PHARYNGEAL MORPHOLOGY AND SPATIAL AND TEMPORAL VARIATION IN TROPHIC COMPOSITION IN THE POLYMORPHIC CICHLID, *HERICTHYS MINCKLEYI* (TELEOSTEI: CICHLIDAE) FROM THE CUATRO CIÉNEGAS BASIN, MEXICO

Thesis

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Chapter 1

Characterizing Polymorphic Variation in

Herichthys minckleyi (Teleostei: Cichlidae) from

Cuatro Ciénegas, Mexico

Abstract

Two trophic morphs are known from the polymorphic cichlid, *Herichthys minckleyi*. The papilliform morph has slender pointed pharyngeal teeth, while the molariform morph has more robust blunt molar pharyngeal teeth. Intermediates between the two have been reported as rare. New characters were used in morphometric analysis to further characterize the morphological variation in *H. minckleyi*, and determine if intermediate morphology could be quantified. Principal component analysis of morphological characters suggests that a third morph, the intermediate, does exist, is more abundant, and is distinguished by the relative area of the lower pharyngeal jaw covered by molars. Possible causes for the intermediates not being described until now are the use of more definitive characters, sampling error, and temporal changes in selective forces favoring intermediate trophic morphology.

Introduction

Fishes in the family Cichlidae are diverse. They can be found in fresh and brackish waters in Africa, South and Central America (one species extending north through Mexico into Texas), the West Indies, Madagascar, Israel, Syria, Coastal India, and Sri Lanka. About 105 genera and 1,300 species of cichlids are described, with the highest diversity in the African Rift Lakes (Nelson 1994). There is a broad range of morphological and behavioral variation within this family, especially in adaptations related to feeding. In fact, feeding specializations are believed to be the basis for such prolific speciation (Liem 1974, Liem and Osse 1975, Keenleyside 1991, Nelson 1994). Trophic adaptations of the pharyngeal apparatus in particular contributed considerably to cichlids' evolutionary success (Stiassny and Meyer 1999). The pharyngeal apparatus of cichlids is a modification of the most posterior ceratobranchials (gill bones) in the pharynx. It consists of two sets of tooth bearing surfaces (upper and lower) that are manipulated to process food items. Fryer and Iles (1972) hypothesized that the African Rift Lake cichlid species flocks that possess diverse pharyngeal morphologies are a monophyletic group. Later genetic work verified the monophyly of the Lake Malawian and Lake Victorian cichlids (Meyer et al. 1990, Meyer 1993, Meyer et al. 1994), whereas Lake Tanganyika, Africa has several different monophyletic tribes (Kornfield and Smith 2000). Monophyletic flocks also occur in much smaller crater lakes in Africa (Scheileiwin et al. 1994). Within monophyletic species assemblages, species with pharyngeal teeth specialized for feeding on fishes, mollusks, and detritus (or other small food items) are common. Most cichlid species are monomorphic and possess a fixed type of pharyngeal morphology specialized for a particular trophic niche, although they can still be generalist feeders (Fryer and Iles 1972, Liem and Osse 1975, Greenwood 1984). The high degree of trophic specialization has confused biologists because significant overlap of resource use occurs in cichlid communities containing species with multiple pharyngeal morphologies (Greenwood 1984), although recent work also demonstrates resource partitioning in some African cichlid communities (Bootsma et al. 1996). On an evolutionary scale, these large monophyletic species flocks are one of the largest vertebrate radiations. Unsurprisingly, cichlids have been proposed as a model group for the

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study of evolution (Kornfield and Smith, 2000) and numerous studies have documented the morphological variation among cichlid species (Keenleyside 1991, Kornfield and Smith 2000).

One Neotropical cichlid species, *Herichthys minckleyi* (Kornfield and Taylor 1983) is polymorphic, possessing multiple feeding morphologies, and has received considerable attention from evolutionary biologists because of this polymorphism (Liem and Kaufman 1984, Greenwood 1991, Kornfield 1991, Liem 1991). *Herichthys minckleyi* is endemic to the numerous isolated and semi-isolated pools, springs, streams, and rivers found in the Cuatro Ciénegas basin, Coahuila, Mexico (Minckley 1969), and is listed as an endangered species by the Mexican government. In *H. minckleyi*, the polymorphism involves variation in the pharyngeal teeth where individuals possess pharyngeal teeth specialized for feeding on different food resources (Kornfield and Taylor 1983). The different pharyngeal morphologies within this species include one adapted to feeding on detritus/small food items (papilliform), another adapted to feeding on snails (molariform) morphology (Kornfield and Taylor 1983), and a rare piscivore can be either of the two pharyngeal morphologies (La Bounty 1974, Stephens and Hendrickson 2001). Papilliform morphs have small pointed pharyngeal teeth, whereas molariforms have 4-10 or more relatively larger blunt stout molars that increase in number and size with standard length. In addition to these readily observable traits, differences in head width, pharyngeal horn width, and gut length distinguish the papilliforms from molariforms. Differences in morphological characters between papilliforms and molariforms are internal or not externally obvious. The variety of trophic morphologies present within *H. minckleyi* are comparable to those found in several other cichlid species that either possess: 1) molariform pharyngeal teeth (*Cichlasoma labridens*, Taylor and Miller 1983 and *Chilotilapia rhodesia*, Fryer and Iles 1972), 2) papilliform pharyngeal teeth (*Cichlasoma bartoni*, Taylor and Miller 1983), or 3) piscivorous feeding (*Cichlasoma steindachneri*, Taylor and Miller 1983). The high degree of intraspecific pharyngeal morphological variation makes *H. minckleyi* an unusual cichlid species, because nearly all cichlid species are monomorphic for pharyngeal morphology (Liem and Kaufman 1984). In fact, when *H. minckleyi* was first discovered, scientists considered the different morphs to be completely different species (personal communication, W.L. Minckley 1999). Subsequent studies concluded that the different morphs are not

reproductively isolated and therefore constitute a single polymorphic species (Kornfield et al. 1982 and Kornfield and Taylor 1983). These studies distinguished the papilliform morph from the molariform morph by describing two discrete pharyngeal morphologies. Individuals with aberrant intermediate pharyngeal morphology are rare and never constituted more than 5% of fish (Kornfield and Taylor 1983). Little is known about the occurrence of intermediate pharyngeal morphology in *H. minckleyi*. In addition to pharyngeal variation, *H. minckleyi* displays a great deal of variation in color and variation in body shapes that can be recognized visually. It is not clear whether body shape variation is related to a particular pharyngeal morphology. The objective of this study was to characterize and quantify the morphological variation of *H minckleyi* in greater detail than previously done. In particular, this study aimed to determine if there were any characters that can be used to distinguish individuals with pharyngeal morphology intermediate between papilliform and molariform.

Materials and Methods

To characterize and quantify intermediate morphs of *H. minckleyi*, I performed morphometric analyses of 19 continuous, and two meristic measurements on 125 randomly selected museum specimens collected in Cuatro Ciénegas (Texas Natural History Collections catalog numbers; 26192, 26239, 26249, 26252, 26276, 26705, 27331, 27363, 27587, 27611). To verify that my sample represented the SL distribution of wild populations, I tested for significant differences (two tailed paired t tests) in mean morph Standard Length among fish I measured and Standard Length distribution calculated from large scale collections made recently at multiple sites in Cuatro Ciénegas.

The characters I measured are commonly used in studies of fish body shape morphology (Hubbs and Lagler 1947), and cichlid pharyngeal morphology (Barel et al. 1977). I also measured additional characters I suspected of having importance. External variables measured are illustrated in Figure 1 and internal pharyngeal characters in Figure 2.

Morphometric measurements-All morphometric characters were measured in millimeters (to 1/100 of a mm) on the left side of each *H. minckleyi* with a Mitutoyo Digimatic digital caliper measuring the distance between the tips of the calipers touching the points defined by each measurement. A description of each measurement is given below and Table 1 lists abbreviations and references of all the measurements taken. Very small distances were measured using a micrometer and are noted below with an (*). All variables measured were continuous, except the number of hypertrophied teeth (NHT), and medial rows of paired molars (MR), which were meristic counts.

External measurements
- Standard Length (SL)- length from tip of the snout to the hyplural plate.
- Body Depth (BD) – distance between insertion of dorsal fin and insertion of pelvic fins

- Head Width (HW)- greatest width between exterior surfaces of the preoperculum.

- Head Length (HL)-greatest distance from the tip of the snout to the most posterior portion of the operculum.

- Mouth (MW)- greatest width between corners of the mouth at the premaxilla.

- Lower Jaw (LJ)- length of lower jaw from anterior tip of dentary to articular.

- Upper Jaw (UJ)- length from anterior tip of premaxilla to the most posterior part of maxilla.

- Eye Diameter (ED)- largest measurable eye diameter in direction from insertion of dorsal fin to the mouth.

- Snout Length (SN)- shortest distance from edge of the eye to the most anterior part of maxilla.

- Lower Jaw to Articular Process (LJ2AP)- distance from anterior tip of dentary to the protrusion at articular area at the bottom edge of the head.

- Eye to Articular Process (E2AP)- shortest distance from eye perimeter to protrusion at articular area at the bottom edge of the head.

Internal pharyngeal characters

- Horn Width (HRW)- greatest width of lower pharyngeal jaw (LPJ) horn where it attaches to the muscles.

- Occlusal Width (OW)- length of the LPJ

- Occlusal Depth (OD)- depth of the most posterior portion of the LPJ

- *Last medial tooth width 1-3 (LMTW 1-3)- width of most posterior pharyngeal teeth from center row (LMTW 1) outward. The next row containing LMTW 2, and the next containing LMTW 3.

- Hypertrophied teeth width (HTRW)- greatest lateral width of hypertrophied teeth on lower pharyngeal jaw (5th ceratobranchial).

Meristic variables

- Number of Hypertrophied Teeth (NHT)- number of hypertrophied blunt molar pharyngeal teeth on LPJ surface. Slender pointed papilliform teeth are not hypertrophied.

- Medial Row (MR)- number of paired medial rows of hypertrophied teeth. MR is counted from the first inner row going out laterally.

After randomly selecting 125 museum specimens for study, I *a priori* nominally identified individuals as intermediates, molariforms, and papilliforms (Figure 3). Nominal criteria for visual specimen determination was that papilliforms had pointed slender pharyngeal teeth, molariforms had numerous blunt pharyngeal molars that covered most of the LPJ surface, and intermediates had papilliform teeth, and some blunt molars that only cover a small part of the LPJ. The molars in intermediates occurred in a line down the middle of the LPJ and were surrounded by numerous papilliform teeth.

All data were log transformed using the equation Log (X+1). Principal component analysis using covariance matrices was done on the continuous variables using SAS version 8.0. I explored the data with scatter plots of principal component scores, and of raw data.

Results

Differences in the mean SL distribution of wild morphs (n=4,912) and morphs measured for this study were less than 14 mm. The mean SL of wild and study papilliforms was 86.0 mm, and 96.1 mm, and wild and study molariform mean SL was 94.6 mm, 108.8 mm respectively. These differences were statistically significant (p =0.002 for papilliforms, two tailed paired t-test comparing mean morph SL of specimens measured to mean morph SL from large scale sampling efforts conducted throughout the basin (n=4,912)). The SL distribution of individuals with intermediate pharyngeal morphology did not differ significantly from the observed natural distribution (measured specimens mean SL=86.1 mm, wild morph populations mean SL=82.42, p= 0.39). I could not increase my sample size in order to possibly achieve a more similar SL distribution between measured fish and field populations because of a lack of available museum specimens for dissection.

Of the 125 randomly collected specimens examined, I identified 46 specimens as molariform, 78 as papilliform, and 23 as intermediate in pharyngeal morphology. PCA also recognized three groups, which corresponded to my *a priori* nominal criteria. PCA I versus PCA II (Figure 4) shows clear clustering of morphs, with complete separation between papilliforms and molariforms, and slight overlap of intermediates with molariforms in graph space. Seventeen of 23 individuals I identified as intermediates clustered together in a group next to the molariforms. Forty-four of 46 individuals I identified as molariforms clustered in a distinct cloud, and all 78 individuals I identified as papilliforms formed a distinct cloud. Papilliform morphology was characterized by the presence of only small slender pointed pharyngeal teeth, and the entire LPJ was relatively delicate when compared to the other morphs. Intermediate morphology was characterized by having papilliform pharyngeal teeth, and blunt molars in one medial row of paired teeth. Molariforms were characterized by having numerous blunt molars that almost always occur in two or more paired medial rows that covered most of the LPJ surface. The molars of intermediates did not cover most of the LPJ surface. From the bottom up, papilliforms had an inwardly concave LPJ surface while intermediates and molariforms had an

outwardly concave LPJ surface indicative of the increased bone and tooth mass. PCA of continuous characters strongly suggested that intermediates were a distinct group. The first two principal components accounted for 96.1% of the total variance (Table 2). PCA I accounted for 71.8% of total variance with most variables loading evenly around c.a. 0.1 except for HTRW, which had a higher loading at 0.332. Thus much of the variation in the data is explained by the width of the molar surface (HTRW). PCA II accounted for 24.4% of the total variance. LMTW1-3, HTRW, and HRW loaded negatively on PCA II and are indicative of differences in pharyngeal morphology among the different morphs. PCA II also loaded much more on HTRW than other variables measured, again showing that most of the variation occurred in width of molars present on the pharyngeal surface (HTRW).

