered into the data form.

The substrate each tadpole and egg mass is located within should also be recorded. Detectability can then be compared under different habitat characteristics such as open sand, algal mats, cobblestone, etc. i.e. do the tadpoles that are missed occur more frequently in algal mats?

The method described will also allow assessment of observer variability. This can be used to better standardize and improve the search protocol and, possibly, be factored into the analysis.

Site/habitat characteristics that influence detectability should be recorded at all sites, i.e. open sand, algal mats, cobblestone, amount of water.

Other factors that influence detectability should also be recorded at all sites, i.e. cloud cover, rain, water temperature, air temperature, and water depth.

This study should be repeated at 4 times during the season.

<u>P2) Which environmental variables and biological factors are associated with the initiation of breeding?</u> Does breeding start at the same time throughout the watershed? Is it possible to establish a few sentinel sites to signal the start of breeding?

A more rigorous model of when arroyo toad breeding starts is needed in order to increase efficiency of monitoring and to optimize the timing for the tadpole surveys. Variables to consider include rainfall, temperature, degree-days, weather, river & stream flows, change in temperature, moon phase (may be obstructed by marine layer) etc. Therefore, it is likely that initial monitoring efforts will need to be more intensive than what may be needed later in the program. In order to determine when breeding starts, workshop participants suggested using nighttime adult surveys, frog call data loggers, and/or weekly surveys for egg masses.

This study is described above under "*Initiation and timing of sampling".*

<u>P3) Which habitat variables are associated with egg masses and tadpoles?</u>
The habitat variables associated with egg masses and tadpoles will be evaluated from data collected at the 8 intensive sites, but should also be recorded whenever tadpoles and egg masses are located at the more general presence/absence sites.

<u>P4) What is the relationship between # adults, # tadpoles, and # egg masses?</u>
What are relationships between # egg masses, tadpoles, metamorphs, adults? Is there a strong correlation or do number of egg masses and number of tadpoles more directly relate to the amount of rainfall and streamflow? Do trends in Proportion Area Occupied (PAO) in one life stage reflect trends in another life stage?

The PAO tadpole monitoring described above assumes that this metric is a good method of tracking the status of arroyo toad populations. However, the relationships between the number of egg masses, tadpoles, metamorphs and adults should be verified using the 8 intensive monitoring sites. Metamorphs could be targeted only at sites where tadpoles are found. If it turns out to be virtually certain metamorphs are found when you find tadpoles, then one can eventually drop this part . This study is mentioned above under "*Methodology and variables recorded at each "intensive" site"*

P5) How do we distinguish between seasonal changes in population size and actual decreases in population?

It is important to understand how long it takes to initiate the various life stages. This will improve the monitoring and also help us to distinguish between seasonal changes in population size and actual decreases in population size. USFS has 3 years of similar data that could be used as a framework to take us from a conceptual model to a quantifiable model that we can test. The data gathered from monitoring the number of egg masses, tadpoles, metamorphs, juveniles, and adults at the "intensive" sites will assist with answering this question.

P6) Should the 200 meter stretches be sub-divided into smaller units and a proportion occupancy be determined for the entire 200 meter stretch versus just presence/absence?

Determining the proportion occupancy of each 200 meter stretch rather than just presence/absence was suggested at the workshop. This will require more work than just determining presence/absence per 200 meter stretch since it will require walking the entire stretch even if a tadpole is seen within the first 10 meters. The amount of additional work in exchange for the additional specificity of the data will need to be evaluated and probably can be done in conjunction with the search time study mentioned under protocol refinement study P1.

6.0 RESEARCH AND ADAPTIVE MANAGEMENT STUDIES

While commenting on the conceptual model and making recommendations regarding the monitoring protocol, the workshop participants identified several areas for research that would improve understanding to assist with management of the arroyo toad, e.g. improving understanding of toad longevity and understanding of relationships between toads, stressors, habitat, and response to potential management actions.

Of the following research items, only item R1 (toad longevity) had concurrence that it could be started immediately since toad longevity should be fairly easy to assess by taking readings for pit-tags from adult arroyo toads at the 8 intensive sites and comparing them with the original tag numbers from Holland *et al* (2001a), provided that the original pit tag data are accessible. The other research items were suggested as good ideas for investigation, but are not included in the pilot monitoring protocol.

The workshop participants emphasized the need to treat management actions such as exotic weed and fauna control treatments as adaptive management experiments and to set them up using designs such as Before-After-Control-Impact (BACI) studies whenever possible. This is especially important when uncertainty exists either in the effectiveness of the action or the short-term or long-term impacts on arroyo toads. Study sites could be selected to dovetail with monitoring program design.

R1) Longevity: How long do arroyo toads live? Suggestions were made to use the current pit-tagged toads and skeletonchronology. It was pointed out that if skeletonchronology is to be used, it is extremely valuable to validate the technique by having known-age animals to age in a blind experiment. Coordination with other research efforts in southern California would be advisable.

R2) Identify core arroyo toad population areas Some of the regular presence/absence monitoring may reveal this, but additional detail may be needed.

R3) What is the frequency distribution of arroyo toad distance from creek? This could provide useful information for management decision-making. However this important question would probably be considered an “upland” issue and should probably be considered in the upland monitoring effort.

R4) Hydrologic effects on toads.
There is a combination of natural, managed, and experimental flow regimes on the base. Flow amount and timing will likely be changing on the Santa Margarita. Toad habitat may in fact be artificially enhanced by flow changes compared to historical flow patterns. Are there ways to manage flows that meet human needs while minimizing negative impacts on the toads?

What is the effect of alterations in flow and groundwater pumping on arroyo toads? How do changes in flows and flow releases from dams affect flows at river and stream margins where arroyo toad breeding habitat occurs? Are these alterations affecting the amount of breeding habitat? Are they resulting in increased mortality for arroyo toad eggs and tadpoles? Are they associated with an increased presence of exotic species? How do factors such as beaver ponds and vegetation such as arundo affect flows and dry-down rates?

Some suggestions were made regarding how to study this topic, but more work is needed. Some opportunities to evaluate these questions include looking at existing data from Pyramid Dam and subsequent effects on arroyo toads (see USFS).
Most gaging stations measure flows in the middle of the river rather than along margins which are more important for the toads. Data loggers of flow, drying times, and temperature profiles along river margins would potentially be helpful. Experiments using percolation ponds were also suggested.

R5) What is the interaction between microhabitat, predation and desiccation? The interaction between shallow water stream margins, drying rates, and predators may be a more important factor in arroyo toad tadpole and metamorph survivorship than changes in water depth and velocity in the middle of channels. Risk of predation may increase as pools begin to dry.

