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Impacts of Habitat Fragmentation on the Cranial Morphology of a Threatened Desert Fish (Cyprinodon Pecosensis)

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IMPACTS OF HABITAT FRAGMENTATION ON THE CRANIAL MORPHOLOGY OF A THREATENED DESERT FISH (*CYPRINODON PECOSENSIS)*

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Master of Science

By
Michael Chaise Gilbert

August 2016

IMPACTS OF HABITAT FRAGMENTATION ON THE CRANIAL MORPHOLOGY OF A THREATENED DESERT FISH (*CYPRINODON PECOSENSIS)*

Date Recommended 7/11/16

Michael L Collyer, Advisor and Chair

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8/10/16

Dean, Graduate Studies and Research Date

This thesis is dedicated to my parents, my grandparents, and my wife. All have been supportive in everything that I do.

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introducing me to this field. I feel that in the end, Dr. Collyer has not only been a fantastic mentor, but also a valued friend.

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"If I have seen a little further, it is by standing on the shoulders of giants."

- *Bernard of Chartres, Issac Newton*

PREFACE

During the conception of this research, the hope was to evaluate the morphological variation of multiple co-occurring species, namely the plains killifish (*Fundulus zebrinus*), the Pecos gambusia (*Gambusia nobilis*), and the Pecos pupfish (*Cyprinodon pecosensis*). This was to be accomplished through two phases, a gross external morphology (akin to the composition of this thesis) and an internal phase to investigate structural elements (*e.g.*, bone shape; via diaphonization). Due to restrictions with federal permits to sample habitats where these species occur, as a result of the endangered status of the Pecos *Gambusia*, it was not possible to collect sufficient data in a timely, realistic fashion. Therefore, the data were collected from museum specimens for the species previously mentioned. Since the data collected for the Pecos gambusia and plains killifish are only representative of what would be required for the initial study, I have not included these data in the thesis. However, they contribute valuable information for future research and I intend to implement this at a later time. Additionally, since the data were collected from roughly sixty years' worth of museum specimens, it was not feasible, nor appropriate, to proceed with clearing and staining for collecting information on the structural elements of these fish.

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IMPACTS OF HABITAT FRAGMENTATION ON THE CRANIAL MORPHOLOGY OF A THREATENED DESERT FISH (*CYPRINODON PECOSENSIS)*

Michael Chaise Gilbert	August 2016	67 Pages

Directed by: Michael L. Collyer, Steve H. Huskey, and Carl. W. Dick

Department of Biology	Western Kentucky University

Drastic alterations to the North American Southwest's hydrology have highly influenced resident fish communities. In New Mexico and Texas, the Pecos River has been severely altered as a result of water manipulation, isolating backwaters and various habitats that were once connected to the main river. *Cyprinodon pecosensis* (Pecos pupfish) has been highly impacted due to the effects of anthropogenic water manipulation, as well as species introductions. *Cyprinodon pecosensis* populations have become isolated and scattered, residing in sinkholes, remnant lakes, and static backwaters, thus creating numerous micropopulations. The purpose of this study was to assess the morphological variation in cranial features that occur in response to varied habitats, especially in terms of environmental factors and species co-occurrence. Landmark-based geometric morphometrics was used to assess shape variation across the aforementioned micropopulations comprising four general habitat types and 27 different localities, each with varied community structure and salinity. Results from this study suggest that head and mandible morphology vary temporally, with year to year variation, as well as among different localities. The head morphology of *C. pecosensis* was most heavily influenced by habitat type and localities within habitat types, but was largely canalized with the exception of localities classified as deep sinkholes. Year to year variation and localities among habitat types were the most influential factors associated with mandible morphology, but there was strong overlap among the convex hulls that

defined regions of morphospace for habitat types. As *C. pecosensis* is a threatened species, this research has important implications for future conservation and management. Additionally, these results could further aid in the understanding of preserving species in fragmented landscapes.

INTRODUCTION

The North American Southwest is often subjected to frequent and radical environmental change as a response to flash flooding interspersed between long periods of drought. Hydrological alterations can occur over short time scales of a year, or less, and can drastically influence the surrounding waterways through reduced water tables, rising temperatures, or changes in stream dynamics (*e.g.,* streams of regular flow and low turbidity expressing reduced flow and high turbidity) (Miller 1961). Alongside natural environmental changes, anthropogenic manipulation of the environment can significantly alter the landscape and natural processes, stressing native populations or entire communities. In an area where water is already scarce, human involvement in waterway manipulation can result in depreciated water levels that can affect existing aquatic systems (*e.g.,* depleting wetlands) (Miller 1961). Anthropogenic manipulation of waterways (*i.e.,* channelization, reservoir construction, irrigation) can drastically alter the hydrology of a system, as well as greatly influence existing fish communities (Oscoz *et al*. 2005; Lau *et al*. 2006; Wang *et al*. 2011; Liermann *et al*. 2012). While there are several means of habitat manipulation, natural and anthropogenic influences often co-occur and can be a direct response to a previous effect (*i.e.,* human diversion of water due to rising temperatures that result in a reduced water table). Furthermore, as the effects of global climate change are exacerbated, aquatic and terrestrial resources will become less abundant and the biological diversity of these systems will continue to diminish (Vitousek 1994; Malcolm *et al*. 2006; Parmesan 2006; Pounds *et al*. 2006).

These drastic alterations to the environment leave resident fish populations susceptible to local or complete extinction. Habitat fragmentation and destruction are often cited and growing concerns for threatened or endangered species. Remnant populations can be confronted with limited genetic variation, environmental change, and different interspecies interactions. Together, these events can spur the contemporary evolution of impacted populations or hasten their demise (Vitousek 1994; Alò and Turner 2005). Phenotypic plasticity can buffer populations against extinction if it moves phenotypes toward the phenotypic optima associated with increased fitness, therefore facilitating phenotypic evolution (Ghalambor *et al*. 2007). In natural populations, it can be challenging to disentangle phenotypic plasticity from contemporary phenotypic evolution; however, in some cases, it is possible to witness the phenotypic change of a population (as a combination of the two mechanisms) in a changing environment, and infer whether such change is adaptive. Nevertheless, empirical tests require replication of populations experiencing similar environmental change, which is extremely rare in nature.

Trophic polymorphisms are known to be considerable among ichthyofauna in both interspecific and intraspecific contexts (Wimberger 1992, 1994; Rüber and Adams 2001). Food availability has been shown to rapidly alter the functional morphology of the feeding apparatus of a population depending on items that are present or absent (Wainwright *et al*. 1991; Hulsey *et al*. 2005; Whiteley 2007; Martin and Wainwright 2011). Community structures can change drastically as a response to environmental conditions (*e.g.*, salinity, temperature), intra- and interspecific competition, and an interaction of these abiotic and biotic factors (Whitfield 1986; Henderson 2007). Habitat fragmentation can alter population densities, interspecific interactions, and the environmental conditions in remnant habitats. Due to the nature of these events, cranial morphology might be associated with, and predicted by, the variation of ecological roles played by a species in different communities in different environments. By evaluating the development and/or presence of trophic polymorphisms, one would better be able to evaluate an organism's, or population's, ability to respond to intraspecific competition (Swanson *et al*. 2003) and exploit new resources (Skulason and Smith 1995). This intuitive hypothesis is generally intractable in empirical studies, unless habitat fragmentation produces diverse but replicated communities in varied environments. Such is the case for numerous fish communities in the Bitter Lake National Wildlife Refuge (BLNWR) in the Roswell Basin, New Mexico. Following a century of water extraction and alteration of the Pecos River, numerous isolated habitats from the historic backwaters of the Pecos River now occupy the landscape (Hoagstrom and Brooks 1999).

Cyprinodon pecosensis (Cyprinodontiformes: Cyprinodontidae), historically widespread throughout the Pecos river system, has experienced exceedingly isolated populations due to the native habitat being destroyed or removed (Miller 1961; Hoagstrom and Brooks 1999). *Cyprinodon pecosensis* populations have chiefly been confined to isolated sinkholes, man-made impoundments, and sparse spring-fed marshes (Collyer *et al*. 2015a), with varying environmental conditions (*e.g.,* salinity) and community structures (*e.g.,* allopatric and sympatric populations; Hoagstrom and Brooks 1999). After the introduction of *C. variegates* (sheepshead minnow) into the Pecos river in Texas during the early 1980's, hybridization quickly became prevalent, with *C. variegatus* x *C. pecosensis* hybrids occupying an estimated 50% of the historic range of the endemic *C. pecosensis* population by 1985 (Echelle and Connor 1989; Echelle *et al*. 1997). The widespread success of hybridization between these two species has been attributed to a selection mechanism for the hybrid population during a period when the endemic *C. pecosensis* population had already been depleted (Childs *et al*. 1996). *Cyprinodon pecosensis* females showing sexual preference for male *C. variegatus* and hybrids (Rosenfield and Kodric-Brown 2003), superior growth-rate and vigor in hybrids (*i.e.,* heterosis) and pure *C. variegatus* in comparison to pure *C. pecosensis* (Rosenfield *et al*. 2004), and increased anthropogenic manipulation of natural habitat (*e.g.*, impoundment, groundwater withdrawal, channelization, diversion of water resources) have all attributed to the recent decline in *C. pecosensis*. Since *C. pecosensis* populations are prone to numerous ecological conditions, it is imperative that the cranial morphology of this species be better understood. Differing morphotypes often express different behaviors, strategies, and diets (Swanson *et al*. 2003; Whiteley 2007), as well as physiological responses (*e.g.*, growth rates; Skulason and Smith 1995).

The purpose of this study was to assess the morphological variation in the head and mandible of *C. pecosensis* populations within and among different populations that inhabit various habitat types, as well as determine if temporal effects (*e.g.,* seasons) influence the aforementioned variation. I used museum specimens from multiple samples over numerous years, seasons, and populations. Morphological data were collected and subsequently subjected to various analyses to assess which environmental variables were influencing shape. With different *C. pecosensis* populations subjected to different ecologies, I expected that similar populations would occupy different regions of morphospace in respect to interspecific interactions and habitat type colonized. I anticipated localities within sinkhole habitat complexes to be unique when compared to other habitat complexes due to longer periods of isolation, lack of seasonal connectivity to other populations, and higher salinity than other habitat complexes. Additionally, I had expected that temporal effects would be of influence. Since populations have their own unique ecologies, temporal effects would be expected to have some sway on food items available, thus encouraging the populations to adapt in response to items present.

MATERIALS AND METHODS

SPECIMENS USED AND THE STUDY SYSTEM

I collected shape data from 1162 *Cyprinodon pecosensis* individuals (575 males, 565 females, 22 juveniles) which incorporated 63 collections from 26 different locations. Specimens were examined at the Museum of Southwestern Biology (MSB) and comprised subsets or complete samples of populations. Accession numbers from the MSB collections used can be found in Appendix 1 and a map of the region is provided (Fig. 1, 2). *Cyprinodon pecosensis* specimens included both males and females to assess variation in head and mandible shape across populations, in addition to assessing sexual dimorphism. The habitats investigated differed primarily in presence or absence of water flow, community structure, fish assemblage, maximum water depth, and salinity. Samples from the Pecos River were excluded from this analysis in order to focus on isolated populations and eliminate potential effects of hybridization.

Habitat classifications correspond to those in Collyer *et al*. (2015a) to remain consistent with previous work and for future ecological comparison. Of the 26 localities investigated, four habitat classifications were used: Bitter Lake region (BL), Bureau of Land Management (BLM), Sinkhole (SH), and Waterfowl Impoundment (WF).

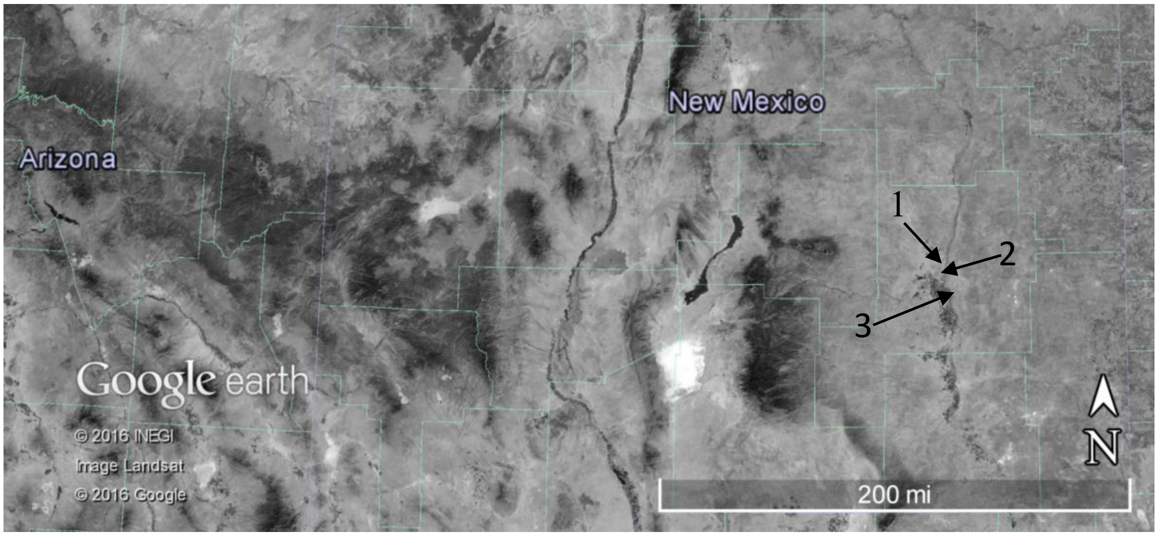

Figure 1. Chaves County, New Mexico. General areas of sampling sites indicated by numbers 1 -3, which correspond to smaller maps in Figure 2, which in turn illustrates individual localities. Locations are too clustered to be identified, but this map is intended to show the relative area.