Allometric scaling of pharyngeal characters with SL was clearly different between papilliforms and fish with pharyngeal molars (intermediates and molariforms), while differences between intermediates and molariforms were less dramatic (Figure 5). Pharyngeal horn width (HRW) increases with SL at different rates (slope) between papilliforms and molariforms, while the difference in rate of increase between intermediates and molariforms was less extreme. The slope of occlusal depth (OD) versus SL shows clear differences between papilliforms and molariforms, while the slope of intermediates was in between. Occlusal width (OW) showed a similar but less pronounced pattern. Size of the last medial tooth (LMTW1) increased with increasing SL in all morphs but did so at different rates.   Intermediates tended to have relatively smaller tooth size to SL ratio than did molariforms. The number of hypertrophied teeth (NHT) appeared to increase at a slower rate in intermediates than in molariforms. The relationship of SL versus the surface of LPJ covered by hypertrophied teeth (HTRW) showed that intermediates had less area covered by molars than did molariforms. Variation observed in body morphology showed differences among fish, but differences were not strictly related any particular morph. Many of the fish with large body depth measurements (large deep bodied fish) were molariforms, whereas papilliforms had both slender and deep body morphologies.

In summary, intermediates and molariforms were clearly different from papilliforms. In general, intermediates and molariforms had hypertrophied molars varying in size and number, while papilliforms did not. In addition, intermediates and molariforms have thicker bones associated with the pharyngeal apparatus than do papilliforms. Molariforms were not as clearly different from intermediates as they were from papilliforms. Morphology of internal pharyngeal characters differed among morphs, especially between those without hypertrophied molar teeth (papilliforms) and those with hypertrophied blunt molars (molariforms and intermediates). Hypertrophied teeth width (HTRW) loaded unevenly on both principal components and therefore was the most important character in distinguishing intermediates from molariforms and papilliforms. Individuals with a relatively thin row of paired pharyngeal molars (MR), i.e. intermediates, are strikingly different from fish with numerous molars spanning most of the lower pharyngeal surface. Finally, variables that measured shape of external morphology did show differences among fish, but this variation was not clearly related to any morph. Long slender fusiform morphology or deep body morphology was observed in individuals with intermediate, molariform, and papilliform pharyngeal morphology.

Discussion

This study shows that there is a recognizable pharyngeal morphology intermediate between the previously described molariform and papilliform morphs and documents even more morphological variation in *H. minckleyi* than previously thought. Before morphometric analysis, I qualitatively classified all specimens measured into three morphs. PCA also recognized three corresponding groups. Of the fish I nominally classified, 74% of intermediates, 95% of molariforms, and 100% of papilliforms clustered into distinct groups with principal components analysis (Figure 4). In addition, intermediates were present at higher frequencies than previously reported (18.4% of specimens sampled here compared to 5% intermediates noted by Kornfield and Taylor (1983). In addition to being morphologically distinct, ancillary work suggests that intermediates are ecologically different from the other morphs. Isotope food web structure analysis suggests that intermediates are an actual intermediate group between papilliforms and molariforms in trophic status (Marks et al. *in prep*). Another line of evidence suggests niche partitioning among morphs, as intermediates may be feeding more frequently on shrimp and invertebrates than other morphs (Hulsey *in prep*). Considering the ecological studies and the morphological results presented here, a strong case for *H. minckleyi* expressing 3 multiple distinct trophic pharyngeal morphologies is presented. There are several explanations why intermediate pharyngeal morphology has not been documented in *H. minckleyi* until now.

Intermediates previously may have been "lumped" with the other recognized trophic morphs. Sage and Selander (1975) described *H. minckleyi* morphology as follows "Cuatro Ciénegas populations are dimorphic in molar number, individuals having none or from four to 31 of these teeth, and tooth number is bimodally distributed. Molar number and body size are correlated ....Herbivorous individuals are similar to snails-eaters in body and head shape, but have more teeth, few if any molars, and longer digestive tracks". Sage and Selander (1975) did not use the terms molariform and papilliform and did not recognize intermediates which were likely lumped with snail eaters or

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herbivores. It is likely that Sage and Selander's snail eaters and herbivores represented molariforms and papilliforms respectively.

Another possibility is that by measuring new characters I revealed variation not previously quantified. Kornfield and Taylor (1983) reported that intermediates were rare, never constituting more than 5% of fish, and therefore postulated two distinct morphs exhibiting a bimodal distribution. Hypertrophied teeth width (HTRW), a character not measured previously, differed among all morphs (Figure 5). Among all the variables measured, PCA showed that HTRW varied most. Other internal pharyngeal characters did differ among morphs, but did not load as heavily on the principal components, and therefore may not have been sufficient to distinguish among the three different morphs. For example one internal character measured, LPJ horn width (HRW), was also used by Kornfield and Taylor (1983). Plotting SL against HRW suggests a third group, the intermediates, which are scattered at the upper distribution of papilliforms, and lower distribution of molariforms. The peak in relative values of HRW at c.a. SL 65 mm or Log SL 1.9 is representative of precocious molariforms (Figure 5).

Perhaps by overlooking intermediates as a morph, previous authors may have also overlooked aspects that affect distribution and abundance of the morphs over space and time. Spatial variation in the abundance of the intermediate morph of *H. minckleyi* could also explain why they were previously undetected. Early collections of *H. minckleyi* from Cuatro Ciénegas could have been made at localities that simply had no individuals with intermediate morphology. Little is known about the spatial distribution of the different morphs, and some sites could have few to no intermediates.

Finally, intermediates could have increased in relative abundance since the time period when earlier authors conducted work on the species. It is possible that previous work did not document them because they were rare. Early studies all used specimens collected in the 1960's and 1970's. I measured specimens collected in 1998-2001 from Cuatro Ciénegas. The polymorphism in *H. minckleyi* could be experiencing temporal dynamics, with different morphs abundance fluctuating through time.

The data from my randomly selected samples showed higher than reported abundance of intermediate fish from more recent collections. Because temporal and spatial dynamics of the trophic morphs in *H. minckleyi* have not yet been characterized, I have also characterized the trophic morph distribution through time and space in my next chapter. Increasing my sample size from more sites in Cuatro Ciénegas at different points in time, should allow me to determine if any of the different morphs have increased in abundance temporally.

Data on the distribution of morph abundance across space and through time could provide insight into mechanisms guiding diversification of pharyngeal morphologies. Future work should investigate temporal and spatial dynamics of the polymorphism and other possible ecological factors affecting *H. minckleyi* as a species. The genetics of the polymorphism are not well understood (Sage and Selander 1975, Kornfield and Taylor 1983, Liem and Kaufman 1984) nor has phenotypic plasticity been investigated in *H. minckleyi*. Plasticity of the pharyngeal apparatus is documented in the pumpkinseed, *Lepomis gibbosus* (Mittlebach et al. 1999) and the African cichlid, *Astatoreochromis alluaudi* (Hoogerhoud 1986, Smitts 1996). Both species are able to develop from delicate pharyngeal morphology to a more sturdy molariform morphology if snail prey are available to eat. Describing and quantifying the morphological variation in *H. minckleyi* lays the groundwork of future work aimed at a greater understanding of the ecological and evolutionary basis of the trophic polymorphism of *H. minckleyi* and perhaps cichlids in general.

Conclusions

Herichthys minckleyi was first described as a species in which two distinct morphologies existed. Those initial studies reported the presence of intermediates, but found few (~5%) in the population. However, a more detailed analysis of numerous morphological characters shows intermediates do exist (at a frequency of 18.4%) and are distinguished from other morphs by the relative area of the Lower Pharyngeal Jaw covered by molars. These intermediates change the perspective from one of nearly complete bimodal intraspecific niche specialization to that of a broad utilization of nearly every variable resource. In fact, intermediates may provide a means to examine the method by which discrete morphs arise from a common ancestor. It is apparent that *Herichthys minckleyi* offers a tractable system to study morphological radiation in vertebrates. This study shows that within one species, *Herichthys minckleyi*, there are multiple pharyngeal morphs (intermediates as well as papilliform and molariform) that are each potentially analogous to different monomorphic cichlid species in the well-known African cichlid species flocks. Thus, pharyngeal morphologies occur in *Herichthys minckleyi* which are similar to the definitive pharyngeal morphologies found in discrete cichlid species.

This study suggests a single cichlid species is capable invading a habitat, and diversifying to occupy multiple trophic niches, and that the divergence is not in dichotomous sequential steps, but as a simultaneous multifurcated process, a nascent explosive radiation. Multiple systematic studies have attempted to reconstruct evolutionary relationships of the species flocks from the African Rift lakes and phylogenies remained unresolved. If the Old World Cichlid lineages have undergone similar processes to those of *Herichthys minckleyi*, it would be predictable that their evolutionary relationships would remain very difficult to resolve into clear dichotomous patterns. The characters that would have supported such a strictly bifurcating solution would have had little time to accrue in a scenario in which multiple morphologies diverged simultaneously.

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Table 1. External and internal pharyngeal morphological variables analyzed with principal component analysis, abbreviations used and the bibliographic reference.

Abbreviation	Measurement	Reference
SL	Standard Length	Hubbs and Lagler 1947
BD	Body Depth	Hubbs and Lagler 1947
HL	Head Length	Hubbs and Lagler 1947
LJ	Lower Jaw	Hubbs and Lagler 1947
UJ	Upper Jaw	Hubbs and Lagler 1947
ED	Eye Diameter	Hubbs and Lagler 1947
SN	Snout Length	Hubbs and Lagler 1947
HW	Head Width	Kornfield and Taylor 1983
MW	Mouth Width	This study
LJ2AP	Lower Jaw to Articular Process	This study
E2AP	Eye to Articular Process	This study
MR	# medial rows of hypertrophied teeth	This study
LMTW 1	Last medial tooth width (1st medial row)	Sage and Selander 1975
LMTW 2	Last medial tooth width (2nd medial row)	This study
LMTW 3	Last medial tooth width (3rd medial row)	This study
OL	Occlusal Surface Length (5th ceratobranchial)	Kornfield and Taylor 1983
OW	Occlusal Surface Width (5th ceratobranchial)	Kornfield and Taylor 1983
HTRW	Hypertrophied Teeth Width	This study
OD	Occlusal Surface Depth (5th ceratobranchial)	Barrel 1978
NHT	# Hypertrophied teeth	Sage and Selander 1975
PHW(HRW)	Pharyngeal Horn Width	Kornfield and Taylor 1983

Table 2. Loading of each character on principal components I and II. The first two principal

components accounted for 96 % of the total variance observed. None of the remaining eight

components accounted for more than 1% per component of the variance, and did not distinguish

morphs.

	Principal component	
	I	II
SL	0.091	0.065
BD	0.101	0.059
HW	0.087	0.061
LJ	0.097	0.071
UJ	0.096	0.072
ED	0.053	0.036
SN	0.101	0.070
HL	0.105	0.054
MW	0.103	0.067
LJ2AP	0.095	0.078
E2AP	0.114	0.078
LMTW1	0.119	-0.043
LMTW2	0.085	-0.020
LMTW3	0.033	-0.003
OL	0.136	0.042
OW	0.114	0.041
HTRW	0.332	-0.220
OD	0.123	0.021
HRW	0.139	-0.012
Percent of total variance explained	71.805	24.350

Figure 1. External morphological characters analyzed by principal component analysis to determine distinct natural groups. The abbreviation and the reference for each variable measured are listed in Table 1. 1=LJ, 2=UJ, 3=HL, 4=LJ2AP, 5=E2AP, 6=SN, 7=ED, 8=BD, 9=SL. X indicates Head Width (HW) and is measured as greatest depth between operculum, x= Mouth Width (MW) and is the distance between outside anterior edges of maxilla. Detailed descriptions of each measurement taken are given in the text.

