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Current Status of the Unarmored Threespine Stickleback (*Gasterosteus aculeatus williamsoni*) along portions of the Santa Clara River drainage

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STATEMENT OF PURPOSE

This report presents the results of a field and laboratory study on the occurence of the Unarmored Threespine Stickleback, Gasterosteus aculeatus williamsoni, in portions of the Santa Clara River drainage owned by Newhall Land and Farming Company. The unarmored subspecies of stickleback is listed as endangered by both state and federal governments. The study was conducted to determine the degree to which the endangered subspecies is represented in the stickleback population found within the study area. Due to the potential for introgression between G. a. williamsoni and the non-endangered subspecies G. a. microcephalus which also occurs in the drainage, a combination of morphologic (lateral plate) and genetic (allozyme) data were used to recognize the subspecies and pattern of gene flow between them. Populations from outside the study area assumed to be non-introgressed were used to establish subspecies characteristics.

The project area consists of: (1) the Santa Clara River from near the confluence with Castaic Creek upstream to near Saugus, (2) Castaic Creek from Highway 5 downstream to Highway 126, and (3) the downstream portion of San Francisquito Creek outside the National Forest (see Figure 1).

This study was conducted to provide data which will assist in the further planning of the Valencia Master Plan within the project area.

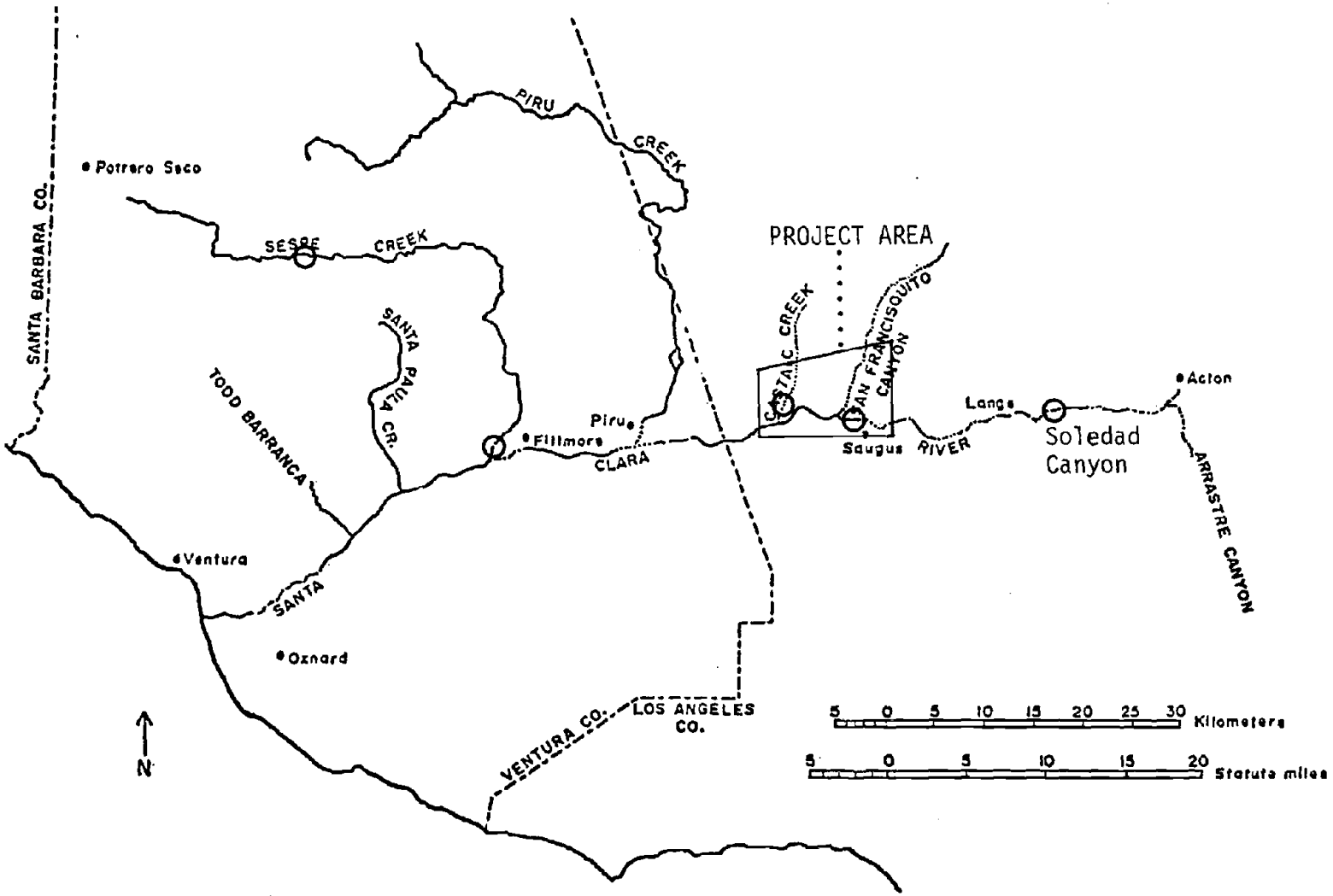


Figure 1. Project area and sample locations (O) used in this study.

INTRODUCTION

The threespine stickleback, Gasterosteus aculeatus, has a circumboreal distribution. The species is renowned for its morphological variability. In particular, lateral bony plates which vary in number (from 0 to about 35) have received considerable attention and have sometimes been used as the primary characteristic in subspecific determination. Along the Pacific coast of North America, three subspecies are commonly recognized based on lateral plate morphology (Miller and Hubbs, 1969). Two of these subspecies, G. a. microcephalus and G. a. williamsoni, are found in southern California. G. a. microcephalus is the "normal" resident freshwater morphological form of the threespine stickleback. It is recognized primarily by the fact that the lateral plates are limited to the anterior portion of the body (see Appendix 1). G. a. williamsoni, the unarmored threespine stickleback, is morphologically unusual in that it totally lacks lateral plates. Although other morphologic characteristics may differentiate these two subspecies, the lack of lateral plates has traditionally been used to define G. a. williamsoni and differentiate it from G. a. microcephalus.

The unarmored form was described by Girard (1854) as Gasterosteus williamsoni. Girard (1854) was unable to specify a type locality but it was later recognized as the upper part of the Santa Clara River in Soledad Canyon, probably between Lang and Acton in Los Angeles County, California (Miller, 1960). Later, other unarmored populations were described from the Los Angeles basin (Los Angeles, San Gabriel and Santa Ana Rivers) in southern California (e.g. Regan, 1909; Gasterosteus santae-annae). In 1925, Jordan and Hubbs united all southern California unarmored stickleback populations as G. a. williamsoni, a view supported by Miller (1960). Therefore,

at one time, G. a. williamsoni was found in at least the Santa Clara River, Los Angeles River, San Gabriel River and Santa Ana River drainages. However, the once abundant (Culver and Hubbs, 1917) Los Angeles basin populations had been extirpated by the mid-1940s (Miller, 1961), leaving G. a. williamsoni restricted to the upper Santa Clara River. The U.S. Fish and Wildlife Service listed the unarmored threespine stickleback as an endangered species in 1970 (Federal Register 35:16047). Critical habitat was proposed in 1980 (Federal Register 45:76012). Appendix 2 contains a description of the essential habitat (proposed critical habitat).

More recently, a population from San Antonio Creek has been recognized as G. a. williamsoni based on a 1976 report by Baskin and Bell, and another population was discovered in the Baldwin Lake basin in 1981 which also qualifies as G. a. williamsoni based on lateral plate number. These populations will not be discussed in this report because genetic data demonstrate they are not G. a. williamsoni (Haglund, 1988). Consequently, the federally endangered threespine stickleback is restricted to the Santa Clara River drainage.

METHODS

Field Work.