R6) Exotic species removal experiments (bullfrogs, exotic fish): Bullfrogs and invasive fish species are found throughout all three watersheds and it is unlikely they can ever be completely eliminated. Opportunities exist in the San Mateo watershed for bullfrog and exotic fish removal during dry years since they are restricted to a few remaining pools. This might successfully reduce numbers for several years at a time. Such removal might also be coordinated with efforts to improve habitat for steelhead. On the remaining watersheds, the workshop participants suggested that bullfrog and exotic fish removal activities might be more effective if they are performed just before arroyo toad breeding and when the arroyo toads could really use assistance (maybe during drier years?). It was also suggested that targeting bull-frog egg masses might be more effective than targeting bullfrog adults.

R7) Arundo removal effects on toads Arundo control activities could be set up as before-after-control-impact experiments to test the effects of arundo removal techniques and timing on control of arundo and on response of arroyo toads to the removal itself and subsequent recovery after removal.

R8) Agriculture tillage effects on arroyo toads: How does agricultural tillage affect arroyo toads? Does this cause increased mortality? Is the timing of tillage an important factor? If there is an impact, is it possible to modify agricultural practices to reduce the impact? Agriculture is limited to a small area in Camp Pendleton, so this may be a research issue for other groups. Davidson *et al* (2002) found that the major variables associated with declines of *B. californicus* were percent of land urbanized and percent land in agriculture within a 5 km radius of a historical site.

R9) Estrogens in treated sewage effluent and agricultural runoff effects on amphibians: Treated sewage effluent is expected to increase in the Santa Margarita. Concerns have been raised that sewage effluent has increased levels of estrogens in it (from birth control pills) and the effects on arroyo toad populations and especially on egg and larval stages is unknown. In addition, the herbicide atrazine has been linked with increased hermaphrodism rates in amphibians (Hayes *et al*, 2002).

7.0 SUMMARY OF ARROYO TOAD MONITORING PROTOCOL AND PROTOCOL REFINEMENT

The following protocol elements, #1-#6, summarize the actual protocol of what surveyors are expected to do in the field. Elements #1-#4a are considered "core" elements of the monitoring protocol. Elements #4b-#6 are considered "protocol refinement" elements. Element #4a contains the minimum required by the protocol. Element #4b would accomplish what is needed for the "core" monitoring program plus provide additional information for protocol refinement.

1) Habitat suitability classification and survey site selection

Purpose: Assess the range of potential arroyo toad habitat on base and what areas can be permanently excluded from sampling. Divide potential arroyo toad habitat in blocks and sites within blocks.

Frequency: First year of protocol and reassess at end of each panel rotation

Sample Period: February/March

Pre-sampling checklist:

Inform base contact of sampling dates in advance (base contact may accompany monitor). The driver will request vehicle pass. Two four-wheel drive vehicles recommended but may not be required for all sampling areas. Check out VHF radio if recommended by the base contact. Be familiar with base regulations and access routes.

Protocol: Riparian habitat on MCB base will be evaluated using GIS coverages and TOPO! maps followed by ground-truthing to determine what areas are not considered potential arroyo toad habitat and should be excluded from the sampling scheme. Areas will be excluded if the slope is greater than 3%, there is a lack of sandy substrate, or the water is brackish due to proximity to the sea. Areas that are off limits due to safety reasons will be also be excluded, i.e. bombing ranges.

Depending on the total length of potential arroyo toad habitat available, the habitat will be divided into approximately 50 blocks of approximately 1.0-1.4 km in length. Each block will be sub-divided into 5 –7 sites approximately 200 meters in length. Within each block, one site will be randomly selected as the permanent site and the other sites will be randomly assigned to years within the rotating panel. 8 blocks of the 50 blocks will be designated as "intensive" blocks.

Personnel: 2 people, at least one with training in assessing potential arroyo toad habitat

Equipment: One to two 4WD vehicles, PDA, field notebook, paper maps, GPS, GIS coverage of MCB Camp Pendleton riparian areas (in lab)

2) Initiation of Breeding

Purpose: Determine when arroyo toad breeding (egg laying) initiates and the environmental conditions that are associated in order to determine when to initiate tadpole surveys.

Sample Period: February/March

Frequency: Weekly until arroyo toad breeding is confirmed

Pre-sampling checklist:
Inform base contact of sampling dates in advance (base contact may accompany monitor). The driver will request vehicle pass. A four-wheel drive vehicle is recommended but may not be required for all sampling areas. Check out VHF radio if recommended by the base contact. Be familiar with base regulations and access routes.
Protocol: One surveyor will visit several (at least one on each watershed) of the intensive sites weekly during beginning in February or March when the environmental conditions approach arroyo toad breeding conditions. The surveyor will listen for adult arroyo toad males calling at night. Once adult males are heard calling, begin weekly surveys for egg masses. After first egg mass is spotted, plan first tadpole surveys approximately two weeks later.
Additional Variables for Protocol refinement: Take air and water temperature readings, water depth readings, moon visibility, and water velocity category. From local weather station get precipitation, high and low air temperatures, and moon phase.
Personnel: 2 people, at least one with training in surveys for arroyo toad adults, tadpoles, and egg masses
Equipment: 4WD vehicle, PDA, field notebook, paper maps, two thermometers (air, water), meter stick, Kohler Wheat lamps, Tidbit temperature loggers

3) Proportion of sites occupied: surveys at regular survey sites (intensive blocks not included)
Purpose: Determine whether arroyo toad egg masses and/or tadpoles are present within the regular survey sites (2 within each of the regular blocks). Take additional variables that will allow assessment of potential causes of change as well as provide cofactors that affect detectability of the protocol,
Sample Period: March - June
Frequency: All sites will be survey 1-2 times. A second visit will occur approximately 1 month after the first if egg masses or tadpoles are not detected at the first visit.
Pre-sampling checklist:
Inform base contact of sampling dates in advance (base contact may accompany monitor). The driver will request vehicle pass. A four-wheel drive vehicle is recommended but may not be required for all sampling areas. Check out VHF radio if recommended by the base contact. Be familiar with base regulations and access routes.
Protocol: Two surveyors will walk in the water and along the banks during the daytime and record the first observation of an arroyo toad tadpole. Location and numbers of arroyo toad egg masses should also be recorded. Additional variables taken include:

- Presence of water at the survey site, wet length of survey site and average wet width
- Time of day, category of substrate where tadpole was found, percent cover, water depth water velocity category, turbidity, are egg masses being covered by excessive sand or silt (i.e. is there excessive turbidity in the water).
- Weather category (i.e. clear, partly cloudy, cloudy/overcast, mist/light drizzle, light rain, heavy rain)
- Water temperature, air temperature
- Presence of invasive fish noted

Presence of bullfrogs, crayfish, or African clawed frogs noted
Signs of recent vehicle disturbance
Comments on other disturbance/threats (e.g. excessive trash)
General comments, Observer names, survey site number, start and ending GPS coordinates, start and end time of transect

The following variables need only be recorded during the first of the two visits
Does site have sandy substrate, sandy terraces, and channel braiding present?
Total number of arroyo toad breeding pools <2 feet in depth and with a sandy bottom
Total number of pools >2 feet in depth (refugia for invasive species)
Dominant vegetation type
Presence of targeted invasive plants within 50 meters of stream, (e.g. arundo, tamarisk, watercress, fennel) and the size category (a few plants, scattered small patches, large contiguous stands)

Personnel: 2 people, at least one with training in surveys for arroyo toad adults, tadpoles, and egg masses

Equipment: 4WD vehicle, PDA, field notebook, paper maps, two thermometers (air, water), GPS, meter stick; Field guide with pictures of arroyo toad tadpoles, egg masses, metamorphs, juveniles and adults, similar looking species such as *Bufo boreas* egg masses and *Hyla cadaverina* tadpoles, and invasive plant and fauna species such as bullfrogs, African clawed frogs, exotic fish, crayfish, Tamarisk, Arundo, fennel, watercress.