50 mm
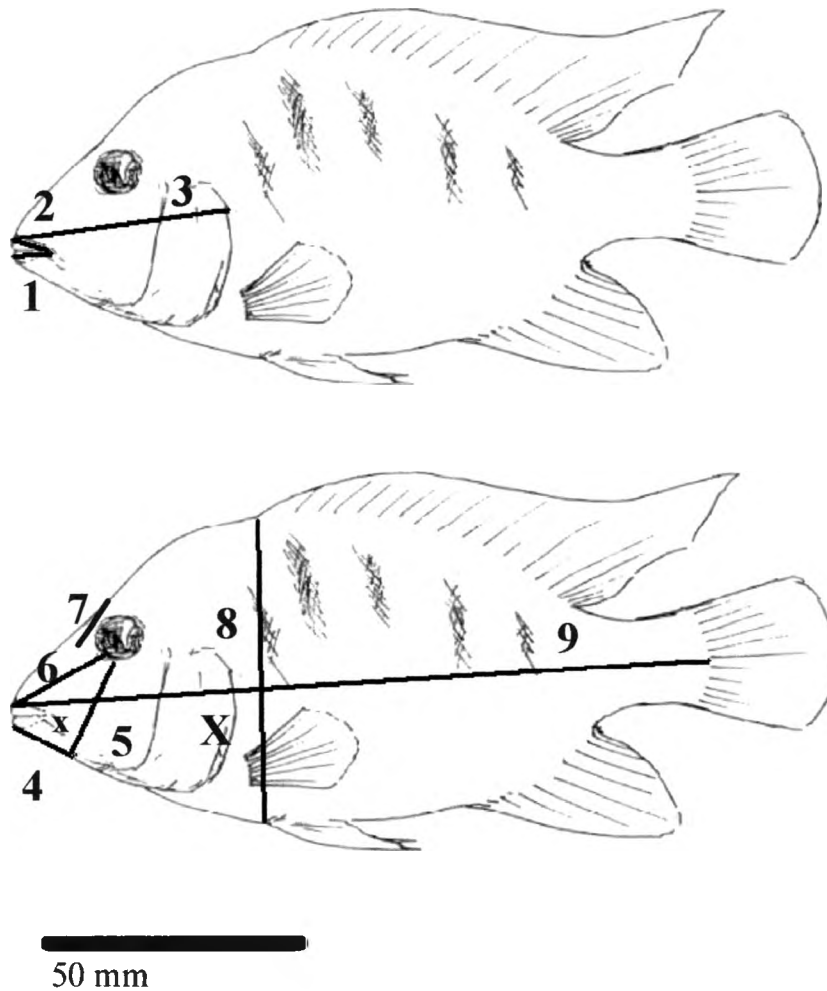
Figure 2. Pharyngeal morphological characters analyzed by principal component analysis to determine distinct natural groups. The Lower Pharyngeal Jaw (LPJ) depicted is a stylized version showing characters of both molariform (left half) and papilliform (right half) pharyngeal morphologies. Papilliform teeth are pointed and slender. Molariform teeth are blunt and much wider. A) View of LPJ from the top, 2=HRW, 3=LMTW 1, 4=LMTW 2, 5=LMTW 3, 6=OL (occlusal length), 7=OW, 8=HTRW measures the widest area of hypertrophied molars on the lower pharyngeal jaw. B) Side view of the LPJ, 1=OD (occlusal depth). Reference bar gives an idea of general size of an adult size lower pharyngeal jaw.
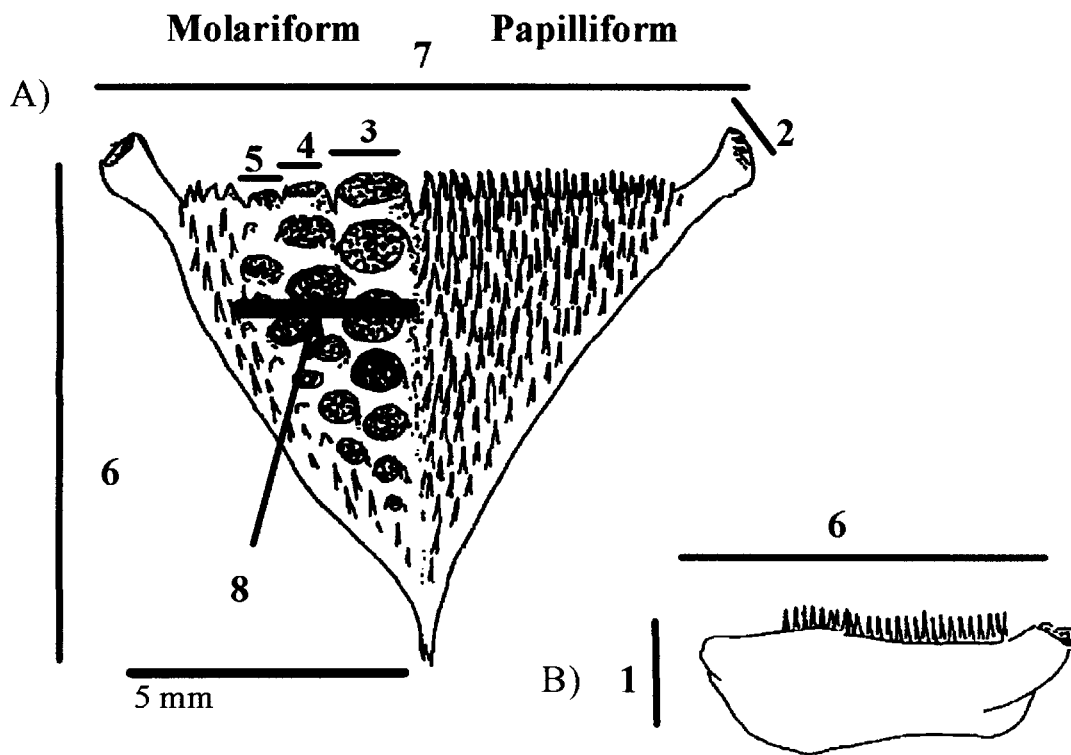
Figure 3. Pharyngeal morphs of *H. minckleyi*. Selected lower pharyngeal jaws (LPJs) of intermediate, molariform, and papilliform morphs are presented in rotated views to show slanted (*) and bottom views (†) of the of the LPJ structure
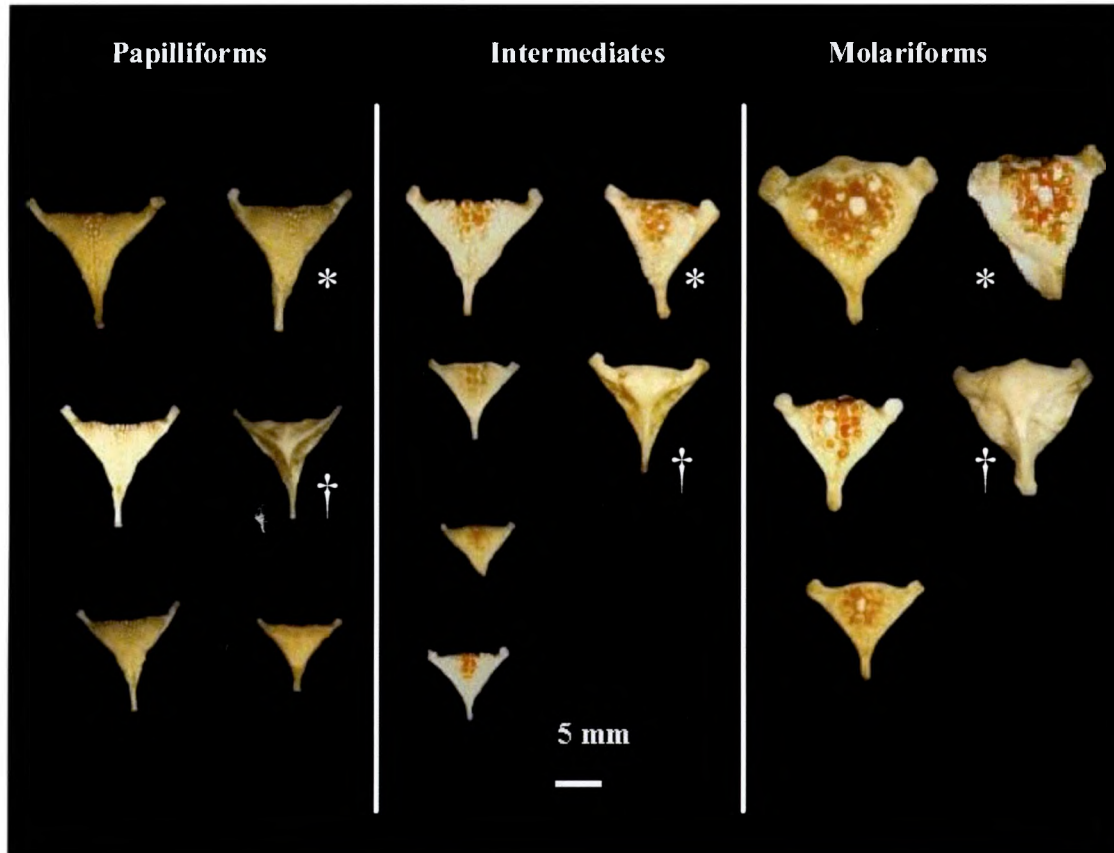
Figure 4. Scatter plot of first two principal component scores from analysis of 10 external and 7 internal morphological characters (n=125). An example of each morphology is superimposed on the graph over their respective groups. (●)= intermediates, (Δ)= molariforms, (□)=papilliforms. The loading of each variable measured on each principal component is given in Table 2. PCA I loaded unevenly on HTRW, which pulls the intermediates and molariforms down away from the papilliforms in graph space. Outliers are indicated with arrows.
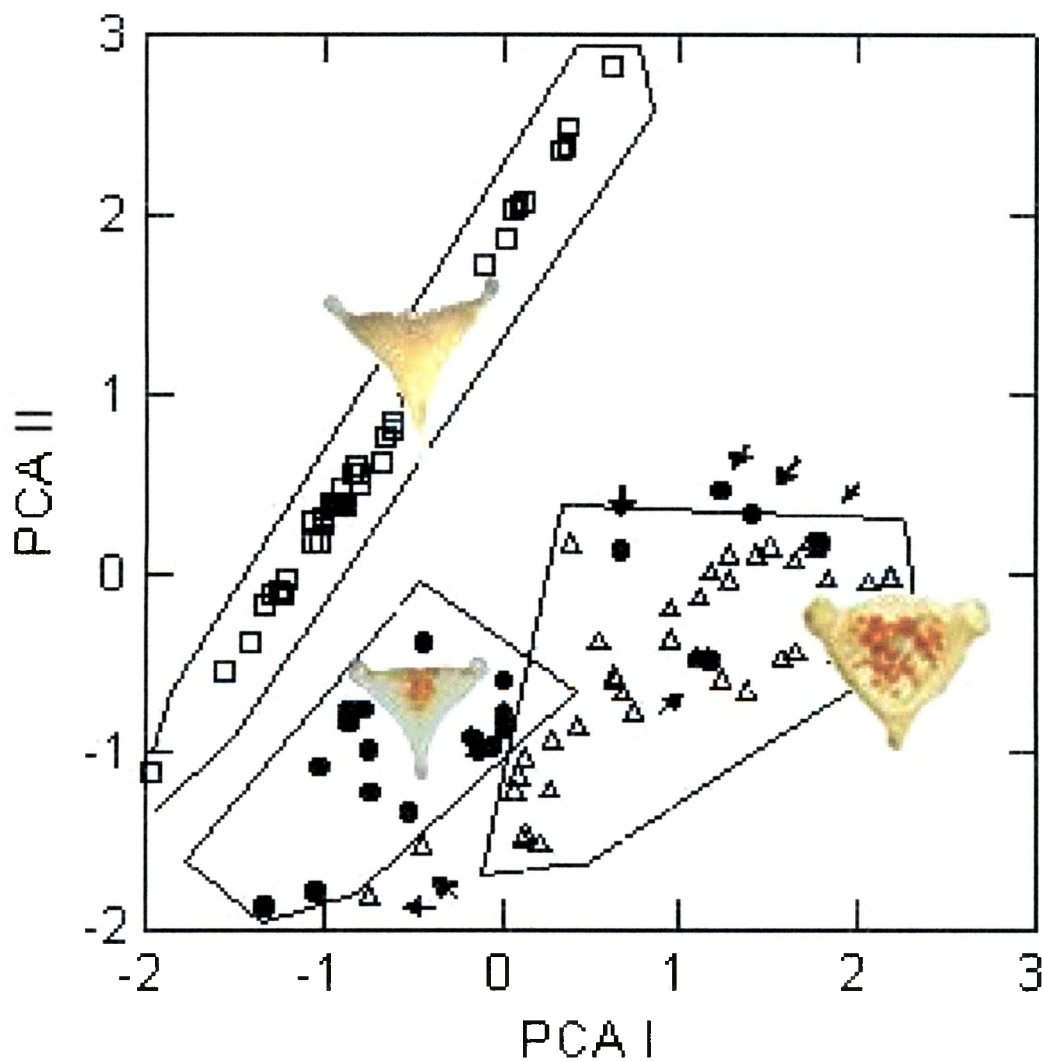
Figure 5. Bivariate regression relationships of morphometric variables among the different morphs of

H. minckleyi. (●)= intermediates, (Δ)= molariforms, (□)=papilliforms. Variables were log

transformed. Differences in slope among morphs are most pronounced between molariforms and

papilliforms, while slopes of intermediates are always in between. Papilliforms had zero values for

NHT and HTRW and therefore graph a straight line with zero slope in graphs representing HTRW and

NHT.