Field work was conducted between May and December, 1988. Although not designed or intended as a habitat survey, most of the stream in the study area was walked by the investigator. Castaic Creek from Interstate 5 downstream to the confluence with the Santa Clara River and all surface flow in San Francisquito Creek downstream of the National Forest boundary were examined. Due to sporadic rains during the study period flow conditions were variable and most stream sections were visited more than once. Although all surface waters were seined in these two areas, no fish were collected in San Francisquito Creek and only 15 Gasterosteus aculeatus were collected in Castaic Creek. Castaic Creek contained Gila orcutti, Cottus asper and Gambusia affinis in addition to the sticklebacks. Sticklebacks were also absent from more upstream areas of Castaic Creek (seined), while they were abundant in the most downstream permanent water within the National Forest (visual survey) in San Francisquito Creek. Within the Santa Clara River most field work occurred just upstream and downstream of McBean Parkway. Sticklebacks in this area are patchily distributed but locally abundant. This stream section contains Gila orcutti and Gambusia affinis in addition to the sticklebacks.

Fish were collected using a 10 foot, 1/8 inch mesh, knitted nylon minnow seine. At sampling localities, an attempt was made to collect 100 sticklebacks, 50 to be frozen and 50 preserved. Complete samples were collected at all localities where sticklebacks occurred except in Castaic Creek.

Lateral Plate Study.

All fish to be used for lateral plate counts were preserved in the field using a 10% solution of formaldehyde. In the laboratory, the samples were soaked in water to remove the formaldehyde. Once this was completed, the specimens were stained in a saturated solution of Alizarin Red S dissolved in 2% KOH (wt/vol). Following staining, the fish were destained in a 2% solution of KOH. Specimens were then placed in a 50% solution of isopropanol where further destaining took place. The destaining process removed the stain from the tissue leaving the bone stained a deep reddish purple.

The phenotype of all specimens was characterized by the total number of lateral plates that each individual possessed. The lateral plate number was obtained by summing the counts from both sides of a specimen. The lateral plates were counted under a dissecting microscope, and all lateral plates were counted regardless of size or position.

Electrophoretic Study.

Specimens to be used for electrophoresis were placed on dry ice immediately following capture and transported to U.C.L.A. where they were stored at -20C until dissected. Electrophoretic methods follow those described by Buth et al. (1984). The five polymorphic loci resolved by Buth et al. (1984) were run by the investigator under the same electrophoretic conditions as those in the aforementioned study. Allelic products were designated on the basis of their relative electrophoretic mobility compared to the origin (0) and that of the same reference (i.e., most common) allele (100) employed by Buth et al. (1984).

LATERAL PLATE DATA

The original description (Girard, 1854) of G. a. williamsoni as well as the present Recovery Plan (1985) diagnosis is based on lateral plate counts. All lateral plate counts presented here were obtained by summing the counts from both sides of the fish; all lateral plates were counted regardless of size or position (Figure 2). Prior to counting lateral plates, specimens were stained with Alizarin Red S, a bone stain which facilitates the examination of lateral plates and increases the accuracy of counts on small fish.

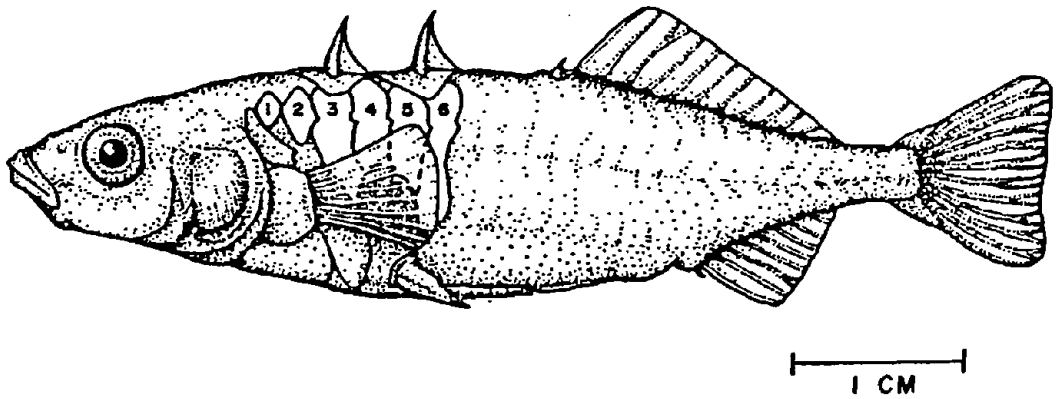


Figure 2. A specimen of the threespine stickleback, Gasterosteus aculeatus, with six lateral plates on its left side. (Reproduced from Bell and Haglund, 1978).

Before presenting the data it is necessary to examine the official method by which G. a. williamsoni is recognized. As would be expected, in order to distinguish subspecies several individuals must be examined. "G. a. williamsoni is readily distinguished from the other two subspecies on the basis of lateral plate counts alone, provided that 10 to 15 morphologically mature (i.e., at least 25mm standard length (SL), Hagen and McPhail,

1970) specimens are available" (Recovery Plan, 1977). Hagen and McPhail (1970) state that lateral plate development is complete by 25mm (SL) but that counts on fish below 30mm (SL) are not accurate unless the specimens are stained with Alizarin. "Samples of G. a. williamsoni generally have an average of 0.06-0.55 lateral plates per individual and G. a. microcephalus has an average of more than six lateral plates per individual (Bell, 1976)" (Recovery Plan, 1977). However, a memorandum (dated 1 February, 1978) from a meeting of the Recovery Team on 27 January, 1978, provides the following amended definition of G. a. williamsoni: "... a sample of 50 specimens 25mm (SL) or more must be used, they must average 2 plates (both sides counted) or less, and/or at least 50% of the specimens must be plateless." The latest Recovery Plan (1985) includes a diagnosis similar to the one contained in the 1977 Recovery Plan. There are, however, two critical revisions in the 1985 definition: (1) 25 specimens must be examined and (2) morphologically mature is defined as at least 32mm (SL) citing Bell (1981). No statement regarding the percentage of the population that must be unarmored is included and samples of G. a. williamsoni are considered to "generally average 0.06 to 0.55 lateral plates per individual" as in the 1977 Recovery Plan. Therefore, the average number of lateral plates per individual is the most consistent recognition criterion; while sample size, specimen size and percent of individuals unarmored are variable considerations. The new size minimum imposed in the 1985 Recovery Plan is based on Bell (1981). However, on page 70 Bell (1981) states, "In addition, plate number stops increasing in low morph specimens at about 21.5mm standard length compared to about 30.3mm in complete morphs." Thus counts on specimens of G. a. microcephalus and G. a. williamsoni greater

than 22mm (SL) would produce accurate counts provided specimens under 30mm (SL) were stained with Alizarin Red S. The sample size consideration can be simply stated: the larger the sample size the more accurate the estimation of average plate count per individual, but the 1985 Recovery Plan provides a minimum sample size of 25 specimens. The percentage of unarmored fish in the population is highly correlated with number of lateral plates per individual and would thus reflect similar trends.

The data supplied here conform to most of the requirements previously stipulated; exceptions will be noted as is appropriate.

Baseline lateral plate data for the entire Santa Clara River system are published in Bell's (1976) dissertation and presented in Appendix 3. These are similar to those supplied in a California Department of Fish and Game unpublished report for contract No. AB-23 (Bell, 1975). All collections were made in 1975. The lateral plate counts were made on specimens greater than 25mm (SL) and therefore do not technically conform to the latest diagnosis. However, as previously indicated, the length requirement in the revised Recovery Plan is apparently in error. Figure 3 shows the percentage of unplated fish in Bell's samples and Figure 4 shows the average number of lateral plates per fish in the same samples graphed against distance upstream in the Santa Clara River. Bell's (1976) data indicate that the values (percent unarmored fish per sample and number of lateral plates per fish) for the Santa Clara River samples adjacent to tributaries are indicative of the values in the tributaries. The data show that G. a. williamsoni is primarily restricted to the upper Santa Clara while G. a. microcephalus occurs in Sespe Creek and dominates downstream populations. Gene flow between the two subspecies creates clinal

variation in lateral plate count values in the Santa Clara River (see Figures 3 and 4). G. a. williamsoni dominates the Santa Clara drainage and its tributaries from just downstream of the Del Valle settlement near the county line upstream to the headwaters of the Santa Clara River and all tributaries upstream of this area in which sticklebacks are found. Bell (1975, 1976) concluded that these morphologic data suggest only a low level of gene flow from G. a. microcephalus to G. a. williamsoni in the upstream portion of the river and hypothesizes that this gene flow occurs at a low level primarily because of long stretches of unsuitable habitat or seasonally dry stretches in the river.