4 (Option A). Proportion of sites occupied: surveys at intensive survey sites – Core protocol only without additional protocol refinement information (see alternate methodology below)

Purpose: To provide 4 repeated surveys for tadpoles and/or egg masses

Sample Period: March - June

Pre-sampling checklist:

Inform base contact of sampling dates in advance (base contact may accompany monitor). The driver will request vehicle pass. A four-wheel drive vehicle is recommended but may not be required for all sampling areas. Check out VHF radio if recommended by the base contact. Be familiar with base regulations and access routes.

Frequency: All sites will be surveyed 4 times, approximately 2-3 weeks apart.

Protocol: The two 200m sites within each of the eight intensive sampling blocks will be surveyed for presence of arroyo toad tadpoles and/or egg masses a total of 4 times during the season approximately 2-3 weeks apart. Two surveyors will walk in the water and along the banks during the daytime and record the first observation of an arroyo toad tadpole. Location and numbers of arroyo toad egg masses should also be recorded. The same variables that are recorded at the regular sites should be recorded here as well. All 4 surveys will be conducted regardless of whether arroyo toad tadpoles are detected during the first three surveys.

Personnel: 2 people, at least one with training in surveys for arroyo toad adults, tadpoles, and egg masses

Equipment: 4WD vehicle, PDA, field notebook, paper maps, two thermometers (air, water), GPS, meter stick; Field guide with pictures of arroyo toad tadpoles, egg masses, metamorphs, juveniles and adults, similar looking species such as *Bufo boreas* egg masses and *Hyla cadaverina* tadpoles, and invasive plant and fauna species such as bullfrogs, African clawed frogs, exotic fish, crayfish, Tamarisk, Arundo, fennel, watercress.

4 (Option B). Protocol refinement – Proportion of sites occupied: surveys at intensive survey sites with additional protocol refinement information

Purpose: To provide 4 repeated surveys for egg masses and/or tadpoles for the proportion of sites occupied analysis. To correlate the number of egg masses, tadpoles, and adult toads across the entire lengths of the intensive blocks to assess the relationship in counts between the different life stages.

Sample Period: March - June

Pre-sampling checklist:

Inform base contact of sampling dates in advance (base contact may accompany monitor). The driver will request vehicle pass. A four-wheel drive vehicle is recommended but may not be required for all sampling areas. Check out VHF radio if recommended by the base contact. Be familiar with base regulations and access routes.

Frequency: All sites will be surveyed 4 times, approximately 2-3 weeks apart.

Protocol: At each "intensive" block, two surveyors will survey the entire block during the daytime and the number and location of egg masses and tadpoles will be counted and recorded. Surveyors will walk in the water and along the banks. The surveyors will then wait until nightfall and then survey the same 1 km for the number of adult toads. Pit tag readers will be run over the adult toads. Pairs in amplexus will not be disturbed. The same variables that are recorded at the regular sites should be recorded here as well.

Personnel: 2 people, at least one with a permit for *handling* arroyo toad adults and training in surveys for arroyo toad adults, tadpoles, and egg masses

Equipment: 4WD vehicle, PDA, field notebook, paper maps, two thermometers (air, water), GPS, meter stick, pit-tag reader, Kohler Wheat lamps; Field guide with pictures of arroyo toad tadpoles, egg masses, metamorphs, juveniles and adults, similar looking species such as *Bufo boreas* egg masses and *Hyla cadaverina* tadpoles, and invasive plant and fauna species such as bullfrogs, African clawed frogs, exotic fish, crayfish, Tamarisk, Arundo, fennel, watercress.

5) Protocol refinment - Metamorph surveys

Purpose: To confirm that arroyo toad tadpoles successfully reach metamorph stage. To determine the mutation rate of metamorphs as an indicator of water quality and disease.

Sample Period: June- August

Frequency: one time at the 8 intensive sites approximately 3 to 3 ½ months after egg masses are observed.

Pre-sampling checklist:

Inform base contact of sampling dates in advance (base contact may accompany monitor). The driver will request vehicle pass. A four-wheel drive vehicle is recommended but may not be required for all sampling areas. Check out VHF radio if

recommended by the base contact. Be familiar with base regulations and access routes.
Protocol: Conduct a daytime survey at the 8 intensive blocks at approximately 3 to 3 ½ months after egg masses were first observed. Count the number of metamorphs seen and estimate the deformity rate in a count of 50 metamorphs within each block.
Personnel: 2 people, at least one with training in surveys for arroyo toad metamorphs
Equipment: 4WD vehicle,PDA, field notebook, paper maps, two thermometers (air, water), GPS, dip nets (?); Field guide with pictures of arroyo toad tadpoles, egg masses, metamorphs, juveniles and adults, similar looking species such as *Bufo boreas* egg masses and *Hyla cadaverina* tadpoles, and invasive plant and fauna species such as bullfrogs, African clawed frogs, exotic fish, crayfish, Tamarisk, Arundo, fennel, watercress.

6) Protocol refinement –Optimizing detectability of tadpoles
Purpose: To determine how the protocol should be standardized across observers, to estimate observer bias, and estimate how detectability changes during different times of the year and across different substrates, i.e. should search time be increased when substrate is dominated by algal mats?
Sample period: March-June
Frequency: 4 times during season at 2-4 of the intensive blocks, possibly in conjunction with the intensive block surveys. Only conducted during first sampling year. The number of blocks sampled will be determined after the first two surveys are done. If large numbers of tadpoles are being detected, then only two blocks will need to be sampled each time. If only a few tadpoles are detected, then more blocks will need to be sampled.
Pre-sampling checklist:
Inform base contact of sampling dates in advance (base contact may accompany monitor). The driver will request vehicle pass. A four-wheel drive vehicle is recommended but may not be required for all sampling areas. Check out VHF radio if recommended by the base contact. Be familiar with base regulations and access routes.
Protocol: Using two observers conduct 2 surveys at two different walking speeds (10 minutes for 200 meter and 30 minutes for 200 meters) of the same 200 meter site. One surveyor is the primary observer and the second is the recorder who records observations of the primary observer and silently records additional observations of egg masses and tadpoles. After 100 meters the role of primary observer and recorder switches. Surveyors will record number and location of egg masses and tadpoles, the size category of the tadpoles, and the substrate they are located within. Surveyors should also make additional comments on how to improve the detectability of the protocol.