25
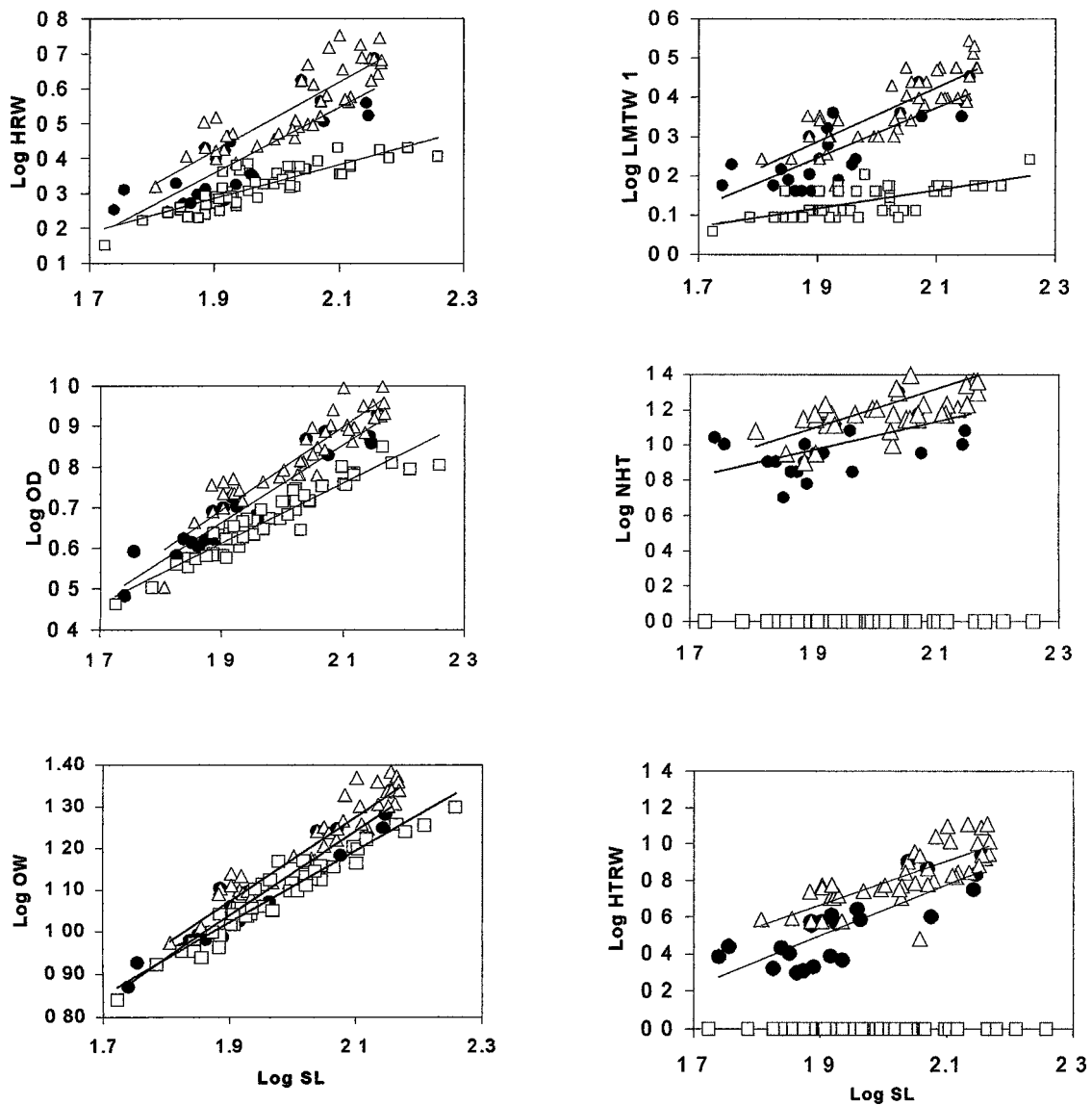
Chapter 2

Temporal and spatial variation

of trophic morphs of *Herichthys minckleyi* (Teleostei: Cichlidae) in Cuatro

Ciénegas, Mexico

Abstract

Dynamics of the 3 trophic morphologies of *Herichthys minckleyi* were investigated. Abundance of the intermediate, molariform, and papilliform feeding morphs were sampled at different localities from two time periods at multiple sites. Abundance of the three trophic morphs were sampled for two time periods for at multiple sites. The pharyngeal morphology of 5,060 *H. minckleyi* was identified (n=566 from historic the time period 1960-1980, n=4,494 for the time period 1998-2001. Abundance of the different morphs varied spatially and temporally. In both time periods (1960-1980 and 1998-2001) morph abundance varied spatially among sites as much as 66%. Temporal dynamics in morph abundance were also detected at some sites, with intermediates and molariform frequency increasing as much as 19% and 25% respectively and corresponding decreases in papilliform as high as 39%. I hypothesize that resource availability and natural and or anthropogenic factors affecting resource availability were involved in the observed differences in the abundance of each feeding morphology. The multiple trophic morphs present in *H. minckleyi* and the natural experiment setting of Cuatro Ciénegas provides a tractable system to study the ecology and evolution of diverse trophic morphologies in *H. minckleyi*. In turn the system may provide insight into the evolution of the numerous trophic morphologies found in larger African cichlid species flocks.

Introduction

Fishes of the family Cichlidae have been proposed as an ideal group for the study of evolution (Greenwood 1984, Baerends 1990, Greenwood 1991, Keenlyside 1991, Stiassney and Meyer 1999, Kornfield and Smith 2000). Within the cichlids there is extensive morphological variation (Greenwood 1984, Smits 1996) and data on how natural selection acts on the variation is central to understanding cichlid radiation. Geographic variation and events leading to it (Gould and Johnston 1972), and resource polymorphism (Smith and Skúllason 1996) are cited as possible factors in speciation/radiation in fish and other vertebrates. Some studies suggest geographic variation as a possible cause for radiation in lithophilic cichlids in African Lakes (Patrick et al. 2000) and it has been suggested to have happened in *Herichthys minckleyi* (Sage and Selander 1975). Resource polymorphisms involve discrete morphs adapted to different resources and occur in diverse taxa. The multiple forms produced by resource polymorphism are likely to be involved in speciation (Smith and Skúllason 1996). Mayr (1982) even suggested that the resource polymorphism in *H. minckleyi* was a rare opportunity to investigate speciation. Despite this, the role of resource polymorphism in cichlid speciation/radiation has received little attention. In chapter 1, I described the intermediate, a new morph in a cichlid with extreme resource polymorphism, *H. minckleyi*. This was a significant discovery because it demonstrated that there was even more morphological variation in *H. minckleyi* than previously thought. In *H. minckleyi* the intermediate, molariform, and papilliform morphologies are each analogous to different trophically specialized monomorphic species in the large species flocks of African Rift Lakes. I hypothesized that differences in characters analyzed in previous studies, systematic "lumping", and temporal and spatial variation in morph distribution could have been responsible for the intermediates not being detected or recognized in Cuatro Ciénegas until now. Here I investigate the temporal and spatial dynamics of the polymorphism in *H. minckleyi* quantified from historic museum and recent collections. The objectives are to determine if temporal and spatial geographic variation exists in the distribution and abundance of the different morphs of *H. minckleyi* in the Cuatro Ciénegas basin to gain a better understanding of the role of resource polymorphism in the trophic diversification in cichlids.

The system

Because *H. minckleyi* is polymorphic and exhibits pharyngeal morphologies found throughout the family Cichlidae, it represents a tractable system to study the ecology and evolution of the pharyngeal apparatus in *H. minckleyi* specifically, and for cichlids in general. Biologists have long been interested in the evolution and ecology of the African Rift Lake's species flocks, but the enormous number of species, and great complexity of those systems has impeded study of their cichlid fauna. In comparison, the Cuatro Ciénegas system provides manageable degree of variation, distributed across numerous habitats geographically. Within the Cuatro Ciénegas basin, there are multiple isolated and semi isolated pozas (desert springs and pools), as well as many streams. *Herichthys minckleyi* is ubiquitous to most of these habitats. The combination of intraspecific morphological variation that is present in *H. minckleyi* at each poza is similar to an evolutionary natural experiment in progress, with different and similar isolated pozas representing contrasting treatments and replicates. Differential selective forces may exert selective pressures on the different morphs in the different habitats. These forces could manifest as changes in food resources, which affect the abundance of the morphs adapted to these resources. Other forces affecting the different morphs could be changes in habitat or species communities. Indeed since the early 1960's, the aquatic environments of the Cuatro Ciénegas Basin have undergone significant anthropological changes. Many pozas have experienced large decreases in size due to channeling of water to other regions outside the basin. In addition, exotic species have recently invaded the basin. The exotic snail *Melanoides tuberculata*, and the African cichlid *Hemichromis guttatus* have established large populations in several pozas and channels. *Hemichromis guttatus* is a small cichlid with papilliform pharyngeal teeth native to west African rivers (P. V. Loiselle pers. comm. 1999) and therefore could be competing with papilliform *H. minckleyi*. Competition from exotic species or variation in environmental factors such as resource availability may be influencing morph abundance in Cuatro Ciénegas. Sage and Selander (1975) first suggested *H. minckleyi* could be tracking snail population abundance in Cuatro Ciénegas. It is likely that as a species, *H. minckleyi* is experiencing ecological

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conditions that favor different morphs at different places and at different times. Thus, dynamics in spatial and temporal patterns of morph relative abundance would be indicative of differential selection.

Study sites

The Cuatro Ciénegas basin is located in Coahuila, Mexico c.a. 270 km southeast of Big Bend Park National Park. It is an arid desert habitat, and is unusual because it is scattered with springs (Pozas) and streams. Water temperatures in Cuatro Ciénegas range from 21 to 32 degrees Celsius or higher, with spring fed pozas being more constant in temperature than channels or rivers. The abundance of gypsum in the soil contributes to the hardness and salty character of the water (Minckley 1969). Water chemistry data for each of the different sites (except Canal de Escobeda where no data was available) is given in Table 1. Below I present a brief overview of the aquatic environments where I sampled *H. minckleyi* morph abundance. The distribution and abundance of riparian (*Salix nigra, Typha domingensis, Phragmites australsi and Eleocharis sp.*) and aquatic vegetation (*Nymphaea ampla, Chara sp., Utricula obtuse, Ludwigia sp., and Potamogoton nodosus*) varies from site to site, however some species like *Typha domingensis* and *Nymphaea ampla* are more common. For a more detailed account of the geology, flora and fauna see Minckley (1969). Sites sampled were distributed throughout the range *H. minckleyi* in the Cuatro Ciénegas Basin (Figure 1). Spring fed pools (Pozas) sampled were Poza Churince, Poza de la Becerra, Poza Juan Santos, Poza Anteojo, Poza Mojarral Este, Poza Mojarral Oeste, Poza Tio Candido, and Poza Escobedo. Rio Mesquites is a river habitat and the Canal de Escobedo is a small stream. The site Tierra Blanca is similar to a river habitat, but there is little to no noticeable current.

Poza Churince is a relatively small spring fed pool located in the western portion of basin. It is oval shaped and about 50 meters long and 10 meters wide. It drains through a small stream to a shallow lake (Laguna Grande) where temperature and water quality conditions are unfavorable to most endemic fishes except for the pupfish *Cyprinadon atruarus*. There are abundant submerged and emergent macrophytes along the banks at Poza Churince. It is next to the main highway leading out of town, frequented by many locals as a swimming hole, and is likely to have experienced some fishing.

Recently it has been invaded by two exotic species: a cichlid, *Hemichromis guttatus*, and a snail, *Melanoides turberculata*. Both are well established and abundant.

The Poza Tio Candido is roughly 2 to 3 times the size of Poza Churince. It is fairly isolated from most public contact. Aquatic macrophytes occupy most of the poza, and it drains to a large shallow marsh.

Poza Escobedo is smaller than Poza Churince and is fed by a small stream, and has a warm spring at the bottom of the pool. There are no aquatic macrophytes, and the bottom has a thick layer of detritus and snail feces. In the upwelling cone of the hot spring there is a colony of suspended algae, which is continuously rising and sinking. Poza Escobedo drains into Canal de Escobedo that flows out into the basin floor for several kilometers. Canal de Escobedo is rarely more than 1 meter deep and 2 meters wide. Emergent macrophytes are present in slower parts of the canal, and the bottom is travertine or detritus.

Poza Anteojo is located on the northern rim of the basin and is about half the size of Poza Churince. It has mostly a silt covered bottom with some emergent macrophytes, seemingly few snails, and drains south into a canal. It has recently undergone significant anthropomorphic changes by the owner who has tried to renovate it into a Balneareo (swimming hole).

Poza de la Becerra is a public swimming site, and is frequented by many swimmers year round. It consists of two main pools connected by a small channel, and is about twice the size of Poza Churince. It has undergone a large decrease in size due to a channel built in the first half of the 1900's that draws water through Canal de la Becerra to outside the Cautro Ciénegas Basin. The bottom is travertine and covered with detritus.

Tierra Blanca is the near the headwaters of the Rio Mesquites, the main river system in the basin. It resembles a river, and has steep travertine banks, and most of the surface water is occupied

with *Nymphaea ampla*. The bottom as well is nearly completely covered with the submerged leafy portion of the *Nymphaea ampla*.

Poza Mojarral Oeste is similar in size to Churince. It consists of a spring on one side and a underwater cave on the other. Water flows from the spring at the bottom of one side of the poza and exits through the underwater cave. There are few aquatic macrophytes and most of the bottom is detritus, and walls are travertine or submerged roots.

Poza Mojarral Este is 10 times the size of Poza Churince and is a heterogeneous poza connected by a small stream to Mojarral Oeste on the west side and to Rio Mesquites by a small stream on the east side. The exotic cichlid, *Hemichromis guttatus*, is well established at Mojarral Este. A *Tilapia sp.* is also present in very small numbers.

Rio Mesquites winds through the center of the basin flowing to the east. It is has many oxbows and is connected to numerous pools as it flows through the desert. For this study all of the current collections from Rio Mesquites were at Las Palapas, a swimming park located not far off the main road out of town.

Poza Juan Santos was possibly the largest pozas sampled in this study. Poza Juan Santos is mostly silt on the bottom with many shallow areas less than a meter deep. It has recently been invaded by the exotic cichlid species, *Hemichromis guttatus*.

Materials and Methods

To quantify changes in temporal abundance of the different trophic morphologies of *H. minckleyi*, I compared past morph frequencies from historic museum collections with current field collections for 9 sites. These sites were: Anteojo, Churince, La Becerra, Tierra Blanca, Rio Mesquites at Las Palapas, Mojarral Este, Mojarral Oeste, and Tio Candido. Spatial analysis was done by comparing relative morph abundance among sites for two time periods, 1960-1980 and 1998-2001. For the time period 1998-2001 spatial analyses from two additional sites (the canal leading off Escobedo, and Poza Juan Santos) were included. Because intermediates were previously uncharacterized, determining if historic collections contained fish which satisfied my criteria as intermediates was crucial to determine if intermediate abundance did increase as suggested by data in chapter one. A direct comparison of specimens examined by Sage and Selander (1975) or Kornfield and Taylor (1983) would have allowed me to determine abundance in both time periods with an identical classification scheme, thus eliminating any ambiguity of what constitutes intermediate pharyngeal morphology. However, specimens examined by Sage and Selander (1975) and Kornfield and Taylor (1983) were unavailable. Therefore I examined *H. minckleyi* from other historic collections made at the same localities and similar points in time in lieu of the specimens collected by Sage and Selander (1975) and Kornfield and Taylor (1983). Data for all collections (including museum catalog numbers) from both time periods used to quantify temporal and spatial abundance are listed in Appendix A.