The pattern of lateral plate counts and conclusions described by Bell (1975, 1976) were used to write the 1977 Recovery Plan and were not significantly modified in the revised 1985 version.

Figures 3 and 4 also show data points from this study, in addition to Bell's (1976) data. It is important to recognize that the more recently collected data (Table 1) are consistent with the pattern of lateral plate distribution discerned by Bell (1976). As a result, Bell's conclusion that only low levels of gene flow exist between the two subspecies is reinforced because the system has remained stable over the 12 years since Bell's work. This also means that populations upstream of Piru Creek are still dominated by the unplated form, G. a. williamsoni.

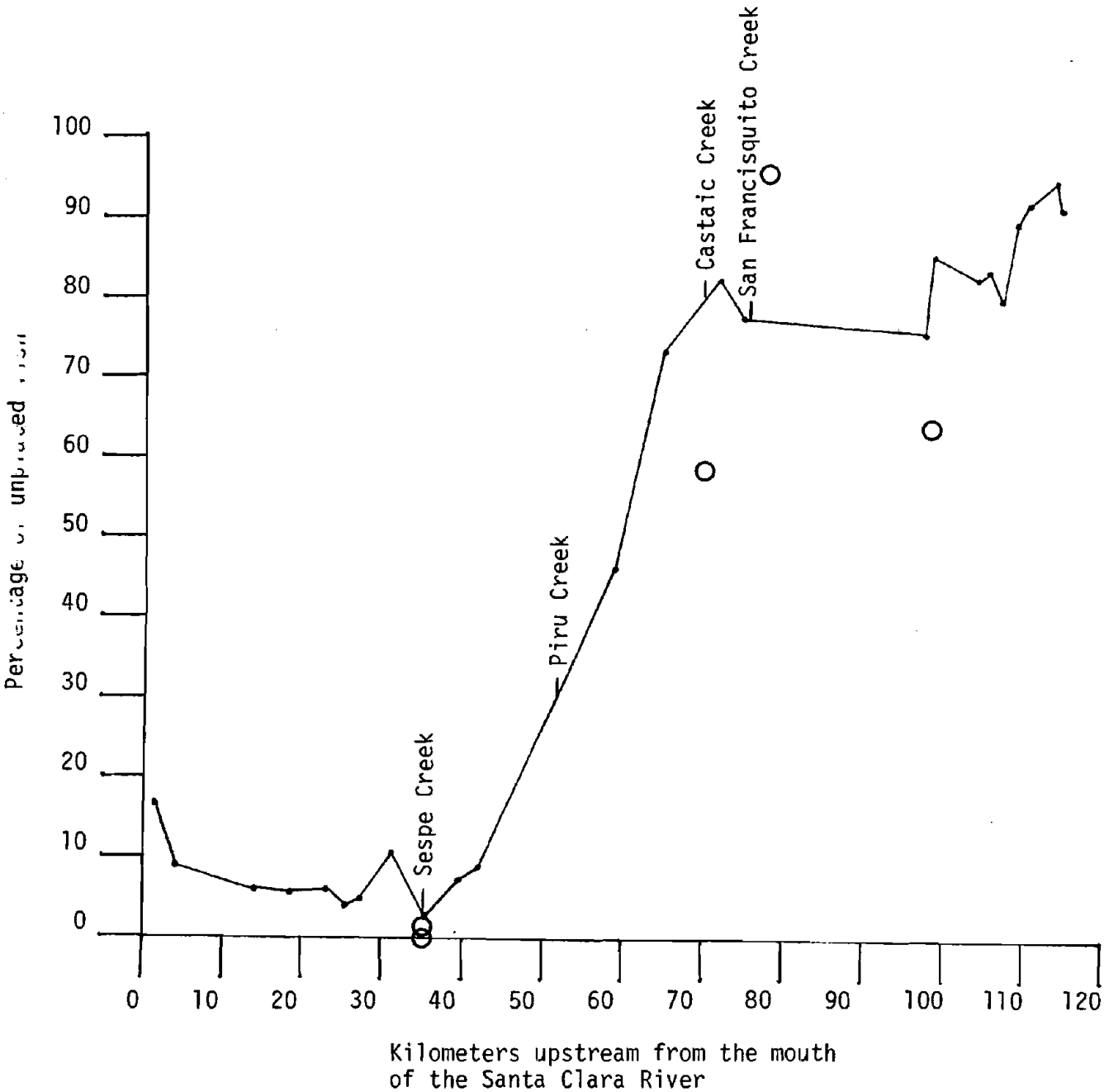


Figure 3. Graph of the percentage of unplated fish versus distance upstream in the Santa Clara River. Data calculated from Bell (1976) are graphed as a series of connected solid points, while the more recent data from Table 1 are graphed as open circles.

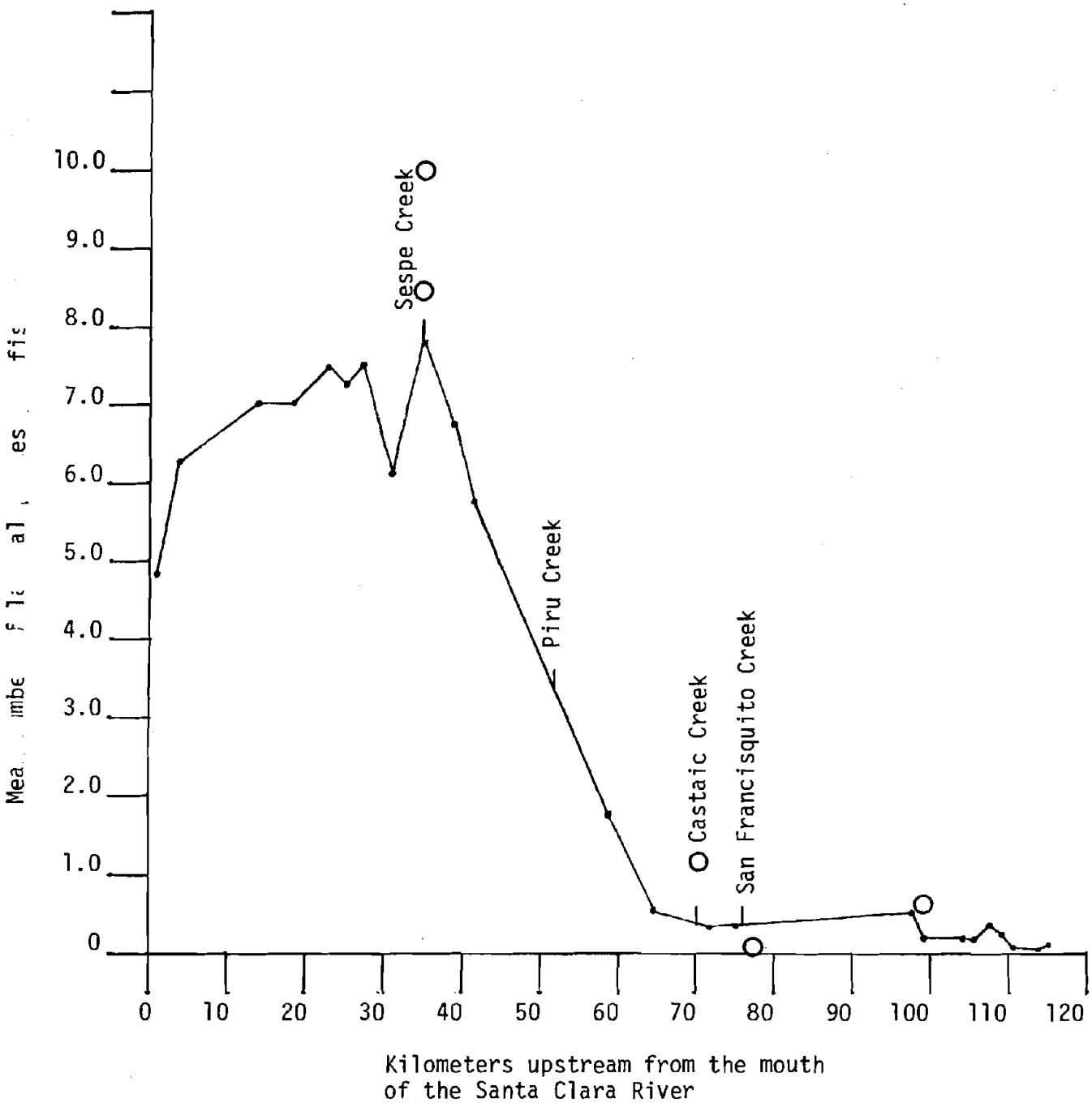


Figure 4. Graph of the mean number of lateral plates per fish versus distance (km) upstream in the Santa Clara River. Data calculated from Bell (1976) are graphed as a series of connected solid points, while the more recent data from Table 1 are graphed as open circles.