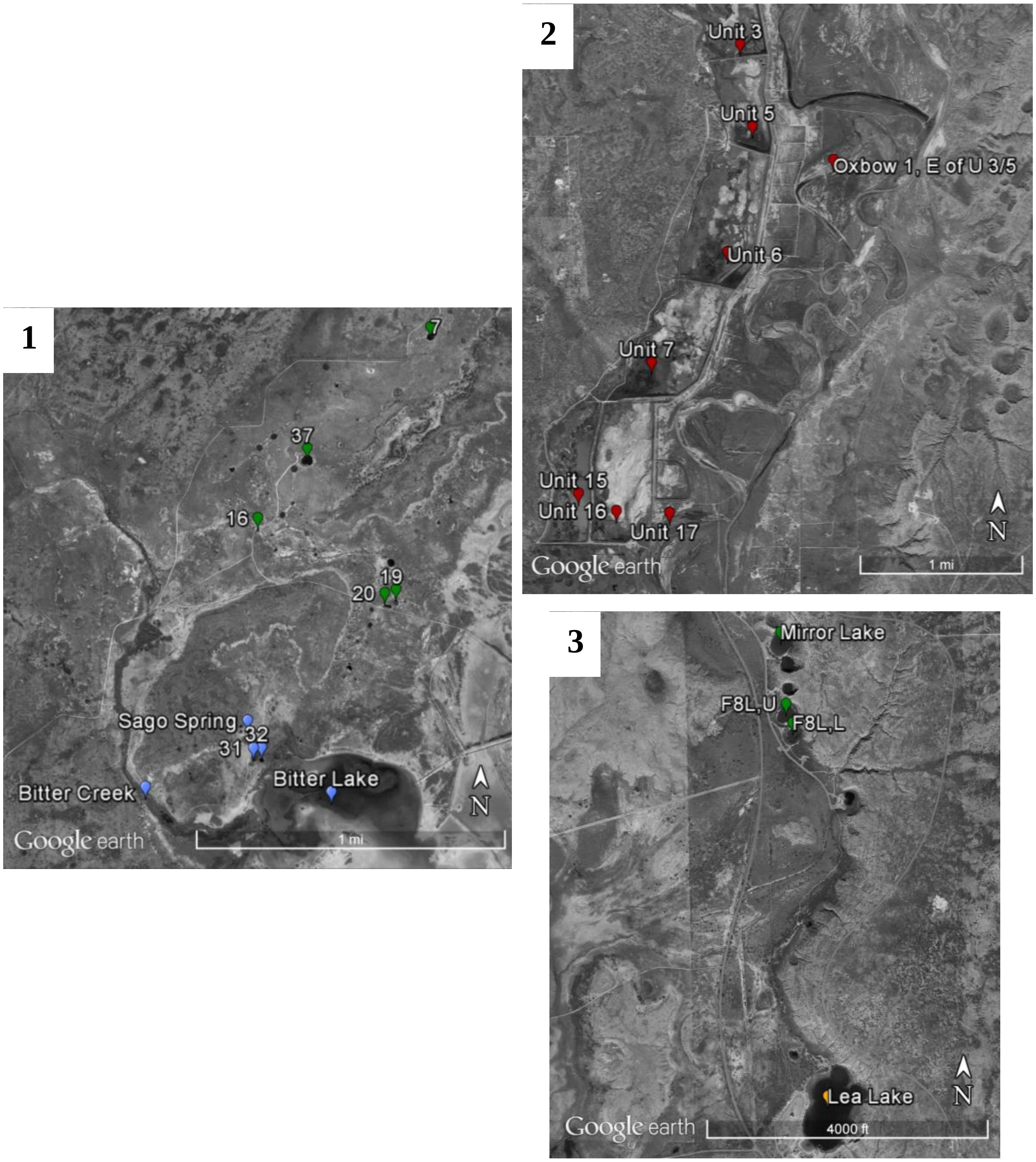

Figure 2. Corresponding maps associated with Figure 1. Sinkholes are listed by associated sinkhole number only, Waterfowl Impoundments are listed as Unit (x). Like colored symbols represent identical habitat complex classifications and include Bitter Lake (BL), Bureau of Land Management (BLM), deep Sinkhole (SH), and Waterfowl Impoundments (WF).

Bitter Lake

Bitter Lake habitat complex (BL) comprises five localities: Bitter Creek (two divisions), Sago Spring, Sinkhole 31, and Sinkhole 32. These locations comprise two systems, which flow directly into Bitter Lake (a spring-fed playa lake) and are roughly located in the same vicinity. Bitter Creek spring complex (confluence and weir collections; BCc, BCw) flows north to south, reaching a confluence with Dragonfly Spring, then Lost River before entering Bitter Lake. Salinity varies along the length of the Bitter Creek spring complex but does not change considerably, ranging from 4.1ppt to 6.8ppt (Hoagstrom and Brooks 1999). The mouth of the Sago Spring complex (Sago Spring, Sinkhole 31, Sinkhole 32) empties into Bitter Lake roughly 100m north of the mouth of Bitter Creek (Collyer *et al*. 2015a). Salinity is relatively consistent among the sinkholes (with minor differences) but is generally lower in Sago Spring itself compared to the adjoining sinkholes (Hoagstrom and Brooks 1999). Since these systems are connected and part of a larger system, the populations are considered part of a single habitat complex.

Bureau of Land Management

The Bureau of Land Management habitat complex (BLM) encompasses two localities, both of which are part of the same outflow. The Lea Lake outflow, and weir, is a spring marsh that flows from Lea Lake to the southwest, resulting in an extensive marsh habitat. Hoagstrom and Brooks (1999) report that as the distance from Lea Lake increases, so does salinity due to effects of evaporation. This wetland is managed by the Bureau of Land Management (BLM) and is associated with the BLM waterfowl complex.

Sinkhole (SH)

Cyprinodon pecosensis populations occupy numerous sinkholes within and outside of the BLNWR. The large majority of sinkholes are isolated, having no connection to other systems, and can vary greatly in community structure and ecological parameters (*e.g.*, salinity, substrate composition; Hoagstrom and Brooks 1999), making each of them inherently unique. The sinkholes that I included in this habitat complex are located within the BLNWR or Bottomless Lakes State Park (BLSP) and are all considered to be isolated sinkholes. Collections from Figure 8 Lakes, upper and lower, were included in this study and were once historically connected. While upper Figure 8 Lake contains both *C. pecosensis* and *Fundulus zebrinus* populations, lower Figure 8 Lake contains only *C. pecosensis*. Other sinkholes included in this study are Sinkholes 7, 16, 19, 20, 37 (*i.q.*, Lake Saint Francis), and Mirror Lake.

Waterfowl Impoundments

The Waterfowl Impoundment habitat complex (WF) contains numerous waterfowl impoundments located within the BLNWR. These impoundments vary in surface area, salinity, and species composition (Hoagstrom and Brooks 1999). While some of the impoundment populations are temporarily isolated during drier months, wetter months result in higher water tables and allow for gene flow among the impoundments. Impoundments are classified as individual units, some of which are connected to oxbow lakes by means of the Pecos River. Collections from Units (Ux) 3, 5, 6, 7, 15, 16, and 17, as well as a collection from an oxbow lake between Units 15 & 16, were incorporated into this study.

DATA COLLECTION

Specimens from each collection were arranged according to sex and size, and 20 individuals (10 males, 10 females) from each collection were chosen to photograph, with the intent of sampling a range of sizes to assess shape allometry. Some collections failed to contain twenty specimens and, when this occurred, all specimens in the collection were included (up to, and not exceeding, 10 of either sex). Specimens expressing a high degree of deformity (*e.g.*, bent or broken due to poor preservation, destroyed tissue from prior use) were omitted.

Photographs were taken using a ShuttlePix P-400R Digital Microscope (Nikon) and landmark-based geometric morphometrics methods were used to assess mandible and head shape (Adams *et al*. 2013). Landmark generation was accomplished using digitizing procedures through TPSDIG2w64 software, version 2.22 (Rohlf 2016). Landmarks can be generally defined as points of homology between two or more specimens. In total, landmark configurations encompassed 61 landmarks (14 fixed, 47 sliding semi-landmarks) and were digitized onto the left lateral surface of the specimen's head (Fig. 2). Fixed landmarks are Cartesian coordinates of the location of specific structural elements, such as the intersection of opercula. Semi-landmarks are points along curves that have less specific meaning, but help to quantify the curvature of structures. Landmarks were generated with the intent of capturing the shape of the mandible (14 total, 4 fixed, 10 semi-landmarks), the pre-maxilla (3 total, 3 fixed), and the head, excluding the aforementioned and including the eye (45 total, 8 fixed, 37 semi-landmarks).

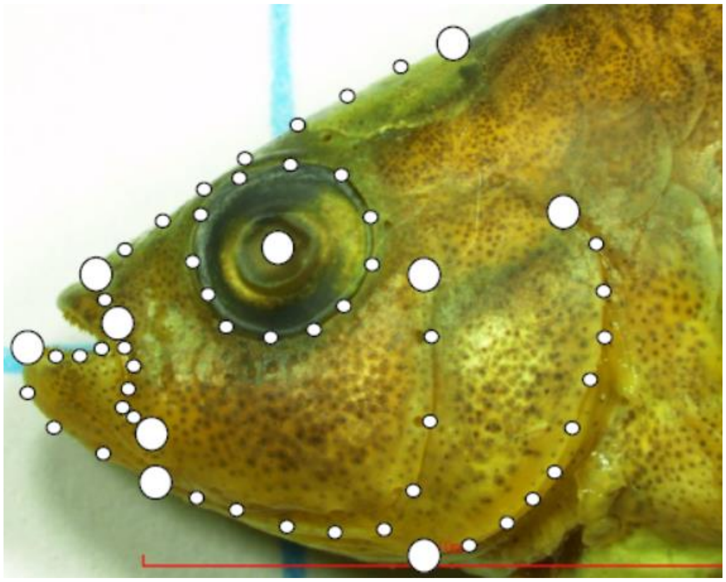

Figure 3. Anatomical landmarks for geometric morphometric analyses. Larger landmarks are fixed landmarks and represent the anterior tip of the maxilla, posterior margin of the first scale on the nape, intersection of the operculum and pre-operculum with the cranium, the "hinge" points of the mandible, the anterior tip of the mandible, the lower margin of the maxilla, and the center of the eye. The smaller landmarks are semi-landmarks (or sliding landmarks) and are used to represent curves along points of interest. Landmarks associated with the premaxilla are not pictured due to exclusion.

Landmark coordinates (X,Y) were subjected to a generalized Procrustes analysis (GPA; Rohlf and Slice 1990), using GEOMORPH, version 3.0.0 (Adams and Otarola-Castillo 2013; Adams *et al.*2016) in R, version 3.2.3 (R Core Team 2016). The procedure centers, rotates, and scales the landmark configurations in such a way that they were invariant of one another in location, orientation, and size, generating Procrustes residuals (the aligned coordinates of individual landmarks and the corresponding mean landmark location). Fixed landmarks were immobile, unlike semi-landmarks which were allowed to slide along a generated curve during GPA in order to minimize squared Procrustes distances among landmarks (Bookstein 1997; Gunz and Mitteroecker 2013).

STATISTICAL ANALYSES

Associations between shape (dependent variable) and various independent variables (*e.g.*, sex, habitat complex, temporal data) were assessed using several models, where the design of each was dictated by the results of a proceeding model. Details of the models can be found in the next section (MODEL DESIGN). For the purpose of significance testing, $\alpha = 0.05$.

Several analyses were performed on the Procrustes residuals using a non-parametric multivariate analysis of variance (MANOVA). All statistical tests and descriptive models utilized a randomized residual permutation procedure (RRPP) and were subjected to 10,000 random permutations. The *post hoc* pairwise comparisons utilized the exact random permutations of the RRPP used in the non-parametric MANOVA. Since the RRPP is performing the same random placement of residuals for all test statistics, the inferences made are not considered to be separate tests and are instead considered to be different statistics from the same test (Collyer *et al*. 2015b). A

RRPP is a resampling technique that employs randomizing the residual shape values from the “reduced” (null) models of each analysis, for evaluating statistics associated with a “full” model. Once randomized, residuals are combined with fitted values from the reduced model to create random pseudo-values for the full model (*e.g.*, calculate sums of squares for effects, given a null hypothesis; Collyer *et al*. 2015b). This approach allowed for the comparison of GPA aligned shape data with a multitude of variables. All models and analyses were designed and conducted using GEOMORPH, version 3.0.0 (Adams and Otarola-Castillo 2013; Adams *et al.*2016) in R, version 3.2.3 (R Core Team 2016).

A principal component analysis (PCA) was conducted on all three units being investigated (*i.e.*, head shape independent of sex, male and female mandible shape) and the PC scores for locality were plotted for visualization. Convex hulls were generated on all principal component plots to visualize the morphospace occupied by each habitat complex. This allows assessment of morphospace overlap among complexes and to determine whether shape change among localities and complex is associated in a particular direction within morphospace.