Pharyngeal morphologies of specimens were identified using a modified otoscope. The use of an otoscope allows examination of the fish's throat and identification of the pharyngeal morphology of living specimens in the field, or the morphology of museum specimens without a damaging dissection. Individuals that possess no hypertrophied blunt or molar like pharyngeal teeth were recorded as papilliforms, individuals that possessed relatively large, blunt, molar-like pharyngeal teeth

in multiple paired medial rows that covered most of the 5th ceratobranchial surface were recorded as molariforms. Individuals that possessed few relatively small, blunt, molar-like teeth that covered a small portion of the 5th ceratobranchial in one row of paired medial teeth were recorded as intermediates. Using the otoscope, I quantified abundance and ratios of the different morphs from numerous museum collections made in the 1960's, 1970's, and 1980 (M. Stephens only). Other workers and I compiled ratios of the different morphs from recent collections (1998, 1999, 2000, and 2001) conducted in Cuatro Ciénegas. Because molariforms and intermediates both have molar pharyngeal teeth, the criteria used to distinguish molariforms from intermediates could have been subject to individual bias. A best effort was made among multiple workers (myself, Dean Hendrickson, Adam Cohen, Eric Dinger, and Brook Swanson) to confer and use consistent criteria to identify intermediates and molariforms. To estimate current morph frequencies, fish were collected using cast nets, gill nets with constant mesh size (25mm), and graduated (25-90mm) mesh sizes, seines, hook and line, and by hand. For each sampling event, method of capture, duration of sampling, time of collection, and workers were recorded. For each fish examined, pharyngeal morphology, determiner (worker determining morphology), standard length, head width, locality, and date, were recorded. All but a few voucher specimens from each site were returned alive after data was recorded.

To compare relative abundance the total n of each morph collected per site per time period was pooled from multiple historical and recent collections for each site (Appendix A). Because no such data currently exists, I created a map showing the distribution of morph abundances for both time periods at each site in the Cuatro Ciénegas basin.

Results

The pharyngeal morphology of 5,060 specimens was identified (n=566 from the time period 1960-1980 (Poza Churince, Poza de la Becerra, Tierra Blanca, Poza Anteojo, Poza Mojarral Este, Poza Mojarral Oeste, Poza Tio Candido, Rio Mesquites and Poza Escobedo), and n=4,494 for the time period 1998-2001 (Poza Churince, Poza de la Becerra, Tierra Blanca, Poza Anteojo, Poza Mojarral Este, Poza Mojarral Oeste, Poza Tio Candido, Rio Mesquites, Poza Escobedo, Poza Juan Santos, and Canal de Escobedo). Comparing all fish sampled (excluding collections from Canal de Escobedo and Poza Juan Santos for an exact comparison), showed that intermediates increased nearly 6% between the time period 1960-1980 and 1998-2001 (Figure 2), while molariforms increased <1%, and papilliforms decreased nearly 6%. The same comparison including fish from Canal de Escobedo and Poza Juan Santos showed similar results.

Pozas and river/channel habitats showed temporal and spatial variation in the abundance of papilliform, intermediate, and molariform trophic morphs of *H. minckleyi* (Figures 3 and 4). Pozas de la Becerra and Churince had increased abundance in molariform, decreased papilliform and little or no change in intermediate trophic morphs between 1960-1980 and 1998-2001. Over the same time interval, molariforms and intermediates increased in Pozas Escobedo, Anteojo, Mojarral Oeste, and Rio Mesquites. Decreases in papilliforms ranged from 4% in Poza de la Becerra to 39% in Rio Mesquites, while increases in intermediates were as high 19% in Poza Anteojo, and 25% in Poza Churince. In contrast, 3 sites increased in papilliforms while decreasing in mainly intermediates (Tierra Blanca) or molariforms (Mojarral Este). Intermediates were detected for the first time at Rio Mesquites and Poza Mojarral Este in the 1998-2001 collections.

The spatial distribution and abundance of trophic morphs of *H. minckleyi* also varied among pozas and riverine habitats for both time periods. For comparison purposes, I arbitrarily ranked sites in order of increasing molariform abundance (Figure 4). Some sites were relatively molariform poor while others had molariforms making up more than 50% of the population. Papilliforms made up a

substantial portion of *H. mincklevi* populations at all sites, but also showed intersite variation by a much as 66%, whereas intermediates varied from 3-22%. The marked change in ordering of sites between 1960-1980 and 1998-2001 relative to molariform abundance also shows the dramatic temporal changes in morph abundance among aquatic habitats in Cuatro Ciénegas. A map of the Cuatro Ciénegas Basin indicating all morphs relative abundance at each site in both time periods is presented in Figure 5.

Discussion

This study is the first to document temporal and spatial dynamics in abundance of the trophic morphs of *H. minckleyi*. It shows that there is variation in the abundance of each morph among habitats spatially, and within habitats temporally. The basin wide ~6% increase in intermediate relative abundance is strongly supported by the data because I used the same criteria to classify intermediates in both time periods to remove any ambiguity of what constitutes intermediate morphology. My estimation of intermediate abundance in historic collections is similar (~5%) to intermediate abundance reported by Kornfield and Taylor (1983), while sampling of the same sites in recent years shows that intermediates are now more abundant on a population level and locally at some sites. Several factors could be responsible for this observed variation in relative abundance of intermediate, molariform and papilliform trophic morphologies in Cuatro Ciénegas.

Spatial variation in morph abundance- The observed spatial variation in morph abundance could be the result of cichlids with different pharyngeal morphologies moving within sites between areas where fish were sampled and adjacent aquatic habitats. Poza Anteojo, Poza Churince, Poza Escobedo, Poza Mojarral Este and Oeste, Tierra Blanca, Poza Tio Candido, Poza Juan Santos, Canal de Escobedo, and Rio Mesquites are sites that have adjacent marshes or connecting canals that cichlids live in. However, there is no evidence showing that morphs migrate or that morph abundance in adjacent habitats differs from the primary poza. Moreover, if migration affects estimation of morph abundance, then we would expect to see variation in morph abundance within relatively short temporal scales. The data suggest the opposite. Many of the sites (Pozas Mojarral Este and Oeste, Poza Escobedo, and Canal Escobedo) were sampled multiple times throughout the year and with the exception of collections with small sample sizes (<20) morph abundance is relatively stable on small time scales (Appendix A).

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Sampling bias or redundancy may also be a factor influencing relative morph abundance especially at sites with low capture rates. Because nearly all fish were returned alive after morph data were taken, calculating pooled morph frequencies ran the risk of some redundancy. Redundancy would have occurred if some trophic morphs were consistently resampled more often than others. This is unlikely. Preliminary mark and recapture results suggest that some individual fish may be more prone to capture (Hendrickson unpublished observation). However there is no evidence to indicate that any particular morphs were more or less prone to capture.

A likely factor causing the observed spatial variation in morph abundance is resource availability differences among sites. Sites like Poza Anteojo, Poza Escobedo, Poza de la Becerra, and Tierra Blanca, where morph abundance is characterized as mostly papilliforms (~70% or more) could have food resource conditions (abundant algae and detritus) that favor papilliforms over intermediates and or molariforms. In contrast, sites like Poza Churince, Poza Juan Santos, Rio Mesquites, and Poza Mojarral Oeste had molariform relative abundance ranging from nearly 40% to 65%, which could be indicative of resource conditions (more abundant snails) at these sites that favor molariforms over papilliforms and intermediates. Spatially, intermediates varied in relative abundance from 0% to 22%. Sites like Tierra Blanca, Poza Anteojo, Poza Tio Candido, Pozas Mojarral Este and Oeste, and Poza Juan Santos which are characterized by intermediate frequencies near or above 10% could have resource conditions that favor cichlids with intermediate morphology more than other sites with relatively low intermediate frequencies. We do not yet know if there are fitness peaks experienced by each morph associated with feeding on the food resource for which they are specialized to feed on. However, it is likely that the intermediate, molariform, and papilliform morphs benefit by having trophic specializations given what is known about trophic specializations in other species. For example, in Lake Victoria, Africa, there are monomorphic trophic specialized species that each possess distinct pharyngeal morphologies that are each specialized to different trophic niches (Smitts 1996). Moreover, in *Cichlasoma citrinellum*, the red devil, which is closely related to *H. minckleyi*, and also exhibits the same trophic polymorphism, Meyer (1989) showed that the molariform and papilliform

trophic morphs showed differences in feeding efficiency between hard and soft food items. This suggests that molariform and papilliform morphologies are differentially efficient at feeding on hard and soft food items. *Herichthys minckleyi* have papilliform and molariform pharyngeal teeth and also feed on hard and soft food (Hulsey 2001). In addition, laboratory experiments suggest differential resource use between molariforms and papilliforms. Liem and Kaufman (1984) found that when food was scarce, molariform *H minckleyi* spent proportionally more time feeding on snails than did papilliforms when food availability was reduced. This may suggest that trophic specializations only are used when resources are rare (Robinson and Wilson (1999). Therefore, it is likely that the different trophic morphs of *H. minckleyi* experience an increase in their relative fitness because of their pharyngeal adaptations to particular food resources. In addition, stable isotope food web analysis shows that the intermediate, molariform, and papilliform morphs are trophically distinct (Marks et al. *in prep*). Recent gut content studies show clear differences between molariforms and papilliforms, while intermediates seem to be eating elements of both molariform and papilliform diets (Hulsey *in press*). What is known about trophic adaptations in diverse taxa and this differential abundance of trophic morphs of *H. minckleyi* in time and space in Cuatro Ciénegas suggests that there is niche partitioning among the trophic morphs and a corresponding fitness benefit in niche space for *H. minckleyi*. Manipulative resource experiments with different trophic morphs of *H. minckleyi* would add support to this hypothesis.

Temporal changes in morph abundance-The temporal changes observed in morph abundance at certain pozas may also be a result of changing resource availability over time. Just as resources may be different among pozas, within a poza resource abundance may vary through time. Other systems have shown that temporal variation in trophic morph abundance is related to changing resource dynamics. For example, the abundance of left and right handed morphs of scale eating cichlids of Lake Tanganyika, Africa occillated over an eleven year period in relation to abundance of prey (Hori 1993) and the abundance of Darwin's finches with contrasting bill morphologies varied with changing environmental conditions (Boag and Grant 1981, Price et al. 1984a, Price et al. 1984b, Schluter and

Grant 1984, and Grant and Grant 1989). The observed increase of intermediates and molariforms and decrease of papilliforms at sites like Poza de la Becerra, Poza Escobedo, Poza Anteojo, Poza Mojarral Oeste, Poza Churince, and Rio Mesquites could indicate an increase in resources such as snails or harder food items for which molariforms and intermediates likely have a superior feeding ability. Likewise, the observed increases in papilliform abundance, and a decrease in either or both relative abundance of molariforms and intermediates at the sites Poza Mojarral Este, Tierra Blanca, and Tio Candido may be the result of opposite changes in resource availability favoring papilliforms over intermediates and molariforms. Sites that are papilliform dominated may have relatively greater abundances of soft foods like detritus or algae which papilliforms are likely to have a superior feeding efficiency. The dramatic temporal changes in abundance of the different trophic of *H. minckleyi* I observed occurred over a span of 20-40 years between sampling events I analyzed. There are presently no corresponding studies of algae, detritus, and snails to determine to what degree morph dynamics are tracking food resources. However, several anthropogenic factors are likely playing a role in the ecology of trophic morph abundance of *H. minckleyi* in different aquatic habitats in Cuatro Ciénegas.

One such change is the lowering of water levels due to the construction of canals. Water levels at Pozas de la Becerra, Escobedo, and Anteojo have been lowered by the construction of canals that drain the water elsewhere for agricultural use. It does not appear that this change in habitat size has affected morph abundance at Pozas de la Becerra, and Escobedo, because relative abundance changed little at these two sites. However, Poza Anteojo has a canal, and the relative abundance of molariforms and intermediates increased temporally there. The owner of Anteojo admits that the fish taste good, and has done considerable work to convert the poza into a swimming spot. His activities (direct removal of fish and habitat alteration) could be factors affecting the morph abundance at Poza Anteojo by changing resource conditions. Anteojo is not the only aquatic habitat impacted directly or indirectly by human activities. Several other sites sampled in this study have been invaded by exotic species.

Morph relative abundance could be influenced by invasive species that affect the endemic hydrobiid snails, a major component of the molariforms diet. *Melanoides tuberculata* is a competitive exotic snail, and has been introduced around the world and at several localities in Cuatro Ciénegas. *Melanoides tuberculata* could be affecting food webs in Cuatro Ciénegas in two ways: it is a strong competitor and could be competing directly with the native snails, or as a prey of the molariform *H. minckleyi*. Crucial factors to determine are: to what extent does *H. minckleyi* prey on *Melanoides tuberculata,* and the competitive interaction between native snails and *Melanoides*. Understanding these relationships would allow us to determine if molariform *H. minckleyi* are losing a food resource or gaining a new one, both are changes that could affect morph abundance. Poza Churince has been invaded by *Melanoides tuberculata,* and become well established. The largest increase in relative molariform abundance was observed at Poza Churince. This could be indicative of an impact of *Melalanoides tuberculata* on the ecosystem, however another exotic may also be playing a role in impacting the native fauna at Poza Churince. The exotic cichlid, *Hemichromis guttatus,* has recently become well established at Poza Churince and several other Pozas in Cuatro Ciénegas. *Hemichromis guttatus* from Poza Churince and elsewhere in Cuatro Ciénegas have papilliform pharyngeal teeth and are likely to be competing with papilliform *H. minckleyi*. If *Hemichromis guttatus* are competing with papilliform *H. minckleyi,* then we would expect to see a decrease in papilliform abundance, and an increase in the abundance at sites where *Hemichromis guttatus* are present. In fact, *Hemichromis guttatus* is now well established at Poza Churince, Poza Mojarral Oeste, and Poza Juan Santos, the sites with the highest observed molariform abundance (38-65%). In addition, molariform abundance showed temporal increases of 25% at Poza Churince. The observed temporal increases and higher relative abundance of molariforms at sites where *Hemichromis guttatus* are established are indicative of a shift in community structure in response to an exotic species. Future work should concentrate on identifying the exact nature of competitive interactions among *Hemichromis guttatus* and the morphs of *H. minckleyi*. Such work could show how a resource polymorphism may act as a buffer against interspecific competition.