TABLE 1. Lateral plate data. Measurements based on specimens greater than 25.00mm (SL). Values for specimens over 32.00mm are indicated in parentheses for those samples which contained specimens between 25.00mm and 32.00mm (SL).

Sample Locality	Equivalent Bell Locality	Percentage Unplated Fish	Mean Number of Lateral Plates/Individual	Sample Size
Upper Sespe Creek	37	0 (0)	10.04 (10.00)	51 (41)
Lower Sespe Creek	28	2	8.48	50
Lower Castaic Creek	None	58.33	1.17	12
Santa Clara River at MacBean Pkwy	16	96.08 (95.92)	0.10 (0.10)	51 (49)
Santa Clara River Soledad Canyon	18	64 (62)	0.68 (0.77)	50 (39)

ELECTROPHORETIC DATA

Electrophoretic analyses of allozyme variation have been successful in clarifying many intraspecific systematic problems. In the early 1980's this technique was finally applied to the study of G. a. williansoni. Buth (1984) analyzed samples from nine southern California river drainages, including a series of samples from the Santa Clara River drainage. In a separate publication, two of the Santa Clara River samples were used to compare G. a. williamsoni and G. a. microcephalus (Buth et al, 1984). Together these two studies contain four very important conclusions:

1). Buth (1984) and Buth et al (1984) both conclude that G. a. williamsoni is genetically differentiated from G. a. microcephalus and worthy of subspecific status.

2). The allozyme data from Buth et al (1984) support the conclusion, of restricted gene flow between the subspecies, that Bell (1976) reached based on lateral plate data.

3). Buth (1984) showed that the unplated population of sticklebacks from San Antonio Creek, Santa Barbara County, is not G. a. williamsoni but is actually an unusual population of G. a. microcephalus that has converged on the plateless condition of G. a. williamsoni. When published in a peer reviewed journal these data will result in the delisting of the San Antonio Creek population.

4). The above conclusion calls into question the use of lateral plate counts as the diagnostic characteristic of G. a. williamsoni. Buth (1984) and Buth et al (1984) provide allozyme data which can be used to recognize G. a. williamsoni.

Buth (1984) surveyed 45 presumptive loci (see Appendix 4). Six of the loci surveyed were polymorphic in the Santa Clara River drainage and thus potentially provide information that may be used to recognize G. a. williamsoni. Buth's (1984) data also serves as baseline data from samples collected in 1982 (see Table 2a). Table 2b provides data from samples collected and analyzed in 1987 and 1988. Clearly the two sets of data are

very similar and little change has occurred in the 5 to 6 years between sampling. It is also obvious that certain loci provide more information than others. Each locus is briefly discussed below.

S-Acon-A (Aconitate hydratase). This system does not provide any useful information. The polymorphism is due to the appearance of a rare allele in the 1982 Sespe Creek sample (Buth) which was not present in either later Sespe Creek sample.

Ada-A (Adenosine deaminase). In this system, G. a. microcephalus is fixed for the 100 allele while two other alleles are found in G. a. williamsoni of the upper Santa Clara River. However, the non-100 alleles are absent from San Francisquito Creek. Therefore the presence of non-100 alleles can be used to recognize G. a. williamsoni but their absence is uninformative.

Est-5 (Esterase). This locus is useful. G. a. williamsoni is fixed for the 100 allele while G. a. microcephalus is dominated by a non-100 allele.

Iddh-A (L-iditol dehydrogenase). Here again G. a. williamsoni is fixed for one allele (100 allele) while in G. a. microcephalus another allele occurs at relatively high frequencies.

Pgm-A (Phosphoglucomutase). The frequencies of the two alleles at this locus do not differentiate the two subspecies.

Pnp-A (Purine-nucleoside phosphorylase). At this locus there is a tendency for G. a. williamsoni populations to have higher frequencies of the 100 allele than G. a. microcephalus populations. But the subspecies are more clearly recognized by the loci where one of the subspecies show fixation.

Thus it can be seen that Ada-A, Est-5 and Iddh-A most readily differentiate the two subspecies because in each case one subspecies is fixed for an allele while the other subspecies is polymorphic. This pattern of allelic differentiation indicates a lack of panmixis and restricted gene flow between the subspecies. The allozyme data show the same pattern of clinal variation as was seen in the lateral plate data (Bell, 1976), (see Figure 5). The cline is very steep in the stream section around Piru

Creek. As in the lateral plate data, the steepness of the cline indicates restricted gene flow between the upper and lower Santa Clara River. The allozyme data are also concordant with the lateral plate data in that both show that the drainage upstream of the Del Valle area is dominated by G. a. williamsoni.

TABLE 2a. Allele frequencies of the polymorphic loci in the Santa Clara River

Locus	Alleles	Buth et al (1984)			
		Sespe Creek	County Line	San Francisquito Creek	Acton
S-Acon-A					
	108	0.17	0	0	0
	100	0.83	1.00	1.00	1.00
Ada-A					
	91	0	0.01	0	0.84
	100	1.00	0.99	1.00	0
	105	0	0	0	0.16
Est-5					
	87	0.74	0.07	0	0
	95	0.05	0	0	0
	100	0.21	0.93	1.00	1.00
Iddh-A					
	45	0.70	0.04	0	0
	100	0.30	0.96	1.00	1.00
Pgm-A					
	93	0.36	0.15	0.06	0.33
	100	0.64	0.85	0.94	0.67
Pnp-A					
	100	0.71	0.64	0.99	0.98
	145	0.29	0.36	0.01	0.02

Table 2b. Allele frequencies of the polymorphic loci in the Santa Clara River

Locus	Alleles	Haglund				
		upper Sespe Cr.	lower Sespe Cr.	Castaic Cr.	Santa Clara R. at San Francisquito	Soledad Cyn.
S-Acon-A						
	108	0	0	0	0	0
	100	1.00	1.00	1.00	1.00	1.00
Ada-A						
	91	0	0	0	0	0.25
	100	1.00	1.00	1.00	1.00	0.70
	105	0	0	0	0	0.05
Est-5						
	87	0	0	0	0	0
	95	0.95	0.53	0	0.01	0
	100	0.05	0.47	1.00	0.99	1.00
Iddh-A						
	45	0.55	0.61	0	0	0
	100	0.45	0.39	1.00	1.00	1.00
Pgm-A						
	93	0.24	0.37	0	0.06	0.22
	100	0.76	0.63	1.00	0.94	0.78
Pnp-A						
	100	0.62	0.88	0.97	0.90	0.78
	145	0.38	0.12	0.03	0.10	0.22