MODEL DESIGN

Numerous models were created in order to tease apart the several potential independent variables that I expected would influence morphology. Variables were assessed broadly at the start and included size (log of centroid size), sex, habitat complex designation, specific localities (populations), month, year, and interactions among these. Centroid size is the square root of summed squared distances of all included landmarks to the centroid (the mean position of all landmarks). Because shape data are multidimensional, typical model selection criteria (*e.g.,* Akaike’s information criterion) are prone to favor over-fitting models (Bedrick and Tsai 1994; Davis *et al*. 2016). With this in mind, the final model chosen was one that included meaningful effects and excluded those with small effect sizes ($R^2 < 0.05$).

Mandible and head shapes were evaluated separately to prevent distortions from specimens that were preserved with open or closed mouths. Several models were designed with the goal of testing for nested effects and will be referenced several times throughout this section. Nested effects are effects where classifications may contain a few levels of one factor, but only within a specific level of a different (primary) factor. Additionally, while premaxilla shape data were collected, I chose to disregard the premaxilla in this study due to difficulty in collecting unobstructed shape data from the coordinates created. This difficulty was due to the individuals preserved in a way that prevented manipulation of the mouth such that all parts of the premaxilla were visible, without damaging the specimens. Thus, two aspects of morphology were considered - mandible and head morphology.

Procrustes residuals were initially subjected to a Procrustes ANOVA, specifically designed to assess the covariation of shape and size with other variables, initially to assess sex and sex-by-habitat interactions. An evaluation of the linear model produced would give insight to whether or not shape was different at various sizes among designated groups (*e.g.,* Sex, Sex/Habitat). An example model for the allometry tests used can be described as:

Mandible Shape ~ log(Centroid size) + Group + log(Centroid size):Group,

where Group refers to a logical stratum within the data, such as sex, habitat, year, etc. The important part of this model is the interaction term (noted in the far right of the formula). This term evaluates whether the levels within the group of interest have different shape allometries (slopes). To ascertain whether this is the case, the preceding full model can be compared to a reduced model:

Mandible Shape ~ log(Centroid size) + Group,

which restricts groups to having a common shape allometry. The sum of squares (SS) for the interaction in the full model is the difference between the two models' sum of squared error (SSE) (Collyer *et al*. 2015b). Thus, one can systematically evaluate the importance of sources of shape variation and arrive at a parsimonious model from which inferences about shape variation can be made.

Preliminary model evaluation indicated sexual dimorphism was a significant source of variation in mandible shape, suggesting these data should subsequently be evaluated for males and females, separately. Results are thus presented separately for males and females for mandible shape, with initial indication of model selection results before evaluating the details of pairwise comparisons among group levels. Because of the hierarchical structure of the data, "habitat" was considered a fixed effect and "sampling date" was considered a random effect, nested within habitat. Sampling date could be further collapsed to month of sampling and year of sampling, to evaluate within-year and among-year sources of variation, in a nested model fashion. Pairwise comparisons either compared means or compared slopes – whichever was warranted, based on model selection – for the group levels that were important. Model selection and evaluation used a combination of the `procD.lm`, `advanced.procD.lm`, `procD.allometry`, and `nested.update` functions of GEOMORPH, version 3.0.0.

RESULTS

HEAD MORPHOLOGY

Of the initial variables and interactions investigated, the only ones with effect sizes large enough to be deemed meaningful were size (log of centroid size), habitat, and locality (Table 1). Consequently, males and females were not separated in the ensuing analyses. Temporal effects were not meaningful with relatively low R^2 (month, 0.0297; year, 0.0186).

A principal component analysis revealed that more than half of the variation could be explained within the first two principal components, PC1 (38.8%) and PC2 (20.3%). With the addition of PC3, this was increased to 75.4%. Shape variation along the first PC axis was associated with the size of the eye and robustness, while length of the head was associated with the second PC axis. A visualization of the data (Fig. 3) illustrates some partitioning of PC space, especially among SH, BL, and BLM hulls, as well as between SH and WF hulls. SH head shape varied but possessed relatively large eyes, a narrow head, and either a small (higher PC2) or large operculum (lower PC2). The BL hull resided primarily in the upper portion of the WF hull, while the BLM hull resided in the middle of the WF hull.

A pairwise test revealed that 340/351 pairwise shape differences in locality means were significant (App. 1). Nearly all localities were different from one another with the exception of 11. Within the BL complexes, a total of four comparisons were not significantly different. Sago Spring was not significantly different than the two other BL localities, Bitter Creek confluence (P = 0.1356) or Sinkhole 32 (P = 0.0955), but was significantly different than the Bitter Creek weir (P = 0.0224). Additionally, Sinkhole 31 was not significantly different than two WF localities, Unit 15 (P = 0.5252) or Unit 17 (P = 0.3635). All comparisons with localities from the BLM complex were significant. Only two intra-habitat comparisons with a deep sinkhole locality (SH) were not found to be significant. Head morphology in Mirror Lake was not significantly different than Sinkhole 16 (P = 0.1420) or Sinkhole 19 (P = 0.0980). WF localities were significantly different than all with the exceptions of the aforementioned, as well as four inter-habitat comparisons. The comparisons of Unit 15 and Unit 17 (P = 0.0539), Unit 5 and Unit 16 (P = 0.0507), Unit 2 and Unit 6 (P = 0.1799), and Unit 2 and Unit 7 (P = 0.0897) were not found to be significant.

Table 1. Results of the Procrustes ANOVA for assessing the effect of habitat complex and the interaction of location (population) among habitat complexes on head morphology. (α = 0.05)

	Df	SS	MS	R^2	Z	P
log(Size)	1	0.0518	0.5182	0.1681	86.322	0.0001
Habitat	3	0.3379	0.1126	0.1096	43.623	0.0001
Habitat : Locality	23	0.4098	0.0178	0.1330	9.059	0.0001
Residuals	1112	1.8153	0.0016			
Total	1139	3.0812				

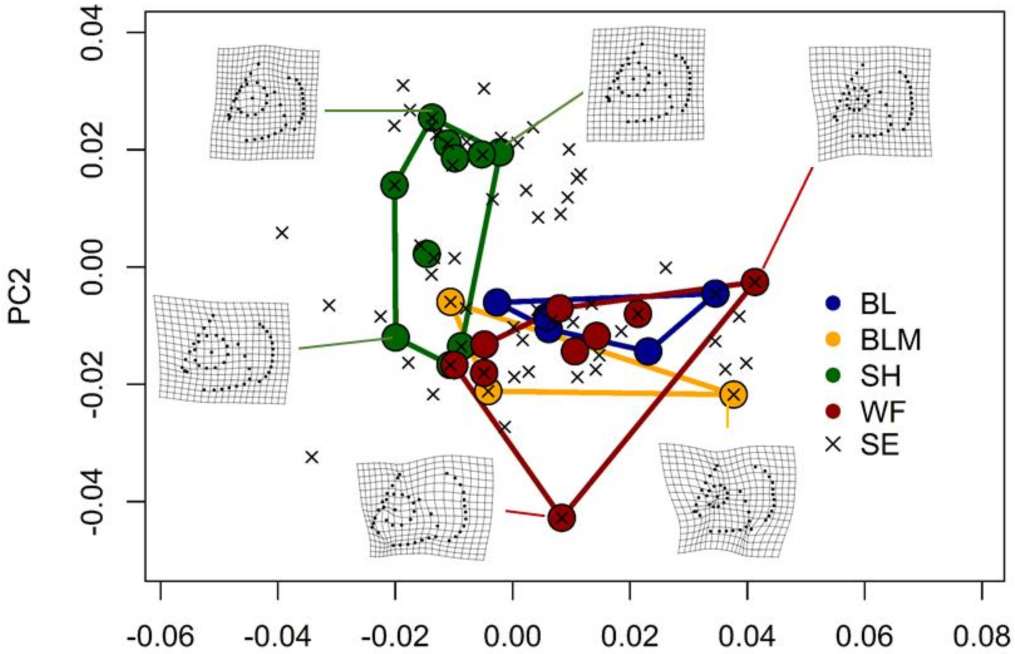

Figure 4. Principal component (PC) plot representing head shape variation among and within morphotypes. Morphotypes include upper Bitter Lake (BL), Bureau of Land Management outflow marsh (BLM), deep sinkholes (SH), and Waterfowl Impoundments (WF). Localities are represented by the colored symbols and convex hulls are displayed to illustrate the most extreme values. Each hull is color coded to match the colors used in Figures 1 and 2 and represents locations within the same habitat complex designation. Small [x] represents sampling events and were determined using the specific MSB lot numbers that were included in this study. [x]s that fall upon a location symbol indicate that only a single collection event occurred for that particular locality. Deformation grids are included to denote the most extreme variations in shape and are representatives of the transformation of mean shape for the connected location and have been magnified by a power of two in order to aid in discerning morphological variation.

MANDIBLE MORPHOLOGY

An initial test of allometry (Procrustes ANOVA) and homogeneity of slopes test revealed both statistical (Table 2) and visual (Fig. 3) evidence that suggests sexual dimorphism is significant. Results from the homogeneity of slopes test between sexes (males, n = 575; females, n = 565) revealed that slopes were not parallel and were therefore separated for subsequent analyses (P =0.0003). While sex explained very little variation in mandible morphology (R^2 = 0.0043), it was still significant (P = 0.0012).

Males

A Procrustes ANOVA examining the effect of habitat complex and the interaction of habitat complex and locality (population; Table 3) on male mandible morphology suggested that habitat complex designations were not significantly different (P = 0.1716) and explained little variation in shape (R^2 = 0.0312). However, individual localities nested within habitat complexes were significantly different (R^2 = 0.1673, P = 0.0001), suggesting that populations could be unique. Results from a Procrustes ANOVA (Table 4) evaluating sampling events (Year/Month; ranging from 1940 to 2003) suggests that location explains a large amount of variation (R^2 =0.1985) but was not statistically significant (P = 0.0980) when compared to the year when sampling occurred (R^2 = 0.0848, P = 0.0001). Further, the effect of months within years was not significant (R^2 = 0.0017, P = 0.1493), and while the effect of month was significant, it explained little shape variation (R^2 = 0.0384). There was a significant interaction of monthly changes within years within localities (P = 0.0001), but this also explained little variation in shape (R^2 = 0.0268).

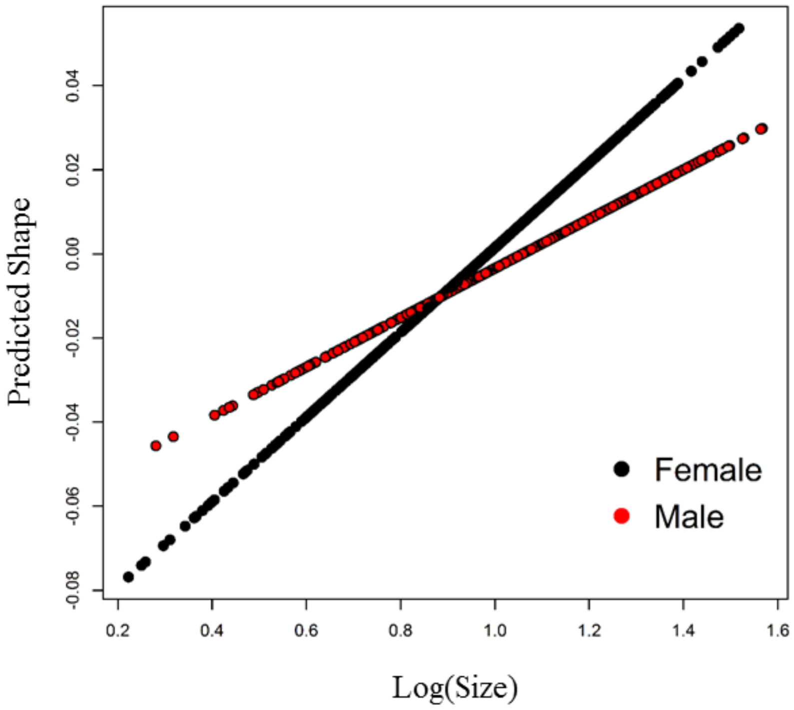

Figure 5. Visualization of allometric effects on GPA aligned shape data among sex groups. The points are representative of the first principal component of the predicted shape of males and females against the log of centroid size to illustrate allometry.

Table 2. Results of the Procrustes ANOVA for assessing the effect of size (allometry) and sex groups. (α = 0.05)

	Df	SS	MS	R^2	Z	P
log(Size)	1	0.3868	0.3867	0.0288	25.9612	0.0001
Sex	1	0.0579	0.0579	0.0043	4.1192	0.0012
log(Size) : Sex	1	0.0460	0.0460	0.0034	3.3358	0.0037
Residuals	1136	12.9392	0.0113			
Total	1139	13.4300				

A principal component analysis revealed that PC1 and PC2 accounted for 80.9% of the variation in the data. Mandible morphotypes among males tended to be mostly associated with PC1 (56.3%), which accounts for variation in mandible length. PC2 (24.6%) tended to represent robustness of the mandible, individuals with higher PC2 scores having a thicker, more robust mandible. A visualization of the data (Fig. 5) shows that morphospace is poorly partitioned among habitat complexes. There is substantial overlap of the SH convex hull with the other three habitat associated hulls, mandible morphology ranging from long and robust to short and narrow. The BLM convex hull was large and overlapped two other convex hulls (SH, WF), but was distinct from the BL hull. Mandible morphology of the BLM and WF convex hulls tended to reside in the center, being neither extremely robust/narrow nor long/short. Mandible morphology with the BL hull tended to be short and narrow when compared to that of BLM and WF hulls.