Site variables recorded: Time of day, Weather category (i.e. clear, partly cloudy, cloudy/overcast, mist/light drizzle, light rain, heavy rain)
Water temperature, air temperature

Variables recorded for each tadpole found: number of tadpoles (if a group), size of tadpoles (or size category), location of tadpole in drainage (on paper map), category of substrate where tadpole was found, percent cover.

Personnel: 2 people, both with training in surveys for arroyo toad adults, tadpoles, and egg masses

Equipment:

4WD vehicle, PDA, field notebook, paper maps, at least 2 detailed TOPO! maps of the 200 meter stream site to be surveyed on clipboard, two thermometers (air, water), GPS, pencils, accurate watch with second hand or stop-watch; Field guide with pictures of arroyo toad tadpoles, egg masses, metamorphs, juveniles and adults, similar looking species such as *Bufo boreas* egg masses and *Hyla cadaverina* tadpoles, and invasive plant and fauna species such as bullfrogs, African clawed frogs, exotic fish, crayfish, Tamarisk, Arundo, fennel, watercress.

APPENDIX A: BACKGROUND READING MATERIALS & ADDITIONAL REFERENCES

Scientific review panel was asked to read the *required* papers and reports prior to the workshop. The *Supplemental Reading* materials were additional literature provided to the reviewers that they might find useful, but are not expected to read.

Required Reading

Gibbs, J.P., H.L. Snell, and C.E. Causton. 1999. Effective Monitoring for Adaptive Wildlife Management: Lessons from the Galápagos Islands. J. Wildl. Management 63(4): 1055-1065.

Holland, D.C., N.R. Sisk, and R.H. Goodman. 2001a. Linear Transect Censusing of the Arroyo Toad (*Bufo californicus*) from 1996-2000 on MCB Camp Pendleton, San Diego County, California.

Sweet, S.S. 1993. Second Report on the Biology and Status of the Arroyo Toad (*Bufo microscaphus californicus*) on the Los Padres National Forest of Southern California.

US Fish and Wildlife Service. 1999. Arroyo Southwestern Toad (*Bufo microscaphus californicus*) Recovery Plan.

US Forest Service. 2002. DRAFT Population Monitoring Plan for Amphibians

Supplemental Reading (not required)

Griffin, P.C. and T.J. Case. 2001. Terrestrial habitat preferences of adult arroyo southwestern toads. Journal of Wildlife Management. 65(4):633-644.

Holland, D.C. and R.H. Goodman, Jr. 1998. A Guide to the Amphibians and Reptiles of MCB Camp Pendleton, San Diego County, California.

Holland, D.C. and N.R. Sisk. 2001b. Habitat Use and Population Demographics of the Arroyo Toad (*Bufo californicus*) on MCB Camp Pendleton, San Diego County, California: Final Report for 1998-1999.

---------. 2001c. Linear Transect Censusing of the Arroyo Toad (*Bufo californicus*) on two streams on the Cleveland National Forest, San Diego County, California 1998-1999.

Sweet, S.S. 1991. Initial report on the Ecology and Status of the Arroyo Toad ((*Bufo microscaphus californicus*) on the Los Padres National Forest of Southern California, with Management Recommendations.

US Fish and Wildlife Service. 2000. Federal Register: Endangered and Threatened Wildlife and Plants; Proposed Designation of Critical Habitat for the Arroyo Southwestern Toad; Proposed Rule.

Additional References

Blaustein A.R. and P. TJ Johnson. 2003. The complexity of deformed amphibians. *Frontiers in Ecology and the Environment*. 1(2):87-94.

Davidson, C., H. B. Shaffer, and M. R. Jennings. 2002. Spatial tests of the pesticide drift, habitat destruction, UV-B, and climate-change hypotheses for California amphibian declines. *Conservation Biology* 16(6):1588-1601.

Hayes, T., K. Haston, M. Tsui, A. Hoang, C. Haeffele, and A. Vonk. 2002. Herbicides: Feminization of male frogs in the wild. *Nature* 419:895-896.

MacKenzie, D. I., J. D. Nichols, G. B. Lachman, S. Droege, J. A. Royle and C. A. Langtimm. 2002. Estimating Site Occupancy When Detection Probabilities Are Less Than One. *Ecology* 83(8): 2248-2255.

APPENDIX B: ARROYO TOAD CONCEPTUAL MODEL

Camp Pendleton

Camp Pendleton occupies approximately 125,000 acres of largely undeveloped land, with more than 17 miles of coastline, in northwestern San Diego County. It is the Marine Corps' premier amphibious training Base and its only west coast amphibious assault training center. Camp Pendleton's mission is "to operate an amphibious training Base that promotes the combat readiness of operating forces by providing facilities, services, and support responsive to the needs of Marines, Sailors, and their families" *(MCB Camp Pendleton Strategic Plan 2002)*.

MCB Camp Pendleton has a distinct Mediterranean climate with cool summers and mild winters. Rainfall occurs primarily between November and April. The average annual precipitation on the study area varies with distance from the coast, with the majority occurring during major winter storms. The average summer and winter temperatures are 65°F and 55°F, respectively. Heavy fog is common, especially along the coastline, although it usually dissipates by mid-morning. Wind speed usually ranges from 5 and 15 mph, although periods of higher velocity may be experienced, especially in the late afternoon.

The base is managed for multiple uses, the primary use being military training exercises. This constraint affects wildlife management decision-making. Camp Pendleton's approach is primarily to minimize effects and to mitigate where necessary.

Flexibility in timing of monitoring may be needed to allow for military exercises to proceed. Some areas of the base are off limits due to the types of activities that occur there (i.e. bombing exercises). The monitoring design should allow for extra sampling sites in case some sites need to be dropped at a later point due to military activities.

Large areas of high quality arroyo toad habitat exist on the base and sufficient background information exists to identify where arroyo toads occur. (Holland and Goodman, 1998; Rob Lovich, personal communication) The populations on the base should be considered "core" breeding populations rather than peripheral populations to upstream breeding populations. Upstream populations are small because the gradient changes and there is little available breeding habitat. The Santa Margarita population is thought to be genetically distinct from the populations along the San Mateo and San Onofre watersheds.

In addition to natural fluctuations in populations due to annual rainfall, the primary stressors / threats to arroyo toads at Camp Pendleton appear to be invasive predators (bass, bullfrogs, crayfish, fire ants, possibly argentine ants), invasive plants (lots of arundo infestation, limited tamarisk infestation), some potential native plant problems (water cress), military operations disturbance of riparian zone by both people and vehicles (note that the base has attempted to minimize disturbance while maintaining some use of area), impacts of sewage effluent on water quality, and hydrological alterations due to diversion of streamflows and groundwater pumping.