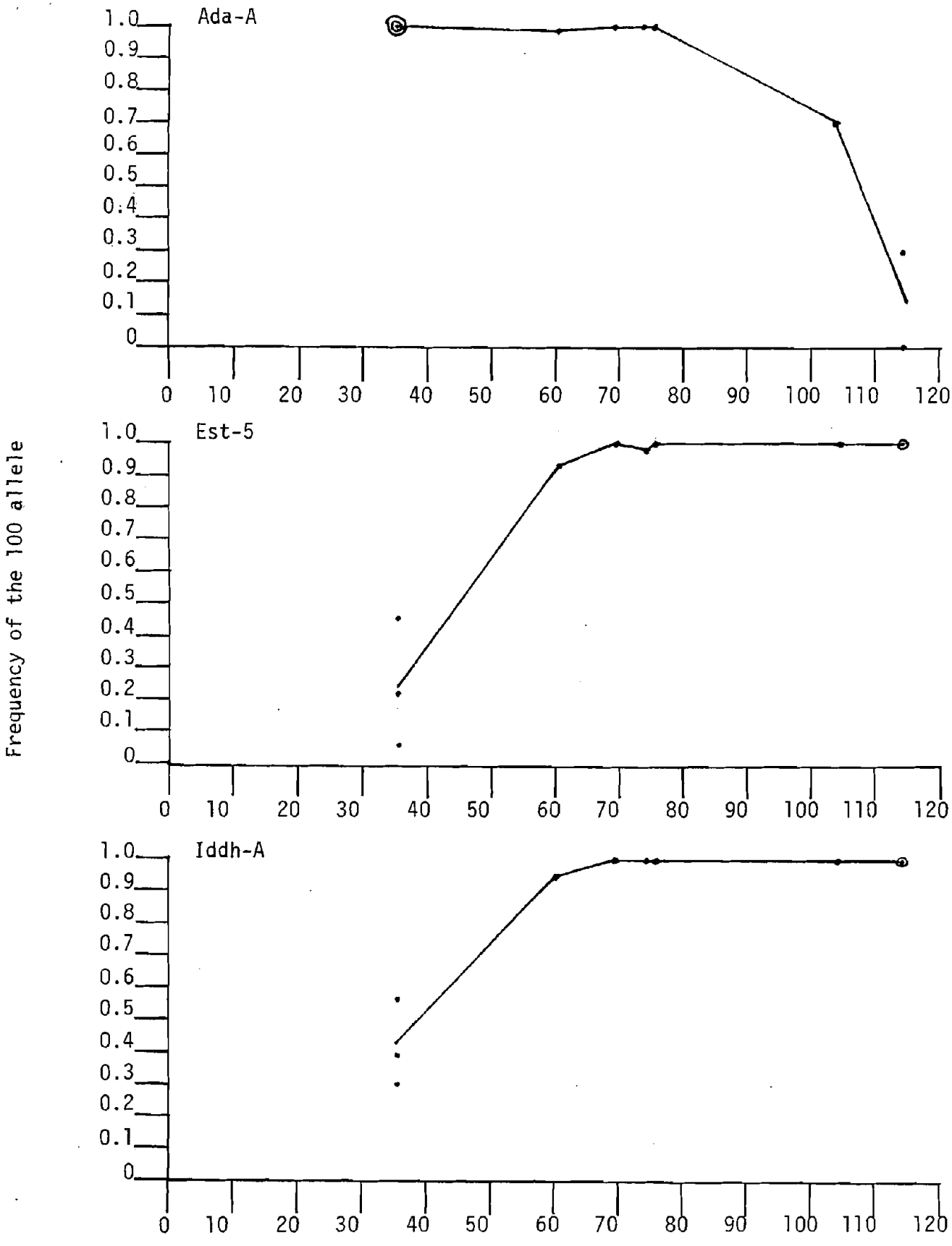


FIGURE 5. Graphs of the frequency of the 100 allele for three loci (Ada-A, Est-5 and Iddh-A). For values from tributaries, the upstream distance graphed is the distance from the mouth of the Santa Clara River to the confluence with the tributary.

STATUS OF *GASTEROSTEUS ACULEATUS WILLIAMSONI* IN THE PROJECT AREA

For the purposes of this discussion the project area will be broken into three units, each of which will be treated separately: (1) Castaic Creek, (2) San Francisquito Creek, and (3) Santa Clara River.

In the Castaic Creek project area, surface flow is intermittent from Interstate Highway 5 downstream to Highway 126. At the lower part of this section, there are a few isolated permanent pools. From Highway 126 downstream to the confluence with the Santa Clara River there is permanent surface flow. All surface water in Castaic Creek was surveyed for three-spine sticklebacks, but only 15 specimens of *Gasterosteus aculeatus* were collected. This stream section is not heavily utilized by sticklebacks. The small stickleback population that was collected is dominated by *G. a. williamsoni* (see Tables 1 and 2b). However, sticklebacks may not permanently occupy this section of stream; Bell was unable to collect a sample from this area in 1975. Upstream of the project area, there is usually no surface flow on the correctional facility property, but permanent water is found below the Castaic dam spillway and for a short distance downstream. In 1975, Bell found a large stickleback population (locality 45) dominated by *G. a. williamsoni*, but no sticklebacks were found in this area in 1988.

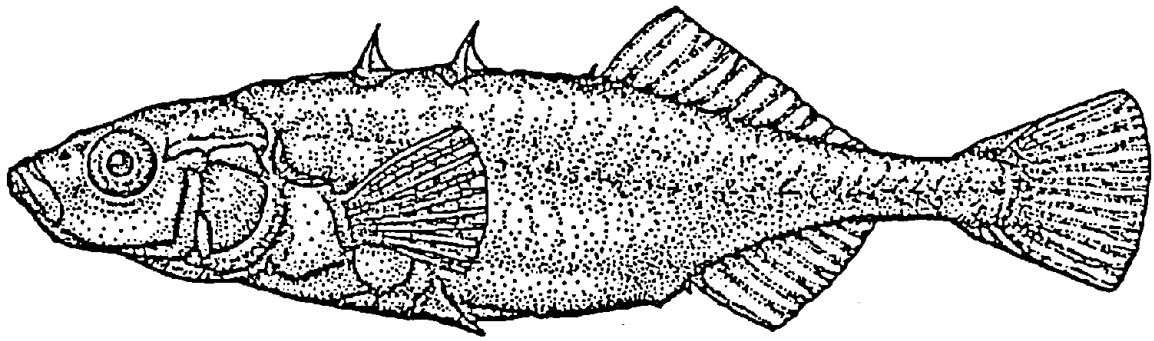
San Francisquito Creek is dry from above the Angeles National Forest boundary downstream into the project area. Incidental water from a vegetable packing plant initiates surface flow approximately 0.4 km upstream of the confluence with the Santa Clara River. The water quality appears poor and there are no aquatic animals in the flow. Near the

confluence there is habitat that appears suitable for sticklebacks but no fish were found. Gasterosteus does not occur in the San Francisquito Creek project area. Upstream of the dry area, sticklebacks are abundant where Bell collected sticklebacks in 1975 (locality 46). This is apparently the most downstream locality of sticklebacks in the San Francisquito drainage; it is over 10 km upstream of the confluence with the Santa Clara River.

The Santa Clara River from the Del Valle essential habitat upstream into the project area has permanent surface flow, although flow is minimal above McBean Parkway and little to no suitable stickleback habitat exists in this upstream area. There is no surface flow upstream of the project area until the Soledad Canyon essential habitat. Sticklebacks are found from McBean Parkway downstream. The sticklebacks in this area are predominantly G. a. williamsoni (see Tables 1 and 2b). In this area the river has many channels, isolated stretches and pools. Sticklebacks are found wherever the habitat is suitable. Sticklebacks were particularly abundant in some of the isolated stretches and pools. Sticklebacks were uncommon and scattered in the main channel due to the lack of suitable habitat (high flows) at the time of the survey.