A pairwise test revealed that 235/351 pairwise differences among locality means of male mandible shape were significant (App.3). Five localities were identified as being associated with the BL habitat complex and across 110 inter-habitat and 10 intra-habitat comparisons, 63 and 5 comparisons were significant, respectively. Three localities fell within the BLM complex and pairwise comparisons among localities resulted in a total of 70 inter-habitat and 3 intra-habitat comparisons. Of these comparisons with other localities, 49 inter-habitat comparisons were significant while all three BLM associated locality comparisons were significant. Ten localities fell within the deep sinkhole (SH) complex, resulting in a total of 170 inter-habitat and 45 intra-habitat comparisons, 113 and 34 were significant, respectively. The last habitat complex, WF, consisted

Table 3. Results of the Procrustes ANOVA for assessing the effect of complex and the interaction of location (population) among habitat complexes on male mandible morphology. Bolded P-values are significant (α = 0.05).

	Df	SS	MS	R^2	Z	P
log(Size)	1	0.1202	0.1202	0.0181	8.4293	**0.0001**
Habitat	3	0.2073	0.0690	0.0312	1.2883	0.1716
Habitat : Locality	23	1.1090	0.0482	0.1673	4.3433	**0.0001**
Residuals	547	5.1913	0.0094			
Total	574	6.6278				

Table 4. Results of the Procrustes ANOVA assessing the effects of location and sampling period on male mandible shape, as well as the effect of sampling periods within locations. Bolded P-values are significant ($\alpha = 0.05$).

	Df	SS	MS	R^2	Z	P
log(Size)	1	0.1202	0.1202	0.0181	8.4293	**0.0001**
Location	26	1.3163	0.0506	0.1985	1.2846	0.0980
Year	9	0.5627	0.0625	0.0848	6.7151	**0.0001**
Month	7	0.2549	0.0364	0.0384	4.3586	**0.0001**
Location : Year	12	0.2980	0.0248	0.0449	3.1985	**0.0001**
Year : Month	1	0.0013	0.0113	0.0017	1.3190	0.1493
Location : Year : Month	5	0.1179	0.0355	0.0268	4.8148	**0.0001**
Residuals	513	3.8864	0.0075			
Total	574	6.6278				

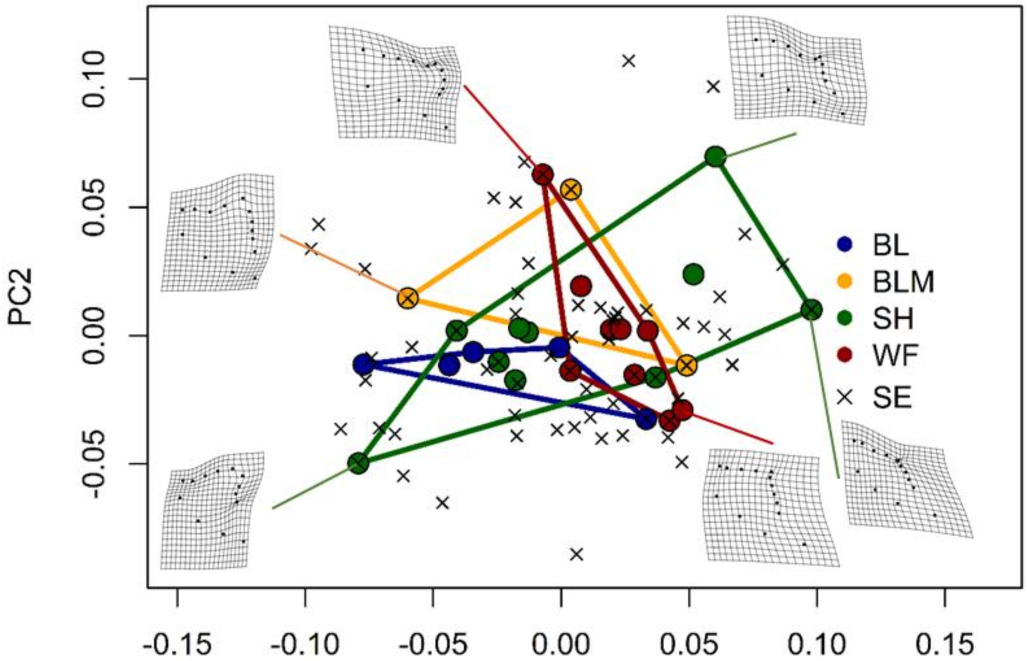

Figure 6. Principal component (PC) plot representing male mandible shape variation among and within morphotypes. Morphotypes and symbol representations are the same as in Fig.3.

of nine localities. Inter-habitat comparisons with WF associated localities resulted in 109 of 162 and 12 of 35 significant comparisons.

The three localities associated with Figure 8 Lakes were significantly different than all other localities, with the exception of three. When compared to upper Figure 8 Lake, the Lea Lake outflow (P = 0.0552), Oxbow 1: East of Units 3 and 5 (P = 0.1078), and Unit 15 (P = 0.0577) were not significant. The combined collection of Unit 15/16 was significantly different from all other localities, with the exception of Oxbow 1: Unit 3 (P = 0.6729).

Females

A Procrustes ANOVA examining the effect of habitat complex and the interaction of habitat complex and population (location; Table 5) on female mandible morphology revealed that habitat complex designations were significantly different (P =0.0364), but explained a minute amount of variation in shape (R^2 = 0.0404). It further suggested that individual populations among habitat complexes were significantly different (R^2 = 0.1514, P = 0.0001).

Results from a Procrustes ANOVA (Table 6) evaluating sampling events (Year/Month; ranging from 1940 to 2003) suggests that location explains a large amount of variation (R^2 = 0.1919) but was not statistically significant (P = 0.1305) when compared to the year when sampling occurred (R^2 = 0.0566, P = 0.0001). The effect of months nested in years was found to be significant but not meaningful (R^2 = 0.0031, P = 0.0216), and while the effect of month alone was significant (P = 0.0001), it poorly explained variation in shape (R^2 = 0.0361).

Table 5. Results of the Procrustes ANOVA for assessing the effect of habitat complex and the interaction of location (population) among habitat complexes on female mandible morphology. Bolded P-values are significant (α = 0.05).

	Df	SS	MS	R^2	Z	P
log(Size)	1	0.3339	0.3338	0.0491	22.1869	**0.0001**
Habitat	3	0.2736	0.0911	0.0404	1.7695	**0.0364**
Habitat : Locality	23	1.0234	0.0445	0.1514	4.0247	**0.0001**
Residuals	537	5.1719	0.0096			
Total	564	6.7582				

Table 6. Results of the Procrustes ANOVA assessing the effects of location and sampling period on female mandible shape, as well as the effect of sampling periods within locations. Bolded P-values are significant ($\alpha = 0.05$).

	Df	SS	MS	R^2	Z	P
log(Size)	1	0.3252	0.3251	0.0481	21.7741	**0.0001**
Location	26	1.2970	0.0498	0.1919	1.2387	0.1305
Year	9	0.3828	0.0425	0.0566	4.5539	**0.0001**
Month	7	0.2441	0.0348	0.0361	4.0222	**0.0001**
Location : Year	11	0.2888	0.0262	0.0427	3.2188	**0.0001**
Year : Month	1	0.0213	0.0213	0.0031	2.3700	**0.0216**
Location: Year : Month	4	0.1389	0.0497	0.029	6.3218	**0.0001**
Residuals	505	4.0002	0.0079			
Total	564	6.7582				

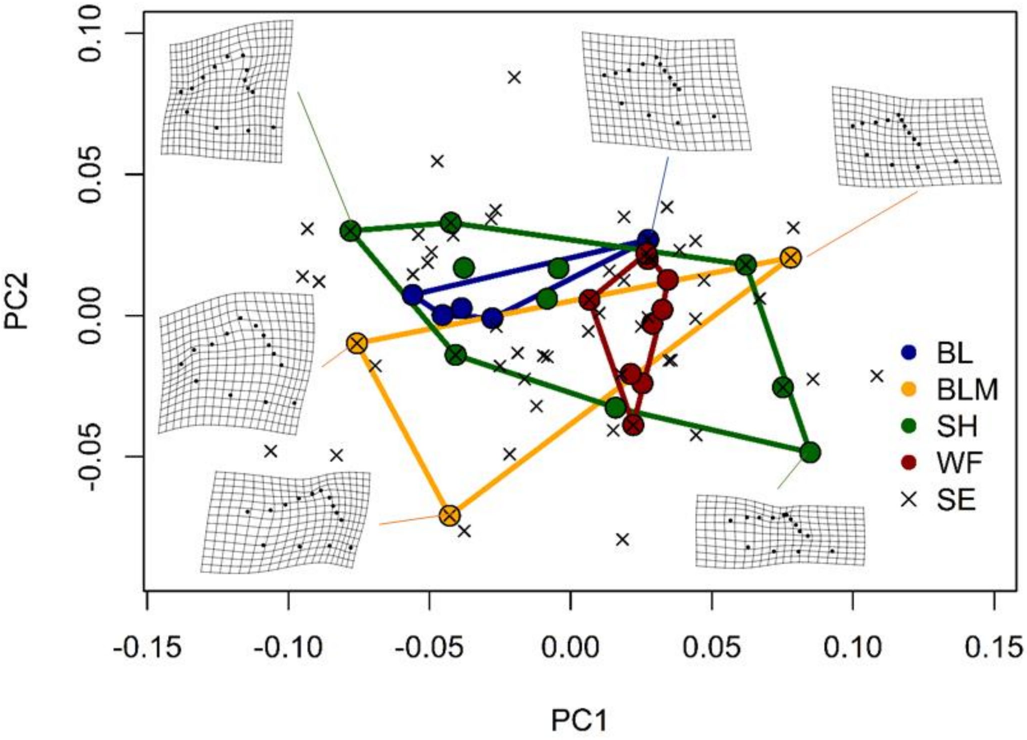

Figure 7. Principal component (PC) plot representing female mandible shape variation among and within morphotypes. Morphotypes and symbol representations are the same as in Fig.3.

A principal component analysis revealed that PC1 and PC2 accounted for 82.1% of the variation in the data. In general, mandible morphotypes among females tended to be mostly associated with PC1 (60.0%), which is associated with the length of the mandible. Conversely, PC2 (22.1%) tended to represent robustness of the mandible, individuals with higher PC2 scores having a thicker, broader mandible. A visualization of the data (Fig. 6) suggests that PC space is poorly partitioned among habitat complexes. While BL and WF habitat complexes are partitioned from one another, the SH habitat complex expresses a high degree of overlap with the other three habitat complex convex hulls. Mandible morphology ranged from short and narrow to long and robust. The BLM hull also had a high degree of overlap with the WF hull, in addition to SH. Two of the three localities comprising the BLM hull tended to represent mandible morphologies that were relatively short yet expressive of low to moderate robustness. Localities comprising the BL hull tended to express a relative high degree of robustness with moderate relative length.

Pairwise tests revealed that 249/351 pairwise comparisons of female mandible shape among localities were significant (App. 4). Five localities were identified as being associated with the BL habitat complex and across 110 inter-habitat and 10 intra-habitat comparisons, 76 and 4 comparisons were significant, respectively. Three localities fell within the BLM complex and pairwise comparisons among localities resulted in a total of 72 inter-habitat and 3 intra-habitat comparisons. Of these comparisons with other localities, 53 of the inter-habitat comparisons were significant while all three BLM associated locality comparisons were significant. Ten localities fell within the deep sinkhole (SH) complex, resulting in a total of 170 inter-habitat and 45 intra-habitat comparisons, 127 and 35 of which were significant, respectively. The WF habitat complex consisted of nine localities. Inter-habitat comparisons with WF associated localities resulted in 128 of 162 and 7 of 35 significant comparisons.

The three localities associated with Figure 8 Lakes (upper, lower, and unknown sample) were significantly different than the majority of other localities investigated. The unknown sample from Figure 8 Lakes was not significantly different than lower Figure 8 Lake (P = 0.1108) or the combined Unit 15/16 sample (P = 0.0648), but was different than Units 15 (P = 0.0253) and 16 (P = 0.0196). Lower Figure 8 Lake was significantly different from all other comparisons with the exception of Oxbow 1 (E. of Unit 3 and 5; P = 0.0805), while upper Figure 8 Lake did not significantly differ from Units 6 (P = 0.06), 15 (P = 0.061), 16 (P = 0.089), nor the combined 15/16 sample (P = 0.378).

DISCUSSION

This is the first study of comparative cranial morphology within *Cyprinodon pecosensis*. Functional mouth-parts of *Cyprinodon* species vary and can depend greatly on the food items that are present (Humphries and Miller 1981; Stevenson 1992; Martin and Wainwright 2011). Studies of trophic level and diet of *Cyprinodon* fishes typically report generalist feeding behaviors and benthic foraging, ingesting primarily diatoms, invertebrates, and detritus (Kennedy 1977; Stevenson 1992; Horstkotte and Strecker 2005). This is consistent with stomach content analyses of *C. pecosensis* consisting of a 45 – 88% diatom/detritus mix, with the remaining being primarily composed of “animal material” (Davis 1981). Though, this was limited to five localities, none of which were among the localities investigated in this study. However, additional populations have more recently been found to consume dinoflagellates, gypsum (presumably by mistake), algae, and pollen within the various sinkhole populations (Swaim and Boeing 2008).