Non-indigenous predators: Bullfrogs, bass, and crayfish are found throughout all three Camp Pendleton watersheds and persist in deeper pools over the summer. Currently an eradication effort is underway on the San Mateo watershed because the extremely dry year (2002) has left the bullfrogs and bass restricted to a few pools which should make control easier and reduce predator populations for a few years. Fire ants may be come a problem in the future as there is a potential for them to invade from the north side of the base. It is unclear to what extent argentine ants are a stressor for arroyo toads since there is some evidence that toads eat the ants. Argentine ants are found on base and especially along the Santa Margarita.

Non-indigenous plants: Arundo is a severe problem on the base and is found in high densities along the lower Santa Margarita. Currently intensive arundo removal activities are underway there. Tamarisk is also found on base but not in high densities. Fennel is a problem in upland areas on the sandy terraces. Native species such as watercress can occasionally restrict the amount of quality arroyo toad breeding habitat in some areas.

Crushing & roadkill: Some problems of crushing by vehicles and people is a concern on Camp Pendleton, although the base tries to minimize the effects. Knowledge of arroyo toad breeding hotspots might be helpful here. Some crushing could also occur due to bison on the base. No other livestock should be a concern. A highway may be built near Cristianitos in the future and it is unclear what effect this will have (noise disturbance, increased trespassing, crushing?)

Altered hydrology: Up to 80-90% of the Santa Margarita flows are diverted upstream. However Santa Margarita continues to have perennial flows year round. The San Onofre and San Mateo watersheds tend to be more seasonal in nature with some pools that remain wet year round. Tidal influx can reach as far inland as Jardine canyon on the San Onofre. 2002 has been an exceedingly dry year and both these latter watersheds were dry in July 2002. San Mateo and Santa Margarita have agricultural diversions near their mouth, and in arroyo toad habitat. Cristianitos, a tributary of the San Mateo, has water diverted to Orange County developed areas. The San Mateo watershed is one of the few remaining watersheds in southern California to still have steelhead. There are some water diversions and ground-water pumping in the upper San Mateo, but it is unclear if there is enough to impact arroyo toad eggs and tadpoles. Los Pulgas creek does not have arroyo toads.

Beavers are present on a small portion of the Santa Margarita but currently affect only a very few acres of habitat.

Water quality problems are unclear in the various watersheds, but may become a bigger issue soon. Development upstream of the base along the Santa Margarita and increases in treated sewage effluent may become more of a concern in the future. Treated sewage effluent may also become an issue on the San Mateo watershed.

Questions about the effect of high estrogens contaminants in sewage effluent on amphibians are unresolved.

Disease was mentioned as a possible concern, but it is unclear if disease is a problem for arroyo toads.

General Conceptual Model

A general conceptual model is presented below and in Figure 6 (last page of document). Elements are drawn primarily from the *Recovery Plan for the Arroyo Southwestern Toad* (USFWS, 1999), Sweet (1993, 1991), Holland *et al* (1998, 2001a, 2001b, 2001c), and Griffin and Case (2001) as well as comments from the workshop participants. The conceptual model is divided into five major life stages (**Breeding, Eggs, Tadpoles, Metamorphs, Juveniles,** and **Adults**). The characteristics of each life stage, the habitat conditions, risk factors and important influencing factors are listed. The major risk factors relevant to Camp Pendleton are underlined. Other risk factors are included for completeness, but are not necessarily relevant to Camp Pendleton at the current time. After the description of all the life stages is a section describing **Possible Management Actions**. Actions that are particularly relevant to Camp Pendleton are underlined, although other potential actions are included for completeness.

BREEDING

Breeding Adult stage characteristics

- Breeding is nocturnal in spring after water temperatures reach at least 14 ^{o}C and water levels (<30 cm deep) and speed (<5 cm/sec) are appropriate for breeding
- females are assumed to lay only one egg mass (USFWS, 1999)
- males may mate with multiple females
- prefer darker nights

Habitat Conditions

- Clear still to slow-moving water with shallow, exposed clean, sandy bottom and open canopy [see influencing factors]

Risk factors (Stressors) (Note: Risks relevant to Camp Pendleton are underlined)

- Breeding habitat loss due to urbanization; lack of flushing flows and sediment supply causes habitat loss due to natural plant succession
- Breeding habitat quality degradation and loss due to exotic plants (arundo, tamarisk) or to native plants (water cress); fennel is a problem in upland habitats where it is dense and grows on sandy terraces
- Lack of water in pools due to low annual rainfall, excessive water diversions and/or groundwater pumping
- Roadkill / crushing by vehicles, people, and livestock
- Predation by raccoons, crows, bullfrogs, bass, crayfish, fire ants, Argentine ants (unclear if argentine ants have a negative impact on toads?)
- Light pollution
- Noise pollution does not appear to affect calling males but may have an effect on female response

- Aquatic contaminants: treated sewage effluent, agricultural chemicals
- Aerial contaminants, fire retardant?
- Disease?

Influencing Factors

- Episodic flushing flows & floods are needed to naturally disturb riparian habitat, clear vegetation on sandy terraces and maintain toad habitat
- Variability in climate, amount of rainfall, and timing of rainfall strongly affect available habitat and breeding. Breeding is limited or may not occur at all in drier years
- Beaver dams block sediment supply and alter river and stream hydrology and create bullfrog habitat.
- Water diversions, and groundwater pumping can reduce flows
- Dams alter the amount and timing of flushing flows and sediment supply
- Excessive urban runoff can increase peak flows and contain contaminants
- Weeds like arundo can slow flows and increase siltation
- Ephemeral water habitats that are occasionally dry have lower concentrations of non-native fish and bullfrogs and perennial habitats have higher concentrations
- Erosion after fires can cause siltation of breeding habitat

EGGS

Egg Life Stage Characteristics

- Strings of 2,000-10,000 eggs on sand, gravel, cobble or mud along pool margins away from vegetation

Habitat Conditions

- Same as breeding habitat; require lack of sediment/turbidity, although can tolerate it for a few days

Risk factors (stressors)

- Desiccation due to lack of rainfall, ground water pumping, and water diversions
- Disturbance/Siltation/Mechanical destruction due to humans, vehicles, livestock, floods, run-off, fires
- Unseasonal flooding can wash eggs downstream
- Aquatic Contaminants: pesticides/herbicides, heavy metals, estrogen in treated effluent
- Disease? Very little known.

TADPOLES

Tadpole Life Stage Characteristics (65-85 days)

- Active during day; very cryptic; tadpoles can disperse downstream

Habitat Conditions

- Similar to breeding habitat, also need detritus, moss, periphyton

Risk factors (stressors)

- Predation: exotic fishes, garter snakes, birds, bullfrogs, etc. Impact of exotic fishes and bullfrogs depends on how much shallow refugia are available for the tadpoles to hide in.
- Crushing, disturbance, and siltation from humans, vehicles, livestock (bison)
- Poorly timed flushing events can wash tadpoles downstream into poor habitats
- Desiccation due to lack of rainfall, ground water pumping, and water diversions
- Disease? not sure if chytrid fungus causes arroyo toad die offs; however, chytrids have been found in *Bufo boreas*
- Contaminants: pesticides, heavy metals, treated effluent (estrogens), urban runoff, herbicides from agricultural runoff, etc.