Finally, the observed temporal changes in morph relative abundance could be due to phenotypic plasticity of the pharyngeal apparatus. Meyer (1990) reported that the relative abundance of molariform and papilliform morphs of the red devil, *Cichlasoma citrinellum,* were also spatially and temporally variable. He suggested that the observed variation in morph relative abundance was due to fish actually changing morphologies. He infers this from temporal differences in relative molariform and papilliform abundance in collections made during the wet and dry seasons, but presents no direct evidence of individual fish switching morphologies. *Herichthys minckleyi* individuals are probably not changing from molariform to papilliform. This transformation would involve major anatomical changes involving reabsorption of bone and tooth mass, and regrowing new papilliform pharyngeal teeth. Little is known about the tooth growth in *H. minckleyi*, but ontogeny of molariform morphology in the African cichlid, *Astatoreochromis alluaudi* is characterized by having larger replacement molars developing under existing molars (Huysseune 1995, Huysseune et al. 1994). This appears to be the condition in *H. minckleyi,* based on limited investigation of internal morphology in resin embedded lower pharyngeal jaws, in which all individuals with molars had larger teeth developing beneath emergent teeth (M. Stephens unpublished observation). The transition from papilliform to molariform is more plausible. All fish develop papilliform pharyngeal teeth from hatching, and at c.a. 25-30 mm SL some individuals start to develop more robust wider blunt molars (Stephens and Hendrickson 2000). Therefore, changing from papilliform to molariform already occurs in the species. Little is known about the ontogenetic onset of switching from papilliform to molariform in *H. minckleyi.* Several other fish species, *Lepomis gibbosus* (Mittlebach et al. 2000) and *Astatoreochromis alluaudi* (Hoogerhoud 1986, Smits 1996), have a phenotypically plastic response to hard food diets, and will develop from a relatively weak pharyngeal morphology to a robust stronger molariform pharyngeal morphology when snails are available as prey. Because of the morphological plasticity for snail consumption exists in other fish species including a cichlid, it is possible that phenotypic plasticity may also have a role in trophic morph abundance of *H. minckleyi.* Current studies by myself using lab-rearing experiments are aimed at determining heritability of pharyngeal morphology, and the role of phenotypic plasticity in pharyngeal morphology of *H. minckleyi.* These experiments will hopefully provide data on how morphology is inherited, and if phenotypic plasticity is a proximate mechanism

involved in pharyngeal morphological development. Dissecting the interactions of genetic and environmental influences will be essential to building a more complete picture of the trophic polymorphism in *H. minckleyi* and the ecological and evolutionary factors, guiding relative morph abundance in Cuatro Ciénegas.

Conclusions

Here I document for the first time temporal and spatial dynamics in the abundance of different trophic morphs in the polymorphic cichlid species *H. minckleyi*. Resource polymorphisms are cited as a possible mechanism of adaptive radiations in fish and other vertebrates. Studies of diverse taxa all suggest that resource polymorphism is important in creating the variation in trophic morphologies involved in trophic radiation and or speciation. The results of my analyses have implications to both evolutionary processes, and the conservation of species.

Addressing the former, the dynamic character of the polymorphism suggests that the morphs are reacting in response to their local environment, and that the response varies on temporal and spatial scales. In an evolutionary context the situation in Cuatro Ciénegas may be similar to density dependent mechanisms operating in Darwin's finches of the Galapagos Islands, an evolutionary icon cited in most biology texts. Long term studies have shown that the abundance of each trophically specialized finch is maintained under the influence of resource dynamics (Boag and Grant 1981, Price et al. 1984b). While Schluter and Grant (1984) correlated specific trophic bill morphologies to specific food resources, and then showed that species abundance tracks the abundance of the particular resource to which they are adapted. Within the numerous pozas and riverine habitats each morph of *H. minckleyi* may be experiencing increased or decreased fitness in response to local resource conditions available at several trophic levels. In the absence of interspecific competition with other cichlid species, *H. minckleyi* is taking advantage of resources at every trophic level via polymorphism. In *H. minckleyi* this adaptation is producing a range of pharyngeal morphologies analogous to those found in distinct monomorphic species of better known African Cichlid species flocks. The Cuatro Ciénegas system provides a rare opportunity to study the evolutionary ecology of trophic adaptations in the cichlid *Herichthys minckleyi*. However more generally such studies may, in turn, provide clues to how similar trophic adaptations in much larger explosive radiations (i.e. Lake Victoria) have evolved. It may be that the diverse trophic forms found in the African Rift lakes have also experienced temporal and spatial dynamics in abundance like the trophic morphs of *H. minckleyi*. Differences between the

African Rift lakes and Cuatro Ciénegas could suggest why the African cichlids have diversified trophically to distinct species and *H. minckleyi* has not. In African Rift lakes, lake level fluctuations may have provided situations conducive to allopatric speciation and subsequent rejoining of derived species back into sympatry resulting in the diverse species flocks observed today (Kornfield and Smith 2000). While in Cuatro Ciénegas there have been no isolation and reconnection of populations. An isolating event could eventually result in speciation of *H. minckleyi* into new species. Alternatively we are just now at the cusp of seeing that isolation occur as the area is drying.

Cuatro Ciénegas provides a rare glimpse into the workings of speciation, adaptation, and the origin of specializations. Unfortunately, like most of the once isolated wilderness areas, Cuatro Ciénegas has undergone potentially radical changes in the past few decades. Consequently, this study has implications to conservation of endangered species like *H. minckleyi*. By performing detailed morphometrics I characterized morphological variation in *H. minckleyi* that was previously not reported in the literature. Then extensive field surveys showed this newly characterized variation is found throughout the range of *H. minckleyi*. Future conservation efforts in Cuatro Ciénegas should aim to protect the diversity of morphs within *H. minckleyi*. All of the lab and field studies conducted here suggest that the true structure of variation in a species is complex, and that without extensive sampling, that complexity may not be recognized or even detected. This should be a warning when management decisions are made with a paucity of information which may then affect the long-term survival of endangered species. In Cuatro Ciénegas, protecting and managing the ecosystem as a whole is likely to be the only scale appropriate to conservation efforts in this rare example of biology and evolution in the process. After all, the health of a species is directly related to the amount of diversity in the species and once that diversity is lost it is quite likely unrecoverable.

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Table 1. Depth and water chemistry characteristics of 10 sites in Cuatro Ciénegas between 1999 and 2000. Data presented are average values calculated from multiple samples over a year (Hendrickson unpublished data).

Site	Depth m	Temp. Degrees C	pH	Conductivity microS/cm	Salinity ppt	DO %sat	DO mg/l	Alkalinity mg/l CaCO3	Turbidity NTUs
Poza Anteojo	0.55	29.29	7.01	1690.50	0.90	47.25	3.30	166.0	2.19
Poza de la Becerra	1.32	33.29	7.06	2557.27	1.32	162.0	0.59	179.1	1.3
Poza Churince	0.68	27.76	7.17	2531.00	1.38	62.02	4.76	156.4	0.4
Poza Escobedo	1.66	33.72	7.15	2668.40	1.44	43.89	2.96	197.3	1.1
Poza Juan Santos	1.52	25.38	7.66	2859.75	1.54	83.01	6.98	171.5	0.7
Poza Mojarral Este	0.98	31.96	7.23	2686.25	1.42	60.29	4.59	176.3	0.6
Poza Mojarral Oeste	1.63	32.64	7.15	2667.32	1.43	53.0	3.45	179.0	0.6
Rio Mesquites	0.50	27.16	7.86	3056.00	1.65	78.2	6.16	159.0	0.65
Tierra Blanca	0.20	28.90	7.18	2850.00	1.50	5.6	75.00	196.0	0.54
Tio Candido	0.50	27.27	7.12	2575.00	1.39	75.4	6.04	167.5	1.00

Figure 1. Sites in the Cuatro Ciénegas basin where different morphs of *H. minckleyi* were sampled.

The number for each site is indicated in the legend.
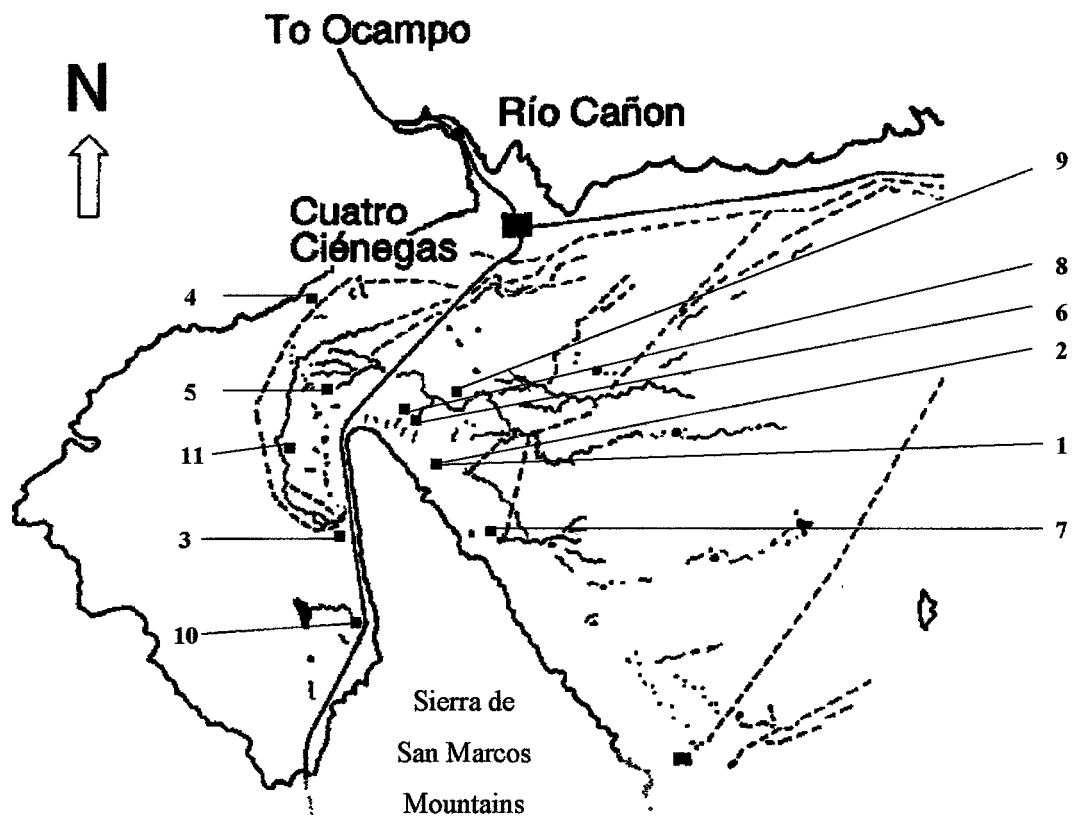
Sites

4=Anteojo	9=Rio Mesquites at
5=Tierra Blanca	las Palapas
11=Juan Santos	8=Mojarral Oeste
3= Poza de la	6=Mojarral Este
Becerra	2=Escobedo
10=Churince	1=Canal de Escobedo
	7=Tio Candido