Head morphologies (Fig. 4) among the SH and BL habitat complex tended to be separately clustered, each expressing more similarity with their related inter-habitat localities. The SH convex hull had minor overlap with two other complexes, consisting of a single BLM locality in the same region of morphospace, but is ultimately occupying a different region. Deep sinkholes had somewhat larger eyes and much narrower heads than did the other three complexes, on average, and occupies a region of morphospace isolated from the other complexes. Large heads may be adaptations reducing risk of hypoxia (Rutjes *et al*. 2009), a consequence of the highly saline environments characteristic of deep sinkholes (Hoagstrom and Brooks 1999). Similar *Cyprinodon* species have been observed to have larger eyes and a larger head, proportional to overall body size, when food is limited and undergo reduced growth rates (Lema and Nevitt 2006), suggesting that drastic environmental factors could be associated with the differences observed in head morphology.

While head morphology was largely canalized among habitats and within habitat localities (with the exception of SH), the morphospace of mandible morphologies within habitats among both males and females was poorly partitioned. All convex hulls had at least some degree of overlap with at least two other convex hulls. Within the morphospace, the SH and BLM convex hulls covered the greatest expanse and BL and WF convex hulls covered the least, having little to no overlap with one another. Contrary to head morphology, mandible morphology was most heavily associated with year to year variation and locality within habitat complex. The large region of morphospace coverage in mandible morphology within SH and BLM habitats could be associated with the respective localities expressing varied sensitivity to changes in environmental conditions, thus potentially influencing the availability of food items present from year to year.

Many of the differences in morphology may be attributed to phenotypic plasticity, especially within the deep sinkhole habitats, where the morphological expanse of mandible shape is vast but head shape is largely associated with large eyes and a small head. Plasticity in head and mandible shape could be greatly beneficial to these populations, where there is little to no connectedness with other bodies of water. Evaporation in these localities (*i.e.*, deep sinkholes) can lead to high salinities, increased temperature, reduced dissolved oxygen, and reduced food availability which leaves residents with little cover and few food options. These types of changes in microhabitat and salinity can additionally lead to fluctuations in invertebrate community structure (Verschuren *et al*. 2000). Additionally, previous research investigating plasticity among geographically isolated populations of *Salvelinus alpinus* (Arctic charr) demonstrated that the differences in cranial morphology can be greatly reduced when both populations are exposed to like environmental conditions (Alexander and Adams 2004).

Cyprinodon pecosensis populations could be adapting to various diets (*e.g.*, from carnivorous to herbivorous) within their locality, depending on how food availability changes temporally, and may explain the significant variation in morphology that I examined on a yearly basis. A closely related and readily hybridizing congener, *C. variegatus,* undergoes temporal dietary shifts depending on the food items available (Harrington and Harrington 1961), alternating from primarily *Aedes* larvae in September to vegetation in October. Another sympatric species, *Lucania parva*

(Cyprinodontiformes: Fundulidae), shifts from carnivory to herbivory, similar to *C. variegatus* (Harrington and Harrington 1961). *Chara*, an algae that is present in numerous localities in this study, has been reported in the gut contents of other *Cyprinodon* fishes (Stevenson 1992; Horstkotte and Strecker 2005) and could potentially be utilized by some populations of *C. pecosensis*.

In other herbivorous fishes, the pharyngeal jaw structure and muscles are key components comprised of hypertrophied pharyngeal jaws and muscles (Hulsey *et al*. 2005; Gidmark *et al*. 2014). A larger head in *C. pecosensis* may be correlated with larger pharyngeal jaws and muscles to facilitate the extra room needed to masticate vegetation. Tricuspid teeth may also benefit *C. pecosensis* when concerning a variable or high vegetative diet. Other fishes that possess tricuspid teeth, such as the cichlid genus *Hemitilapia* (Liem 1980) and some serrasalmid fishes (Machado-Allison and Garcia 1986), indicate that tricuspid teeth may be better suited to herbivory or omnivory. This is further supported by terrestrial reptiles that are herbivorous. The genus *Iguana*, a strong herbivore and opportunistic omnivore (Govender *et al*. 2012), is known to have polycuspid dentition (Sokol 1967). The development of polycuspid teeth could be the product of convergent evolution, facilitated by the need for tearing plant material or as a highly adaptable structure for being an opportunistic feeder.

While it is well known that *Cyprinodon* species are able to withstand high salinity, a modified head may in part be due to differences in bone structure for supporting additional musculature or adaptations for specialized feeding. Fishes with hypertrophied jaw adductor muscles have greater bite force (Herrel *et al*. 2002; Grubich *et al*. 2012), presumably for excising flesh. While fishes that consume primarily zooplankton through means of suction feeding, benefit from adaptations (*e.g.,* a smaller mouth opening) to generate greater suction and acceleration (Carroll *et al*. 2004; Holzman *et al*. 2008). Whereas fishes that consume primarily gastropods and mollusks benefit from modified molariform teeth (Hulsey *et al*. 2005) or modifications to the pharyngeal muscles and jaw (Wainwright *et al*. 1991). Contrary to a generalist benthic feeding behavior, Martin and Wainwright (2011) found that unique morphologies (*i.e.,* greatly modified adductor muscle mass, tooth length, mandible length) arose rapidly in response to substantial resource partitioning within sympatric clades of *Cyprinodon*. *Cyprinodon* species that consumed hard-shelled prey (*i.e.,* ostracods, gastropods) possessed relatively shorter jaws and a larger closing lever ratios for crushing prey, while the sympatric scale-eating *Cyprinodon* species developed longer jaws, increased size of the adductor muscles, and shortened opening and closing lever ratios for executing quick, powerful strikes. The lack of data associated with the community structure and diet of *C. pecosensis* populations in distinct, isolated localities creates difficulty in determining what is directly influencing the cranial morphology of these populations.

Variable fish assemblages of *C. pecosensis* populations have likely influenced the evolutionary trajectories among the populations differently. Assemblages vary in number of co-occurring species (App. 1), ranging from allopatric to sympatric with closely related fundulid (*e.g., Lucania parva, Fundulus zebrinus*), poeciliid (*e.g., Gambusia affinis, G. nobilis*), and other cyprinodontid (*e.g., C. variegatus*) species across all four habitat complexes and inter-habitat localities. It is likely, that *Cyprinodon* species existing in sympatry with competitors mitigate antagonism over food resources by partitioning trophic niches in their community (Echelle *et al*. 1972; Martin and

Wainwright 2011), a phenomenon supported by previous research evaluating stomach contents of sympatric *C. pecosensis* and *G. nobilis* (Swaim and Boeing 2008). In connected populations, a generalist approach may be more beneficial for *Cyprinodon* populations existing in systems where food items may not be as limiting. However, in deep sinkhole communities where competition may be high, the ability to partition resources would be greatly beneficial and can lead to alterations to the functional morphology involved in prey detection and acquisition (Rüber *et al*. 1999; Ferry-Graham *et al*. 2002).

Climate change is perhaps the greatest current threat to biodiversity with many communities, populations, and species already realizing the consequences (Vitousek 1994; Both *et al*. 2006; Pratchett *et al*. 2008; Bellard *et al*. 2012; Doney *et al*. 2012). Anthropogenic water consumption is expected to increase as the human population and their demand for water continues to increase, resulting in local water resources becoming critically reduced (Barnett *et al*. 2008; Elliott *et al*. 2014; Diffenbaugh *et al*. 2015). Fragmented populations of *C. pecosensis* may have an increased likelihood for speciation (Dias *et al*. 2013), but also hold a high risk of extinction (Fagen *et al*. 2002; Rybicki and Hanski 2013; Schnell *et al*. 2013). It is apparent that the morphological variation within localities included in this study is diverse in both head and mandible morphology. Continued isolation could create a scenario maladaptive to the survival of *C. pecosensis* in a system undergoing such drastic anthropogenic alteration, in addition to natural environmental fluctuation.

The habitat of *C. pecosensis* was not always fragmented, once a large, interconnected wetland system that served as floodplain and riparian for the Pecos river.

The same sinkholes once interconnected yearly or seasonally are now isolated, confining resident fish species to a series of micro-populations that vary greatly in abiotic parameters and community composition (Hoagstrom and Brooks 1999). Given the history of this system, along with the contemporaneous hydrology and climate, these results could be capturing historic adaptations for phenotypic plasticity, a trait that may explain why members of *Cyprinodon* are successful in desert environments. The remarkable evolutionary history of *Cyprinodon* has resulted in new radiations in relatively short time frames (Martin and Wainwright 2011) when in allopatry (Turner *et al*. 2008), presumably due to lack of gene flow. The same could be true for isolated populations of *C. pecosensis*, where several populations are currently confined to isolated sinkholes and would suggest that these results are capturing a more recent trend in *C. pecosensis*, one associated with local adaptation, unique for each population. The implications for both scenarios are only now becoming clearer, but this research has made the initial first step into better understanding the evolutionary responses of these fish in isolated systems.

This study provides evidence for a strong association with both head and mandible shape to individual localities within habitat complexes, as well as year to year variation. In small isolated systems (*i.e.*, deep sinkholes), variation in abiotic factors (*e.g.*, temperature and salinity) can result in altered fish assemblages (Kushlan 1980; Marchetti and Moyle 2001; Ostrand and Wilde 2001) and community composition (Verschuren *et al*. 2000). Localities need to be further investigated to elucidate how they vary from one another and to what magnitude. It is understood that the localities investigated in this study are free of *C. variegatus* where, to date, none have been collected (Hoagstrom and Brooks 1999). Management tactics associated with reconnecting isolated populations of

C. pecosensis with historic populations may lead to the introduction of *C. variegatus* into naïve Pecos pupfish populations, an occurrence which should be avoided due to heterosis (Rosenfield *et al*. 2004) and the general preference of females for hybrid males and pure *C. variegatus* (Rosenfield and Kodric-Brown 2003). Populations of Pecos pupfish are known to encounter significant reduction in numbers during harsh conditions (*i.e.*, 122 ppt salinity), but then improve once conditions become favorable (Swaim and Boeing 2008). The rebound of these isolated populations that undergo such reduced numbers (bottlenecking event) is dependent on the improvement of the system, a phenomena that is not always guaranteed, and can typically lead to the elimination of rare (low frequency) alleles and reduced genetic variation (Nei *et al*. 1975). Continued habitat maintenance and restoration, in addition to studies that monitor *C. pecosensis* populations and the abiotic changes in localities over time, is paramount to improving the status of *C. pecosensis*, and can be implemented to better assess and improve other threatened desert fish species.

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APPENDIX

Appendix 1. Information regarding the collections used in this study. Accession numbers (Cat#) indicate specific collections from which data were collected, proceeded by information pertaining to the collection locality. Species names are abbreviated as follows: *Fundulus zebrinus* (Fz), *Gambusia affinis* (Ga), *Gambusia nobilis* (Gn), *Cyprinella lutrensis* (Cl), *Dionda episcopa* (De), *Lucania parva* (Lp), *Etheostoma lepidum* (El), *Astyanax mexicanus* (Am), *Lepomis cyanellus* (Lc), *Dorosoma cepidianum* (Dc), *Cyprinus carpio* (Cc).

Cat#	*Locality*	*Habitat*	*Mon./Year*	*Likely Co-Occurring Species*
948	Figure Eight Lakes	SH	7, 1940	**Unknown**
3132	Units 15 & 16, Waterfowl Lake	WF	9, 1944	**Unknown**
5141	Unit 17 Waterfowl Lake	WF	5, 1987	Fz, Ga, Lp
5163	Unit 3 Waterfowl Lake	WF	5, 1987	Ga, Lp, Dc, Cc
5165	Sinkhole 31	BL	5, 1987	Gn
5166	Sinkhole 37	SH	5, 1987	Gn
5173	Unit 16 Waterfowl Lake	WF	5, 1987	Fz, Ga
5177	Sago Spring	BL	5, 1987	Gn, De, Lp, El,
5179	Marsh Outflow, weir.	BLM	5, 1987	**Unknown**
5185	Unit 5 Waterfowl Lake	WF	5, 1986	Fz, Ga, Lp, El, Cc
5189	Unit 15 Waterfowl Lake	WF	5, 1987	Fz, Ga, Cl, Lp, El, Lc, Cc
5206	Unit 6 Waterfowl Lake	WF	5, 1987	Fz, Ga, Lp, El
5221	Sinkhole 20	SH	5, 1987	Ga, Gn, Cl
30006	Figure Eight Lakes	SH	6, 1988	**Unknown**
43659	Sinkhole 20	SH	5, 1999	Ga, Gn, Cl
43661	Sinkhole 37	SH	5, 1999	Fz, Gn
43664	Sinkhole 7	SH	5, 1999	Fz, Gn
43668	Bitter Creek, weir	BL	11, 1999	Ga, Gn, De, Lp, El
44649	Bitter Creek, weir.	BL	5, 1999	Ga, Gn, De, Lp, El
44652	Bitter Creek, confluence	BL	11, 1999	Ga, Gn, De, Lp, El
44656	Sago Spring	BL	11, 1999	Gn, De, Lp, El
44659	Sinkhole 32	SH	11, 1999	Ga, Gn, De, Lp
46795	Sinkhole 20	SH	4, 2000	Ga, Gn, Cl
46815	Sinkhole 20	SH	5, 2000	Ga, Gn, Cl
46816	Sinkhole 37	SH	5, 2000	Fz, Gn
46818	Sinkhole 7	SH	5, 2000	Fz, Gn
46821	Bitter Creek, weir.	BL	5, 2000	Ga, Gn, De, Lp, El
46831	Sinkhole 32	SH	5, 2000	Ga, Gn, De, Lp
46836	Sinkhole 20	SH	6, 2000	Ga, Gn, Cl
46859	Sinkhole 20	SH	7, 2000	Ga, Gn, Cl
46904	Sinkhole 20	SH	9, 2000	Ga, Gn, Cl
46928	Sinkhole 20	SH	10, 2000	Ga, Gn, Cl
46930	Sinkhole 37	SH	10, 2000	Fz, Gn
46933	Sinkhole 7	SH	10, 2000	Fz, Gn