Influencing Factors

- There is an interaction between microhabitat, dessication and predation. The availability of shallow refugia may allow arroyo toad tadpoles to escape predation. However predation risk may increase as streams and pools dry and tadpoles and predators become more concentrated in space.

METAMORPHS

Metamorph Life stage characteristics (limited knowledge)

- Active during day on sandy benches, still fairly clustered together, Feed on native ants and possibly other invertebrates

Habitat Conditions

- Soft, exposed, sand and moist sandy benches with partial shading adjacent to pools

Risk factors (stressors)

- Crushing from vehicles and humans (when still clustered they are especially vulnerable)
- Fire ants and Argentine ants displacing native ants (effect of argentine ants is unclear since they are eaten by arroyo toads)
- Predation from garter snakes, bullfrogs, birds (killdeer, herons)
- Contaminants: pesticides, heavy metals, urban runoff, etc.
- Water releases can wash metamorphs into marginal habitat
- Habitat loss e.g Arundo can wipeout metamorph habitat
- compaction of sand prevents metamorph burrowing

JUVENILES

Juvenile Life Stage Characteristics (limited knowledge)

- Assume moving into upland but may remain by pools for up to 6 months, more dispersed than metamorph stage, nocturnal, assume eat native ants & beetles; also eat Argentine ants but it is unknown how well they do on that diet. Upland movement is close and parallel to stream and appears to be influenced by topography (low gradient and distance from the creek) and suitable microhabitat (e.g., desert/coast).

Habitat Conditions & risk factors

- Similar to adults

<u>ADULTS</u>

Adult Life Stage Characteristics (limited knowledge)

- Lifespan about 5 years (?);
- Favor nights for activity, burrow in sand during day;
- typically do not go more than 0.5-0.75 miles from breeding pools but may travel over 1 mile, very dispersed;
- distance is influenced by gradient (relief) away from creek and by desert/coast microclimate. Note: the frequency distribution of dispersal is a question at Pendleton because this affects management and use of nearby habitats
- feed on native ants and other invertebrates (?)

Habitat Conditions

- Coastal sage scrub, chapparal, oak woodland, but not grasslands (may travel through grasslands); Require friable soils & permeable plant understory for burrowing.

Risk factors (Stressors)

- Habitat loss
- Lack of connectivity between breeding habitat and uplands
- <u>Roadkill / crushing by vehicles</u>
- <u>Non-native ants (Argentine & fire ants);</u> toads do routinely eat Argentine ants, but it is unclear how well they do on them.
- Predation- by native species (raccoons, snakes) and <u>non-native species</u> (bullfrogs, house cats)
- <u>Fire and fire retardant</u>
- Pesticides
- <u>Drought (starvation)</u>
- Toads are attracted by friable soils in agricultural areas, it is unclear how agricultural practices and different methods and timing of tillage impact toad mortality

<u>POSSIBLE MANAGEMENT ACTIONS</u>

- Protect and maintain breeding habitat and connectivity with upland habitats. Maintain sandy soil next to rivers.
- Manage natural hydrology and sediment supply to extent possible to allow natural creation and maintenance of toad habitat. Maintain flushing flows during winter and avoid unseasonal floods during spring.
- <u>Control invasive predators</u> such as bullfrogs, African clawed frogs, non-native fishes in and around breeding areas. Control beavers. Control of bullfrogs and non-native fish may be most useful just prior to the arroyo toad breeding season unless opportunity exists to remove them from an entire watershed such as the San Mateo during dry years. It was suggested that it might be better to target

bullfrog egg mass removal than adults. Using an off-channel alternate lake might be a possibility to allow maintenance of fishing in the area while removing bass from toad breeding sites.

- Control invasive plants in and around breeding areas (arundo, tamarisk, water cress). Note: since arundo is largely propagated vegetatively, controlling from the top of the watershed down may be the most efficient approach.
- Avoid disturbance, crushing, & siltation of breeding areas by vehicles/humans/livestock during breeding season
- Minimize contaminants. Treated sewage effluent inputs concerns

APPENDIX C: ANALYSIS OF NUMBER OF SITES NEEDED

Trends in proportion area occupied by arroyo toad tadpoles are planned to be analyzed using a proportion area occupied estimator developed by Mackenzie et al (2002). The estimator is available in a trial version of computer software called “PRESENCE” available on the web site: http://www.proteus.co.nz/

Since the math involved is complicated, rather than directly calculating a recommended sample size, a variety of scenarios were run using the “PRESENCE” simulator feature. This simulator conducts a monte-carlo simulation in which the user specifies the total number of sites, the number of visits, the number of intensive sites and number of intensive site visits, the true proportion of sites occupied and the detectability of the protocol (ability to detect presence of a species, if it is there, after just one visit). A variety of scenarios were evaluated in which the total number of sites was varied, the protocol detectability was varied and the true proportion of sites occupied was varied. The program then calculates the estimated occupancy and standard error.

Starting numbers used in the simulator for the experimental design and other variables were somewhat arbitrarily chosen. 100 sites with 2 visits was used as a starting point because these numbers were tentatively suggested at the workshop with the understanding that they needed to be revised based upon a power analysis. 8 intensive sites were used as a starting point because Holland *et* al (2001a) already had 8 sites. The true proportion of sites occupied by tadpoles was estimated at 60% because the true occupancy was not known. The detectability of the protocol is critical and is unknown at this point. However USGS researchers felt it should be at least 60%. This will be a critical point to test during the pilot study.

The resulting standard error given by the program was used to calculate the decrease in occupancy that would be detectable with 80% power and 5% alpha (1 tailed). These results are reported in Table 6.

Three different approaches are possible:

1) in the first approach the same number of repeat visits occur at all sites regardless of whether tadpoles and/or egg masses are detected at the first visit;
2) in the second approach, repeat visits only occur at sites where the tadpoles and/or egg masses were not detected during the first visit.
3) the third approach is a combination of 1 & 2 in which the “intensive” sites receive the complete number of visits regardless of whether tadpoles are detected in earlier visits, and the regular sites only receive repeat visits if tadpoles and/or egg masses are not detected on the first visit.

The first approach requires more sampling effort for the same number of sites than the second, but provides a better estimate of the proportion area occupied with a smaller standard error. The second approach can allow a greater number of sampling sites for the same amount of effort. However, the amount of sampling effort in the second approach can vary from year to year and the standard error of the proportion area occupancy estimate is higher under low detectabilities than the first approach. The third approach provides a good compromise by having all repeat visits occur at the intensive sites to provide a better estimate of detectability while minimizing repeat visits at the regular sites.