Figure 2. Relative morph abundance of *H. minckleyi* from 1960-1980, and 1998-2001. Morph abundance from current collections at Canal de Escobedo, and Poza Juan Santos were not included in order to make a strict basin wide comparison of the same sites from historic and recent collections. Results including data from Canal de Escobedo and Poza Juan Santos showed similar results.
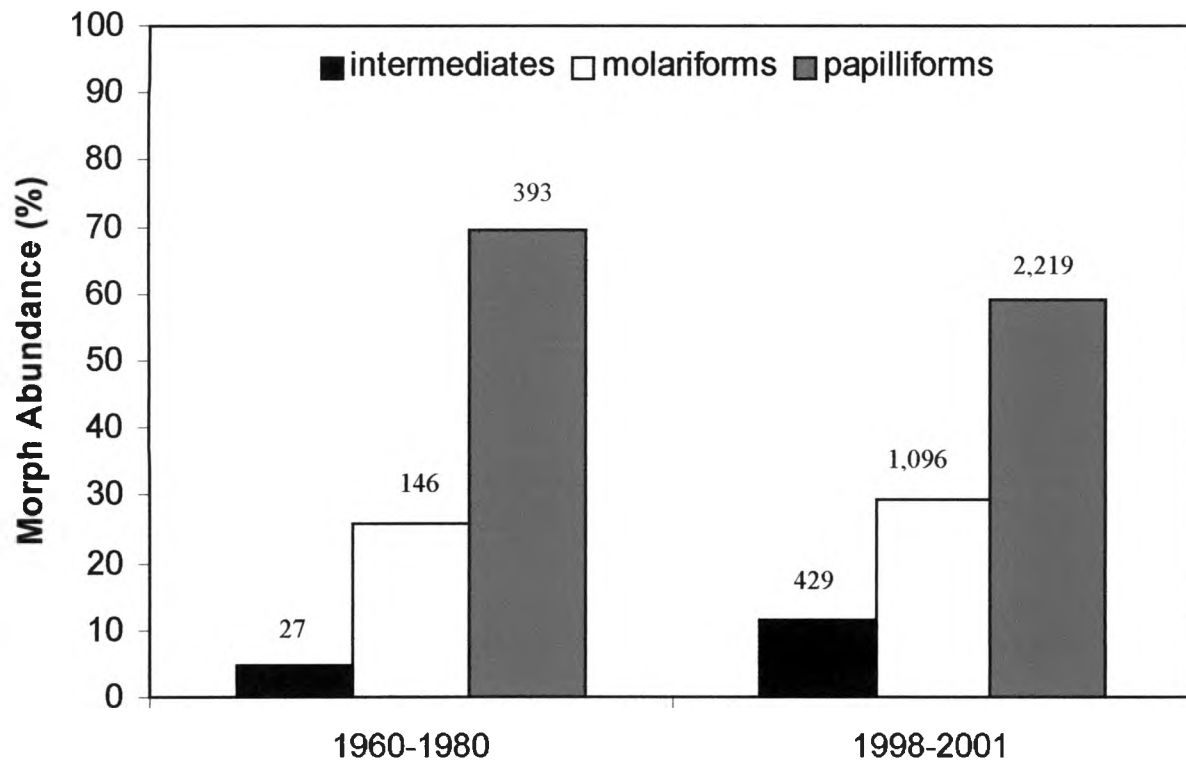
Figure 3. Temporal distribution in relative abundance of the papilliform, intermediate, and molariform morphs of *H. minckleyi* from 9 sites in Cuatro Ciénegas.
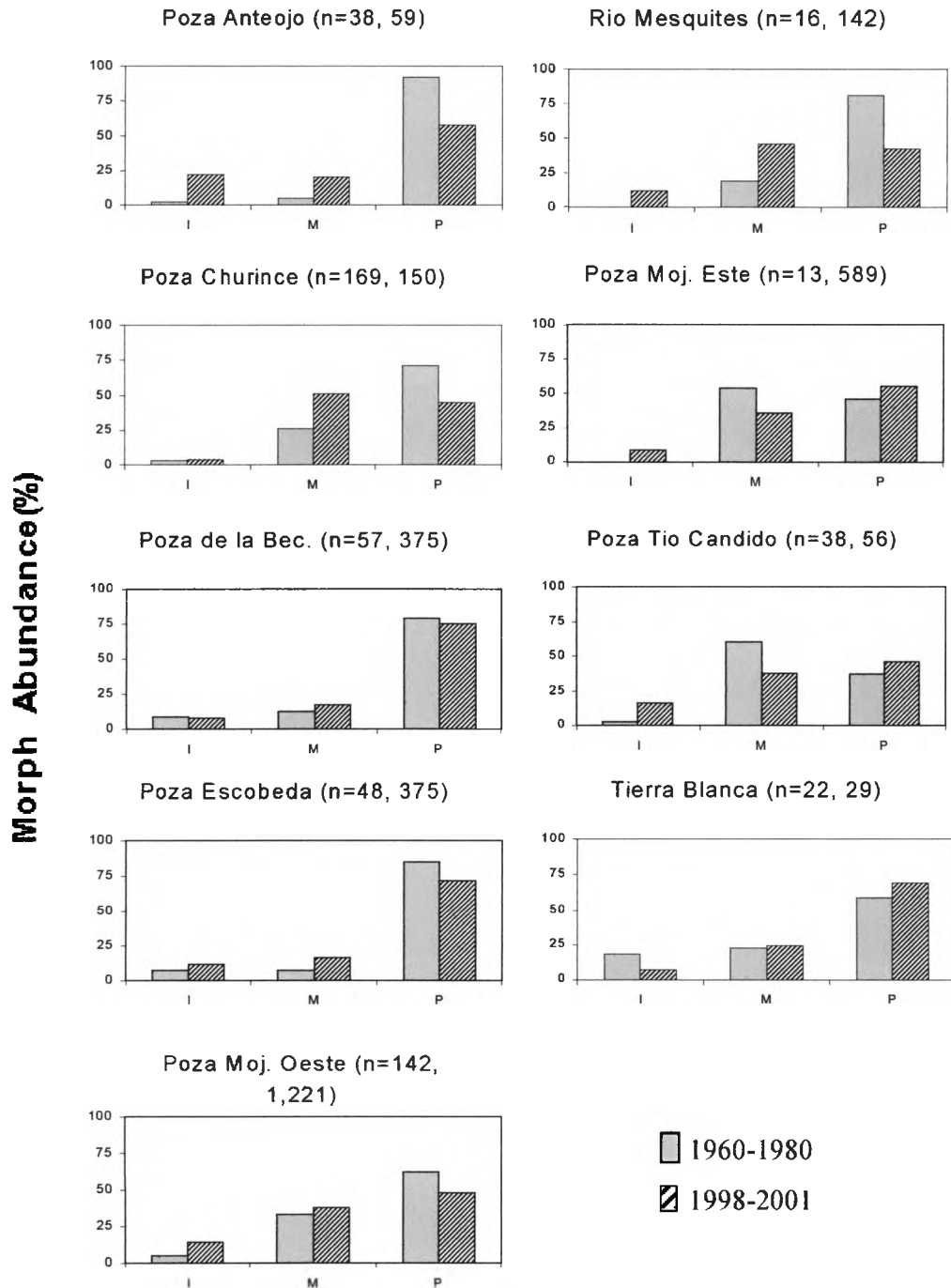
Figure 4. Spatial distribution of the intermediate, molariform and papilliform morphologies of *H. minckleyi* from collections made during the time period 1960-80, and 1998-2001. For each time period sites were ranked according to molariform frequency increasing from left to right. Sites are indicated by their ranked number and are 1=Canal de Escobeda, 2=Poza Escobeda, 3=Poza de la Escobedo, 4=Poza Anteojo, 5=Tierra Blanca, 6=Poza Mojarral Este, 7=Poza Tio Candido, 8=Poza Mojarral Oeste, 9=Rio Mesquites, 10=Poza Churince, and 11=Canal de Escobedo. Note ranking of sites changed between 1960-1980 to 1998-2001.
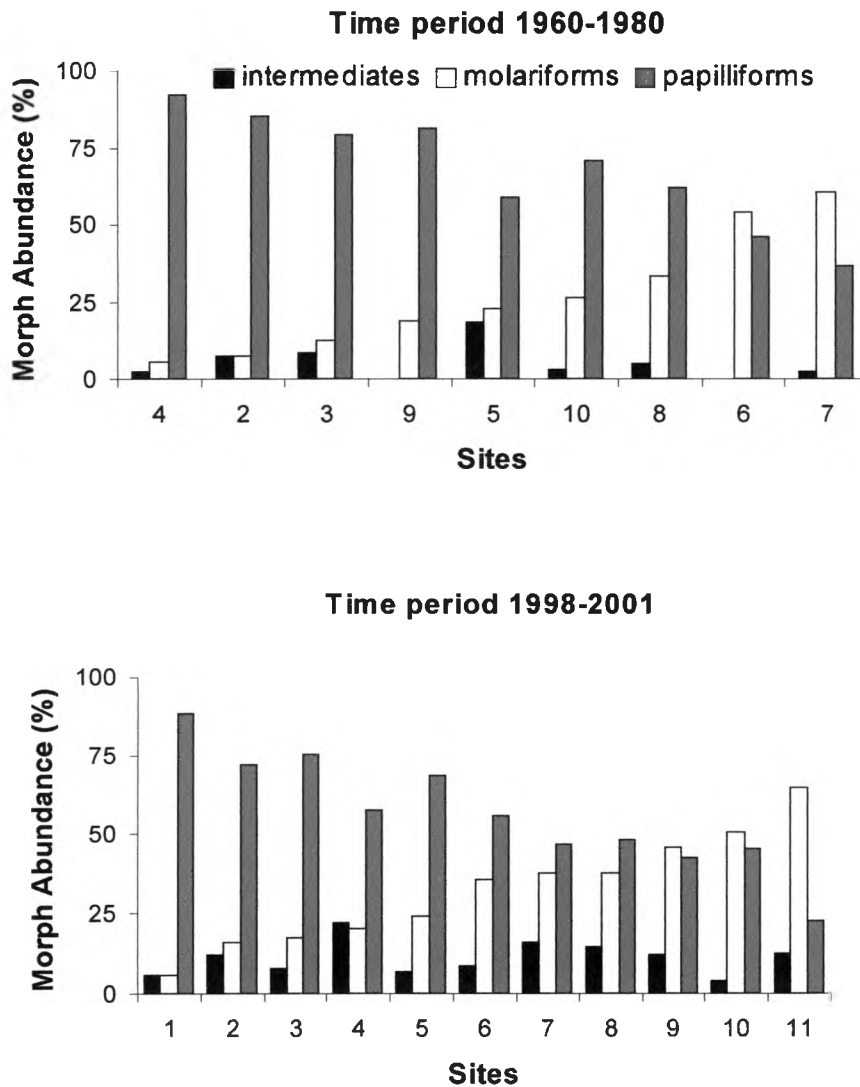
Figure 5. Map of Cuatro Ciénegas Basin showing morph frequencies at sites from the time period, 1960-80 (left pie chart), 1998-2001 (right pie chart). Blanks indicate sites where no historic collections were available (Poza Juan Santos and Canal de Escobedo).

Sites

4=Anteojo	9=Rio Mesquites at
5=Tierra Blanca	las Palapas
11=Juan Santos	8=Mojarral Oeste
3= Poza de la	6=Mojarral Este
Becerra	2=Escobedo
10=Churince	1=Canal de Escobedo
	7=Tio Candido
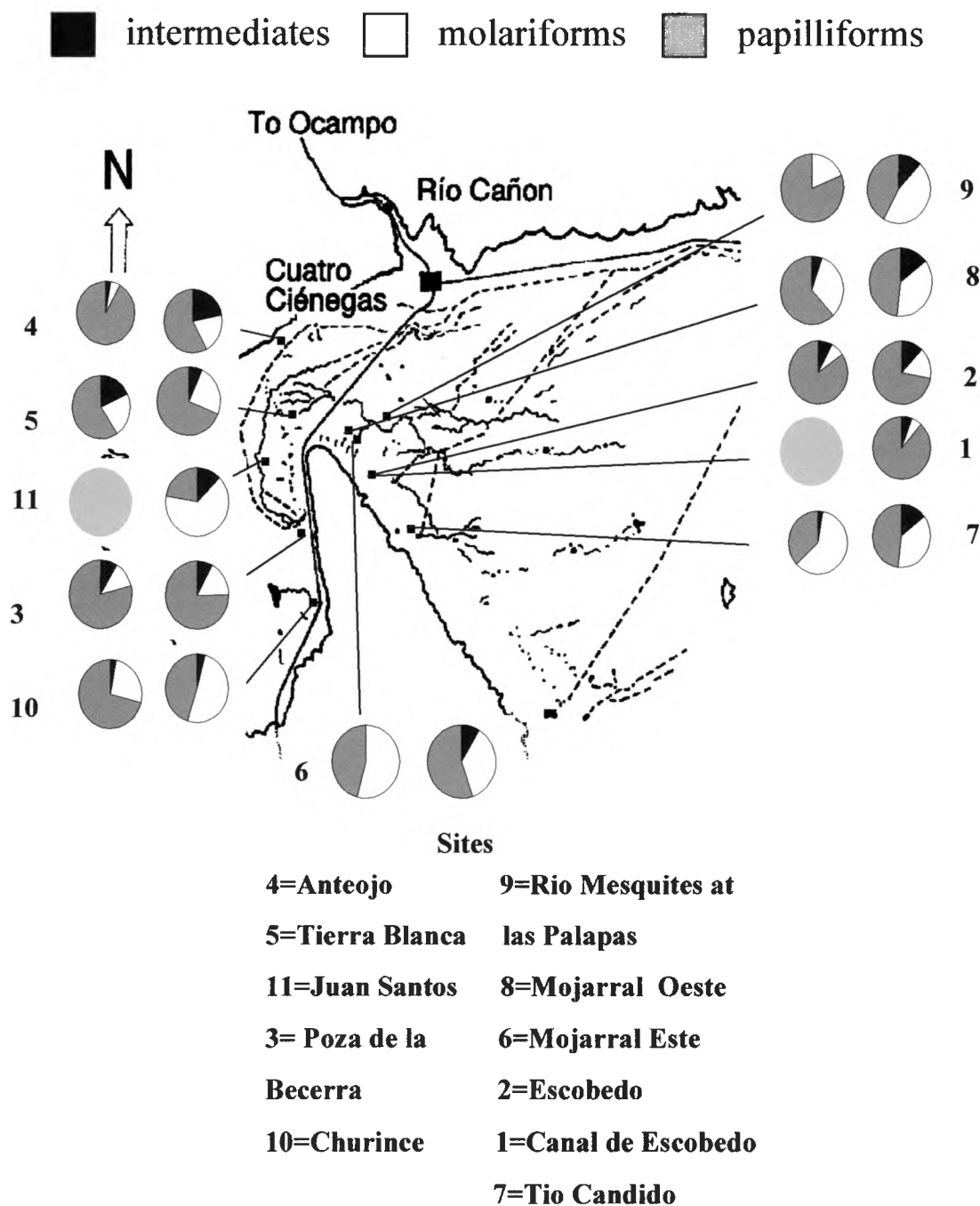
Appendix A

Appendix A.