Appendix 1. *Continued*

Cat#	*Locality*	*Habitat*	*Mon./Year*	*Likely Co-Occurring Species*
46951	Sinkhole 20	SH	11, 2000	Ga, Gn, Cl
46954	Sinkhole 7	SH	11, 2000	Fz, Gn
46958	Bitter Creek, weir.	BL	11, 2000	Ga, Gn, De, Lp, El
46961	Bitter Creek, confluence	BL	11, 2000	Ga, Gn, De, Lp, El
46964	Sinkhole 32	BL	11, 2000	Ga, Gn, De, Lp
49238	Lea Lake outflow	SH	4, 2002	Fz, Ga, Lp, Am
49725	Figure Eight Lakes, upper	SH	6, 2003	Fz
55269	Unit 2 Waterfowl Lake	WF	10, 1999	**Unknown**
55270	Unit 3 Waterfowl Lake	WF	10, 1999	Ga, Lp, Dc, Cc
55273	Unit 5 Waterfowl Lake	WF	10, 1999	Fz, Ga, Lp, El, Cc
55281	Unit 6 Waterfowl Lake	WF	10, 1999	Fz, Ga, Lp, El
55288	Unit 7 Waterfowl Lake	WF	10, 1999	Fz, Ga, Cl, Lp, El, Lc, Cc
55294	Unit 16 Waterfowl Lake	WF	10, 1999	Fz, Ga, Lp,
55305	Oxbow 1, east of Unit 3 and 5	BLM	10, 1999	**Unknown**
56946	Unit 7 Waterfowl Lake	WF	10, 2001	Fz, Ga, Cl, Lp, El, Lc, Cc
56954	Unit 7 Waterfowl Lake	WF	10, 2001	Fz, Ga, Cl, Lp, El, Lc, Cc
56956	Unit 6 Waterfowl Lake	WF	10, 2001	Fz, Ga, Lp, El
56958	Unit 3 Waterfowl Lake	WF	10, 2001	Ga, Lp, Dc, Cc
56962	Oxbow 1, Unit 3	BLM	10, 2001	Ga, Lp, Dc, Cc
62527	Unit 3 Waterfowl Lake	WF	10, 2002	Ga, Lp, Dc, Cc
62539	Figure Eight Lakes, upper	SH	7, 1994	Fz
62643	Figure Eight Lakes, upper	SH	12, 1994	Fz
78596	Sinkhole 37	SH	5, 1987	Fz, Gn
79478	Sinkhole 20	SH	10, 1998	Ga, Gn, Cl
79479	Sinkhole 19	SH	10, 1998	**Unknown**
79482	Sinkhole 16	SH	10, 1998	**Unknown**
79493	Mirror Lake, sinkhole	SH	10, 1998	Fz, Ga, Gn
79496	Figure Eight Lakes, upper	SH	10, 1998	Fz
85034	Figure Eight Lakes, lower	SH	12, 1994	**Unknown**

Appendix 2. P-values (below horizontal) and pairwise Procrustes distances (above horizontal) from a randomization test performed simultaneously with a MANOVA comparing head morphology across localities. Bolded P-values values are significant (α=0.05)

	BCc	*BCw*	*F8L*	*F8Ll*	*F8Lu*	*Lea Lake outflow*
BCc		0.0164	0.0417	0.0335	0.0449	0.0420
BCw	**0.0108**		0.0373	0.0392	0.0472	0.0389
F8L	**0.0001**	**0.0001**		0.0424	0.0353	0.0295
F8Ll	**0.0001**	**0.0001**	**0.0001**		0.0361	0.0424
F8Lu	**0.0001**	**0.0001**	**0.0001**	**0.0001**		0.0440
Lea Lake outflow	**0.0001**	**0.0001**	**0.0002**	**0.0001**	**0.0001**	
Marsh Outflow, weir	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Mirror Lake, sinkhole	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Oxbow 1, E of U 3/5	**0.0003**	**0.0001**	**0.0001**	**0.0004**	**0.0001**	**0.0001**
Oxbow 1, Unit 3	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sago Spring	0.1356	**0.0224**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 16	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 19	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 20	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 31	**0.0001**	**0.0002**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 32	0.0991	**0.0002**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 37	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 7	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U15	**0.0002**	**0.0021**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U16	**0.003**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U17	**0.0031**	**0.0013**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U2	**0.0298**	**0.0011**	**0.0001**	**0.0015**	**0.0001**	**0.0001**
U3	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U5	**0.0002**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U6	**0.0098**	**0.0056**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U7	**0.0004**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U 15/16	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

Appendix 2. Continued

	Marsh Outflow, weir	*Mirror Lake, sinkhole*	*Oxbow 1, E of U 3/5*	*Oxbow 1, Unit 3*	*Sago Spring*
BCc	0.0542	0.0409	0.0272	0.0503	0.0147
BCw	0.0449	0.0474	0.0301	0.0460	0.0148
F8L	0.0575	0.0421	0.0296	0.0413	0.0362
F8Ll	0.0692	0.0453	0.0279	0.0554	0.0353
F8Lu	0.0740	0.0408	0.0324	0.0432	0.0440
Lea Lake outflow	0.0681	0.0513	0.0350	0.0471	0.0396
Marsh Outflow, weir		0.0716	0.0641	0.0585	0.0495
Mirror Lake, sinkhole	**0.0001**		0.0316	0.0616	0.0377
Oxbow 1, E of U 3/5	**0.0001**	**0.0001**		0.0465	0.0258
Oxbow 1, Unit 3	**0.0001**	**0.0001**	**0.0001**		0.0497
Sago Spring	**0.0001**	**0.0001**	**0.0005**	**0.0001**	
Sinkhole 16	**0.0001**	0.142	**0.0002**	**0.0001**	**0.0001**
Sinkhole 19	**0.0001**	0.098	**0.0001**	**0.0001**	**0.0001**
Sinkhole 20	**0.0001**	**0.0142**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 31	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0007**
Sinkhole 32	**0.0001**	**0.0001**	**0.0011**	**0.0001**	0.0955
Sinkhole 37	**0.0001**	**0.0055**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 7	**0.0001**	**0.0011**	**0.0001**	**0.0001**	**0.0001**
U15	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0012**
U16	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0002**
U17	**0.0001**	**0.0001**	**0.0004**	**0.0001**	**0.0119**
U2	**0.0001**	**0.0001**	**0.0414**	**0.0001**	**0.0195**
U3	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0002**
U5	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U6	**0.0001**	**0.0001**	**0.0155**	**0.0001**	0.0914
U7	**0.0001**	**0.0001**	**0.0006**	**0.0001**	**0.0001**
U 15/16	**0.0001**	**0.0001**	**0.0001**	**0.0002**	**0.0001**

Appendix 2. Continued

	Sinkhole 16	*Sinkhole 19*	*Sinkhole 20*	*Sinkhole 31*	*Sinkhole 32*
BCc	0.0410	0.0472	0.0387	0.0299	0.0143
BCw	0.0468	0.0506	0.0419	0.0253	0.0183
F8L	0.0358	0.0426	0.0359	0.0466	0.0361
F8Ll	0.0431	0.0549	0.0449	0.0470	0.0320
F8Lu	0.0361	0.0429	0.0329	0.0497	0.0373
Lea Lake outflow	0.0473	0.0552	0.0447	0.0549	0.0372
Marsh Outflow, weir	0.0714	0.0732	0.0699	0.0427	0.0566
Mirror Lake, sinkhole	0.0193	0.0201	0.0187	0.0500	0.0363
Oxbow 1, E of U 3/5	0.0306	0.0390	0.0296	0.0419	0.0239
Oxbow 1, Unit 3	0.0553	0.0632	0.0567	0.0561	0.0490
Sago Spring	0.0389	0.0428	0.0355	0.0264	0.0138
Sinkhole 16		0.0216	0.0221	0.0530	0.0366
Sinkhole 19	**0.0497**		0.0190	0.0520	0.0407
Sinkhole 20	**0.0007**	**0.0063**		0.0444	0.0301
Sinkhole 31	**0.0001**	**0.0001**	**0.0001**		0.0277
Sinkhole 32	**0.0001**	**0.0001**	**0.0001**	**0.0004**	
Sinkhole 37	**0.0001**	**0.0051**	**0.0149**	**0.0001**	**0.0001**
Sinkhole 7	**0.0001**	**0.0012**	**0.0001**	**0.0001**	**0.0001**
U15	**0.0001**	**0.0001**	**0.0001**	0.5252	**0.0005**
U16	**0.0001**	**0.0001**	**0.0001**	**0.0055**	**0.0006**
U17	**0.0001**	**0.0001**	**0.0001**	0.3635	**0.0024**
U2	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0057**
U3	**0.0001**	**0.0001**	**0.0001**	**0.0005**	**0.0001**
U5	**0.0001**	**0.0001**	**0.0001**	**0.0027**	**0.0005**
U6	**0.0001**	**0.0001**	**0.0001**	**0.0002**	**0.0034**
U7	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0005**
U 15/16	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

Appendix 2. Continued

	Sinkhole 37	*Sinkhole 7*	*U15*	*U16*	*U17*	*U2*
BCc	0.0404	0.0435	0.0324	0.0203	0.0243	0.0202
BCw	0.0420	0.0447	0.0250	0.0201	0.0231	0.0217
F8L	0.0362	0.0360	0.0440	0.0455	0.0413	0.0335
F8Ll	0.0466	0.0512	0.0502	0.0366	0.0360	0.0271
F8Lu	0.0345	0.0332	0.0500	0.0444	0.0440	0.0400
Lea Lake outflow	0.0456	0.0477	0.0508	0.0491	0.0501	0.0334
Marsh Outflow, weir	0.0660	0.0648	0.0428	0.0551	0.0474	0.0555
Mirror Lake, sinkhole	0.0207	0.0242	0.0509	0.0500	0.0444	0.0424
Oxbow 1, E of U 3/5	0.0329	0.0355	0.0422	0.0324	0.0320	0.0210
Oxbow 1, Unit 3	0.0564	0.0489	0.0536	0.0520	0.0526	0.0448
Sago Spring	0.0355	0.0392	0.0282	0.0223	0.0214	0.0201
Sinkhole 16	0.0258	0.0266	0.0519	0.0510	0.0460	0.0415
Sinkhole 19	0.0200	0.0226	0.0510	0.0541	0.0490	0.0508
Sinkhole 20	0.0091	0.0171	0.0428	0.0441	0.0408	0.0401
Sinkhole 31	0.0420	0.0452	0.0165	0.0227	0.0166	0.0352
Sinkhole 32	0.0313	0.0357	0.0291	0.0204	0.0234	0.0211
Sinkhole 37		0.0147	0.0404	0.0451	0.0402	0.0420
Sinkhole 7	**0.0003**		0.0437	0.0482	0.0438	0.0444
U15	**0.0001**	**0.0001**		0.0279	0.0235	0.0365
U16	**0.0001**	**0.0001**	**0.0015**		0.0182	0.0245
U17	**0.0001**	**0.0001**	0.0539	0.0597		0.0249
U2	**0.0001**	**0.0001**	**0.0002**	**0.0006**	**0.0068**	
U3	**0.0001**	**0.0001**	**0.0185**	**0.0001**	**0.0018**	**0.0001**
U5	**0.0001**	**0.0001**	**0.0015**	0.0507	**0.01**	**0.0025**
U6	**0.0001**	**0.0001**	**0.0009**	**0.0001**	**0.0164**	0.1799
U7	**0.0001**	**0.0001**	**0.0001**	**0.0017**	**0.0007**	0.0897
U 15/16	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**	**0.0001**

Appendix 2. Continued

	U3	*U5*	*U6*	*U7*	*U 15/16*
BCc	0.0263	0.0229	0.0180	0.0209	0.0565
BCw	0.0186	0.0205	0.0153	0.0222	0.0503
F8L	0.0276	0.0402	0.0286	0.0381	0.0550
F8Ll	0.0404	0.0355	0.0329	0.0265	0.0635
F8Lu	0.0391	0.0387	0.0390	0.0369	0.0625
Lea Lake outflow	0.0389	0.0413	0.0355	0.0360	0.0594
Marsh Outflow, weir	0.0435	0.0557	0.0505	0.0618	0.0578
Mirror Lake, sinkhole	0.0395	0.0475	0.0383	0.0456	0.0791
Oxbow 1, E of U 3/5	0.0272	0.0301	0.0195	0.0233	0.0584
Oxbow 1, Unit 3	0.0420	0.0466	0.0452	0.0486	0.0323
Sago Spring	0.0197	0.0239	0.0140	0.0228	0.0561
Sinkhole 16	0.0393	0.0490	0.0375	0.0447	0.0727
Sinkhole 19	0.0410	0.0513	0.0430	0.0513	0.0812
Sinkhole 20	0.0328	0.0400	0.0339	0.0391	0.0752
Sinkhole 31	0.0241	0.0245	0.0282	0.0333	0.0601
Sinkhole 32	0.0218	0.0198	0.0172	0.0173	0.0584
Sinkhole 37	0.0310	0.0403	0.0351	0.0414	0.0753
Sinkhole 7	0.0324	0.0432	0.0382	0.0455	0.0701
U15	0.0216	0.0273	0.0281	0.0349	0.0585
U16	0.0266	0.0152	0.0217	0.0177	0.0554
U17	0.0220	0.0218	0.0203	0.0247	0.0563
U2	0.0264	0.0232	0.0150	0.0158	0.0511
U3		0.0237	0.0156	0.0278	0.0521
U5	**0.0001**		0.0207	0.0172	0.0542
U6	**0.0028**	**0.0005**		0.0189	0.0524
U7	**0.0001**	**0.0029**	**0.0002**		0.0553
U 15/16	**0.0001**	**0.0001**	**0.0001**	**0.0001**	