The simulator in PRESENCE could only simulate approaches #1 and #2, although the main program can perform the calculations for all three approaches. However the estimate of the standard error for approach #3 can be bounded by substituting the standard error for the intensive sites alone in which all visits are performed (e.g. 16 sites with 4 repeat visits). The actual standard error should theoretically be smaller for approach #3, but this provided an estimate.

The simulations showed, not unexpectedly, that the detectability of the protocol is critical. Based upon these simulation results, approach #1 designs that range from 75 to 100 sites with 2 repeat visits plus 8 –16 intensive sites with 4 repeat visits seem appropriate. However, approach #3 with 100 sites, 16 of which are intensive sites with 2 and 4 visits respectively would also be appropriate. *However these conclusions assume that the probability of detection of arroyo toads by the protocol if they are actually present does not fall below 50-60%.* This sampling strategy will allow detection of declines in the proportion of sites occupied of approximately 45-50%% with a power of 80% even if detectability is indeed only 60%. Hopefully the detectability of the protocol will be much higher. If the detectability of the protocol falls below 50% then the number of revisits to the sites will need to be increased or the total number of sites visited every year will need to be increased.

Since using a scenario with 80 sites (40 blocks) would require a 8 or 9 year rotation to cover all the potential habitat on base and a scenario with 100 sites (50) blocks would require a 5 or 6 year rotation, the later scenario is preferred (see scenario 20 in Table 6). The third sampling approach, in which intensive sites receive all visits but regular sites only receive a second visit if tadpoles are not detected on the first visit, is recommended in order to minimize sampling effort while maintaining good estimates of detectability.

FINAL RECOMMENDATION: Thus the final recommended design is 100 sites (50 blocks) with 16 intensive sites (8 blocks). Regular sites will receive 1-2 visits. If tadpoles or egg masses are detected at the first visit, a second visit will not be required. Intensive sites will receive 4 visits regardless of whether tadpoles or egg masses are detected at earlier visits.

Table 6. Percent Area Occupied (PAO) by arroyo toad tadpoles: sampling design scenarios and resulting standard error (SE) and proportion difference detectable at alpha=0.05 (one-tailed) and power of 0.80 using Program PRESENCE. Proportion differences detectable of less than 50% are highlighted.

		Experimental design				Other variables			Simulator results		Difference Detectable		
	Design Scenario	Total # sites	# intensive sites	# revisits to regular sites	# revisits to intensive sites	True # sites occupied	True Proportion sites occupied	Protocol detection probability if tadpole present	Estimated occupancy	SE	Proportion difference detectable	# sites that lose occupancy for significant decline	Total # surveys
Number of Intensive Sites and Number of Visits varied	1	100	8	2	6	60	0.6	0.8	0.6003	0.052	0.305	18	232
							0.6	0.6	0.6033	0.0657	0.385	23	
							0.6	0.4	0.6129	0.1026	0.601	36	
							0.6	0.3	0.6293	0.1417	0.831	50	
	2	100	8	2	4	60	0.6	0.8	0.6003	0.0522	0.306	18	216
							0.6	0.6	0.6068	0.0685	0.402	24	
							0.6	0.4	0.6234	0.1183	0.693	42	
	3	100	8	2	2	60	0.6	0.8	0.6011	0.0529	0.310	19	200
							0.6	0.6	0.6073	0.074	0.434	26	
							0.6	0.4	0.6307	0.1349	0.791	47	
							0.6	0.3	0.6536	0.1794	1.052	63	
	4	100	8	3	3	60	0.6	0.8	0.6006	0.0493	0.289	17	300
							0.6	0.6	0.6017	0.0549	0.322	19	
							0.6	0.4	0.6094	0.0813	0.477	29	
							0.6	0.3	0.6235	0.118	0.692	42	
	5	100	2	2	2	60	0.6	0.8	0.6015	0.0529	0.310	19	200
							0.6	0.6	0.6079	0.0744	0.436	26	
							0.6	0.4	0.6303	0.1344	0.788	47	
	6	100	16	2	4	60	0.6	0.8	0.6004	0.0516	0.302	18	296
							0.6	0.6	0.6048	0.0637	0.373	22	
							0.6	0.4	0.618	0.1082	0.634	38	
True proportion of sites occupied varied	7	100	8	2	6	80	0.8	0.8	0.8008	0.0448	0.197	16	232
							0.8	0.6	0.8047	0.0627	0.276	22	
							0.8	0.4	0.8087	0.0958	0.421	34	
							0.8	0.3	0.8109	0.1228	0.540	43	
							0.8	0.2	0.8071	0.1566	0.688	55	
	8	100	8	2	6	60	0.6	0.8	0.6003	0.052	0.305	18	232
							0.6	0.6	0.6033	0.0657	0.385	23	
							0.6	0.4	0.6129	0.1026	0.601	36	
							0.6	0.3	0.6293	0.1417	0.831	50	
	9	100	8	2	6	40	0.4	0.8	0.4016	0.0511	0.449	18	232
							0.4	0.6	0.4026	0.0614	0.540	22	
							0.4	0.4	0.4144	0.0934	0.821	33	
Total number of sites varied	10	20	2	2	6	12	0.6	0.8	0.6067	0.1156	0.678	8	48
							0.6	0.6	0.6180	0.1481	0.868	10	
							0.6	0.4	0.6519	0.2351	1.378	17	
	11	30	3	2	6	18	0.6	0.8	0.6019	0.0945	0.554	10	72
							0.6	0.6	0.6144	0.1204	0.706	13	
							0.6	0.4	0.6292	0.174	1.020	18	
	12	50	3	2	6	30	0.6	0.8	0.6023	0.0739	0.433	13	112
							0.6	0.6	0.6111	0.0969	0.568	17	
							0.6	0.4	0.6335	0.1519	0.890	27	
	13	50	8	2	6	30	0.6	0.8	0.6017	0.0725	0.425	13	132
							0.6	0.6	0.6043	0.0874	0.512	15	
							0.6	0.4	0.6175	0.1254	0.735	22	
	14	75	8	2	6	45	0.6	0.8	0.5996	0.0598	0.351	16	182
							0.6	0.6	0.6055	0.0741	0.434	20	
							0.6	0.4	0.6152	0.1123	0.658	30	
							0.6	0.3	0.6263	0.1515	0.888	40	
	15	80	8	2	4	48	0.6	0.6	0.6085	0.0759	0.445	21	208
	16	100	8	2	6	60	0.6	0.8	0.6003	0.052	0.305	18	232
							0.6	0.6	0.6033	0.0657	0.385	23	
							0.6	0.4	0.6129	0.1026	0.601	36	
							0.6	0.3	0.6293	0.1417	0.831	50	
8 intensive blocks only		16	16	0	4	12.8	0.8	0.8	0.8019	0.0994	0.437	6	64
	17	16	16	0	4	12.8	0.8	0.6	0.8161	0.1119	0.492	6	64
		16	16	0	4	12.8	0.8	0.4	0.8159	0.1376	0.605	8	64
Sampling halted after first detection of arroyo toads	18	100	8	2	6	60	0.6	0.8	0.6037	0.0547	0.321	19	188
		100	8	2	6		0.6	0.6	0.611	0.0835	0.489	29	199
		100	8	2	6		0.6	0.4	0.6275	0.1474	0.864	52	210
	19	100	8	3	3	60	0.6	0.8	0.6015	0.0496	0.291	17	164
		100	8	3	3		0.6	0.6	0.6071	0.0624	0.366	22	175
		100	8	3	3		0.6	0.4	0.6397	0.1354	0.794	48	186
	20	100	16	2	4	60	0.6	0.8	0.6032	0.0532	0.312	19	192
		100	16	2	4		0.6	0.6	0.6128	0.0842	0.494	30	202
		100	16	2	4		0.6	0.4	0.6448	0.1692	0.992	60	212
Final Choice	**20**	100	16	2	4	60	0.6	0.6	0.6128	0.0842	0.494	30	202