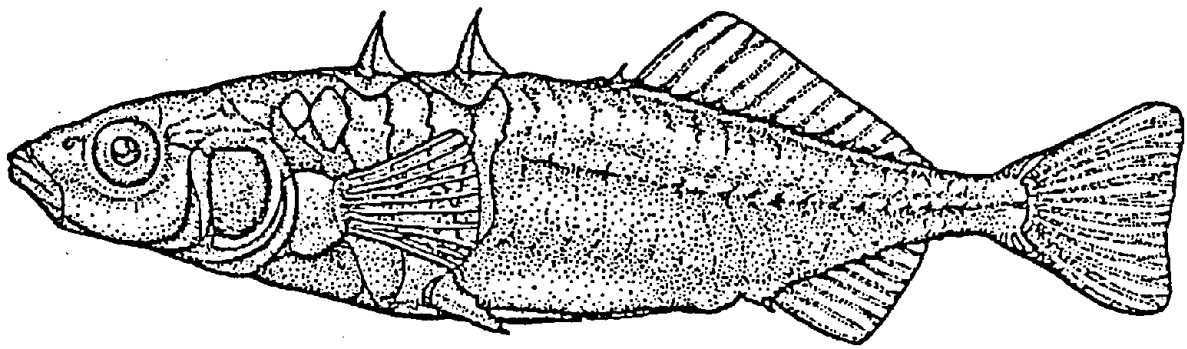
When sticklebacks are found in the project area, the populations are dominated by the endangered subspecies G. a. williamsoni. However, significant numbers of sticklebacks are found only in the Santa Clara River. Sticklebacks are absent from San Francisquito Creek and are now rare to absent in Castaic Creek. Furthermore, based on their rarity and Bell's inability to locate sticklebacks in lower Castaic Creek in 1975, it is possible that this stream section is not regularly used by stickle-

backs. Therefore, only the Santa Clara River downstream of McBean Parkway provides important habitat for the endangered unarmored three-spine stickleback within the project area.



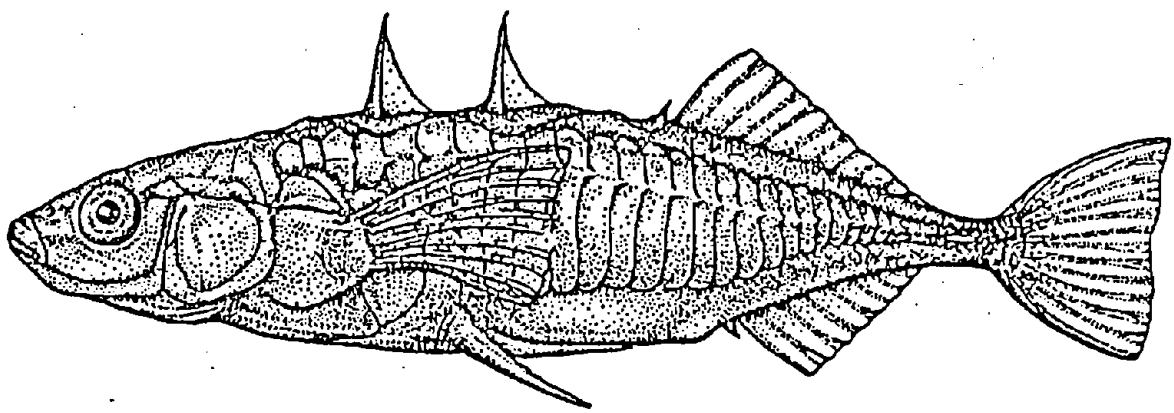
G. a. williamsoni

1 CM



G. a. microcephalus

1 CM



G. a. aculeatus

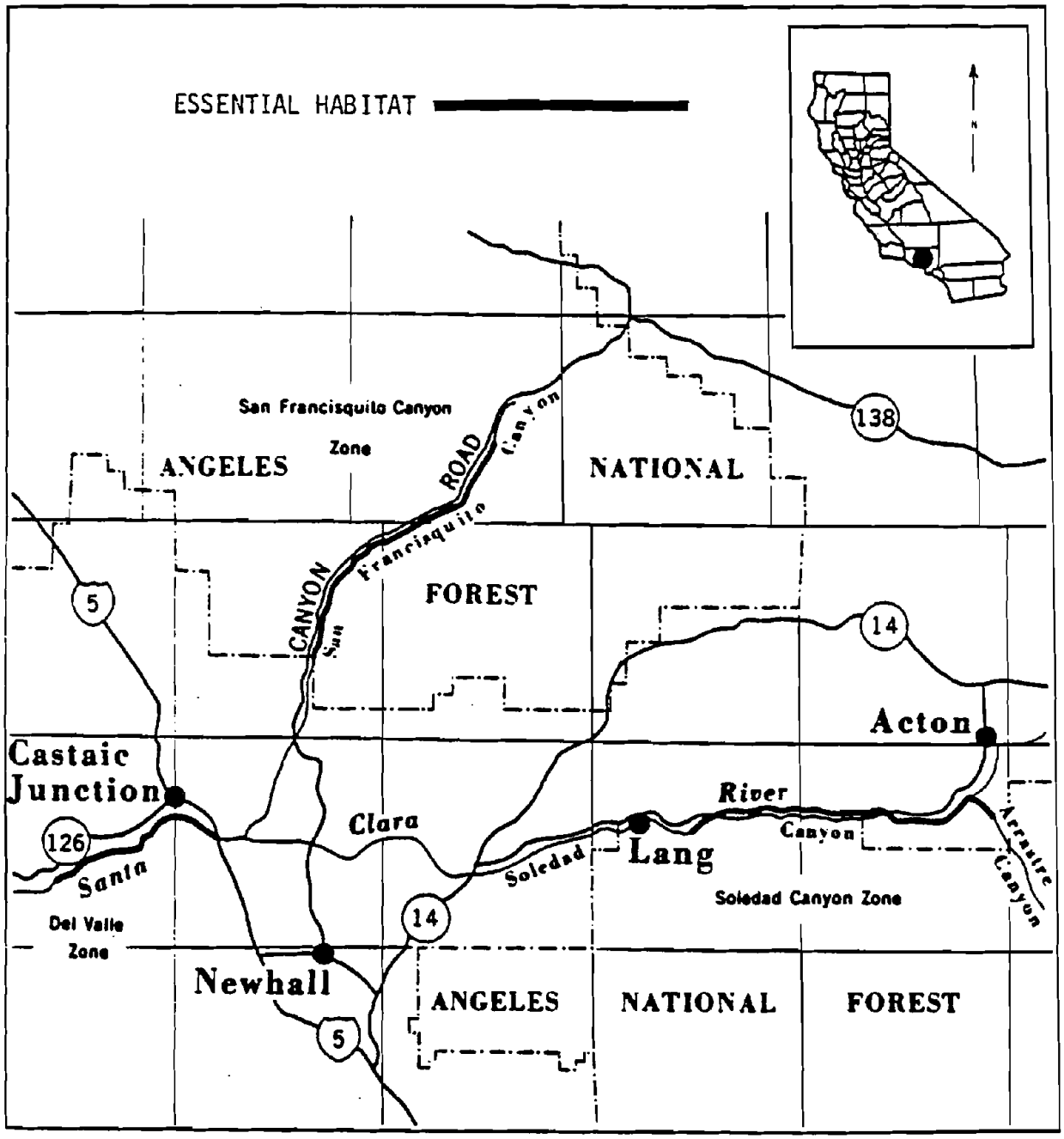
1 CM

APPENDIX 2

Appendix A (pages 68 and 69) from the 1985 revised Unarmored Threespine Stickleback Recovery Plan. The essential habitat (proposed critical habitat) for Gasterosteus aculeatus williamsoni.

APPENDIX A

Essential habitat for the unarmored threespine stickleback in Los Angeles County, California.