Appendix 3. P-values (below horizontal) and pairwise Procrustes distances (above horizontal) from a randomization test performed simultaneously with a MANOVA comparing mandible morphology in males across localities. Bolded P-values values are significant (α=0.05)

	BCc	*BCw*	*F8L*	*F8Ll*	*F8Lu*	*Lea Lake outflow*
BCc		0.0588	0.1598	0.1953	0.1362	0.1242
BCw	**0.0165**		0.1233	0.1461	0.0939	0.0821
F8L	**0.0001**	**0.0001**		0.1033	0.0590	0.0936
F8Ll	**0.0001**	**0.0001**	**0.0008**		0.0781	0.0880
F8Lu	**0.0001**	**0.0001**	**0.0161**	**0.0071**		0.0578
Lea Lake outflow	**0.0001**	**0.0024**	**0.0024**	**0.0185**	0.0552	
Marsh Outflow, weir	0.2508	0.0886	**0.0001**	**0.0001**	**0.0001**	**0.0003**
Mirror Lake, sinkhole	0.0729	0.1845	**0.0002**	**0.0001**	**0.0011**	**0.0494**
Oxbow 1, E of U 3/5	**0.0001**	**0.0032**	**0.0042**	**0.0227**	0.1078	0.9657
Oxbow 1, Unit 3	**0.0005**	**0.0029**	**0.024**	**0.0001**	**0.0102**	**0.0035**
Sago Spring	**0.0027**	0.2009	**0.0006**	**0.0002**	**0.0085**	0.2498
Sinkhole 16	0.1973	0.0898	**0.0002**	**0.0001**	**0.0002**	**0.0045**
Sinkhole 19	0.1932	**0.0096**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 20	**0.0003**	0.1344	**0.0001**	**0.0001**	**0.0001**	**0.0171**
Sinkhole 31	**0.0003**	**0.0106**	**0.0014**	**0.0091**	**0.0333**	0.7580
Sinkhole 32	0.1311	0.2195	**0.0001**	**0.0001**	**0.0001**	**0.0013**
Sinkhole 37	**0.0010**	0.2525	**0.0001**	**0.0001**	**0.0001**	**0.0129**
Sinkhole 7	**0.0011**	0.0593	**0.0001**	**0.0001**	**0.0001**	**0.0103**
U 15	**0.0134**	0.2682	**0.0055**	**0.0037**	0.0577	0.6252
U 16	**0.0002**	**0.0290**	**0.0007**	**0.0003**	**0.0064**	0.2278
U 17	**0.0003**	**0.0048**	**0.0001**	**0.0052**	**0.0026**	0.1809
U 2	**0.0004**	**0.0144**	**0.0006**	**0.0014**	**0.0185**	0.3538
U 3	**0.0001**	**0.0057**	**0.0006**	**0.0001**	**0.0002**	**0.034**
U 5	**0.0001**	**0.0003**	**0.0001**	**0.0109**	**0.0052**	0.3469
U 6	**0.0001**	**0.0054**	**0.001**	**0.0004**	**0.0027**	0.3305
U 7	**0.0001**	**0.0013**	**0.0005**	**0.0005**	**0.0031**	0.5246
U 15/16	**0.0002**	**0.0001**	**0.0039**	**0.0001**	**0.0006**	**0.0007**

Appendix 3. Continued

	Marsh Outflow, weir	*Mirror Lake*	*Oxbow 1, E of U 3/5*	*Oxbow 1, Unit 3*	*Sago Spring*
BCc	0.0486	0.0633	0.1275	0.1080	0.0837
BCw	0.0546	0.0472	0.0815	0.0866	0.0421
F8L	0.1380	0.1181	0.0896	0.0767	0.1003
F8Ll	0.1835	0.1506	0.0856	0.1428	0.1190
F8Lu	0.1219	0.0900	0.0517	0.0739	0.0657
Lea Lake outflow	0.1188	0.0746	0.0243	0.1000	0.0512
Marsh Outflow, weir		0.0701	0.1169	0.0907	0.0806
Mirror Lake, sinkhole	0.0727		0.0761	0.0790	0.0404
Oxbow 1, E of U 3/5	**0.0007**	**0.0399**		0.0947	0.0513
Oxbow 1, Unit 3	**0.0116**	**0.0398**	**0.0086**		0.0788
Sago Spring	**0.0155**	0.5137	0.2513	**0.0283**	
Sinkhole 16	0.2611	0.8409	**0.0044**	**0.0497**	0.1564
Sinkhole 19	**0.0251**	0.0706	**0.0002**	**0.0001**	**0.005**
Sinkhole 20	**0.0097**	0.2066	**0.0135**	**0.0005**	0.3697
Sinkhole 31	**0.0013**	0.0943	0.9378	**0.0057**	0.2174
Sinkhole 32	0.1787	0.4288	**0.001**	**0.0044**	0.0961
Sinkhole 37	0.061	0.2648	**0.018**	**0.0105**	0.3592
Sinkhole 7	**0.041**	0.5114	**0.0137**	**0.0407**	0.2568
U 15	**0.0405**	0.4754	0.6976	0.0674	0.8121
U 16	**0.0047**	0.1632	0.4246	**0.0264**	0.5569
U 17	**0.0006**	**0.0188**	0.374	**0.001**	0.0748
U 2	**0.0062**	0.1449	0.5737	**0.0109**	0.3328
U 3	**0.012**	0.092	0.0585	0.0805	0.2094
U 5	**0.0001**	**0.0027**	0.6218	**0.0003**	**0.0393**
U 6	**0.0016**	0.0879	0.4213	**0.0167**	0.3859
U 7	**0.0003**	**0.0382**	0.6958	**0.0058**	0.245
U 15/16	**0.002**	**0.0082**	**0.0007**	0.6729	**0.0029**

Appendix 3. Continued

	Sinkhole 16	*Sinkhole 19*	*Sinkhole 20*	*Sinkhole 31*	*Sinkhole 32*	*Sinkhole 37*
BCc	0.0520	0.0521	0.0714	0.1245	0.0469	0.0712
BCw	0.0548	0.0724	0.0280	0.0829	0.0354	0.0273
F8L	0.1228	0.1796	0.1166	0.1101	0.1314	0.1067
F8Ll	0.1683	0.2024	0.1344	0.1019	0.1659	0.1370
F8Lu	0.1026	0.1483	0.0842	0.0725	0.1056	0.0811
Lea Lake outflow	0.0958	0.1276	0.0628	0.0379	0.0925	0.0686
Marsh Outflow, weir	0.0534	0.0812	0.0671	0.1182	0.0505	0.0560
Mirror Lake, sinkhole	0.0310	0.0705	0.0420	0.0734	0.0394	0.0416
Oxbow 1, E of U 3/5	0.0967	0.1310	0.0647	0.0291	0.0944	0.0660
Oxbow 1, Unit 3	0.0768	0.1323	0.0893	0.1072	0.0887	0.0754
Sago Spring	0.0578	0.0910	0.0319	0.0589	0.0528	0.0342
Sinkhole 16		0.0696	0.0559	0.0964	0.0386	0.0510
Sinkhole 19	0.0789		0.0744	0.1228	0.0525	0.0837
Sinkhole 20	**0.0457**	**0.0043**		0.0638	0.0375	0.0248
Sinkhole 31	**0.0125**	**0.0008**	0.0533		0.0937	0.0702
Sinkhole 32	0.4551	0.1496	0.0762	**0.0054**		0.0396
Sinkhole 37	0.1073	**0.0021**	0.1292	**0.0354**	0.0883	
Sinkhole 7	0.2675	**0.003**	**0.0338**	**0.033**	0.1558	0.3044
U 15	0.1664	**0.0169**	0.6245	0.7808	0.155	0.5449
U 16	**0.0427**	**0.0001**	0.0529	0.3571	**0.0103**	0.2185
U 17	**0.0025**	**0.0002**	**0.0091**	0.6093	**0.0013**	**0.0106**
U 2	**0.0278**	**0.0006**	**0.0282**	0.5809	**0.0066**	0.0661
U 3	**0.0478**	**0.0001**	**0.0007**	0.0539	**0.0013**	0.0803
U 5	**0.0003**	**0.0001**	**0.0005**	0.7005	**0.0001**	**0.0002**
U 6	**0.0236**	**0.0001**	**0.019**	0.3785	**0.0026**	0.0527
U 7	**0.0045**	**0.0001**	**0.0019**	0.3951	**0.0003**	**0.0136**
U 15/16	**0.0146**	**0.0001**	**0.0001**	**0.0005**	**0.0006**	**0.0004**

Appendix 3. Continued

	Sinkhole 7	*U 15*	*U 16*	*U 17*	*U 2*	*U 3*
BCc	0.0706	0.0962	0.0994	0.1333	0.1075	0.0893
BCw	0.0388	0.0530	0.0529	0.0870	0.0705	0.0496
F8L	0.1031	0.1054	0.0863	0.1254	0.1034	0.0801
F8Ll	0.1408	0.1161	0.1130	0.1085	0.1126	0.1209
F8Lu	0.0772	0.0718	0.0595	0.0915	0.0672	0.0604
Lea Lake outflow	0.0717	0.0448	0.0491	0.0627	0.0493	0.0613
Marsh Outflow, weir	0.0606	0.0887	0.0835	0.1242	0.0975	0.0688
Mirror Lake, sinkhole	0.0340	0.0509	0.0530	0.0896	0.0621	0.0527
Oxbow 1, E of U 3/5	0.0693	0.0422	0.0408	0.0509	0.0408	0.0571
Oxbow 1, Unit 3	0.0635	0.0846	0.0745	0.1260	0.0923	0.0566
Sago Spring	0.0389	0.0357	0.0336	0.0705	0.0475	0.0402
Sinkhole 16	0.0423	0.0692	0.0670	0.1114	0.0801	0.0588
Sinkhole 19	0.0809	0.0981	0.1080	0.1331	0.1167	0.1092
Sinkhole 20	0.0333	0.0364	0.0419	0.0771	0.0598	0.0455
Sinkhole 31	0.0723	0.0416	0.0489	0.0454	0.0447	0.0673
Sinkhole 32	0.0373	0.0637	0.0652	0.1063	0.0827	0.0619
Sinkhole 37	0.0249	0.0403	0.0348	0.0778	0.0560	0.0322
Sinkhole 7		0.0440	0.0406	0.0903	0.0615	0.0359
U 15	0.4621		0.0308	0.0627	0.0478	0.0441
U 16	0.1247	0.8899		0.0603	0.0402	0.0283
U 17	**0.0028**	0.3028	0.1309		0.0501	0.0807
U 2	**0.0373**	0.5611	0.4379	0.4023		0.0522
U 3	0.0635	0.4472	0.4498	**0.0085**	0.0938	
U 5	**0.0002**	0.2324	**0.0436**	0.305	0.2211	**0.0009**
U 6	**0.0226**	0.8388	0.7202	0.0705	0.2434	0.3463
U 7	**0.0049**	0.7436	0.6638	0.1213	0.2956	0.0806
U 15/16	**0.0034**	**0.0134**	**0.001**	**0.0001**	**0.0005**	**0.0022**

Appendix 3. Continued

	U 5	*U 6*	*U 7*	*U 15/16*
BCc	0.1334	0.1015	0.1121	0.1184
BCw	0.0885	0.0591	0.0653	0.1058
F8L	0.1061	0.0848	0.0828	0.0898
F8Ll	0.0833	0.1084	0.1002	0.1592
F8Lu	0.0639	0.0600	0.0553	0.0894
Lea Lake outflow	0.0444	0.0427	0.0352	0.1149
Marsh Outflow, weir	0.1239	0.0866	0.0972	0.1044
Mirror Lake, sinkhole	0.0894	0.0570	0.0636	0.0923
Oxbow 1, E of U 3/5	0.0350	0.0391	0.0301	0.1123
Oxbow 1, Unit 3	0.1139	0.0766	0.0817	0.0382
Sago Spring	0.0638	0.0371	0.0412	0.0964
Sinkhole 16	0.1080	0.0705	0.0811	0.0873
Sinkhole 19	0.1367	0.1127	0.1202	0.1387
Sinkhole 20	0.0730	0.0438	0.0506	0.1050
Sinkhole 31	0.0365	0.0468	0.0447	0.1236
Sinkhole 32	0.1052	0.0710	0.0780	0.0978
Sinkhole 37	0.0797	0.0418	0.0466	0.0948
Sinkhole 7	0.0850	0.0482	0.0545	0.0797
U 15	0.0590	0.0322	0.0353	0.1014
U 16	0.0575	0.0252	0.0254	0.0954
U 17	0.0497	0.0651	0.0576	0.1486
U 2	0.0506	0.0463	0.0425	0.1141
U 3	0.0717	0.0286	0.0374	0.0798
U 5		0.0537	0.0536	0.1324
U 6	0.0531		0.0224	0.0953
U 7	**0.0433**	0.7235		0.1005
U 15/16	**0.0001**	**0.0006**	**0.0003**	