Figure 6. Arroyo Toad Conceptual Model*

Possible Management Actions

•Protect and maintain breeding habitat and connectivity with upland habitats. Maintain sandy soil next to rivers.

•Manage natural hydrology and sediment supply to extent possible to allow natural creation and maintenance of toad habitat. Maintain flushing flows during winter and avoid unseasonal floods during spring

•Control invasive predators such as bullfrogs, African clawed frogs, non-native fish in and around breeding areas. Control invasive plants in and around breeding areas (arundo, tamarisk, water cress). Control beavers.

•Avoid disturbance, crushing, & siltation of breeding areas by vehicles/humans/livestock during breeding season

•Minimize contaminants

Breeding Adult Stage Characteristics

Breeding is nocturnal in spring after water temperatures reach at least 14 ºC and water levels (<30 cm deep) and speed (<5 cm/sec) are appropriate for breeding; females assumed to lay only one egg mass, males may mate with multiple females; prefer darker nights

Habitat Conditions

Clear still to slow-moving water with shallow, exposed clean, sandy bottom and open canopy [see influencing factors]

Risk Factors (Stressors)

Breeding habitat loss due to urbanization; lack of flushing flows and sediment supply causes habitat loss due to natural plant plant succession • Breeding habitat quality degradation and loss due to exotic plants (arundo, tamarisk) or to native plants (water cress) • Lack of water in pools due to low annual rainfall, excessive water diversions and/or groundwater pumping • roadkill / crushing by vehicles, people, livestock • predation by raccoons,crows,bullfrogs, bass, crayfish, fire ants, Argentine ants? • light pollution • noise pollution does not appear to affect calling males but may have an effect on female response • aquatic contaminants (sewage effluent, pesticides) • aerial contaminants? fire retardant? • disease?

Influencing Factors

Episodic flushing flows & floods are needed to naturally disturb riparian habitat, clear vegetation on sandy terraces and maintain toad habitat; • Variability in climate, amount of rainfall, and timing of rainfall strongly affect available habitat and breeding. Breeding is limited or may not occur at all in drier years • Water diversions, and groundwater pumping can reduce flows • Dams alter the amount and timing of flushing flows and sediment supply • Beaver dams block sediment supply and alter river and stream hydrology • Excessive urban runoff can increase peak flows and contain contaminants • Weeds like arundo can slow flows and increase siltation • Ephemeral water habitats that are occasionally dry have lower concentrations of non-native fish and bullfrogs and perennial habitats have higher concentration • Erosion after fires can cause siltation of breeding habitat

Adult Life Stage Characteristics (limited knowledge)

Lifespan about 5 years (?); Favor nights for activity, burrow in sand during day; typically do not go more than 0.5-0.75 miles from breeding pools but may travel over 1 mile, distance influenced by topgraphy and microclimate; very dispersed; feed on native ants and other invertebrates

Habitat Conditions

Coastal Sage Scrub, Chapparal, oak woodland, but not grasslands (may travel thru grasslands); Require friable soils & permeable plant understory for burrowing.

Risk Factors (Stressors)

Habitat loss • Lack of connectivity between breeding habitat and uplands • Roadkill / crushing by vehicles • Non-native ants (argentine & fire ants) • Predation-native and house cats • Fire • Pesticides • drought (starvation)

Females mature: 2-3 years
Males: 1-2 years

BREEDING
Jan – Early July**

ADULTS
Uplands

EGGS
Feb-Early July**

12-20 days

TADPOLES
March-July**

JUVENILES
Uplands

METAMORPHS
(10-17mm)
May-August**

65-85 days

Egg Life Stage Characteristics

Strings of 2,000-10,000 eggs on sand, gravel, cobble or mud along pool margins away from vegetation

Habitat Conditions

Same as breeding habitat; require lack of sediment/turbidity (but can tolerate it for a few days)

Risk Factors (stressors)

Desiccation due to lack of rainfall, ground water pumping, and water diversions • *Disturbance/Siltation* due to humans, vehicles, livestock, floods, run-off, fires • Unseasonal flooding can wash eggs downstream • *Predation*: exotic fishes, crayfish, invertebrates • disease? • *Contaminants*: pesticides, heavy metals, treated effluent

Juvenile Life Stage Characteristics (limited knowledge)

Assume moving into upland but may remain by pools for up to 6 months, more dispersed than metamorphs, nocturnal, assume eat native ants & beetles; upland movement is close and parallel to stream and influenced by topography and availability of suitable microhabitat

Habitat Conditions & Risk Factors

Similar to adults

Metamorph Life Stage Characteristics

Active during day on sandy benches; still fairly clustered together; feed on native ants and possibly other invertebrates;

Habitat Conditions

Soft, exposed, sand and moist sandy benches with partial shading adjacent to pools

Risk Factors (stressors)

Crushing from vehicles and humans (when still clustered they are especially vulnerable) • Fire ants and Argentina ant displacing native ants • Predation from garter snakes, bullfrogs, birds (killdeer, herons) • Contaminants: pesticides, heavy metals, urban runoff, etc. • Habitat loss (arundo) • compaction of sand prevents metamorph burrowing

Tadpole Life Stage Characteristics (65-85 days)

Active during day; very cryptic; can disperse downstream

Habitat Conditions

Similar to breeding habitat, also need detritus, moss, periphyton

Risk Factors (stressors)

Predation: exotic fishes, garter snakes,birds,bullfrogs,etc. • Crushing, disturbance, & siltation from humans vehicles, livestock (bison)• Poorly timed flushing events can wash tadpoles downstream into poor habitats • Desiccation due to lack of rainfall, ground water pumping, and water diversions • Disease? • Contaminants: pesticides, heavy metals, treated effluent, urban runoff, etc.

* For details see arroyo toad recovery plan (USFWS, 1999)

** These dates may shift in some years depending on rainfall. Dates also shift in montane or inland desert areas.