Del Valle, San Francisquito Canyon and Soledad Canyon Zones

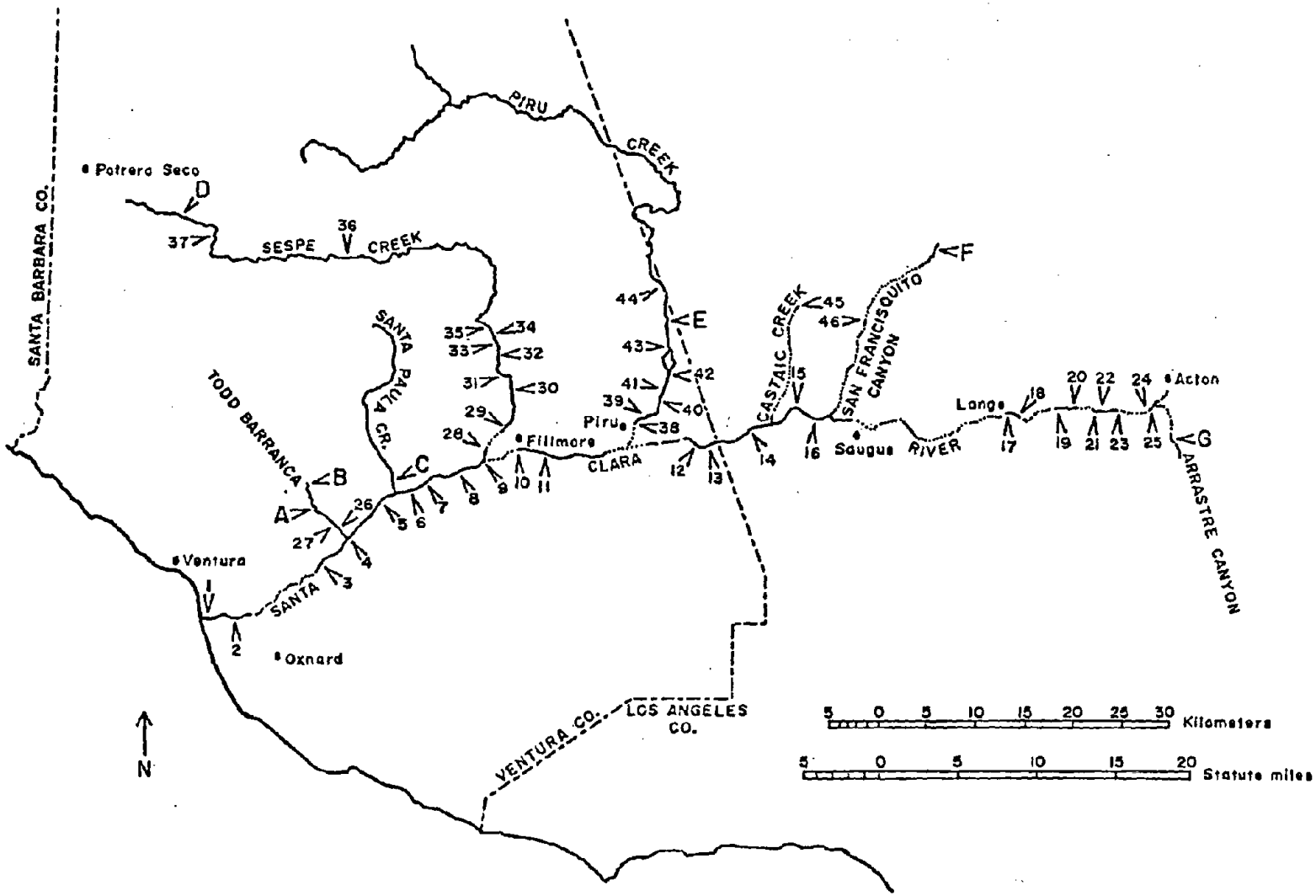
Los Angeles County, CALIFORNIA

Description of the three zones of essential habitat for the unarmored threespine stickleback in Los Angeles County.

1. Del Valle zone. An area of land and water with the following components (San Bernardino meridian): Santa Clara River within T4N, R16W and R17W, beginning at its confluence with San Martinez Grande Canyon, at a point 0.9 of a mile (1.5 kilometers) southwest of Del Valle settlement, and extending upstream approximately 5.6 miles (8.8 kilometers) to the overcrossing of Interstate Highway 5.

2. San Francisquito Canyon zone. An area of land and water with the following components (San Bernardino meridian): San Francisquito Canyon watercourse, within T5N, R16W and T6N, R15W, beginning at a point where the Angeles National Forest boundary intersects the San Francisquito Canyon watercourse approximately 2½ miles southwest of San Francisquito Powerhouse No. 2, and extending upstream in San Francisquito Canyon approximately 8.4 miles (13.5 kilometers) to San Francisquito Powerhouse No. 1, near its junction with Clearwater Canyon.

3. Soledad Canyon zone. An area of land and water in Los Angeles County, with the following components (San Bernardino meridian): Santa Clara River within T4N, R13W and R14W, beginning at a point 1.4 miles (2.3 kilometers) upstream in Soledad Canyon from the community of Lang, at the downstream end of the area called River's End Park, at 34° 26' 7" N, 118° 21' 51" W, thence extending upstream approximately 8.5 miles (13.7 kilometers) to its confluence with Arrastre Canyon, at a point located about 0.6 of a mile (1 kilometer) southwest of Los Angeles County Rehabilitation Camp, thence upstream in Arrastre Canyon approximately 0.8 of a mile (1.4 kilometers) to 34° 26' 7" N, 118° 11' 51" W.



A. The numbers represent localities collected by Bell (1976).

B. Table 1. Lateral plate counts in the Santa Clara River system.

STATION	LATERAL PLATES																		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	N	-H	x
1	35	13	6	11	16	12	20	16	25	11	9	8	5	1			199	2.46	5.43
2	17	11	3	8	14	7	28	13	25	16	25	9	6				182	2.41	6.31
3	14	8	2	5	14	19	27	22	37	19	36	10	6	4	1		224	2.42	7.01
4	12	7	5	11	14	17	18	21	24	23	37	12	12	1			214	2.47	7.02
5	6	0	3	3	3	6	10	10	11	12	18	7	4	2			95	2.37	7.52
6	9	6	5	7	11	11	21	25	25	30	29	12	10	2			203	2.44	7.24
7	9	1	3	7	5	12	12	22	38	20	33	15	6				186	2.29	7.53
8	20	9	6	7	11	11	26	17	23	16	25	5	2	1			179	2.42	6.06
9	6	1	3	7	12	12	19	25	42	27	50	15	9	2			230	2.23	7.96
10	14	7	5	12	10	12	24	15	22	17	18	10	12	5			183	2.42	6.72
11	17	6	9	4	18	20	32	18	21	9	17	6	2				179	2.38	5.79
12	22	6	5	2	4	4	2	1	1								47	1.70	1.91
14	71	11	7	2	3	2											96	0.93	0.55
15	48	5	2	2	0	1											58	0.67	0.34
16	52	10	2	1	1	1											67	0.77	0.41
17	160	18	15	8	7	2											210	0.89	0.53
18	83	8	3	1	1												96	0.53	0.22
19	153	13	8	3	2												184	0.57	0.25
20	154	19	8	1	1												183	0.57	0.23
21	176	21	6	12	2	2	1										220	0.77	0.42
22	154	9	11	4	3												172	0.63	0.31
23	193	7	6	1	2												209	0.36	0.14
24	197	6	3														206	0.21	0.06
25	167	9	1	2	0	0	1										180	0.32	0.12
28	6	2	2	4	11	7	16	22	36	24	42	15	12	2			201	2.29	7.98
29	6	2	6	3	4	11	10	11	21	20	18	6	3	1			122	2.26	7.16
30	1	1	5	1	2	4	20	16	22	10	14	9	4				109	2.19	7.59
31			1	1	2	2	8	6	19	26	61	39	43	8	4		220	2.22	10.06
32	4	1	1	2	8	5	16	10	20	22	44	27	28	9	3		200	2.30	9.09
33		1	0	2	2	2	8	10	18	23	37	24	42	9	16	1	195	2.20	10.19
34	4	1	1	2	7	8	16	17	39	26	34	12	7	4	1		179	2.25	8.15
35	1	0	0	1	2	5	6	12	33	27	53	25	26	18	5		214	2.14	9.76
36					1	0	7	3	18	27	47	40	20	9	2	1	175	1.94	10.09
37									3	0	18	9	13	3	5	1	52	1.70	11.21
26	3	2	2	2	5	2	2	5	2	5	14	2	3	1			50		7.22
27	4	1	0	2	1	4	8	3	6	4	4	3	2				42		6.65
38	21	5	4	2	5	4	4	2	0	0	3						50		2.54
39	19	7	5	5	6	4	2	1	1								50		2.09
40	21	3	9	4	4	3	3	3									50		2.06
41	16	9	4	5	8	3	3	2									50		2.24
42	14	7	7	4	7	2	6	2	0	1							50		2.62
45	36	4	5	4	1												50		1.09