Appendix 4. P-values (below horizontal) and pairwise Procrustes distances (above horizontal) from a randomization test performed simultaneously with a MANOVA comparing mandible morphology in females across localities. Bolded P-values values are significant (α=0.05)

	BCc	*BCw*	*F8L*	*F8Ll*	*F8Lu*	*Lea Lake outflow*
BCc		0.0446	0.1447	0.1530	0.0905	0.1382
BCw	0.1625		0.1138	0.1185	0.0658	0.1037
F8L	**0.0001**	**0.0001**		0.0608	0.0624	0.0793
F8Ll	**0.0001**	**0.0001**	0.1108		0.0815	0.0787
F8Lu	**0.0001**	**0.0001**	**0.0149**	**0.0028**		0.0894
Lea Lake outflow	**0.0001**	**0.0001**	**0.0221**	**0.0317**	**0.0006**	
Marsh Outflow, weir	0.4366	**0.0274**	**0.0001**	**0.0001**	**0.0006**	**0.0001**
Mirror Lake, sinkhole	0.3414	0.3705	**0.0001**	**0.0001**	**0.0013**	**0.0012**
Oxbow 1, E of U 3/5	**0.0001**	**0.0002**	**0.0171**	0.0805	**0.0003**	0.7154
Oxbow 1, Unit 3	**0.0081**	**0.0011**	**0.0001**	**0.0001**	**0.0031**	**0.0001**
Sago Spring	0.2146	0.4749	**0.0002**	**0.0001**	**0.001**	**0.0002**
Sinkhole 16	0.2935	0.092	**0.0001**	**0.0001**	**0.0013**	**0.0002**
Sinkhole 19	0.4781	**0.0284**	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 20	**0.0018**	**0.0218**	**0.0001**	**0.0003**	**0.0001**	**0.0019**
Sinkhole 31	**0.001**	**0.0175**	**0.0036**	**0.0219**	**0.0066**	0.256
Sinkhole 32	0.4231	0.1625	**0.0001**	**0.0001**	**0.0001**	**0.0001**
Sinkhole 37	**0.0101**	0.1301	**0.0001**	**0.0001**	**0.0001**	**0.0003**
Sinkhole 7	0.2166	0.1419	**0.0001**	**0.0001**	**0.0001**	**0.0001**
U15	**0.0079**	0.093	**0.0253**	**0.0163**	0.0613	0.2231
U16	**0.0006**	**0.0083**	**0.0196**	**0.0055**	0.089	**0.0082**
U17	**0.0007**	**0.0166**	**0.006**	**0.0116**	**0.0067**	0.3859
U2	**0.0015**	**0.0109**	**0.0027**	**0.0028**	**0.003**	0.1876
U3	**0.0004**	**0.0015**	**0.0033**	**0.0048**	**0.0041**	**0.0346**
U5	**0.0001**	**0.0006**	**0.0038**	**0.0074**	**0.0057**	0.1853
U6	**0.0031**	**0.0369**	**0.0073**	**0.0025**	0.0608	**0.0043**
U7	**0.0003**	**0.0035**	**0.0021**	**0.0046**	**0.0035**	**0.049**
U 15/16	**0.0053**	**0.0148**	0.0648	**0.0029**	0.3786	**0.0062**

Appendix 4. Continued

	Marsh Outflow, weir	*Mirror Lake, sinkhole*	*Oxbow 1, E of U 3/5*	*Oxbow 1, Unit 3*	*Sago Spring*
BCc	0.0437	0.0528	0.1405	0.0887	0.0459
BCw	0.0642	0.0444	0.1018	0.0887	0.0274
F8L	0.1485	0.1434	0.0787	0.1208	0.1130
F8Ll	0.1575	0.1491	0.0688	0.1449	0.1223
F8Lu	0.0937	0.1003	0.0892	0.0792	0.0651
Lea Lake outflow	0.1506	0.1168	0.0358	0.1556	0.1054
Marsh Outflow, weir		0.0834	0.1526	0.0912	0.0638
Mirror Lake, sinkhole	**0.0401**		0.1184	0.1163	0.0506
Oxbow 1, E of U 3/5	**0.0001**	**0.0013**		0.1569	0.1098
Oxbow 1, Unit 3	**0.0103**	**0.0014**	**0.0001**		0.0788
Sago Spring	0.0522	0.2971	**0.0003**	**0.0095**	
Sinkhole 16	0.0966	0.2414	**0.0002**	0.0784	0.4477
Sinkhole 19	0.0681	0.5174	**0.0001**	**0.0006**	0.053
Sinkhole 20	**0.0007**	0.1032	**0.0043**	**0.0001**	**0.0075**
Sinkhole 31	**0.0002**	**0.0329**	0.3641	**0.0002**	**0.0148**
Sinkhole 32	0.088	0.2509	**0.0001**	**0.0059**	0.5013
Sinkhole 37	**0.0057**	0.1557	**0.0006**	**0.0003**	**0.0442**
Sinkhole 7	0.0533	0.466	**0.0001**	**0.0005**	0.17
U15	**0.0061**	0.0848	0.2387	**0.0012**	0.0509
U16	**0.0014**	**0.0075**	**0.0103**	**0.0019**	**0.0108**
U17	**0.0005**	**0.0488**	0.4954	**0.0003**	**0.0111**
U2	**0.0001**	**0.0463**	0.201	**0.0001**	**0.0056**
U3	**0.0002**	**0.0087**	0.0549	**0.0001**	**0.0012**
U5	**0.0003**	**0.0058**	0.123	**0.0001**	**0.0014**
U6	**0.0028**	**0.0248**	**0.0074**	**0.0029**	**0.0385**
U7	**0.0002**	**0.0186**	0.0836	**0.0001**	**0.0041**
U 15/16	**0.0033**	**0.0119**	**0.0037**	0.0979	0.0576

Appendix 4. Continued

	Sinkhole 16	*Sinkhole 19*	*Sinkhole 20*	*Sinkhole 31*	*Sinkhole 32*	*Sinkhole 37*
BCc	0.0529	0.0432	0.0729	0.1060	0.0362	0.0655
BCw	0.0591	0.0666	0.0356	0.0672	0.0349	0.0304
F8L	0.1343	0.1730	0.1001	0.0930	0.1306	0.1037
F8Ll	0.1528	0.1795	0.0984	0.0819	0.1398	0.1098
F8Lu	0.0886	0.1233	0.0652	0.0742	0.0825	0.0622
Lea Lake outflow	0.1330	0.1556	0.0761	0.0538	0.1261	0.0904
Marsh Outflow, weir	0.0706	0.0723	0.0865	0.1162	0.0571	0.0742
Mirror Lake, sinkhole	0.0636	0.0480	0.0576	0.0867	0.0515	0.0548
Oxbow 1, E of U 3/5	0.1401	0.1582	0.0722	0.0487	0.1275	0.0876
Oxbow 1, Unit 3	0.0734	0.1151	0.1136	0.1342	0.0784	0.1003
Sago Spring	0.0422	0.0659	0.0508	0.0750	0.0291	0.0445
Sinkhole 16		0.0640	0.0843	0.1115	0.0467	0.0705
Sinkhole 19	0.1753		0.0915	0.1224	0.0513	0.0867
Sinkhole 20	**0.0038**	**0.0006**		0.0400	0.0638	0.0267
Sinkhole 31	**0.0015**	**0.0005**	0.26		0.0920	0.0575
Sinkhole 32	0.3055	0.1717	**0.0001**	**0.0013**		0.0572
Sinkhole 37	**0.0214**	**0.0012**	0.088	0.0509	**0.0019**	
Sinkhole 7	0.099	0.0697	**0.0003**	**0.0031**	0.0733	**0.0143**
U15	**0.0173**	**0.0015**	0.5086	0.6366	**0.0059**	0.4828
U16	**0.0065**	**0.0001**	**0.0088**	0.0736	**0.0002**	**0.047**
U17	**0.0036**	**0.0002**	0.2629	0.8028	**0.0004**	0.0969
U2	**0.0028**	**0.0001**	0.16	0.3359	**0.0005**	0.0689
U3	**0.0018**	**0.0001**	**0.0202**	0.2872	**0.0001**	**0.0242**
U5	**0.0004**	**0.0001**	**0.0116**	0.5548	**0.0001**	**0.004**
U6	**0.0202**	**0.0002**	**0.0308**	**0.0458**	**0.0025**	0.0774
U7	**0.0026**	**0.0001**	**0.0361**	0.3868	**0.0001**	**0.0239**
U 15/16	**0.0495**	**0.0006**	**0.0035**	**0.0201**	**0.0085**	**0.0104**

Appendix 4. Continued

	Sinkhole 7	*U15*	*U16*	*U17*	*U2*	*U3*
BCc	0.0415	0.0972	0.0940	0.1055	0.1025	0.0950
BCw	0.0320	0.0612	0.0582	0.0672	0.0701	0.0565
F8L	0.1305	0.0869	0.0668	0.0902	0.0954	0.0734
F8Ll	0.1382	0.0935	0.0826	0.0880	0.0991	0.0771
F8Lu	0.0781	0.0656	0.0413	0.0745	0.0788	0.0493
Lea Lake outflow	0.1176	0.0616	0.0800	0.0475	0.0584	0.0623
Marsh Outflow, weir	0.0585	0.1044	0.0965	0.1198	0.1186	0.1034
Mirror Lake, sinkhole	0.0409	0.0814	0.0914	0.0815	0.0813	0.0841
Oxbow 1, E of U 3/5	0.1180	0.0604	0.0772	0.0435	0.0571	0.0579
Oxbow 1, Unit 3	0.0941	0.1240	0.0920	0.1323	0.1353	0.1103
Sago Spring	0.0369	0.0719	0.0628	0.0765	0.0821	0.0656
Sinkhole 16	0.0578	0.0998	0.0875	0.1051	0.1078	0.0947
Sinkhole 19	0.0579	0.1192	0.1209	0.1222	0.1216	0.1198
Sinkhole 20	0.0530	0.0372	0.0509	0.0403	0.0448	0.0367
Sinkhole 31	0.0807	0.0422	0.0609	0.0326	0.0499	0.0417
Sinkhole 32	0.0401	0.0891	0.0803	0.0940	0.0969	0.0848
Sinkhole 37	0.0419	0.0395	0.0440	0.0519	0.0542	0.0391
Sinkhole 7		0.0742	0.0755	0.0823	0.0827	0.0738
U15	**0.0279**		0.0421	0.0324	0.0325	0.0240
U16	**0.0003**	0.5205		0.0566	0.0628	0.0275
U17	**0.0022**	0.8854	0.1094		0.0296	0.0340
U2	**0.0021**	0.871	0.0529	0.8755		0.0417
U3	**0.0001**	0.9446	0.4749	0.5138	0.2699	
U5	**0.0003**	0.7084	**0.0409**	0.4824	0.3447	0.1927
U6	**0.0008**	0.332	0.643	0.1003	0.0603	0.1639
U7	**0.0001**	0.6464	0.228	0.5317	0.1236	0.4721
U 15/16	**0.0043**	0.0783	0.1888	**0.0245**	**0.0123**	**0.0316**

Appendix 4. Continued

	U5	*U6*	*U7*	*U 15/16*
BCc	0.1035	0.0819	0.0965	0.0961
BCw	0.0707	0.0486	0.0565	0.0734
F8L	0.0794	0.0732	0.0755	0.0687
F8Ll	0.0814	0.0894	0.0809	0.1021
F8Lu	0.0585	0.0438	0.0529	0.0395
Lea Lake outflow	0.0509	0.0840	0.0612	0.0969
Marsh Outflow, weir	0.1122	0.0903	0.1064	0.1032
Mirror Lake, sinkhole	0.0936	0.0798	0.0795	0.1018
Oxbow 1, E of U 3/5	0.0553	0.0805	0.0560	0.1014
Oxbow 1, Unit 3	0.1242	0.0859	0.1114	0.0674
Sago Spring	0.0758	0.0545	0.0626	0.0648
Sinkhole 16	0.1081	0.0772	0.0917	0.0809
Sinkhole 19	0.1285	0.1074	0.1157	0.1233
Sinkhole 20	0.0492	0.0445	0.0374	0.0798
Sinkhole 31	0.0360	0.0656	0.0389	0.0866
Sinkhole 32	0.0949	0.0676	0.0819	0.0794
Sinkhole 37	0.0586	0.0404	0.0423	0.0735
Sinkhole 7	0.0816	0.0699	0.0723	0.0832
U15	0.0357	0.0497	0.0362	0.0780
U16	0.0539	0.0272	0.0361	0.0522
U17	0.0384	0.0574	0.0348	0.0844
U2	0.0429	0.0622	0.0516	0.0899
U3	0.0360	0.0369	0.0246	0.0660
U5		0.0612	0.0457	0.0716
U6	**0.0142**		0.0389	0.0521
U7	0.0735	0.1599		0.0647
U 15/16	**0.031**	0.1873	**0.0434**	