Site	Date		I	M	P	total	Source
Canal de Escobedo	07/03/99		0	3	0	3	Current collections
Canal de Escobedo	08/11/99		1	0	6	7	Current collections
Canal de Escobedo	01/13/00		0	0	1	1	Current collections
Canal de Escobedo	03/15/00		0	2	19	21	Current collections
Canal de Escobedo	03/16/00		4	3	30	37	Current collections
Canal de Escobedo	03/18/00		3	2	96	101	Current collections
Canal de Escobedo	04/07/00		2	0	26	28	Current collections
Canal de Escobedo	04/08/00		2	2	64	68	Current collections
Canal de Escobedo	09/23/00		0	1	2	3	Current collections
Canal de Escobedo	01/11/01		1	1	111	113	Current collections
Canal de Escobedo	01/14/01		5	2	43	50	Current collections
Canal de Escobedo	03/14/01		6	7	72	85	Current collections
Canal de Escobedo	03/17/01		4	4	117	125	Current collections
Canal de la Becerra	06/29/99		3	3	5	11	Current collections
Canal de la Becerra	07/04/99		7	8	2	17	Current collections
		Sum	**38**	**38**	**594**	**670**	
		Pooled rel. abun.	**0.06**	**0.06**	**0.89**		

Site	Date		I	M	P	total	Source
Poza Anteojo	08/20/66		1	1	4	6	ASU 4557,4559
Poza Anteojo	11/09/80		0	1	31	32	ASU 8498
		Sum	**1**	**2**	**35**	**38**	
		Pooled rel. abun.	**0.03**	**0.05**	**0.92**		

Site	Date		I	M	P	total	Source
Poza Anteojo	09/14/98		0	1	1	2	Current collections
Poza Anteojo	12/05/98		5	1	11	17	Current collections
Poza Anteojo	06/20/99		8	10	22	40	Current collections
		Sum	**13**	**12**	**34**	**59**	
		Pooled rel. abun.	**0.22**	**0.20**	**0.58**		

Site	Date		I	M	P	total	Source
Poza Churince	12/23/65		0	2	15	17	ASU 2329, 2331
Poza Churince	12/23/66		4	13	35	52	ASU 2857-58
Poza Churince	07/10/68		1	17	56	74	ASU 3663-64
Poza Churince	09/03/70		0	3	14	17	ASU 15958-59
Poza Churince	04/22/73		0	9	33	42	ASU 15952-55
		Sum	**5**	**44**	**120**	**169**	
		Pooled rel. abun.	**0.03**	**0.26**	**0.71**		

Site	Date		I	M	P	total	Source
Poza Churince	05/24/98		0	8	1	9	Current collections
Poza Churince	09/11/98		1	13	18	32	Current collections
Poza Churince	06/21/99		4	18	13	35	Current collections
Poza Churince	01/08/00		0	1	8	9	Current collections
Poza Churince	01/09/00		1	10	12	23	Current collections
Poza Churince	07/10/00		0	22	13	35	Current collections
Poza Churince	07/28/00		0	4	3	7	Current collections
		Sum	**6**	**76**	**68**	**150**	
		Pooled rel. abun.	**0.04**	**0.51**	**0.45**		

Site	Date		I	M	P	total	Source
Poza de la Becerra	12/28/64		1	5	18	24	ASU 1655-56
Poza de la Becerra	08/12/79		5	4	39	48	USNM 23195
		Sum	**6**	**9**	**57**	**72**	
		Pooled rel. abun.	**0.08**	**0.13**	**0.79**		

Site	Date		I	M	P	total	Source
Poza de la Becerra	05/24/98		2	11	12	25	Current collections
Poza de la Becerra	09/12/98		5	14	30	49	Current collections
Poza de la Becerra	12/03/98		1	1	1	3	Current collections
Poza de la Becerra	12/04/98		3	4	38	45	Current collections
Poza de la Becerra	04/22/99		1	6	23	30	Current collections
Poza de la Becerra	04/23/99		2	11	62	75	Current collections
Poza de la Becerra	07/23/99		2	8	29	39	Current collections
Poza de la Becerra	01/08/00		9	19	84	112	Current collections
Poza de la Becerra	07/13/00		13	11	96	120	Current collections
		Sum	**38**	**85**	**375**	**498**	
		Pooled rel. abun.	**0.08**	**0.17**	**0.75**		

Site	Date		I	M	P	total	Source
Poza Escobedo	12/31/64		0	0	7	7	ASU 1681-82
Poza Escobedo	12/20/65		0	3	14	17	ASU 2339-41
Poza Escobedo	09/03/70		3	0	13	16	ASU 5966
		Sum	**3**	**3**	**34**	**40**	
		Pooled rel. abun.	**0.08**	**0.08**	**0.85**		

Site	Date		I	M	P	total	Source
Poza Escobedo	12/04/98		7	7	47	61	Current collections
Poza Escobedo	06/26/99		13	11	41	65	Current collections
Poza Escobedo	07/03/99		1	4	6	11	Current collections
Poza Escobedo	08/11/99		8	3	54	65	Current collections

Poza Escobedo	01/13/00		0	4	13	17	Current collections
Poza Escobedo	03/14/00		10	9	40	59	Current collections
Poza Escobedo	04/07/00		11	5	37	53	Current collections
Poza Escobedo	07/23/00		8	9	27	44	Current collections
Poza Escobedo	08/01/00		4	2	6	12	Current collections
Poza Escobedo	09/21/00		19	34	154	207	Current collections
Poza Escobedo	09/23/00		5	17	70	92	Current collections
Poza Escobedo	01/10/01		7	18	74	99	Current collections
Poza Escobedo	01/14/01		11	14	58	83	Current collections
Poza Escobedo	03/15/01		14	24	94	132	Current collections
		Sum	**118**	**161**	**721**	**1000**	
		Pooled rel. abun.	**0.12**	**0.16**	**0.72**		

Site	Date		I	M	P	total	Source
Poza Juan Santos	06/25/99		0	30	6	36	Current collections
Poza Juan Santos	01/07/00		1	11	8	20	Current collections
Poza Juan Santos	07/08/00		0	1	0	1	Current collections
Poza Juan Santos	07/09/00		9	10	4	23	Current collections
		Sum	**10**	**52**	**18**	**80**	
		Pooled rel. abun.	**0.13**	**0.65**	**0.23**		

Site	Date		I	M	P	total	Source
Poza Mojarral Este	04/14/65		0	3	3	6	ASU 2313,1740-41
Poza Mojarral Este	08/11/67		0	4	3	7	ASU 4508-9
		Sum	**0**	**7**	**6**	**13**	
		Pooled rel. abun.	**0**	**0.538**	**0.462**		

Site	Date		I	M	P	total	Source
Poza Mojarral Este	09/14/98		3	13	18	34	Current collections
Poza Mojarral Este	06/19/99		5	16	25	46	Current collections
Poza Mojarral Este	05/29/00		8	19	38	65	Current collections
Poza Mojarral Este	05/30/00		6	27	28	61	Current collections
Poza Mojarral Este	06/04/00		1	22	18	41	Current collections
Poza Mojarral Este	06/11/00		2	13	22	37	Current collections
Poza Mojarral Este	06/18/00		0	10	15	25	Current collections
Poza Mojarral Este	06/19/00		0	13	4	17	Current collections
Poza Mojarral Este	06/20/00		0	8	5	13	Current collections
Poza Mojarral Este	06/24/00		0	3	4	7	Current collections
Poza Mojarral Este	07/17/00		4	13	27	44	Current collections
Poza Mojarral Este	07/18/00		6	8	25	39	Current collections
Poza Mojarral Este	01/09/01		2	26	35	63	Current collections
Poza Mojarral Este	03/16/01		14	19	64	97	Current collections
		Sum	**51**	**210**	**328**	**589**	

| | | | Pooled rel. abun. | 0.09 | 0.36 | 0.56 | | |

Site	Date			I	M	P	total	Source
Poza Mojarral Oeste	12/27/65			0	25	73	98	ASU 2321-23
Poza Mojarral Oeste	03/23/67			7	22	15	44	ASU 3705-07
		Sum		7	47	88	142	
		Pooled rel. abun.		0.05	0.33	0.62		

Site	Date			I	M	P	total	Source
Poza Mojarral Oeste	05/25/98			12	24	15	51	Current collections
Poza Mojarral Oeste	09/12/98			4	23	20	47	Current collections
Poza Mojarral Oeste	06/18/99			7	31	24	62	Current collections
Poza Mojarral Oeste	08/05/99			2	14	11	27	Current collections
Poza Mojarral Oeste	08/07/99			3	20	26	49	Current collections
Poza Mojarral Oeste	08/08/99			1	5	13	19	Current collections
Poza Mojarral Oeste	10/21/99			3	14	10	27	Current collections
Poza Mojarral Oeste	10/22/99			8	26	25	59	Current collections
Poza Mojarral Oeste	01/05/00			2	7	5	14	Current collections
Poza Mojarral Oeste	01/06/00			6	13	20	39	Current collections
Poza Mojarral Oeste	01/07/00			6	8	21	35	Current collections
Poza Mojarral Oeste	03/12/00			5	21	14	40	Current collections
Poza Mojarral Oeste	03/13/00			5	13	22	40	Current collections
Poza Mojarral Oeste	06/02/00			9	18	31	58	Current collections
Poza Mojarral Oeste	06/03/00			5	9	14	28	Current collections
Poza Mojarral Oeste	07/22/00			4	10	21	35	Current collections
Poza Mojarral Oeste	07/23/00			10	15	27	52	Current collections
Poza Mojarral Oeste	07/28/00			8	9	20	37	Current collections
Poza Mojarral Oeste	09/20/00			7	36	37	80	Current collections
Poza Mojarral Oeste	09/22/00			13	70	69	152	Current collections
Poza Mojarral Oeste	01/07/01			18	35	54	107	Current collections
Poza Mojarral Oeste	01/08/01			7	10	19	36	Current collections
Poza Mojarral Oeste	01/12/01			17	9	35	61	Current collections
Poza Mojarral Oeste	03/13/01			13	19	34	66	Current collections
		Sum		175	459	587	1221	
		Pooled rel. abun.		0.14	0.38	0.48		

Site	Date			I	M	P	total	Source
Poza Tierra Blanca	07/04/65			3	2	0	5	ASU 2262-64
Poza Tierra Blanca	07/07/68			1	3	13	17	ASU 3647-49
		Sum		4	5	13	22	
		Pooled rel. abun.		0.18	0.23	0.59	1	

Site	Date			I	M	P	total	Source

Site	Date		I	M	P	total	Source
Poza Tierra Blanca	12/04/98		2	7	20	29	Current collections
		Sum	**2**	**7**	**20**	**29**	
		Pooled rel. abun.	**0.07**	**0.24**	**0.69**		

Site	Date		I	M	P	total	Source
Poza Tio Candido	06/06/64		1	8	9	18	ASU 921,1419-23
Poza Tio Candido	12/30/64		0	15	5	20	ASU 1667-68
		Sum	**1**	**23**	**14**	**38**	
		Pooled rel. abun.	**0.03**	**0.61**	**0.37**		

Site	Date		I	M	P	total	Source
Poza Tio Candido	09/13/98		2	10	2	14	Current collections
Poza Tio Candido	06/23/99		1	4	7	12	Current collections
Poza Tio Candido	07/21/00		6	7	17	30	Current collections
		Sum	**9**	**21**	**26**	**56**	
		Pooled rel. abun.	**0.16**	**0.38**	**0.46**		

Site	Date		I	M	P	total	Source
Rio Mesquites	07/06/68		0	1	7	8	ASU 3640-41, 15961
Rio Mesquites	07/10/68		0	2	6	8	ASU 3696-97
		Sum	**0**	**3**	**13**	**16**	
		Pooled rel. abun.	**0.00**	**0.19**	**0.81**		

Site	Date		I	M	P	total	Source
Rio Mesquites	09/12/98		6	19	14	39	Current collections
Rio Mesquites	04/23/99		8	27	25	60	Current collections
Rio Mesquites	07/01/99		3	19	21	43	Current collections
		Sum	**17**	**65**	**60**	**142**	
		Pooled rel. abun.	**0.12**	**0.46**	**0.42**		

VITA

Matthew J. Stephens was born on November 4, 1970 in Austin, Texas, the son of Joan Griffith, and Michael King Stephens. At an early age he enjoyed aquatic animals, especially fish. He got his first aquarium at age 8, and went on to keep and breed all types of native and aquarium fish. In 1990, he enrolled in Austin Community College, and two years later transferred to the University of Texas at Austin where he received the degree of Bachelor of Science in Aquatic Biology in December of 1995. In the summer of 1995, he began work at the Texas Memorial Museum in collaboration with the Curator of Fishes, Dean Hendrickson, to study the biology of the Mexican cichlid *Herichthys minckleyi*. In 1997, he received the Guy Jordan Endowment from the American Cichlid Association to study the dynamics of trophic morphs in *Herichthys minckleyi*, which enabled him to conduct fieldwork in Mexico. This greatly facilitated his ability to combine several important things: tacos, beer, and catching fish. During the next seven years, Matt made over twenty trips to Mexico to conduct his own research and assist in the research of collaborators. During that same time period Matt married his wife Iektje van Bolhuis, constructed live fish laboratory facilities at the Texas Memorial Museum, maintained over 100 aquariums for his research, published a description of the ontogeny in *H. minckleyi* in the Journal of the Southwestern Naturalist and several magazine articles, collaborated with scientists from: the University of Texas at Austin, the University of California at Davis, Boston University at Woods Hole, and Northern Arizona State University. In 1999 he entered Graduate School at Southwest Texas State University in San Marcos, Texas, and finished the Master's program in 2002.

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