C. Calculations from Bell's (1976) data.

sample number	kilometers upstream	percentage of unplated fish	mean number of plates per fish
Santa Clara River			
1	1.1	17.7	4.87
2	3.9	9.3	6.35
3	14.2	6.3	7.00
4	18.0	5.6	7.03
5	23.3	6.3	7.49
6	25.6	4.4	7.27
7	27.7	4.8	7.51
8	32.0	11.2	6.07
9	35.3	2.6	7.84
10	39.3	7.7	6.72
11	42.3	9.5	5.78
12	58.8	46.8	1.81
13	60.4		
14	64.7	74.0	0.55
15	71.8	82.8	0.34
16	74.5	77.6	0.39
17	97.6	76.2	0.52
18	98.1	86.5	0.22
19	103.7	83.2	0.25
20	105.3	84.2	0.23
21	107.4	80.0	0.42
22	108.2	89.5	0.32
23	110.5	92.3	0.14
24	114.2	95.6	0.06
25	114.6	92.8	0.13

Todd Barranca (at 17.3km)			
26	1.1	6.0	7.22
27	2.6	9.5	6.69
Sespe Creek (at 35.3km)			
28	1.6	3.0	7.99
29	4.3	4.9	7.16
30	8.0	0.9	7.59
31	11.7	0	10.06
32	13.1	2.0	9.11
33	14.2	0	10.19
34	15.2	2.2	8.16
35	16.5	0.5	9.74
36	49.0	0	10.08
37	70.9	0	11.21
Piru Creek (at 51.8km)			
38	2.0	42.0	2.54
39	3.5	38.0	2.06
40	5.9	42.0	2.06
41	7.5	32.0	2.22
42	9.1	28.0	2.60
43	12.0		
44	18.7		
Castaic Creek (at 69.8km)			
45	8.1	72.0	0.60
San Francisquito Creek (at 75.4km)			
46	13.9		

APPENDIX 4

Table 1 from Buth et al (1984) showing the enzyme systems surveyed in G. a. williamsoni and G. a. microcephalus from the Santa Clara River.

TABLE 1. ENZYME SYSTEMS EXAMINED AND ELECTROPHORETIC CONDITIONS EMPLOYED

Enzyme	Enzyme commission number	Locus	Number of allelic products resolved*	Tissue source	Electrophoretic conditions†
Acid phosphatase	3.1.3.2	Acp-A	1	liver	A
Adenosine deaminase	3.5.4.4	Ada-A	3	muscle	B
Adenylate kinase	2.7.4.3	Ak-A	1	muscle	C
Alcohol dehydrogenase	1.1.1.1	Adh-A	1	liver	D
Aminopeptidase	3.4.11.1	Ap-A	1	muscle	B
Aspartate aminotransferase (mitochondrial)	2.6.1.1	M-Aat-A	1	liver	E
Aspartate aminotransferase (supernatant)	2.6.1.1	S-Aat-A	1	liver	E
Creatine kinase	2.7.3.2	Ck-A	1	muscle	D
Creatine kinase	2.7.3.2	Ck-B	1	brain	D
Dihydrolipoamide reductase	1.6.4.3	Dlr-B	1	brain	B
Dipeptidase	3.4.13.11	Pept-A	1	muscle	D
Dipeptidase	3.4.13.11	Pept-S	1	muscle	D
Esterase	-	Est-3	1	brain	B
Esterase	-	Est-4	1 (+1)	brain	B
Esterase	-	Est-5	2 (+1)	brain	B
Fructose-bisphosphate	3.1.3.11	Fbp-A	1	liver	D
Fructose-bisphosphate aldolase	4.1.2.13	Ald-A	1	muscle	B
Fructose-bisphosphate aldolase	4.1.2.13	Ald-B	1	liver	B
Fructose-bisphosphate aldolase	4.1.2.13	Ald-C	1	brain	B
Fumarate hydratase	4.2.1.2	Fum-A	1	liver	B
Glucose dehydrogenase	1.1.1.47	Gcdh-A	1	liver	B
Glucosephosphate isomerase	5.3.1.9	Gpi-A	1	muscle	F
Glucosephosphate isomerase	5.3.1.9	Gpi-B	1	muscle	F
Glucose-6-phosphate dehydrogenase	1.1.1.49	G-6-pdh-A	1	muscle	B
Glutamate dehydrogenase	1.4.1.2	Gdh-A	1	liver	B
Glyceraldehyde-phosphate dehydrogenase	1.2.1.12	Gapdh-A	1	muscle	D
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G-3-pdh-A	1	muscle	C
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G-3-pdh-B	1	liver	A
L-Iditol (sorbitol) dehydrogenase	1.1.1.14	Iddh-A	2	liver	D
Isocitrate dehydrogenase (mitochondrial)	1.1.1.42	M-Icdh-A	1	muscle, liver	B
Isocitrate dehydrogenase (supernatant)	1.1.1.42	S-Icdh-A	2	liver, muscle	B
Lactate dehydrogenase	1.1.1.27	Ldh-A	1	muscle	F
Lactate dehydrogenase	1.1.1.27	Ldh-B	1	heart	F
Lactate dehydrogenase	1.1.1.27	Ldh-C	1	eye	F
Malate dehydrogenase (NAD; mitochondrial)	1.1.1.37	M-Mdh-A	1	muscle	E
Malate dehydrogenase (NAD; supernatant)	1.1.1.37	S-Mdh-A	1	muscle	E
Malate dehydrogenase (NAD; supernatant)	1.1.1.37	S-Mdh-B	1	muscle	E
Malate dehydrogenase (NADP; mitochondrial)	1.1.1.40	M-Me-A	1	muscle	E
Mannosephosphate isomerase	5.3.1.8	Mpi-A	1	muscle	F
Phosphoglucomutase	2.7.5.1	Pgm-A	2	muscle	F
Phosphogluconate dehydrogenase	1.1.1.44	Pgdh-A	1	liver	D
Purine-nucleoside phosphorylase	2.4.2.1	Pnp-A	2	muscle	E
Pyruvate kinase	2.7.1.40	Pk-A	1	muscle	E
Superoxide dismutase (supernatant)	1.15.1.1	S-Sod-A	1	liver	D
Xanthine dehydrogenase	1.2.1.37	Xdh-A	1	liver	D

*The number of allelic products resolved are those appearing in the initial comparison of 20 specimens per subspecies. The numbers of additional products resolved when the sampling was increased to 50 specimens per subspecies are shown in parentheses.

†A, Citrate pH 7.0 [13] 5.5 V/cm for 6 hr; B, phosphate-citrate pH 7.0 [14] 4.4 V/cm for 9 hr; C, citrate pH 8.0 [13] 5.5 V/cm for 6 hr; D, Tris–citrate pH 8.0 [14] 6 V/cm for 8 hr; E, Tris–citrate pH 7.0 [15] 5 V/cm for 24 hr; F, 'Poulik' system [14] 11 V/cm for 5 hr.

APPENDIX 5

Allele frequencies of the five polymorphic loci in G. a. microcephalus and G. a. williamsoni. Modified from Buth et al (1984).

Locus	Alleles	Allele frequencies	
		G. a. microcephalus	G. a. williamsoni
Ada-A	105	Ø	0.16
	100	1.00	Ø
	91	Ø	0.84
Est-5	100	0.21	1.00
	95	0.05	Ø
	87	0.74	Ø
Iddh-A	100	0.30	1.00
	45	0.70	Ø
Pgm-A	100	0.64	0.67
	45	0.36	0.33
Pnp-A	145	0.29	0.02
	100	0.71	0.98

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